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Valores hormonales en el síndrome de ovario poliquístico

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DEDICATORIA

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RESUMEN

El síndrome de ovario poliquístico (SOP) es una patología común en las mujeres que se encuentran en la edad reproductiva, entre sus manifestaciones clínicas están los ciclos menstruales irregulares, hirsutismo, acné y alopecia androgénica; presentan una relación con factores como la resistencia a la insulina, factores hereditarios, diabetes mellitus tipo 2, sobrepeso y obesidad. El propósito principal de esta investigación se basó en estimar los valores hormonales en el síndrome de ovario poliquístico para el diagnóstico oportuno. Esta fue de carácter descriptivo, documental, no experimental, de tipo transversal y retrospectivo. Se obtuvo una muestra de 54 artículos científicos de diferentes bases de datos científicas como Pubmed, Google Académico, ScienceDirect, Elsevier, Scielo, los que fueron recopilados, analizados y organizados en tablas. Los datos más significativos se presentaron así, se encontró que el fenotipo A en conjunto con los ciclos menstruales irregulares y las alteraciones hormonales se presentan en mayor frecuencia en las pacientes, en cuanto a los factores de riesgo, el sobrepeso y obesidad tiene una alta probabilidad de desarrollar el síndrome de ovario poliquístico. Por otro lado, los valores hormonales evidenciaron que la hormona testosterona total posee gran relevancia a comparación de las demás y entre las manifestaciones clínicas que comúnmente se pueden observar en mujeres con síndrome de ovario poliquístico están los ciclos menstruales irregulares, el hirsutismo, el acné y la alopecia androgénica. En conclusión, los datos recabados ayudaron a determinar que los valores de ciertas hormonas tienen gran importancia clínica para el diagnóstico oportuno de síndrome de ovario poliquístico.

Palabras clave: síndrome de ovario poliquístico, SOP, fenotipos, valores hormonales, ciclos menstruales irregulares.

ABSTRACT

Polycystic ovary syndrome (PCOS) is a common pathology in women of reproductive age; among its clinical manifestations are irregular menstrual cycles, hirsutism, acne, and androgenic alopecia; it is related to factors such as insulin resistance, hereditary factors, type 2 diabetes mellitus, overweight and obesity. The main purpose of this research was based on estimating hormonal values in polycystic ovary syndrome for timely diagnosis. This was a descriptive, documentary, non-experimental, cross-sectional, and retrospective study. A sample of 54 scientific articles was obtained from different scientific databases such as Pubmed, Google Scholar, ScienceDirect, Elsevier, and Scielo, which were collected, analyzed, and organized in tables. The most significant data were presented as follows, it was found that phenotype A together with irregular menstrual cycles and hormonal alterations occur more frequently in patients; as for risk factors, overweight and obesity have a high probability of developing polycystic ovary syndrome. On the other hand, the hormonal values showed that the total testosterone hormone has great relevance compared to the others and among the clinical manifestations that can be commonly observed in women with polycystic ovary syndrome are irregular menstrual cycles, hirsutism, acne, and androgenic alopecia. In conclusion, the data collected helped to determine that the values of certain hormones are of great clinical importance for the timely diagnosis of polycystic ovary syndrome.

Keywords: polycystic ovary syndrome, PCOS, phenotypes, hormone values, irregular menstrual cycles.

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CAPÍTULO I. INTRODUCCION.

El síndrome de ovario poliquístico (SOP) también conocido como hiperandrogenismo ovárico funcional o anovulación crónica hiperandrogénica, es una patología causada por la disfunción endocrino-metabólica la cual presenta una alta prevalencia en la población femenina, esta manifiesta signos y síntomas diversos con origen fisiopatológico variado, por lo tanto, su estudio, diagnóstico, tratamiento y evaluación se torna un tanto difícil[1].

En el año 1935, el SOP fue descrito por Stein y Leventhal, quienes encontraron un vínculo entre el hirsutismo, la obesidad, amenorrea en relación con la oligomenorrea y/o morfología de ovarios poliquísticos (MOP), y se conoció que este síndrome se asocia a un alto riesgo de padecer infertilidad, enfermedades cardiovasculares y alteraciones metabólicas, además para realizar un buen diagnóstico es necesario implementar la exclusión de criterios pertenecientes a otros trastornos endocrinos[2].

Algunas de las características que se presenta en los pacientes con SOP son el aumento de hormona luteinizante (LH) con respecto a la hormona folículo estimulante (FSH), un aumento de la producción de testosterona dependiente de LH en ovarios, profunda resistencia a la insulina, obesidad en ocasiones también se produce acné, hirsutismo, seborrea y alopecia androgénica[3,4].

Para el diagnóstico del SOP hay una variedad de guías que se basan en la combinación de tres elementos como son: la morfología ovárica, disfunción ovulatoria, hiperandrogenismo clínico o bioquímico. También existen varios criterios extra ováricos que están ampliamente relacionados a la fisiopatología de SOP, pero se centran en la disfunción ovárica en cada paciente.

Azziz y colaboradores realizaron un estudio en que señala una prevalencia de 6,6% para paciente pertenecientes a la raza negra y un 4,8% en pacientes de raza blanca, y no existen diferencias estadísticas significativas entre estos grupos[5].

A nivel mundial se considera que la causa más común de hiperandrogenismos se da tanto en las mujeres adolescentes como en las adultas con una incidencia del 20%, aproximadamente

el 75% de las mujeres hirsutas y el 10% de las mujeres premenopáusicas presentan este síndrome[1]. También se conoce que hasta el 70% de las mujeres con SOP pueden presentar oligo-anovulación, además que las cifras por infertilidad por anovulación producida por SOP abarca el 80% de los casos[6].

En un estudio se demostró que en Reino Unido la prevalencia de SOP en las mujeres caucásicas es de aproximadamente el 22%, mientras que en mujeres asiáticas con origen en la India esta prevalencia es de aproximadamente el 52%, tomando en cuenta criterios ecográficos[7]. En España se conoce que existe una prevalencia de 6,5% [5].

Se conoce que América Latina es la región más afectada por el síndrome de ovario poliquístico en donde abarca un índice de 5-10% de la población femenina en etapa fértil. La prevalencia del SOP va a depender de los criterios diagnósticos que se utilicen debido a que cada uno incluye in tipo diferente de fenotipo[4].

En la ciudad de Lima-Perú en el año 2017 se realizó un estudio de 152 historias clínicas en la unidad de ginecología del Hospital Nacional Sergio Enrique Bernales donde se mostró los siguientes resultados: existe un 8,55% de prevalencia del SOP, de los cuales con más frecuencia se da en mujeres de 27-30 años con un porcentaje de 3,90%, mujeres solteras con un 4,60%, mujeres que acuden a la secundaria con un 5,90%, amas de casa un 5,30%, nulíparas 5,30% y pacientes con obesidad tipo 1 con un 3,90%[8].

En Centroamérica como específicamente en Cuba se conoce que la prevalencia del SOP alcanza el 46,2% de acuerdo con un estudio realizado en el Instituto Nacional de Endocrinología de la Habana, en donde 140 mujeres fueron atendidas debido a sospechas de desarrollo de este síndrome. También se conoce que en países de Sudamérica como Chile la prevalencia de desarrollar SOP alcanza el 12%[9].

En la ciudad de Cuenca en el año 2017 se realizó un estudio en donde se mostró los siguientes resultados, la prevalencia de SOP en mujeres de parroquias urbanas analizadas fue de 13,3% en donde su total de residentes fueron 248 personas, también se estableció que uno de los factores asociados es el aumento de esteroides sexuales, especialmente los estrógenos y que otros factores no fueron tan significativos con el mencionado[10].

En el año 2019 se realizó una recolección de datos en el Hospital Regional Docente Ambato sobre pacientes que presentaban SOP desde el año 2015 hasta el 2019, en donde se determinó que la mayor incidencia de este síndrome se da en mujeres jóvenes de entre los 20 a 39 años y seguidas de las mujeres adultas de 40 a 60 años. Estos datos ayudaron a determinar que de las pacientes estudiadas el 73,2% fueron diagnosticadas con SOP quienes se encuentran en los dos grupos mencionados[7].

De acuerdo con los datos encontrados en Ecuador, se menciona que no existen más datos referentes al Síndrome de ovario poliquístico, especialmente en la provincia de Chimborazo y los cantones que lo conforman, debido a falta de estudio o a situaciones que comprometan la falta de recursos en los centros de salud de dichos cantones.

El SOP se ha convertido en una de las patologías más frecuentes en mujeres en la etapa fértil de la vida, esta se produce por un desbalance de las hormonas femeninas, la cual se caracteriza por no presentar sus manifestaciones clínicas de manera simultánea por lo que se dificulta mucho llegar a su diagnóstico, por este motivo es de suma importancia realizar un estudio laboratorial que nos ayude a conocer el estado hormonal femenino y poder controlar o evitar la progresión de signos y síntomas como es el caso del hirsutismo, el acné y sobre todo la dificultad de fecundación la cual puede provocar una infertilidad.

El SOP es un problema a gran escala que afecta de manera significativa a la mujer, en la actualidad a pesar de los avances médicos y tecnológicos se conoce que los criterios para el diagnóstico de este síndrome no son específicos y esto impide que las mujeres que padecen o presentan sospecha del problema no tengan una valoración y tratamiento adecuado. Además, en los periodos de la adolescencia y la perimenopausia, la evaluación médica es más compleja debido a que se debe realizar un abordaje diferente.

Entre las patologías que se conoce con frecuencia en la consulta ginecológica es el desarrollo de SOP o la sospecha de este, este síndrome presenta un alto impacto en la salud de las mujeres quienes lo padecen y por ende en su estilo de vida cotidiano, esta disfunción ovárica es causada por un sinnúmero de factores como son hormonales, genéticos, patológicos, entre otros, además tanto la clínica como la edad en la que empieza a desarrollarse se puede decir que presenta un abordaje diagnóstico complejo de mujer a mujer.

Lo expuesto hace notar que entre las consecuencias que se presenta debido a la alteración de las hormonas y la predisposición a ciertos factores en el caso del SOP están: la infertilidad, el hirsutismo, disfunción menstrual, anormalidades ováricas, obesidad, desarrollo de diabetes mellitus 2, resistencia a la insulina, cáncer de endometrio, etc[3]. De esta manera nos planteamos la siguiente interrogante de investigación:

¿Cuáles son los valores hormonales alterados que conducen al desarrollo del SOP?

El SOP es un trastorno de gran importancia en la población femenina a partir de la edad reproductiva, ya que afecta a la calidad de vida de las mujeres que lo padecen, es decir, a su aspecto físico, al nivel hormonal, con un sinnúmero de manifestaciones clínicas como el hirsutismo, la anovulación, la resistencia a la insulina, etc. y en el nivel de vida que tiene las mujeres en su entorno social, sin infravalorar aspectos como la fertilidad, en donde el instinto maternal juega un gran papel.

El motivo esencial para conocer los valores hormonales que se asocian al desarrollo de SOP es debido a la aparición de enfermedades o problemas de salud que aparecen cuando la mujer no tiene un control adecuado de síndrome, es decir es importante conocer si los valores hormonales han aumentado o han disminuido cuando la persona diagnosticada con SOP presenta un tratamiento prescrito por un médico endocrinólogo, ginecólogo o médico tratante, debido que de esta manera se conocerá si es efectivo el tratamiento o es necesario el cambio de los medicamentos.

Los malos hábitos alimenticios y la falta actividad física son algunos de los factores que intervienen directa o indirectamente en el desarrollo de SOP, es decir, hormonas como los estrógenos, testosterona, etc. se verán afectadas y provocaran la aparición de este síndrome, por lo mismo, el análisis hormonal en las pacientes con sospecha de SOP o ya diagnosticadas deben realizarse de manera regular de dos a tres meses, dependiendo las indicaciones del médico.

Entre los diferentes métodos de diagnósticos existentes para conocer si una paciente presenta el desarrollo de SOP, tanto en estadios tempranos o avanzados junto a sus manifestaciones clínicas, se encuentran el examen físico, en donde se evidencia el desarrollo de vello en

ciertas zonas del cuerpo donde no es característico, el examen de laboratorio en el mismo que se puede evidenciar el desarrollo de las hormonas y también el aumento o disminución de ciertos valores bioquímicos, para complementación están los exámenes de imagenología en donde se puede comprobar si los ovarios presentan quistes, la cantidad y el tamaño de estos.

OBJETIVOS

General

- Estimar los valores hormonales en el síndrome de ovario poliquístico para su diagnóstico oportuno por medio de revisión bibliográfica.

Específicos

- Describir los fenotipos de síndrome de ovario poliquístico para reconocer el más frecuente e identificar sus causas mediante revisión bibliográfica.
- Destacar factores de riesgo que pueden conllevar al desarrollo del síndrome de ovario poliquístico para el diagnóstico oportuno mediante la búsqueda de información bibliográfica.
- Comparar datos de valores hormonales normales y alterados para que sirva como guía diagnóstica mediante revisión bibliográfica.

CAPÍTULO II. MARCO TEÓRICO

SÍNDROME DE OVARIO POLIQUISTICO

Definición

El síndrome de ovario Poliquístico (SOP) conocido también como anovulación crónica hiperandrogénica o hiperandrogenismo ovárico funcional es un trastorno endocrino complejo debido a que su aparición se da por la interacción de variantes genéticas así también como factores ambientales[11]. Este síndrome afecta a aproximadamente al 6-10% de las mujeres en edad fértil y las manifestaciones clínicas que se presentan son muy variadas[12,13].

Etiología

Se conoce que la etiología referente al SOP es multifactorial, es decir, para la aparición de este síndrome se pueden incluir procesos metabólicos, suprarrenales, neurológicos, ováricos y ambientales [14,15]. Las causas ambientales y genéticas todavía no se han podido establecer las más precisas o una específica por lo que se menciona que esté síndrome presenta etiología incierta. Uno de los factores en común que presentan las mujeres con este síndrome es la alteración de la concentración de los andrógenos desde etapas muy tempranas[4].

Fisiopatología

Debido a que la etiología compleja del SOP y a los factores que afectan al desarrollo de este se destacan tres tipos de alteraciones que se relacionan entre sí, estos son:

a) **Disfunción neuroendocrina:** es la primera anomalía que se relaciona al desarrollo de SOP, se caracteriza por la alteración a nivel del eje hipotálamo-hipófisis-ovario generándose de esta manera un aumento de la producción de hormona luteinizante (LH), y una secreción de hormona folículo estimulante (FSH) en concentraciones normales o disminuidas[12]. Estas alteraciones se dan debido a un aumento en la amplitud y frecuencia de pulsos de LH provocándose así una elevación de pulsos de la hormona liberadora de gonadotropina (GnRH) y por consiguiente un exceso de andrógenos circulantes[15,16].

b) **Trastorno metabólico:** se da debido a presentar una resistencia a la insulina la cual provoca una hipersecreción de esta y a la vez la insulina estimula la producción de andrógenos, esto se debe a que los ovarios poliquísticos son sensibles o hipersensibles a la insulina[15,16]. El SOP también es asociado con trastornos del perfil lipídico como es el caso de la dislipidemia aterogénica, así también presentan más probabilidad de presentar Diabetes mellitus 2[12].

c) **Disfunción de la esteroidogénesis y foliculogénesis ovárica:** se da una alteración en la síntesis de los andrógenos tanto en el ovario como en las glándulas suprarrenales, es decir , se da un aumento de los andrógenos ováricos y como consecuencia se altera el desarrollo folicular y la ovulación[12,15]. Entre las alteraciones están la oligo/anovulación, hiperandrogenismos, ovarios poliquísticos[16].

Manifestaciones clínicas

Entre las manifestaciones clínicas que se ven implicadas en el síndrome de ovario poliquístico están el hirsutismo, la perdida de cabello, la disfunción ovárica, el hiperandrogenismo, entre otras. Además, en las mujeres que están en la etapa de la adolescencia pueden presentar cambios o trastornos psiquiátricos como la ansiedad y la depresión (Anexo 1)[17,18].

a) **Hirsutismo:** es conocido como el exceso de pelo o vello especialmente en las zonas dependientes de andrógenos, esto mostraría la interacción entre el exceso de los andrógenos, la sensibilidad de los folículos pilosos a los andrógenos y la concentración local. La escala de Ferriman-Gallwey es utilizada para la valoración de las mujeres con sospecha de SOP (Anexo 2)[19].

b) **Hiperandrogenismo:** es el aumento de las concentraciones tanto en testosterona libre, testosterona libre y androstenediona. Entre algunas de las manifestaciones debido al hiperandrogenismo están el acné, pero solo en casos severos, así también la alopecia androgénica [18,19].

c) **Disfunción ovulatoria:** se conoce que los ciclos en los primeros años después de la menarquia son anovulatorios y también presenta irregularidades menstruales, pero cuando estas irregularidades persisten, es decir se da una oligomenorrea o ciclos menstruales de más de 35 días, menorrea secundaria o ausencia del ciclo menstrual por más de 3 meses, sangrado uterino disfuncional, menorrea es necesaria una investigación por alteración de las concentraciones de andrógenos[19].

d) **Morfología poliquística ovárica:** debido a que muchas de las mujeres con SOP son asintomáticas se deben realizar estudios ecográficos para determinar la presencia de ovarios poliquísticos, la presencia de 12 o más folículos de 2-9 mm se define como presencia de ovarios poliquísticos [19].

e) **Alopecia androgénica:** es conocida como la pérdida progresiva del cabello, misma que se da de manera común en los hombres, pero es infradiagnosticada en las mujeres especialmente en aquella que presentan SOP. La presencia de esta manifestación clínica se debe a la concentración elevada de andrógenos en la sangre y también puede estar acompañada de una predisposición familiar[20].

f) **Sobrepeso y obesidad:** esta manifestación clínica se presenta en casi el 50% de las mujeres con SOP, por lo que contribuye al desarrollo de la resistencia a la insulina, el hiperandrogenismo, los trastornos menstruales así también como a las comorbilidades asociadas. Para la prevención del sobrepeso y la obesidad puede variar debido a muchos factores como: la etnia, el fenotipo que haya desarrollado cada mujer y los hábitos diarios[21,22].

g) **Acné:** es conocido como un desorden causado por la inflamación del folículo piloso y la glándula sebácea y apocrina asociada. En las mujeres con SOP existe un incremento de secreción sebácea y esto se puede presenciar en un tercio de las mujeres con dicho síndrome. A diferencia de otras manifestaciones clínicas, los metabolitos androgénicos que están en la sangre no se encuentran necesariamente elevados[20].

FENOTIPOS DEL SÍNDROME DE OVARIO POLIQUÍSTICO

Para el diagnóstico de SOP es necesario un diagnóstico en conjunto con los exámenes de imagenología, de laboratorio y también se toma en cuenta las manifestaciones clínicas y examen físico de la paciente. En cuanto a la clasificación del SOP se menciona que los criterios más claros sobre el SOP son los de Rotterdam del 2003, en donde se encuentra descrito 4 fenotipos que se basan en ciertos hallazgos clínicos, además esto ayuda a la dirección del diagnóstico final y al tratamiento. Estos fenotipos son[11,23]:

- **Subfenotipo A:** las mujeres presentan disfunción ovulatoria, morfología ovárica poliquística al ultrasonido e hiperandrogenismo.
- **Subfenotipo B:** las mujeres presentan disfunción ovulatoria e hiperandrogenismo.
- **Subfenotipo C:** se presenta hiperandrogenismo, y morfología ovárica poliquística.
- **Subfenotipo D:** se presenta morfología ovárica poliquística y disfunción ovulatoria.

Los fenotipos A y B son considerados clásicos y estos se caracterizan por presentar una mayor presencia de los signos y síntomas tal como: resistencia a la insulina, obesidad, disfunción menstrual y riesgo de síndrome metabólico. El fenotipo C también es conocido como SOP ovulatorio en el que se presentan síntomas intermedios entre los fenotipos clásicos y el fenotipo D o no hiperandrogénico, este último se diferenciara de los demás debido a que se presentan niveles hormonales más bajos y también fisiopatologías diferentes tales como baja presentación de diabetes gestacional, obesidad, hipertensión, etc[11].

HORMONAS QUE INTERVIENEN EN EL SOP

Entre las hormonas que intervienen en el eje hipotálamo-hipofisario-gonadal están las siguientes (Anexo 3):

Hormona liberadora de gonadotropina

La hormona liberadora de gonadotropina (GnRH) es conocida como el regulador principal en el eje hipotálamo-hipofisario-gonadal. La secreción de manera pulsátil de esta hormona

regula el ciclo menstrual, es decir regula la secreción de las hormonas luteinizante (LH) y de la hormona folículo estimulante (FSH), también regula la función endócrina y la maduración de los gametos. La GnRH es secretada desde el hipotálamo hacia la hipófisis y actuara en esta producción la secreción de LH y FSH[24,25].

Hormona luteinizante

La hormona luteinizante (LH) es conocido por ser la hormona que estimula la secreción de esteroides sexuales, también ayuda en la maduración y folicular. Se conoce que la LH estimula la síntesis de andrógenos en las células teca, e induce la latinización sobre las células de la granulosa y la síntesis de progesterona[24,25].

Hormona folículo estimulante

Se describe que la hormona folículo estimulante (FSH) está encargada del desarrollo y la maduración de los folículos ováricos, esta hormona actúa en las células granulosos de los ovarios, que a su vez estimula la esteroidogénesis[24,25].

Prolactina

La prolactina es considerada una hormona multifactorial que es secretada por la glándula pituitaria, mediante el eje hipotálamo-pituitario-suprarrenal, esta ayuda en el desarrollo y crecimiento de las glándulas mamarías, la síntesis y mantenimiento de la secreción de leche, etc[26,27]. Se conoce también que ciertos fármacos como antidepresivos, medicamentos antieméticos y antipsicóticos son los responsables del aumento de la prolactina y se lo conoce como hiperprolactinemia[28].

Testosterona

La testosterona es conocida por ser una hormona tanto femenina como masculina, que actúa como un andrógeno y en el caso de las mujeres es un precursor obligatorio de la biosíntesis

del estradiol. Presenta efectos fisiológicos tanto en tejidos reproductivos y no reproductivos femeninos. Esta hormona es producida en los ovarios y en las glándulas suprarrenales y es el primer marcador de SOP[29].

Se considera que la testosterona es un andrógeno importante en cuanto al diagnóstico de hirsutismo y SOP, debido a que esta hormona y también la Dihidrotestosterona (DHT) influyen en las alteraciones del vello corporal y en el ciclo de este, es decir, causa que este vello se torne más oscuro y espeso en ciertas zonas en las que actúan los andrógenos por ejemplo el rostro, tórax, pubis y cuello. Se menciona que el análisis más sensible al momento de la detección de hiperandrogenismo en mujeres con sospecha de SOP es la testosterona libre[30].

Androstenediona

Este andrógeno presenta un origen ovárico y se conoce que puede ser el único andrógeno que se eleva en las mujeres que presentan SOP. Presenta elevación en etapas tardías de la transición menopáusica a comparación con la testosterona, además al no generar variabilidad en los resultados se puede realizar la determinación de esta en un solo tipo de ensayo. Este andrógeno al no ser de primera línea puede ayudar a la aclaración de ciertas dudas diagnósticas[31].

En las mujeres la androstenediona se convierte en estrógenos y los niveles normales de esta hormona oscilan entre 26 – 214 ng/dL. Los valores superiores a estos los presentan las mujeres que desarrollaron SOP[30].

Cortisol

El cortisol es una hormona que se sintetiza del colesterol y es metabolizado en el hígado y excretado en la orina, este no se ve afectado por el estadio puberal, ni por la edad de los pacientes. Por otro lado, el estrés tanto físico como mental activa el eje hipotálamo-hipófisis-adrenal por medio de la hormona adrenocorticótropa y como consecuencia de presenta el

aumento del cortisol[32]. El cortisol al ser estimulado por medio del estrés este aumenta en la producción de andrógenos y además forma quistes ováricos[30].

El cortisol por ser conocido como la hormona del estrés se conoce en mujeres adolescentes la afectación que provoca el estrés es la aparición de hirsutismo y acné. Y en mujeres en edad reproductiva se relaciona con la infertilidad debido a una gran carga de estrés por depresión[30].

Hormona antimülleriana

La hormona antimülleriana (AMH) es una proteína inhibidora de los conductos Müller, esta es producida por los folículos ováricos. Presenta funciones específicas como ser regulador del crecimiento folicular para obtener una respuesta ovárica en mujeres con alteraciones de la fertilidad. Otra función que presenta esta hormona es la influencia de la producción de testosterona por las células de la teca[33,34].

La AMH es utilizada como herramienta complementaria para el diagnóstico de causas de amenorrea secundaria, como es SOP, la falla ovárica precoz y otras disfunciones del eje hipotálamo-hipofisiario. También se puede decir que es un marcador importante de la reserva y función ovárica, el envejecimiento ovárico y la falla de este[34,35].

DIAGNÓSTICO DE LABORATORIO

Indicaciones al paciente

Entre las indicaciones previas y como regla general se encuentran las siguiente[36,37]:

- Realizar ayudo con un mínimo de 8 horas y máximo 10 horas.
-
- No realizar actividad física antes de la toma de muestra, debido a que esta genera cambios en las concentraciones hormonales.
- No fumar antes de la extracción sanguínea.
- Evitar el estrés, ansiedad, fatiga, etc., antes de acudir al laboratorio
- Si el paciente requiere tomar medicamentos, los debe ingerir con normalidad a menos que exista indicaciones contradictorias de parte del médico o el laboratorio.

Indicaciones especificas

Para realizar los análisis hormonales de debe tomar en cuenta ciertas indicaciones de acuerdo con la hormona a analizarse, como son[38–40]:

- Para el análisis de LH y FSH, estradiol, progesterona y testosterona, se recomienda tomar en cuenta el día del ciclo menstrual en el que se encuentra o seguir indicaciones dadas por el médico o el analista clínico.
- Cuando las pacientes presentan ciclos menstruales regulares, la toma de muestra es recomendable hacerlo entre el día 12 y 14 del ciclo.
- Se recomienda realizarse las pruebas de LH, FSH y estradiol en el tercer día del ciclo menstrual.
- El análisis de la hormona progesterona debe realizarse entre el día 21 y el 23 del ciclo menstrual.
- Para conocer la concentración de la hormona antimülleriana, la toma de muestra se puede realizar en cualquier día del ciclo menstrual.
- En cuanto a los niveles de prolactina en sangre es necesario que la paciente no tenga relaciones sexuales el día anterior, también debe permanecer en reposo hasta el momento de la extracción y esta se lo debe realizar antes de las 10 am.

Muestras

Para el análisis de las hormonas antimülleriana, testosterona total, progesterona es necesario la utilización de suero o plasma heparinizado[41–43]. Mientras que para el análisis de FSH, LH y testosterona libre, prolactina se utiliza suero que debe ser recolectado en tubos de venopunción de tapa roja mismos que no contengas anticoagulantes, aditivos o gel separador[44–46].

Una vez recolectada la muestra y separar sus componentes, se debe tomar en cuenta que, si el procedimiento no se lo realizara de manera inmediata, estas deben almacenarse a una temperatura de 2-8°C como máximo por 7 días o -20°C por un máximo de 30 días.

Pruebas de laboratorio

Testosterona total

Cuando se presenta un hiperandrogenismo es recomendable medir los niveles de testosterona total. Los valores normales en suero de testosterona total se encuentran entre 45 – 60 ng/dL, en el caso de presentarse un valor mayor a 150 ng/dL en mujeres podría indicar patologías como hiperandrogenismo, tumores secretores de andrógenos que pueden ser de origen ovario o adrenal[47]. Valores normales en Anexo 4[48].

Principio de la prueba

El análisis de la testosterona total se lo realiza por medio de un ensayo inmunoenzimático competitivo, en el cual se produce una reacción de competencia entre antígeno presente en la muestra y el antígeno presente en el conjugado enzimático al querer unirse al anticuerpo biotinilado limitado que esta inmovilizado en el pocillo, el proceso de decantación ayuda a la eliminación de antígenos no unidos a los anticuerpos. Finalmente, la actividad enzimática en la fracción que se une al anticuerpo resulta ser inversamente proporcional a la concentración del antígeno presente en la muestra del paciente. Ver Anexo 5[42].

Testosterona libre

Este parámetro no es considerado un examen de rutina, pero en algunas literaturas se recomienda realizar la medición de testosterona libre en lugar de la testosterona total sin embargo la testosterona libre tiene una mayor sensibilidad para evidenciar la presencia de hiperandrogenemia por lo que es considerada una prueba complementaria[47]. Se considera que las concentraciones normales de la testosterona libre van desde 0,8 hasta 9,2 pg/mL. Valores normales en Anexo 6[48].

Principio de la prueba

El procedimiento de análisis de la testosterona libre se basa en ser un ensayo inmunoenzimático competitivo, en el cual al mezclarse el anticuerpo inmovilizado, el antígeno del conjugado enzimático y en antígeno presente en la muestra se da una reacción

de competencia por un número limitado de sitios de unión, posterior se realiza un proceso de lavado y decantación para la eliminación de los antígenos no unidos a los anticuerpos, la actividad enzimática en la fracción unida al anticuerpo es inversamente proporcional a la concentración de antígeno presente en la muestra. Ver Anexo 7[46]

Sulfato de dehidroepiandrosterona (S-DHEA)

Esta prueba no es recomendable para pacientes que presenten SOP ya que generalmente no se presenta un aumento del marcador, por otro lado, esta prueba cobra utilidad en carcinomas adrenocorticales donde sus valores se encontraran aumentados, se conoce que los valores normales de esta hormona van desde 35 a 430 µg/dL[47]. Valores normales en Anexo 8[48].

Principio de la prueba

Al igual que muchas hormonas, la prueba de S-DHEA se lo realiza mediante un ensayo inmunoenzimático competitivo, en el que se mezclan el anticuerpo biotinilado inmovilizado en el pocillo, el antígeno del conjugado enzimático y al antígeno de la muestra creando una reacción de competencia por los sitios de unión limitados. Luego se procede a la decantación para la eliminación de antígenos no unidos. Finalmente, la actividad enzimática que se encuentra única al anticuerpo es inversamente proporcional a la concentración del antígeno de la muestra. Ver Anexo 9[49].

17-hidroxiprogesterona

Este parámetro generalmente se utiliza para descartar hiperplasia suprarrenal congénita por lo que se recomienda realizarlo en personas con sospecha de SOP cuando se encuentra en una fase folicular temprana para poder descartar esta patología que se debe a la deficiencia de 21-hidroxilasa[47]. El rango de referencia de esta prueba va desde 22 a 469 ng/dL .Valores normales en Anexo 10[48].

Principio de la prueba

En esta prueba se la realiza por medio de un ensayo inmunoenzimático competitivo, en donde se mezclan el anticuerpo biotinilado que esta inmovilizado en el pocillo, junto con dos antígenos, el uno presente en el conjugado enzimático y el otro en la muestra a estudio, estos producen una reacción de competencia entre sí por los sitios de unión limitados que existen, se realiza un proceso de decantación para eliminar los antígenos que no se unieron a los anticuerpos y finalmente se conoce que la actividad enzimática del complejo Ag/Ac es inversamente proporcional a la concentración de antígeno presente en la muestra. Ver Anexo 11[50].

Hormona antimülleriana (AMH)

Cuando un paciente presenta un diagnóstico de SOP las concentraciones de AMH se van a encontrar en valores elevados, es decir sobre pasan el rango normal que se encuentra entre 0,0 a 6,9 ng/mL, sin embargo, estos análisis serán limitados por no contar con un estándar internacional[47,51]. Valores normales en Anexo 12[48].

Principio de la prueba

En la determinación de la concentración de hormona antimülleriana, se utiliza el ensayo inmunoenzimático de tipo sándwich, en donde existe una interacción entre el anticuerpo inmovilizado, el anticuerpo marcado con enzima y el antígeno presente en la muestra, sin competencia y formando un complejo tipo sándwich. Después de un tiempo suficiente se realiza el proceso de decantación y se eliminan los antígenos que no se unieron al anticuerpo, posteriormente se conoce que la actividad enzimática que se une al anticuerpo es directamente proporcional a la concentración del antígeno en la muestra. Ver Anexo 13[41].

Hormona folículo estimulante (FSH)

En el SOP las concentraciones de esta hormona pueden presentarse dentro de los valores normales, mismos que en la fase folicular media van de 3,85 a 8,78 mUI/mL o pueden estar por debajo de estos. A pesar de que en la actualidad no es utilizada como parte de los criterios

de diagnóstico de este síndrome, es una prueba orientadora al diagnóstico final[31,47]. Valores normales en el Anexo 14[48]

Principio de la prueba

El principio de la prueba para conocer la concentración de FSH, se realiza un ensayo inmunoenzimático tipo sándwich en donde se mezclan el anticuerpo biotinilado monoclonal, el anticuerpo marcado con enzima y el antígeno que se encuentra presente en el suero del paciente, produciéndose una reacción en donde no existe competencia y creando un complejo tipo sándwich. Luego de un tiempo se decanta para eliminar los antígenos no unidos, finalmente la actividad enzimática que se encuentra unida al anticuerpo es directamente proporcional a la concentración del antígeno presente en la muestra. Ver Anexo 15[44].

Hormona luteinizante (LH)

Las concentraciones de LH ayudan a si la paciente son SOP presentan hiperandrogenismo o pueden desarrollar hiperinsulinemia. Se conoce que los valores normales de esta hormona se dividen según su fase ovulatoria dando como resultado los siguientes, en la fase folicular media van desde 2,12 a 10,89 mUI/mL; en la fase máxima central del ciclo van desde 19,18 a 103,03 mUI/mL; en la fase lútea el rango es desde 1,2 a 12,86 mUI/mL; finalmente en la posmenopausia es de 10,87 a 58,64 mUI/mL. Ver valores normales en Anexo 16[48].

Principio de la prueba

para conocer la concentración de LH, se realiza un ensayo inmunoenzimático tipo sándwich en donde se mezclan el anticuerpo biotinilado monoclonal, el anticuerpo marcado con enzima y el antígeno que está presente en el suero del paciente, se produce una reacción entre estos sin competencia para crear el complejo sándwich. Luego de un tiempo se decanta para eliminar los antígenos no unidos, finalmente la actividad enzimática que se encuentra unida al anticuerpo es directamente proporcional a la concentración del antígeno presente en la muestra. Ver Anexo 17[45].

Progesterona

La mejor manera de evaluar la ovulación es mediante la determinación de progesterona, misma que se recomienda realizarse en el día 21 a 23 del ciclo menstrual. Los niveles >2,5 ng/mL indican que hay ovulación, concentraciones ≥7 ng/mL muestran una adecuada función lútea y valores ≥15 ng/mL en tres pruebas consecutivas son utilizadas para conocer la función lútea normal. Mientras que los valores normales en la fase folicular van de 0,31 a 1,52 ng/mL. Ver valores normales en el Anexo 18[48].

Principio de la prueba

Para conocer la concentración de progesterona, se utiliza un ensayo inmunoenzimático competitivo, en donde se mezcla un anticuerpo biotinilado inmovilizado en el pocillo, un antígeno del conjugado enzimático y el antígeno presente en la muestra, los cuales reaccionan generando una acción de competencia por los sitios de unión limitados del anticuerpo, luego se procede a realizar la decantación respectiva en donde se elimina los antígenos que no se unieron a los anticuerpos. Finalmente, la actividad enzimática que se encuentra unida al anticuerpo será inversamente proporcional a la concentración del antígeno presente en la muestra. Ver anexo 19[43].

Prolactina

Para la determinación de prolactina en mujeres con presencia o sospecha de SOP, la detección en el aumento de esta hormona se lo puede hacer en la fase folicular como en la fase lútea, dicha acción puede dar como consecuencias la reducción de los folículos ováricos y con esto también la reducción o falta de ovulación[28]. Se conoce que los valores normales de prolactina van de 3,34 a 26,72 µg/L en mujeres < 50 años, mientras que los valores de 2,74 a 19,64 µg/L se observan en mujeres > 50 años. Ver valores normales en el Anexo 20[48].

Principio de la prueba

El principio del ensayo inmunoenzimático tipo sándwich que se desarrolla para conocer la concentración de prolactina menciona que al mezclar el anticuerpo biotinilado inmovilizado

en el pocillo, el anticuerpo marcado con la enzima y el antígeno presente en la muestra, se produce una reacción no competitiva y de esta manera formar el complejo tipo sándwich. Posterior del tiempo necesario se decanta la solución para eliminar los anticuerpos y antígenos que no se unieron o no formaron el complejo sándwich. Finalmente, la actividad enzimática que se encuentra unidad al anticuerpo será directamente proporcional a la concentración del antígeno presente en la muestra. Ver Anexo 21[52].

Estradiol

Bajo el control de las hormonas LH y FHS, el estradiol se produce en los ovarios, este junto a las gonadotropinas ayudan a la evaluación de la fertilidad de las mujeres y los problemas menstruales[48]. Se conoce que el estradiol se encuentra de manera excesiva en todas las fases de maduración folicular en las pacientes que presentan SOP[53]. Los valores normales de estradiol en pacientes sanas son los siguientes: en la fase folicular media de 27 a 122 pg/ml; en la fase peri ovulatoria de 95 a 433 pg/ml y en la fase lútea media de 49 a 291 pg/ml. Ver valores normales en el Anexo 22[48].

Principio de la prueba

Para la determinación de la concentración de estradiol en la muestra del paciente, se utiliza el método del ensayo inmunoenzimático competitivo, en donde se mezcla el anticuerpo marcado con biotina y el antígeno presente en la muestra. Luego de una pequeña incubación se adiciona la enzima combinada generando una reacción de competencia entre la enzima y el antígeno de la muestra por los sitios de unión del anticuerpo. Posteriormente se decanta para la eliminación de los antígenos no unidos. La actividad enzimática que se encuentra unida al anticuerpo es inversamente proporcional a la concentración del antígeno presente en la muestra. Ver Anexo 23[54].

CAPÍTULO III. METODOLOGÍA.

TIPO DE INVESTIGACIÓN

Esta investigación fue de carácter descriptivo pues se analizaron las variables de estudio propuestas en donde se detalló información sobre los valores hormonales en el síndrome de ovario poliquístico, sus fenotipos y sus manifestaciones clínicas , mediante información que fue recolectada de bases de datos confiables.

Se presentó con un diseño documental y no experimental porque toda la información que se recopiló se basó en revisiones bibliográficas obtenidas de diferentes bases de datos, además las variables de estudio que conforman el tema principal no fueron manipuladas o modificadas.

Según la secuencia fue de tipo transversal ya que esta se efectuó en un periodo determinado de tiempo, es decir datos recopilados que corresponden a los años de 2012 al 2022 sobre los valores hormonales en el síndrome de ovario poliquístico en diferentes fuentes bibliográficas.

Según la cronología, fue de tipo retrospectivo porque se refiere a información ya publicada previamente haciendo relevancia a los datos mostrados en tablas de resultados y conclusiones de artículos científicos originales, revisiones bibliográficas, tesis y libros.

POBLACIÓN DE ESTUDIO

El presente trabajo investigativo contó con 280 artículos científicos de diversas revistas digitales científicas a través de la utilización de palabras claves como: valores hormonales, fenotipos, manifestaciones clínicas y factores de riesgo del SOP; además, estos fueron tomados en cuenta al identificarse con el tema, en particular con los objetivos de investigación planteados y que su año de publicación resulte desde el 2014 al 2022. La búsqueda se la realizo en bases de datos relevantes, de libre acceso como: Scielo, Pubmed, Elsevier, ScienceDirect y Google Académico.

TAMAÑO DE MUESTRA

Se aplico criterios de selección (inclusión y exclusión), tomando en cuenta que cubra la mayor parte del tema de estudio y se identifique con las variables de interés. En definitiva, se logró seleccionar 54 publicaciones, con la información estrictamente requerida, lo que constituye la muestra, distribuyéndose de la siguiente manera: 28 Pubmed, 5 Scielo, 6 ScienceDirect, 1 Elsevier, 14 Google Académico.

Criterios de inclusión

En la investigación se tuvo en cuenta ciertos criterios de inclusión que debían ser relevantes para poder considerar importante la información de ciertos artículos científicos como se encuentren dentro de la temática sobre el síndrome de ovario poliquístico, los fenotipos de este, así también como las manifestaciones clínicas y sus factores de riesgo; entre los tipos de documentos de los que se podían recolectar la información estaban artículos originales, de revisión, tesis de repositorios. Todos los archivos que se utilizaron debían estar dentro de los años 2014 y 2022, también todos estos artículos podían estar tanto en inglés como en español.

Criterios de exclusión

Al momento de realizar la investigación se pudo descartar ciertos artículos científicos debido a que se sometieron bajo criterios de exclusión, mismos que ayudaban a restar importancia a estos artículos. Entre los criterios de exclusión que se utilizaron se tomó en cuenta las páginas web como blogs mismos que presentan información poco confiable, todos los artículos que describían sobre tratamiento farmacológico del síndrome de ovario poliquístico, también aquellos artículos que no tengan información como autores y año de publicación. Las publicaciones científicas que se presenten en idiomas diferentes al español e inglés también fueron descartadas.

MÉTODOS DE ANÁLISIS

El método utilizado en el presente trabajo investigativo fue teórico, analizando y deduciendo la información que se ajusta a las variables de estudio obtenidas de artículos científicos, tesis, etc. de la temática planteada sobre los valores hormonales en el síndrome de ovario poliquístico.

PROCESAMIENTOS DE DATOS

Las técnicas utilizadas en la investigación fueron la búsqueda, el análisis, la recopilación y la organización de información sobre los valores hormonales del síndrome de ovario poliquístico. En esta búsqueda se incluyeron temáticas referentes a los fenotipos, los factores de riesgo, las manifestaciones clínicas y sobre todo los valores hormonales alterados que se involucran en el SOP.

Se reviso varias bases de datos científicas como: Pubmed, Google Académico, ScienceDirect, Elsevier, Scielo donde se analizó diferentes artículos científicos de los cuales se seleccionó los de más relevancia acorde a la temática, además se tomó en cuenta los criterios de inclusión y exclusión para poder lograr una población y muestra adecuada para la investigación.

Los datos recolectados de los diferentes artículos científicos se los organizo por medio de tablas, estas muestras de manera adecuada la información referente a los subtemas tratados en la investigación sobre el SOP, además del lugar y el año de publicación de dichos artículos

Procesamientos estadísticos

Para la presente investigación se recopilo, analizo y organizo datos cuantitativos y cualitativos en Microsoft Excel, de los distintos artículos científicos, es decir información sobre los fenotipos, valores hormonales, factores de riesgo y manifestaciones clínicas del síndrome de ovario poliquístico. Esta información se encuentra expuesta en tablas, estas se encuentras en el capítulo IV sobre resultados y discusiones.

CONSIDERACIONES ÉTICAS

El trabajo de investigación tiene carácter bibliográfico que se basará en la recolección de datos de fuentes de investigación confiables por lo que respeta los principios bioéticos y no requiere de la aprobación del comité de bioética.

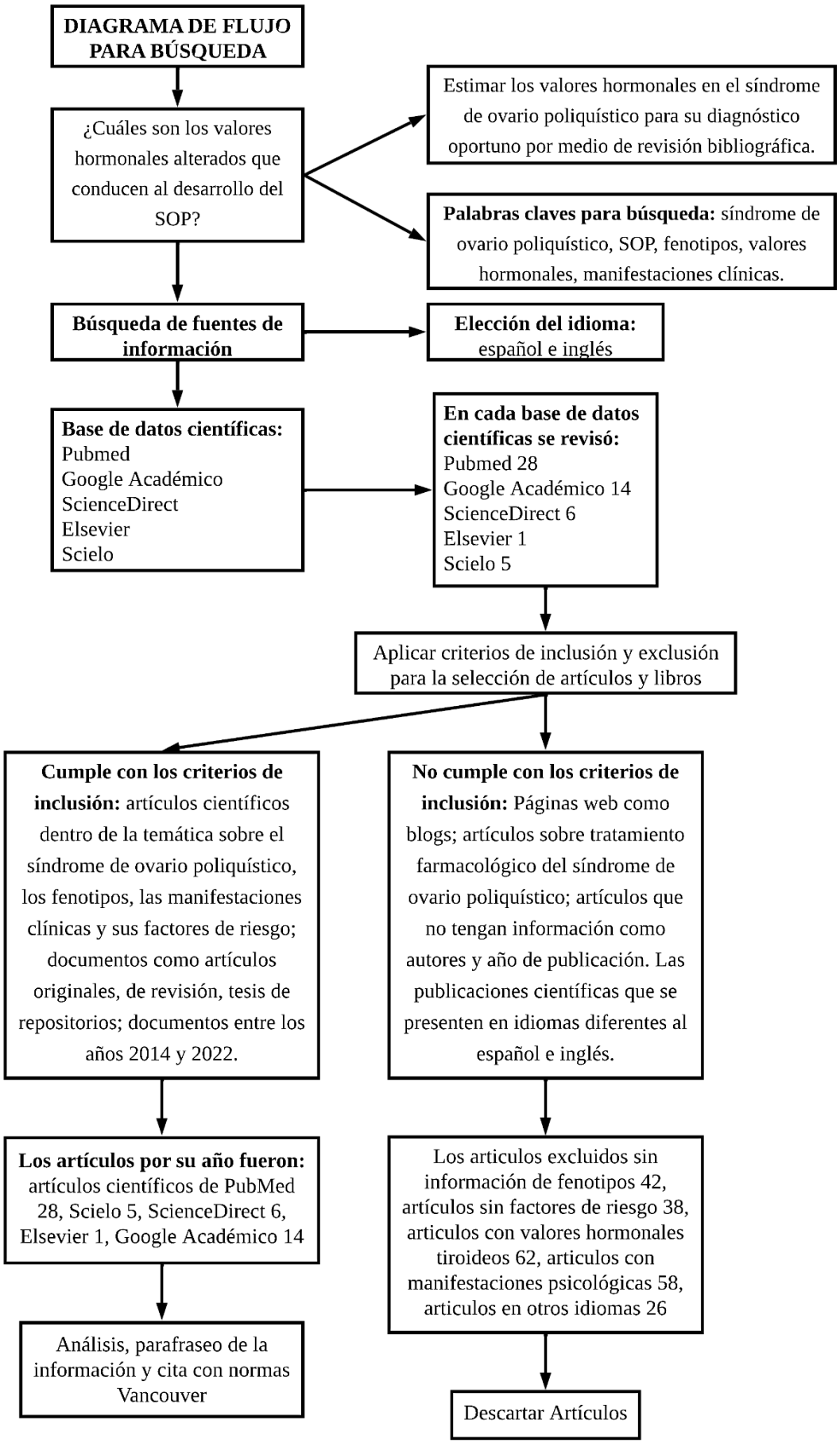


DIAGRAMA DE FLUJO PARA BÚSQUEDA
¿Cuáles son los valores hormonales alterados que conducen al desarrollo del SOP?
Estimar los valores hormonales en el síndrome de ovario poliquístico para su diagnóstico oportuno por medio de revisión bibliográfica.
Palabras claves para búsqueda: síndrome de ovario poliquístico, SOP, fenotipos, valores hormonales, manifestaciones clínicas.
Búsqueda de fuentes de información
Elección del idioma: español e inglés
Base de datos científicas: Pubmed Google Académico ScienceDirect Elsevier Scielo
En cada base de datos científicas se revisó: Pubmed 28 Google Académico 14 ScienceDirect 6 Elsevier 1 Scielo 5
Aplicar criterios de inclusión y exclusión para la selección de artículos y libros
Cumple con los criterios de inclusión: artículos científicos dentro de la temática sobre el síndrome de ovario poliquístico, los fenotipos, las manifestaciones clínicas y sus factores de riesgo; documentos como artículos originales, de revisión, tesis de repositorios; documentos entre los años 2014 y 2022.
No cumple con los criterios de inclusión: Páginas web como blogs; artículos sobre tratamiento farmacológico del síndrome de ovario poliquístico; artículos que no tengan información como autores y año de publicación. Las publicaciones científicas que se presenten en idiomas diferentes al español e inglés.
Los artículos por su año fueron: artículos científicos de PubMed 28, Scielo 5, ScienceDirect 6, Elsevier 1, Google Académico 14
Los articulos excluidos sin información de fenotipos 42, artículos sin factores de riesgo 38, articulos con valores hormonales tiroideos 62, articulos con manifestaciones psicológicas 58, articulos en otros idiomas 26
Análisis, parafraseo de la información y cita con normas Vancouver
Descartar Artículos

CAPÍTULO IV. RESULTADOS Y DISCUSIÓN

Con la finalidad de encontrar los valores hormonales que indican alteraciones sobre la temática tratada, se expone a continuación tablas y gráficos en donde se compara el estudio de varios investigadores. La información bibliográfica está principalmente enfocada en la frecuencia de cada uno de los fenotipos, los factores de riesgo más relevantes, las alteraciones hormonales y las manifestaciones clínicas más evidentes para el diagnóstico de SOP. Para poder plasmar estos datos se tomó en cuenta los criterios de inclusión y exclusión la cual proporciono información que ayudo a desarrollar el tema sobre el síndrome de ovario poliquístico.

FENOTIPOS DE SÍNDROME DE OVARIO POLIQUÍSTICO

En la *Tabla 1* se especifica sobre los fenotipos del SOP, los cuales se dividen en cuatro (A, B, C y D), la frecuencia de cada uno de estos y las principales causas que desarrollan este síndrome. Además, en base a esta tabla se describe el fenotipo con mayor frecuencia en el *Grafico 1*.

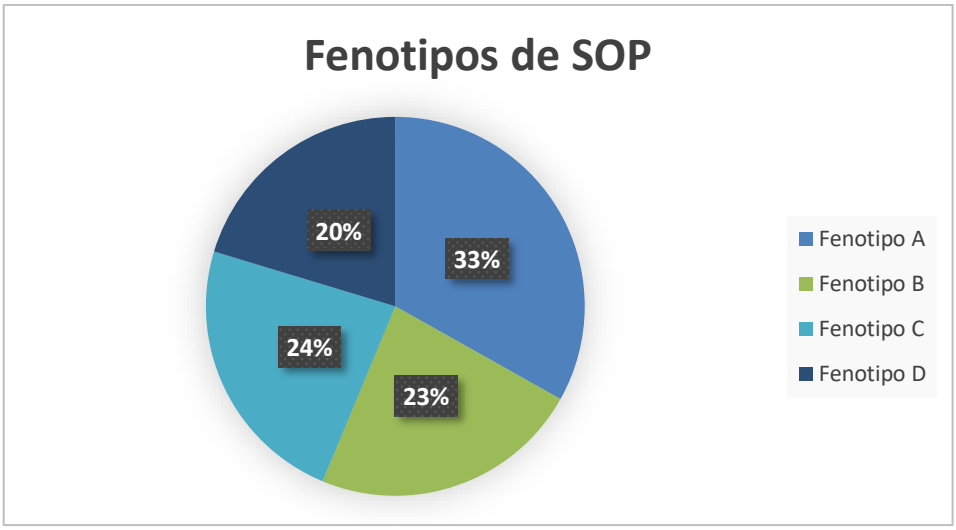
Tabla 1. *Frecuencia de los fenotipos del síndrome de ovario poliquístico y sus principales causas*

N°	Fenotipos				Causas	País	Primer autor y año
	A (H+O+P)	**B (H+O)**	**C (H+P)**	**D (O+P)**			
1	50,00%	9,00%	10,00%	31,00%	Sobrepeso, ciclos menstruales irregulares, etc.	Dinamarca	Pelanis, 2017[55]
2	25,50%	42,60%	27,70%	4,30%		Perú	Quispe, 2019[56]
3	58,00%	19,00%	8,00%	15,00%	Antecedentes familiares, hormonas alteradas, obesidad	Ecuador	Solís, 2018[57]
4	40,95%	19,05%	40,00%	0,00%		Colombia	Mantilla, 2022[58]
5	54,10%	11,70%	14,40%	19,80%	Alteraciones hormonales y metabólicas	Brasil	Tavares, 2019[59]
6	67,70%	11,00%	17,70%	3,60%		India	Sachdeva, 2019[60]
7	27,30%	14,90%	16,80%	41,00%	Sobrepeso y alteraciones hormonales	India	Pikee, 2016[61]
8	39,20%	50,40%	6,00%	4,40%		India	Kaur, 2021[62]
9	4,65%	4,65%	72,10%	18,60%	Alteraciones hormonales y ciclos menstruales irregulares	Dinamarca	Lauritsen. 2014[63]
10	12,94%	22,35%	49,41%	15,30%		Irán	Ramezani, 2014[64]
11	7,60%	22,60%	18,20%	51,60%		Sudan	Elasam, 2022[65]
12	24,70%	4,50%	6,50%	64,30%		China	Wang, 2022[66]
13	23,90%	46,30%	21,60%	8,20%		Irán	Farhadi, 2022[67]
14	22,20%	55,60%	11,10%	10,10%	Ciclos menstruales irregulares, alteraciones hormonales, obesidad	Cuba	Ovies, 2015[68]
15	57,70%	9,20%	30,00%	3,10%		Italia	Carmina, 2019[69]
16	26,60%	27,10%	23,60%	22,90%	Antecedentes familiares	Turquía	Altintas, 2017[70]
17	27,15%	24,50%	21,85%	26,50%	Hormonas alteradas, obesidad, falta de actividad física	Irán	Zaeemzadeh, 2020[71]
18	26,10%	21,70%	26,10%	26,10%	Antecedentes familiares y hormonas alteradas	Irán	Yarjanli, 2022[72]
PT	33,13%	23,12%	23,39%	20,32%			

Notas: *se considera la coma (,) como separador decimal*

Abreviaturas: *H: hiperandrogenismo; O: oligo-ovulación; P: morfología poliquística ovárica; PT: promedio total*

Gráfico 1. *Frecuencia de los fenotipos del SOP*



Análisis

En los resultados obtenidos en la tabla 1 se evidencia los diferentes fenotipos que se presentan en el SOP, así también como la frecuencia y las causas más comunes de desarrollarse. Al organizar la información recopilada se pudo notar que el más frecuente es el fenotipo A (33.13%) en donde se desarrolla hiperandrogenismos, oligo-ovulación y morfología poliquística ovárica, seguido del fenotipo C (23,39%) que se caracteriza por presentar oligo-ovulación y morfología poliquística ovárica, el fenotipo B (23,12%) con el desarrollo de hiperandrogenismo y oligo-ovulación y el fenotipo D (20.32%) en cual se desarrolla oligo-ovulación y morfología poliquística ovárica siendo estos dos últimos los menos frecuentes.

Entre las causas más comunes del desarrollo de SOP se encuentran las alteraciones hormonales, obesidad y los ciclos menstruales irregulares. Por otro lado, las causas que presentan una menor frecuencia son los antecedentes familiares, alteraciones en el metabolismo y la falta de ejercicio.

Discusión

Según Solís[57] en su investigación realizada en Ecuador en el año 2018, menciona que el fenotipo más frecuente entre las mujeres con SOP fue el fenotipo A con un promedio de 58%

a comparación de los demás fenotipos. En otra investigación realizada por Mantilla[58] en Colombia en el año 2022 se evidencia que los resultados obtenidos concuerdan con los de autor antes mencionado, es decir, el resultado obtenido en la investigación fue de 40,95% para el fenotipo A, mostrándose, así como el más frecuente en esta investigación.

Por otro lado, Ovies, et al[68] en su investigación elaborada en el año 2015 en Cuba, menciona que existieron pacientes tanto con alteraciones aisladas como con alteraciones conjuntas que hacen referencia a los fenotipos que se pueden desarrollar en el SOP, en el grupo del pacientes con SOP se encontraban 18 de los cuales el 55,60% de los pacientes presentaban el fenotipo B. este estudio concuerda con la investigación de Kaur, et al[5762] en el 2021 que se realizó en la India, en el que se obtuvo como resultado que el 50,40% de población de estudio desarrollo el fenotipo B.

En el 2014, Lauritsen, et al[63] realizaron un estudio en Dinamarca en el cual se evidencia que el grupo de pacientes que presentaban SOP, el 72,10% desarrollarlo el fenotipo C. Ramezani, et al[64] en el año 2014 concuerda con este estudio debido a que en la investigación que realizo también encontró que el fenotipo C es el más frecuente entre los fenotipos hallados en su población.

Elasam, et al[65] en el año 2022 en Sudan, manifiesta que el fenotipo más frecuente en su investigación fue el fenotipo D con un resultado de 51,60% en su población de estudio, esta concuerda con la investigación realizada en China en el año 2022 de Wang, et al[66], en donde se menciona que el resultado del fenotipo más frecuente es de 64,30% correspondiente al fenotipo D.

En conjunto con los datos recopilados y los promedios obtenidos de la investigación se puede mencionar que el fenotipo A es el más frecuente entre los cuatro existentes, de igual manera, las causas que se presentan más a menudo en mujeres con SOP son los ciclos menstruales irregulares y alteraciones a nivel hormonal, sin embargo, sin dejar de lado otros factores predisponentes como la obesidad y los antecedentes familiares que de igual manera participan en el desarrollo de este síndrome.

ANÁLISIS DE FACTORES DE RIESGO

Existes varios factores de riesgo que ayudan al desarrollo del SOP , en la Tabla 2 se detalla sobre estos, como el sobrepeso y la obesidad; la resistencia a la insulina; los factores hereditarios y la diabetes mellitus tipo 2, y también su frecuencia. En el Grafico 2 se observa el porcentaje de la frecuencia de cada factor de riesgo.

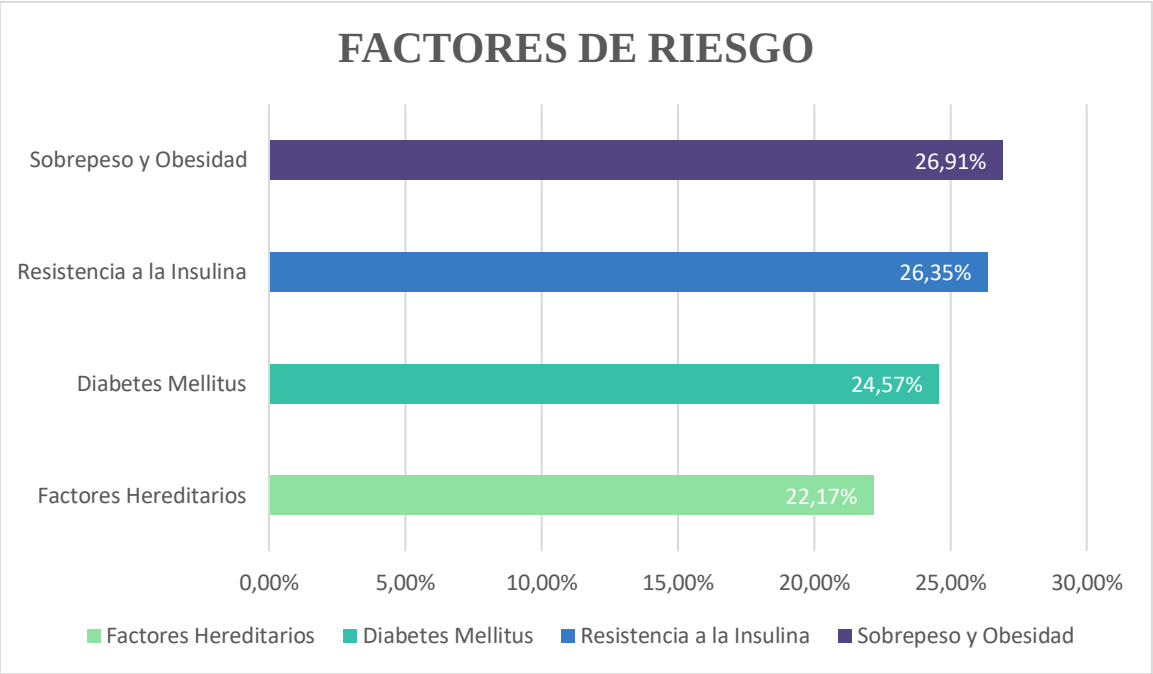
Tabla 2. *Factores de riesgo en el síndrome de ovario poliquístico*

Factor de Riesgo	Edad (años)	Población de estudio	Frecuencia	Promedio	%	País	Primer autor y año
Sobrepeso y Obesidad	18 a 39	57	94,73%	48,63%	26,91%	México	Pulido, 2016[73]
	31 a 46	1758	10,30%			EE.UU	Ollila, 2016[74]
	12 a 13	47	80,00%			México	Vital, 2017[75]
	20 a 40	248	8,46%			Ecuador	Peña, 2018[76]
	18 a 39	111	67,56%			Brasil	Tavares, 2019[59]
	40 a 44	30	53,30%			Cuba	Carmenate, 2021[77]
	27 a 37	169	26,04%			Colombia	Mantilla, 2022[58]
	19 a 33	368	42.40%			Sudan	Elasam, 2022[65]
Resistencia a la Insulina	18 a 40	152	70,39%	47,62%	26,35%	Cuba	Monteagudo, 2019[78]
	30 a 34	60	56,67%			Cuba	Tamayo, 2019[79]
	40 a 44	30	36,70%			Cuba	Carmenate, 2021[77]
	15 a 35	60	26,70%			India	Singh, 2022[80]

Factores Hereditarios	18 a 49	48	37,50%	40,07%	22,17%	Cuba	Ovies, 2015[68]
	29 a 35	285	35,00%			China	Shan, 2015[81]
	13 a 17	3334	56,00%			Ecuador	Ponce, 2018[82]
	19 a 26	140	31,76%			Perú	Yallico, 2018[83]
Diabetes Mellitus	25 a 34	876	3.00%	44,39%	24,57%	Dinamarca	Pelanis, 2017[55]
	21 a 30	74	13,50%			Ecuador	Hernández, 2017[84]
	30 a 39	81	9,88%			Paraguay	Giménez, 2020[85]
	15 a 49	116	65,50%			Perú	Iturrizaga, 2020[86]
	21 a 29	221	58,82%			Ecuador	Moyano, 2020[87]
	20 a 39	132	74,24%			Ecuador	García, 2021[88]
Total				180,71%	100,00%		

Nota: se considera la coma (,) como separador decimal

Gráfico 2. *Porcentaje total de frecuencia de factores de riesgo en el SOP*



Análisis

Los datos expuestos en la *Tabla 2* muestras la frecuencia que presentan factores de riesgo más representativos que contribuyen al desarrollo de SOP. El sobrepeso y la obesidad resulto en un 26,91% siendo el más frecuente en pacientes que presentan SOP, este factor puede darse debido a alteraciones hormonales tiroideos, falta de actividad física, ansiedad y depresión que provocan atracones de comida, a este le sigue la resistencia a la insulina con 26,35%; con un menor porcentaje, pero no con menos importancia se encuentra la diabetes mellitus tipo 2 con 24.57% y los factores hereditarios con 22,17%.

Discusión

Pulido et al[73] en su estudio realizado en el año 2016 menciona que el sobrepeso y la obesidad se encuentran en una frecuencia alta en las mujeres que presentan SOP, en especial en el grupo de 18 a 39 años, mostrando un porcentaje de 94,73% en la población de estudio de su investigación, Tavares et al[59] en su estudio en el año 2019, en Brasil concuerda con este al decir que el porcentaje de frecuencia más alto se encuentra en las mujeres con edades de entre los 18 a 39 años con un 67,56%. Por otro lado, Carmenate et al[77] en el año 2021 menciona que el grupo con mayor frecuencia de sobrepeso y obesidad son las mujeres de entre los 40 a 44 años con un porcentaje de 53,30%.

En cuanto a la resistencia a la insulina, Monteagudo et al[78] en el año 2019, en su investigación realizada en Cuba menciona que el 70,39% de su población de estudio presentaba este factor de riesgo, mismas que pertenecen al grupo de edad entre 18 a 40 años; Singh et al[80], en su estudio realizado en La India en el año 2022 presenta un rango de edad similar al anterior, que van desde los 15 a los 35 años, pero en cuanto a su frecuencia, esta se muestra disminuida (26,70%). Tamayo et al[79] en el año 2019, presenta un rango de edades más corto que van desde los 30 a 34 años, sin embargo, la frecuencia de este factor de riesgo es alto, presentándose con un 56,67%.

Ponce et al[82] en su estudio realizado en Ecuador en el año 2018, menciona que los factores hereditarios son un factor de riesgo importante para el desarrollo de SOP, obteniendo un 56% del total de su población con este factor. Por otro parte, Ovies et al[68], Shan et al[81] en el 2015 y Yallico et al [83] en el 2018 muestran que es sus estudios los resultados no sobrepasaron el 50%, es decir obtuvieron resultados de 37,5%; 35% y 31,76% respectivamente, por lo que se puede evidenciar este factor no presenta una gran relevancia para dichos autores. Hay que tomar en cuenta que cada autor presenta un rango diferente en cuanto a las edades de los pacientes.

Pelanis et al [55] en 2017 en Dinamarca, Hernández[84] en el 2017 en Ecuador y Giménez et al[85] en el 2020 en Paraguay, presentan resultados de sus investigaciones con porcentajes demasiado bajos haciendo referencia a que la Diabetes mellitus tipo 2 no es un factor de riesgo con gran relevancia. Pero Iturrizaga[86] en Perú en el 2020, Moyano[87] en Ecuador en el 2020 y García[88] en Ecuador en el 2021 presentan estudios en donde refutan los anteriores estudios, demostrando que la diabetes mellitus tipo 2 es un problema grave que ayuda al desarrollo de SOP, es decir en sus resultados mostraron que las poblaciones con este factor representaban el 65,5%; 58,82% y 74,24% respectivamente.

Los datos recopilados ayudaron a determinar la frecuencia de los factores de riegos para el desarrollo de este síndrome, obteniéndose como resultado que el sobrepeso y la obesidad es el factor más común, seguido de la resistencia a la insulina que lleva una mínima diferencia con el anteriormente mencionado, sin embargo, también se debe considerar importante la diabetes mellitus y los factores hereditarios como coadyuvantes al desarrollo del SOP.

VALORES HORMONALES REFERENCIALES INDICATIVO DE SOP

En la Tabla 3 se observa la comparación de los valores hormonales normales frente a los alterados, además de la media de estos últimos, así también en el porcentaje de artículos y cuantos autores hablan sobre una hormona específica.

Tabla 3. *Valores normales y alterados en el síndrome de ovario poliquístico*

Hormona	Valores Hormonales		Media de valores	N° de autores	Promedio de artículos N=54	País	Primer autor y año
	Normales	Alterados					
AMH	0 – 6,9 ng/ml	9,3 ± 3,1	9,05	7	12,96	China	Yue, 2018[89]
		12,4 ± 8,1				Turquía	Nigar, 2021[90]
		11,2 ± 3,7				Suiza	Khashchenko, 2020[91]
T. Total	9 – 55 ng/dl	1,09 ± 1,10	1,28	31	57,40	Paquistán	Khattak. 2021[92]
		0,43 ± 0,19				Turquía	Gülücü, 2022[93]
		1,77 ± 0,43				China	Wei, 2022[94]
T. Libre	1,2 – 9,2 pg/ml	4,93 ± 3,45	9,81	9	16,7	Turquía	Bostanci, 2015[95]
		14,91 ± 6,59				Australia	Broskey, 2018[96]
		9,6 ± 0,7				EE.UU	Ezeh. 2021[97]
LH	1,79 – 5,12 mIU/ml	6,1 ± 4,6	7,92	23	42,60	Polonia	Franik, 2018[98]
		8,67 ± 2,05				Turquía	Gursu, 2021[99]
		8,98 ± 6,63				Turquía	Eroglu, 2021[100]
FSH	1,2 – 12,86 mIU/ml	15,5 ± 14,8	9,62	23	42,60	Cuba	Vázquez, 2016[101]
		6,65 ± 1,25				Suiza	Khashchenko, 2020[91]
		6,70 ± 1,23				Turquía	Gursu, 2021[99]

Nota: se considera la coma (,) como separador decimal

Abreviaturas: AMH: hormona antimülleriana; T: testosterona; LH: hormona luteinizante; FSH: hormona folículo estimulante

Análisis

Luego de analizar y organizar la información se compararon los valores hormonales normales y alterados y se consiguieron los siguientes resultados, para la AMH en el 12.96% de los artículos se obtuvo una media de valores alterados de 9,05 ng/ml en los valores alterado; en cuanto a la T. Total el 57,40% de los artículos la media obtenida fue de 1,28 ng/dL; la T. libre se obtuvo una media de 9,81 pg/ml del 16,70% de artículos; por ultimo para la LH y FSH se obtuvo una media de 7,92 mIU/ml y 9,62 mIU/ml respectivamente en el 42,60%de los artículos.

Discusión

Yue et al[89] en el 2018; Nigar et al[90] en 2021, Khashchenko et al[91] en el 2020 en sus investigaciones mencionan que de la población estudiada los valores de la AMH fueron superiores al rango de normalidad, denotándose de esta manera que los resultados de esta hormona son importantes para el diagnóstico de SOP, debido a que estos valores se encuentran elevados cuando existen problemas a nivel hormonal de los ovarios, o cuando la paciente presenta quistes en estos.

Los estudios realizados de Khattak et al[92], Gülücü et al[93], Wei en los años 2021 y 2022 muestran que los resultados de la hormona T. Total se encuentran inferiores al rango de valores normales, es decir, las pacientes de las respectivas investigaciones pueden presentar algún trastorno de los ovarios. Cuando los valores hormonales se encuentran superiores a los rangos normales puede ser indicador de patologías como el hiperandrogenismo.

Se conoce que la hormona T. Libre no es un examen de rutina, pero es utilizado debido a su sensibilidad, por lo que Bostanci et al[95] en el año 2015; Broskey et al[96] en el 2018 y Ezeh et al[97]en el 2021, utilizaron este parámetro para considerar la población que presentaba SOP en sus respectivas investigaciones obteniendo resultados que superar a los normales, es decir, en las pacientes se puede evidenciar la presencia de hiperandrogenemia.

Franik et al[98] en el 2018, Gursu et al[99]en el 2021, Eroglu et al [100]en el 2021 mencionan que una hormona importante también es la LH debido a que estimula la síntesis de andrógenos,

y los resultados obtenidos de sus respectivas investigaciones fueron superiores a los rangos normales, por lo que puede decir que esta hormona debe ser considerada al momento de un diagnóstico de SOP.

En cuanto a la hormona FSH se conoce que valores superiores a los normales indican que las mujeres entran en estadio de climaterio también conocido como menopausia o presentan el síndrome de ovarios poliquísticos, por lo que Vázquez et al[101] en su investigación en el 2016 obtuvo resultados superiores a los normales y denotándose así su población son dicho síndrome, pero Khashchenko et al[91] en el 2020, Gursu et al[99] en el 2021 en sus investigaciones obtuvieron resultados de la población con SOP dentro de los valores normales.

Con este análisis se puede evidenciar que los valores hormonales alterados pueden ser superiores o inferiores al rango de valores normales, a diferencia de la hormona FSH en donde su media se encuentra dentro del rango de normalidad

MANIFESTACIONES CLÍNICAS

En el SOP pueden presentarse una variedad de manifestaciones clínicas debido a la gravedad y el fenotipo que este muestre, sin embargo, entre las más relevantes y comunes se encuentran: los ciclos menstruales irregulares, el hirsutismo, el acné y la alopecia androgénica. En *Gráfico 3* se muestra el porcentaje total de acuerdo con la frecuencia de cada una de las manifestaciones clínicas.

Tabla 4. *Frecuencia de las manifestaciones clínicas en el síndrome de ovario poliquístico*

Manifestación clínica	Frecuencia	Promedio	%	País	Primer autor y año
Ciclos menstruales irregulares	63,20%	63,32%	32,07%	China	Shan, 2015[81]
	68,50%			Cuba	Vázquez, 2016[101]
	62,86%			Colombia	Castillo, 2019[102]
	83,00%			República Dominicana	Romero, 2020[103]
	39,05%			Colombia	Mantilla, 2022[58]
Hirsutismo	50,00%	54,96%	27,84%	Cuba	Ovies, 2015[68]
	7,00%			Perú	Zamudio, 2016[104]
	54,00%			Ecuador	Hernández, 2017[84]
	78,40%			Brasil	Tavares, 2019[59]
	63,33%			Cuba	Tamayo, 2019[79]
	77,00%			Ecuador	García, 2021[88]
Acné	28,00%	40,59%	20,56%	Ecuador	Solís, 2018[57]
	18,20%			Polonia	Franik, 2[98]
	25,88%			Perú	Yallico, 2018[83]
	65,71%			Colombia	Castillo, 2019[102]
	59,25%			Paraguay	Giménez, 2020[85]
	46,50%			Sudan	Elasam, 2022[65]

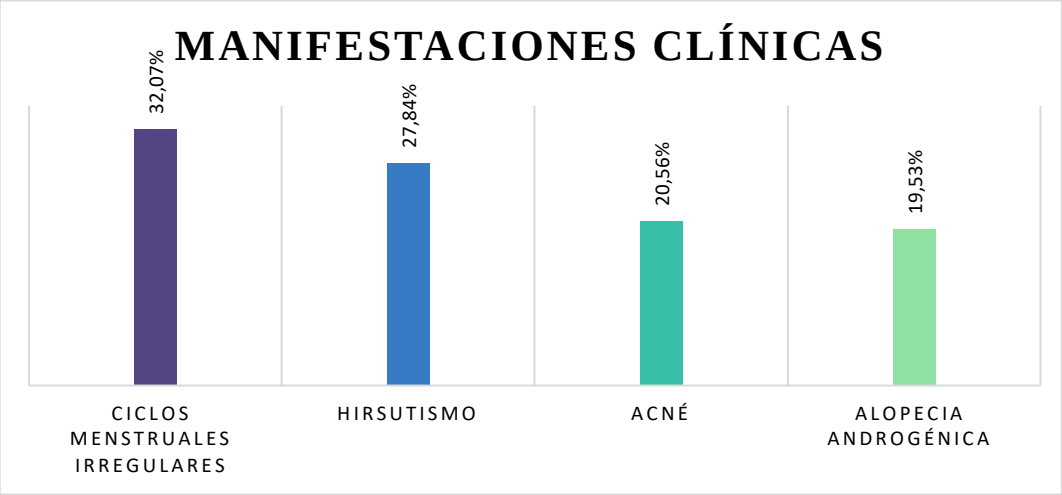
Alopecia androgénica	3,60%	38,56%	19,53%	Cuba	Vázquez, 2016[101]
	25,00%			República Dominicana	Romero, 2020[103]
	42,50%			Jordania	Abusailik, 2021[105]
	20,30%			Arabia saudita	Alijefri, 2021[106]
	48,97%			India	Vishnubhotla, 2022[107]
	91,00%			Turquía	Artar, 2022[108]
Total		197,43	100%		

Nota: se considera la coma (,) como separador decimal

Análisis

En la tabla 4 se muestran las manifestaciones clínicas más comunes desarrolladas en pacientes con SOP, la frecuencia de esta y el país en donde se realizó el estudio. Entre las manifestaciones clínicas analizadas están las siguientes: ciclos menstruales irregulares (32,07%) es la característica más notable entre las pacientes con esta patología, seguido del hirsutismo (27,84%), este hace referencia al crecimiento del vello dando una característica masculina; el acné (20.56%) también es una manifestación que se puede observar en las pacientes, finalmente y con menor frecuencia se puede evidenciar la alopecia androgénica (19,53%), es decir, las mujeres pueden padecer de calvicie.

Gráfico 3. *Porcentaje total de frecuencia de manifestaciones clínicas en el SOP*



Con la comparación entre los estudios analizados se nota que ciertas manifestaciones clínicas han aumentado con el tiempo, es decir, en la actualidad en las mujeres que presentan SOP pueden observar que pueden presentar una o más manifestaciones clínicas, sin dejar de lado las alteraciones de acuerdo con el fenotipo que presentan.

Discusión

Según Shan, et al[81]; Vázquez[101], et al; Castillo, et al[102] y Romero, et al[103] en sus investigaciones respectivas mencionan que los ciclos menstruales irregulares es una de las manifestaciones clínicas más frecuentes además en investigaciones realizadas en años anteriores hasta años recientes muestran que la frecuencia de esta manifestación clínica ha tenido un incremento en la población con SOP. Mantilla[58] en su investigación realizada en el año 2022 discrepa de los demás autores debido a que los resultados de su investigación son relativamente bajos a comparación de años anteriores.

En los años 2017 y 2021, Hernández[84] y García[88] respectivamente realizaron investigaciones en Ecuador en donde los dos autores concuerda que el hirsutismo es una manifestación clínica con gran importancia debido a que esta se muestra en más de la mitad de la población estudiada en cada investigación. Por otro lado, una investigación realizada en Perú por

Zamudio[104] indica que, en la población de estudio, esta manifestación clínica no fue tan relevante a comparación de otras, debido a que solo el 7% de la población la presenta.

Solís[44]s, Franik et al[98], y Yallico[83] en el 2018, realizaron investigaciones en diferentes países en las cuales concuerdan entre si porque indican que el acné no es una manifestación clínica de gran importancia debido a que se muestra en un porcentaje bajo en las poblaciones de estudio. Los estudios de Castillo et al[102] en el 2019, Giménez et al[85], en el 2020 y Elasam et al[65] 2022 están en desacuerdo con los autores anteriores, ya que en sus investigaciones esta manifestación clínica tiene un porcentaje que abarca casi la mitad de la población de estudio o supera esta, por lo cual la convierte en una manifestación de gran importancia entre las pacientes con SOP.

La alopecia androgénica es otra manifestación que se evidencia en las pacientes con SOP, el estudio de Artar et al[108] en el 2022 realizado en Turquía, señala que el 91% de pacientes en su población presentaba esta complicación, Abusailik et al[105] concuerda con el autor anterior debido a que en su estudio casi la mitad de su población desarrollo esta manifestación y la convierte en un problema relevante al diagnosticar SOP. Mientras que Vázquez et al[101] en su estudio realizado en Cuba en el 2016 menciona la discrepancia sobre la alopecia androgénica porque esta solo muestra que el 3.60% de la población tiene esta complicación.

Las investigaciones analizadas, los datos recopilados y los promedios obtenidos ayudaron a determinar que las manifestaciones clínicas más frecuente entre las pacientes que presentan SOP son los ciclos menstruales irregulares y el hirsutismo, mismo que se presentan en un gran número de dichas pacientes, las demás manifestaciones clínicas como: el acné y la alopecia androgénica también afectan a una parte de la población que han desarrollado SOP, pero su porcentaje en mucho menor.

CAPÍTULO V. CONCLUSIONES Y RECOMENDACIONES

CONCLUSIONES

- En el síndrome de ovario poliquístico se pueden presentar varios fenotipos como lo es el A, B, C y D de los cuales por medio de la recopilación y organización de datos se concluye que el tipo A con un 33,13% es el más frecuente en comparación a los otros y se puede presentar en la mayoría de mujeres que sufren este síndrome, por otro lado se evidencia que entre las causas más comunes son alteraciones hormonales, los ciclos menstruales irregulares y la obesidad siendo estos uno de los principales pilares para el desarrollo de este síndrome.
- Para el desarrollo síndrome de ovario poliquístico existen varios factores de riesgo, por consiguiente, al organizar la información se constató que los más destacables son la diabetes mellitus tipo 2; factores hereditarios; resistencia a la insulina; sobrepeso y obesidad siendo este el último el más frecuente en mujeres de edad reproductiva temprana, es decir presentó un mayor porcentaje a comparación de los demás. Por consiguiente, este también ayuda al desarrollo de otros factores como trastornos menstruales e hiperandrogenismo.
- El SOP es una afección en la cual la paciente presenta un desbalance en su estado hormonal, a partir del análisis realizado, se menciona que las hormonas más relevantes que sirven como guía para el diagnóstico de SOP son la antimülleriana, testosterona total, testosterona libre, luteinizante y folículo estimulante, debido a que los resultados de los análisis de estas, sean estos superiores o inferiores a los rangos normales presentan un riesgo de desarrollar patologías como trastornos ováricos, hiperandrogenismos, etc.

RECOMENDACIONES

- Debido a la necesidad de continuar con las investigaciones sobre el diagnóstico del síndrome de ovario poliquístico en la población ecuatoriana y al no tener un número amplio de artículos científicos sobre el tema, se recomienda al Ministerio de Salud Pública que se implementen más campañas a nivel nacional en donde se explique de manera adecuada sobre las manifestaciones clínicas, los fenotipos, los factores de riesgos y además cuales son los análisis de laboratorio más adecuados para conocer si porta o no dicho síndrome o porta otra afección.
- Se recomienda a la población en general que a partir del inicio de su edad reproductiva se realicen chequeos médicos periódicamente, así también en el caso de presentar alguna irregularidad, signo o síntoma acudir a un médico especialista mismo que le indique los exámenes hormonales pertinentes que conjunto con la clínica ayuden al diagnóstico de SOP y su posterior tratamiento
- Se recomienda a la población en general a cambiar sus hábitos alimenticios e incrementar actividad física en su diario vivir, debido a que uno de los factores más comunes que ayuda al desarrollo de SOP es el sobrepeso y la obesidad, este a su vez causa otras patologías como la resistencia a la insulina, enfermedades coronarias, etc.

BIBLIOGRAFÍA

1. Teresa Sir P, Jessica Preisler R, Amiram Magendzo N. Síndrome de ovario poliquístico. diagnóstico y manejo. Revista Médica Clínica Las Condes [Internet]. el 1 de septiembre de 2013 [citado el 25 de junio de 2022];24(5):818–26. Disponible en: https://www.elsevier.es/es-revista-revista-medica-clinica-las-condes-202-articulo-sindrome-ovario-poliquistico-diagnostico-manejo-S0716864013702293
2. García M, Ferreira A, Lobaton M. Proinflammatory cytokines in polycystic ovarian syndrome. Rev Med Inst Mex Seguro Soc [Internet]. el 31 de mayo de 2022;60(5):569–76. Disponible en: http://revistamedica.imss.gob.mx/
3. Zarnowski A, Salazar A. Síndrome de ovario poliquístico | Revista Médica Sinergia. Revista Médica Sinergia [Internet]. el 20 de septiembre de 2022 [citado el 25 de junio de 2022];7(1). Disponible en: https://revistamedicasinergia.com/index.php/rms/article/view/748/1695
4. Alfaro G, Salas B, Zúñiga A. Evaluación del síndrome de ovario poliquístico: herramientas diagnósticas y nuevas terapias. Revista Médica Sinergia [Internet]. el 17 de diciembre de 2020 [citado el 25 de junio de 2022];6(1). Disponible en: https://www.medigraphic.com/pdfs/sinergia/rms-2021/rms211k.pdf
5. del Castillo Tirado FJ, Martínez Ortega AJ, del Castillo Tirado RA. Guía de práctica clínica de síndrome de ovario poliquístico. iMedPub [Internet]. 2014 [citado el 16 de octubre de 2022];10(2:3). Disponible en: https://www.archivosdemedicina.com/medicina-de-familia/gua-de-prctica-clnica-de-sndrome-de-ovario-poliqustico.pdf
6. Vanhauwaert PS. Síndrome de ovario poliquístico e infertilidad. Revista Médica Clínica Las Condes [Internet]. el 1 de marzo de 2021 [citado el 25 de junio de 2022];32(2):166–72. Disponible en: https://www.elsevier.es/es-revista-revista-medica-clinica-las-condes-202-articulo-sindrome-ovario-poliquistico-e-infertilidad-S0716864021000195
7. García M, Viteri J. Síndrome de ovario poliquístico. Revista Experimental Médica [Internet]. marzo de 2021 [citado el 25 de junio de 2022];7(1). Disponible en: https://rem.hrlamb.gob.pe/index.php/REM/article/view/442/290
8. Fernández C. PREVALENCIA DEL SÍNDROME DE OVARIO POLIQUÍSTICO EN PACIENTES QUE ACUDEN AL HOSPITAL NACIONAL SERGIO ENRIQUE

BERNALES EN EL AÑO 2017 [Internet]. [Lima]: Universidad Privada San Juan Bautista; 2018 [citado el 25 de junio de 2022]. Disponible en: https://renati.sunedu.gob.pe/handle/sunedu/2842943

9. Becerra Quevedo KD, Patricio B, Loyola G, Fierro BC, Esther M, Rodríguez R, et al. Prevalence of obesity in women of childbearing age who have polycystic ovarian syndrome. Revista del Centro de Estudio y Desarrollo de la Amazonia. 2019;09(01):23–6.
10. Peña S, Salazar Z, Cárdenas F, Ochoa S, Cumbe K, Crespo C. Prevalencia y factores asociados al Ovario Poliquístico en adultas de las parroquias urbanas de la ciudad de Cuenca-Ecuador, 2017. Sociedad Venezolana de Farmacología y de Farmacología Clínica y Terapéutica [Internet]. 2018 [citado el 25 de junio de 2022];37(3):184–9. Disponible en: https://www.redalyc.org/journal/559/55963208003/html/
11. Guzmán López J, Robles Lara P, Rivera Contreras O, Ramírez Velandia F, Sepúlveda Sanguino A, Sepúlveda Agudelo J. Revisión de los criterios diagnósticos para el síndrome de ovario poliquístico. Revista apoyada por los estudiantes de medicina de la Universidad Industrial de Santander [Internet]. el 2 de junio de 2020 [citado el 26 de junio de 2022];33(2):21–8. Disponible en: https://doi.org/10.18273/revmed.v33n3-2020002
12. Sánchez E. Actualización del manejo de síndrome de ovario poliquístico. Revista Médica Sinergia [Internet]. diciembre de 2019 [citado el 26 de junio de 2022];4(12). Disponible en: https://revistamedicasinergia.com/index.php/rms/article/view/322/667
13. Pérez J, Maroto K. Síndrome de Ovarios Poliquísticos . Asociación Costarricense de Medicina Legal y Disciplinas Afines [Internet]. el 1 de marzo de 2018 [citado el 26 de junio de 2022];35(1). Disponible en: https://www.scielo.sa.cr/pdf/mlcr/v35n1/1409-0015-mlcr-35-01-94.pdf
14. Fonseca Villanea C. SÍNDROME DE OVARIO POLIQUÍSTICO (Polycystic ovary sindrome). Revista Médica Sinergia [Internet]. junio de 2018 [citado el 26 de junio de 2022];3(6):9–15. Disponible en: http://revistamedicasinergia.com
15. Orias M. Actualización del síndrome de ovario poliquístico. Revista Médica Sinergia [Internet]. febrero de 2021 [citado el 26 de junio de 2022];6(2). Disponible en: https://revistamedicasinergia.com/index.php/rms/article/view/648/1143

16. Azziz R. Síndrome de ovario poliquístico. Obstet Gynecol [Internet]. 2018 [citado el 26 de junio de 2022];132:321–57. Disponible en: www.greenjournal.orgReconocimientodeeducaciónmédicacontinuadisponibleparaesteartículoenhttp://links.lww.com/AOG/B111
17. Ganie M, Vasudevan V, Wani I, Baba M, Arif T, Rashid A. Epidemiology, pathogenesis, genetics & management of polycystic ovary syndrome in India. Indian J Med Res [Internet]. el 1 de octubre de 2019 [citado el 27 de junio de 2022];150(4):333. Disponible en: /pmc/articles/PMC6902362/
18. Hernández R. Síndrome de ovario poliquístico. Revista Médica SINERGIA. octubre de 2017;2(11):17–20.
19. Roldán M, Corredor A. Síndrome de ovario poliquístico en la adolescente. Pediatría Integral [Internet]. 2020 [citado el 27 de junio de 2022];24(5):258–67. Disponible en: https://www.pediatriaintegral.es/wp-content/uploads/2020/xxiv05/03/n5-258-267_BelenRoldan.pdf
20. Balceiro Batista L, Inda Pichardo D, Garay Crespo MI, Álvarez Escobar M del C, Domínguez Suárez N, Quiñones Berrío A. Síndrome de ovarios poliquísticos. Entidad que demanda diagnóstico temprano. Revista Médica Electrónica [Internet]. agosto de 2019 [citado el 7 de marzo de 2023];41(4):940–58. Disponible en: https://www.medigraphic.com/pdfs/revmedele/me-2019/me194j.pdf
21. Hernández A, Gómez Tabares G. Hirsutismo y estados hiperandrogénicos. REV COL DE MENOPAUSIA [Internet]. 2020 [citado el 7 de marzo de 2023];26(3). Disponible en: https://fi-admin.bvsalud.org/document/view/445qw#:~:text=Es%20un%20crecimiento%20excesivo%20no,Hirsurtismo%20idiop%C3%A1tico.
22. Winnykamien I, Dalibón A, Knoblovits P. Síndrome de ovario poliquístico. Revista del Hospital Italiano de Buenos Aires [Internet]. el 10 de marzo de 2017 [citado el 7 de marzo de 2023];37(1):10–20. Disponible en: https://www1.hospitalitaliano.org.ar/multimedia/archivos/noticias_attachs/47/documentos/31087_10-20-Winnykamien-D.pdf
23. Pereira J, Pereira Y, Quirós L. Manejo del síndrome de ovario poliquístico . Revista Médica Sinergia [Internet]. abril de 2020 [citado el 27 de junio de 2022];5(4). Disponible en: https://revistamedicasinergia.com/index.php/rms/article/view/438/800

24. Merck. Fisiología y fisiopatología de la LH [Internet]. 2019 [citado el 27 de junio de 2022]. p. 4–15. Disponible en: https://www.siicsalud.com/pdf/lh_fisiologia_d2019.pdf
25. Bosch E, Alviggi C, Lispi M, Conforti A, Hanyaloglu AC, Chuderland D, et al. Reduced FSH and LH action: implications for medically assisted reproduction. Hum Reprod [Internet]. el 1 de junio de 2021 [citado el 27 de junio de 2022];36(6):1469. Disponible en: /pmc/articles/PMC8129594/
26. Borba VV, Zandman-Goddard G, Shoenfeld Y. Prolactin and autoimmunity [Internet]. Vol. 9, Frontiers in Immunology. Frontiers Media S.A.; 2018 [citado el 3 de diciembre de 2022]. Disponible en: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5816039/
27. Hathaway CA, Rice MS, Townsend MK, Hankinson SE, Arslan AA, Buring JE, et al. Prolactin and risk of epithelial ovarian cancer. Cancer Epidemiology Biomarkers and Prevention [Internet]. el 1 de septiembre de 2021 [citado el 3 de diciembre de 2022];30(9):1652–9. Disponible en: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8419083/
28. Davoudi Z, Araghi F, Vahedi M, Mokhtari N, Gheisari M. Prolactin level in polycystic ovary syndrome (Pcos): An approach to the diagnosis and management. Acta Biomedica [Internet]. el 3 de noviembre de 2021 [citado el 3 de diciembre de 2022];92(5). Disponible en: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8689332/
29. Davis S, Wahlin S. La testosterona en las mujeres. Lancet Diabetes Endocrinol [Internet]. el 1 de febrero de 2016 [citado el 3 de diciembre de 2022];3:980–92. Disponible en: https://www.sasharg.com.ar/descargas/Actualizaciones/La%20testosterona%20en%20las%20mujeres.pdf
30. Viteri JA, Romero HL, García A, Salinas MJ. Aspectos genéticos y hormonales del síndrome de ovario poliquístico (SOP). Metanoia [Internet]. 2019 [citado el 7 de marzo de 2023];6(1). Disponible en: http://45.238.216.13/ojs/index.php/METANOIA/article/view/2180/1538
31. Sir T, Preisler J, Magendzo A. Síndrome de ovario poliquístico. diagnóstico y manejo. Revista Médica Clínica Las Condes [Internet]. el 1 de septiembre de 2013 [citado el 7 de marzo de 2023];24(5):818–26. Disponible en: https://www.elsevier.es/es-revista-

revista-medica-clinica-las-condes-202-articulo-sindrome-ovario-poliquistico-diagnostico-manejo-S0716864013702293

32. Hernández Quiceno S, Uribe Bojanini E, Alfaro Velásquez JM, Campuzano Maya G, Salazar Peláez LM. Endocrinología Cortisol: mediciones de laboratorio y aplicación clínica Cortisol. Medicina & Laboratorio. 2016;22(3–4):147–64.
33. Capecce E, Pelanda M, Dicugno M, De Sampaio EG, Buongiorno G, Corazza N, et al. La hormona antimülleriana como marcador de función ovárica. Rev Argent Endocrinol Metab [Internet]. el 1 de julio de 2016 [citado el 21 de marzo de 2023];53(3):106–13. Disponible en: https://www.elsevier.es/es-revista-revista-argentina-endocrinologia-metabolismo-185-articulo-la-hormona-antimulleriana-como-marcador-S0326461016300377
34. González M, Noffal V. Hormona antimülleriana: nueva herramienta para la evaluación de la fertilidad. Rev Invest Med Sur Mex [Internet]. junio de 2013 [citado el 21 de marzo de 2023];20(2):110–1013. Disponible en: https://www.medigraphic.com/pdfs/medsur/ms-2013/ms132e.pdf
35. Henao Ochoa C, Lasprilla Tovar JD, Alfaro Velásquez JM, Campuzano Maya G. Utilidad clínica de la medición de la hormona antimülleriana en las mujeres. Medicina & Laboratorio [Internet]. 2017 [citado el 21 de marzo de 2023];23(5–6):213–36. Disponible en: https://www.medigraphic.com/pdfs/medlab/myl-2017/myl175-6b.pdf
36. IABIM. Recomendaciones e indicaciones previas a la toma de muestra [Internet]. IABIM. 2019 [citado el 8 de abril de 2023]. Disponible en: https://www.iabim.com/indicaciones-previas/
37. Laboratorios Eugenio Sue. Perfil Ovario Poliquístico [Internet]. [citado el 8 de abril de 2023]. Disponible en: https://www.geneticaeugeniosue.com.mx/estudio/perfiles-y-check-ups/perfil-ovario-poliquistico/
38. Rodrigo A, Santiago E, Barranquero M, Salvador Z. Análisis hormonal en la mujer: ¿cuáles son los niveles normales? [Internet]. Reproducción Asistida ORG. 2021 [citado el 8 de abril de 2023]. Disponible en: https://www.reproduccionasistida.org/valores-hormonales-en-la-mujer/
39. Hospital Universitario. Instructivos para análisis [Internet]. [citado el 8 de abril de 2023]. Disponible en: https://hospital.uncuyo.edu.ar/instructivos-para-analisis36
40. René T, Jesús Véliz L, Nelson Wohllk G. LABORATORIO DE HORMONAS: ASPECTOS PRÁCTICOS. Revista Médica Clínica Las Condes [Internet]. el 1 de

noviembre de 2015 [citado el 8 de abril de 2023];26(6):776–87. Disponible en: https://www.elsevier.es/es-revista-revista-medica-clinica-las-condes-202-articulo-laboratorio-de-hormonas-aspectos-practicos-S0716864015001534

41. Monobind Inc. Anti-Müllerian Hormone (AMH) [Internet]. 2019 [citado el 8 de abril de 2023]. p. 1–2. Disponible en: https://system.na7.netsuite.com/core/media/media.nl?id=1399856&c=445858&h=be090d1abd4de54e2938&_xt=.pdf&vid=XaGNFcoVA3W58GJF&chrole=17&ck=7171x8oVA3K58PLb&cktime=202051&promocode=&promocodeaction=overwrite&sj=wJj7OykkXYw2rtWxGI9gS8CI9%3B1681093999%3B774762631
42. Monobind Inc. Testosterone Test System [Internet]. 2019 [citado el 8 de abril de 2023]. p. 1–2. Disponible en: https://www.monobind.com/core/media/media.nl?id=1261&c=445858&h=d44ba212e14bc11e6b2a&_xt=.pdf
43. Monobind Inc. Progesterone [Internet]. 2019 [citado el 8 de abril de 2023]. p. 1–2. Disponible en: https://system.netsuite.com/core/media/media.nl?id=1231&c=445858&h=2067525b897ba1561e21&_xt=.pdf&addrcountry=US
44. Monobind Inc. Follicle Stimulating Hormone (FSH). 2019. p. 1–2.
45. Monobind Inc. Luteinizing Hormone (LH) [Internet]. 2019 [citado el 8 de abril de 2023]. p. 1–2. Disponible en: https://system.netsuite.com/core/media/media.nl?id=1231&c=445858&h=2067525b897ba1561e21&_xt=.pdf&addrcountry=US
46. Monobind Inc. Free Testosterone [Internet]. 2018 [citado el 8 de abril de 2023]. p. 1–3. Disponible en: https://www.monobind.com/core/media/media.nl?id=8577&c=445858&h=dc383ff5b9bc405f1706&_xt=.pdf
47. Espinosa L. Síndrome de ovario poliquístico Diagnóstico y farmacoterapia [Internet]. [Madrid]: UNIVERSIDAD COMPLUTENSE; 2020 [citado el 27 de junio de 2022]. Disponible en: http://147.96.70.122/Web/TFG/TFG/Memoria/LAURA%20ESPINOSA%20GARCIA%20DE%20LA%20SANTA.pdf
48. Williamson M, Snyder M. WALLACH interpretación clínica de pruebas diagnósticas. Novena. Barcelona: Lippincott William s & Wilkins; 2012.

49. Monobind Inc. Dehydroepiandrosterone Sulfate (DHEA-S) [Internet]. 2019 [citado el 8 de abril de 2023]. Disponible en: https://system.netsuite.com/core/media/media.nl?id=2155&c=445858&h=107eae05199f6ae2a1a8&_xt=.pdf
50. Monobind Inc. 17α-OH Progesterone (17-OHP) [Internet]. 2019 [citado el 8 de abril de 2023]. p. 1–2. Disponible en: https://system.netsuite.com/core/media/media.nl?id=531432&c=445858&h=76386bc98712e06f4673&_xt=.pdf&addrcountry=US
51. Capecce E, Pelanda M, Dicugno M, de Sampaio EG, Buongiorno G, Corazza N, et al. La hormona antimülleriana como marcador de función ovárica. Rev Argent Endocrinol Metab [Internet]. el 1 de julio de 2016 [citado el 29 de junio de 2022];53(3):106–13. Disponible en: https://www.elsevier.es/es-revista-revista-argentina-endocrinologia-metabolismo-185-articulo-la-hormona-antimulleriana-como-marcador-S0326461016300377
52. Monobind Inc. Prolactin Hormone (PRL) [Internet]. 2019 [citado el 8 de abril de 2023]. p. 1–2. Disponible en: https://system.netsuite.com/core/media/media.nl?id=1232&c=445858&h=26aaa06e53e8bfaf7998&_xt=.pdf&addrcountry=US
53. Ventura-Arizmendi E, Hernández-Valencia M, Ventura-Arizmendi E, Hernández-Valencia M. La esteroidogénesis en el síndrome de ovarios poliquísticos. Gac Med Mex [Internet]. 2019 [citado el 11 de abril de 2023];155(2):184–90. Disponible en: https://www.scielo.org.mx/scielo.php?script=sci_arttext&pid=S0016-38132019000200184
54. Monobind Inc. Estradiol (E2) [Internet]. 2019 [citado el 11 de abril de 2023]. p. 1–2. Disponible en: https://www.monobind.com/core/media/media.nl?id=1464&c=445858&h=4d7e12b7e1c80c4047de&_xt=.pdf
55. Pelanis R, Mellembakken JR, Sundström-Poromaa I, Ravn P, Morin-Papunen L, Tapanainen JS, et al. The prevalence of Type 2 diabetes is not increased in normal-weight women with PCOS. Human Reproduction [Internet]. el 1 de noviembre de 2017 [citado el 12 de marzo de 2023];32(11):2279–86. Disponible en: https://academic.oup.com/humrep/article/32/11/2279/4282625?login=false

56. Quispe K. Síndrome de ovario poliquístico en el Hospital Militar - 2018. Universidad Peruana de los Andes [Internet]. marzo de 2019 [citado el 11 de marzo de 2023]; Disponible en: 1. http://www.repositorio.upla.edu.pe/bitstream/handle/20.500.12848/554/QUISPE%20USCUVILCA%2c%20KATHERINE%20HILARIA.pdf?sequence=1&isAllowed=y
57. Solís Zambrano SM. Prevalencia de ovario poliquístico como causa de infertilidad en mujeres de 18 a 35 años de edad el estudio se ha realizado en el Hospital Regional Teodoro Maldonado Carbo en el período 2015 - 2016. Universidad de Guayaquil [Internet]. 2018 [citado el 12 de marzo de 2023];1(1). Disponible en: http://repositorio.ug.edu.ec/handle/redug/31223
58. Mantilla Villabona LY. Prevalencia del síndrome de ovario poliquístico en mujeres colombianas con acné. Universidad Autónoma de Bucaramanga [Internet]. abril de 2022 [citado el 11 de marzo de 2023];2(1). Disponible en: https://repository.unab.edu.co/bitstream/handle/20.500.12749/16709/2022_Tesis_Leydi_Yurani_Mantilla.pdf?sequence=1&isAllowed=y
59. Tavares A, Rêgo Barros RC. The Prevalence of Metabolic Syndrome in the Different Phenotypes of Polycystic Ovarian Syndrome. Revista Brasileira de Ginecologia e Obstetricia [Internet]. el 14 de enero de 2019 [citado el 11 de marzo de 2023];41(1):37–43. Disponible en: http://www.thieme-connect.com/products/ejournals/html/10.1055/s-0038-1676568
60. Sachdeva G, Gainder S, Suri V, Sachdeva N, Chopra S. Comparison of the Different PCOS Phenotypes Based on Clinical Metabolic, and Hormonal Profile, and their Response to Clomiphene. Indian J Endocrinol Metab [Internet]. junio de 2019 [citado el 11 de marzo de 2023];23(3):331. Disponible en: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6683693/
61. Pikee S, Shivani S, Jayshree B. Endocrine and Metabolic Profile of Different Phenotypes of Polycystic Ovarian Syndrome. J Obstet Gynaecol India [Internet]. el 1 de octubre de 2016 [citado el 11 de marzo de 2023];66(1):566. Disponible en: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5016474/
62. Kaur R, Kaur M, Suri V. Phenotypic presentation of PCOS with respect to BMI in a north Indian population. Clinica Terapeutica [Internet]. el 9 de octubre de 2021

[citado el 11 de marzo de 2023];172(5):435–7. Disponible en: https://www.clinicaterapeutica.it/2021/172/5/11_KAUR.pdf

63. Lauritsen M, Bentzen J, Pinborg A, Loft A, Forman J, Thuesen L, et al. The prevalence of polycystic ovary syndrome in a normal population according to the Rotterdam criteria versus revised criteria including anti-Müllerian hormone. Human Reproduction [Internet]. el 1 de abril de 2014 [citado el 11 de marzo de 2023];29(4):791–801. Disponible en: https://academic.oup.com/humrep/article/29/4/791/604232?login=false
64. Ramezani F, Rashidi H, Bahri M, Tohidi M, Azizi F. The prevalence of metabolic disorders in various phenotypes of polycystic ovary syndrome: A community based study in Southwest of Iran. Reproductive Biology and Endocrinology [Internet]. el 16 de septiembre de 2014 [citado el 11 de marzo de 2023];12(89):1–6. Disponible en: https://rbej.biomedcentral.com/articles/10.1186/1477-7827-12-89
65. Elasam A, Ahmed M, Ahmed A, Sharif M, Abusham A, Hassan B, et al. The prevalence and phenotypic manifestations of polycystic ovary syndrome (PCOS) among infertile Sudanese women: a cross-sectional study. BMC Womens Health [Internet]. el 13 de mayo de 2022 [citado el 11 de marzo de 2023];22(165). Disponible en: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9102290/
66. Wang Q, Wang H, Li P, Li X, Wang Z, Yan L, et al. Association of Polycystic Ovary Syndrome Phenotypes with Adverse Pregnancy Outcomes After In-Vitro Fertilization/Intracytoplasmic Sperm Injection. Front Endocrinol (Lausanne) [Internet]. el 3 de junio de 2022 [citado el 11 de marzo de 2023];13(889029). Disponible en: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9203834/
67. Farhadi-Azar M, Behboudi-Gandevani S, Rahmati M, Mahboobifard F, Khalili Pouya E, Ramezani Tehrani F, et al. The Prevalence of Polycystic Ovary Syndrome, Its Phenotypes and Cardio-Metabolic Features in a Community Sample of Iranian Population: Tehran Lipid and Glucose Study. Front Endocrinol (Lausanne) [Internet]. el 1 de marzo de 2022 [citado el 11 de marzo de 2023];13(825528). Disponible en: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8920974/
68. Ovies G, Sardiñas I, Monteaguno G, Martínez A, Lardoext R. Agregación familiar de causa genética en familiares de primer grado de mujeres con síndrome de ovarios poliquísticos. Revista Cubana de Endocrinología [Internet]. 2015 [citado el 12 de

marzo de 2023];26(1):21–32. Disponible en: http://scielo.sld.cu/scielo.php?script=sci_arttext&pid=S1561-29532015000100003

69. Carmina E, Nasrallah M, Guastella E, Lobo R. Characterization of metabolic changes in the phenotypes of women with polycystic ovary syndrome in a large Mediterranean population from Sicily. Clin Endocrinol (Oxf) [Internet]. el 1 de octubre de 2019 [citado el 11 de marzo de 2023];91(4):553–60. Disponible en: https://onlinelibrary.wiley.com/doi/10.1111/cen.14063
70. Altintas KZ, Dilbaz B, Cirik DA, Ozelci R, Zengin T, Erginay ON, et al. The incidence of metabolic syndrome in adolescents with different phenotypes of PCOS. Ginekol Pol [Internet]. 2017 [citado el 11 de marzo de 2023];88(6):289–95. Disponible en: https://journals.viamedica.pl/ginekologia_polska/article/view/48979
71. Zaeemzadeh N, Sadatmahalleh SJ, Ziaei S, Kazemnejad A, Mottaghi A, Mohamadzadeh N, et al. Prevalence of metabolic syndrome in four phenotypes of PCOS and its relationship with androgenic components among Iranian women: A cross-sectional study. Int J Reprod Biomed [Internet]. el 1 de abril de 2020 [citado el 11 de marzo de 2023];18(4):264. Disponible en: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7218672/
72. Yarjanli M, Jahanian S, Mirzaei N, Azarbajani K. Female sexual function in different phenotypes of polycystic ovarian syndrome: a comparative cross-sectional study. Sci Rep [Internet]. el 11 de noviembre de 2022 [citado el 11 de marzo de 2023];12(19317). Disponible en: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9652450/
73. Pulido DI, Scott ML, Barreras C, Soto F, Barrios C, López CM. Síndrome de ovario poliquístico en mujeres portadoras de síndrome metabólico. Revista Médica Clínica Las Condes [Internet]. el 1 de julio de 2016 [citado el 13 de marzo de 2023];27(4):540–4. Disponible en: https://www.elsevier.es/es-revista-revista-medica-clinica-las-condes-202-articulo-sindrome-de-ovario-poliquistico-en-S0716864016300633
74. Ollila MM, Piltonen T, Puukka K, Ruokonen A, Järvelin MR, Tapanainen J, et al. Weight Gain and Dyslipidemia in Early Adulthood Associate with Polycystic Ovary Syndrome: Prospective Cohort Study. J Clin Endocrinol Metab [Internet]. el 1 de febrero de 2016 [citado el 13 de marzo de 2023];101(2):739–47. Disponible en: https://academic.oup.com/jcem/article/101/2/739/2811201

75. Vital V, López M, Inda P, Márquez C. Alteraciones metabólicas sutiles en adolescentes con obesidad y síndrome de ovario poliquístico. Gac Med Mex [Internet]. el 7 de septiembre de 2017 [citado el 13 de marzo de 2023];153(2):34–41. Disponible en: https://www.researchgate.net/profile/Patricia-Inda-Icaza/publication/321136421_Alteraciones_metabolicas_sutiles_en_adolescentes_con_obesidad_y_sindrome_de_ovario_poliquistico/links/5e0913d2a6fdcc2837485807/Alteraciones-metabolicas-sutiles-en-adolescentes-con-obesidad-y-sindrome-de-ovario-poliquistico.pdf
76. Peña S, Salazar Z, Cárdenas F, Ochoa S, Cumbe K, Sánchez G. Prevalencia y factores asociados al Ovario Poliquístico en adultas de las parroquias urbanas de la ciudad de Cuenca-Ecuador, 2017. Archivos Venezolanos de Farmacología y Terapéutica [Internet]. 2018 [citado el 13 de marzo de 2023];37(3). Disponible en: https://www.revistaavft.com/images/revistas/2018/avft_3_2018/3_prevalencia_factores_asociados_ovario.pdf
77. Carmenate J, Cabrera Rode E, Monteagudo Peña G, Peix González A, González Sánchez R, González Domínguez N, et al. Asociación entre el fenotipo hipertrigliceridemia-obesidad abdominal, la resistencia a la insulina y las variables cardiometabólicas en el síndrome de ovario poliquístico. Revista Cubana de Endocrinología [Internet]. 2021 [citado el 13 de marzo de 2023];30(2):271. Disponible en: http://scielo.sld.cu/pdf/end/v32n1/1561-2953-end-32-01-e271.pdf
78. Monteagudo Peña G, González Suárez R, Gómez Alzugaray M, Ovies Carballo G, Menocal Alayón A, Rodríguez Martínez K, et al. Resistencia a la insulina en mujeres con síndrome de ovario poliquístico. Revista Cubana de Endocrinología [Internet]. 2019 [citado el 13 de marzo de 2023];30(2):179. Disponible en: http://scielo.sld.cu/pdf/end/v30n2/1561-2953-end-30-02-e179.pdf
79. Tamayo R, Betancourt E, Urquiza Y, García M, Betancourt J. Infertilidad por síndrome de ovario poliquístico en mujeres del municipio Las Tunas . Revista Electrónica Dr Zoilo E Marinello Vidaurreta [Internet]. 2019 [citado el 12 de marzo de 2023];44(5). Disponible en: https://revzoilomarinello.sld.cu/index.php/zmv/article/view/1926
80. Singh JR, Jain A, Wadhwa N, H.r. T, Ahirwar AK. La resistencia a la insulina como factor etiológico en el síndrome del ovario poliquístico: un estudio de casos y controles. Advances in Laboratory Medicine [Internet]. el 1 de junio de 2022 [citado

el 13 de marzo de 2023];3(2):205–9. Disponible en: https://www.degruyter.com/document/doi/10.1515/almed-2022-0050/html#Vancouver

81. Shan B, Cai J hong, Yang SY, Li ZR. Risk factors of polycystic ovarian syndrome among Li People. Asian Pac J Trop Med [Internet]. el 1 de julio de 2015 [citado el 13 de marzo de 2023];8(7):590–3. Disponible en: https://www.sciencedirect.com/science/article/pii/S1995764515000577?via%3Dihub#tbl2
82. Ponce Morán AM, Alonso Madero JL. Factores de riesgo del síndrome de ovario poliquístico en mujeres entre las edades de 15 a 30 años del Hospital Teodoro Maldonado Carbo durante el período 2016 - 2018. Universidad de Guayaquil [Internet]. 2019 [citado el 13 de marzo de 2023];1(1). Disponible en: http://repositorio.ug.edu.ec/handle/redug/43457
83. Yallico I. Acné tardío en relación a síndrome de ovario poliquístico en pacientes de la consulta dermatológica del Hospital Belén de Trujillo. Universidad Privada Antenor Orrego [Internet]. 2018 [citado el 12 de marzo de 2023];1(1). Disponible en: http://repositorio.upao.edu.pe/bitstream/20.500.12759/4360/1/REP_MED.HUMA_IRIS.%2cYALLICO_ACN%c3%89.TARD%c3%8dO.RELACI%c3%93N.S%c3%8dNDROME.OVARIO.POLIQU%c3%8dSTICO.PACIENTES.CONSULTA.DERMATOL%c3%93GICA.HOSPITAL.BEL%c3%89N.TRUJILLO.pdf
84. Hernández J. Prevalencia y factores asociados en los casos de síndrome de ovario poliquístico diagnosticados en el centro de salud Tulcán sur periodo 2014-2016. Universidad Técnica del Norte [Internet]. 2017 [citado el 12 de marzo de 2023];1(1). Disponible en: 6. http://repositorio.utn.edu.ec/bitstream/123456789/6540/1/06%20NUT%20210%20TRABAJO%20DE%20GRADO.pdf
85. Giménez SR, Ríos CM. Características clínicas y epidemiológicas del Síndrome de Ovario Poliquístico en un Hospital de referencia de Paraguay. Revista científica ciencias de la salud [Internet]. el 28 de mayo de 2020 [citado el 12 de marzo de 2023];2(1):18–26. Disponible en: http://scielo.iics.una.py/scielo.php?pid=S2664-28912020000100018&script=sci_arttext
86. Iturrizaga C. Síndrome de ovario poliquístico como factor asociado para diabetes mellitus 2 en un Hospital Nacional. Universidad Peruana Los Andes [Internet]. 2020

[citado el 13 de marzo de 2023];1(1). Disponible en: https://repositorio.upla.edu.pe/handle/20.500.12848/1432

87. Moyano Gómez VA. Síndrome de ovario poliquístico y su correlación la diabetes mellitus. Universidad de Guayaquil [Internet]. 2020 [citado el 13 de marzo de 2023];1(1). Disponible en: http://repositorio.ug.edu.ec/handle/redug/52361
88. García L. Prevalencia del síndrome de ovarios poliquísticos y factores de riesgos asociados en mujeres atendidas en el centro de salud Santa Rosa. 2018-2020. Universidad Regional Autónoma de los Andes "Uniandes" [Internet]. 2021 [citado el 13 de marzo de 2023];1(1). Disponible en: https://dspace.uniandes.edu.ec/bitstream/123456789/14196/1/UA-ENF-EAC-008-2021.pdf
89. Yue CY, Lu LKY, Li M, Zhang QL, Ying CM. Threshold value of anti-Mullerian hormone for the diagnosis of polycystic ovary syndrome in Chinese women. PLoS One [Internet]. el 28 de agosto de 2018 [citado el 12 de marzo de 2023];13(8). Disponible en: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6112651/
90. Nigar A, Birge Ö, Uysal A, Görar S, Tekeli F. Hashimoto's thyroiditis worsens ovaries in polycystic ovary syndrome patients compared to Anti-Müllerian hormone levels. BMC Endocr Disord [Internet]. el 1 de diciembre de 2021 [citado el 13 de marzo de 2023];21(1). Disponible en: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7941903/
91. Khashchenko E, Uvarova E, Vysokikh M, Ivanets T, Krechetova L, Tarasova N, et al. The Relevant Hormonal Levels and Diagnostic Features of Polycystic Ovary Syndrome in Adolescents. J Clin Med [Internet]. el 1 de junio de 2020 [citado el 12 de marzo de 2023];9(6):1–12. Disponible en: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7355484/
92. Khattak M, Usman R, Sultana N, Khattak A. Comparison of free androgen index in polycystic ovary syndrome and non-polycystic ovary syndrome infertile patients. J Ayub Med Coll Abbottabad [Internet]. 2021 [citado el 12 de marzo de 2023];33(4):577–81. Disponible en: https://jamc.ayubmed.edu.pk/jamc/index.php/jamc/article/view/8121/3171
93. Gülücü S, Can İS. Total, cholesterol/high-density lipoprotein and inflammatory parameters in patients with polycystic ovary syndrome. Rev Assoc Med Bras

[Internet]. el 28 de noviembre de 2022 [citado el 12 de marzo de 2023];68(11):1503. Disponible en: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9720768/

94. Wei L, Wu F, Zhang J, Li J, Yang D, Wen G. Evaluation of Endocrine and Metabolic Changes in Polycystic Ovary Syndrome by Ultrasonic Imaging Features under an Intelligent Algorithm. Comput Math Methods Med [Internet]. el 26 de abril de 2022 [citado el 12 de marzo de 2023];2022(1411943). Disponible en: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9064505/
95. Bostanci M, Akdemir N, Cinemre A, Cevrioglu S, Özden S, Ünal O. Serum irisin levels in patients with polycystic ovary syndrome. Eur Rev Med Pharmacol Sci [Internet]. 2015 [citado el 12 de marzo de 2023];19(23):4462–8. Disponible en: https://www.europeanreview.org/article/9925
96. Broskey NT, Tam CS, Sutton EF, Altazan AD, Burton JH, Ravussin E, et al. Metabolic inflexibility in women with PCOS is similar to women with type 2 diabetes. Nutr Metab (Lond) [Internet]. el 20 de octubre de 2018 [citado el 12 de marzo de 2023];15(75). Disponible en: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6195988/
97. Ezeh U, Ezeh C, Pisarska M, Azziz R. Menstrual dysfunction in polycystic ovary syndrome: association with dynamic state insulin resistance rather than hyperandrogenism. Fertil Steril [Internet]. el 1 de junio de 2021 [citado el 12 de marzo de 2023];115(6):1557–68. Disponible en: https://www.fertstert.org/article/S0015-0282(20)32758-8/fulltext
98. Franik G, Bizoń A, Włoch S, Kowalczyk K, Biernacka-Bartnik A, Madej P. Hormonal and metabolic aspects of acne vulgaris in women with polycystic ovary syndrome. Eur Rev Med Pharmacol Sci [Internet]. 2018 [citado el 12 de marzo de 2023];22(1):4411–8. Disponible en: https://www.europeanreview.org/wp/wp-content/uploads/4411-4418-1.pdf
99. Gursu T, Cevik H, Desteli G, Yilmaz B, Bildaci T, Eraslan A. Diagnostic value of shear wave velocity in polycystic ovarian syndrome. J Ultrason [Internet]. el 29 de noviembre de 2021 [citado el 12 de marzo de 2023];21(87):277–81. Disponible en: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8678642/
100. Eroglu S, Cakmakliogullari EK. Decreased serum profile of the interleukin-36α in polycystic ovary syndrome. Taiwan J Obstet Gynecol [Internet]. el 1 de noviembre de 2021 [citado el 12 de marzo de 2023];60(6):1018–22. Disponible en:

https://www.sciencedirect.com/science/article/pii/S1028455921002527?via%3Dihub

101. Vázquez J, Calero J, Carías J, Monteagudo G. Correspondencia clínica, hormonal y ecográfica en el diagnóstico del síndrome de ovarios poliquísticos. Revista Cubana de Endocrinología [Internet]. 2016 [citado el 12 de marzo de 2023];27(1):4–17. Disponible en: http://scielo.sld.cu/pdf/end/v27n1/end02116.pdf
102. Castillo T, Marín J, Moreno H, Villalba G, Forero M. Caracterización clínica de una muestra de pacientes colombianas con síndrome de ovario poliquístico (SOP) . Universidad Pedagógica y Tecnológica de Colombia [Internet]. el 30 de septiembre de 2019 [citado el 12 de marzo de 2023];1(1). Disponible en: https://repositorio.uptc.edu.co/bitstream/handle/001/5307/Caracterizacion_clinica_de_una_muestra_de_pacientes_colombianas_con_sindrome_de_ovario_poliquistico.pdf;jsessionid=5094FA45D33518E46E8B74934786D150?sequence=1
103. Romero S, Pérez J. Relación en la presentación de manifestaciones clínicas del hiperandrogenismo en mujeres normopeso y sobrepeso con síndrome de ovario poliquístico, septiembre 2019 - febrero 2020. Universidad Nacional Pedro Henríquez Ureña [Internet]. 2020 [citado el 12 de marzo de 2023];1(1). Disponible en: 3. https://repositorio.unphu.edu.do/bitstream/handle/123456789/3615/Relaci%c3%b3n%20en%20la%20presentaci%c3%b3n%20de%20manifestaciones%20cl%c3%adnicas-%20Sharina%20M.%20Romero%20y%20Jirandy%20J.%20P%c3%a9rez.pdf?sequence=1&isAllowed=y
104. Zamudio A. Hallazgos clínicos de hiperandrogenismo en lesbianas de la ciudad de Trujillo. Universidad Privada Antenor Orrego [Internet]. el 30 de enero de 2016 [citado el 12 de marzo de 2023];1(1). Disponible en: http://repositorio.upao.edu.pe/bitstream/20.500.12759/3918/1/REP_MED.HUMA_ANDREA.ZAMUDIO_HALLAZGOS.CL%c3%8dNICOS.HIPERANDROGENISMO.LESBIANAS.CIUDAD.TRUJILLO.pdf
105. Abusailik M, Muhanna A, Almuhisen A, Alhasanat A, Alshamaseen A, Bani Mustafa S, et al. Cutaneous manifestation of polycystic ovary syndrome. Dermatol Reports [Internet]. el 8 de agosto de 2021 [citado el 12 de marzo de 2023];13(2). Disponible en: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8451069/

106. Aljefri Y, Alahmadi R, Alajmi R, Alkhamisi T, Maaddawi H, Alraddadi A, et al. Cutaneous Manifestations and Hormonal Changes Among Polycystic Ovary Syndrome Patients at a Tertiary Care Center. Cureus [Internet]. el 22 de diciembre de 2021 [citado el 12 de marzo de 2023];13(12). Disponible en: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8777386/
107. Vishnubhotla DS, Tenali SN, Fernandez M, Madireddi S. Evaluation of Prevalence of PCOS and Associated Depression, Nutrition, and Family History: A Questionnaire-based Assessment. Indian J Endocrinol Metab [Internet]. el 20 de septiembre de 2022 [citado el 12 de marzo de 2023];26(4):347. Disponible en: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9519834/
108. Artar G, Tas B, Turan G, Uckan H. Evaluation of androgen-dependent skin findings of polycystic ovary syndrome (PCOS). Gynecological Endocrinology [Internet]. el 6 de junio de 2022 [citado el 12 de marzo de 2023];38(12):1104–8. Disponible en: https://www.tandfonline.com/doi/full/10.1080/09513590.2022.2162496

ANEXOS

Anexo 1. *Criterios para el diagnóstico de síndrome de ovario poliquístico.*

Tabla I. Criterios diagnósticos del síndrome de ovario poliquístico según el consenso de Rotterdam, avalados por el Instituto Nacional de Salud de Estados Unidos en el año 2012[15,17]

Disfunción ovulatoria:

- Amenorrea primaria: ausencia de menarquia a los 15 años o >3 años tras inicio de telarquia
- Irregularidades menstruales:
  - Normal, durante el primer año postmenarquia
  - Oligomenorrea: ciclos >45 días de 1-3 años postmenarquia o >35 días a partir del tercer año
  - <8 ciclos al año a partir del tercer año tras menarquia
  - Polimenorrea: ciclos menstruales <21 días
  - Ciclo menstrual >90 días después de 1 año postmenarquia (amenorrea secundaria)

Hiperandrogenismo clínico y/o bioquímico:

- Hirsutismo, acné y alopecia androgénica
- Elevación de testosterona total y libre calculada y/o otros andrógenos (androstenediona, DHEAS)

Morfología poliquística ovárica

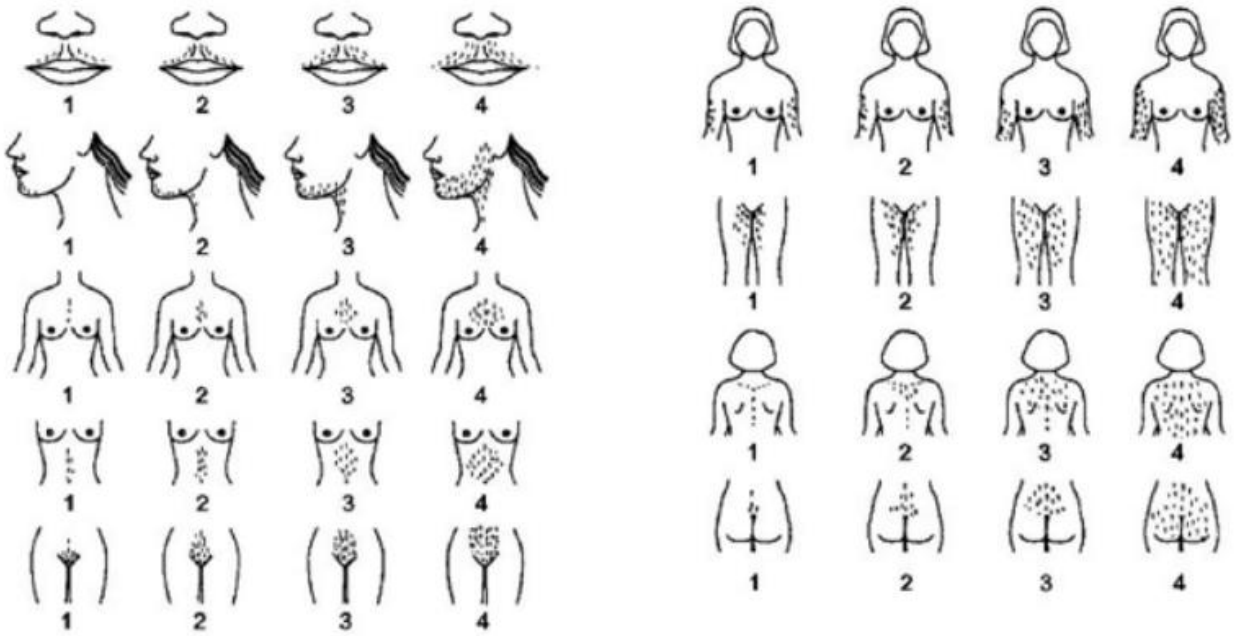
Exclusión de otras causas de exceso androgénico:

Hiperplasia suprarrenal congénita no clásica, tumores productores de andrógenos, disfunción tiroidea, hiperprolactinemia, síndrome de Cushing y fármacos con actividad androgénica

DHEAS: dehidroepiandrosterona sulfato.

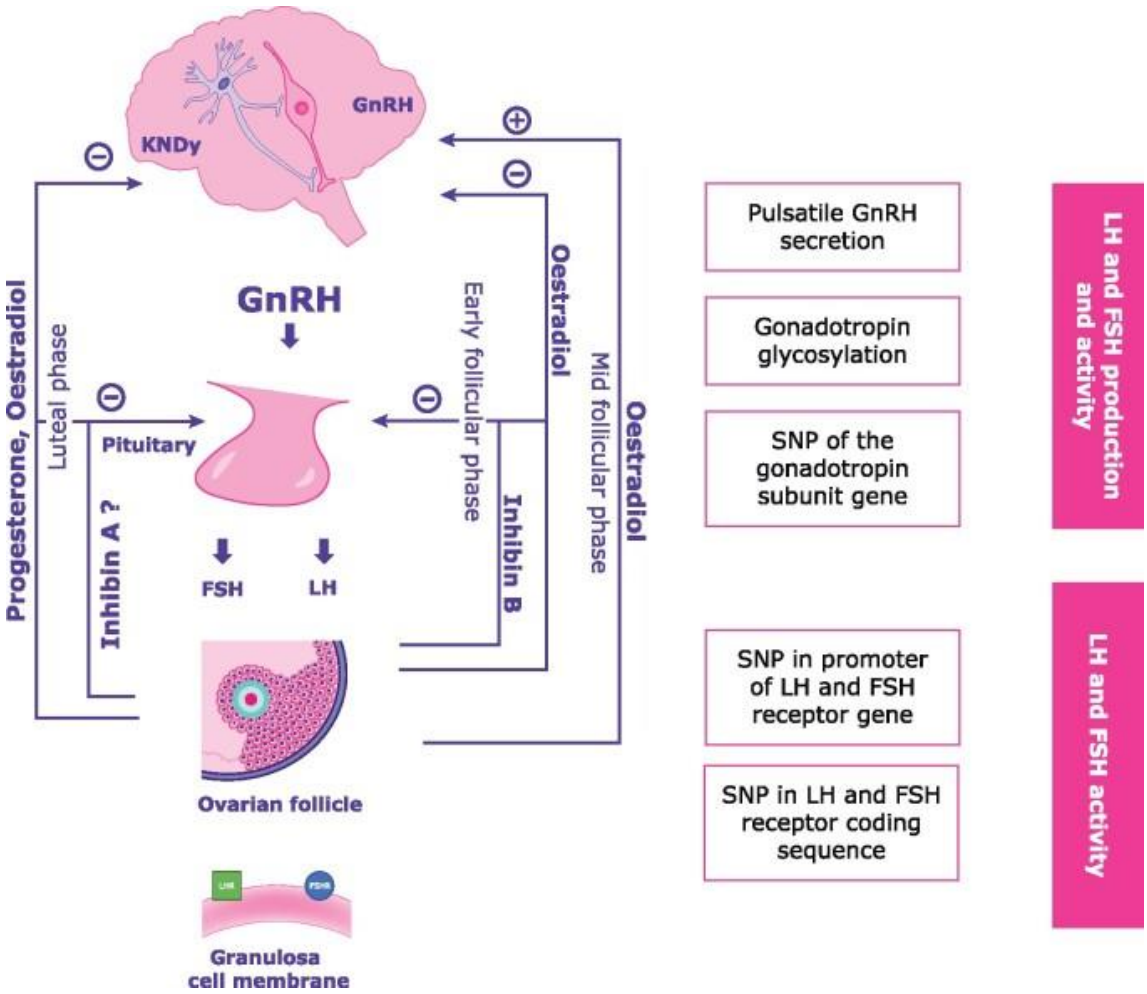
Fuente: *Roldan, M. & Corredor, B. 2020.*

Anexo 2. *Escala de Ferriman-Gallwey modificada.*



Fuente: *Roldán, M. & Corredor, B. 2020.*

Anexo 3. *Sistema de retroalimentación negativa del eje hipotálamo-hipofisario-gonadal.*



Fuente: *Bosch,E. et al. 2021.*

Anexo 4. *Valores normales de Testosterona Total.*

TESTOSTERONA TOTAL VALORES DE REFERENCIA	
Edades	**Valor**
7 – 13 años	2 – 64 ng/dL
14 – 17 años	9 – 63 ng/dL
18 – 40 años	11 – 59 ng/dL
41- 51 años	9 – 55 ng/dL
Posmenopáusica	6 – 25 ng/dL

Fuente: *Williamson, M. & Snyder, L. 2012.*

Anexo 5. *Inserto de la hormona Testosterona Total.*

Monobind Inc.
Lake Forest, CA 92630, USA

AccuBind
ELISA Microwells

Testosterone Test System
Product Code: 3725-300

1.0 INTRODUCTION

Intended Use: The Quantitative Determination of Total Testosterone Concentration in Human Serum or Plasma by a Microplate Enzyme Immunoassay, Colorimetric

2.0 SUMMARY AND EXPLANATION OF THE TEST

Testosterone, (17β-Hydroxy-4-androstene-3-one), a C_{19} steroid, is the most potent naturally secreted androgen.[1] In normal post pubertal males, testosterone is secreted primarily by the testes with only a small amount derived from peripheral conversion of 4-Androstene-3, 17-dione (ASD).[2] In adult women, it has been estimated that over 50% of serum testosterone is derived from peripheral conversion of ASD secreted by the adrenal and ovary, with the remainder from direct secretion of testosterone by these glands.

In the male, testosterone is mainly synthesized in the interstitial Leydig cells and the testis, and is regulated by the interstitial cell stimulating hormone (ICSH), or luteinizing hormone (LH) of the anterior pituitary (the female equivalent of ICSH).[3] Testosterone is responsible for the development of secondary sex characteristics, such as the accessory sex organs, the prostate, seminal vesicles and the growth of facial, pubic and auxiliary hair. Testosterone measurements have been very helpful in evaluating hypogonadal states. Increased testosterone levels in males can be found in complete androgen resistance (testicular feminization). Common causes of decreased testosterone levels in males include: hypogonadism, orchidectomy, estrogen therapy, Klinefelter's syndrome, hypopituitarism, and hepatic cirrhosis.[2,4]

In the female, testosterone levels are normally found to be much lower than those encountered in the healthy male. Testosterone in the female comes from three sources. It is secreted in small quantities by both the adrenal glands and the ovaries, and in healthy women 50–60% of the daily testosterone production arises from peripheral metabolism of prohormone, chiefly androstenedione. Common causes of increased serum testosterone levels in females include polycystic ovaries (Stein-Leventhal syndrome), ovarian tumors, adrenal tumors and adrenal hyperplasia. Virilization in women is associated with the administration of androgens and endogenous overproduction of testosterone. There appears to be a correlation between serum testosterone levels and the degree of virilization in women, although approximately 25% of women with varying degrees of virilism have serum testosterone levels that fall within the female reference range.

3.0 PRINCIPLE

Competitive Enzyme Immunoassay (TYPE 7):
The essential reagents required for an enzyme immunoassay include antibody, enzyme-antigen conjugate and native antigen.

Upon mixing biotinylated antibody, enzyme-antigen conjugate and a serum containing the native antigen, a competition reaction results between the native antigen and the enzyme-antigen conjugate for a limited number of antibody binding sites. The interaction is illustrated by the followed equation:

$$^{Enz}Ag + Ag + Ab_{Btn} \underset{k_{-a}}{\overset{k_a}{\rightleftharpoons}} AgAb_{Btn} + {}^{Enz}AgAb_{Btn}$$

Ab_{Btn} = Biotinylated Antibody (Constant Quantity)
Ag = Native Antigen (Variable Quantity)
^{Enz}Ag = Enzyme-antigen Conjugate (Constant Quantity)
$AgAb_{Btn}$ = Antigen-Antibody Complex
$^{Enz}AgAb_{Btn}$ = Enzyme-antigen Conjugate -Antibody Complex
k_a = Rate Constant of Association
k_{-a} = Rate Constant of Disassociation
$K = k_a / k_{-a}$ = Equilibrium Constant

A simultaneous reaction between the biotin attached to the antibody and the streptavidin immobilized on the microwell occurs. This effects the separation of the antibody bound fraction after decantation or aspiration.
$AgAb_{Btn} + {}^{Enz}AgAb_{Btn} + \underline{Streptavidin}_{cw} \Rightarrow \underline{immobilized\ complex}$
$\underline{Streptavidin}_{cw}$ = Streptavidin immobilized on well
Immobilized complex = sandwich complex bound to the solid surface

The enzyme activity in the antibody bound fraction is inversely proportional to the native antigen concentration. By utilizing several different serum references of known antigen concentration, a dose response curve can be generated from which the antigen concentration of an unknown can be ascertained.

4.0 REAGENTS

Materials Provided:

A. Testosterone Calibrators – 1ml/vial – Icons A-G
Seven (7) vials of serum reference for Testosterone at concentrations of 0 (**A**), 0.1 (**B**), 0.5 (**C**), 1.0 (**D**), 2.5 (**E**), 5.0 (**F**) and 12.0 (**G**) in ng/ml Store at 2-8°C. A preservative has been added. The calibrators can be expressed in molar concentrations (nM/L) by multiplying by 3.47.
For example: 1ng/ml x 3.47 = 3.47 nM/L

B. Testosterone Enzyme Reagent – 6.0 ml/vial – Icon Ⓔ
One (1) ready to use vial of Testosterone (Analog)-horseradish peroxides (HRP) conjugate in a protein stabilizing matrix with buffer, red dye, preservative, and binding protein inhibitors. Store 2-8°C.

C. Testosterone Biotin Reagent – 6.0 ml – Icon ∇
One (1) vial containing anti-Testosterone biotinylated purified rabbit IgG conjugate in buffer, dye and preservative. Store at 2-8°C.

D. Streptavidin Coated Plate – 96 wells – Icon ⇓
One 96-well microplate coated with 1.0 µg/ml streptavidin and packaged in an aluminum bag with a drying agent. Store at 2-8°C.

E. Wash Solution Concentrate – 20ml/vial – Icon 🔥
One (1) vial containing a surfactant in buffered saline. A preservative has been added. Store at 2-8°C.

F. Substrate A – 7ml/vial – Icon S^A
One (1) vial containing tetramethylbenzidine (TMB) in buffer. Store at 2-8°C.

G. Substrate B – 7ml/vial – Icon S^B
One (1) vial containing hydrogen peroxide (H_2O_2) in buffer. Store at 2-8°C.

H. Stop Solution – 8ml/vial – Icon STOP
One (1) vial containing a strong acid (1N HCl). Store at 2-8°C.

I. Product Instructions.

Note 1: Do not use reagents beyond the kit expiration date.
Note 2: Avoid extended exposure to heat and light. **Opened reagents are stable for sixty (60) days when stored at 2-8°C. Kit and component stability are identified on the label.**
Note 3: Above reagents are for a single 96-well microplate.

4.1 Required But Not Provided:
1. Pipette capable of delivering 0.010ml (10µl), 0.050ml(50µl), 0.100ml (100µl) volumes with a precision of better than 1.5%.
2. Dispenser(s) for repetitive deliveries of 0.50ml (50µl) .0.100ml (100 µl) and 0.350ml (350µl) volumes with a precision of better than 1.5%.
3. Microplate washer or a squeeze bottle (optional).
4. Microplate Reader with 450nm and 620nm wavelength absorbance capability.
5. Absorbent Paper for blotting the microplate wells.
6. Plastic wrap or microplate covers for incubation steps.
7. Vacuum aspirator (optional) for wash steps.
8. Timer.
9. Quality control materials.

5.0 PRECAUTIONS

For In Vitro Diagnostic Use
Not for Internal or External Use in Humans or Animals

All products that contain human serum have been found to be non-reactive for Hepatitis B Surface Antigen, HIV 1&2 and HCV Antibodies by FDA required tests. Since no known test can offer complete assurance that infectious agents are absent, all human serum products should be handled as potentially hazardous and capable of transmitting disease. Good laboratory procedures for handling blood products can be found in the Center for Disease Control / National Institute of Health, "Biosafety in Microbiological and Biomedical Laboratories," 2nd Edition, 1988, HHS Publication No. (CDC) 88-8395.

Safe Disposal of kit components must be according to local regulatory and statutory requirement.

6.0 SPECIMEN COLLECTION AND PREPARATION

The specimens shall be blood; serum or plasma in type and the usual precautions in the collection of venipuncture samples should be observed. For accurate comparison to established normal values, a fasting morning serum sample should be obtained. The blood should be collected in a plain redtop venipuncture tube or (for plasma) in evacuated tube(s) containing heparin. Allow the blood to clot for serum samples. Centrifuge the specimen to separate the serum or plasma from the cells.

In patients receiving therapy with high biotin doses (i.e. >5mg/day), no sample should be taken until at least 8 hours after the last biotin administration, preferably overnight to ensure fasting sample.

Samples may be refrigerated at 2-8°C for a maximum period of five (5) days. If the specimen(s) cannot be assayed within this time, the sample(s) may be stored at temperatures of -20°C for up to 30 days. Avoid use of contaminated devices. Avoid repetitive freezing and thawing. When assayed in duplicate, 0.020ml (20µl) of the specimen is required.

7.0 QUALITY CONTROL

Each laboratory should assay controls at levels in the low, normal and high range for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control charts should be maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends. The individual laboratory should set acceptable assay performance limits. In addition, maximum absorbance should be consistent with past experience. Significant deviation from established performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.

8.0 REAGENT PREPARATION

1. **Wash Buffer**
Dilute contents of wash solution concentrate to 1000ml with distilled or deionized water in a suitable storage container. Diluted buffer can be stored at 2-30°C for up to 60 days.
3. **Working Substrate Solution** - Stable for 1 year.
Pour the contents of the amber vial labeled Solution 'A' into the clear vial labeled Solution 'B'. Place the yellow cap on the clear vial for easy identification. Mix and label accordingly. Store at 2 - 8°C.

Note 1: Do not use the working substrate if it looks blue.
Note 2: Do not use reagents that are contaminated or have bacteria growth.

9.0 TEST PROCEDURE

Before proceeding with the assay, bring all reagents, serum calibrators and controls to room temperature (20 - 27°C).
*****Test Procedure should be performed by a skilled individual or trained professional*****

1. Format the microplates' wells for each serum reference, control and patient specimen to be assayed in duplicate. **Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8°C.**
2. Pipette 0.010 ml (10µL) of the appropriate serum reference, control or specimen into the assigned well.
3. Add 0.050 ml (50µl) of the ready to use Testosterone Enzyme Reagent to all wells.
4. Swirl the microplate gently for 20-30 seconds to mix.
5. Add 0.050 ml (50µl) of Testosterone Biotin Reagent to all wells.
6. Swirl the microplate gently for 20-30 seconds to mix.
7. Cover and incubate for 60 minutes at room temperature.
8. Discard the contents of the microplate by decantation or aspiration. If decanting, blot the plate dry with absorbent paper.
9. Add 0.350ml (350µl) of wash buffer (see Reagent Preparation Section), decant (tap and blot) or aspirate. Repeat two (2) additional times for a total of three (3) washes. **An automatic or manual plate washer can be used. Follow the manufacturer's instruction for proper usage. If a squeeze bottle is employed, fill each well by depressing the container (avoiding air bubbles) to dispense the wash. Decant the wash and repeat two (2) additional times.**
10. Add 0.100 ml (100µl) of working substrate solution to all wells (see Reagent Preparation Section). **Always add reagents in the same order to minimize reaction time differences between wells.**
DO NOT SHAKE THE PLATE AFTER SUBSTRATE ADDITION
11. Incubate at room temperature for fifteen (15) minutes.
12. Add 0.050ml (50µl) of stop solution to each well and gently mix for 15-20 seconds. **Always add reagents in the same order to minimize reaction time differences between wells.**
13. Read the absorbance in each well at 450nm (using a reference wavelength of 620-630nm to minimize well imperfections) in a microplate reader. **The results should be read within thirty (30) minutes of adding the stop solution.**

Note: Dilute the samples suspected of concentrations higher than 12 ng/ml 1:5 and 1:10 with Testosterone '0' ng/ml calibrator or female patient sera with a known low value for testosterone.

10.0 CALCULATION OF RESULTS

A dose response curve is used to ascertain the concentration of Testosterone in unknown specimens.
1. Record the absorbance obtained from the printout of the microplate reader as outlined in Example 1.
2. Plot the absorbance for each duplicate serum reference versus the corresponding Testosterone concentration in ng/ml on linear graph paper (do not average the duplicates of the serum references before plotting).
3. Connect the points with a best-fit curve.
4. To determine the concentration of Testosterone for an unknown, locate the average absorbance of the duplicates for each unknown on the vertical axis of the graph, find the intersecting point on the curve, and read the concentration (in ng/ml) from the horizontal axis of the graph (the duplicates of the unknown may be averaged as indicated). In the following example, the average absorbance (1.764) intersects the dose response curve at (0.57ng/ml) Testosterone concentration (See Figure 1).

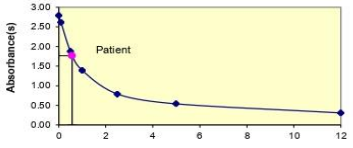
Note: Computer data reduction software designed for ELISA assays may also be used for the data reduction. If such software is utilized, the validation of the software should be ascertained.

EXAMPLE 1

Sample I.D.	Well Number	Abs (A)	Mean Abs (B)	Value (ng/ml)
Cal A	A1	2.780	2.787	0
	B1	2.794		
Cal B	C1	2.576	2.611	0.1
	D1	2.646		
Cal C	E1	1.789	1.877	0.5
	F1	1.965		
Cal D	G1	1.391	1.392	1.0
	H1	1.393		
Cal E	A2	0.780	0.788	2.5
	B2	0.796		
Cal F	C2	0.530	0.538	5.0
	D2	0.547		
Cal G	E2	0.301	0.308	12.0
	F2	0.314		
Ctrl 1	G2	1.040	0.760	**1.61**
	H2	1.045		
Patient	A3	1.751	1.764	**0.57**
	B3	1.778		

*The data presented in Example 1 and Figure 1 is for illustration only and **should not** be used in lieu of a standard curve prepared with each assay.

Figure 1



11.0 Q.C. PARAMETERS

In order for the assay results to be considered valid the following criteria should be met:

1. The absorbance (OD) of calibrator 0 ng/ml should be ≥ 1.3
2. Four out of six quality control pools should be within the established ranges.

12.0 RISK ANALYSIS

The MSDS and Risk Analysis Form for this product are available on request from Monobind Inc.

12.1 Assay Performance

1. It is important that the time of reaction in each well is held constant to achieve reproducible results.
2. Pipetting of samples should not extend beyond ten (10) minutes to avoid assay drift.
3. Highly lipemic, hemolyzed or grossly contaminated specimen(s) should not be used.
4. If more than one (1) plate is used, it is recommended to repeat the dose response curve.
5. The addition of substrate solution initiates a kinetic reaction, which is terminated by the addition of the stop solution. Therefore, the substrate and stop solution should be added in the same sequence to eliminate any time-deviation during reaction.
6. Plate readers measure vertically. Do not touch the bottom of the wells.
7. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
8. Use components from the same lot. No intermixing of reagents from different batches.
9. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from Monobind's IFU may yield inaccurate results.
10. All applicable national standards, regulations and laws, including, but not limited to, good laboratory procedures, must be strictly followed to ensure compliance and proper device usage.
11. It is important to calibrate all the equipment e.g. Pipettes, Readers, Washers and/or the automated instruments used with this device, and to perform routine preventative maintenance.
12. Risk Analysis- as required by CE Mark IVD Directive 98/79/EC - for this and other devices, made by Monobind, can be requested via email from Monobind@monobind.com.

12.2 Interpretation

1. ***Measurements and interpretation of results must be performed by a skilled individual or trained professional.***
2. Laboratory results alone are only one aspect for determining patient care and should not be the sole basis for therapy, particularly if the results conflict with other determinants.
3. The reagents for the procedure have been formulated to eliminate maximal interference; however, potential interaction between rare serum specimens and test reagents can cause erroneous results. Heterophilic antibodies often cause these interactions and have been known to be problems for all kinds of immunoassays. *(Boscato LM Stuart MC.'Heterophilic antibodies: a problem for all immunoassays' Clin. Chem 1988;3427-33).* For diagnostic purposes the results from this assay should be used in combination with clinical examination, patient's history and, all other clinical findings.
4. For valid test results, adequate controls and other parameters must be within the listed ranges and assay requirements.
5. If test kits are altered, such as by mixing parts of different kits, which could producefalse test results, or if results are incorrectly interpreted, Monobind shall have no liability.
6. If computer controlled data reduction is used to interpret the results of the test, it is imperative that the predicted values for the calibrators fall within 10% of the assigned concentrations.

13.0 EXPECTED RANGES OF VALUES

In agreement with established reference intervals[3] for a "normal" adult population, the expected ranges for the Testosterone AccuBind® ELISA Test System are detailed in Table 1.

TABLE I
Expected Values for Testosterone EIA Test System (ng/ml)

Boys Before Puberty	0.1 – 3.7
Male	2.5 – 10.0
Female	0.2 – 0.95

It is important to keep in mind that establishment of a range of values, which can be expected to be found by a given method for a population of "normal" persons, is dependent upon a multiplicity of factors: the specificity of the method, the population tested and the precision of the method in the hands of the analyst. For these reasons, each laboratory should depend upon the range of expected values established by the Manufacturer only until an in-house range can be determined by the analysts using the method with a population indigenous to the area in which the laboratory is located.

14.0 PERFORMANCE CHARACTERISTICS

14.1 Precision

The within and between assay precision of the Testosterone AccuBind® ELISA Test System were determined by analyses on three different levels of pool control sera. The number, mean values, standard deviation and coefficient of variation for each of these control sera are presented in Table 2 and Table 3.

TABLE 2
Within Assay Precision (Values in ng/ml)

Sample	N	X	σ	C.V.
Low	22	1.63	0.16	9.8%
Normal	22	9.14	0.44	4.8%
High	22	14.22	0.79	5.6%

TABLE 3
Between Assay Precision (Values in ng/ml)

Sample	N	X	σ	C.V.
Low	24	1.72	0.16	9.1%
Normal	24	7.06	0.69	9.7%
High	24	13.08	1.03	7.9%

*As measured in several experiments in duplicate over a ten day period.

14.2 Sensitivity

The Testosterone AccuBind® ELISA Test System has a sensitivity of 0.576 pg. This is equivalent to a sample containing a concentration of 0.0576 ng/ml. The sensitivity was ascertained by determining the variability of the 0 ng/ml serum calibrator and using the 2σ (95% certainty) statistic to calculate the minimum dose.

14.3 Accuracy

The Testosterone AccuBind® ELISA Test System was compared with a chemiluminescence immunoassay method. Biological specimens from low, normal and high Testosterone level populations were used. The values ranged from 0.29 ng/ml – 21.9ng/ml. The total number of such specimens was 58. The least square regression equation and the correlation coefficient were computed for this Testosterone EIA in comparison with the reference method. The data obtained is displayed in Table 4.

TABLE 4

Method	Mean (x)	Least Square Regression Analysis	Correlation Coefficient
Monobind (y)	3.12	y= -0.265+0.944(x)	0.985
Reference (X)	3.02		

Only slight amounts of bias between this method and the reference method are indicated by the closeness of the mean values. The least square regression equation and correlation coefficient indicates excellent method agreement.

14.4 Specificity

The % cross reactivity of the testosterone antibody to selected substances was evaluated by adding the interfering substance to a serum matrix at various concentrations. The cross-reactivity was calculated by deriving a ratio between dose of interfering substance to dose of Testosterone needed to displace the same amount of labeled analog.

Substance	Cross Reactivity
Testosterone	1.0000
Androstenedione	0.0009
Dihydotestosterone	0.0178
Cortisone	<0.0001
Corticosterone	<0.0001
Cortisol	<0.0001
Spirolactone	<0.0001
Progesterone	<0.0001
17α-OH Progesterone	<0.0001
DHEA sulfate	<0.0001
Estradiol	<0.0001
Estrone	<0.0001
Estriol	<0.0001
Hemolysis	<0.0001
Rubella	<0.0001
Lipemia	<0.0001

15.0 REFERENCES

1. Dorfman, RI and Shipley, RA, ED: Androgens, New York,: John Wiley and Sons, 1956.
2. Horton R, Tait JF: Androstenedione production and interconversion rates measured in peripheral blood and studies on the possible site of conversion to testosterone. J.Clin Invest 45: 301-303, 1966.
3. Faiman C and Winter, JSD, Reyes, FI, Clin *Obstet Gynaecol,* 3, 467 (1976).
4. Sizonenka, PC, *Pediatrician,* 14, 191 (1987).
5. Cummings DC, Wall SR: Non sex hormone binding globulin bound testosterone as a marker for hyperandrogenism. J. Clin Endocrinol Metab. 61:873-876, 1985.
6. Lashansky, G, et. al., *J Clin Endocrinol Metab,*58, 674 (1991)
7. Tietz, NW, ED: Clinical Guide to Laboratory Tests, 3rd ed. Philadelphia, WA Saunders Co, 1995.

Effective Date: 2019-Jul-16 Rev. 5 **DCO: 1353**
MP3725 **Product Code: 3725-300**

Size		96(A)	192(B)
Reagent (fill)	A)	1ml set	1ml set
	B)	1 (6ml)	2 (6ml)
	C)	1 (6ml)	2 (6ml)
	D)	1 plate	2 plates
	E)	1 (20ml)	1 (20ml)
	F)	1 (7ml)	2 (7ml)
	G)	1 (7ml)	2 (7ml)
	H)	1 (8ml)	2 (8ml)

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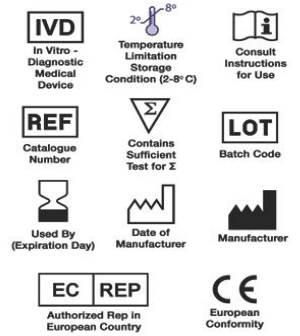
Please visit our website to learn more about our products and services.

Glossary of Symbols
(EN 980/ISO 15223)



Fuente: *Monobind Inc.*

Anexo 6. *Valores normales de Testosterona libre.*

TESTOSTERONA LIBRE VALORES DE REFERENCIA

Edades	Valor
7 – 13 años	0,6 – 6,8 pg/mL
14 – 17 años	1,2 – 9,9 pg/mL
18 – 40 años	0,8 – 9,2 pg/mL
41- 51 años	1,1 – 5,8 pg/mL
Posmenopáusica	0,6 – 3,8 pg/mL

Fuente: *Williamson, M. & Snyder, L. 2012.*

Anexo 7. Inserto de Testosterona libre.

Monobind Inc.
Lake Forest, CA 92630, USA

AccuBind
ELISA Microwells

Free Testosterone AccuBind® ELISA Test System

Product Code: 5325-300

Rx Only

1.0 INTRODUCTION

Indications for Use: The device is an Enzyme Immunoassay (EIA) for the quantitative measurement of free testosterone in human serum. Measurement of free testosterone is used in the diagnosis and treatment of disorders involving the male sex hormones (androgens), including primary and secondary hypogonadism, impotence in males and in females; hirsutism (excessive hair) and virilization (masculinization) due to tumors, polycystic ovaries and adrogenital syndromes.

2.0 SUMMARY AND EXPLANATION OF THE TEST

Testosterone, (17β-Hydroxy-4-androstene-3-one), a C_{19} steroid, is the most potent naturally secreted androgen.[1] In normal post pubertal males, testosterone is secreted primarily by the testes, with only a small amount derived from peripheral conversion of 4-Androstene-3, 17-dione (ASD).[2] In adult women, it has been estimated that over 50% of serum testosterone is derived from peripheral conversion of ASD secreted by the adrenal and ovary, with the remainder from direct secretion of testosterone by these glands.

In the male, testosterone is mainly synthesized in the interstitial Leydig cells and the testis, and is regulated by the interstitial cell stimulating hormone (ICSH), or luteinizing hormone (LH) of the anterior pituitary (the female equivalent of ICSH).[3] Testosterone is responsible for the development of secondary sex characteristics, such as the accessory sex organs, the prostate, seminal vesicles and the growth of facial, pubic and auxiliary hair. Testosterone measurements have been very helpful in evaluating hypogonadal states. Increased testosterone levels in males can be found in complete androgen resistance (testicular feminization). Common causes of decreased testosterone levels in males include: hypogonadism, orchidectomy, estrogen therapy, Klinefelter's syndrome, hypopituitarism, and hepatic cirrhosis.[2,4]

In the female, testosterone levels are normally found to be much lower than those encountered in the healthy male. Testosterone in the female comes from three sources. It is secreted in small quantities by both the adrenal glands and the ovaries, and in healthy women, 50–60% of the daily testosterone production arises from peripheral metabolism of prohormone, chiefly androstenedione. Common causes of increased serum testosterone levels in females include polycystic ovaries (Stein-Leventhal syndrome), ovarian tumors, adrenal tumors and adrenal hyperplasia. Virilization in women is associated with the administration of androgens and endogenous overproduction of testosterone. There appears to be a correlation between serum testosterone levels and the degree of virilization in women, although approximately 25% of women with varying degrees of virilism have serum testosterone levels that fall within the female reference range.

The majority of testosterone is bound to transport proteins: weakly bound to albumin and cortisol binding protein (25-65% females; 45-85% males) and tightly bound to sex hormone-binding globulin (SHBG) (35-75% females; 14-50% males).[5] A small fraction exist as unbound or free testosterone; however, this form is biologically active. Therefore, the free hormone concentration is a better indicator of biological activity than total testosterone.

3.0 PRINCIPLE

Competitive Enzyme Immunoassay (TYPE 5)
The essential reagents required for a solid phase enzyme immunoassay include immobilized antibody, enzyme-antigen conjugate and native antigen.

Upon mixing immobilized antibody, enzyme-antigen conjugate and a serum containing the native antigen, a competition reaction results between the native antigen and the enzyme-antigen conjugate for a limited number of insolubulized binding sites. The interaction is illustrated by the equation in the following below.

$$^{Enz}Ag + Ag + Ab_{c.w.} \underset{k_a}{\overset{k_a}{\rightleftharpoons}} AgAb_{c.w.} + {}^{Enz}AgAb_{c.w.}$$

$Ab_{c.w}$ = Monospecific Immobilized Antibody (Constant Quantity)
Ag = Native Antigen (Variable Quantity)
^{Enz}Ag = Enzyme-antigen Conjugate (Constant Quantity)
$AgAb_{c.w.}$ = Antigen-Antibody Complex
$^{Enz}AgAb_{c.w.}$ = Enzyme-antigen Conjugate -Antibody Complex
k_a = Rate Constant of Association
k_a = Rate Constant of Disassociation
$K = k_a / k_a$ = Equilibrium Constant

After equilibrium is attained, the antibody-bound fraction is separated from unbound antigen by decantation or aspiration. The enzyme activity in the antibody-bound fraction is inversely proportional to the native antigen concentration. By utilizing several different serum references of known antigen concentration, a dose response curve can be generated from which the antigen concentration of an unknown can be ascertained.

4.0 REAGENTS

Materials Provided:

A. Free Testosterone Calibrators – 1ml/vial – Icons A-G
Seven (7) vials of serum reference for Free Testosterone at concentrations of 0 (**A**), 0.2 (**B**), 1.0 (**C**), 2.5 (**D**), 7.5 (**E**), 20 (**F**) and 60 (**G**) in pg/ml. Store at 2-8°C. A preservative has been added. The calibrators can be expressed in molar concentrations (pM/L) by multiplying by 3.47.
For example: 1pg/ml x 3.47 = 3.47 pM/L

B. Free Testosterone Controls – 1ml/vial – Icons L, M, N
Three (3) vials of serum reference for Free Testosterone at low, middle, and high established concentrations (range values listed on labels). A preservative has been added. Store at 2-8 °C.

C. Free Testosterone Enzyme Reagent – 13ml/vial – Icon
One (1) vial of Testosterone (Analog)-horseradish peroxides (HRP) conjugate in a protein stabilizing matrix with dye. Store at 2-8°C.

D. Free Testosterone Coated Plate – 96 wells – Icon
One 96-well microplate coated with testosterone antibody and packaged in an aluminum bag with a drying agent. Store at 2-8°C.

E. Wash Solution Concentrate – 20ml/vial – Icon
One (1) vial containing a surfactant in buffered saline. A preservative has been added. Store at 2-8°C.

F. Substrate A – 7ml/vial – Icon S^A
One (1) vial contains tetramethylbenzidine (TMB) in buffer. Store at 2-8°C. See "Reagent Preparation."

G. Substrate B – 7ml/vial – Icon S^B
One (1) vial contains hydrogen peroxide (H_2O_2) in buffer. Store at 2-8°C. See "Reagent Preparation."

H. Stop Solution - 8ml/vial – Icon
One (1) vial contains a strong acid (1N HCl). Store at 2-8°C.

I. Product Instructions

Note 1: Do not use reagents beyond the kit expiration date.
Note 2: Avoid extended exposure to heat and light. **Opened reagents are stable for sixty (60) days when stored at 2-8°C. Kit and component stability are identified on label.**
Note 3: Above reagents are for a single 96-well microplate.

4.1 Required But Not Provided:
1. Pipette capable of delivering 0.020 & 0.050ml (20µl & 50µl) volumes with a precision of better than 1.5%.
2. Dispenser(s) for repetitive deliveries of 0.100 & 0.350ml (100 & 350µl) volumes with a precision of better than 1.5%.
3. Adjustable volume (200-1000µl) dispenser(s) for conjugate.
4. Microplate washer or a squeeze bottle (optional).
5. Microplate Reader with 450nm and 620nm wavelength absorbance capability.
6. Absorbent Paper for blotting the microplate wells.
7. Plastic wrap or microplate cover for incubation steps.
8. Vacuum aspirator (optional) for wash steps.
9. Timer.
10. Quality control materials.

5.0 PRECAUTIONS

For In Vitro Diagnostic Use
Not for Internal or External Use in Humans or Animals

All products that contain human serum have been found to be non-reactive for Hepatitis B Surface Antigen, HIV 1&2 and HCV Antibodies by FDA approved tests. Since no known test can offer complete assurance that infectious agents are absent, all human serum products should be handled as potentially hazardous and capable of transmitting disease. Good laboratory procedures for handling blood products can be found in the Center for Disease Control / National Institute of Health, "Biosafety in Microbiological and Biomedical Laboratories," 2nd Edition, 1988, HHS Publication No. (CDC) 88-8395.

Safe Disposal of kit components must be according to local regulatory and statutory requirement.

6.0 SPECIMEN COLLECTION AND PREPARATION

The specimens shall be blood serum in type, and the usual precautions in the collection of venipuncture samples should be observed. The blood should be collected in a plain redtop venipuncture tube. Allow the blood to clot for serum samples. Centrifuge the specimen to separate the serum from the cells.

Samples may be refrigerated at 2-8°C for a maximum period of five (5) days. If the specimen(s) cannot be assayed within this time, the sample(s) may be stored at temperatures of -20°C for up to 30 days. Avoid use of contaminated devices. Avoid repetitive freezing and thawing. When assayed in duplicate, 0.040ml (40µl) of the specimen is required.

7.0 QUALITY CONTROL

Each laboratory should assay controls at levels in the low, normal and high range for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control charts should be maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends. The individual laboratory should set acceptable assay performance limits in accordance with local, state, and federal quality control regulations. In addition, maximum absorbance should be consistent with past experience. Significant deviation from established performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.

8.0 REAGENT PREPARATION

1. **Wash Buffer**
   Dilute contents of wash solution to 1000ml with distilled or deionized water in a suitable storage container. Diluted buffer can be stored at 2-30°C for up to 60 days.
2. **Working Substrate Solution** - Stable for 1 year.
   Pour the contents of the amber vial labeled Solution 'A' into the clear vial labeled Solution 'B'. Place the yellow cap on the clear vial for easy identification. Mix and label accordingly. Store at 2 - 8°C.

Note 1: Do not use reagents that are contaminated or have bacteria growth.
Note 2: Do not use the working substrate if it looks blue.

9.0 TEST PROCEDURE

Before proceeding with the assay, bring all reagents, serum reference calibrators and controls to room temperature (20- 27°C).
***Test Procedure should be performed by a skilled individual or trained professional**

1. Format the microplates' wells for each serum reference, control and patient specimen to be assayed in duplicate. **Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8°C.**
2. Pipette 0.020ml (20µL) of the appropriate serum reference, control or specimen into the assigned well.
3. Add 0.100ml (100µl) of the Free Testosterone Enzyme Reagent to all wells.
4. Swirl the microplate gently for 20-30 seconds to mix.
5. Cover and incubate for 60 minutes at room temperature.
6. Discard the contents of the microplate by decantation or aspiration. If decanting, blot the plate dry with absorbent paper.
7. Add 0.350ml (350µl) of wash buffer (see Reagent Preparation Section), decant (tap and blot) or aspirate. Repeat two (2) additional times for a total of three (3) washes. **An automatic or manual plate washer can be used. Follow the manufacturer's instruction for proper usage. If a squeeze bottle is employed, fill each well by depressing the container (avoiding air bubbles) to dispense the wash. Decant the wash and repeat two (2) additional times.**
8. Add 0.100ml (100µl) of working substrate solution to all wells (see Reagent Preparation Section). **Always add reagents in the same order to minimize reaction time differences between wells.**
   DO NOT SHAKE THE PLATE AFTER SUBSTRATE ADDITION
9. Incubate at room temperature for fifteen (15) minutes.
10. Add 0.050ml (50µl) of stop solution to each well and gently mix for 15-20 seconds. **Always add reagents in the same order to minimize reaction time differences between wells.**
11. Read the absorbance in each well at 450nm (using a reference wavelength of 620-630nm to minimize well imperfections) in a microplate reader. **The results should be read within thirty (30) minutes of adding the stop solution.**

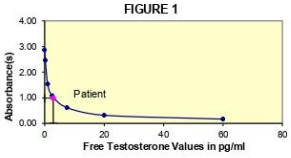
10.0 CALCULATION OF RESULTS

A dose response curve is used to ascertain the concentration of Free Testosterone in unknown specimens within the analytical measuring range of 0.11-60 pg/ml.

1. Record the absorbance obtained from the printout of the microplate reader as outlined in Example 1.
2. Plot the absorbance for each duplicate serum reference versus the corresponding Free Testosterone concentration in pg/ml on linear graph paper.
3. Connect the points with a best-fit curve.
4. To determine the concentration of Free Testosterone for an unknown, locate the average absorbance of the duplicates for each unknown on the vertical axis of the graph, find the intersecting point on the curve, and read the concentration (in pg/ml) from the horizontal axis of the graph (the duplicates of the unknown may be averaged as indicated). In the following example, the average absorbance 0.989 intersects the dose response curve at 2.87pg/ml Free Testosterone concentration.

Note: Computer data reduction software designed for ELISA assays may also be used for the data reduction. If such software is utilized, the validation of the software should be ascertained.

FIGURE 1



EXAMPLE 1

Sample I.D.	Well Number	Abs (A)	Mean Abs (B)	Value (pg/ml)
Cal A	A1	2.867	2.867	0.0
	B1	2.867		
Cal B	C1	2.489	2.470	0.2
	D1	2.451		
Cal C	E1	1.509	1.533	1.0
	F1	1.556		
Cal D	G1	1.071	1.084	2.5
	H1	1.097		
Cal E	A2	0.620	0.614	7.5
	B2	0.608		
Cal F	C2	0.333	0.318	20
	D2	0.303		
Cal G	E2	0.171	0.168	60
	F2	0.165		
Ctrl L	G2	1.333	1.384	1.339
	H2	1.434		
Ctrl M	A3	0.734	0.737	5.284
	B3	0.739		
Ctrl N	C3	0.192	0.187	47.107
	D3	0.182		
Patient	C4	0.997	0.989	2.870
	D4	0.980		

*The data presented in Example 1 and Figure 1 is for illustration only and **should not** be used in lieu of a standard curve prepared with each assay.

11.0 Q.C. PARAMETERS

In order for the assay results to be considered valid the following criteria should be met:

1. The absorbance (OD) of calibrator 0 pg/ml should be ≥ 1.3.

12.0 RISK ANALYSIS

The MSDS and Risk Analysis Form for this product is available on request from Monobind Inc.

12.1 Assay Performance

1. It is important that the time of reaction in each well is held constant to achieve reproducible results.
2. Pipetting of samples should not extend beyond ten (10) minutes to avoid assay drift.
3. Highly lipemic, hemolyzed or grossly contaminated specimen(s) should not be used.
4. If more than one (1) plate is used, it is recommended to repeat the dose response curve.
5. The addition of substrate solution initiates a kinetic reaction, which is terminated by the addition of the stop solution. The substrate and stop solution should be added in the same sequence to eliminate any time-deviation during reaction.
6. Plate readers measure vertically. Do not touch the bottom of the wells.
7. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
8. Use components from the same lot. No intermixing of reagents from different batches.
9. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from IFU may yield inaccurate results.
10. All applicable national standards, regulations and laws, including, but not limited to, good laboratory procedures, must be followed to ensure compliance and proper device usage.
11. It is important to calibrate all the equipment e.g. Pipettes, Readers, Washers and/or the automated instruments used with this device, and to perform routine preventative maintenance.
12. Risk Analysis- as required by CE Mark IVD Directive 98/79/EC - for this and other devices, made by Monobind, can be requested via email from Monobind@monobind.com.

12.2 Interpretation

1. ***Measurements and interpretation of results must be performed by a skilled individual or trained professional.***
2. Laboratory results alone are only one aspect for determining patient care and should not be the sole basis for therapy, particularly if the results conflict with other determinants.
3. The reagents for the test system procedure have been formulated to eliminate maximal interference; however, potential interaction between rare serum specimens and test reagents can cause erroneous results. Heterophilic antibodies often cause these interactions and have been known to be problems for all kinds of immunoassays. (*Boscato LM Stuart MC. 'Heterophilic antibodies: a problem for all immunoassays' Clin.Chem. 1988:3427-33*). For diagnostic purposes, the results from this assay should be used in combination with clinical examination, patient history, and other clinical findings.
4. For valid test results, adequate controls and other parameters must be within the listed ranges and assay requirements.
5. If test kits are altered, such as by mixing parts of different kits, which could produce false test results, or if results are incorrectly interpreted, Monobind shall have no liability.
6. If computer controlled data reduction is used to interpret the results of the test, it is imperative that the predicted values for the calibrators fall within 10% of the assigned concentrations.

13.0 EXPECTED RANGES OF VALUES

In agreement with established reference intervals for a "normal" adult population, the expected ranges for the Free Testosterone AccuBind® ELISA Test System are detailed in Table 1.

TABLE I

Population	Range (in pg/ml)
Male / 20-39	9.2-34.6
Male / 40-59	6.1-30.3
Male / ≥60	6.1-27.9
Female / 20-39	0.2-6.1
Female / 40-59	0.3-4.4
Female / ≥60	0.5-3.4

It is important to keep in mind that establishment of a range of values, which can be expected to be found by a given method for a population of "normal" persons, is dependent upon a multiplicity of factors: the specificity of the method, the population tested and the precision of the method in the hands of the analyst. For these reasons, each laboratory should depend upon the range of expected values established by the Manufacturer only until an in-house range can be determined by the analysts using the method with a population indigenous to the area in which the laboratory is located.

14.0 PERFORMANCE CHARACTERISTICS

14.1 Accuracy

The Free Testosterone AccuBind® ELISA Test System was compared with a reference ELISA method. Biological specimens from from low, normal, and elevated concentrations were assayed. The total number of such specimens was 137. The least square regression equation and the correlation coefficient were computed.

TABLE 2

	Least Square Regression Analysis	Correlation Coefficient
Monobind (y)	y=1.017x-0.24	0.997
Reference (x)		

14.2 Precision

This study was conducted during 20 days of testing. The human serum and control sample were tested in duplicate, two times per day for a total of 80 measurements per sample. Three (3) different reagent lots, three (3) serum pools, and three (3) controls were used for the study (low, medium, and high concentration). The results of a representative lot are shown below:

TABLE 3

Lot 1 N=32	Mean (pg/ml)	Within-Run		Total	
		SD	CV%	SD	CV%
Ctrl 1	2.51	0.09	3.7%	0.20	7.8%
Ctrl 2	10.98	0.40	3.6%	0.96	8.7%
Ctrl 3	22.72	0.83	3.6%	2.18	9.6%
Serum 1	0.98	0.06	5.9%	0.12	12.4%
Serum 2	4.53	0.26	5.7%	0.36	8.0%
Serum 3	53.62	4.24	7.9%	4.32	8.1%

TABLE 4

	Mean (pg/ml)	Within-Run Precision		Within-Kit Precision		Total Precision (n=80)	
		SD	CV%	SD	CV%	SD	CV%
Ctrl 1	2.48	0.11	4.57	0.20	8.20	0.21	8.51
Ctrl 2	11.04	0.47	4.23	0.84	2.60	0.87	8.00
Ctrl 3	23.24	1.00	4.31	1.80	7.73	1.83	7.95
Pnt 1	0.97	0.05	4.88	0.09	9.14	0.08	9.43
Pnt 2	4.62	0.23	4.88	0.32	6.89	0.33	7.20
Pnt 3	54.66	3.25	5.95	3.92	7.17	4.13	7.55

14.3 Detection Limits

The LOB (limit of the blank), the LOD (limit of detection) and the LOQ (limit of quantitation) were determined in accordance with CLSI EP 17-A A guideline, Protocols for Determination of Limits of Detection).

TABLE 5

LoB	LoD	LoQ
0.0295 pg/ml	0.0519 pg/ml	0.0519 pg/ml

14.4 Cross Reactivity

Cross reactivity was determined by testing those compounds most likely to interfere with the Monobind Free Testosterone ELISA Test System. The specificity of the assay was determined in accordance with CLSI EP07-A2. The results of the cross-reactivity study are as follows.

TABLE 6

Sample	Conc. (ng/ml)	Cross Reactivity	
		Spiked Serum	Blank Serum
11-Deoxycortisol	1000	0.000%	ND
11-KetoTestosterone	10	0.647%	0.519%
11β-Hydroxytestosterone	100	0.065%	0.054%
17α-ethynyl estradiol	1000	0.000%	ND
17α-Estradiol	1000	0.000%	0.000%
17β-Estradiol	100	0.000%	ND
17-Hydroxypregnenolone	1000	0.000%	ND
17-Hydroxyprogesterone	10	0.000%	0.000%
3-EstriolGluc	1000	0.000%	ND
3-EstriolSul	1000	0.000%	ND
3β-Androstanediol	500	0.000%	ND
5α-Dihydrotestosterone	100	0.054%	0.042%
Aldosterone	8000	0.000%	0.000%
Amitriptyl HCl	1000	0.000%	ND
Androsterone	1000	0.000%	ND
Androstenedione	1000	0.004%	0.002%
Clomiphene Citrate	1000	0.000%	ND
Corsticosterone	1000	0.000%	0.000%
Corstisone	1000	0.000%	0.000%
Cortisol	1000	0.000%	0.000%
Cyproterone acetate	1000	0.000%	ND
D-5-Androstene-3β,17β-diol	1000	0.000%	ND
Danazol	1000	0.000%	ND
DHEA	100000	0.000%	0.000%
DHEA-S	1000	0.000%	0.000%
Desogestrel	100	0.000%	ND
Dexamethasone	1000	0.000%	ND
Epistestosterone	1000	0.001%	0.001%
Estriol	1000	0.000%	0.000%
Estrone	1000	0.000%	0.000%
Ethisterone	1000	0.000%	0.000%
Ethynodiol	1000	0.000%	0.000%
Ethynodiol diacetate	50	0.000%	ND
Flunisolide	1000	0.000%	ND
Fluoxymesterone	1000	0.000%	ND
Lynestrol	1000	0.000%	ND
Medoxyprogesterone acetate	1000	0.000%	ND
Methyl Testosterone	100	0.000%	ND
Mestranol	1000	0.000%	ND
Norethindrone	50	0.000%	ND
Norethinodrone acetate	50	0.000%	ND
Norgestimate	1000	0.000%	ND
Norgestrel (Levonorgestrel)	50	0.000%	ND
Norethynodrel	50	0.000%	ND
Oxymetholone	100	0.000%	ND
Prednisolone	1000	0.000%	ND
Prednisone	800	0.000%	0.000%
Progesterone	1000	0.000%	0.000%
Salbutamol	1000	0.000%	ND
Spironolactone	1000	0.000%	0.000%
Stanozolol	1000	0.000%	0.000%
Testosterone Cypionate	12	0.002%	0.000%
Testosterone enanthate	100	0.000%	0.000%
Testosterone SO4	1000	0.004%	0.003%
Testosterone Propionate	1000	0.000%	0.000%
Testosterone Undecanoate	12	0.011%	0.053%
Triamcinolone	50	0.000%	0.000%

14.5 Interference

Using CLSI-A2 Interference Testing in Clinical Chemistry as a guide, potential interferents were tested utilizing charcoal-stripped human serum spiked with known concentrations of interferent. The following results of % binding values even at higher than normal interferent levels indicate that there is no significant binding on the free testosterone-HRP conjugate.

TABLE 7

Substance	Highest concentration at which no significant interference was observed
Acetaminophen	20 mg/dl
Acetylcysteine	150 mg/dl
Ascorbic Acid	6 mg/dl
Bilirubin Conjugated	15 mg/dl
Bilirubin Unconjugated	20 mg/dl
Biotin	100 ng/ml
Caffeine	6 mg/dl
Cholesterol	503 mg/dl
Creatine	30 mg/dl
Dextran	5000 mg/dl
Digoxin	6.1 ng/ml
Doxycycline	50 mg/L
Erythromycin	6 mg/dl
Gentamicin	1 mg/dl
HAMA	440 ng/ml
Heparin	3 U/ml
Hemoglobin	500 mg/dl
Human Serum Albumin	2.5 g/dl
Ibuprofen	50 mg/dl
Immunoglobulin G	4 g/dl
Levodopa	20 mg/L
Lidocaine	1.2 mg/dl
Lipemia (glycerides)	1000 mg/dl
Methyldopa	20 mg/L
Nicotine	0.1 mg/dl
Phenobarbital	15 mg/dl
Protein: Total	10.5 g/dl
Rheumatoid Factor	1110 IU/ml
Salicylic Acid	60 mg/dl
SHBG	200 µg/ml
Triglycerides	900 mg/dl
Urea	500 mg/dl

15.0 REFERENCES

1. Dorfman, RI and Shipley, RA, ED: Androgens, New York,: John Wiley and Sons, 1956.
2. Horton R, Tait JF: Androstenedione production and interconversion rates measured in peripheral blood and studies on the possible site of conversion to testosterone. J.Clin Invest 45: 301-303, 1966.

Fuente: *Monobind Inc.*

Anexo 8. *Valores normales de S-DHEA.*

S-DHEA VALORES DE REFERENCIA	
Mujer	35 – 430 µg/dL

Fuente: *Williamson, M. & Snyder, L. 2012.*

Anexo 9. inserto de la hormona Sulfato de dehidroepiandrosterona

Monobind Inc.
Lake Forest, CA 92630, USA

AccuBind
ELISA Microwells

Dehydroepiandrosterone Sulfate (DHEA-S) Test System

Product Code: 5125-300

1.0 INTRODUCTION

Intended Use: The Quantitative Determination of Dehydroepiandrosterone Sulfate Concentration in Human Serum or Plasma by a Microplate Enzyme Immunoassay, Colorimetric

2.0 SUMMARY AND EXPLANATION OF THE TEST

Dehydroepiandrosterone sulfate (DHEA-S) is the major C19 steroid secreted by the adrenal cortex, and is a precursor in testosterone and estrogen biosynthesis. DHEA-S, the sulfate ester of DHEA, is derived from sulfated precursors and by enzymatic conversion of DHEA in adrenal and extradrenal tissues. Due to the presence of a 17-oxo [rather than hydroxyl] group, DHEA-S possesses relatively weak androgenic activity, which for unsulfated DHEA has been estimated at ~10% that of testosterone.[1] However, the bioactivity of DHEA-S may be increased by its relatively high serum concentrations, approximately 100 to 1000-fold higher than DHEA or testosterone, and its weak affinity for sex-hormone binding globulin.[2]

The physiologic role of DHEA-S is not well-defined. Serum levels are relatively high in the fetus and neonate, low during childhood, and increase during puberty.[3,4] Increased levels of DHEA-S during adrenarche may contribute to the development of secondary sexual hair. DHEA-S levels show a progressive decline after the third decade of life.[3] Unlike DHEA, DHEA-S levels do not show significant diurnal variation and little day-to-day variation. DHEA-S levels are not responsive to acute corticotropin administration,[4] and do not vary significantly during the normal menstrual cycle.[2] This may be due to the slower metabolic clearance rate of DHEA-S as compared to DHEA.[6]

Measurement of serum DHEA-S is a useful marker of adrenal androgen synthesis. Abnormally low levels have been reported in hypoadrenalism,[3] while elevated levels occur in several conditions; including virilizing adrenal adenoma and carcinoma,[7] 21-hydroxylase and 3β-hydroxysteroid dehydrogenase deficiencies[2,5] and some cases of female hirsutism.[2] Since very little DHEA-S is produced by the gonads,[2,3] measurement of DHEA-S may aid in the localization of the androgen source in virilizing conditions. Methods for measurement of DHEA-S include gas-liquid chromatography, double-isotope derivative techniques, competitive protein-binding assays, and radioimmunoassay. Although significant cross-reactivity occurs with DHEA, androstenedione and androsterone, the relative concentrations of these competing substances in most normal and pathologic samples predicts a minimal effect on assay performance.

The Monobind DHEA-S ELISA Kit uses a specific anti-DHEA-S antibody, and does not require prior sample extraction of serum or plasma. Cross-reactivity to other naturally occurring and structurally related steroids is low. The employment of several serum references of known DHEA-S concentration permits construction of a graph of activity and concentration. From comparison to the dose response curve, an unknown specimen's activity can be correlated with DHEA-S concentration.

3.0 PRINCIPLE

Competitive Enzyme Immunoassay (TYPE 7):
The essential reagents required for an enzyme immunoassay include antibody, enzyme-antigen conjugate and native antigen. Upon mixing biotinylated antibody, enzyme-antigen conjugate and a serum containing the native antigen, a competition reaction results between the native antigen and the enzyme-antigen conjugate for a limited number of antibody binding sites. The interaction is illustrated by the following equation:

$$^{Enz}Ag + Ag + Ab_{Btn} \underset{k_d}{\overset{k_a}{\rightleftharpoons}} AgAb_{Btn} + {}^{Enz}AgAb_{Btn}$$

Ab_{Btn} = Biotinylated x-DHEA-S IgG Antibody (Constant Quantity)
Ag = Native Antigen (Variable Quantity)
^{Enz}Ag = Enzyme-antigen Conjugate (Constant Quantity)
$AgAb_{Btn}$ = Antigen-Antibody Complex
$^{Enz}Ag\ Ab_{Btn}$ = Enzyme-antigen Conjugate -Antibody Complex
k_a = Rate Constant of Association
k_d = Rate Constant of Disassociation
$K = k_a / k_d$ = Equilibrium Constant

A simultaneous reaction between the biotin attached to the antibody and the streptavidin immobilized on the microwell occurs. This effects the separation of the antibody bound fraction after decantation or aspiration.

$AgAb_{Btn} + {}^{Enz}AgAb_{Btn} + \underline{Streptavidin}_{cw} \Rightarrow$ immobilized complex

$\underline{Streptavidin}_{cw}$ = Streptavidin immobilized on well
Immobilized complex = sandwich complex bound to the solid surface

The enzyme activity in the antibody bound fraction is inversely proportional to the native antigen concentration. By utilizing several different serum references of known antigen concentration, a dose response curve can be generated from which the antigen concentration of an unknown can be ascertained.

4.0 REAGENTS

Materials Provided:

A. DHEA-S Calibrators – 1ml/vial - Icons A-F
Six (6) vials of serum reference for DHEA-S at concentrations of 0 (**A**), 0.2 (**B**), 1.0 (**C**), 2.0 (**D**), 4.0 (**E**) and 8.0 (**F**) in µg/ml. Store at 2-8°C. A preservative has been added. The calibrators can be expressed in molar concentrations (nM/L) by using 2.71 as a conversion factor.
For example: 1µg/ml x 2.71 = 2.71 µM/L

B. DHEA-S Enzyme Reagent – 6.0 ml/vial
One (1) vial of DHEA-S (Analog)-horseradish peroxides (HRP) conjugate in a protein-stabilizing matrix with red dye. Store at 2-8°C.

C. DHEA-S Biotin Reagent – 6.0 ml - Icon ▽
One (1) bottle of reagent contains anti-DHEA-S biotinylated purified rabbit IgG conjugate in buffer, blue dye and preservative. Store at 2-8°C.

D. Streptavidin Coated Plate – 96 wells –Icon ⇩
One 96-well microplate coated with 1.0 µg/ml streptavidin and packaged in an aluminum bag with a drying agent. Store at 2-8°C.

E. Wash Solution Concentrate – 20ml/vial - Icon
One (1) vial contains a surfactant in buffered saline. A preservative has been added. Store at 2-8°C.

F. Substrate A – 7ml/vial - Icon S[A]
One (1) vial contains tetramethylbenzidine (TMB) in buffer. Store at 2-8°C.

G. Substrate B – 7ml/vial - Icon S[B]
One (1) vial contains hydrogen peroxide (H_2O_2) in buffer. Store at 2-8°C.

H. Stop Solution – 8ml/vial - Icon
One (1) vial contains a strong acid (1N HCl). Store at 2-8°C.

I. Product Instructions

Note 1: Do not use reagents beyond the kit expiration date.
Note 2: Avoid extended exposure to heat and light. **Opened reagents are stable for sixty (60) days when stored at 2-8°C. Kit and component stability are identified on the label.**
Note 3: Above reagents are for a single 96-well microplate.

4.1 Required But Not Provided:
1. Pipette capable of delivering 0.010ml (10µl) and 0.050ml (50µl) with a precision of better than 1.5%.
2. Dispenser(s) for repetitive deliveries of 0.100ml (100µl) and 0.350ml (350µl) volumes with a precision of better than 1.5%.
3. Adjustable volume (200-1000µl) dispenser(s) for conjugate.
4. Microplate washer or a squeeze bottle (optional).
5. Microplate Reader with 450nm and 620nm wavelength absorbance capability.
6. Absorbent Paper for blotting the microplate wells.
7. Plastic wrap or microplate cover for incubation steps.
8. Vacuum aspirator (optional) for wash steps.
9. Timer.
10. Quality control materials.

5.0 PRECAUTIONS

For In Vitro Diagnostic Use
Not for Internal or External Use in Humans or Animals

All products that contain human serum have been found to be non-reactive for Hepatitis B Surface Antigen, HIV 1&2 and HCV Antibodies by FDA required tests. Since no known test can offer complete assurance that infectious agents are absent, all human serum products should be handled as potentially hazardous and capable of transmitting disease. Good laboratory procedures for handling blood products can be found in the Center for Disease Control / National Institute of Health, "Biosafety in Microbiological and Biomedical Laboratories," 2nd Edition, 1988, HHS Publication No. (CDC) 88-8395.

Safe Disposal of kit components must be according to local regulatory and statutory requirement.

6.0 SPECIMEN COLLECTION AND PREPARATION

The specimens shall be blood serum or heparanised plasma in type, and taken with the usual precautions in the collection of venipuncture samples. For accurate comparison to establish normal values, a fasting morning serum sample should be obtained. The blood should be collected in a redtop veni-puncture tube with or without additives or anti-coagulants (for serum) or evacuated tube(s) containing EDTA or heparin (for plasma). Allow the blood to clot for serum samples. Centrifuge the specimen to separate the serum or plasma from the cells.

In patients receiving therapy with high biotin doses (i.e. >5mg/day), no sample should be taken until at least 8 hours after the last biotin administration, preferably overnight to ensure fasting sample.

Samples may be refrigerated at 2-8°C for a maximum period of five (5) days. If the specimen(s) cannot be assayed within this time, the sample(s) may be stored at temperatures of -20°C for up to 30 days. Avoid use of contaminated devices. Avoid repetitive freezing and thawing. When assayed in duplicate, 0.020ml (20µl) of the specimen is required.

7.0 QUALITY CONTROL

Each laboratory should assay controls at levels in the low, normal and high range for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control charts should be maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends. The individual laboratory should set acceptable assay performance limits. In addition, maximum absorbance should be consistent with past experience. Significant deviation from established performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.

8.0 REAGENT PREPARATION

1. **Wash Buffer**
Dilute contents of wash solution to 1000ml with distilled or deionized water in a suitable storage container. Diluted buffer can be stored at 2-30°C for up to 60 days.

2. **Working Substrate Solution - Stable for 1 year**
Pour the contents of the amber vial labeled Solution 'A' into the clear vial labeled Solution 'B'. Place the yellow cap on the clear vial for easy identification. Mix and label accordingly. Store at 2 - 8°C.

Note1: Do not use the working substrate if it looks blue.
Note 2: Do not use reagents that are contaminated or have bacteria growth.

9.0 TEST PROCEDURE

Before proceeding with the assay, bring all reagents, serum reference calibrators and controls to room temperature (20-27°C).
*****Test Procedure should be performed by a skilled individual or trained professional*****

1. Format the microplates' wells for each serum reference calibrator, control and patient specimen to be assayed in duplicate. **Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8°C.**
2. Pipette 0.010 ml (10 µL) of the appropriate serum reference calibrator, control or specimen into the assigned well.
3. Add 0.050 ml (50µl) of the DHEA-S Enzyme Reagent to all wells.
4. Swirl the microplate gently for 20-30 seconds to mix.
5. Add 0.050 ml (50µl) of Anti- DHEA-S Biotin Reagent to all wells.
6. Swirl the microplate gently for 20-30 seconds to mix.
7. Cover and incubate for 30 minutes at room temperature.
8. Discard the contents of the microplate by decantation or aspiration. If decanting, blot the plate dry with absorbent paper.
9. Add 0.350ml (350µl) of wash buffer (see Reagent Preparation Section), decant (tap and blot) or aspirate. Repeat two (2) additional times for a total of three (3) washes. **An automatic or manual plate washer can be used. Follow the manufacturer's instruction for proper usage. If a squeeze bottle is employed, fill each well by depressing the container (avoiding air bubbles) to dispense the wash. Decant the wash and repeat two (2) additional times.**
10. Add 0.100 ml (100µl) of working substrate solution to all wells (see Reagent Preparation Section). **Always add reagents in the same order to minimize reaction time differences between wells.**

DO NOT SHAKE THE PLATE AFTER SUBSTRATE ADDITION

11. Incubate at room temperature for fifteen (15) minutes.
12. Add 0.050ml (50µl) of stop solution to each well and gently mix for 15-20 seconds. **Always add reagents in the same order to minimize reaction time differences between wells.**
13. Read the absorbance in each well at 450nm (using a reference wavelength of 620-630nm.. **The results should be read within thirty (30) minutes of adding the stop solution.**

Note: Dilute the samples suspected of concentrations higher than 8.0 ug/ml 1:5 and 1:10 with DHEA-S '0' µg/ml calibrator or patient serum pools with a known low value for DHEA-S.

10.0 CALCULATION OF RESULTS

A dose response curve is used to ascertain the concentration of DHEA-S in unknown specimens.
1. Record the absorbance obtained from the printout of the microplate reader as outlined in Example 1.
2. Plot the absorbance for each duplicate serum reference versus the corresponding DHEA-S concentration in ug/ml on linear graph paper (do not average the duplicates of the serum references before plotting).
3. Connect the points with a best-fit curve.
4. To determine the concentration of DHEA-S for an unknown, locate the average absorbance of the duplicates for each unknown on the vertical axis of the graph, find the intersecting point on the curve, and read the concentration (in ug/ml) from the horizontal axis of the graph (the duplicates of the unknown may be averaged as indicated). In the following example, the average absorbance in the patient sample (1.078) intersects the dose response curve at (1.21 µg/ml) DHEA-S concentration (See Figure 1).

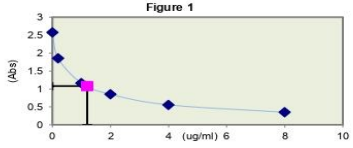
Note: Computer data reduction software designed for ELISA assays may also be used for the data reduction. If such

software is utilized, the validation of the software should be ascertained.

EXAMPLE 1

Sample I.D.	Well Number	Abs (A)	Mean Abs (B)	Value (µg/ml)
Cal A	A1	2.562	2.572	0.0
	B1	2.582		
Cal B	C1	1.865	1.847	0.2
	D1	1.829		
Cal C	E1	1.186	1.163	1.0
	F1	1.140		
Cal D	G1	0.855	0.850	2.0
	H1	0.845		
Cal E	A2	0.555	0.556	4.0
	B2	0.557		
Cal F	C2	0.355	0.349	8.0
	D2	0.344		
Cont 1	G2	1.394	1.387	0.62
	H2	1.380		
Pat# 1	A3	1.065	1.078	**1.21**
	B3	1.091		

Figure 1



*The represented in Example 1 and Firgure 1 is for illustration only and **should NOT** be used in lieu of a dose response curve prepared with each assay.

11.0 Q.C. PARAMETERS

In order for the assay results to be considered valid the following criteria should be met:

1. The absorbance (OD) of calibrator 0 ug/ml should be ≥ 1.3
2. Four out of six quality control pools should be within the established ranges.

12.0 RISK ANALYSIS

The MSDS and Risk Analysis Form for this product are available on request from Monobind Inc.

12.1 Assay Performance

1. It is important that the time of reaction in each well is held constant to achieve reproducible results.
2. Pipetting of samples should not extend beyond ten (10) minutes to avoid assay drift.
3. Highly lipemic, hemolyzed or grossly contaminated specimen(s) should not be used.
4. If more than one (1) plate is used, it is recommended to repeat the dose response curve.
5. The addition of substrate solution initiates a kinetic reaction, which is terminated by the addition of the stop solution. Therefore, the substrate and stop solution should be added in the same sequence to eliminate any time-deviation during reaction.
6. Plate readers measure vertically. Do not touch the bottom of the wells.
7. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
8. Use components from the same lot. No intermixing of reagents from different batches.
9. Patient specimens with DHEA-S concentrations above 8.0 µg/mL may be diluted (1/5, 1/10 or higher) with DHEA-S '0' calibrator and re-assayed. The sample's concentration is obtained by multiplying the result by the dilution factor.
10. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from Monobind' IFU may yield inaccurate results.
11. All applicable national standards, regulations and laws, including, but not limited to, good laboratory procedures, must be strictly followed to ensure compliance and proper device usage.
12. It is important to calibrate all the equipment e.g. Pipettes, Readers, Washers and/or the automated instruments used with this device, and to perform routine preventative maintenance.
13. Risk Analysis- as required by CE Mark IVD Directive 98/79/EC - for this and other devices, made by Monobind, can be requested via email from Monobind@monobind.com.

12.2 Interpretation

1. ***Measurements and interpretation of results must be performed by a skilled individual or trained professional.***
2. Laboratory results alone are only one aspect for determining patient care and should not be the sole basis for therapy, particularly if the results conflict with other determinants.
3. The reagents for the test system have been formulated to eliminate maximal interference; however, potential interaction between rare serum specimens and test reagents can cause erroneous results. Heterophilic antibodies often cause these interactions and have been known to be problems for all kinds of immunoassays (Boscato LM, Stuart MC. 'Heterophilic antibodies: a problem for all immunoassays' Clin. Chem. 1988;3427-33). For diagnostic purposes, the results from this assay should be in combination with clinical examination, patient history and all other clinical findings.
4. For valid test results, adequate controls and other parameters must be within the listed ranges and assay requirements.
5. If test kits are altered, such as by mixing parts of different kits, which could produce false test results, or if results are incorrectly interpreted, Monobind shall have no liability.
6. If computer controlled data reduction is used to interpret the results of the test, it is imperative that the predicted values for the calibrators fall within 10% of the assigned concentrations.
7. Clinically, **a DHEA-S value alone is not of diagnostic value** and should only be used in conjunction with other clinical manifestations (observations) and diagnostic procedures.

13.0 EXPECTED RANGES OF VALUES

In agreement with established reference intervals for a "normal" adult population, the expected ranges for the DHEA-S AccuBind® ELISA Test System are detailed in Table 1.

TABLE I
Expected Values for the DHEA-S Test System

POPULATION	RANGE (µg/ml)
Male	0.06 – 4.58
Female	0.03 – 5.88

It is important to keep in mind that establishment of a range of values which can be expected to be found by a given method for a population of "normal" persons is dependent upon a multiplicity of factors: the specificity of the method, the population tested and the precision of the method in the hands of the analyst. For these reasons each laboratory should depend upon the range of expected values established by the manufacturer only until an in-house range can be determined by the analysts using the method with a population indigenous to the area in which the laboratory is located.

14.0 PERFORMANCE CHARACTERISTICS

14.1 Precision

The within and between assay precision of the DHEA-S AccuBind® ELISA Test System were determined by analyses on three different levels of pool control sera. The number, mean values, standard deviation and coefficient of variation for each of these control sera are presented in Table 2 and Table 3.

TABLE 2
Within Assay Precision (Values in µg/ml)

Sample	N	X	σ	C.V.
Low	16	0.66	0.06	9.8%
Normal	16	1.14	0.05	4.9%
High	16	4.84	0.21	4.3%

TABLE 3
Between Assay Precision (Values in µg/ml)

Sample	N	X	σ	C.V.
Low	10	0.61	0.06	9.5%
Normal	10	1.36	0.04	3.1%
High	10	4.73	0.16	3.4%

*As measured in ten experiments in duplicate over a ten day period.

14.2 Sensitivity

The DHEA-S AccuBind® ELISA Test System has a sensitivity of 0.042 ug/ml. The sensitivity was ascertained by determining the variability of the 0 ug/ml serum calibrator and using the 2σ (95% certainty) statistic to calculate the minimum dose.

14.3 Accuracy

The DHEA-S AccuBind® ELISA Test System was compared with a chemiluminescence immunoassay method. Biological specimens from low, normal and relatively high DHEA-S level populations were used (The values ranged from 0.2 ug/ml – 7.7 ug/ml). The total number of such specimens was 77. The least square regression equation and the correlation coefficient were computed for this DHEA-S EIA in comparison with the reference method. The data obtained is displayed in Table 4.

TABLE 4

Method	Mean (x)	Least Square Regression Analysis	Correlation Coefficient
Monobind (y)	1.12	y= 0.1448+0.986x)	0.983
Reference (X)	1.18		

Only slight amounts of bias between this method and the reference method are indicated by the closeness of the mean values. The least square regression equation and correlation coefficient indicates excellent method agreement.

14.4 Specificity

The % cross reactivity of the DHEA-S antibody to selected substances was evaluated by adding the interfering substance to a serum matrix at various concentrations. The cross-reactivity was calculated by deriving a ratio between dose of interfering substance to dose of DHEA-S needed to displace the same amount of labeled analog.

Substance	Cross Reactivity
DHEA-S	1.0000
DHEA	0.0004
Androstenedione	0.0003
Dihydotestosterone	0.0008
Cortisone	<0.0001
Corticosterone	<0.0001
Cortisol	0.0004
Spirolactone	<0.0001
Estriol	<0.0001
Estradiol	<0.0001
Estrone	<0.0001
Testosterone	<0.0001

15.0 REFERENCES

1. Dorfman RI, Shipley RA: *Androgens*, John Wiley and Sons, New York, 1956, pp. 116-128.
2. Pang S, Riddick L: Hirsutism. IN Lifshitz F (Ed): *Pediatric Endocrinology*, A Clinical Guide, second edition. Marcel Dekker, Inc., New York, 1990, pp. 259-291.
3. De Peretti E, Forest MG: Pattern of plasma dehydroepiandrosterone sulfate levels in humans from birth to adulthood: evidence for testicular production. *J Clin Endocrinol Metab*, 47:572-577 (1978).
4. Lashansky G, Saenger P, Fishman K, Gautier T, Mayes D, Berg G, Di Martino-Nardi J, Reiter E, "Normative data for adrenal steroidogenesis in a healthy pediatric population: age and sex-related changes after adrenocorticotropin stimulation", *J Clin Endocrinol Metab*, 73:674-686 (1991).
5. Zumoff B, Roenfeld RS, Stain GW, Levin J, Fukushima DK," Sex differences in twenty-four hour mean plasma concentration of dehydroepiandrosterone (DHEA) and dehydroepiandrosterone sulfate (DHEAS) and the DHEA to DHEAS ratio in normal adults", *J Clin Endocrinol Metab* 51:330-333 (1980).
6. Pang S, Lerner A, Stoner E, Oberfield S, Engle I, New M, "Late-onset adrenal steroid 3"-hydroxysteroid dehydrogenase deficiency: A cause of hirsutism in pubertal and postpubertal women", *J Clin Endocrinol Metab*, 60:428-439 (1985).
7. Lee PDK, Winter RJ, and Green OC, "Virilizing adrenocortical tumors in childhood: eight cases and a review of the literature", *Pediatrics*, 76:437-444 (1985).
8. Rittmaster RS, "Differential suppression of testosterone and estradiol in hirsute women with the superactive gonadotropin-releasing hormone agonist leuprolide"' *J Clin Endocrinol Metab*, 67:651-655,(1988).
9. Beer NA, Jakubowicz DJ, Beer RM, Arocha IR, and Nestler JE "Effects of ntrendipine on glucose tolerance and serum insulin dehydroepiandrostereone sulfate levels in insulin-resistant obese and hypertensive men", *J Clin Endocrinol Metab*, 76:178-163 (1993).
10. Tietz, NW, *Textbook of clinical chemistry*, 2nd ed. Philadelphia: W.B. Saunders, 1994.

Revision: 5 Date: 2019-Jul-16 DCO: 1353
MP5125 Product Code: 5125-*300*

Size		96(A)	192(B)
Reagent (fill)	A)	1ml set	1ml set
	B)	1 (6ml)	2 (6ml)
	C)	1 (6ml)	2 (6ml)
	D)	1 plate	2 plates
	E)	1 (20ml)	1 (20ml)
	F)	1 (7ml)	2 (7ml)
	G)	1 (7ml)	2 (7ml)
	H)	1 (8ml)	2 (8ml)

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Please visit our website to learn more about our products and services.

Glossary of Symbols
(EN 980/ISO 15223)

IVD — In Vitro - Diagnostic Medical Device
2°–8° — Temperature Limitation Storage Condition (2-8° C)
Consult Instructions for Use
REF — Catalogue Number
Contains Sufficient Test for Σ
LOT — Batch Code
Used By (Expiration Day)
Date of Manufacturer
Manufacturer
EC REP — Authorized Rep in European Country
CE — European Conformity

Fuente: *Monobind Inc.*

Anexo 10. *Valores normales de 17-Hidroxiprogesterona.*

17-HIDROXIPROGESTERONA VALORES DE REFERENCIA	
Fase Ovulatoria	**Valor**
Fase folicular	19 – 182 ng/dL
Fase lútea	22 – 469 ng/dL
Uso de anticonceptivos orales	18 – 251 ng/dL
Posmenopáusica	20 – 72 ng/dL

Fuente: *Williamson, M. & Snyder, L. 2012.*

Anexo 11. *Inserto de la hormona 17-hidroxiprogesterona*

Monobind Inc.
Lake Forest, CA 92630, USA

Accu-Bind
ELISA Microwells

17α-OH Progesterone (17-OHP) Test System

Product Code: 5225-300

1.0 INTRODUCTION

Intended Use: The Quantitative Determination of 17α-OH Progesterone Concentration in Human Serum or Plasma by a Microplate Enzyme Immunoassay, Colorimetric

2.0 SUMMARY AND EXPLANATION OF THE TEST

Plasma/Serum concentrations of 17α-hydroxyprogesterone (17-OHP) are valuable in the initial diagnosis of congenital adrenal hyperplasia (CAH).[1,2] This common inborn error of metabolism is usually characterized by deficiency in the C21-hydroxylase enzyme system, and necessitates steroid replacement therapy. Adequacy of treatment has been monitored by determining circulating 17α-OHP concentrations.[3,4]

The incidence is roughly estimated to be 1 in 15,000 newborns and can reach as high as 1 in 1480 in native Alaskans. Early diagnosis is valuable to detect CAH in newborns afflicted with the disease, not clinically recognizable, but which will lead to life threatening adrenal crisis in the neonatal period and to determine the cause of infants with ambiguous genitalia. Delayed diagnosis may also lead to further virilization in female children, acceleration of skeletal maturation and premature development of secondary sex characteristics in male children. Prompt treatment can save the life of infants and allow afflicted children to attain normal growth.

17P is a steroid produced in the adrenal cortex and the gonads. It is the immediate precursor to 11-desoxycortisol (CpS), which is converted to cortisol. Because CpS is produced by 21-hydroxylation of 17P, measurement of 17P is an indirect indicator of 21-hydroxylase activity. CAH occurs where there is a deficiency of this enzyme. The result is a decrease in the conversion of 17P to CpS, which blocks the normal synthesis of cortisol. Due to the feed back mechanism, a decrease in cortisd causes an increase in ACTH secretion, resulting in adrenal hyperplasia. As 17P is not being converted, increased concentrations of this steroid will be found.

17P concentration increases during pregnancy in the maternal and fetal blood. After birth, values decline rapidly to reach normal adult values in 2 to 7 days. Thus, it is advisable not to collect samples before the third day of life. Premature and sick term infants exhibit 2 to 3 fold 17P values with no CAH disorder. It is suggested that a different cut off be adapted to pre-term and sick infants.

In this method, a sample containing 17α-OH progesterone is dispensed into a microplate well. An enzyme labeled 17OH progesterone derivative and biotinylated anti-17OH-progesterone are then added. After a suitable incubation, the antibody fraction is separated from unbound enzyme reagent.

The employment of several serum references of known 17α-OH Progesterone concentration permits construction of a graph of activity and concentration. From comparison to the dose response curve, an unknown specimen's activity can be correlated with 17-OH Progesterone concentration.

3.0 PRINCIPLE

Competitive Enzyme Immunoassay (TYPE 7):
The essential reagents required for an enzyme immunoassay include antibody, enzyme-antigen conjugate and native antigen. Upon mixing biotinylated antibody, enzyme-antigen conjugate and a serum containing the native antigen, a competition reaction results between the native antigen and the enzyme-antigen conjugate for a limited number of antibody binding sites. The interaction is illustrated by the followed equation:

$$^{Enz}Ag + Ag + Ab_{Btn} \underset{k_{-a}}{\overset{k_a}{\rightleftharpoons}} AgAb_{Btn} + {}^{Enz}AgAb_{Btn}$$

Ab_{Btn} = Biotinylated Antibody (Constant Quantity)
Ag = Native Antigen (Variable Quantity)
^{Enz}Ag = Enzyme-antigen Conjugate (Constant Quantity)
$AgAb_{Btn}$ = Antigen-Antibody Complex
$^{Enz}AgAb_{Btn}$ = Enzyme-antigen Conjugate -Antibody Complex
k_a = Rate Constant of Association
k_{-a} = Rate Constant of Disassociation
$K = k_a / k_{-a}$ = Equilibrium Constant

A simultaneous reaction between the biotin attached to the antibody and the streptavidin immobilized on the microwell occurs. This effects the separation of the antibody bound fraction after decantation or aspiration.
$AgAb_{Btn} + {}^{Enz}AgAb_{Btn} + \underline{Streptavidin}_{cw} \Rightarrow \underline{immobilized\ complex}$
$\underline{Streptavidin}_{cw}$ = Streptavidin immobilized on well
Immobilized complex = sandwich complex bound to the solid surface

The enzyme activity in the antibody bound fraction is inversely proportional to the native antigen concentration. By utilizing several different serum references of known antigen concentration, a dose response curve can be generated from which the antigen concentration of an unknown can be ascertained.

4.0 REAGENTS

Materials Provided:

A. 17 -OHP Calibrators – 1ml/vial - Icons A-F
Six (6) vials of serum reference for 17α-OH Progesterone at concentrations of 0 (**A**), 0.1 (**B**), 0.5 (**C**), 1.0 (**D**), 2.5 (**E**), and 10 (F) ng/ml. Store at 2-8°C. A preservative has been added. The calibrators can be expressed in molar concentrations (nM/L) by multiplying by 3.03.
For example: 1ng/ml x 3.03 = 3.03 nM/L

B. 17-OHP Enzyme Reagent – 6ml/vial – Icon Ⓔ
One (1) ready to use vial containing 17-OH Progesterone (Analog)-horseradish peroxides (HRP) conjugate in a protein stabilizing matrix with buffer, preservative, binding protein inhibitors, and dye. Store at 2-8°C.

C. 17-OHP Biotin Reagent – 6ml/vial – Icon ∇
One (1) vial containing anti-17α-OH Progesterone biotinylated purified rabbit IgG conjugate in buffer, blue dye and preservative. Store at 2-8°C.

D. Streptavidin Coated Plate – 96 wells – Icon ⇓
One 96-well microplate coated with 1.0 µg/ml streptavidin and packaged in an aluminum bag with a drying agent. Store at 2-8°C.

E. Wash Solution Concentrate – 20ml/vial – Icon ♦
One (1) vial containing a surfactant in buffered saline. A preservative has been added. Store at 2-8°C.

F. Substrate Solution – 12ml/vial – Icon S^N
One (1) vial containing tetramethylbenzidine (TMB) and hydrogen peroxide (H_2O_2) in buffer. Store at 2-8°C.

G. Stop Solution – 8ml/vial - Icon ⊖
One (1) vial containing a strong acid (0.5M H_2SO_4). Store at 2-8°C.

H. Product Instructions

Note 1: Do not use reagents beyond the kit expiration date.
Note 2: Above reagents are for a single 96-well Microplate.

Note 3: Avoid extended exposure to heat and light. **Opened reagents are stable for sixty (60) days when stored at 2-8°C. Kit and component stability are identified on label.**

4.1 Required But Not Provided:
1. Pipette capable of delivering 0.025 & 0.050 ml (25 & 50 µl) with a precision of better than 1.5%.
2. Dispenser(s) for repetitive deliveries of 0.100 & 0.350 ml (100 & 350 µl) volumes with a precision of better than 1.5%.
3. Adjustable volume (200-1000µl) dispenser(s) for conjugate.
4. Microplate washer or a squeeze bottle (optional).
5. Microplate Reader with 450nm and 620nm wavelength absorbance capability.
6. Absorbent Paper for blotting the microplate wells.
7. Plastic wrap or microplate cover for incubation steps.
8. Vacuum aspirator (optional) for wash steps.
9. Timer.
10. Quality control materials.

5.0 PRECAUTIONS

For In Vitro Diagnostic Use
Not for Internal or External Use in Humans or Animals

All products that contain human serum have been found to be non-reactive for Hepatitis B Surface Antigen, HIV 1&2 and HCV Antibodies by FDA required tests. Since no known test can offer complete assurance that infectious agents are absent, all human serum products should be handled as potentially hazardous and capable of transmitting disease. Good laboratory procedures for handling blood products can be found in the Center for Disease Control / National Institute of Health, "Biosafety in Microbiological and Biomedical Laboratories," 2nd Edition, 1988, HHS Publication No. (CDC) 88-8395.

Safe Disposal of kit components must be according to local regulatory and statutory requirement.

6.0 SPECIMEN COLLECTION AND PREPARATION

The specimens shall be blood serum or heparanised plasma in type and taken with the usual precautions in the collection of venipuncture samples. For accurate comparison to establish normal values, a fasting morning serum sample should be obtained. The blood should be collected in a redtop (with or without gel additives) venipuncture tube or for plasma use evacuated tube(s) containing heparin. Allow the blood to clot for serum samples. Centrifuge the specimen to separate the serum or plasma from the cells.

In patients receiving therapy with high biotin doses (i.e. >5mg/day), no sample should be taken until at least 8 hours after the last biotin administration, preferably overnight to ensure fasting sample.

Samples may be refrigerated at 2-8ₒC for a maximum period of five (5) days. If the specimen(s) cannot be assayed within this time, the sample(s) may be stored at temperatures of -20ₒC for up to 30 days. Avoid use of contaminated devices. Avoid repetitive freezing and thawing. When assayed in duplicate, 0.050 ml (50 µl) of the specimen is required.

7.0 QUALITY CONTROL

Each laboratory should assay controls at levels in the low, normal and high range for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control charts should be maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends. The individual laboratory should set acceptable assay performance limits. In addition, maximum absorbance should be consistent with past experience. Significant deviation from established performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.

8.0 REAGENT PREPARATION

1. **Wash Buffer Solution**
Dilute contents of wash concentrate solution to 1000 ml with distilled or deionized water in a suitable storage container. Diluted buffer can be stored at 2-30°C for up to 60 days.

Note 1: Do not use the working substrate if it looks blue.
Note 2: Do not use reagents that are contaminated or have bacteria growth.

9.0 TEST PROCEDURE

Before proceeding with the assay, bring all reagents, serum reference calibrators and controls to room temperature (20-27°C).
"Test Procedure should be performed by a skilled individual or trained professional"

1. Format the microplates' wells for each serum reference calibrator, control and patient specimen to be assayed in duplicate. **Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8°C.**
2. Pipette 0.025 ml (25 µl) of the appropriate serum reference calibrator, control or specimen into the assigned well.
3. Add 0.050 ml (50 µl) of ready to use 17α-OH Progesterone Enzyme Reagent to all wells.
4. Swirl the microplate gently for 20-30 seconds to mix.
5. Add 0.050 ml (50 µl) of the 17α-OH Progesterone Biotin Reagent to all wells.
6. Swirl the microplate gently for 20-30 seconds to mix
7. Cover and incubate for 60minutes at room temperature.
8. Discard the contents of the microplate by decantation or aspiration. If decanting, blot the plate dry with absorbent paper.
9. Add 0.350 ml (350 µl) of wash buffer (see Reagent Preparation Section), decant (tap and blot) or aspirate. Repeat two (2) additional times for a total of three (3) washes. **An automatic or manual plate washer can be used. Follow the manufacturer's instruction for proper usage. If a squeeze bottle is employed, fill each well by depressing the container (avoiding air bubbles) to dispense the wash. Decant the wash and repeat two (2) additional times.**
10. Add 0.100 ml (100 µl) of substrate solution to all wells (see Reagent Preparation Section). **Always add reagents in the same order to minimize reaction time differences between wells.**
DO NOT SHAKE THE PLATE AFTER SUBSTRATE ADDITION
11. Incubate at room temperature for twenty (20) minutes.
12. Add 0.050 ml (50 µl) of stop solution to each well and gently mix for 15-20 seconds. **Always add reagents in the same order to minimize reaction time differences between wells.**
13. Read the absorbance in each well at 450nm (using a reference wavelength of 620-630nm. **The results should be read within fifteen (15) minutes of adding the stop solution.**

Note: Dilute the samples suspected of concentrations higher than 10ng/ml 1:1 and 1:5 with 17-OH Progesterone '0' ng/ml calibrator or male patient serum pools with a known low value for 17-OH Progesterone.

10.0 CALCULATION OF RESULTS

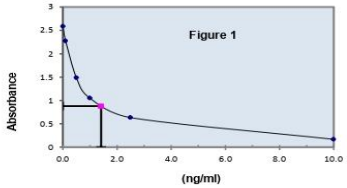
A dose response curve is used to ascertain the concentration of 17 α -OH Progesterone in unknown specimens.
1. Record the absorbance obtained from the printout of the microplate reader as outlined in Example 1.
2. Plot the absorbance for each duplicate serum reference versus the corresponding 17-OHP concentration in ng/ml on linear graph paper (do not average the duplicates of the serum references before plotting).
3. Connect the points with a best-fit curve.
4. To determine the concentration of 17-OHP for an unknown, locate the average absorbance of the duplicates for each unknown on the vertical axis of the graph, find the intersecting point on the curve, and read the concentration (in ng/ml) from the horizontal axis of the graph (the duplicates of the unknown may be averaged as indicated). In the following example, the average absorbance (0.880) intersects the dose response curve at 1.41 ng/ml 176-OHP concentration (See Figure 1).

Note: Computer data reduction software designed for ELISA assays may also be used for the data reduction. If such software is utilized, the validation of the software should be ascertained.

*The data presented in Example 1 and Figure is for illustration only and **should NOT** be used in lieu of a dose response curve prepared with each assay.

EXAMPLE 1

Sample I.D.	Well Number	Abs (A)	Mean Abs (B)	Value (ng/ml)
Cal A	A1	2.586	2.586	0
	B1	2.586		
Cal B	C1	2.276	2.275	0.1
	D1	2.274		
Cal C	E1	1.509	1.486	0.5
	F1	1.463		
Cal D	G1	1.069	1.049	1.0
	H1	1.030		
Cal E	A2	0.642	0.634	2.5
	B2	0.626		
Cal F	C2	0.172	0.169	10
	D2	0.166		
Pat# 1	A3	0.876	0.880	1.41
	B3	0.884		



11.0 Q.C. PARAMETERS

In order for the assay results to be considered valid the following criteria should be met:

1. The absorbance (OD) of calibrator 0 ng/ml should be ≥ 1.3.
2. Four out of six quality control pools should be within the established ranges.

12.0 RISK ANALYSIS

The MSDS and Risk Analysis Form for this product are available on request from Monobind Inc.

12.1 Assay Performance

1. It is important that the time of reaction in each well is held constant to achieve reproducible results.
2. Pipetting of samples should not extend beyond ten (10) minutes to avoid assay drift.
3. Highly lipemic, hemolyzed or grossly contaminated specimen(s) should not be used.
4. If more than one (1) plate is used, it is recommended to repeat the dose response curve.
5. The addition of substrate solution initiates a kinetic reaction, which is terminated by the addition of the stop solution. Therefore, the substrate and stop solution should be added in the same sequence to eliminate any time-deviation during reaction.
6. Plate readers measure vertically. Do not touch the bottom of the wells.
7. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
8. Use components from the same lot. No intermixing of reagents from different batches.
9. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from Monobind's IFU may yield inaccurate results.
10. All applicable national standards, regulations and laws, including, but not limited to, good laboratory procedures, must be strictly followed to ensure compliance and proper device usage.
11. It is important to calibrate all the equipment e.g. Pipettes, Readers, Washers and/or the automated instruments used with this device, and to perform routine preventative maintenance.
12. Risk Analysis- as required by CE Mark IVD Directive 98/79/EC - for this and other devices, made by Monobind, can be requested via email from Monobind@monobind.com.

12.2 Interpretation

1. ***Measurements and interpretation of results must be performed by a skilled individual or trained professional.***
2. Laboratory results alone are only one aspect for determining patient care and should not be the sole basis for therapy, particularly if the results conflict with other determinants.
3. The reagents for the test system have been formulated to eliminate maximal interference; however, potential interaction between rare serum specimens and test reagents can cause erroneous results. Heterophilic antibodies often cause these interactions and have been known to be problems for all kinds of immunoassays (Boscato LM, Stuart MC. "Heterophilic antibodies: a problem for all immunoassays" *Clin. Chem.* 1988:3427-33). For diagnostic purposes, the results from this assay should be used in combination with clinical examination, patient history and all other clinical findings.
4. For valid test results, adequate controls and other parameters must be within the listed ranges and assay requirements.
5. If test kits are altered, such as by mixing parts of different kits, which could produce false test results, or if results are incorrectly interpreted, Monobind shall have no liability.
6. If computer controlled data reduction is used to interpret the results of the test, it is imperative that the predicted values for the calibrators fall within 10% of the assigned concentrations.

13.0 EXPECTED RANGES OF VALUES

In agreement with established reference intervals for a "normal" adult population and females during gestation the expected ranges for the 17α-OH Progesterone AccuBind® ELISA Test System are detailed in Table 1.

TABLE I
Expected Values for 17-OHP AccuBind® ELISA Test System

	(ng/ml)	(nmol/l)
Prepubertal Child (1-10 yr)	0.2 – 0.8	0.64 – 2.54
Adult man	0.2 – 3.1	0.64 – 9.86
Adult woman		
Follicular phase	0.20-1.30	0.64 – 4.13
Luteal phase	1.00 – 4.51	3.18 – 14.34
Postmenopausal woman	0.2 – 0.9	0.64 – 2.86

It is important to keep in mind that establishment of a range of values, which can be expected to be found by a given method for a population of "normal" persons, is dependent upon a multiplicity of factors: the specificity of the method, the population tested and the precision of the method in the hands of the analyst. For these reasons, each laboratory should depend upon the range of expected values established by the manufacturer only until an in-house range can be determined by the analysts using the method with a population indigenous to the area in which the laboratory is located.

14.0 PERFORMANCE CHARACTERISTICS

14.1 Precision

The within and between assay precision of the 17α-OHP AccuBind® ELISA Test System were determined by analyses on three different levels of pool control sera. The number, mean values, standard deviation and coefficient of variation for each of these control sera are presented in Table 2 and Table 3.

TABLE 2
Within Assay Precision (Values in ng/ml)

Sample	N	X	σ	C.V.
Low	20	0.94	0.06	8.5%
Normal	20	3.25	0.22	6.7%
High	20	7.38	0.43	5.8%

TABLE 3
Between Assay Precision (Values in ng/ml)

Sample	N	X	σ	C.V.
Low	10	0.88	0.07	8.0%
Normal	10	3.12	0.24	7.7%
High	10	7.55	0.48	6.4%

*As measured in ten experiments in duplicate over a ten day period.

14.2 Sensitivity

The 17-OHP AccuBind® ELISA Test System has a sensitivity of 0.077ng/ml. The sensitivity was ascertained by determining the variability of the 0 ng/ml serum calibrator and using the 2σ (95% certainty) statistic to calculate the minimum dose.

14.3 Accuracy

The 17-OHP AccuBind® ELISA Test System was compared with a reference method. Biological specimens from low, normal and high 17-OH Progesterone level populations were used (values ranged from < 0.15 ng/ml – 128 ng/ml). The total number of such specimens was 66. The least square regression equation and the correlation coefficient were computed for this method in comparison with the reference method. The data obtained is displayed in Table 4.

TABLE 4

Method	Mean (x)	Least Square Regression Analysis	Correlation Coefficient
Method (Y)	3.49	y= 0.2232+1.065(x)	0.957
Reference (X)	3.19		

Only slight amounts of bias between this method and the reference method are indicated by the closeness of the mean values. The least square regression equation and correlation coefficient indicates excellent method agreement.

14.4 Specificity

The % cross reactivity of the 17OH-progesterone antibody to selected substances was evaluated by adding the interfering substance to a serum matrix at various concentrations. The cross-reactivity was calculated by deriving a ratio between dose of interfering substance to dose of 17α-OH Progesterone needed to displace the same amount of labeled analog.

Substance	Cross Reactivity
17α-OH Progesterone	100.000
Progesterone	0.375
Androstenedione	0.158
Cortisone	0.014
Corticosterone	0.347
Cortisol	0.005
Danazol	0.003
Dihydotestosterone	0.006
DHEA sulfate	0.002
Estradiol	0.004
Estrone	0.003
Estriol	0.002
Prednisone	0.023
Testosterone	0.015
RF	<0.001

15.0 REFERENCES

1. Strott, C.A., Yoshimi, T., and Lipsett, M.B: "Plasma progesterone and 17α-hydroxyprogesterone in normal men and children with *congenital adrenal hyperplasia* (CAH)". J. Clin. Invest. 48,930(1969).
2. Yousseff, N, David R. "Early diagnosis of *congenital adrenal hyperplasia* by measurement of 17α-OH Progesterone". Clin. Endocrinol. 4,451 (1975).
3. Lippe B.M, LaFranchi, S.H, Lavin, N. "Serum 17α-OH progesterone, progesterone and testosterone and Estradiol in the diagnosis and management of *congenital adrenal Hyperplasia*". J. Pediatrics 85,782.(1974).
4. Abraham GE. "The application of natural steroid radioimmunoassay to gynecologic endocrinology". In: Abraham GE, editor. *Radioassay Systems in Clinical Endocrinology*, Basel: Marcel Dekker,: 475-529 (1981).
5. Aufrere MB, Benson H. Progesterone: an overview and recent advances. 65:783-800 (1976).
6. Walker R.F, Read GF., and Fahmy D.R. "Adrenal status assessed by direct radioimmunassay of Cortisol in whole saliva or parotid fluid". *Clin. Chem. 24; 1460 (1978).*
7. Bacon G.E, Spencer M.L., and Kelch R.P. "Effect of Cortisol therapy on hormonal relationships in congenital adrenal hyperplasia". Clin. Endocrinol. 6, 115 (1977).
8. David M, Foresr M.G., "Prenatal treatment of *congenital adrenal hyperplasia* resulting from 21-hydroxylase deficiency". *J. Pediat.105:799, 1984.*
9. August G.P: Growth and development in the normal infant and child. Ibid.p 79.
10. BIO-ED slide/seminar educational program, Rochester: Bioeducational Publications (1981).
11. Tietz, *Textbook of clinical chemistry*, 2nd ed. Philadelphia: W.B. Saunders, (1994).

Effective Date: 2019-Jul-16 Rev. 5 **DCO: 1353**
MP5225 **Product Code: 5225-300**

Size		96(A)	192(B)
Reagent (fill)	A)	1ml set	1ml set
	B)	1 (6ml)	2 (6ml)
	C)	1 (6ml)	2 (6ml)
	D)	1 plate	2 plates
	E)	1 (20ml)	1 (20ml)
	F)	1 (12ml)	2 (12ml)
	G)	1 (8ml)	2 (8ml)

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Please visit our website to learn more about our products and services.

Glossary of Symbols
(EN 980/ISO 15223)

IVD — In Vitro - Diagnostic Medical Device
2°–8° — Temperature Limitation Storage Condition (2-8° C)
Consult Instructions for Use
REF — Catalogue Number
Contains Sufficient Test for Σ
LOT — Batch Code
Used By (Expiration Day)
Date of Manufacturer
Manufacturer
EC REP — Authorized Rep in European Country
CE — European Conformity

Fuente: *Monobind Inc.*

Anexo 12. *Valores normales de la Hormona Antimülleriana.*

ANTIMÜLLERIANA VALORES DE REFERENCIA

Edad	Valor
0 – 8 años	0,0 – 7,1 ng/mL
9 años a adulto	0,0 – 6,9 ng/mL

Fuente: *Williamson, M. & Snyder, L. 2012.*

Anexo 13. *Inserto de la hormona Antimülleriana.*

Monobind Inc.
Lake Forest, CA 92630, USA

AccuBind
ELISA Microwells

Anti-Müllerian Hormone (AMH) Test System

Product Code: 9725-300

1.0 INTRODUCTION

Intended Use: The Quantitative Determination of AMH Concentration in Human Serum or Plasma by a Microplate Enzyme Immunoassay, Colorimetric

2.0 SUMMARY AND EXPLANATION OF THE TEST

Anti-Müllerian Hormone (AMH) is a disulfide-linked homodimeric 140kDa glycoprotein from the trans-forming growth factor-β (TGF-β) superfamily of growth and differentiation factors. It is primarily produced by the gonads in both males and females.

In fetal males, AMH is produced by the Sertoli cells and induces regression of the Müllerian duct and therefore promotes development of the male reproductive tract.[1] Infant males have very high levels of AMH (>30 ng/ml) that slowly decreases until post-pubescence where it remains at a low level (<10 ng/ml).[2-3]

In females, AMH begins to be produced near the time of birth with levels increasing until puberty. After puberty, blood AMH levels decrease until menopause where it becomes nearly undetectable (<0.1 ng/ml). AMH concentration in female blood has repeatedly been linked to ovarian reserve, thereby giving an indication to patients' remaining reproductive lifespans.[4] Additionally, high levels of AMH (>4.7 ng/ml, 80% CI) in females are an indication of polycystic ovarian syndrome (PCOS).[4]

When AMH levels drop below 1.0 ng/ml in females, they are considered to have low ovarian reserves. Patients in these ranges are advised to not delay family planning or to undergo infertility treatments such as in vitro fertilization.[4-3]

The AccuBind® AMH test kit is a highly sensitive assay that can be used to measure blood AMH levels in order to monitor progress of patients' infertility treatments and approximate the onset of menopause.

3.0 PRINCIPLE

Sandwich Equilibrium Method (TYPE 2):
The essential reagents required for an immunoenzymometric assay include high affinity and specificity antibodies (enzyme and immobilized), with different and distinct epitope recognition, **in excess,** and native antigen. In this procedure, the immobilization takes place during the assay at the surface of a microplate well through the interaction of x-AMH antibody coated on the well.

Upon mixing the enzyme-labeled x-AMH antibody (separate epitope) and serum containing the native antigen, a reaction results between the native antigen and the antibodies without competition or steric hindrance to form a sandwich complex. The interaction is illustrated by the following equation:

$$^{Enz}Ab + Ag_{AMH} + Ab_{(well)} \underset{k_{-a}}{\overset{k_a}{\rightleftharpoons}} {}^{Enz}Ab - Ag_{AMH} - Ab_{(well)}$$

$Ab_{(well)}$ = Antibody coated on well (Excess Quantity)
Ag_{AMH} = Native Antigen (Variable Quantity)
^{Enz}Ab = Enzyme labeled Antibody (Excess Quantity)
$^{Enz}Ab - Ag_{AMH} - Ab_{(well)}$ = Antigen-Antibodies Sandwich Complex
k_a = Rate Constant of Association
k_{-a} = Rate Constant of Dissociation

After sufficient time results, the antibody-bound fraction is separated from unbound antigen by decantation or aspiration. The enzyme activity in the antibody-bound fraction is directly proportional to the native antigen concentration. By utilizing several different serum references of known antigen values, a dose response curve can be generated from which the antigen concentration of an unknown can be ascertained.

4.0 REAGENTS

Materials Provided:

A. AMH Calibrators – 1.0 ml/vial (Lyophilized) Icons [A – F]
Six (6) vials of references for AMH at levels of 0(**A**), 0.2(**B**), 0.5(C), 1.0(**D**), 5.0(E) and 15.0(**F**) ng/ml. Store at 2-8 °C. ***Reconstitute each vial with 1.0ml of distilled or deionized water.*** The reconstituted calibrators are stable for 10 days at 2-8 °C. To store for a longer period, aliquot the reconstituted calibrators into cryo vials and store at -20 °C. ***DO NOT FREEZE/ THAW MORE THAN TWICE.*** A preservative has been added.
Note: The calibrators, human serum based, were calibrated using a reference preparation and are traceable against NIBSC code 16/190.

B. AMH Controls – 1.0 ml/vial (Lyophilized) Icons [M&N]
Two (2) vials of reference controls for AMH. Store at 2-8 °C. ***Reconstitute each vial with 1.0ml of distilled or deionized water.*** The reconstituted controls are stable for 10 days at 2-8 °C. To store for a longer period, aliquot the reconstituted calibrators into cryo vials and store at -20°C. ***DO NOT FREEZE/ THAW MORE THAN TWICE.*** A preservative has been added.

C. AMH Enzyme Reagent – 6 ml/vial – Icon
One (1) vial contains anti-AMH conjugate reagent. Store at 2-8 °C.

D. AMH Antibody Coated Plate – 96 wells – Icon
One 96-well microplate coated with x-AMH antibody. Store at 2-8 °C.

E. Wash Solution Concentrate – 20 ml/vial – Icon
One (1) vial contains a surfactant in buffered saline. A preservative has been added. Store at 2-8 °C. *See Reagent Preparation section.*

F. Substrate Reagent – 12 ml/vial – Icon S^N
One (1) vial contains tetramethylbenzidine (TMB) and hydrogen peroxide (H_2O_2) in buffer. Store at 2-8 °C.

G. Stop Solution – 8 ml/vial – Icon
One (1) vial contains a strong acid (0.5 M H_2SO_4). Store at 2-8°C.

H. Product Instructions.

Note 1: Do not use reagents beyond the kit expiration date.
Note 2: Do not expose reagents to heat, sun, or strong light.
Note 3: The above components are for one 96-well microplate. For other kit configurations, refer to table at the end of the instructions.

4.1 Required But Not Provided:
1. Pipette capable of delivering 0.050ml (50µl) and 0.100ml (100µl) volumes with a precision of better than 1.5%.
2. Dispenser(s) for repetitive deliveries of 0.100ml (100µl) and 0.350ml (350µl) volumes with a precision of better than 1.5%.
3. Microplate washers or a squeeze bottle (optional).
4. Microplate reader with 450nm and 620nm wavelength absorbance capability.
5. Absorbent paper for blotting the microplate wells.
6. Plastic wrap or microplate cover for incubation steps.
7. Vacuum aspirator (optional) for wash steps.
8. Timer.
9. Quality control materials.

5.0 PRECAUTIONS

For In Vitro Diagnostic Use
Not for Internal or External Use in Humans or Animals

All products that contain human serum have been found to be non-reactive for Hepatitis B Surface Antigen, HIV 1&2 and HCV Antibodies by FDA licensed reagents. Since no known test can offer complete assurance that infectious agents are absent, all human serum products should be handled as potentially hazardous and capable of transmitting disease. Good laboratory procedures for handling blood products can be found in the Center for Disease Control / National Institute of Health, "Biosafety in Microbiological and Biomedical Laboratories," 2nd Edition, 1988, HHS Publication No. (CDC) 88-8395.

Safe Disposal of kit components must be according to local regulatory and statutory requirement.

6.0 SPECIMEN COLLECTION AND PREPARATION

The specimens shall be blood serum or plasma in type and the usual precautions in the collection of venipuncture samples should be observed. For accurate comparison to established normal values, a fasting morning serum sample should be obtained. The blood should be collected in a plain redtop venipuncture tube without additives or anti-coagulants for serum or EDTA/heparin containing tubes for plasma. Allow the blood to clot for serum samples. Centrifuge the specimen to separate the serum or plasma from the cells.

If the specimen(s) cannot be assayed immediately after blood withdrawal, the sample(s) may be stored at temperatures of 2-8 °C for up to seven (7) days or -20 °C for up to 30 days. Avoid use of contaminated devices. Avoid repetitive freezing and thawing (a maximum of two freeze/thaw cycles prior to use). When assayed in duplicate, 0.100 ml (100 µl) of the specimen is required.

7.0 QUALITY CONTROL

Each laboratory should assay controls at levels in the low, medium and high ranges of the dose response curve for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control charts should be maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends. Significant deviation from established performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.

8.0 REAGENT PREPARATION

1. **Wash Buffer**
Dilute contents of wash solution to 1000ml with distilled or deionized water in a suitable storage container. Diluted buffer can be stored at 2-30°C for up to 60 days.

Note: Do not use reagents that are contaminated or have bacterial growth.

9.0 TEST PROCEDURE

Before proceeding with the assay, bring all reagents, serum reference calibrators and controls to room temperature (20-27°C).
*****Test Procedure should be performed by a skilled individual or trained professional*****
1. Format the microplates' wells for each serum reference, control and patient specimen to be assayed in duplicate. **Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8 °C.**
2. Pipette 0.050 ml (50 µl) of the appropriate serum reference calibrator, control or specimen into the assigned well.
3. Add 0.050 ml (50 µl) of the AMH Enzyme Reagent to each well. **It is very important to dispense all reagents close to the bottom of the coated well.**
4. Swirl the microplate gently for 20-30 seconds, cover and incubate for 60 minutes at room temperature.
5. Discard the contents of the microplate by decantation or aspiration. If decanting, tap and blot the plate dry with absorbent paper.
6. Add 0.350 ml (350 µl) of wash buffer (see Reagent Preparation Section), decant (tap and blot) or aspirate. Repeat two (2) additional times for a total of three (3) washes. **An automatic or manual plate washer can be used. Follow the manufacturer's instruction for proper usage. If a squeeze bottle is employed, fill each well by depressing the container (avoiding air bubbles) to dispense the wash. Decant the wash and repeat two (2) additional times.**
7. Add 0.100 ml (100 µl) of TMB Substrate to all wells. **Always add reagents in the same order to minimize reaction time differences between wells.**
DO NOT SHAKE PLATE AFTER SUBSTRATE ADDITION
8. Incubate at room temperature for twenty (20) minutes.
9. Add 0.050 ml (50 µl) of stop solution to each well and mix gently for 15-20 seconds. **Always add reagents in the same order to minimize reaction time differences between wells.**
10. Read the absorbance in each well at 450nm (using a reference wavelength of 630nm to minimize well imperfections) in a microplate reader. **The results should be read within fifteen (15) minutes of adding the stop solution.**

Note: For re-assaying specimens with concentrations greater than 15 ng/ml, the samples should be diluted and multiplied accordingly. Acceptable diluents are serum from postmenopausal females (<0.1 ng/ml AMH), the "0" calibrator, and other diluent solutions sold by Monobind.

10.0 CALCULATION OF RESULTS

A dose response curve is used to ascertain the concentration of AMH in unknown specimens.
1. Plot the absorbance for each duplicate serum reference versus the corresponding AMH concentration in ng/ml on linear graph paper (do not average the duplicates of the serum references before plotting).
2. Draw the best-fit curve through the plotted points.
3. To determine the concentration of AMH for an unknown, locate the average absorbance of the duplicates for each unknown on the vertical axis of the graph, find the intersecting point on the curve, and read the concentration (in ng/ml) from the horizontal axis of the graph (the duplicates of the unknown may be averaged as indicated). In the following example, the average absorbance (0.299) intersects the dose response curve at 1.33 ng/ml AMH concentration (See Figure 1).

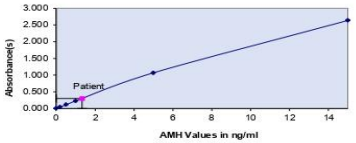
Note: Computer data reduction software designed for ELISA assays may also be used for the data reduction. If such software is utilized, the validation of the software should be ascertained.

EXAMPLE 1

Sample I.D.	Well Number	Abs (A)	Mean Abs (B)	Value (ng/ml)
Cal A	A1	0.005	0.005	0
	B1	0.005		
Cal B	C1	0.041	0.043	0.20
	D1	0.045		
Cal C	E1	0.109	0.112	0.50
	F1	0.115		
Cal D	G1	0.215	0.225	1.00
	H1	0.234		
Cal E	A2	1.047	1.068	5.00
	B2	1.097		
Cal F	C2	2.570	2.635	15.00
	D2	2.678		
Patient	E2	0.300	0.299	**1.33**
	F2	0.298		

*The data presented in Example 1 and Figure 1 is for illustration only and **should not** be used in lieu of a dose response curve prepared with each assay.

FIGURE 1



*If the absorbance readout is off-scale or higher than the average absorbance of the highest calibrator, sample should be repeated with dilution.

11.0 Q.C. PARAMETERS

In order for the assay results to be considered valid the following criteria should be met:

1. Maximum Absorbance (Calibrator 'F') ≥1.3
2. Four out of six quality control pools should be within the established ranges.

12.0 RISK ANALYSIS

The MSDS and Risk Analysis Form for this product are available on request from Monobind Inc.

12.1 Assay Performance

1. It is important that the time of reaction in each well is held constant to achieve reproducible results.
2. Pipetting of samples should not extend beyond ten (10) minutes to avoid assay drift.
3. Highly lipemic, hemolyzed or grossly contaminated specimen(s) should not be used.
4. If more than one (1) plate is used, it is recommended to repeat the dose response curve.
5. The addition of substrate solution initiates a kinetic reaction, which is terminated by the addition of the stop solution. Therefore, the substrate and stop solution should be added in the same sequence to eliminate any time-deviation during reaction.
6. Plate readers measure vertically. Do not touch the bottom of the wells.
7. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
8. Use components from the same lot. No intermixing of reagents from different batches.
9. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from Monobind's IFU may yield inaccurate results.
10. All applicable national standards, regulations and laws, including, but not limited to, good laboratory procedures, must be strictly followed to ensure compliance and proper device usage.
11. It is important to calibrate all the equipment e.g. pipettes, readers, washers and/or the automated instruments used with this device, and to perform routine preventative maintenance.
12. Risk Analysis- as required by CE Mark IVD Directive 98/79/EC - for this and other devices, made by Monobind, can be requested via email from Monobind@monobind.com.

12.2 Interpretation

1. ***Measurements and interpretation of results must be performed by a skilled individual or trained professional.***
2. Laboratory results alone are only one aspect for determining patient care and should not be the sole basis for therapy, particularly if the results conflict with other determinants.
3. For valid test results, adequate controls and other parameters must be within the listed ranges and assay requirements.
4. If test kits are altered, such as by mixing parts of different kits, which could produce false test results, or if results are incorrectly interpreted, Monobind shall have no liability.
5. If computer controlled data reduction is used to interpret the results of the test, it is imperative that the predicted values for the calibrators fall within 10% of the assigned concentrations.
6. The reagents for AccuBind® ELISA procedure have been formulated to eliminate maximal interference; however, potential interaction between rare serum specimens and test reagents can cause erroneous results. Heterophilic antibodies often cause these interactions and have been known to be problems for all kinds of immunoassays. *(Boscato LM Stuart MC.'Heterophilic antibodies: a problem for all immunoassays' Clin. Chem 1988:3427-33).* For diagnostic purposes the results from this assay should be used in combination with clinical examination, patient's history, and, all other clinical findings.

13.0 EXPECTED RANGES OF VALUES

AMH levels were measured by the AMH Accubind® Test System in apparently normal females of different age groups. The values obtained are given in Table 2.

Table 2
Female Reference Ranges for the AMH Test System

Age Group	N	Mean (ng/ml)	Median (ng/ml)	Minimum (ng/ml)	Maximum (ng/ml)
20-29	24	5.95	5.94	1.77	12.41
30-39	80	2.83	2.47	0.11	12.67
40-49	38	1.33	0.59	0.02	9.77

The expected values for males and females of untested age groups as determined by literature sources are collected in Table 3.[5,6]

Table 3
Additional Reference Ranges for the AMH Test System

Males	Range (ng/ml)
<24 months	14-466
2-12 years	7.4-243
>12 years	0.7-19
Females	
<24 months	<4.7
2-12 years	<8.8
13-19 years	0.9-9.5

It is important to keep in mind that establishment of a range of values, which can be expected to be found by a given method for a population of "normal" persons, is dependent upon a multiplicity of factors: the specificity of the method, the population tested and the precision of the method in the hands of the analyst. For these reasons, each laboratory should depend upon the range of expected values established by the manufacturer only until an in-house range can be determined by the analysts using the method with a population indigenous to the area in which the laboratory is located.

14.0 PERFORMANCE CHARACTERISTICS

14.1 Precision

The within assay precision of the AMH AccuBind® Microplate EIA Test System were determined by analyses on six different levels of pool control and patient sera. The mean values, standard deviation and coefficient of variation for each of these control sera are presented in Table 4.

TABLE 4
Precision data for the AMH Test System

Sample	Mean Value (pg/ml)	Within-Run Precision		Total Precision (n=80)	
		SD	CV%	SD	CV%
Control 1	0.80	0.02	2.87	0.04	5.32
Control 2	4.54	0.12	2.60	0.24	5.24
Control 3	14.16	0.30	2.11	0.77	5.47
Patient 1	1.42	0.03	2.20	0.08	5.79
Patient 2	0.30	0.02	5.69	0.03	9.46
Patient 3	9.57	0.26	2.73	0.51	5.36

*As measured in forty experiments in duplicate over a 20 day period.

14.2 Sensitivity

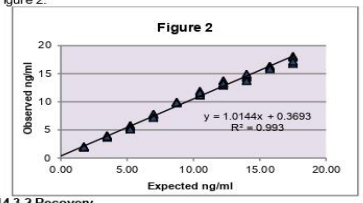
The AMH Accubind® ELISA test system has a LoB=0.024 ng/ml and a LoD=LoQ=0.044 ng/ml.

14.3 Accuracy

14.3.1 Linearity

The linearity of the AMH Accubind® ELISA test system was tested by diluting human serum samples containing high levels of AMH (2 to 17.5 ng/ml) with low AMH (<0.2 ng/ml) human serum samples or the "0" Calibrator. The system demonstrates excellent linearity through the range of the test up to 17.5 ng/ml as shown in Figure 2.

Figure 2



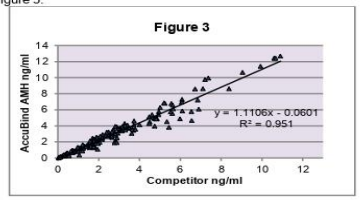
14.3.2 Recovery

The recovery of the AMH AccuBind® Microplate ELISA Test System was calculated for five patient samples spiked with 0.5, 1.0, 2.0, 4.0, and 8.0 ng/ml AMH. Recoveries were determined to be within 15% of the expected values for all samples.

14.3.3 Method Comparison

The AMH AccuBind® Microplate ELISA Test System was initially evaluated on 167 patients with known AMH concentrations calculated by a different AMH test system from a different manufacturer. Correlation between the two methods is excellent with a R^2 coefficient = 0.951. A graph of the data is shown in Figure 3.

Figure 3



14.4 Cross-Reactivity

The following analytes were tested and found to be non-reactive.

Analyte	Concentration	% Reactivity
Follicle Simulating Hormone	100 mIU/ml	<0.001
Human Chorionic Gonadotropin	1000 mIU/ml	<0.001
Leutenizing Hormone	200 mIU/ml	<0.001
Prolactin	100 ng/ml	<0.001

15.0 REFERENCES

1. Wilson, J.D.; George, F.W.; Griffin, J.E. The hormonal control of sexual development. *Science* 1981, 211, *4488*, 1278-1284.
2. Jopling, H.; Yates, A.; Burgoyne, N.; Hayden, K.; Chaloner, C.; Tetlow, L. Paediatric Anti-Müllerian Hormone measurement: Male and Female reference intervals established… *Endocrinal Diab Metab.* 2018, 1, *4*, e00021.
3. Matuszczak, E.; Hermanowicz, A.; Komarowska, M.; Debek, W. Serum AMH in Physiology and Pathology of Male Gonads. *Int. J. of Endocrin.* 2013, 2013, Article ID 128907, 6 pgs.
4. Broer, S.L.; Broekmans, F.J.M.; Laven, J.S.E.; Fauser, B.C.J.M. Anti-Müllerian hormone: ovarian reserve testing and its potential clinical implications. *Human Reproduction Update*, 2014, 20, *5*, 688-701.
5. Lee, J.Y.; Ahn, S.; Lee, J.R.; Jee, B.C.; Kim, C.H.; Seo, S.; Suh, C.S.; Kim, S.H. Reference Values for the Revised Anti-Müllerian Hormone Generation II Assay: Infertile Population-based Study. *J Korean Med Sci* 2017, 32, 825-829.
6. Test ID: AMH Antimullerian Hormone (AMH), Serum. https://www.mayocliniclabs.com/test-catalog/Clinical and Interpretive/89711 (accessed Aug 16, 2019).

Effective Date: 2022-OCT-27 Rev. 4 **DCO: 1578**
MP9725 **Product Code: 9725-300**

Size		96(A)	192(B)
Reagent (fill)	A)	1.0ml (dry) set	1.0ml (dry) set
	B)	1.0ml (dry) set	1.0ml (dry) set
	C)	1 (6ml)	2 (6ml)
	D)	1 plate	2 plates
	E)	1 (20ml)	1 (20ml)
	F)	1 (12ml)	2 (12ml)
	G)	1 (8ml)	2 (8ml)

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Please visit our website to learn more about our products and services.

Glossary of Symbols
(EN 980/ISO 15223)

IVD — In Vitro - Diagnostic Medical Device
2°–8° — Temperature Limitation Storage Condition (2-8°C)
Consult Instructions for Use
REF — Catalogue Number
Contains Sufficient Test for Σ
LOT — Batch Code
Used By (Expiration Day)
Date of Manufacturer
Manufacturer
EC REP — Authorized Rep in European Country
CE — European Conformity

Fuente: *Monobind Inc.*

Anexo 14. *Valores normales de la Hormona Folículo Estimulante*

FSH VALORES DE REFERENCIA

Fase Ovulatoria	Valor
Fase folicular media	3,85 – 8,78 mUI/mL
Máxima central del ciclo	4,54 – 22,51 mUI/mL
Fase lútea	1,79 – 5,12 mUI/mL
Posmenopáusica	6,74 – 13,59 mUI/mL

Fuente: *Williamson, M. & Snyder, L. 2012.*

Anexo 15. *Inserto de la hormona folículo estimulante*

Monobind Inc.
Lake Forest, CA 92630, USA

AccuBind
ELISA Microwells

Follicle Stimulating Hormone (FSH) Test System

Product Code: 425-300

1.0 INTRODUCTION

Intended Use: The Quantitative Determination of Follicle Stimulating Hormone Concentration in Human Serum by a Microplate Enzyme Immunoassay, Colorimetric.

2.0 SUMMARY AND EXPLANATION OF THE TEST

Follicle Stimulating hormone (FSH) is a glycoprotein consisting of two subunits with an approximate molecular mass of 35,500 daltons. The α-subunit is similar to other pituitary hormones [luteinizing stimulating hormone (LH), thyroid stimulating hormone (TSH) and chorionic gonadotropin (CG)] while the β-subunit is unique. The β-subunit confers the biological activity to the molecule. Stimulation by gonadotropin-releasing hormone (GnRH) causes release of FSH, as well as LH, from the pituitary and is transported by the blood to their sites of action, the testes or ovary.

In men, FSH acts on the Sertoli cells of the testis, stimulating the synthesis of inhibin, which appears to specifically inhibit further FSH secretion, and androgen-binding protein. Thus, it indirectly supports spermatogenesis. In women, FSH acts on the granulosa cells of the ovary, stimulating steroidogensis. All ovulatory menstrual cycles have a characteristic pattern of FSH, as well as LH, secretion. The menstrual cycle is divided into a follicular phase and a luteal phase by the midcycle surge of the gonadotropins (LH and FSH). As the follicular phase progresses, FSH concentration decreases. Near the time ovulation occurs, about midcycle, FSH peaks (lesser in magnitude than LH) to its highest level.

The clinical usefulness of the measurement of Follicle Stimulating hormone (FSH) in ascertaining the homeostasis of fertility regulation via the hypothalamic - pituitary - gonadal axis has been well established.[1,2]

In this method, FSH calibrator, patient specimen or control is first added to a streptavidin coated well. Biotinylated monoclonal and enzyme labeled antibodies (directed against distinct and different epitopes of FSH) are added and the reactants mixed. Reaction between the various FSH antibodies and native FSH forms a sandwich complex that binds with the streptavidin coated to the well.

After the completion of the required incubation period, the enzyme-Follicle Stimulating Hormone antibody bound conjugate is separated from the unbound enzyme-follicle stimulating hormone conjugate by aspiration or decantation. The activity of the enzyme present on the surface of the well is quantitated by reaction with a suitable substrate to produce color.

The employment of several serum references of known Follicle Stimulating Hormone levels permits construction of a dose response curve of activity and concentration. From comparison to the dose response curve, an unknown specimen's activity can be correlated with Follicle Stimulating Hormone concentration.

3.0 PRINCIPLE

Immunoenzymometric assay (TYPE 3):
The essential reagents required for an immunoenzymometric assay include high affinity and specificity antibodies (enzyme and immobilized), with different and distinct epitope recognition, **in excess,** and native antigen. In this procedure, the immobilization takes place during the assay at the surface of a microplate well through the interaction of streptavidin coated on the well and exogenously added biotinylated monoclonal anti-FSH antibody. Upon mixing monoclonal biotinylated antibody, the enzyme-labeled antibody and a serum containing the native antigen, reaction results between the native antigen and the antibodies without competition or steric hindrance to form a soluble sandwich complex. The interaction is illustrated by the following equation:

$$^{E\rightarrow}Ab_{(\beta)} + Ag_{FSH} + {}^{Btn}Ab_{(m)} \underset{k_{-a}}{\overset{k_a}{\rightleftharpoons}} {}^{E\rightarrow}Ab_{(\beta)} - Ag_{FSH} - {}^{Btn}Ab_{(m)}$$

$^{Btn}Ab_{(m)}$ = Biotinylated Monoclonal Antibody (Excess Quantity)
Ag_{FSH} = Native Antigen (Variable Quantity)
$^{E\rightarrow}Ab_{(\beta)}$ = Enzyme labeled Antibody (Excess Quantity)
$^{E\rightarrow}Ab_{(\beta)} - Ag_{FSH} - {}^{Btn}Ab_{(m)}$ = Antigen-Antibodies Sandwich Complex
k_a = Rate Constant of Association
k_{-a} = Rate Constant of Dissociation

Simultaneously, the complex is deposited to the well through the high affinity reaction of streptavidin and biotinylated antibody. This interaction is illustrated below:
$^{E\rightarrow}Ab_{(\beta)} - Ag_{FSH} - {}^{Btn}Ab_{(m)} + Streptavidin_{c.w.} \Rightarrow$ Immobilized complex
$Streptavidin_{c.w.}$ = Streptavidin immobolized on well
Immobilized complex = sandwich complex bound to the solid surface

After equilibrium is attained, the antibody-bound fraction is separated from unbound antigen by decantation or aspiration. The enzyme activity in the antibody-bound fraction is directly proportional to the native antigen concentration. By utilizing several different serum references of known antigen values, a dose response curve can be generated from which the antigen concentration of an unknown can be ascertained.

4.0 REAGENTS

Materials Provided:

A. FSH Calibrators – 1 ml/vial - Icons A-F
Six (6) vials of references for FSH Antigen at levels of 0(**A**), 5(**B**), 10(**C**), 25(**D**), 50(**E**) and 100(**F**) mIU/ml. Store at 2-8°C. A preservative has been added.
Note: The calibrators, human serum based, were calibrated using a reference preparation, which was assayed against the WHO 2nd IRP (78/549).

B. FSH Enzyme Reagent – 13 ml/vial - Icon
One (1) vial-containing enzyme labeled antibody, biotinylated monoclonal mouse IgG in buffer, dye, and preservative. Store at 2-8°C.

C. Streptavidin Coated Plate – 96 wells - Icon ⇓
One 96-well microplate coated with streptavidin and packaged in an aluminum bag with a drying agent. Store at 2-8°C.

D. Wash Solution Concentrate – 20 ml/vial - Icon
One (1) vial containing a surfactant in buffered saline. A preservative has been added. Store at 2-8°C.

E. Substrate A – 7.0ml/vial - Icon S^A
One (1) vial containing tetramethylbenzidine (TMB) in buffer. Store at 2-8°C.

F. Substrate B – 7.0ml/vial - Icon S^B
One (1) vial containing hydrogen peroxide (H_2O_2) in buffer. Store at 2-8°C.

G. Stop Solution – 8ml/vial - Icon (stop)
One (1) vial containing a strong acid (1N HCl). Store at 2-8°C.

H. Product Instructions.

Note 1: Do not use reagents beyond the kit expiration date.
Note 2: Avoid extended exposure to heat and light. **Opened reagents are stable for sixty (60) days when stored at 2-8°C. Kit and component stability are identified on the label.**
Note 3: Above reagents are for a single 96-well microplate.

4.1 Required But Not Provided:
1. Pipette capable of delivering 0.050ml (50µl) and 0.100ml (100µl) volumes with a precision of better than 1.5%.
2. Dispenser(s) for repetitive deliveries of 0.100ml (100µl) and 0.350ml (350µl) volumes with a precision of better than 1.5%.
3. Microplate washers or a squeeze bottle (optional).
4. Microplate Reader with 450nm and 620nm wavelength absorbance capability.
5. Absorbent Paper for blotting the microplate wells.
6. Plastic wrap or microplate cover for incubation steps.
7. Vacuum aspirator (optional) for wash steps.
8. Timer.
9. Quality control materials

5.0 PRECAUTIONS

For In Vitro Diagnostic Use
Not for Internal or External Use in Humans or Animals

All products that contain human serum have been found to be non-reactive for Hepatitis B Surface Antigen, HIV 1&2 and HCV Antibodies by FDA licensed reagents. Since no known test can offer complete assurance that infectious agents are absent, all human serum products should be handled as potentially hazardous and capable of transmitting disease. Good laboratory procedures for handling blood products can be found in the Center for Disease Control / National Institute of Health, "Biosafety in Microbiological and Biomedical Laboratories," 2nd Edition, 1988, HHS Publication No. (CDC) 88-8395.

Safe Disposal of kit components must be according to local regulatory and statutory requirement.

6.0 SPECIMEN COLLECTION AND PREPARATION

The specimens shall be blood, serum in type and the usual precautions in the collection of venipuncture samples should be observed. For accurate comparison to established normal values, a fasting morning serum sample should be obtained. The blood should be collected in a plain redtop venipuncture tube without additives or anti-coagulants. Allow the blood to clot. Centrifuge the specimen to separate the serum from the cells.

In patients receiving therapy with high biotin doses (i.e. >5mg/day), no sample should be taken until at least 8 hours after the last biotin administration, preferably overnight to ensure fasting sample.

Samples may be refrigerated at 2-8°C for a maximum period of five (5) days. If the specimen(s) cannot be assayed within this time, the sample(s) may be stored at temperatures of -20°C for up to 30 days. Avoid use of contaminated devices. Avoid repetitive freezing and thawing. When assayed in duplicate, 0.100ml (100µl) of the specimen is required.

7.0 QUALITY CONTROL

Each laboratory should assay controls at levels in the low, normal and elevated range for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control charts should be maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends. Significant deviation from established performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.

8.0 REAGENT PREPARATION

1. **Wash Buffer**
Dilute contents of wash concentrate to 1000ml with distilled or deionized water in a suitable storage container. Store at 2-30°C for up to 60 days.
2. **Working Substrate Solution** – Stable for one year
Pour the contents of the amber vial labeled Solution 'A' into the clear vial labeled Solution 'B'. Place the yellow cap on the clear vial for easy identification. Mix and label accordingly. Store at 2 - 8°C.

Note1 : Do not use the working substrate if it looks blue.
Note 2: Do not use reagents that are contaminated or have bacteria growth.

9.0 TEST PROCEDURE

Before proceeding with the assay, bring all reagents, serum reference calibrators and controls to room temperature (20-27°C).
*****Test Procedure should be performed by a skilled individual or trained professional*****

1. Format the microplate wells for each serum reference calibrator, control and patient specimen to be assayed in duplicate. **Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8°C.**
2. Pipette 0.050 ml (50µl) of the appropriate serum reference calibrator, control or specimen into the assigned well.
3. Add 0.100 ml (100µl) of FSH-Enzyme Reagent solution to all wells.
4. Swirl the microplate gently for 20-30 seconds to mix and cover.
5. Incubate 60 minutes at room temperature.
6. Discard the contents of the microplate by decantation or aspiration. If decanting, blot the plate dry with absorbent paper.
7. Add 350µl of wash buffer (see Reagent Preparation Section) decant (tap and blot) or aspirate. Repeat two (2) additional times for a total of three (3) washes. **An automatic or manual plate washer can be used. Follow the manufacturer's instruction for proper usage. If a squeeze bottle is employed, fill each well by depressing the container (avoiding air bubbles) to dispense the wash. Decant the wash and repeat two (2) additional times.**
8. Add 0.100 ml (100µl) of working substrate solution to all wells (see Reagent Preparation Section). **Always add reagents in the same order to minimize reaction time differences between wells**

DO NOT SHAKE THE PLATE AFTER SUBSTRATE ADDITION

9. Incubate at room temperature for fifteen (15) minutes.
10. 10.Add 0.050ml (50µl) of stop solution to each well and gently mix for 15-20 seconds). **Always add reagents in the same order to minimize reaction time differences between wells**
11. Read the absorbance in each well at 450nm (using a reference wavelength of 620-630nm to minimize well imperfections) in a microplate reader. **The results should be read within thirty (30) minutes of adding the stop solution.**

10.0 CALCULATION OF RESULTS

A dose response curve is used to ascertain the concentration of follicle stimulating hormone in unknown specimens.
1. Record the absorbance obtained from the printout of the microplate reader as outlined in Example 1.
2. Plot the absorbance for each duplicate serum reference versus the corresponding FSH concentration in mIU/ml on linear graph paper (do not average the duplicates of the serum references before plotting).
3. Draw the best-fit curve through the plotted points.
4. To determine the concentration of FSH for an unknown, locate the average absorbance of the duplicates for each unknown on the vertical axis of the graph, find the intersecting point on the curve, and read the concentration (in mIU/ml) from the horizontal axis of the graph (the duplicates of the unknown may be averaged as indicated). In the following example, the average absorbance (1.214) intersects the dose response curve at 43.2mIU/ml FSH concentration (See Figure 1).

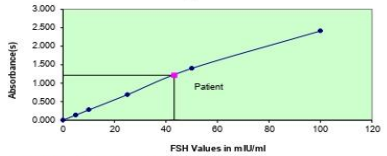
Note: Computer data reduction software designed for ELISA assays may also be used for the data reduction. If such software is utilized, the validation of the software should be ascertained.

*The data presented in Example 1 and Figure 1 are for illustration only and **should not** be used in lieu of a dose response curve prepared with each assay.

EXAMPLE 1

Sample I.D.	Well Number	Abs (A)	Mean Abs (B)	Value (mIU/ml)
Cal A	A1	0.001	0.001	0
	B1	0.001		
Cal B	C1	0.146	0.139	5
	D1	0.133		
Cal C	E1	0.276	0.277	10
	F1	0.278		
Cal D	G1	0.680	0.689	25
	H1	0.698		
Cal E	A2	1.444	1.399	50
	B2	1.354		
Cal F	C2	2.471	2.412	100
	D2	2.354		
Ctrl 1	E2	0.162	0.157	*5.6*
	F2	0.152		
Ctrl 2	G2	0.545	0.546	*19.9*
	H2	0.547		
Patient	A3	1.173	1.214	*43.2*
	B3	1.255		

Figure 1



11.0 Q.C. PARAMETERS

In order for the assay results to be considered valid the following criteria should be met:

1. The absorbance (OD) of calibrator F should be ≥ 1.3
2. Four out of six quality control pools should be within the established ranges.

12.0 RISK ANALYSIS

The MSDS and Risk Analysis Form for this product is available on request from Monobind Inc.

12.1 Assay Performance

1. It is important that the time of reaction in each well is held constant to achieve reproducible results.
2. Pipetting of samples should not extend beyond ten (10) minutes to avoid assay drift.
3. Highly lipemic, hemolyzed or grossly contaminated specimen(s) should not be used.
4. If more than one (1) plate is used, it is recommended to repeat the dose response curve.
5. The addition of substrate solution initiates a kinetic reaction, which is terminated by the addition of the stop solution. Therefore, the substrate and stop solution should be added in the same sequence to eliminate any time-deviation during reaction.
6. Plate readers measure vertically. Do not touch the bottom of the wells.
7. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
8. Use components from the same lot. No intermixing of reagents from different batches.
9. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from Monobind IFU may yield inaccurate results.
10. All applicable national standards, regulations and laws, including, but not limited to, good laboratory procedures, must be strictly followed to ensure compliance and proper device usage.
11. It is important to calibrate all the equipment e.g. Pipettes, Readers, Washers and/or the automated instruments used with this device, and to perform routine preventative maintenance.
12. Risk Analysis- as required by CE Mark IVD Directive 98/79/EC - for this and other devices, made by Monobind, can be requested via email from Monobind@monobind.com.

12.2 Interpretation

1. ***Measurements and interpretation of results must be performed by a skilled individual or trained professional.***
2. Laboratory results alone are only one aspect for determining patient care and should not be the sole basis for therapy, particularly if the results conflict with other determinants.
3. The reagents for the test system have been formulated to eliminate maximal interference; however, potential interaction between rare serum specimens and test reagents can cause erroneous results. Heterophilic antibodies often cause these interactions and have been known to be problems for all kinds of immunoassays (Boscato LM, Stuart MC. 'Heterophilic antibodies: a problem for all immunoassays' Clin. Chem. 1988:3427-33). For diagnostic purposes, the results from this assay should be in combination with clinical examination, patient history and all other clinical findings.For valid test results, adequate controls and other parameters must be within the listed ranges and assay requirements.
4. If test kits are altered, such as by mixing parts of different kits, which could produce false test results, or if results are incorrectly interpreted, Monobind shall have no liability.
5. If computer controlled data reduction is used to interpret the results of the test, it is imperative that the predicted values for the calibrators fall within 10% of the assigned concentrations.
6. FSH is suppressed by estrogen but in woman taking oral contraceptives the level may be low or normal. Excessive dieting and weight loss may lead to low gonadotropin concentrations.
7. Follicle Stimulating Hormones are dependent upon diverse factors other than pituitary homeostasis. Thus, the determination alone is not sufficient to assess clinical status.

13.0 EXPECTED RANGES OF VALUES

A study of an apparent normal adult population was undertaken to determine expected values for the FSH Accubind® ELISA Test System. The expected values are presented in Table 1.

TABLE 1
Expected Values for the FSH Accubind® ELISA Test System (in mIU/ml 2nd IRP 78/549)

Women		
Follicular phase	3.0 --	12.0
Midcycle	8.0 --	22.0
Luteal phase	2.0 --	12.0
Postmenopausal	35.0 --	151.0
Men		
1.0 -- 14.0		

It is important to keep in mind that establishment of a range of values which can be expected to be found by a given method for a population of "normal"-persons is dependent upon a multiplicity of factors: the specificity of the method, the population tested and the precision of the method in the hands of the analyst. For these reasons each laboratory should depend upon the range of expected values established by the manufacturer only until an in-house range can be determined by the analysts using the method with a population indigenous to the area in which the laboratory is located.

14.0 PERFORMANCE CHARACTERISTICS

14.1 Precision

The within and between assay precisions of the FSH Accubind® ELISA test system were determined by analyses on three different levels of control sera. The number (N), mean value (X), standard deviation (σ) and coefficient of variation (C.V) for each of these control sera are presented in Table 2 and Table 3.

TABLE 2
Within Assay Precision (Values in mIU/ml)

Sample	N	X	σ	C.V.
Level 1	20	5.0	0.25	5.4%
Level 2	20	25.0	0.94	3.8%
Level 3	20	40.6	1.64	4.0%

TABLE 3
Between Assay Precision* (Values in mIU/ml)

Sample	N	X	σ	C.V.
Level 1	20	4.7	0.42	9.0%
Level 2	20	23.1	1.99	8.6%
Level 3	20	37.8	3.2	8.4%

*As measured in ten experiments in duplicate.

14.2 Sensitivity

The Follicle Stimulating Hormone procedure has a sensitivity of 0.006 mIU/well. This is equivalent to a sample containing 0.134mIU/ml FSH concentration.The sensitivity (detection limit) was ascertained by determining the variability of the '0 mIU/ml' calibrator and using the 2σ (95% certainty) statistic to calculate the minimum dose.

14.3 Accuracy

This FSH Accubind® ELISA test system was compared with a reference radioimmunoassay. Biological specimens from low, normal, and elevated concentrations were assayed. The total number of such specimens was 106. The least square regression equation and the correlation coefficient were computed for the FSH Accubind® ELISA test system in comparison with the reference method. The data obtained is displayed in Table 4.

TABLE 4

Method	Mean (x)	Least Square Regression Analysis	Correlation Coefficient
Monobind	17.4	y = 0.98(x) – 1.7	0.978
Reference	19.5		

Only slight amounts of bias between the FSH Accubind® ELISA test method and the reference method are indicated by the closeness of the mean values. The least square regression equation and correlation coefficient indicates excellent method agreement.

14.4 Specificity

The cross-reactivity of the FSH Accubind® ELISA test system to selected substances was evaluated by adding the interfering substance to a serum matrix at various concentrations. The cross-reactivity was calculated by deriving a ratio between dose of interfering substance to dose of Follicle Stimulating Hormone needed to produce the same absorbance.

Substance	Cross Reactivity	Concentration
Follitropin (FSH)	1.0000	--
Lutropin Hormone (hLH)	< 0.0001	1000ng/ml
Chorionic Gonadotropin (hCG)	< 0.0001	1000ng/ml
Thyrotropin (TSH)	< 0.0001	1000ng/ml

15.0 REFERENCES

1. Odell WD, Parlow AF et al, *J Clin Invest,* 47, 2551 (1981).
2. Saxena B.B., Demura H.M., et al. *J Clin Endocrinol Metab.* 28, 591 (1968).
3. Wennink JM, Delemarre-van de Waal HA, Schoemaker R, Schoemaker H, Schoemaker J. "Luteinizing hormone and follicle stimulating hormone secretion patterns in girls throughout puberty measured using highly sensitive immunoradiometric assays", *Clin Endocrinol (Oxf),* 33, 333–344 (1990).
4. Winter JS, Faiman C, "The development of cyclic pituitary-gonadal function in adolescent females", *J Clin Endocrinol Metab*, 37, 714–718 (1973).
5. Simoni M, Gromoll J, Nieschlag E, "The follicle stimulating hormone receptor: biochemistry, molecular biology, physiology and pathophysiology", *Endocr Rev*, 18, 739–773 (1997).
6. Vitt UA, Kloosterboer HJ, Rose UM, Mulders JW, Kiesel PS, Bete S, Nayudu PL, "Isoforms of human recombinant follicle-stimulating hormone: comparison of effects on murine follicle development in vitro", *Biol Reprod* 59, 854–861 (1998).
7. Layman LC, Lee EJ, Peak DB, Namnoum AB, Vu KV, van Lingen BL, Gray MR, McDonough PG, Reindollar RH, Jameson JL, "Delayed puberty and hypogonadism caused by mutations in the follicle-stimulating hormone ß subunit gene", *N Engl J Med,* 337, 607–611 (1997).
8. Robertson DR, "Circulating half-lives of follicle stimulating hormones and luteinizing hormone in pituitary extracts and isoform fractions of ovariectomized and intact ewes", *Endocrinology*.,129, 1805–1813 (1991).
9. Wide L, "Electrophoretic and gel chromatographic analyses of follicle stimulating hormone in human serum", *Ups J Med Sci,* 86, 249–258 (1981).
10. Berger P, Bidart JM, Delves PS, Dirnhofer S, Hoermann R, Isaacs N, Jackson A, Klonisch T, Lapthorn A, Lund T, Mann K, Roitt I, Schwarz S, Wick G, "Immunochemical mapping of gonadotropins", *Mol Cell Endocrinol*, **125,** 33–43 (1996).

Revision: 4 Date: 2019-Jul-16 DCO: 1353
MP425 Cat #: *425-300*

Size	96(A)	192(B)
Reagent (fill) A)	1ml set	1ml set
B)	1 (13ml)	2 (13ml)
C)	1 plate	2 plates
D)	1 (20ml)	1 (20ml)
E)	1 (7ml)	2 (7ml)
F)	1 (7ml)	2 (7ml)
G)	1 (8ml)	2 (8ml)

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IVD 2°-8° CE
EC REP **CEpartner4U,** Esdoornlaan 13 3951 DB Maarn, The Neatherlands **www.cepartner4u.eu**

Please visit our website to learn more about our products and services.

Glossary of Symbols
(EN 980/ISO 15223)

IVD — In Vitro - Diagnostic Medical Device
2°-8° — Temperature Limitation Storage Condition (2-8°C)
Consult Instructions for Use
REF — Catalogue Number
Σ — Contains Sufficient Test for Σ
LOT — Batch Code
Used By (Expiration Day)
Date of Manufacturer
Manufacturer
EC REP — Authorized Rep in European Country
CE — European Conformity

Fuente: *Monobind Inc.*

Anexo 16. *Valores de referencia de la hormona luteinizante*

LH VALORES DE REFERENCIA

Fase Ovulatoria	Valor
Fase folicular media	2,12 – 10,89 mUI/mL
Máxima central del ciclo	19,18 – 103,03 mUI/mL
Fase lútea	1,2 – 12,86 mUI/mL
Posmenopáusica	10,87 – 58,64 mUI/mL

Fuente: *Williamson, M. & Snyder, L. 2012.*

Anexo 17. Inserto de la hormona luteinizante

Monobind Inc.
Lake Forest, CA 92630, USA

AccuBind
ELISA Microwells

Luteinizing Hormone (LH) Test System
Product Code: 625-300

1.0 INTRODUCTION

Intended Use: The Quantitative Determination of Luteinizing Hormone Concentration in Human Serum by a Microplate Enzyme Immunoassay, Colorimetric

2.0 SUMMARY AND EXPLANATION OF THE TEST

Luteinizing hormone (LH) is a glycoprotein consisting of two subunits with a molecular mass of 30,000 daltons. The α-subunit is similar to other pituitary hormones follicle stimulating hormone (FSH), thyroid stimulating hormone (TSH) and chorionic gonadotropin (CG)] while the β-subunit is unique. The β-subunit confers the biological activity to the molecule. The α-subunit consists of 89 amino acid residues while the β-subunit contains 129 amino acids. The carbohydrate content is between 15% and 30%.

The clinical usefulness of the measurement of luteinizing hormone (LH) in ascertaining the homeostasis of fertility regulation via the hypothalamic - pituitary - gonadal axis has been well established.[1,2] In addition, the advent of *in vitro* fertilization (IVF) technology to overcome infertility-associated problems has provided the impetus for rapid improvement in LH assay methodology from the technically demanding bioassay[3] to the procedurally simple and rapid immunoenzymometric assays.

In this method, LH calibrator, patient specimen or control is first added to a streptavidin coated well. Biotinylated monoclonal and enzyme labeled antibodies (directed against distinct and different epitopes of LH) are added and the reactants mixed. Reaction between the various LH antibodies and native LH forms a sandwich complex that binds with the streptavidin coated to the well.

After the completion of the required incubation period, the enzyme-luteinizing hormone antibody bound conjugate is separated from the unbound enzyme-luteinizing hormone conjugate by aspiration or decantation. The activity of the enzyme present on the surface of the well is quantitated by reaction with a suitable substrate to produce color.

The employment of several serum references of known luteinizing hormone levels permits construction of a dose response curve of activity and concentration. From comparison to the dose response curve, an unknown specimen's activity can be correlated with luteinizing hormone concentration.

3.0 PRINCIPLE

Immunoenzymometric assay (TYPE 3):
The essential reagents required for an immunoenzymometric assay include high affinity and specificity antibodies (enzyme and immobilized), with different and distinct epitope recognition, **in excess,** and native antigen. In this procedure, the immobilization takes place during the assay at the surface of a microplate well through the interaction of streptavidin coated on the well and exogenously added biotinylated monoclonal anti-LH antibody.

Upon mixing monoclonal biotinylated antibody, the enzyme-labeled antibody and a serum containing the native antigen, reaction results between the native antigen and the antibodies without competition or steric hindrance to form a soluble sandwich complex. The interaction is illustrated by the following equation:

$$^{Enz}Ab_{(p)} + Ag_{LH} + {}^{Btn}Ab_{(m)} \underset{k_{-a}}{\overset{k_a}{\rightleftharpoons}} {}^{Enz}Ab_{(p)}\text{-}Ag_{LH}\text{-}{}^{Btn}Ab_{(m)}$$

$^{Btn}Ab_{(m)}$ = Biotinylated Monoclonal Antibody (Excess Quantity)
Ag_{LH} = Native Antigen (Variable Quantity)
$^{Enz}Ab_{(p)}$ = Enzyme labeled Antibody (Excess Quantity)
$^{Enz}Ab_{(p)}\text{-}Ag_{LH}\text{-}{}^{Btn}Ab_{(m)}$ = Antigen-Antibodies Sandwich Complex
k_a = Rate Constant of Association
k_{-a} = Rate Constant of Dissociation

Simultaneously, the complex is deposited to the well through the high affinity reaction of streptavidin and biotinylated antibody. This interaction is illustrated below:
$^{Enz}Ab_{(p)}\text{-}Ag_{LH}\text{-}{}^{Btn}Ab_{(m)} + Streptavidin_{C.W.} \Rightarrow$ Immobilized complex
$Streptavidin_{C.W.}$ = Streptavidin immobilized on well
Immobilized complex = Antibodies-Antigen sandwich bound

After equilibrium is attained, the antibody-bound fraction is separated from unbound antigen by decantation or aspiration. The enzyme activity in the antibody-bound fraction is directly proportional to the native antigen concentration. By utilizing several different serum references of known antigen values, a dose response curve can be generated from which the antigen concentration of an unknown can be ascertained.

4.0 REAGENTS

Materials Provided

A. LH Calibrators – 1ml/vial – Icons A-F
Six (6) vials of references for LH Antigen at levels of 0(**A**), 5(**B**), 25(**C**), 50(**D**), 100(**E**) and 200(**F**) mIU/ml. Store at 2-8°C. A preservative has been added.
Note: The calibrators, human serum based, were calibrated using a reference preparation, which was assayed against the WHO 2nd IS 80/552.

B. LH Enzyme Reagent – 13 ml/vial – Icon Ⓔ
One (1) vial containing enzyme labeled affinity purified antibody, biotinylated monoclonal mouse IgG in buffer, dye, and preservative. Store at 2-8°C.

C. Streptavidin Coated Plate – 96 wells – Icon ⇓
One 96-well microplate coated with streptavidin and packaged in an aluminum bag with a drying agent. Store at 2-8°C.

D. Wash Solution Concentrate – 20 ml/vial - Icon ♦
One (1) vial containing a surfactant in buffered saline. A preservative has been added. Store at 2-8°C.

E. Substrate A – 7ml/vial – Icon S^A
One (1) vial containing tetramethylbenzidine (TMB) in buffer. Store at 2-8°C.

F. Substrate B – 7ml/vial – Icon S^B
One (1) vial containing hydrogen peroxide (H_2O_2) in buffer. Store at 2-8°C.

G. Stop Solution – 8ml/vial – Icon STOP
One (1) vial containing a strong acid (1N HCl). Store at 2-8°C.

H. Product Instructions.

Note 1: Do not use reagents beyond the kit expiration date.
Note 2: Avoid extended exposure to heat and light. **Opened reagents are stable for sixty (60) days when stored at 2-8°C. Kit and component stability are identified on the label.**
Note 3: Above reagents are for a single 96-well microplate

4.1 Required But Not Provided:
1. Pipette capable of delivering 0.050ml (50µl) volumes with a precision of better than 1.5%.
2. Dispenser(s) for repetitive deliveries of 0.100 and 0.350ml (100 and 350µl) volumes with a precision of better than 1.5%.
3. Microplate washers or a squeeze bottle (optional).
4. Microplate Reader with 450nm and 620nm wavelength absorbance capability.
5. Absorbent Paper for blotting the microplate wells.
6. Plastic wrap or microplate cover for incubation steps.
7. Vacuum aspirator (optional) for wash steps.
8. Timer.
9. Quality control materials

5.0 PRECAUTIONS

For In Vitro Diagnostic Use
Not for Internal or External Use in Humans or Animals

All products that contain human serum have been found to be non-reactive for Hepatitis B Surface Antigen, HIV 1&2 and HCV Antibodies by FDA licensed reagents. Since no known test can offer complete assurance that infectious agents are absent, all human serum products should be handled as potentially hazardous and capable of transmitting disease. Good laboratory procedures for handling blood products can be found in the Center for Disease Control / National Institute of Health, "Biosafety in Microbiological and Biomedical Laboratories," 2nd Edition, 1988, HHS Publication No. (CDC) 88-8395.

Safe Disposal of kit components must be according to local regulatory and statutory requirement.

6.0 SPECIMEN COLLECTION AND PREPARATION

The specimens shall be blood serum in type and the usual precautions in the collection of venipuncture samples should be observed. For accurate comparison to established normal values, a fasting morning serum sample should be obtained. The blood should be collected in a plain redtop venipuncture tube without additives or gel barrier. Allow the blood to clot. Centrifuge the specimen to separate the serum from the cells.

In patients receiving therapy with high biotin doses (i.e. >5mg/day), no sample should be taken until at least 8 hours after the last biotin administration, preferably overnight to ensure fasting sample.

Samples may be refrigerated at 2-8°C for a maximum period of five (5) days. If the specimen(s) cannot be assayed within this time, the sample(s) may be stored at temperatures of -20°C for up to 30 days. Avoid use of contaminated devices. Avoid repetitive freezing and thawing. When assayed in duplicate, 0.100 ml (100 µl) of the specimen is required.

7.0 QUALITY CONTROL

Each laboratory should assay controls at levels in the low, normal and elevated range for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control charts should be maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends. Significant deviation from established performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.

8.0 REAGENT PREPARATION

1. **Wash Buffer**
   Dilute contents of wash concentrate to 1000ml with distilled or deionized water in a suitable storage container. Store at 2-30°C for up to 60 days.
2. **Working Substrate Solution** – Stable for one year
   Pour the contents of the amber vial labeled Solution 'A' into the clear vial labeled Solution 'B'. Place the yellow cap on the clear vial for easy identification. Mix and label accordingly. Store at 2 - 8°C.

Note1: Do not use the working substrate if it looks blue.
Note 2: Do not use reagents that are contaminated or have bacteria growth.

9.0 TEST PROCEDURE

Before proceeding with the assay, bring all reagents, serum reference calibrators and controls to room temperature (20-27°C).

*****Test Procedure should be performed by a skilled individual or trained professional*****

1. Format the microplate wells for each serum reference calibrator, control and patient specimen to be assayed in duplicate. **Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8°C**
2. Pipette 0.050 ml (50µl) of the appropriate serum reference calibrator, control or specimen into the assigned well.
3. Add 0.100 ml (100µl) of LH-Enzyme Reagent to all wells.
4. Swirl the microplate gently for 20-30 seconds to mix and cover.
5. Incubate 60 minutes at room temperature.
6. Discard the contents of the microplate by decantation or aspiration. If decanting, blot the plate dry with absorbent paper.
7. Add 0.350ml (350µl) of wash buffer (see Reagent Preparation Section) decant (tap and blot) or aspirate. Repeat two (2) additional times for a total of three (3) washes. **An automatic or manual plate washer can be used. Follow the manufacturer's instruction for proper usage. If a squeeze bottle is employed, fill each well by depressing the container (avoiding air bubbles) to dispense the wash. Decant the wash and repeat two (2) additional times.**
8. Add 0.100 ml (100µl) of working substrate solution to all wells (see Reagent Preparation Section). **Always add reagents in the same order to minimize reaction time differences between wells**
   DO NOT SHAKE THE PLATE AFTER SUBSTRATE ADDITION
9. Incubate at room temperature for fifteen (15) minutes.
10. Add 0.050ml (50µl) of stop solution to each well and gently mix for 15-20 seconds). **Always add reagents in the same order to minimize reaction time differences between wells**
11. Read the absorbance in each well at 450nm (using a reference wavelength of 620-630nm to minimize well imperfections) in a microplate reader. **The results should be read within thirty (30) minutes of adding the stop solution.**

10.0 CALCULATION OF RESULTS

A dose response curve is used to ascertain the concentration of luteinizing hormone (LH) in unknown specimens.
1. Record the absorbance obtained from the printout of the microplate reader as outlined in Example 1.
2. Plot the absorbance for each duplicate serum reference versus the corresponding LH concentration in mIU/ml on linear graph paper (do not average the duplicates of the serum references before plotting).
3. Draw the best-fit curve through the plotted points.
4. To determine the concentration of LH for an unknown, locate the average absorbance of the duplicates for each unknown on the vertical axis of the graph, find the intersecting point on the curve, and read the concentration (in mIU/ml) from the horizontal axis of the graph (the duplicates of the unknown may be averaged as indicated). In the following example, the average absorbance (1.005) intersects the dose response curve at 42.7 mIU/ml LH concentration (See Figure 1).

Note: Computer data reduction software designed for ELISA assays may also be used for the data reduction. If such software is utilized, the validation of the software should be ascertained.

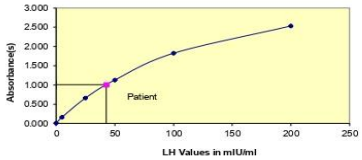
*The data presented in Example 1 and Figure 1 is for illustration only and **should not** be used in lieu of a dose response curve prepared with each assay.

EXAMPLE 1

Sample I.D.	Well Number	Abs (A)	Mean Abs (B)	Value (mIU/ml)
Cal A	A1	0.009	0.009	0
	B1	0.009		
Cal B	C1	0.161	0.162	5
	D1	0.163		
Cal C	E1	0.677	0.662	25
	F1	0.647		
Cal D	G1	1.155	1.130	50
	H1	1.106		
Cal E	A2	1.852	1.825	100
	B2	1.797		
Cal F	C2	2.556	2.534	200
	D2	2.512		
Ctrl 1	E2	0.077	0.072	**1.9**
	F2	0.067		
Ctrl 2	G2	0.582	0.575	**20.5**
	H2	0.568		
Patient	A3	0.998	1.005	**42.7**
	B3	1.112		

*The data presented in Example 1 and Figure 1 is for illustration only and **should not** be used in lieu of a dose response curve prepared with each assay.

Figure 1



11.0 Q.C. PARAMETERS

In order for the assay results to be considered valid the following criteria should be met:

1. The absorbance (OD) of the calibrator 'F' should be ≥ 1.3.
2. Four out of six quality control pools should be within the established ranges

12.0 RISK ANALYSIS

The MSDS and Risk Analysis Form for this product is available on request from Monobind Inc.

12.1 Assay Performance

1. It is important that the time of reaction in each well is held constant to achieve reproducible results.
2. Pipetting of samples should not extend beyond ten (10) minutes to avoid assay drift.
3. Highly lipemic, hemolyzed or grossly contaminated specimen(s) should not be used.
4. If more than one (1) plate is used, it is recommended to repeat the dose response curve.
5. The addition of substrate solution initiates a kinetic reaction, which is terminated by the addition of the stop solution. Therefore, the substrate and stop solution should be added in the same sequence to eliminate any time-deviation during reaction.
6. Plate readers measure vertically. Do not touch the bottom of the wells.
7. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
8. Use components from the same lot. No intermixing of reagents from different batches.
9. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from Monobind's IFU may yield inaccurate results.
10. All applicable national standards, regulations and laws, including, but not limited to, good laboratory procedures, must be strictly followed to ensure compliance and proper device usage.
11. It is important to calibrate all the equipment e.g. Pipettes, Readers, Washers and/or the automated instruments used with this device, and to perform routine preventative maintenance.
12. Risk Analysis- as required by CE Mark IVD Directive 98/79/EC - for this and other devices, made by Monobind, can be requested via email from Monobind@monobind.com.

12.2 Interpretation

1. ***Measurements and interpretation of results must be performed by a skilled individual or trained professional.***
2. Laboratory results alone are only one aspect for determining patient care and should not be the sole basis for therapy, particularly if the results conflict with other determinants.
3. "The reagents for the test system procedure have been formulated to eliminate maximal interference; however, potential interaction between rare serum specimens and test reagents can cause erroneous results. Heterophilic antibodies often cause these interactions and have been known to be problems for all kinds of immunoassays. (*Boscato LM Stuart MC. 'Heterophilic antibodies: a problem for all immunoassays' Clin.Chem. 1988:3427-33).* For diagnostic purposes, the results from this assay should be used in combination with clinical examination, patient history and all other clinical findings."
4. For valid test results, adequate controls and other parameters must be within the listed ranges and assay requirements.
5. If test kits are altered, such as by mixing parts of different kits, which could produce false test results, or if results are incorrectly interpreted, Monobind shall have no liability.
6. If computer controlled data reduction is used to interpret the results of the test, it is imperative that the predicted values for the calibrators fall within 10% of the assigned concentrations.
7. LH is suppressed by estrogen but in woman taking oral contraceptives the level may be low or normal. Excessive dieting and weight loss may lead to low gonadotropin concentrations.
8. Luteinizing hormone is dependent upon diverse factors other than pituitary homeostasis. Thus, the determination alone is not sufficient to assess clinical status.

13.0 EXPECTED RANGES OF VALUES

A study of an apparent normal adult population was undertaken to determine expected values for the LH AccuBind® ELISA Test System. The expected values are presented in Table 1.

TABLE I
Expected Values for the LH ELISA Test System (in mIU/ml)

Women			
Follicular phase	0.5	--	10.5
Midcycle	18.4	--	61.2
Luteal phase	0.5	--	10.5
Postmenopausal	8.2	--	40.8
Men			
0.7 -- 7.4			

It is important to keep in mind that establishment of a range of values, which can be expected to be found by a given method for a population of "normal" persons, is dependent upon a multiplicity of factors: the specificity of the method, the population tested and the precision of the method in the hands of the analyst. For these reasons, each laboratory should depend upon the range of expected values established by the Manufacturer only until an in-house range can be determined by the analysts using the method with a population indigenous to the area in which the laboratory is located.

14.0 PERFORMANCE CHARACTERISTICS

14.1 Precision

The within and between assay precisions of the LH AccuBind® ELISA Test System were determined by analyses on three different levels of control sera. The number (N), mean value (X), standard deviation (σ) and coefficient of variation (C.V.) for each of these control sera are presented in Table 2 and Table 3.

TABLE 2
Within Assay Precision (Values in mIU/ml)

Sample	N	X	σ	C.V.
Level 1	20	1.4	0.10	6.8%
Level 2	20	21.6	0.85	3.9%
Level 3	20	58.3	2.10	3.6%

TABLE 3
Between Assay Precision* (Values in mIU/ml)

Sample	N	X	σ	C.V.
Level 1	20	1.6	0.12	7.8%
Level 2	20	21.5	2.32	10.8%
Level 3	20	55.4	5.34	9.6%

*As measured in ten experiments in duplicate.

14.2 Sensitivity

The LH AccuBind® ELISA Test System has a sensitivity of 0.003mIU/well. This is equivalent to a sample containing 0.054 mIU/ml LH concentration. The analytical sensitivity (detection limit) was ascertained by determining the variablity of the '0 mIU/ml' calibrator and using the 2σ (95% certainty) statistic to calculate the minimum dose.

14.3 Accuracy

This LH AccuBind® ELISA Test System system was compared with a reference radioimmunoassay. Biological specimens from normal, and pregnant populations were assayed. The total number of such specimens was 110. The least square regression equation and the correlation coefficient were computed for the LH ELISA method in comparison with the reference method. The data obtained is displayed in Table 4.

TABLE 4

Method	Mean (x)	Least Square Regression Analysis	Correlation Coefficient
This Method	14.8	y = 0.081 + 0.93(x)	0.989
Reference	15.1		

Only slight amounts of bias between the LH AccuBind® ELISA Test System and the reference method are indicated by the closeness of the mean values. The least square regression equation and correlation coefficient indicates excellent method agreement.

14.4 Specificity

The cross-reactivity of the LH AccuBind® ELISA Test System to selected substances was evaluated by adding the interfering substance to a serum matrix at various concentrations. The cross-reactivity was calculated by deriving a ratio between dose of interfering substance to dose of Luteinizing Hormone needed to produce the same absorbance.

Substance	Cross Reactivity	Concentration
Lutropin (LH)	1.0000	---
β-LH subunit	1.0800	---
Follitropin (FSH)	< 0.0001	1000ng/ml
Chorionic gonadotropin (CG)	< 0.0001	1000ng/ml
Thyrotropin (TSH)	< 0.0001	1000ng/ml

15.0 REFERENCES

1. Kosasa T.S., "Measurement of Human Luteinizing Hormone", *Journal of Reproductive Medicine,* 26, 201-6 (1981).
2. Danzer H, Braunstein GD, et al, "Maternal Serum Human Chorionic Gonadotropic Concentrations and Fetal Sex Predictions", *Fertility and Sterility,* 34, 336-40 (1980).
3. Braunstein GD, et al, "Serum Human Luteinizing Hormone Levels through Normal Pregnancy", *American Journal of Obstetrics and Gynecology,* 126, 678-81 (1976).
4. Goldstein DP, and Kosasa T, "The Subunit Radiammunoassay for LH Clinical Application", *Gynecology,* 6, 45-84 (1975).
5. Batzer F, "Hormonal Evaluation of Early Pregnancy", *Fertility and Sterility, 34,* 1-12 (1980).
6. Braunstein, G.D., et al., "First-Trimester Luteinizing Hormone Measurements as an Aid to the Diagnosis of Early Pregnancy Disorders", *American Journal of Obstetrics and Gynecology* 131, 25-32 (1978).
7. Lenton E, Neal L and Sulaiman R, "Plasma Concentrations of Human Gonadotropin from the time of Implantation until the Second Week of Pregnancy", *Fertility and Sterility,* 37, 773-78 (1982).

Revision: 5 Date: 2019-Jul-16 DCO: 1353
Product Code: *625-300*
MP625

Size		96(A)	192(B)
Reagent (fill)	A)	1ml set	1ml set
	B)	1 (13ml)	2 (13ml)
	C)	1 plate	2 plates
	D)	1 (20ml)	1 (20ml)
	E)	1 (7ml)	2 (7ml)
	F)	1 (7ml)	2 (7ml)
	G)	1 (8ml)	2 (8ml)

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IVD 2°-8° CE
EC REP **CEpartner4U,** Esdoornlaan 13 3951 DB Maarn, The Neatherlands **www.cepartner4u.eu**

Please visit our website to learn more about our products and services.

Glossary of Symbols
(EN 980/ISO 15223)

IVD — In Vitro - Diagnostic Medical Device
2°-8° — Temperature Limitation Storage Condition (2-8° C)
Consult Instructions for Use
REF — Catalogue Number
Σ — Contains Sufficient Test for Σ
LOT — Batch Code
Used By (Expiration Day)
Date of Manufacturer
Manufacturer
EC REP — Authorized Rep in European Country
CE — European Conformity

Fuente: *Monobind Inc.*

Anexo 18. *Valores normales de la Hormona Progesterona*

PROGESTERONA VALORES DE REFERENCIA	
Fase Ovulatoria	**Valor**
Mitad de la fase folicular	0,31 – 1,52 ng/mL
Mitad de la fase lútea	5,16 – 18,56 ng/mL
Posmenopáusica	<0,08 – 0,78 ng/mL

Fuente: *Williamson, M. & Snyder, L. 2012.*

Anexo 19. Inserto de la Hormona Progesterona

Monobind Inc.
Lake Forest, CA 92630, USA

AccuBind
ELISA Microwells

Progesterone Test System
Product Code: 4825-300

1.0 INTRODUCTION

Intended Use: The Quantitative Determination of Progesterone Concentration in Human Serum or Plasma by a Microplate Enzyme Immunoassay, Colorimetric

2.0 SUMMARY AND EXPLANATION OF THE TEST

Measurement of progesterone in serum or plasma is considered to be the most reliable way to assess its rate of production.

Progesterone is a steroid hormone, which plays an important role in the preparation for and maintenance of pregnancy. It is synthesized from cholesterol via pregnenolone — then rapidly metabolized to pregnanediol primarily in the liver.[2, 9,13] The ovary and placenta are the major production sites, but a small amount is also produced by the adrenal cortex in both men and women. Circulating progesterone levels, which are characteristically low during the follicular phase, increase sharply during the luteal phase of menstrual cycles, reaching a maximum approximately 5 to 10 days after the midcycle LH peak.[12] Unless pregnancy occurs, a steep decline to follicular levels sets in about 4 days before the next menstrual period. This pattern constitutes the rationale behind the well established use of serum progesterone measurements as a simple and reliable method for ovulation detection.[3,4,16]

For routine measurements, immunoassays using steroid specific antibodies are preferred. Initial immunoassays for serum progesterone used organic solvents to remove the steroid from endogenous binding proteins such as corticosteroid binding globulin (CBG) and albumin. Direct measurement of progesterone in serum or plasma is considered to be the method of choice for routine applications. Both RIA and EIA (and some FIA) are available in the market. Since RIA involves handling radioactivity and causes radioactive waste disposal issues, various non-isotopic methods have replaced the RIA. These methods use very specific antibodies to determine levels of progesterone in circulation.

The Monobind Progesterone ELISA kit uses a specific anti-progesterone antibody, and does not require sample extraction of serum or plasma. Cross-reactivity to other naturally occurring and structurally related steroids is low.

The employment of several serum references of known progesterone concentration permits construction of a graph of activity and concentration. From comparison to the dose response curve, an unknown specimen's activity can be correlated with progesterone concentration.

3.0 PRINCIPLE

Competitive Enzyme Immunoassay (TYPE 7):
The essential reagents required for an enzyme immunoassay include antibody, enzyme-antigen conjugate and native antigen. Upon mixing biotinylated antibody, enzyme-antigen conjugate and a serum containing the native antigen, a competition reaction results between the native antigen and the enzyme-antigen conjugate for a limited number of antibody binding sites. The interaction is illustrated by the followed equation:

$$^{Enz}Ag + Ag + Ab_{Btn} \underset{k_{-a}}{\overset{k_a}{\rightleftharpoons}} AgAb_{Btn} + {}^{Enz}AgAb_{Btn}$$

Ab_{Btn} = Biotinylated Antibody (Constant Quantity)
Ag = Native Antigen (Variable Quantity)
^{Enz}Ag = Enzyme-antigen Conjugate (Constant Quantity)
$AgAb_{Btn}$ = Antigen-Antibody Complex
$^{Enz}AgAb_{Btn}$ = Enzyme-antigen Conjugate -Antibody Complex
k_a = Rate Constant of Association
k_{-a} = Rate Constant of Disassociation
$K = k_a / k_{-a}$ = Equilibrium Constant

A simultaneous reaction between the biotin attached to the antibody and the streptavidin immobilized on the microwell occurs. This effects the separation of the antibody bound fraction after decantation or aspiration.
$AgAb_{Btn} + {}^{Enz}AgAb_{Btn} + Streptavidin_{cw} \Rightarrow$ immobilized complex
$Streptavidin_{cw}$ = Streptavidin immobilized on well
Immobilized complex = sandwich complex bound to the solid surface

The enzyme activity in the antibody bound fraction is inversely proportional to the native antigen concentration. By utilizing several different serum references of known antigen con-centration, a dose response curve can be generated from which the antigen concentration of an unknown can be ascertained.

4.0 REAGENTS

Materials Provided:
A. Progesterone Calibrators – 1ml/vial - Icons A-G
Seven (7) vials of serum reference for progesterone at concentrations of 0 (**A**), 0.3 (**B**), 2.0 (**C**), 5.0 (**D**), 15 (**E**), 30 (**F**) and 60.0 (**G**) ng/ml. Store at 2-8°C. A preservative has been added. The calibrators can be expressed in molar concentrations (nM/L) by multiplying by 3.18.
For example: 1ng/ml x 3.18 = 3.18 nM/L
B. Progesterone Enzyme Reagent – 6ml/vial – Icon
One (1) vial of Progesterone (Analog)-horseradish peroxides (HRP) conjugate in a protein stabilizing matrix with red dye. Store at 2-8°C.
C. Progesterone Biotin Reagent – 6ml/vial - Icon ∇
One (1) vial of reagent contains anti-Progesterone biotinylated purified rabbit IgG conjugate in buffer, yellow dye and preservative. Store at 2-8°C.
D. Streptavidin Coated Plate – 96 wells –Icon ⇓
One 96-well microplate coated with 1.0 µg/ml streptavidin and packaged in an aluminum bag with a drying agent. Store at 2-8°C.
E. Wash Solution Concentrate – 20ml/vial – Icon
One (1) vial contains a surfactant in buffered saline. A preservative has been added. Store at 2-8°C.
F. Substrate Reagent – 12ml/vial - Icon S^N
One (1) vial contains tetramethylbenzidine (TMB) and hydrogen peroxide (H_2O_2) in buffer. Store at 2-8°C.
G. Stop Solution – 8ml/vial - Icon
One (1) vial contains a strong acid (H_2SO_4). Store at 2-8°C.
H. Product Instructions

Note 1: Do not use reagents beyond the kit expiration date.
Note 2: Avoid extended exposure to heat and light. **Opened reagents are stable for sixty (60) days when stored at 2-8°C. Kit and component stability are identified on label.**
Note 3: Above reagents are for a single 96-well Microplate.

4.1 Required But Not Provided:
1. Pipette capable of delivering 0.025 & 0.050ml (25 & 50µl) with a precision of better than 1.5%.
2. Dispenser(s) for repetitive deliveries of 0.100 & 0.350ml (100 & 350µl) volumes with a precision of better than 1.5%.
3. Adjustable volume (200-1000µl) dispenser(s) for conjugate.
4. Microplate washer or a squeeze bottle (optional).
5. Microplate Reader with 450nm and 620nm wavelength absorbance capability.
6. Absorbent Paper for blotting the microplate wells.
7. Plastic wrap or microplate cover for incubation steps.
8. Vacuum aspirator (optional) for wash steps.
9. Timer.
10. Quality control materials.

5.0 PRECAUTIONS

For In Vitro Diagnostic Use
Not for Internal or External Use in Humans or Animals

All products that contain human serum have been found to be non-reactive for Hepatitis B Surface Antigen, HIV 1&2 and HCV Antibodies by FDA required tests. Since no known test can offer complete assurance that infectious agents are absent, all human serum products should be handled as potentially hazardous and capable of transmitting disease. Good laboratory procedures for handling blood products can be found in the Center for Disease Control / National Institute of Health, "Biosafety in Microbiological and Biomedical Laboratories," 2nd Edition, 1988, HHS Publication No. (CDC) 88-8395.

Safe Disposal of kit components must be according to local regulatory and statutory requirement.

6.0 SPECIMEN COLLECTION AND PREPARATION

The specimens shall be blood, serum or heparanised plasma in type, and taken with the usual precautions in the collection of venipuncture samples. For accurate comparison to establish normal values, a fasting morning serum sample should be obtained. The blood should be collected in a redtop (with or without gel additives) venipuncture tube or for plasma use evacuated tube(s) containing heparin. Allow the blood to clot for serum samples. Centrifuge the specimen to separate the serum or plasma from the cells.

In patients receiving therapy with high biotin doses (i.e. >5mg/day), no sample should be taken until at least 8 hours after the last biotin administration, preferably overnight to ensure fasting sample.

Samples may be refrigerated at 2-8oC for a maximum period of five (5) days. If the specimen(s) cannot be assayed within this time, the sample(s) may be stored at temperatures of -20oC for up to 30 days. Avoid use of contaminated devices. Avoid repetitive freezing and thawing. When assayed in duplicate, 0.050ml (50µl) of the specimen is required.

7.0 QUALITY CONTROL

Each laboratory should assay controls at levels in the low, normal and high range for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control charts should be maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends. The individual laboratory should set acceptable assay performance limits. In addition, maximum absorbance should be consistent with past experience. Significant deviation from established performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.

8.0 REAGENT PREPARATION

1. **Wash Buffer**
Dilute contents of wash solution to 1000ml with distilled or deionized water in a suitable storage container. Diluted buffer can be stored at 2-30°C for up to 60 days.

Note: Do not use reagents that are contaminated or have bacteria growth.

9.0 TEST PROCEDURE

Before proceeding with the assay, bring all reagents, serum reference calibrators and controls to room temperature (20 -27°C).
Test Procedure should be performed by a skilled individual or trained professional*

1. Format the microplates' wells for each serum reference calibrator, control and patient specimen to be assayed in duplicate. **Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8°C.**
2. Pipette 0.025ml (25µl) of the appropriate serum reference calibrator, control or specimen into the assigned well.
3. Add 0.050ml (50µl) of Progesterone Enzyme Reagent to all wells.
4. Swirl the microplate gently for 10-20 seconds to mix.
5. Add 0.050ml (50µl) Progesterone Biotin Reagent to all wells.
6. Swirl the microplate gently for 10-20 seconds to mix.
7. Cover and incubate for 60 minutes at room temperature.
8. Discard the contents of the microplate by decantation or aspiration. If decanting, blot the plate dry with absorbent paper.
9. Add 0.350ml (350µl) of wash buffer (see Reagent Preparation Section), decant (tap and blot) or aspirate. Repeat two (2) additional times for a total of three (3) washes. **An automatic or manual plate washer can be used. Follow the manufacturer's instruction for proper usage. If a squeeze bottle is employed, fill each well by depressing the container (avoiding air bubbles) to dispense the wash. Decant the wash and repeat two (2) additional times.**
10. Add 0.100ml (100µl) of Substrate reagent to all wells. **Always add reagents in the same order to minimize reaction time differences between wells.**

DO NOT SHAKE THE PLATE AFTER SUBSTRATE ADDITION

11. Incubate at room temperature for twenty (20) minutes.
12. Add 0.050ml (50µl) of stop solution to each well and gently mix for 15-20 seconds. **Always add reagents in the same order to minimize reaction time differences between wells.**
13. Read the absorbance in each well at 450nm (using a reference wavelength of 620-630nm. **The results should be read within fifteen (15) minutes of adding the stop solution.**

Note: Dilute the samples suspected of concentrations higher than 60ng/ml 1:5 and 1:10 with progesterone '0' ng/ml calibrator or male patient serum pools with a known low value for progesterone.

10.0 CALCULATION OF RESULTS

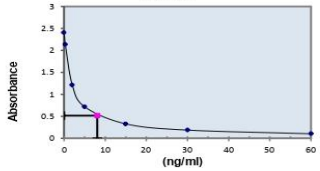
A dose response curve is used to ascertain the concentration of progesterone in unknown specimens.
1. Record the absorbance obtained from the printout of the microplate reader as outlined in Example 1.
2. Plot the absorbance for each duplicate serum reference versus the corresponding progesterone concentration in ng/ml on linear graph paper.
3. Connect the points with a best-fit curve.
4. To determine the concentration of progesterone for an unknown, locate the average absorbance of the duplicates for each unknown on the vertical axis of the graph, find the intersecting point on the curve, and read the concentration (in ng/ml) from the horizontal axis of the graph (the duplicates of the unknown may be averaged as indicated). In the following example, the average absorbance (0.517) intersects the dose response curve at 8.1ng/ml progesterone concentration.

Note: Computer data reduction software designed for ELISA assays may also be used for the data reduction. If such software is utilized, the validation of the software should be ascertained.

EXAMPLE 1

Sample I.D.	Well Number	Abs (A)	Mean Abs (B)	Value (ng/ml)
Cal A	A1	2.420	2.406	0
	B1	2.391		
Cal B	C1	2.155	2.137	0.3
	D1	2.119		
Cal C	E1	1.248	1.215	2.0
	F1	1.183		
Cal D	G1	0.721	0.719	5.0
	H1	0.717		
Cal E	A2	0.338	0.330	15.0
	B2	0.322		
Cal F	C2	0.187	0.188	30.0
	D2	0.190		
Cal G	G2	0.107	0.105	60.0
	H2	0.104		
Pat# 1	A3	0.525	0.517	8.1
	B3	0.510		

Figure 1



*The data presented in Example 1 and Figure 1 are for illustration only and **should not** be used in lieu of a dose response curve prepared with each assay.

11.0 Q.C. PARAMETERS

In order for the assay results to be considered valid the following criteria should be met:

1. The absorbance (OD) of calibrator 0 ng/ml should be ≥ 1.3
2. Four out of six quality control pools should be within the established ranges.

12.0 RISK ANALYSIS

The MSDS and Risk Analysis Form for this product are available upon request from Monobind, Inc.

12.1 Assay Performance

1. It is important that the time of reaction in each well is held constant to achieve reproducible results.
2. Pipetting of samples should not extend beyond ten (10) minutes to avoid assay drift.
3. Highly lipemic, hemolyzed or grossly contaminated specimen(s) should not be used.
4. If more than one (1) plate is used, it is recommended to repeat the dose response curve.
5. The addition of substrate solution initiates a kinetic reaction, which is terminated by the addition of the stop solution. Therefore, the substrate and stop solution should be added in the same sequence to eliminate any time-deviation during reaction.
6. Plate readers measure vertically. Do not touch the bottom of the wells.
7. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
8. Use components from the same lot. No intermixing of reagents from different batches.
9. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from Monobind's IFU may yield inaccurate results.
10. Patient specimens with Progesterone levels higher than 60ng/ml may be diluted (1:5 or 1:10) with progesterone '0 ng/ml' calibrator or male patient serum pools with a known low value for progesterone.
11. All applicable national standards, regulations and laws, including, but not limited to, good laboratory procedures, must be strictly followed to ensure compliance and proper device usage.
12. It is important to calibrate all the equipment e.g. Pipettes, Readers, Washers and/or the automated instruments used with this device, and to perform routine preventative maintenance.
13. Risk Analysis- as required by CE Mark IVD Directive 98/79/EC - for this and other devices, made by Monobind, can be requested via email from Monobind@monobind.com.

12.2 Interpretation

1. ***Measurements and interpretation of results must be performed by a skilled individual or trained professional.***
2. Laboratory results alone are only one aspect for determining patient care and should not be the sole basis for therapy, particularly if the results conflict with other determinants.
3. The reagents for the test system procedures have been formulated to eliminate maximal interference; however, potential interaction between rare serum specimens and test reagents can cause erroneous results. Heterophilic antibodies often cause these interactions and have been known to be problems for all kinds of immunoassays (Boscato LM, Stuart MC. "Heterophilic antibodies: a problem for all immunoassays" *Clin.Chem.* 1988:3427-33). For diagnostic purposes, results from this assay should be used in combination with clinical examination, patient history and all other clinical findings.
4. For valid test results, adequate controls and other parameters must be within the listed ranges and assay requirements.
5. If test kits are altered, such as by mixing parts of different kits, which could produce false test results, or if results are incorrectly interpreted, Monobind shall have no liability.
6. If computer controlled data reduction is used to interpret the results of the test, it is imperative that the predicted values for the calibrators fall within 10% of the assigned concentrations.

13.0 EXPECTED RANGES OF VALUES

In agreement with established reference intervals for a "normal" adult population and females during gestation the expected ranges for the Progesterone AccuBind® ELISA Test System are detailed in Table 1. During pregnancy the progesterone serum levels rise rapidly till the end of third trimester.[17]

TABLE I
Expected Values for the Progesterone Test System

	(ng/ml)	(nmol/L)
Prepubertal Child (1-10 yr)	0.07 – 0.52	0.2-1.7
Adult man	0.13 – 1.22	0.4 – 3.88
Adult woman		
Follicular phase	0.15 – 1.40	0.5 – 4.4
Luteal phase	2.0 – 25.0	6.4 – 79.5
Pregnant woman		
First trimester	7.25 – 90.0	23 – 286
Second trimester	19.5 – 91.0	62 – 289
Third trimester	49.0 – 422.0	153 – 1342
Postmenopausal woman	0.0 – 0.80	0.0 – 2.55

It is important to keep in mind that establishment of a range of values, which can be expected to be found by a given method for a population of "normal" persons, is dependent upon a multiplicity of factors: the specificity of the method, the population tested and the precision of the method in the hands of the analyst. For these reasons, each laboratory should depend upon the range of expected values established by the manufacturer until an in-house range can be determined by analysts using the method with a population indigenous to the area in which the laboratory is located.

14.0 PERFORMANCE CHARACTERISTICS

14.1 Precision

The within and between assay precision of the Progesterone AccuBind® ELISA Test System were determined by analyses on three different levels of pool control sera. The number, mean values, standard deviation and coefficient of variation for each of these control sera are presented in Table 2 and Table 3.

TABLE 2
Within Assay Precision (Values in ng/ml)

Sample	N	X	σ	C.V.%
Low	20	0.65	0.100	15.3
Normal	20	10.77	0.405	3.8
High	20	24.94	1.528	6.1

TABLE 3
Between Assay Precision (Values in ng/ml)

Sample	N	X	σ	C.V.%
Low	20	0.72	0.065	8.9
Normal	20	10.88	0.846	7.5
High	20	24.05	1.534	6.4

*As measured in ten experiments in duplicate over a ten day period.

14.2 Sensitivity

The Progesterone AccuBind® ELISA Test System has a sensitivity of 0.105 ng/ml. The sensitivity was ascertained by determining the variability of the 0 ng/ml serum calibrator and using the 2σ (95% certainty) statistic to calculate the minimum dose.

14.3 Accuracy

The Progesterone AccuBind® ELISA Test System was compared with a chemiluminescence immunoassay method. Biological specimens from low, normal and high progesterone level populations were used (values ranged from < 0.15 ng/ml – 128 ng/ml). The total number of such specimens was 60. The least square regression equation and the correlation coefficient were computed for this method in comparison with the reference method. The data obtained is displayed in Table 4.

TABLE 4

Method	Mean (x)	Least Square Regression Analysis	Correlation Coefficient
This Method (y)	14.59	y= -1.223+1.018(x)	0.989
Reference (X)	15.53		

Only slight amounts of bias between this method and the reference method are indicated by the closeness of the mean values. The least square regression equation and correlation coefficient indicates excellent method agreement.

14.4 Specificity

The % cross reactivity of the progesterone antibody to selected substances was evaluated by adding the interfering substance to a serum matrix at various concentrations. The cross-reactivity was calculated by deriving a ratio between dose of interfering substance to dose of progesterone needed to displace the same amount of labeled analog.

Substance	Cross Reactivity
Progesterone	100.000
17OH-Progesterone	0.375
Androstenedione	0.158
Cortisone	0.014
Corticosterone	0.347
Cortisol	0.005
Danazol	0.003
Dihydotestosterone	0.006
DHEA sulfate	0.002
Estradiol	0.004
Estrone	0.003
Estriol	0.002
Prednisone	0.023
Testosterone	0.015

15.0 REFERENCES

1. Abraham GE. The application of natural steroid radioimmunoassay to gynecologic endocrinology. In: Abraham GE, editor. *Radioassay Systems in Clinical Endocrinology*, Basel: Marcel Dekker,: 475-529 (1981).
2. Aufrere MB, Benson H. Progesterone: an overview and recent advances. , 65:783-800 (1976).
3. Bauman J, "Basal body temperature: unreliable method of ovulation detection", *Fertility Sterility*, 36:729-33, (1981).
4. Brown JB," Timing of ovulation", *Med J Austral*, 2:780-3 (1977).
5. Gautray JP, et al, "Clinical investigation of the menstrual cycle: clinical, endometrial and endocrine aspects of luteal defects",. *Fertility Sterility*, 35:296-303 (1981).
6. Hensleigh PA, Fainstat T, "Corpus luteum dysfunction: serum progesterone levels in diagnosis and assessment of therapy for recurrent and threatened abortion", *Fertility Sterility*, 32:396-9. (1979).
7. Hernandez JL, et al," Direct evidence of luteal insufficiency in women with habitual abortion", *Obstetric Gynecology*, 49:705-8 (1977).
8. Jones G. Luteal phase defects. In: Behrman SJ, Kistner RW, editors. Progress in Infertility. Boston: Little, Brown and Company, 2nd ed., 1975: 299-324.
9. Klopper A, Fuchs F. Progestagens. In: Fuchs F, Klopper A, editors. *Endocrinology of Pregnancy*. Hagerstown: Harper & Row,: 99-122 (1977).
10. Lehmann F, Bettendorf G," The endocrine shift from a normal cycle to anovulation",: Insler V, Bettendorf G, editors. Advances in Diagnosis and Treatment of Infertility. Amsterdam: Elseveir/North Holland, 105 -13 (1981).
11. March CM. Luteal phase defects. In: Mishell DR, Davajan V, editors. *Reproductive Endocrinology*, Infertility and Contraception. Philadelphia: F. A. Davis Company, 469-76, (1979).
12. March CM, Goebelsmann U, Nakamura RM, Mishell Dr. Roles of estradiol and progesterone in eliciting the midcycle luteinizing hormone and follicle stimulating hormone surges. *J Clin Endocrinol Metab*, 49:507-13 (1979).
13. BIO-ED slide/seminar educational program, Rochester: Bioeducational Publications (1981).
14. Radwanska E, et al, "Plasma progesterone and estradiol estimations in the diagnosis and treatment of luteal insufficiency in menstruating infertile women", *Acta Eur Fertility*, 7:39-47(1976).
15. Radwanska E, et al,"Plasma progesterone levels in normal and abnormal early human pregnancy", *Fertility Sterility* 30:398-402 (1978).
16. Radwanska E, et al, "Single midluteal progesterone assay in the management of ovulatory infertility". *J Reprod Med*, 26:85-9 (1981).
17. Tietz, Reference Information for the Clinical Laboratory. In *Textbook of Clinical Chemistry*, 3rd Ed Burtis, C.A., Ashwood, R.A. W.B. Saunders: Philadelphia, 1999; 1831.

Revision: 7 Date: 2019-Jul-16 DCO: 1353
MP4825 Product Code: 4825-300

Size		96(A)	192(B)
Reagent (fill)	A)	1ml set	1ml set
	B)	1 (6ml)	2 (6ml)
	C)	1 (6ml)	2 (6ml)
	D)	1 plate	2 plates
	E)	1 (20ml)	1 (20ml)
	F)	1 (12ml)	2 (12ml)
	G)	1 (8ml)	2 (8ml)

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IVD 2°-8° CE
EC REP **CEpartner4U,** Esdoornlaan 13 3951 DBMaarn, The Neatherlands **www.cepartner4u.eu**

Please visit our website to learn more about our products and services.

Glossary of Symbols
(EN 980/ISO 15223)

IVD In Vitro - Diagnostic Medical Device	2°-8° Temperature Limitation Storage Condition (2-8° C)	Consult Instructions for Use
REF Catalogue Number	Σ Contains Sufficient Test for Σ	LOT Batch Code
Used By (Expiration Day)	Date of Manufacturer	Manufacturer
EC REP Authorized Rep in European Country	CE European Conformity	

Fuente: *Monobind Inc.*

Anexo 20. *Valores normales de la Hormona Prolactina*

PROLACTINA VALORES DE REFERENCIA

Edad	Valor
< 50 años	3,34 – 26,72 µg/L
> 50 años	2,74 – 19,64 µg/L

Fuente: *Williamson, M. & Snyder, L. 2012.*

Anexo 21. Inserto de la Hormona Prolactina

Monobind Inc.
Lake Forest, CA 92630, USA

AccuBind
ELISA Microwells

Prolactin Hormone (PRL) Test System
Product Code: 725-300

1.0 INTRODUCTION

Intended Use: The Quantitative Determination of Prolactin Hormone Concentration in Human Serum by a Microplate Enzyme Immunoassay, Colorimetric

2.0 SUMMARY AND EXPLANATION OF THE TEST

Prolactin hormone (PRL), secreted from the lactotrophs of the anterior pituitary, is a protein consisting of a single polypeptide chain containing approximately 200 amino acids. The primary biological action of the hormone is on the mammary gland where it is involved in the growth of the gland and in the induction and maintenance of milk production. There is evidence to suggest that prolactin may be involved in steroidogenesis in the gonad, acting synergistically with luteinizing hormone (LH). High levels of prolactin appear to inhibit steroidogenesis as well as inhibiting LH and follicle stimulating hormone (FSH) synthesis at the pituitary gland.[1,2]

The clinical usefulness of the measurement of prolactin hormone (PRL) in ascertaining the diagnosis of hyperprolactinemia and for the subsequent monitoring the effectiveness of the treatment has been well established.[3,4]

In this method, PRL calibrator, patient specimen or control is first added to a streptavidin coated well. Biotinylated monoclonal and enzyme labeled antibodies (directed against distinct and different epitopes of PRL) are added and the reactants mixed. Reaction between the various PRL antibodies and native PRL forms a sandwich complex that binds with the streptavidin coated to the well.

After the completion of the required incubation period, the enzyme-prolactin hormone antibody bound conjugate is separated from the unbound enzyme-prolactin hormone conjugate by aspiration or decantation. The activity of the enzyme present on the surface of the well is quantitated by reaction with a suitable substrate to produce color.

The employment of several serum references of known prolactin hormone levels permits the construction of a dose response curve of activity and concentration. From comparison to the dose response curve, an unknown specimen's activity can be correlated with prolactin hormone concentration.

3.0 PRINCIPLE

Immunoenzymometric assay (TYPE 3):
The essential reagents required for an immunoenzymometric assay include high affinity and specificity antibodies (enzyme labeled and immobilized), with different and distinct epitope recognition, **in excess,** and native antigen. In this procedure, the immobilization takes place during the assay at the surface of a microplate well through the interaction of streptavidin coated on the well and exogenously added biotinylated monoclonal anti-PRL antibody.

Upon mixing monoclonal biotinylated antibody, the enzyme-labeled antibody and a serum containing the native antigen, reaction results between the native antigen and the antibodies, without competition or steric hindrance, to form a soluble sandwich complex. The interaction is illustrated by the following equation:

$$^{Enz}Ab_{(f)} + Ag_{PRL} + {}^{Btn}Ab_{(m)} \underset{k_{-a}}{\overset{k_a}{\rightleftharpoons}} {}^{Enz}Ab_{(f)} - Ag_{PRL} - {}^{Btn}Ab_{(m)}$$

$^{Btn}Ab_{(m)}$ = Biotinylated Monoclonal Antibody (Excess Quantity)
Ag_{PRL} = Native Antigen (Variable Quantity)
$^{Enz}Ab_{(f)}$ = Enzyme labeled Antibody (Excess Quantity)
$^{Enz}Ab_{(f)} - Ag_{PRL} - {}^{Btn}Ab_{(m)}$ = Antigen-Antibodies Sandwich Complex
k_a = Rate Constant of Association
k_{-a} = Rate Constant of Dissociation

Simultaneously, the complex is deposited to the well through the high affinity reaction of streptavidin and biotinylated antibody. This interaction is illustrated below:

$^{Enz}Ab_{(m)} - Ag_{PRL} - {}^{Btn}Ab_{(m)} + Streptavidin_{C.W.} \Rightarrow$ immobilized complex

$Streptavidin_{C.W.}$ = Streptavidin immobilized on well
Immobilized complex = sandwich complex bound to the well

After equilibrium is attained, the antibody-bound fraction is separated from unbound antigen by decantation or aspiration. The enzyme activity in the antibody-bound fraction is directly proportional to the native antigen concentration. By utilizing several different serum references of known antigen values, a dose response curve can be generated from which the antigen concentration of an unknown can be ascertained.

4.0 REAGENTS

Materials Provided:

A. PRL Calibrators – 1 ml/vial - Icons A-F
Six (6) vials of references for PRL antigen in human serum at levels of 0(**A**), 5(**B**), 10(C), 25(**D**), 50(E) and 100(**F**) ng/ml. Store at 2-8°C. A preservative has been added.
Note: The calibrators, human serum based, were calibrated using a reference preparation, which was assayed against the WHO 3rd IS (84/500).

B. PRL Enzyme Reagent – 13ml/vial - Icon Ⓔ
One (1) vial containing enzyme labeled antibody, biotinylated monoclonal mouse IgG in buffer, dye, and preservative. Store at 2-8°C.

C. Streptavidin Coated Plate – 96 wells – Icon ⇓
One 96-well microplate coated with streptavidin and packaged in an aluminum bag with a drying agent. Store at 2-8°C.

D. Wash Solution Concentrate – 20 ml - Icon ♦
One (1) vial containing a surfactant in buffered saline. A preservative has been added. Store at 2-8°C.

E. Substrate A – 7ml/vial - Icon S^A
One (1) vial containing tetramethylbenzidine (TMB) in buffer. Store at 2-8°C.

F. Substrate B – 7ml/vial - Icon S^B
One (1) vial containing hydrogen peroxide (H_2O_2) in buffer. Store at 2-8°C.

G. Stop Solution – 8ml/vial – Icon STOP
One (1) vial containing a strong acid (1N HCl). Store at 2-8°C.

H. Product Instructions

Note 1: Do not use reagents beyone the kit expiration date.
Note 2: Avoid extended exposure to heat and light. **Opened reagents are stable for sixty (60) days when stored at 2-8°C. Kit and component stability are identified on the label.**
Note 3: Above reagents are for a single 96-well microplate.

4.1 Required But Not Provided:
1. Pipette capable of delivering 0.025 and 0.050ml (25 and 50µl) volumes with a precision of better than 1.5%.
2. Dispenser(s) for repetitive deliveries of 0.100 and 0.350ml (100 and 350µl) volumes with a precision of better than 1.5%.
3. Microplate washers or a squeeze bottle (optional).
4. Microplate reader with 450 & 620nm filters.
5. Absorbent Paper for blotting the microplate wells.
6. Plastic wrap or microplate cover for incubation steps.
7. Vacuum aspirator (optional) for wash steps.
8. Timer.
9. Quality control materials

5.0 PRECAUTIONS

For In Vitro Diagnostic Use
Not for Internal or External Use in Humans or Animals

All products that contain human serum have been found to be non-reactive for Hepatitis B Surface Antigen, HIV 1&2 and HCV Antibodies by FDA licensed reagents. Since no known test can offer complete assurance that infectious agents are absent, all human serum products should be handled as potentially hazardous and capable of transmitting disease. Good laboratory procedures for handling blood products can be found in the Center for Disease Control / National Institute of Health, "Biosafety in Microbiological and Biomedical Laboratories," 2nd Edition, 1988, HHS Publication No. (CDC) 88-8395.

Safe Disposal of kit components must be according to local regulatory and statutory requirement.

6.0 SPECIMEN COLLECTION AND PREPARATION

The specimens shall be blood, serum in type and the usual precautions in the collection of venipuncture samples should be observed. For accurate comparison to established normal values, a fasting morning serum sample should be obtained. The blood should be collected in a plain redtop venipuncture tube without additives or anti-coagulants. Allow the blood to clot. Centrifuge the specimen to separate the serum from the cells.

In patients receiving therapy with high biotin doses (i.e. >5mg/day), no sample should be taken until at least 8 hours after the last biotin administration, preferably overnight to ensure fasting sample.

Samples may be refrigerated at 2-8°C for a maximum period of five (5) days. If the specimen(s) cannot be assayed within this time, the sample(s) may be stored at temperatures of -20°C for up to 30 days. Avoid use of contaminated devices. Avoid repetitive freezing and thawing. When assayed in duplicate, 0.050ml (50µl) of the specimen is required.

7.0 QUALITY CONTROL

Each laboratory should assay controls at levels in the low, normal and elevated range for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control charts should be maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends. Significant deviation from established performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.

8.0 REAGENT PREPARATION

1. **Wash Buffer**
Dilute contents of wash concentrate to 1000ml with distilled or deionized water in a suitable storage container. Store at room temperature 20-27°C for up to 60 days.
2. **Working Substrate Solution** – Stable for one year
Pour the contents of the amber vial labeled Solution 'A' into the clear vial labeled Solution 'B'. Place the yellow cap on the clear vial for easy identification. Mix and label accordingly. Store at 2 - 8°C.

Note1: Do not use the working substrate if it looks blue.
Note 2: Do not use reagents that are contaminated or have bacteria growth.

9.0 TEST PROCEDURE

Before proceeding with the assay, bring all reagents, serum reference calibrators and controls to room temperature (20-27°C).
*****Test Procedure should be performed by a skilled individual or trained professional*****

1. Format the microplate wells for each serum reference calibrator, control and patient specimen to be assayed in duplicate. **Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8°C**
2. Pipette 0.025 ml (25µl) of the appropriate serum reference calibrator, control or specimen into the assigned well.
3. Add 0.100 ml (100µl) of PRL Enzyme Reagent solution to all wells.
4. Swirl the microplate gently for 20-30 seconds to mix and cover.
5. Incubate 60 minutes at room temperature.
6. Discard the contents of the microplate by decantation or aspiration. If decanting, blot the plate dry with absorbent paper.
2. Add 0.350ml (350µl) of wash buffer (see Reagent Preparation Section), decant (tap and blot) or aspirate. Repeat two (2) additional times for a total of three (3) washes. **An automatic or manual plate washer can be used. Follow the manufacturer's instruction for proper usage. If a squeeze bottle is employed, fill each well by depressing the container (avoiding air bubbles) to dispense the wash. Decant the wash and repeat two (2) additional times.**
7. Add 0.100 ml (100µl) of working substrate solution to all wells (see Reagent Preparation Section). **Always add reagents in the same order to minimize reaction time differences between wells**
DO NOT SHAKE PLATE AFTER SUBSTRATE ADDITION
8. Incubate at room temperature for fifteen (15) minutes.
9. Add 0.050ml (50µl) of stop solution to each well and gently mix for 15-20 seconds). **Always add reagents in the same order to minimize reaction time differences between wells**
10. Read the absorbance in each well at 450nm (using a reference wavelength of 620-630nm to minimize well imperfections) in a microplate reader. **The results should be read within thirty (30) minutes of adding the stop solution.**

10.0 CALCULATION OF RESULTS

A dose response curve is used to ascertain the concentration of prolactin hormone (PRL) in unknown specimens.
1. Record the absorbance obtained from the printout of the microplate reader as outlined in Example 1.
2. Plot the absorbance for each duplicate serum reference versus the corresponding PRL concentration in ng/ml on linear graph paper (do not average the duplicates of the serum references before plotting).
3. Draw the best-fit curve through the plotted points.
4. To determine the concentration of PRL for an unknown, locate the average absorbance of the duplicates for each unknown on the vertical axis of the graph, find the intersecting point on the curve, and read the concentration (in ng/ml) from the horizontal axis of the graph (the duplicates of the unknown may be averaged as indicated). In the following example, the average absorbance (1.374) intersects the dose response curve at (36.1 ng/ml) PRL concentration (See Figure 1).

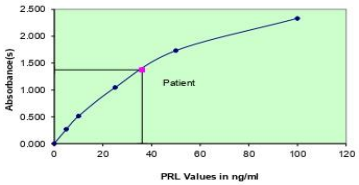
Note: Computer data reduction software designed for ELISA assays may also be used for the data reduction. If such software is utilized, the validation of the software should be ascertained.

EXAMPLE 1

Sample I.D.	Well Number	Abs (A)	Mean Abs (B)	Value (ng/ml)
Cal A	A1	0.001	0.003	0
	B1	0.005		
Cal B	C1	0.278	0.269	5
	D1	0.260		
Cal C	E1	0.502	0.513	10
	F1	0.524		
Cal D	G1	1.065	1.045	25
	H1	1.024		
Cal E	A2	1.730	1.732	50
	B2	1.733		
Cal F	C2	2.359	2.333	100
	D2	2.307		
Ctrl 1	E2	0.292	0.311	**5.8**
	F2	0.330		
Ctrl 2	G2	0.715	0.714	**14.9**
	H2	0.713		
Patient	A3	1.407	1.374	**36.1**
	B3	1.341		

*The data presented in Example 1 and Figure 1 are for illustration only and **should not** be used in lieu of a dose response curve prepared with each assay.

Figure 1



11.0 Q.C. PARAMETERS

In order for the assay results to be considered valid the following criteria should be met:

1. The absorbance (OD) of calibrator 100 ng/ml should be ≥ 1.3.
2. Four out of six quality control pools should be within the established ranges.

12.0 RISK ANALYSIS

The MSDS and Risk Analysis Form for this product are available on request from Monobind Inc.

12.1 Assay Performance

1. It is important that the time of reaction in each well is held constant to achieve reproducible results.
2. Pipetting of samples should not extend beyond ten (10) minutes to avoid assay drift.
3. Highly lipemic, hemolyzed or grossly contaminated specimen(s) should not be used.
4. If more than one (1) plate is used, it is recommended to repeat the dose response curve.
5. The addition of substrate solution initiates a kinetic reaction, which is terminated by the addition of the stop solution. Therefore, the substrate and stop solution should be added in the same sequence to eliminate any time-deviation during reaction.
6. Plate readers measure vertically. Do not touch the bottom of the wells.
7. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
8. Use components from the same lot. No intermixing of reagents from different batches.
9. Patient specimens with abnormally high prolactin levels can cause a hook effect, that is, paradoxical low absorbance results. If this is suspected, dilute the specimen 1/100 with '0' calibrator; reassay (multiply the result by 100). However, values as high as 3000ng/ml have been found to absorb greater than the absorbance of the highest calibrator
10. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from Monobind IFU may yield inaccurate results.
11. All applicable national standards, regulations and laws, including, but not limited to, good laboratory procedures, must be strictly followed to ensure compliance and proper device usage.
12. It is important to calibrate all the equipment e.g. Pipettes, Readers, Washers and/or the automated instruments used with this device, and to perform routine preventative maintenance.
13. Risk Analysis- as required by CE Mark IVD Directive 98/79/EC - for this and other devices, made by Monobind, can be requested via email from Monobind@monobind.com.

12.2 Interpretation

1. ***Measurements and interpretation of results must be performed by a skilled individual or trained professional.***
2. Laboratory results alone are only one aspect for determining patient care and should not be the sole basis for therapy, particularly if the results conflict with other determinants.
3. The reagents for the test system have been formulated to eliminate maximal interference; however, potential interaction between rare serum specimens and test reagents can cause erroneous results. Heterophilic antibodies often cause these interactions and have been known to be problems for all kinds of immunoassays (Boscato LM, Stuart MC. 'Heterophilic antibodies: a problem for all immunoassays' Clin. Chem. 1988:3427-33). For diagnostic purposes, the results from this assay should be in combination with clinical examination, patient history and all other clinical findings.For valid test results, adequate controls and other parameters must be within the listed ranges and assay requirements.
4. If test kits are altered, such as by mixing parts of different kits, which could produce false test results, or if results are incorrectly interpreted, Monobind shall have no liability.
5. If computer controlled data reduction is used to interpret the results of the test, it is imperative that the predicted values for the calibrators fall within 10% of the assigned concentrations.
6. Patients receiving preparations of mouse monoclonal antibodies for diagnosis or therapy may contain human anti-mouse antibodies (HAMA) and may show either falsely elevated or depressed values when assayed.
7. Pregnancy, lactation, and the administration of oral contraceptives can cause an increase in the level of Prolactin.
8. Drugs such as morphine, reserpine and the psychotropic drugs increase prolactin secretion.[5,6,7]
9. Since Prolactin hormone concentration is dependent upon diverse factors other than pituitary homeostasis, the determination alone is not sufficient to assess clinical status.

13.0 EXPECTED RANGE OF VALUES

A study of an apparent normal adult population was undertaken to determine expected values for the PRL AccuBind® ELISA test system. The expected values (95% confidence intervals) are presented in Table 1.

TABLE 1
Expected Values for the PRL AccuBind® ELISA (in ng/ml)

Women	
Adult (Number = 70)	1.2 -- 19.5
Postmenopausal (Number = 10)	1.5 -- 18.5
Men	
Adult (Number = 50)	1.8 -- 17.0

It is important to keep in mind that establishment of a range of values which can be expected to be found by a given method for a population of "normal"-persons is dependent upon a multiplicity of factors: the specificity of the method, the population tested and the precision of the method in the hands of the analyst. For these reasons each laboratory should depend upon the range of expected values established by the manufacturer only until an in-house range can be determined by the analysts using the method with a population indigenous to the area in which the laboratory is located.

14.0 PERFORMANCE CHARACTERISTICS

14.1 Precision

The within and between assay precisions of the PRL AccuBind® ELISA test system were determined by analyses on three different levels of control sera. The number (N), mean value (X), standard deviation (σ) and coefficient of variation (C.V.) for each of these control sera are presented in Table 2 and Table 3.

TABLE 2
Within Assay Precision (Values in ng/ml)

Sample	N	X	σ	C.V.
Level 1	20	5.4	0.23	4.3%
Level 2	20	18.4	0.67	3.6%
Level 3	20	40.8	2.78	6.8%

TABLE 3
Between Assay Precision* (Values in ng/ml)

Sample	N	X	σ	C.V.
Level 1	20	5.8	0.57	9.8%
Level 2	20	19.8	1.73	8.8%
Level 3	20	43.8	2.97	6.8%

*As measured in ten experiments in duplicate.

14.2 Sensitivity

The PRL AccuBind® ELISA test system has a sensitivity of 0.004 ng/well. This is equivalent to a sample containing 0.150 ng/ml PRL concentration. The analytical sensitivity (detection limit) was ascertained by determining the variability of the '0 ng/ml' calibrator and using the 2σ (95% certainty) statistic to calculate the minimum dose.

14.3 Accuracy

The Prolactin AccuBind® ELISA test system was compared with a reference chemiluminometric (ICMA) method. Biological specimens from normal and pregnant populations were assayed. The total number of such specimens was 65. The least square regression equation and the correlation coefficient were computed for the PRL AccuBind® ELISA in comparison with the reference method. The data obtained is displayed in Table 4.

TABLE 4

Method	Mean (x)	Least Square Regression Analysis	Correlation Coefficient
Monobind	15.5	y = 0.83 + 0.97(x)	0.956
Reference	14.8		

Only slight amounts of bias between the PRL AccuBind® ELISA method and the reference method are indicated by the closeness of the mean values. The least square regression equation and correlation coefficient indicates excellent method agreement.

14.4 Specificity

The cross-reactivity of the PRL AccuBind® ELISA test system to selected substances was evaluated by adding the interfering substance to a serum matrix at various concentrations. The cross-reactivity was calculated by deriving a ratio between dose of interfering substance to dose of prolactin hormone needed to produce the same absorbance.

Substance	Cross Reactivity	Concentration
Prolactin Hormone (PRL)	1.0000	--
Luteinizing Hormone (LH)	< 0.0001	1000ng/ml
Follitropin (FSH)	< 0.0001	1000ng/ml
Chorionic gonadotropin (CG)	< 0.0001	1000ng/ml
Thyrotropin (TSH)	< 0.0001	1000ng/ml
Growth Hormone (GH)	< 0.0001	1000ng/ml

15.0 REFERENCES

1. Maddox PR, Jones DL, Mansel RE, *Acta Endocrinol,* 125, 621 (1991).
2. Gonzales E.R., *JAMA*, 242, 401 (1979).
3. Tolis G., *Hosp Pract*, 15, 85 (1980).
4. Balagura S, Frantz AG, Houseplan EM, *J Neurosurg,* 51, 42 (1979).
5. Friesen H, Hwang P, *Ann Rev Med,* 24, 251 (1973).
6. Frantz A. G., *N Eng J Med.* 298, 201 (1978).
7. Parkes DN, *J Med,* 301, 873 (1979).
8. Tietz N, *Clinical Guide to Laboratory Tests*, WB Saunders, Philadelphia, London, 2nd Ed. (1992).
9. Jackson RD, Wortsman J, Malarky WB, "Persistence of large molecular weight prolactin secretions during pregnancy in women with macroprolactenemia and its presence in fetal cord blood", *J Clin Endo & Metabol,* 68, 1046-50 (1989).
10. Fraser IS, Lun ZG, Zhou JP, Herrington AC, McCarron G, Caterson I, et al, "Detailed assessment of big prolactin in women with hyperprolactenemia and normal ovary function", *J Clin Endo & Metabol*, 69, 585-592 (1989).
11. Pasini F, Bergamini CM, Malfaccini M, Cocilovo G, Linciano M, Jacobs M, Bagni B, "Multiple molecular forms of prolactin during pregnancy in women", *J Endocrinol* , 106, 81-86 (1985).

Revision: 4 Date: 2019-Jul-16 DCO: 1353
MP725 Product Code: *725-300*

Size		96(A)	192(B)
Reagent (fill)	A)	1ml set	1ml set
	B)	1 (13ml)	2 (13ml)
	C)	1 plate	2 plates
	D)	1 (20ml)	1 (20ml)
	E)	1 (7ml)	2 (7ml)
	F)	1 (7ml)	2 (7ml)
	G)	1 (8ml)	2 (8ml)

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IVD 2°-8° CE
EC REP **CEpartner4U,** Esdoornlaan 13
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www.cepartner4u.eu

Please visit our website to learn more about our products and services.

Glossary of Symbols
(EN 980/ISO 15223)

IVD — In Vitro - Diagnostic Medical Device
2°-8° — Temperature Limitation Storage Condition (2-8° C)
Consult Instructions for Use
REF — Catalogue Number
Contains Sufficient Test for Σ
LOT — Batch Code
Used By (Expiration Day)
Date of Manufacturer
Manufacturer
EC REP — Authorized Rep in European Country
CE — European Conformity

Fuente: *Monobind Inc.*

Anexo 22. *Valores normales de la Hormona Estradiol*

ESTRADIOL VALORES DE REFERENCIA

Fase Ovulatoria	Valor
Fase folicular media	27 – 122 pg/mL
Fase preovulatoria	95 – 433 pg/mL
Fase lútea media	49 – 291 pg/mL
Posmenopáusica	<20 – 40 pg/mL

Fuente: *Williamson, M. & Snyder, L. 2012.*

Anexo 23. Inserto de la Hormona Estradiol

Monobind Inc.
Lake Forest, CA 92630, USA

AccuBind
ELISA Microwells

Estradiol (E2) Test System
Product Code: 4925-300

1.0 INTRODUCTION

Intended Use: The Quantitative Determination of Estradiol Concentration in Human Serum or Plasma by a Microplate Enzyme Immunoassay, Colorimetric

2.0 SUMMARY AND EXPLANATION OF THE TEST

Measurement of estradiol in serum or plasma is considered to be the most reliable way to assess its rate of production.

Estradiol (17β-estradiol) is a steroid hormone (molecular weight of 272.3 daltons), which circulates predominantly protein-bound. In addition to estradiol, other natural steroidal estrogens include estrone, estriol and their metabolites. Natural estrogens are hormones secreted principally by the ovarian follicles and also by the adrenals, corpus luteum, and placenta and, in males, by the testes. Exogenous estrogens (natural or synthetic) elicit, to varying degrees, all the pharmacologic responses usually produced by endogenous estrogens.

Estrogenic hormones are secreted at varying rates during the menstrual cycle throughout the period of ovarian activity. During pregnancy, the placenta becomes the main source of estrogens. At menopause, ovarian secretion of estrogens declines at varying rates. The gonadotropins of the anterior pituitary regulate secretion of the ovarian hormones, estradiol and progesterone; hypothalamic control of pituitary gonadotropin production is in turn regulated by plasma concentrations of the estrogens and progesterone. This complex feedback system results in the cyclic phenomenon of ovulation and menstruation.

Estradiol determinations have proved of value in a variety of contexts, including the investigation of precocious puberty in girls and gynecomastia in men. Its principal uses have been in the differential diagnosis of amenorrhea and in the monitoring of ovulation induction.

This kit uses a specific anti-estradiol antibody, and does not require prior sample extraction of serum or plasma. Cross-reactivity to other naturally occurring and structurally related steroids is low.

The employment of several serum references of known estradiol concentration permits construction of a graph of activity and concentration. From comparison to the dose response curve, an unknown specimen's activity can be correlated with estradiol concentration.

3.0 PRINCIPLE

Delayed Competitive Enzyme Immunoassay (TYPE 9):
The essential reagents required for an enzyme immunoassay include antibody, enzyme-antigen conjugate and native antigen. Upon mixing the biotinylated antibody with a serum containing the antigen, a reaction results between the antigen and the antibody. The interaction is illustrated by the following equation:

$$Ag + Ab_{Btn} \rightleftharpoons AgAb_{Btn}$$

Ab_{Btn} = Biotinylated antibody
Ag = Antigen (Variable Quantity) $AgAb_{Btn}$ = Immune Complex

After a short incubation, the enzyme conjugate is added (This delayed addition permits an increase in sensitivity for low concentration samples). Upon the addition of the enzyme conjugate, competition reaction results between the enzyme analog and the antigen in the sample for a limited number of antibody binging sites (not consumed in the first incubation).

$$^{Enz}Ag + Ag + rAb_{Btn} \underset{k_{-a}}{\overset{k_a}{\rightleftharpoons}} AgAb_{Btn} + {}^{Enz}AgAb_{Btn}$$

^{Enz}Ag = Enzyme-antigen Conjugate (Constant Quantity)
$^{Enz}AgAb_{Btn}$ = Enzyme-antigen Conjugate -Antibody Complex
rAb_{Btn} = Biotinylated antibody not reacted in first incubation
k_a = Rate Constant of Association
k_{-a} = Rate Constant of Disassociation
$K = k_a / k_{-a}$ = Equilibrium Constant

A simultaneous reaction between the biotin attached to the antibody and the streptavidin immobilized on the microwell occurs. This effects the separation of the antibody bound fraction after decantation or aspiration.
$AgAb_{Btn} + {}^{Enz}AgAb_{Btn} + \underline{Streptavidin_{CW}} \Rightarrow \underline{immobilized\ complex}$
$\underline{Streptavidin_{CW}}$ = Streptavidin immobilized on well
Immobilized complex = sandwich complex bound to the solid surface

The enzyme activity in the antibody bound fraction is inversely proportional to the native antigen concentration. By utilizing several different serum references of known antigen concentration, a dose response curve can be generated from which the antigen concentration of an unknown can be ascertained.

4.0 REAGENTS

Materials Provided

A. Estradiol Calibrators – 1ml/vial - Icons A-G
Seven (7) vials of serum reference for estradiol at concentrations of 0 (**A**), 20 (**B**), 100 (**C**), 250 (**D**), 500 (**E**), 1500 (**F**) and 3000 (**G**) in pg/ml. Store at 2-8°C. A preservative has been added. The calibrators can be expressed in molar concentrations (pM/L) by multiplying by 3.67.
For example: 1pg/ml x 3.67= 3.67 pM/L

B. Estradiol Enzyme Reagent – 6.0 ml/vial
One (1) vial of Estradiol (Analog)-horseradish peroxides (HRP) conjugate in a protein-stabilizing matrix red with dye. Store at 2-8°C.

C. Estradiol Biotin Reagent – 6.0 ml - Icon ▽
One (1) bottle of reagent contains anti-estradiol biotinylated purified rabbit IgG conjugate in buffer, green dye and preservative. Store at 2-8°C.

D. Streptavidin Coated Plate – 96 wells –Icon ⇓
One 96-well microplate coated with 1.0 µg/ml streptavidin and packaged in an aluminum bag with a drying agent. Store at 2-8°C.

E. Wash Solution Concentrate – 20ml/vial - Icon
One (1) vial contains a surfactant in buffered saline. A preservative has been added. Store at 2-8°C.

F. Substrate Reagent – 12ml/vial - Icon S^N
One (1) vial contains tetramethylbenzidine (TMB) and hydrogen peroxide (H_2O_2) in buffer. Store at 2-8°C.

G. Stop Solution – 8ml/vial - Icon
One (1) vial contains a strong acid (0.5M H_2SO_4). Store at 2-8°C.

H. Product Instructions

Note 1: Do not use reagents beyond the kit expiration date.
Note 2: Avoid extended exposure to heat and light. **Opened reagents are stable for sixty (60) days when stored at 2-8°C. Kit and component stability are identified on label.**
Note 3: Above reagents are for a single 96-well microplate.

4.1 Required But Not Provided:
1. Pipette capable of delivering 0.025ml (25µl) and 0.050ml (50µl) with a precision of better than 1.5%.
2. Dispenser(s) for repetitive deliveries of 0.100ml (100µl) and 0.350ml (350µl) volumes with a precision of better than 1.5%.
3. Microplate washer or a squeeze bottle (optional).
4. Absorbent Paper for blotting the microplate wells.
5. Microplate Reader with 450nm and 620nm wavelength absorbance capability.
6. Plastic wrap or microplate cover for incubation steps.
7. Vacuum aspirator (optional) for wash steps.
8. Timer.
9. Quality control materials.

5.0 PRECAUTIONS

For In Vitro Diagnostic Use
Not for Internal or External Use in Humans or Animals

All products that contain human serum have been found to be non-reactive for Hepatitis B Surface Antigen, HIV 1&2 and HCV Antibodies by FDA required tests. Since no known test can offer complete assurance that infectious agents are absent, all human serum products should be handled as potentially hazardous and capable of transmitting disease. Good laboratory procedures for handling blood products can be found in the Center for Disease Control / National Institute of Health, "Biosafety in Microbiological and Biomedical Laboratories," 2nd Edition, 1988, HHS Publication No. (CDC) 88-8395.

Safe Disposal of kit components must be according to local regulatory and statutory requirement.

6.0 SPECIMEN COLLECTION AND PREPARATION

The specimens shall be blood, serum or heparanised plasma in type, and taken with the usual precautions in the collection of venipuncture samples. For accurate comparison to establish normal values, a fasting morning serum sample should be obtained. The blood should be collected in a redtop (with or without gel additives) venipuncture tube(s) or for plasma use evacuated tube(s) containing heparin.. Allow the blood to clot for serum samples. Centrifuge the specimen to separate the serum or plasma from the cells.

In patients receiving therapy with high biotin doses (i.e. >5mg/day), no sample should be taken until at least 8 hours after the last biotin administration, preferably overnight to ensure fasting sample.

Samples may be refrigerated at 2-8oC for a maximum period of five (5) days. If the specimen(s) cannot be assayed within this time, the sample(s) may be stored at temperatures of -20oC for up to 30 days. Avoid use of contaminated devices. Avoid repetitive freezing and thawing. When assayed in duplicate, 0.050ml of the specimen is required.

7.0 QUALITY CONTROL

Each laboratory should assay controls at levels in the low, normal and high range for monitoring assay performance. These controls should be treated as unknowns and values determined in every test procedure performed. Quality control charts should be maintained to follow the performance of the supplied reagents. Pertinent statistical methods should be employed to ascertain trends. The individual laboratory should set acceptable assay performance limits. In addition, maximum absorbance should be consistent with past experience. Significant deviation from established performance can indicate unnoticed change in experimental conditions or degradation of kit reagents. Fresh reagents should be used to determine the reason for the variations.

8.0 REAGENT PREPARATION

1. **Wash Buffer**
Dilute contents of wash solution to 1000ml with distilled or deionized water in a suitable storage container. Diluted buffer can be stored at 2-30°C for up to 60 days.

Note: Do not use reagents that are contaminated or have bacteria growth.

9.0 TEST PROCEDURE

Before proceeding with the assay, bring all reagents, serum reference calibrators and controls to room temperature (20-27°C).

*****Test Procedure should be performed by a skilled individual or trained professional*****

1. Format the microplates' wells for each serum reference calibrator, control and patient specimen to be assayed in duplicate. **Replace any unused microwell strips back into the aluminum bag, seal and store at 2-8°C.**
2. Pipette 0.025 ml (25 µL) of the appropriate serum reference calibrator, control or specimen into the assigned well.
3. Add 0.050 ml (50µl) of the Estradiol Biotin Reagent to all wells.
4. Swirl the microplate gently for 20-30 seconds to mix.
5. Cover and incubate for 30 minutes at room temperature.
6. Add 0.050 ml (50µl) of Estradiol Enzyme Reagent to all wells. **Add directly on top the reagents dispensed in the wells**.
7. Swirl the microplate gently for 20-30 seconds to mix.
8. Cover and incubate for 90 minutes at room temperature.
9. Discard the contents of the microplate by decantation or aspiration. If decanting, blot the plate dry with absorbent paper.
10. Add 0.350ml (350µl) of wash buffer (see Reagent Preparation Section), decant (tap and blot) or aspirate. Repeat two (2) additional times for a total of three (3) washes. **An automatic or manual plate washer can be used. Follow the manufacturer's instruction for proper usage. If a squeeze bottle is employed, fill each well by depressing the container (avoiding air bubbles) to dispense the wash. Decant the wash and repeat two (2) additional times.**
11. Add 0.100 ml (100µl) of substrate solution to all wells. **Always add reagents in the same order to minimize reaction time differences between wells.**

DO NOT SHAKE THE PLATE AFTER SUBSTRATE ADDITION

12. Incubate at room temperature for twenty (20) minutes.
13. Add 0.050ml (50µl) of stop solution to each well and gently mix for 15-20 seconds. **Always add reagents in the same order to minimize reaction time differences between wells.**
14. Read the absorbance in each well at 450nm (using a reference wavelength of 620-630nm. **The results should be read within fifteen (15) minutes of adding the stop solution.**

Note: Dilute the samples suspected of concentrations higher than 3000pg/ml 1:5 and 1:10 with estradiol '0' pg/ml calibrator or male patient serum pools with a known low value for estradiol.

10.0 CALCULATION OF RESULTS

A dose response curve is used to ascertain the concentration of estradiol in unknown specimens.
1. Record the absorbance obtained from the printout of the microplate reader as outlined in Example 1.
2. Plot the absorbance for each duplicate serum reference versus the corresponding estradiol concentration in pg/ml on linear graph paper (do not average the duplicates of the serum references before plotting).
3. Connect the points with a best-fit curve.
4. To determine the concentration of estradiol for an unknown, locate the average absorbance of the duplicates for each unknown on the vertical axis of the graph, find the intersecting point on the curve, and read the concentration (in pg/ml) from the horizontal axis of the graph (the duplicates of the unknown may be averaged as indicated). In the following example, the average absorbance (1.202) intersects the dose response curve at (160pg/ml) estradiol concentration (See Figure 1).

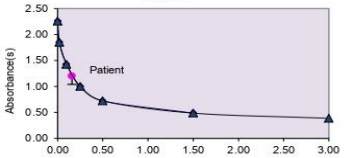
Note: Computer data reduction software designed for ELISA assay may also be used for the data reduction. If such software is utilized, the validation of the software should be ascertained.

EXAMPLE 1

Sample I.D.	Well Number	Abs (A)	Mean Abs (B)	Value (pg/ml)
Cal A	A1	2.268	2.256	0
	B1	2.244		
Cal B	C1	1.839	1.849	20
	D1	1.860		
Cal C	E1	1.409	1.426	100
	F1	1.443		
Cal D	G1	1.017	1.003	250
	H1	0.989		
Cal E	A2	0.698	0.723	500
	B2	0.748		
Cal F	C2	0.480	0.487	1500
	D2	0.493		
Cal G	E2	0.390	0.388	3000
	F2	0.385		
Pat# 1	G2	1.202	1.202	**160**
	H2	1.203		

*The data presented in Example 1 and Figure 1 is for illustration only and **should not** be used in lieu of a dose response curve prepared with each assay.

Figure 1



Note: Multiply the horizontal values by 1000 to convert into pg/ml.

11.0 Q.C. PARAMETERS

In order for the assay results to be considered valid the following criteria should be met:

1. The absorbance (OD) of calibrator 0 pg/ml should be ≥ 1.3
2. Four out of six quality control pools should be within the established ranges.

12.0 RISK ANALYSIS

The MSDS and Risk Analysis Form for this product are available on request from Monobind Inc.

12.1 Assay Performance

1. It is important that the time of reaction in each well is held constant to achieve reproducible results.
2. Pipetting of samples should not extend beyond ten (10) minutes to avoid assay drift.
3. Highly lipemic, hemolyzed or grossly contaminated specimen(s) should not be used.
4. If more than one (1) plate is used, it is recommended to repeat the dose response curve.
5. The addition of substrate solution initiates a kinetic reaction, terminated by the addition of the stop solution. Therefore, the substrate and stop solution should be added in the same sequence to eliminate any time-deviation during reaction.
6. Plate readers measure vertically. Do not touch the bottom of the wells.
7. Failure to remove adhering solution adequately in the aspiration or decantation wash step(s) may result in poor replication and spurious results.
8. Use components from the same lot. No intermixing of reagents from different batches.
9. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from Monobind IFU may yield inaccurate results.
10. All applicable national standards, regulations and laws, including, but not limited to, good laboratory procedures, must be strictly followed to ensure compliance and proper usage.
11. It is important to calibrate all the equipment e.g. Pipettes, Readers, Washers and/or the automated instruments used with this device, and to perform routine preventative maintenance.
12. Risk Analysis- as required by CE Mark IVD Directive 98/79/EC - for this and other devices, made by Monobind, can be requested via email from Monobind@monobind.com.

12.2 Interpretation

1. ***Measurements and interpretation of results must be performed by a skilled individual or trained professional.***
2. Laboratory results alone are only one aspect for determining patient care and should not be the sole basis for therapy, particularly if the results conflict with other determinants.
3. The reagents for the test system have been formulated to eliminate maximal interference; however, potential interaction between rare serum specimens and test reagents can cause erroneous results. Heterophilic antibodies often cause these interactions and have been known to be problems for all kinds of immunoassays (Boscato LM, Stuart MC. 'Heterophilic antibodies: a problem for all immunoassays' Clin. Chem. 1988:3427-33). For diagnostic purposes, the results from this assay should be in combination with clinical examination, patient history and all other clinical findings.
4. For valid test results, adequate controls and other parameters must be within the listed ranges and assay requirements.
5. If test kits are altered, such as by mixing parts of different kits, which could produce false test results, or if results are incorrectly interpreted, Monobind shall have no liability.
6. If computer controlled data reduction is used to interpret the results of the test, it is imperative that the predicted values for the calibrators fall within 10% of the assigned concentrations.

13.0 EXPECTED RANGES OF VALUES

In agreement with established reference intervals for a "normal" adult population and females during gestation the expected ranges for the Estradiol AccuBind® ELISA Test System are detailed in Table 1.

TABLE 1
Expected Values for the Estradiol Test System

	Median	Range
Females	-	-
Follicular Phase	48	9-175
Luteal Phase	103	44-196
Periovulatory	209	107-281
Treated Menopausal	122	42-289
Untreated Menopausal	7.3	ND-20
Oral Contraceptives	13	ND-103
Males	19	4-94

During pregnancy the Estradiol serum levels rise rapidly till the end of third trimester.[19]

It is important to keep in mind that establishment of a range of values which can be expected to be found by a given method for a population of "normal" persons is dependent upon a multiplicity of factors: the specificity of the method, the population tested and the precision of the method in the hands of the analyst. For these reasons each laboratory should depend upon the range of expected values established by the manufacturer only until an in-house range can be determined by the analysts using the method with a population indigenous to the area in which the laboratory is located.

14.0 PERFORMANCE CHARACTERISTICS

14.1 Precision

The within and between assay precision of the estradiol AccuBind® Microplate ELISA Test System were determined by analyses on three different levels of pool control sera. The number, mean values, standard deviation and coefficient of variation for each of these control sera are presented in Table 2 and Table 3.

TABLE 2
Within Assay Precision (Values in pg/ml)

Sample	N	X	σ	C.V.
Low	20	81.9	8.1	9.9%
Normal	20	242.7	20.5	8.5%
High	20	423.7	7.5	7.5%

TABLE 3
Between Assay Precision (Values in pg/ml)

Sample	N	X	σ	C.V.
Low	20	106.1	5.1	4.8%
Normal	20	261.5	10.0	3.8%
High	20	436.7	13.5	8.2%

*As measured in ten experiments in duplicate over a ten day period.

14.2 Sensitivity

The estradiol AccuBind® EIA Test System has a sensitivity of 8.2 pg/ml. The sensitivity was ascertained by determining the variability of the 0 pg/ml serum calibrator and using the 2σ (95% certainty) statistic to calculate the minimum dose.

14.3 Accuracy

The Estradiol AccuBind® ELISA Test System was compared with a reference method. Biological specimens from low, normal and relatively high estradiol level populations were used (The values ranged from 10 pg/ml – 4300 pg/ml). The total number of such specimens was 65. The least square regression equation and the correlation coefficient were computed for this estradiol EIA in comparison with the reference method. The data obtained is displayed in Table 4.

TABLE 4

Method	Mean (x)	Least Square Regression Analysis	Correlation Coefficient
Monobind (y)	336.8	y= 36.50+1.023(x)	0.989
Reference (X)	293.4		

Only slight amounts of bias between this method and the reference method are indicated by the closeness of the mean values. The least square regression equation and correlation coefficient indicates excellent method agreement.

14.4 Specificity

The % cross reactivity of the estradiol antibody to selected substances was evaluated by adding the interfering substance to a serum matrix at various concentrations. The cross-reactivity was calculated by deriving a ratio between dose of interfering substance to dose of estradiol needed to displace the same amount of labeled analog.

Substance	Cross Reactivity
Androstenedione	0.0003
Dihydotestosterone	0.0008
Cortisone	<0.0001
Corticosterone	<0.0001
Cortisol	0.0004
Estriol	<0.0001
DHEA sulfate	<0.0001
Estradiol	<0.0001
Estrone	<0.0001
Testosterone	<0.0001

15.0 REFERENCES

1. Abraham GE. The application of natural steroid radioimmunoassay to gynecologic endocrinology. In: Abraham GE, editor. *Radioassay Systems in Clinical Endocrinology*, Basel: Marcel Dekker,: 475-529 (1981).
2. Batzer F,"Hormonal evaluation of early pregnancy", *Fertility Sterility*, 34:1-13 (1980).
3. Bauman J, "Basal body temperature: unreliable method of ovulation detection", *Fertility Sterility*, 36:729-33, (1981).
4. Bergquist C, Nillius SJ, Wide L "Human gonadotropin therapy: Serum estradiol and progesterone patterns during conceptual cycles", *Fertility Sterility*; 39:761-65 (1983).
5. Gautray JP, et al, "Clinical investigation of the menstrual cycle: clinical, endometrial and endocrine aspects of luteal defects", *Fertility Sterility*, 35:296-303 (1981).
6. Hensleigh PA, Fainstat T, "Corpus luteum dysfunction: serum progesterone levels in diagnosis and assessment of therapy for recurrent and threatened abortion", *Fertility Sterility*, 32:396-9. (1979)
7. Hernandez JL, et al,"Direct evidence of luteal insufficiency in women with habitual abortion", *Obstetric Gynecology* , 49:705-8.(1977).
8. Goldstein D, et al, "Correlation between Estradiol and Progesterone in cycles with luteal phase deficiency", *Fertility Sterility*, 37:348-54 (1982).
9. Klopper A, Fuchs F. Progestagens. In: Fuchs F, Klopper A, editors. *Endocrinology of Pregnancy*. Hagerstown: Harper & Row, 99-122 (1977).
10. Lehmann F, Bettendorf G,"The endocrine shift from a normal cycle to anovulation", Insler V, Bettendorf G, editors. *Advances in Diagnosis and Treatment of Infertility*. Amsterdam: Elseveir/North Holland, 105 -13 (1981).
11. March CM. Luteal phase defects. In: Mishell DR, Davajan V, editors. *Reproductive Endocrinology, Infertility and Contraception*. Philadelphia: F. A. Davis Company, 1979: 469-76.
12. March CM, Goebelsmann U, Nakamura RM, Mishell Dr. Roles of estradiol and progesterone in eliciting the midcycle luteinizing hormone and follicle stimulating hormone surges. *J Clin Endocrinol Metab*, 49:507-13 (1979).
13. BIO-ED slide/seminar educational program. Rochester: Bioeducational Publications, 1981.
14. Radwanska E, et al,"Plasma progesterone levels in normal and abnormal early human pregnancy", *Fertility Sterility* 30:398-402 (1978).
15. Tietz, *Textbook of clinical chemistry*, 2nd ed. Philadelphia: W.B. Saunders, (1994).

Revision: 5 Date: 2019-Jul-16 DCO: 1353
MP4925 Product Code: 4925-*300*

Size		96(A)	192(B)
Reagent (fill)	A)	1ml set	1ml set
	B)	1 (6ml)	2 (6ml)
	C)	1 (6ml)	2 (6ml)
	D)	1 plate	2 plates
	E)	1 (20ml)	1 (20ml)
	F)	1 (12ml)	2 (12ml)
	G)	1 (8ml)	2 (8ml)

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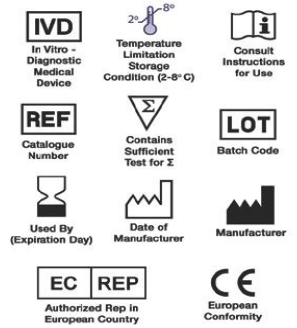
Glossary of Symbols
(EN 980/ISO 15223)



Fuente: *Monobind Inc.*