16 samples whose LC/MS MS values have been obtained by a reference lab when tested with ELECTRA 25-OH Vitamin D and following are the results

Total Samples -16	ELECTRA 25-OH Vitamin D	Reference Method
Deficient	1	1
Insufficient	12	13
Sufficient	3	2
Toxicity	0	0

Important Note:

1. The ELECTRA 25-OH Vitamin D assay is a temperature sensitive assay. The best temperature condition for this assay is from 18°C to 22°C.
2. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.
3. It is recommended to use the multi channel pipettes to avoid time effect. A full plate of 96 wells may be used if automated pipetting is available.
4. 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 results.

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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:

Qualpro Diagnostics

A Division of Tulip Diagnostics (P) Ltd.

88/89, Phase II C, 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

0620/VER-01

electra™

25-OH Vitamin D CLIA

Chemiluminensence Assay for Quantitative Determination of total 25-OH Vitamin D in Human Serum.

FOR IN VITRO DIAGNOSTIC USE ONLY

Store at 2°C to 8°C

INTENDED USE

ELECTRA™ 25-OH Vitamin D CLIA test is intended for the quantitative determination of total 25-hydroxy (25-OH) Vitamin D in human serum. For In Vitro Diagnostic Use only.

INTRODUCTION

Vitamin D is a steroid hormone responsible for enhancing intestinal absorption of calcium and the regulation of its homeostasis. There are two common forms of Vitamin D: Vitamin D2 and D3. Vitamin D3 is naturally produced in the human skin through the exposure to ultraviolet light and Vitamin D2 is mainly obtained from plant foods. Vitamin D is transported to the liver where it is metabolized to 25-hydroxy Vitamin D. In medicine, a total 25-hydroxy Vitamin D test is used to determine Vitamin D concentration in the body. The blood concentration of 25-hydroxy Vitamin D (including D2 and D3) is considered the best indicator of Vitamin D status.

PRINCIPLE

ELECTRA™ 25-OH Vitamin D CLIA Quantitative CLIA assay is for use on **ELECTRA** analyzers. **ELECTRA™ 25-OH Vitamin D 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.

The **ELECTRA™ 25-OH Vitamin D CLIA** test employs a pair of monoclonal agglutinating sera, first one is immobilized on solid phase (Microwells) and another monoclonal agglutinating sera is in the liquid phase. In the assay procedure, samples along with standards are added to the coated Microwells & incubated together with the first & second agglutinating sera. The wells are then washed to remove the unbound components. The resulted Vitamin D-antibody immunocomplex is detected with a third agglutinating sera conjugated with horseradish peroxidase (HRPO). 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 proportional to the amount of enzyme present and is directly related to the amount of Vitamin D in the sample. By reference to a series of Vitamin D calibrators assayed in the same way, the concentration of Vitamin D in the unknown sample is quantified.

MATERIALS & COMPONENTS

Materials provided with the test kits:

- Coated Microwells: Microwells coated with monoclonal anti-25-OH Vitamin D – antibody.
- Vitamin D Sample Diluent: Buffered solution containing monoclonal agglutinating sera stabilizing proteins and preservatives.
- Vitamin D Enzyme Conjugate. Ready to use. Agglutinating sera conjugated with Horse Raddish Peroxidase.
- Substrate A: Chemiluminescent substrate containing enhanced luminol solution.
- Substrate B: Chemiluminescent substrate containing stabilized peroxide solution.
- Two levels of controls (Control values are provided in the kit)
- Vitamin D Standard set of 6 calibrators labeled as A to F in liquid 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™ 25-OH Vitamin D CLIA** 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

- 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.
- Dilute Wash Buffer 20 times (for example add 5ml concentrated buffer to 95 ml distilled or deionized water). Mix well before use.
- Prepare a Working Substrate by Mixing Substrate A and Substrate B in equal volume (1:1 ratio) before addition to the micro-wells.

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 **10 µl** of Calibrators and Serum into the appropriate wells.
2. Dispense **200 µl** of Sample Diluent into each well. Gently shake the plate to mix the contents. Incubate at room temperature (18-25°C) for **10 minutes**.
3. Wash each well by filling approximately **350 µl** diluted Wash Buffer and aspirating / flicking off five times. Blot dry.
4. Dispense **100 µl** of Enzyme Conjugate into each well. Incubate at room temperature (18-25°C) for **10 minutes.**
5. Wash each well by filling approximately **350 µl** diluted Wash Buffer and aspirating / flicking off five times. Blot dry.
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™** micro plate 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 **10 µl** Calibrators/Samples into the respective Microwell

↓

Add **200 µl** Sample Diluent in each well

↓

Apply plate sealer and incubate for **10 minutes** at **18-25°C**

↓

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

↓

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

↓

Apply plate sealer and incubate for **10 minutes** at 18-25°C

↓

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

↓

Add **50 µl** working 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

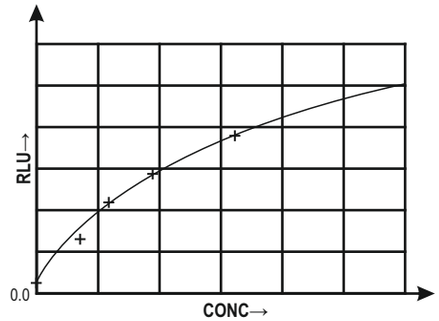
Construct a calibrator curve by plotting the mean RLU obtained from each reference calibrator against its concentration in ng/ml on the graph paper, with RLU values on the vertical or Y axis and concentrations on the horizontal or X axis. Use the RLU values for each specimen to determine the corresponding concentration of Vitamin D in ng/ml from the standard curve. Any diluted specimens must be corrected by the appropriate dilution factor.

Example of Standard curve

Results of a typical calibrator run with RLU's shown in the Y axis against 25-OH Vitamin D concentrations shown in the X axis.

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

Vitamin D Values	RLU's
A	45826
B	196822
C	1001323
D	3071220
E	5414161
F	7869859



This calibrator curve is for the purpose of illustration only, and should not be used to calculate samples. Each user should obtain his or her own Calibrator curve and data.

Expected Ranges of values

Multiple guidelines for Vitamin D deficiency have been published. Recent literature has suggested the following ranges for the classification of Vitamin D status:

25-OH Vitamin D Level	Reference Range (ng/ml)
Deficient	0 – 10
Insufficient	10 – 30
Sufficient	30 – 100
Toxicity	>100

PERFORMANCE CHARACTERISTICS

External Evaluation:

Electra™ 25-OH Vitamin D CLIA has been evaluated by a NABL accredited lab against their reference method. In this evaluation **Electra™ 25-OH Vitamin D CLIA** has demonstrated 100% correlation with the reference method.

*Data file: Qualpro Diagnostics (A Division of Tulip Diagnostics Pvt. Ltd).

Internal Evaluation:

In an internal evaluation, 300 random samples collected from local lab were evaluated against a reference method & following is our observations:

Total samples = 300	ELECTRA™ 25-OH Vitamin D	Reference Method
Deficient	26	29
Insufficient	239	236
Sufficient	34	34
Toxicity	1	1

On the basis of the above evaluation data **ELECTRA™ 25-OH Vitamin D** has demonstrated 99.0% correlation with reference method.

electra™ Chemiluminescence assay
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