1 lipca 2013 r.

PILNY KOMUNIKAT DOTYCZĄCY BEZPIECZEŃSTWA STOSOWANIA AMH Gen II ELISA (REF A79765)

Część numer	Numer partii zestawów
A79765	Wszystkie partie ≤ 326119

Drodzy Klienci firmy Beckman Coulter, użytkownicy produktu AMH Gen II ELISA!

Firma Beckman Coulter kontynuuje akcję dotyczącą bezpieczeństwa stosowania produktu wymienionego powyżej. Niniejsza wiadomość stanowi kontynuację „Pilnego komunikatu dotyczącego bezpieczeństwa stosowania FSN-20434-2" z dnia 21 czerwca 2013 roku. Komunikat ten zawiera ważne informacje, z którymi muszą się Państwo niezwłocznie zapoznać i dotyczące oznaczania poziomu hormonu anty-Mullerowskiego (ang. Anti-Müllerian Hormone, AMH) za pomocą zestawu odczynników AMH Gen II ELISA.

PROBLEM:
Firma Beckman Coulter potwierdziła, że zastosowanie zestawu odczynników AMH Gen II ELISA do nierozcieńczonych próbek testowych może skutkować uzyskaniem wyników niższych od spodziewanych, z uwagi na oddziaływanie dopełniacza.

WPŁYW:

- Wyniki oznaczeń hormonu AMH z użyciem nierozcieńczonych próbek mogą być niższe od oczekiwanych.
- Na podstawie wewnętrznych testów można stwierdzić, że zaobserwowana wielkość przesunięcia wartości wynosiła około siedemdziesiąt procent (70%) w odniesieniu do próbek poddanych analizie w ciągu 1–2 godzin od pobrania. Obserwowane przesunięcie wartości jest uzależnione od próbki oraz warunków jej przechowywania. Próbki świeżo pobrane lub zamrożone bezpośrednio po pobraniu odznaczają się większym ryzykiem oddziaływania dopełniacza. W miarę starzenia się próbki ryzyko oddziaływania dopełniacza maleje.
- Zmiana wielkości nie dotyczy wartości kontrolnych.

DZIAŁANIE/POSTANOWIENIE:

- We wcześniejszym powiadomieniu o numerze FSN-20434-2 z dnia 21 czerwca 2013 roku firma Beckman Coulter informowała, że należy:
  - Wycofać z użycia wszystkie zestawy odczynników AMH Gen II ELISA o numerach partii niższych niż lub równych 326119.
- Instrukcje dotyczące produktu przedstawione w niniejszym liście zastępują instrukcje opisane w poprzednich komunikatach o numerach: FSN-20434 i FSN-20434-2.
- Zmieniono procedurę stosowania zestawu odczynników AMH Gen II ELISA i obecnie można korzystać z zestawu odczynników AMH Gen II ELISA (REF A79765) z poprawioną

instrukcją użycia AMH Gen II ELISA (IFU – REF A92268D) załączoną do niniejszej wiadomości. Do zmienionej procedury badania włączono dodatkowy etap testowania, który należy wykonać, zanim na mikropłytkę zostaną naniesione: odczynniki do kalibracji AMH Gen II, substancja kontrolna lub próbki hormonu AMH. Ten dodatkowy etap pozwoli wyeliminować skutki oddziaływania dopełniacza.

PROCEDURA OCENY:

Można posłużyć się niniejszą procedurą, aby określić, czy powyższa kwestia dotyczy badania próbek hormonu AMH w danym laboratorium.

- Należy pobrać zestaw próbek do badania AMH **zgodnie ze standardową procedurą pozyskiwania takich próbek, stosowaną w danym laboratorium i na sposób określony w obowiązującej instrukcji użycia**. Zaleca się przetestowanie co najmniej 40 próbek. Należy przygotować wystarczającą objętość próbek, tak aby przeprowadzić dwa identyczne testy w dwóch osobnych próbach.
- Przeprowadzić badanie próbek za pomocą testu AMH Gen II ELISA zgodnie z instrukcjami zawartymi w obowiązującej instrukcji użycia AMH Gen II ELISA IFU.
- Przeprowadzić badanie tego samego zestawu próbek za pomocą testu AMH Gen II ELISA zgodnie z instrukcjami zawartymi w poprawionej instrukcji użycia AMH Gen II ELISA IFU (REF A92268D). Do niniejszego powiadomienia załączono poprawioną instrukcję użycia.

    1. Przed naniesieniem próbek na mikropłytkę AMH Gen II ELISA należy przygotować odczynniki do kalibracji, substancję kontrolną i próbki z **AMH Gen II Assay Buffer** (REF A56021) w sposób opisany poniżej.
    2. Należy przygotować w odpowiednich probówkach mieszaninę odczynnika do kalibracji, substancji kontrolnej i próbki testowej (w tym rozcieńczone próbki pobrane od chłopców) z AMH Gen II Assay Buffer w stosunku 1:5 (np. 60 µl odczynników do kalibracji, substancji kontrolnej lub próbki testowej do 300 µl AMH Gen II Assay Buffer) na probówkę.

        UWAGA: Procedura przygotowania mieszaniny odczynników do kalibracji AMH, substancji kontrolnej i próbki testowej z AMH Gen II Assay Buffer: czynnik rozcieńczenia nie jest wymagany,

    3. Wymieszać dokładnie,

- W ciągu godziny należy za pomocą automatycznej pipety rozpipetować do odpowiednich studzienek 120 µl wcześniej wymieszanych odczynników do kalibracji, substancji kontrolnych i próbek, porównać wyniki badań uzyskane obiema metodami, aby ocenić wpływ omawianej kwestii na badanie próbek AMH.
- Jeśli wyniki badań sugerują, że wystąpił wpływ omawianej kwestii na testowany zestaw próbek AMH, należy zastosować poprawioną instrukcję użycia AMH Gen II ELISA IFU do przeprowadzenia ponownych badań.

    UWAGA: W poprawionej procedurze przywrócono możliwość rozcieńczania próbek klinicznych AMH przed rozpoczęciem testu. Należy rozcieńczać każdą próbkę, której odczyty są wyższe niż najwyższe odczyty odczynników do kalibracji z

rozpuszczalnikiem, a następnie zbadać ponownie rozcieńczoną próbkę. Przed przystąpieniem do badania próbek pobranych od chłopców, należy je rozcieńczyć w stosunku 1:9 z rozcieńczalnikiem.

Lokalny właściwy urząd został powiadomiony o tej akcji dotyczącej bezpieczeństwa stosowania produktu.

Prosimy przekazać tę informację personelowi laboratorium i zachować niniejsze powiadomienie jako element dokumentacji Systemu Jakości Twojego laboratorium. Jeśli przekazali Państwo do innego laboratorium jakikolwiek produkt, którego dotyczy powyższa informacja, proszę przekazać do tego laboratorium również kopię tego listu.

Proszę także wypełnić i zwrócić załączony Formularz odpowiedzi w ciągu dziesięciu dni, tak abyśmy mieli pewność, że otrzymali Państwo powyższe ważne informacje.

Jeśli macie Państwo jakiekolwiek pytania dotyczące powyższej informacji, proszę kontaktować się z lokalnym przedstawicielem firmy Beckman Coulter.Polska Sp Z O O pod numerem telefonu 22 355 15 00.

Przepraszamy za wszelkie niedogodności, jakie powyższa kwestia mogła spowodować w Państwa laboratoriach.

Z poważaniem,

Zanna Bartel

Zanna Bartel
z upowaznienia Reulatory Specialist

Załączniki: Poprawiona instrukcja użycia i formularz odpowiedzi

POTWIERDZENIE KLIENTA

AMH Gen II ELISA (REF A79765)

Numer części	Numer partii zestawów
A79765	Wszystkie partie ≤ 326119

Wskazówki: Prosimy wypełnić poniższe rubryki, potwierdzając otrzymanie ww. informacji i przesłać wypełniony formularz faksem w terminie 10 dni od daty otrzymania niniejszej informacji pod numer 22 355 15 39 lub pocztą elektroniczną na adres: opowilajtis@beckman.com
Jeśli Państwo nie posiadają ww. produktu, prosimy również o odesłanie wypełnionego formularza, umożliwi to zaktualizowanie naszej bazy danych.

Nazwa systemu: _____

Prosimy zaznaczyć właściwe:

☐ Przeczytałam/łem i zrozumiałam ważną informację dotyczącą bezpieczeństwa produktu oraz podjęłam/łem odpowiednie działania w nim wskazane.

☐ Nie rozumiem otrzymanej informacji i proszę o dalsze wyjasnienia.

☐ Nie posiadam wyżej wymienionego produktu.

Prosimy o wysłanie niniejszego formularza:

- Faksem na numer: **22 355 15 39**

  lub

- Pocztą e-mail: **opowilajtis@beckman.com**

Nazwa Podmiotu: _____

Imię i nazwisko: _____

Tytuł/Stanowisko:_____ Miejscowość_____

Nr telefonu:_____

Podpis:_____Data:_____

AMH Gen II ELISA

REF A79765

CAUTION

Not for sale in U.S.A.

INTENDED USE

The Anti-Mullerian Hormone (AMH) Gen II enzyme linked immunosorbent assay (ELISA) kit provides materials for the quantitative measurement of AMH in human serum and lithium heparin plasma. This assay is intended for *in vitro* diagnostic use.

SUMMARY AND EXPLANATION

AMH is a glycoprotein dimer composed of two 72 kDa monomers linked by disulfide bridges.[1,2,3,4,5,6,7,8] It belongs to the transforming growth factor-β family. AMH performs various physiological functions. In males, AMH is secreted by the Sertoli cells. During embryonic development, AMH is responsible for Mullerian duct regression. AMH continues to be produced by the testicles until puberty and then decreases slowly to residual post-puberty values. In females, AMH is produced in small amounts by ovarian granulosa cells after birth until menopause, and then becomes undetectable.

PRINCIPLE OF THE TEST

The AMH Gen II ELISA is an enzymatically amplified two-site immunoassay. In the assay, calibrators, controls and samples are incubated in microtitration wells which have been coated with anti-AMH antibody. After incubation and washing, anti-AMH detection antibody labeled with biotin is added to each well. After a second incubation and washing step, streptavidin-horseradish peroxidase (HRP) is added to the wells. After a third incubation and washing step, the substrate tetramethylbenzidine (TMB) is added to the wells. Lastly, an acidic stopping solution is added. The degree of enzymatic turnover of the substrate is determined by dual wavelength absorbance measurement at 450 nm and between 600 and 630 nm. The absorbance measured is directly proportional to the concentration of AMH in the samples. A set of AMH calibrators is used to plot a calibration curve of absorbance versus AMH concentration. The AMH concentrations in the samples can then be calculated from this calibration curve.

MATERIALS SUPPLIED

Ab PLATE Anti-AMH Gen II Antibody Coated Microtitration strips:
A56025

- One stripholder, containing 96 polystyrene microtitration wells with mouse monoclonal anti-AMH IgG immobilized to the inside wall of each well.
- Store at 2 to 8°C until expiration date in the resealable pouch with a desiccant to protect from moisture.

SAMPLE DIL AMH Gen II Sample Diluent:
A56026

- One bottle, 13 mL, containing buffer with bovine serum albumin (BSA), < 0.5% ProClin* 300 and sodium azide.
- Store unopened at 2 to 8°C until the expiration date.

BIO CONJ RTU AMH Gen II Antibody-Biotin Conjugate:
A56023

- Provided ready to use.
- One bottle, 13 mL, containing biotinylated anti-AMH antibody in buffer with protein (bovine, mouse), < 0.3% ProClin 300 and sodium azide.
- Store at 2 to 8°C until expiration date.

STREP CONJ RTU Streptavidin-Enzyme Conjugate:
A56024

- Provided ready to use.
- One bottle, 13 mL, containing streptavidin-HRP in buffer with protein (mouse, fish) and < 10% methanol.
- Store at 2 to 8°C until expiration date.

ASSAY BUFFER AMH Gen II Assay Buffer:
A56021

- Two bottles, 13 mL, containing buffer with BSA, protein (bovine, mouse), < 0.3% ProClin 300 and sodium azide.
- Store at 2 to 8°C until expiration date.

TMB SOLN TMB Chromogen Solution:
DSL-10-9755

- One bottle, 11 mL, containing a solution of TMB in citrate buffer with hydrogen peroxide.
- Store at 2 to 8°C until expiration date.

WASHCONC I Wash Concentrate I:
DSL-10-4030

- One bottle, 100 mL, containing buffered saline with a nonionic detergent.
- Store at 2 to 8°C or room temperature (~25°C) until expiration date.
- Dilute 10-fold with deionized water prior to use.

STOP SOLN A Stopping Solution A:
DSL-10-9780

- One bottle, 11 mL, containing 0.2 M sulfuric acid.
- Store at 2 to 8°C or room temperature (~25°C) until expiration date.

MATERIALS REQUIRED BUT NOT SUPPLIED

1. **AMH Gen II Calibrators and Controls A79766**
2. Appropriate size tube (for sample premix)
3. Microtitration plate reader capable of absorbance measurement at 450/405 nm and preferentially capable of dual wavelength (reference filter) at 600 to 630 nm
4. Deionized water
5. Precision pipette(s) to deliver 10–1000 µL
6. Microtitration plate shaker capable of 600–800 orbital revolutions per minute (rpm)
7. Microtitration plate washer
8. Vortex mixer
9. Absorbent materials for blotting the strips
10. Graph paper for manual data reduction

WARNINGS AND PRECAUTIONS

- **For *in vitro* diagnostic use.**
- Use good laboratory practices.[9]
- Samples and blood-derived products may be routinely processed with minimum risk using the procedure described. However, handle these products as potentially infectious according to universal precautions and good clinical laboratory practices, regardless of their origin, treatment or prior certification.[10] Use an appropriate disinfectant for decontamination. Store and dispose of these materials and their containers in accordance with local regulations and guidelines.
- Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. On disposal of liquids, flush with a large volume of water to prevent azide build-up.[11]
- Xi: Irritant: < 0.5% ProClin 300.

R 43: May cause sensitization by skin contact.
S 28-37: After contact with skin, wash immediately with plenty of soap and water. Wear suitable gloves.

- Xn: Harmful: < 10% Methanol.

R 20/21/22: Harmful by inhalation, in contact with skin and if swallowed.
R 68/20/21/22: Harmful: possible risk of irreversible effects through inhalation, in contact with skin and if swallowed.
S 36/37: Wear suitable protective clothing and gloves.
S 45: In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible).

- The Material Safety Data Sheet (MSDS) is available upon request.

SAMPLE COLLECTION AND PREPARATION

- Serum and lithium heparin plasma are the recommended samples.
- Observe the following recommendations for handling, processing and storing blood samples:[12]
  - a.) Collect all blood samples observing routine precautions for venipuncture.
  - b.) Allow serum samples to clot completely before centrifugation.
  - c.) Keep tubes stoppered at all times.
  - d.) Within two hours after centrifugation, transfer at least 500 µL of cell-free sample to a storage tube. Tightly stopper the tube immediately.
  - e.) Serum and lithium heparin plasma may be stored at 2 to 8°C for 48 hours.
  - f.) If the assay will not be completed within 48 hours, or for shipment of samples, freeze at -20°C.
- Use the following guidelines when preparing samples:
  - a.) Ensure residual fibrin and cellular matter have been removed prior to analysis.
  - b.) Follow blood collection tube manufacturer's recommendations for centrifugation.
- Each laboratory should determine the acceptability of its own blood collection tubes and serum separation products. Variations in these products may exist between manufacturers and, at times, from lot-to-lot.
- Avoid repeated freezing and thawing of samples.
- Avoid assaying lipemic or hemolyzed samples.

PROCEDURAL NOTES

- A thorough understanding of this package insert is necessary for successful use of the AMH Gen II ELISA.
- It is the responsibility of the customer to validate the assay for their use.
- Reliable results will only be obtained by using precise laboratory techniques and accurately following the package insert.
- A calibration curve must be included with each assay.
- Bring all kit reagents to room temperature (~25ºC) before use.
- Thoroughly mix the reagents before use by gentle inversion.
- Do not mix various lots of any kit component within an individual assay.
- Do not use any component beyond the expiration date shown on its label.
- Incomplete washing will adversely affect the outcome and assay precision.
- To minimize potential assay drift due to variation in the substrate incubation time, care should be taken to add the stopping solution into the wells in the same order and speed used to add the TMB chromogen solution.
- Avoid microbial contamination of reagents, especially of the conjugate and the assay buffer.
- Avoid contamination of the TMB chromogen solution with the conjugates.
- Use a clean disposable pipette tip for each reagent, calibrator, control or sample.
- For dispensing sulfuric acid and TMB chromogen solution, avoid pipettes with metal parts.
- The enzyme used as the label is inactivated by oxygen, and is highly sensitive to microbial contamination, sodium azide, hypochlorous acid and aromatic chlorohydrocarbons often found in laboratory water supplies.
- Use deionized water.
- Avoid exposure of the reagents to excessive heat or direct sunlight during storage and incubation.

TEST PROCEDURE

Preparation of Reagents

1. ***Wash Solution:*** Dilute 1 part Wash Concentrate I with 9 parts deionized water. The resulting working strength wash solution is stable for one month at room temperature (~25°C) when stored in a tightly sealed bottle.
2. ***Microtitration Wells:*** Select the number of coated wells required for the assay. The remaining unused wells should be placed in the resealable pouch with a desiccant. The pouch must be resealed to protect from moisture.

Assay Procedure

Allow all samples and reagents to reach room temperature (~25°C). Mix reagents thoroughly by gentle inversion before use. After reconstitution of reagents, mix thoroughly, avoiding foam. Calibrators, controls and samples should be assayed in duplicate.

NOTE: All samples reading higher than the highest calibrator should be thoroughly mixed and diluted in the AMH Gen II Sample Diluent prior to assay. For pediatric male samples: Dilute 1 part sample with 9 parts Sample Diluent before testing.

1. Before adding sample to the AMH Gen II ELISA microplate, you must prepare all calibrators, controls, and samples with the **AMH Gen II Assay Buffer** (REF A56021).
   In a sample tube, prepare 1 part of each calibrator, control, or test sample respectively (including diluted pediatric male samples) with 5 parts AMH Gen II Assay Buffer (for example, 60 µL calibrator, control, or sample + 300 µL AMH Gen II Assay Buffer). Mix thoroughly.
   NOTE: This is a preparation of the AMH calibrators, controls and test samples with the AMH Gen II Assay Buffer. No dilution factor is required.
2. Mark the microtitration strips to be used.
3. Within 1 hour, pipet 120 µL of the premixed calibrators, controls and samples to the appropriate wells using a precision pipette.
4. Incubate the wells, shaking at 600–800 rpm on an orbital microplate shaker, for one hour at room temperature (~25°C).
5. Prepare the wash solution as described under the "Preparation of Reagents" section of this package insert.
6. Aspirate and wash each well five times with the wash solution using an automatic microplate washer or manually using a precision pipette. Blot and dry by inverting plate on absorbent material.
   NOTE: Use of an automatic microplate washer is strongly recommended. Incomplete washing will adversely affect assay precision. If a microplate washer is not available, follow these steps to wash the plate manually:
   (a) Completely aspirate the liquid from each well
   (b) Dispense 400 µL of the wash solution into each well using a precision pipette
   (c) Aspirate the liquid again
   (d) Repeat steps (b) and (c) four times
7. Add 100 µL of the antibody-biotin conjugate solution to each well using a precision pipette.
8. Incubate the wells, shaking at 600–800 rpm on an orbital microplate shaker, for one hour at room temperature (~25°C).
9. Aspirate and wash each well five times with the wash solution using an automatic microplate washer. Blot dry by inverting plate on absorbent material.
10. Add 100 µL of the streptavidin-enzyme conjugate to each well using a precision pipette.
11. Incubate the wells, shaking at 600–800 rpm on an orbital microplate shaker, for 30 minutes at room temperature (~25°C).
12. Aspirate and wash each well five times with the wash solution using an automatic microplate washer. Blot dry by inverting plate on absorbent material.
13. Add 100 µL of the TMB chromogen solution to each well using a precision pipette.
    Avoid exposure to direct sunlight.
14. Incubate the wells, shaking at 600–800 rpm on an orbital microplate shaker, for 8–12 minutes at room temperature (~25°C).
    NOTE: Be aware that the color may develop more quickly or more slowly than the recommended incubation time depending on the localized room temperature. Visually monitor the color development to optimize the incubation time.

15. Add 100 µL of the stopping solution to each well using a precision pipette.
16. Read the absorbance of the solution in the wells within 30 minutes, using a microplate reader set to 450 nm.

NOTE: 1) While reading the absorbance of the microtitration well, it is necessary to program the zero calibrator as a "Blank".
2) If wavelength correction is available, set the instrument to dual wavelength measurement at 450 nm with background wavelength correction set between 600 and 630 nm.

RESULTS

1. Calculate the mean absorbance for each calibrator, control or sample.
2. Plot the log of the mean absorbance readings for each of the calibrators along the y-axis versus log of the AMH concentrations in ng/mL along the x-axis, using a cubic regression curve-fit. Use of curve fits other than recommended may cause results to vary.
3. Determine the AMH concentrations of the controls and samples from the calibration curve by matching their mean absorbance readings with the corresponding AMH concentrations.
4. Any sample reading higher than the highest calibrator should be appropriately diluted using sample diluent and reassayed. For pediatric male samples: Dilute 1 part sample with 9 parts Sample Diluent before testing.
5. Any sample reading lower than the analytical sensitivity should be reported as such.
6. Multiply the value by a dilution factor, if required.

NOTE: If the absorbance readings exceed the limitations of the plate reader, a second reading at 405 nm is needed (reference filter between 600 and 630 nm if available). In this case, proceed to construct a second calibration curve as above with the absorbance readings of all calibrators at 405 nm. The concentration of the off-scale samples at 450 nm is then read from the new calibration curve. The readings at 405 nm should not replace the on-scale readings at 450 nm.

LIMITATIONS

- The reagents supplied in this kit are optimized to measure AMH levels in serum and lithium heparin plasma.
- For assays employing antibodies, the possibility exists for interference by heterophile antibodies in the sample. Samples from individuals which have been regularly exposed to animals or have received immunotherapy or diagnostic procedures utilizing immunoglobulins or immunoglobulin fragments may produce antibodies, e.g. HAMA, that interfere with immunoassays. Additionally, other heterophile antibodies such as human anti-goat antibodies may be present in samples.[13,14]
- If there is evidence of microbial contamination or excessive turbidity in a reagent, discard the vial.

QUALITY CONTROL

- Each laboratory should establish mean values and acceptable ranges to assure proper performance.
- AMH Gen II ELISA controls or other commercial controls should fall within established confidence limits.
- The confidence limits for AMH Gen II ELISA controls are provided with the AMH Gen II Calibrator and Control kit.
- A full calibration curve, plus low and high level controls, should be included in each assay.
- The TMB chromogen solution should be colorless to very light yellow. Development of a blue color may indicate reagent contamination or instability.

EXPECTED VALUES

1. Each laboratory should establish its own reference ranges to assure proper representation of specific populations.

SAMPLES	MEDIAN AGE (yrs)	MEDIAN IN (ng/mL)	2.5–97.5TH PERCENTILE IN (ng/mL)
Random Males (N=136)	38	5.7	1.3-14.8
Random Females (N=95)	30	2.4	ND-12.6
Males fertility clinic (N=100)	37	5.3	0.8-14.6
Females 3rd day of cycle (N=106)	-	1.5	ND-10.6
Post Menopausal Females (N=45)†	71	ND	ND
Boys (N=36)†	4.8	56.3	3.8-159.8
Girls (N=36)†	5.0	1.3	ND-8.9

†Non parametric reference at 90% limit.
ND = Non-Detectable

TYPICAL CALIBRATION CURVE

WELL NO.	WELL CONTENTS	MEAN ABSORBANCE	CONC. (ng/mL)
	CALIBRATORS		
A1, A2	A	(Blank)	0
B1, B2	B	0.019	0.16
C1, C2	C	0.053	0.4
D1, D2	D	0.17	1.2
E1, E2	E	0.57	4.0
F1, F2	F	1.47	10.0
G1, G2	G	3.11	22.5

CAUTION: The above data must not be employed in lieu of data obtained by the user in the laboratory.

SPECIFIC PERFORMANCE CHARACTERISTICS

All analytical characteristics are stated in ng/mL. To convert to SI units (International System of Units):

1 ng/mL = 7.14 pM

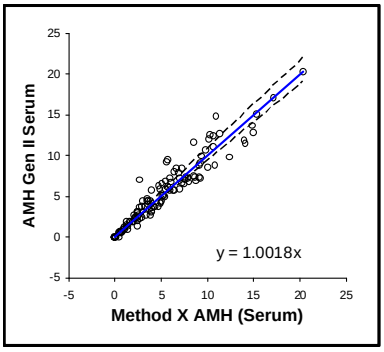
Method Comparison

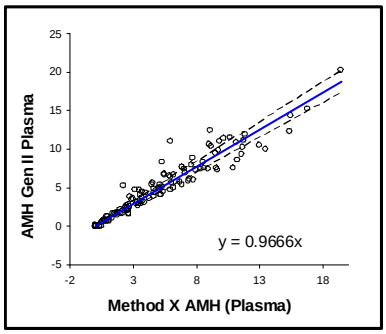
The AMH Gen II ELISA has been compared to another commercially available AMH kit (Method X). One hundred nineteen male and female serum and lithium heparin plasma samples, ranging in age from 20-50 years were assayed and linear regression analysis of the results yielded the following:

Regression:

A. AMH SERUM =1.0 (Method X)
(r = 0.98; 97.5% CI = 0.95-0.98, P <0.0001)

B. AMH PLASMA =0.967 (Method X)
(r = 0.98; 97.5% CI = 0.95-0.98, P <0.0001)





Dilution Recovery (Linearity)

Multiple dilutions of four samples containing various AMH levels with AMH Gen II sample diluent (zero) resulted in the following data.[16]

SAMPLE	DILUTION FACTOR (1:X)	EXPECTED VALUE IN (ng/mL)	OBSERVED VALUE IN (ng/mL)	% RECOVERY
1	Neat value	4.94	N/A	N/A
	2	2.47	2.66	108
	4	1.24	1.37	110
	8	0.62	0.63	102
	16	0.31	0.26	84
2	Neat value	6.47	N/A	N/A
	2	3.24	3.46	107
	4	1.62	1.77	109
	8	0.81	0.88	109
	16	0.41	0.37	90
3	Neat value	10.34	N/A	N/A
	2	5.17	5.23	101
	4	2.58	2.59	100
	8	1.29	1.34	104
	16	0.65	0.59	91
4	Neat value	12.86	N/A	N/A
	2	6.43	6.36	99
	4	3.22	3.11	97
	8	1.61	1.58	98
	16	0.80	0.68	85

Spiking Recovery

Addition of three different levels of AMH to four patient samples with low AMH resulted in the following data:

SAMPLE	ENDOGENOUS CONC. (ng/mL)	EXPECTED CONC. (ng/mL)	OBSERVED CONC. (ng/mL)	% RECOVERY
1	0.67	1.97	1.96	99
		3.16	3.20	101
		4.24	4.45	105
2	1.16	2.44	2.53	104
		3.60	3.81	106
		4.66	5.01	107
3	2.21	3.44	3.86	112
		4.55	4.48	98
		5.57	5.69	102
4	1.47	2.73	2.77	101
		3.88	3.87	100
		4.93	5.07	103

Imprecision:

Reproducibility of the AMH Gen II assay was determined in a study using two in-house serum pool based controls (Q1, Q2) and two kit controls (C1, C2) with two lots of reagents. The study included a total of 40 assays, four replicates per assay.[15]

SAMPLE	MEAN CONC.	WITHIN RUN	BETWEEN RUN	TOTAL
	(ng/mL)	% CV	% CV	% CV
Q1	4.42	5.4	5.6	7.7
Q2	14.03	3.6	4.5	5.8
C1	3.82	3.7	4.4	5.7
C2	16.45	3.4	4.0	5.3

Analytical Specificity

The antibodies used in the assay bind to the mature region of AMH, which is more stable against proteolysis compared to pro-hormone region. This highly characterized dual monoclonal antibody pair is specific to AMH and does not detect inhibin A, activin A, FSH and LH at 2 times their physiological concentrations.

Interference

When potential interferents (hemoglobin, triglycerides and bilirubin), were added at least at two times their physiological concentration, AMH concentrations were within ±10% of the control as represented in the following table.[17]

INTERFERENTS	ANALYTE CONC.	UNSPIKED SAMPLE (ng/mL)	SPIKED SAMPLE (ng/mL)	% DIFFERENCE TO REFERENCE
HEMOGLOBIN	2 mg/mL	2.85	2.96	3.9
TRIGLYCERIDES	20 mg/mL	2.48	2.31	-6.9
BILIRUBIN	0.6 mg/mL	2.5	2.51	0.4

Limit of Detection (LoD):

The lowest amount of AMH in a sample that can be detected with a 95% probability is 0.08 ng/mL. The value was determined by processing a complete seven point calibration curve, controls and seven serum samples in the range of zero to 1.5 ng/mL.[18] Two assay runs per day were performed over 10 days with all samples run in duplicate per run.

Limit of Quantitation (LoQ):

The estimated minimum dose achieved at 20% total imprecision is 0.16 ng/mL. The value was determined by processing a complete seven point calibration curve, controls and eight human serum samples with at least two samples that were less than the median of normal and minimum of three samples that were greater than the median of normal over 20 runs and 10 days in duplicates.[18]

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