Concentrations	No. of testing	Range	SD	CV
Level 1	10	0.1-0.6	0.153	4.7%
Level 2	10	5-15	0.862	5.9%

B) Internal Evaluation:
Accuracy: In an internal study **Electra™ AMH** was evaluated against commercially available licensed kit with 90 random clinical samples & **Electra™ AMH** has demonstrated 100% clinical correlation with the commercially available licensed kit.

C) External Evaluation:
Electra™ AMH CLIA has been evaluated by a NABL accredited lab against their reference method. In this evaluation **Electra™ AMH** has demonstrated 100% correlation with the reference method.
*Data file: Zephyr Biomedicals (A Division of Tulip Diagnostics Pvt. Ltd).

Important Note:
1. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.
2. It is recommended to use the multiple channel pipettes to avoid time effect. A full plate of 96 wells may be used if automated pipetting is available.
3. Duplication of standards & samples is not mandatory but may provide information on reproducibility & application errors.

LIMITATIONS OF THE ASSAY
1. As with all diagnostic tests, a definite clinical diagnosis should not be based on the results of a single test, but should only be made by the physician after all clinical and laboratory findings have been evaluated.
2. The activity of the enzyme used is temperature-dependent and the RLU values may vary. The higher the room temperature (+18°C to +25°C) during substrate incubation, the greater will be the RLU values. Corresponding variations apply also to the incubation times. However, the standards are subject to the same influences, with the result that such variations will be largely compensated in the calculation of the result.
3. Adaptation of this assay for use with automated sample processors and other liquid handling devices, in whole or in part, may yield differences in test results from those obtained using the manual procedure. It is the responsibility of each laboratory to validate that their automated procedure yields test results within acceptable limits.
4. Insufficient washing (e.g., less than 5 wash cycles, too small wash buffer volumes, or shortened reaction times) can lead to incorrect RLU values.

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SYMBOL KEYS

Temperature Limitation	Consult Instructions for use	Date of Manufacture	LOT Batch Number / Lot Number
Manufacturer	IVD *In vitro* Diagnostic Medical Device	This side up	Σ Contains sufficient for <n> tests
Use by	REF Catalogue Number	Do not reuse	

Manufactured by:
Zephyr Biomedicals
A Division of Tulip Diagnostics (P) Ltd.
M 46-47, Phase III B, Verna Industrial Estate, Verna, Goa- 403 722, INDIA.
Regd. Office: Gitanjali, Tulip Block, Dr. Antonio Do Rego Bagh, Alto Santacruz, Bambolim Complex P.O., Goa - 403 202, INDIA.

An ISO 13485 Certified Company

1220/VER-01

electra™ AMH

Chemiluminescence Assay for Quantitative Determination of Anti-Mullerian Hormone (AMH) in Human Serum.
FOR IN VITRO DIAGNOSTIC USE ONLY
Store at 2°C to 8°C

INTENDED USE
ELECTRA™ AMH CLIA test is intended for the quantitative determination of AMH in human serum. For In Vitro Diagnostic Use only.

INTRODUCTION
Anti-Mullerian hormone is a glycoprotein hormone structurally related to inhibin and activin from the transforming growth factor beta superfamily, whose key roles are in growth differentiation and folliculogenesis. AMH expression is critical to sex differentitation at a specific time during fetal development and appears to be tightly regulated by nuclear receptor SF1, transcription GATA factors, sex-reversal gene DAX1, and follicle-stimulating hormone (FSH). AMH is activated by SOX9 in the Sertoli cells of the male fetus thereby arresting the development of fallopian tubes, uterus, and upper vagina. AMH is also a product of granulosa cells of the preantral and small antral follicles in women. As such, AMH is only present in the ovary until menopause. AMH level is also lower and even below the detection limit if women with premature ovarian failure of any cause, including after cancer chemotherapy, etc.

PRINCIPLE
ELECTRA™ AMH CLIA is for use on **ELECTRA** analyzers. **ELECTRA™ AMH** CLIA works on the principle of chemiluminescence wherein light is produced by a chemical reaction from a substance as it returns from an electronically excited state to the ground state. When catalysed by HRP, the oxidation of luminol by hydrogen peroxide produces an electronically excited form of 3-aminophthalate which on relaxation emits light with maximum intensity at λ=425nm.
In this assay, standards and samples are directly incubated in micro titer wells which have been coated with anti-AMH antibody. After the first incubation and washing, then, another anti-AMH detection antibody labeled with horseradish peroxidase (HRP) is added to each well. After a second incubation and washing step, a "sandwich" of solid-phase antibody-human AMH-HRP-conjugated monoclonal antibody is formed. After a short incubation the wells are washed again and bound enzyme is detected by adding the chemiluminescent substrate and Luminescence is measured in RLU. The intensity of the emitting light is directly proportional to the amount of enzymatic activity of the immunocomplex and hence to the amount human AMH in the test samples. By reference to a series of AMH standards assayed in the same way, the AMH concentrations in the unknown samples can then be calculated.

MATERIALS & COMPONENTS
Materials provided with the test kits:
- Coated Microwells: Microwells coated with monoclonal anti- AMH –antibody
- AMH Sample Diluent. Ready to use.
- AMH Enzyme Conjugate (100X)
- AMH Conjugate Diluent
- Substrate A: Chemiluminescent substrate containing enhanced luminol solution.
- Substrate B: Chemiluminesent substrate containing stabilized peroxide solution.
- AMH Standard set of 6 standards labeled as A to F in Lyophilized form. Ready to use. For standard Concentrations refer vial label.
- Wash Buffer Concentrate (20X).

Materials required but not provided:
- Precision pipettes: 10-100µl, 20-200µl, 100-1000µl
- Disposable pipette tips
- Distilled water
- Disposable Gloves
- **ELECTRA™ Analyzer**

STORAGE AND STABILITY
1. **ELECTRA™ AMH** kit is stable at 2-8°C up to the expiry date printed on the label.
2. Coated micro-wells should be used within one month of opening the pouch. Once opened, the pouch must be sealed properly to protect from moisture. In case the desiccant pouch changes color from blue to white, the strips should not be used.
3. Diluted wash buffer is stable up to one week at 2-8°C.
4. Working Substrate (A+B) must be used immediately.

SPECIMEN COLLECTION

1. Collect blood specimen by venipuncture according to the standard procedure.
2. Only serum should be used.
3. Avoid grossly hemolytic, lipemic or turbid samples.
4. Preferably use fresh samples. However, specimens can be stored up to 48 hours at 2-8°C, for short duration.
5. For longer storage, specimens can be frozen at -20°C. Thawed samples must be mixed prior to testing.
6. Do not heat inactivate before use.
7. Specimen containing precipitate or particulate matter should be clarified by centrifugation prior to use.
8. Specimen should be free from particulate matter and microbial contamination.

PRECAUTIONS

1. Bring all reagents and specimen to room temperature before use.
2. Do not pipette any material by mouth.
3. Do not eat, drink or smoke in the area where testing is done.
4. Use protective clothing and wear gloves when handling samples.
5. Use absorbent sheet to cover the working area.
6. Immediately clean up any spills with sodium hypochlorite.
7. All specimens and standards should be considered potentially infectious and discarded appropriately.
8. Neutralize acid containing waste before adding hypochlorite.
9. Do not use kit after the expiry date.
10. Do not mix components of one kit with another.
11. Always use new tip for each specimen and reagent.
12. Do not allow liquid from one well to mix with other wells.
13. Do not let the strips dry in between the steps.

Reagent Preparation

1. All reagents should be brought to room temperature (18-25°C) and mixed by gently inverting or swirling prior to use. Do not induce foaming.
2. Dilute wash buffer 20 times (for example add 5ml concentrated buffer to 95 ml distilled or deionized water). Mix well before use.
3. Dilute enzyme conjugate with Conjugate diluent according to the requirement as shown below. Prepare a fresh dilution for each assay.
4. Prepare a working substrate by mixing substrate A and substrate B in equal volume (1:1 ratio) before addtion to the microwells.
5. Since the reference standards are lyophilized, reconstitute each standard with 1mL distilled water. Allow the reconstituted material to stand for at least 20 minutes. Reconstituted standards should be sealed and stored at 2-8°C.

No. of Strips	1	2	3	4	5	6	7	8	9	10	11	12
Enzyme Conjugate (µl)	10	20	30	35	45	50	60	70	75	85	90	100
Conjugate Diluent (µl)	1000	2000	3000	3500	4500	5000	6000	7000	7500	8500	9000	10000

No. of Strips	1	2	3	4	5	6	7	8	9	10	11	12
Substrate-A µl	250	450	650	850	1050	1250	1450	1650	1850	2050	2250	2450
Substrate-B µl	250	450	650	850	1050	1250	1450	1650	1850	2050	2250	2450

TEST PROCEDURE

1. Secure the desired number of coated wells in the holder. Dispense **50** µl of standards and serums into the appropriate wells.
2. Dispense **50** µl of Sample Diluent into each well. Incubate at room temperature (18-25°C) for **45 mins**.
3. Remove the incubation mixture by emptying the plate content into a waste container. Rinse and empty the microtiter plate 5 times with Wash Buffer (1X). Strike the microtiter plate sharply onto absorbent paper or paper towels to remove all residual water droplets.
4. Dispense **100** µl of diluted Enzyme Conjugate into each well. Incubate at room temperature (18-25°C) for **45 mins**.
5. Remove the incubation mixture by emptying the plate content into a waste container. Rinse and empty the microtiter plate 5 times with Wash Buffer (1X). Strike the microtiter plate sharply onto absorbent paper or paper towels to remove all residual water droplets.
6. Add **50** µl of working Substrate (A+B) in all the micro-wells. Keep away from direct light while adding the substrate.
7. Cover the **ELECTRA™** microplate and incubate for **10** minutes at room temperature (**18-25°C**) in dark.
8. Read the **ELECTRA™** micro-plate exactly at **10** minutes in **ELECTRA™ Analyzer.** If **ELECTRA™** micro-plate is not read between 10-15 minutes the test results should be considered as invalid.

Add **50µl** Standards/Samples into the respective Microwell

↓

Add **50µl** of Sample diluent into each well

↓

Apply plate sealer and incubate for **45** minutes at **18-25°C**

↓

Wash Microwells **5 times** with 350 µl of diluted Wash Buffer

↓

Add **100 µl** of diluted Enzyme Conjugate in each well

↓

Apply plate sealer and incubate for **45** minutes at **18-25°C**

↓

Wash Microwells **5 times** with 350 µl of diluted Wash Buffer

↓

Add **50 µl** Substrate in each well

↓

Incubate for **10** Minutes at **18-25°C in dark**

↓

Read results on **ELECTRA ™** Analyzer exactly at the end of **10** Minutes

CALCULATIONS

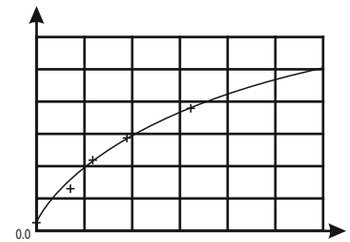
Construct a Standard curve by plotting the mean RLU obtained from each reference standard against its concentration in ng/ml on the graph paper, with RLU values on the vertical or Y axis and concentrations on the horizontal or X axis. Use the RLU values for each specimen to determine the corresponding concentration of AMH in ng/ml from the standard curve.

Example of Standard curve

Results of a typical Standard run with RLU's shown in the Y axis against AMH concentrations in the X axis.

Suggest: Use 4-Parameter Standard curve to calculate sample values.

AMH Values (ng/ml)	RLU's
A	1702
B	6531
C	62092
D	585640
E	1567673
F	3464269



This Standard curve is for the purpose of illustration only and should not be used to calculate unknowns. Each user should obtain their own Standard curve and data.

Expected Ranges of values

Gender	Age	Range (ng/ml)
Males	< 24 months	14~466
	24months~12 years	7.4~243
	> 12 years	0.7~19
Females	< 24 months	<4.7
	24months~12 years	<8.8
	13~45 years	0.9~9.5
	> 45 years	<0.1

The minimum detectable concentration of this assay is 0.073 ng/ml.

PERFORMANCE CHARACTERISTICS

A) Precision data

Two concentration levels of AMH samples were used to determine the inter precision of AMH CLIA assay kit.

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