INSTRUCTIONS FOR USE

tVitD

VITROS Immunodiagnostic Products 25-OH Vitamin D Total Reagent Pack REF 684 2894

VITROS Immunodiagnostic Products 25-OH Vitamin D Total Calibrators REF 684 2893

Intended Use

For *in vitro* diagnostic use only.

VITROS Immunodiagnostic Products 25-OH Vitamin D Total Reagent Pack

For the quantitative measurement of total 25-OH vitamin D in human serum using the VITROS ECi/ECiQ/3600 Immunodiagnostic Systems and the VITROS 5600/XT 7600 Integrated Systems.

The results of the VITROS 25-OH Vitamin D Total test are used in the assessment of Vitamin D sufficiency. Test results may be used in conjunction with other clinical or laboratory data to assist the clinician in patient management.

VITROS Immunodiagnostic Products 25-OH Vitamin D Total Calibrators

For use in the calibration of the VITROS ECi/ECiQ/3600 Immunodiagnostic Systems and the VITROS 5600/XT 7600 Integrated Systems for the quantitative measurement of total 25-OH vitamin D in human serum.

Summary and Explanation of the Test

Vitamin D is a fat soluble steroid hormone that comes in two forms, vitamin D2 (ergocalciferol) and vitamin D3 (cholecalciferol). Vitamin D is synthesized from cholesterol upon skin exposure to UVB sunlight or through dietary intake. Vitamin D is hydroxylated in the liver to form 25-OH Vitamin D which is further hydroxylated in the kidney to form the biologically active form, 1,25-(OH)2 Vitamin D. The active hormone is tightly regulated by plasma parathyroid hormone levels and serum calcium and phosphorous levels. The active form, 1,25-(OH)2 Vitamin D, increases the intestinal absorption of calcium and phosphorous, both are required for regulating bone metabolism.

Vitamin D metabolites are bound to a vitamin D binding protein and are circulated throughout the body. The concentration of 1,25-(OH)2 Vitamin D is 1000 times lower than 25-OH Vitamin D and has a half life of 4 hours. Due to its half life of 2–3 weeks, 25-OH Vitamin D is the metabolite that is the most reliable clinical indicator of vitamin D status. [1] Also, 25-OH Vitamin D levels are indicative of the body's storage levels of vitamin D and correlate with the clinical symptoms of vitamin D deficiency. In the late 18th century, vitamin D was first recognized as an essential dietary component in the prevention of rickets. Recently, research has indicated that vitamin D deficiency may be linked to chronic diseases such as cancer (breast, colon and prostate), cardiovascular disease, osteoporosis, osteomalacia and several autoimmune diseases among others. [2]

Principles of the Procedure

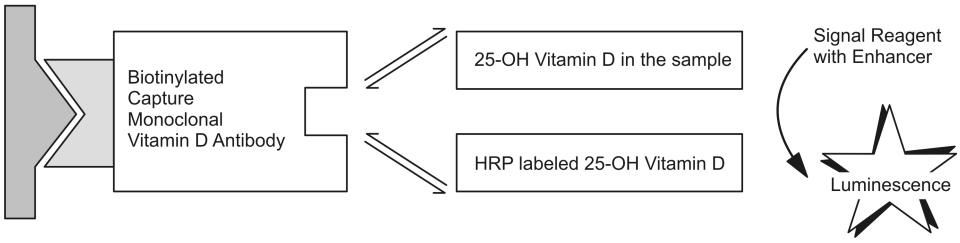
A competitive immunoassay technique is used which involves the release of the 25-OH Vitamin D in the sample from the binding protein using a low pH denaturant and the subsequent competition of the free 25-OH Vitamin D with horseradish peroxidase (HRP) labeled 25-OH Vitamin D reagent for monoclonal anti-Vitamin D bound to the wells. Unbound materials are removed by washing.

The bound HRP conjugate is measured by a luminescent reaction. [3] A reagent containing luminogenic substrates (a luminal derivative and a peracid salt) and an electron transfer agent, is added to the wells. The HRP in the bound conjugate catalyzes the oxidation of the luminol derivative, producing light. The electron transfer agent (a substituted acetanilide) increases the level of light produced and prolongs its emission. The light signals are read by the system. The amount of HRP conjugate bound is indirectly proportional to the concentration of 25-OH vitamin D present.

Test Type	System *	Incubation Time	Time to first result	Test Temperature	Reaction Sample Volume
Competitive Immunoassay	ECi/ECiQ, 3600, 5600, XT 7600	16 minutes	24 minutes	37 °C	60 µL

* Not all products and systems are available in all countries.

Reaction Scheme



Warnings and Precautions

WARNING:	Potentially Infectious Material Human blood products provided as components of the VITROS 25-OH Vitamin D Total Calibrators have been obtained from donors who were tested individually and who were found to be negative for hepatitis B surface antigen, and for antibodies to human immunodeficiency virus (HIV 1+2) and hepatitis C virus (HCV), using FDA approved methods (enzyme immunoassays). Treat as if capable of transmitting infection. Use caution when handling material of human origin. Consider all samples potentially infectious. No test method can offer complete assurance that hepatitis B virus, HCV, HIV 1+2 or other infectious agents are absent. Handle, use, store and dispose of solid and liquid waste from samples and test components in accordance with procedures defined by appropriate national biohazard safety guideline or regulation (e.g. CLSI document M29). [4]
WARNING:	Contains ProClin 950 (CAS 2682-20-4) [5] The VITROS 25-OH Vitamin D Total Reagent Pack and VITROS 25-OH Vitamin D Total Calibrators contain 0.5% ProClin 950. H317: May cause an allergic skin reaction. P280: Wear protective gloves/protective clothing/eye protection/face protection. P302 + P352: IF ON SKIN: Wash with plenty of soap and water. P333 + P313: If skin irritation or rash occurs: Get medical advice/attention. P363: Wash contaminated clothing before reuse. Refer to www.Orthoclinicaldiagnostics.com for the Safety Data Sheets and for Ortho contact information.

WARNING

Reagents

Reagent Pack Contents

1 reagent pack containing:

- 100 coated wells (streptavidin, bacterial; binds ≥0.33 pmol sheep IgG/well)
- 6.9 mL conjugate reagent (HRP-25-OH VitD) with horse serum and bovine gamma globulin
- 10.0 mL dissociation reagent in buffer

Reagent Pack Handling

- The reagent pack is supplied ready for use.
- The reagent pack contains homogeneous liquid reagents that do not require shaking or mixing prior to loading onto the system.

- As with all immunoassay protein-based solutions, inappropriate handling of the reagent pack can cause foam to occur on the surface of the reagent. Avoid agitation, which may cause foaming or the formation of bubbles.
  - If reagent packs are dropped or agitated, small levels of fine foam could be generated that may not be detected by the system.
  - Reagent packs containing fine foam that is not detected by the system, may show a negative bias.
- If you must use a dropped or agitated reagent pack before it has been allowed to settle, you should verify performance by running high and low quality control samples in duplicate after loading the pack on the system.

Reagent Pack Storage and Preparation

Reagent	Storage Condition		Stability
Unopened	Refrigerated	2–8 °C (36–46 °F)	expiration date
Opened	On system	System turned on	≤8 weeks
Opened	Refrigerated	2–8 °C (36–46 °F)	≤8 weeks

- The VITROS 25-OH Vitamin D Total Reagent Pack is suitable for use until the expiration date on the carton when stored and handled as specified. Do not use beyond the expiration date.
- Do not freeze unopened reagent packs.
- Load reagent packs directly from refrigerated storage to minimize condensation.
- Store opened refrigerated reagent packs in a sealed reagent pack storage box that contains dry desiccant.

Calibrator Contents

- 3 sets of VITROS 25-OH Vitamin D Total Calibrators 1 and 2 (freeze-dried, 25-OH Vitamin D in human serum with antimicrobial agent, reconstitution volume 1 mL); nominal values 28 and 120 ng/mL (70 and 300 nmol/L)
- Lot calibration card
- Protocol card
- 16 calibrator bar code labels (8 for each calibrator)

Calibrator Handling

- Use only with reagent packs of the same lot number. Mix thoroughly by inversion and bring to 15–30 °C (59–86 °F) before use. Each pack contains sufficient for a minimum of 6 determinations of each calibrator.
- Handle calibrators in stoppered containers to avoid contamination and evaporation. To avoid evaporation, limit the amount of time calibrators are on the system. Refer to the operating instructions for your system. Return to 2–8 °C (36–46 °F) as soon as possible after use, or load only sufficient for a single determination.

Calibrator Storage and Preparation

Calibrator	Storage Condition		Stability
Unopened	Refrigerated	2–8 °C (36–46 °F)	expiration date
Opened, reconstituted	Refrigerated	2–8 °C (36–46 °F)	≤7 days
Opened, reconstituted	Frozen	≤-20 °C (≤-4 °F)	≤13 weeks

- VITROS 25-OH Vitamin D Total Calibrators are supplied freeze-dried.
- VITROS 25-OH Vitamin D Total Calibrators are suitable for use until the expiration date on the carton when stored and handled as specified. Do not use beyond the expiration date.
- The VITROS 25-OH Vitamin D Total test uses 120 µL of calibrator for each determination. Transfer an aliquot of each calibrator into a sample container (taking account of the minimum fill volume of the container), which may be bar coded with the labels provided. For details on minimum fill volume of sample cups or containers, refer to the operating instructions for your system.
- VITROS 25-OH Vitamin D Total Calibrators are automatically processed in duplicate.
- Reconstitute with 1 mL distilled water.
- Opened, reconstituted calibrators may be stored frozen (with no more than 1 freeze-thaw cycle).

Specimen Collection, Preparation and Storage

Patient Preparation

No special patient preparation is necessary.

Specimens Recommended

Serum

Specimens Not Recommended

- Do not use turbid specimens. Turbidity in specimens may affect test results.
- Plasma:
  - Citrate
  - Heparin
  - EDTA

Special Precautions

IMPORTANT:	Certain collection devices have been reported to affect other analytes and tests. [6] Owing to the variety of specimen collection devices available, Ortho Clinical Diagnostics is unable to provide a definitive statement on the performance of its products with these devices. Confirm that your collection devices are compatible with this test.

Specimen Collection and Preparation

- Collect specimens using standard procedures. [7, 8]
- Samples should be thoroughly separated from all cellular material. Failure to do so may lead to an erroneous result.
- Thoroughly mix samples by inversion and bring to 15–30 °C (59–86 °F) before use.
- The VITROS 25-OH Vitamin D Total test uses 60 µL of sample for each determination. This does not take account of the minimum fill volume of the chosen sample container. For details on minimum fill volume of sample cups or containers, refer to the operating instructions for your system.

Handling and Storage Conditions

- Handle samples in stoppered containers to avoid contamination and evaporation.
- Follow procedures within your laboratory to avoid cross contamination of patient specimens.
- The amount of time samples are on the system prior to analysis should be limited to avoid evaporation. Refer to the operating instructions for your system.
- Return to 2–8 °C (36–46 °F) as soon as possible after use, or load sufficient volume for a single determination.
- Serum samples may be stored for up to 7 days at 2–8 °C (36–46 °F) or 4 weeks at -20 °C (-4 °F).
- Avoid repeated freeze-thaw cycles.

Testing Procedure

Materials Provided

- VITROS Immunodiagnostic Products 25-OH Vitamin D Total Reagent Pack
- VITROS Immunodiagnostic Products 25-OH Vitamin D Total Calibrators

Materials Required but Not Provided

- VITROS Immunodiagnostic Products Signal Reagent
- VITROS Immunodiagnostic Products Universal Wash Reagent
- Quality control materials
- VITROS Immunodiagnostic Products Reagent Pack Storage Box (optional) with desiccant
- Calibrated pipette, distilled water and sample containers for reconstitution of VITROS 25-OH Vitamin D Total Calibrators

Operating Instructions

Check the inventory regularly to aid the management of reagents and ensure that sufficient VITROS Signal Reagent, VITROS Universal Wash Reagent and calibrated reagent lots are available for the work planned. When performing panels of tests on a single sample, ensure that the sample volume is sufficient for the tests ordered.

For detailed information refer to the operating instructions for your system.

Note:	Do not use visibly damaged product.

Sample Dilution

Samples may be manually diluted up to 2-fold with a suitable human serum with a low analyte concentration prior to the test.

Manual Sample Dilution

1. Dilute the sample with a patient serum sample with a low 25-OH vitamin D concentration.
2. Re-analyze.

3. If necessary, correct for 25-OH vitamin D concentration in the diluents.

Multiply the results by the dilution factor and subtract the tVitD concentration from the diluent sample (if applicable) to obtain an estimate of the original sample's 25-OH vitamin D concentration.

Default Test Name

The default test name which will appear on patient reports is VITAMIN D TOTAL. The default short name that will appear on the test selection menus and laboratory reports is tVitD. These defaults may be reconfigured, if required. For detailed information refer to the operating instructions for your system

Calibration

Calibration Procedure

- Calibration is lot specific; reagent packs and calibrators are linked by lot number. Reagent packs from the same lot may use the same calibration.
- A Master Calibration (a dose response curve covering the full calibration range) is established for each new reagent lot. Concentrations for the linked lot of calibrators are determined from the Master Calibration.
- Ensure that the Master Calibration for each new reagent lot is available on your system.
- Process calibrators in the same manner as samples. Calibration need not be programmed if bar code labels are used; load the calibrators in any order, calibration will be initiated automatically.
- When the calibrators are processed the signal expected for each calibrator is compared against the actual signal obtained. The Master Calibration is then rescaled to reflect the differences between the actual and expected signals. The validity of this calibration curve is assessed against a range of quality parameters, and if acceptable, it is stored for use with any reagent pack of that lot.
- The quality of calibration cannot be completely described by a single parameter. The calibration report should be used in conjunction with acceptable control values to determine the validity of the calibration.
- Recalibration is required after a pre-determined calibration interval, or when a different reagent lot is loaded.
- Calibration results are assessed against a range of quality parameters. Failure to meet any of the defined quality parameter ranges will be coded in the calibration report. For actions to be taken following a failed calibration refer to the operating instructions for your system.
- Refer to the operating instructions for your system for detailed instructions on the calibration process.

When to Calibrate

- Calibrate when the reagent pack and calibrator lot changes.
- Calibrate every 28 days.
- After specified service procedures have been performed.
- If quality control results are consistently outside of your acceptable range.

For additional information on when to calibrate, refer to the operating instructions for your system.

Traceability of Calibration

Calibration of the VITROS 25-OH Vitamin D Total test is traceable to in house reference calibrators, which have been value assigned to correlate to samples measured by LC-MS-MS.

Calibration Model

A modified four-parameter logistic curve fit function is used to construct the Master Calibration. The calibration process rescales the Master Calibration to establish a valid stored curve for the VITROS Immunodiagnostic and VITROS Integrated Systems.

Measuring (Reportable) Range

System	Measuring (Reportable) Range
ECi/ECiQ, 3600, 5600, XT 7600	8.00*–150 ng/mL (20.0–375 nmol/L)

* lower limit of measuring range reported by the system software is based on the Limit of Quantitation.

Quality Control

Quality Control Material Selection

Controls containing suitable levels of 25-OH vitamin D are recommended for use with the VITROS Immunodiagnostic and VITROS Integrated Systems. The performance of commercial control fluids should be evaluated for compatibility with this test before they are used for quality control.

Control materials may show a difference when compared with other 25-OH vitamin D methods if they contain high concentrations of preservatives, stabilizers, or other nonphysiological additives, or otherwise depart from a true human sample matrix.

Appropriate quality control value ranges must be established for all quality control materials used with the VITROS 25-OH Vitamin D Total test.

Quality Control Procedure Recommendations

- Good laboratory practice requires that controls be processed to verify the performance of the test.
- Choose control levels that check the clinically relevant concentrations.
- To verify system performance, analyze control materials:
  - After calibration
  - According to local regulations or at least once each day that the test is being performed
  - After specified service procedures are performed

If quality control procedures within your laboratory require more frequent use of controls, follow those procedures.

- Analyze quality control materials in the same manner as patient specimens.
- If control results fall outside your acceptable range, investigate the cause before deciding whether to report patient results.
- Refer to published guidelines for general quality control recommendations. [9]

For more detailed information, refer to the operating instructions for your system.

Quality Control Material Preparation and Storage

Refer to the manufacturer's product literature for preparation, storage, and stability information.

Results

Results are automatically calculated by the VITROS Immunodiagnostic and VITROS Integrated Systems.

Reporting Units and Unit Conversion

Analyte results are quoted in units of ng/mL or nmol/L. To configure the units, refer to the operating instructions for your system.

Conventional	Alternate
ng/mL (nmol/L× 0.4)	nmol/L (ng/mL× 2.5)

Limitations of the Procedure

Known Interferences

The VITROS 25-OH Vitamin D Total test was evaluated for interference consistent with CLSI document EP7. [10] Commonly encountered substances were tested on 2 lots of reagents. Of the compounds tested, Paricalcitol (Zemplar) may interfere with the VITROS 25-OH Vitamin D Total test. Paricalcitol (Zemplar), when tested, caused a positive bias at the concentration indicated.

Refer to Substances that do not Interfere for a list of other compounds tested that did not show interference.

			Units = ng/mL		Units = nmol/L	
Interferent	Interferent Concentration		Analyte Conc*	Bias**	Analyte Conc*	Bias**
Paricalcitol (Zemplar)	24 ng/mL	57.6 nmol/L	10.5	125	26.3	313

* Average test concentration of replicate determinations using 2 different lots of reagent.

** Estimate of the average difference observed.

Note: These results are representative. The degree of interference at concentrations other than those listed might not be predictable from these results. Other interfering substances may be encountered in the patient population.

Other Limitations

- The results from this or any other diagnostic test should be used and interpreted only in the context of the overall clinical picture.
- Certain drugs and clinical conditions are known to alter 25-OH Vitamin D concentrations *in vivo*. For additional information, refer to one of the published summaries. [11- 13]

- Heterophilic antibodies in serum or plasma samples may cause interference in immunoassays. [14] These antibodies may be present in blood samples from individuals regularly exposed to animals or who have been treated with animal serum products. Results that are inconsistent with clinical observations indicate the need for additional testing.

Expected Values

It is recommended that each laboratory establish its own expected values for the population it serves.

A review of the most recent literature [15] suggests the recommendation for 25-OH Vitamin D levels are:

Level	Range	
	ng/mL	nmol/L
Deficient	<20	<50
Insufficient	20–<30	50–<75
Sufficient	30–100	75–250
Potential Toxicity	>100	>250

A study was conducted using 399 apparently healthy adults between the ages of 21–79. Samples came from individuals who live in the North, South and Central regions of the United States and were collected in both summer and winter. These samples were tested using the VITROS 25-OH Vitamin D Total test and the observed values are summarized below:

Observed Values	ng/mL	nmol/L
Median 25 OH Vitamin D	33.4	83.5
Observed Range 2.5th to 97.5th Percentile	14.7–68.3	36.8–171

Performance Characteristics

Limit of Detection

The Limit of Detection (LoD) for VITROS 25-OH Vitamin D Total test is 7.43 ng/mL (18.6 nmol/L), determined consistent with CLSI document EP17 [16] and with proportions of false positives (α) less than 5% and false negatives (β) less than 5%; based on 600 determinations, with 1 blank and 5 low-level samples. The Limit of Blank (LoB) is 2.64 ng/mL (6.60 nmol/L). The Limit of Quantitation (LoQ) is 8.00 ng/mL (20.0 nmol/L) as determined by the lowest concentration at which precision design requirements are still met and within the linear range of the test.

At 8.00 ng/mL (20.0 nmol/L), the observed imprecision (%CV) ranged from 23.0–30.0% across lots and analyzers.

Limit of Blank, Limit of Detection and Limit of Quantitation

LoB		LoD*		LoQ	
ng/mL	nmol/L	ng/mL	nmol/L	ng/mL	nmol/L
2.64	6.60	7.43	18.6	8.00	20.0

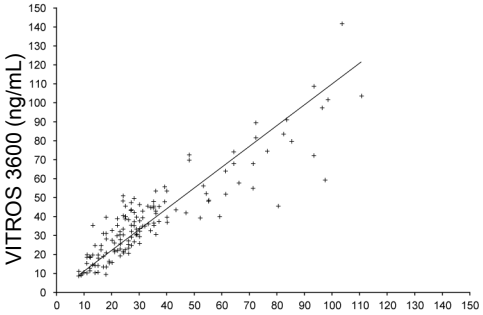
* Proportions of false positives (α) and false negatives (β) were less than 5%; based on 600 determinations, with 1 blank and 5 low-level samples.

Accuracy (Method Comparison)

Accuracy was evaluated consistent with CLSI document EP9. [17] The plots and tables show the results of a method comparison study using patient serum samples analyzed on the VITROS 3600 Immunodiagnostic System compared with those analyzed using the LC-MS/MS test and the DiaSorin LIAISON test. The relationship between the VITROS Immunodiagnostic and Integrated Systems and comparator methods was determined by Passing and Bablok regression. [18]

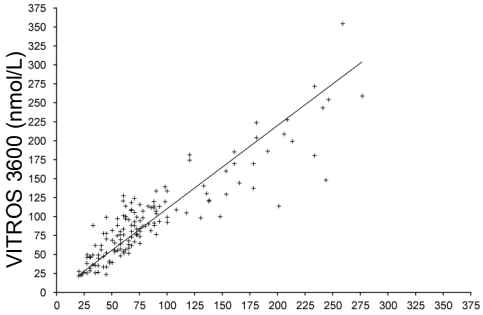
The table also shows the results of method comparison studies using patient serum samples analyzed on the VITROS 3600 Immunodiagnostic System compared with those analyzed using the VITROS ECi/ECiQ Immunodiagnostic System and the VITROS 5600 Integrated System. The relationship between the 2 methods was determined by Passing and Bablok regression. [18]

Conventional Units



Comparative Method: LC-MS/MS test (ng/mL)

Alternate Units

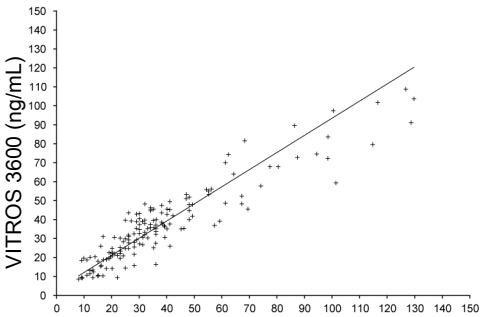


Comparative Method: LC-MS/MS test (nmol/L)

System	n	Slope	Correlation Coefficient	Conventional Units (ng/mL) Range of Samples	Conventional Units (ng/mL) Intercept	Alternate Units (nmol/L) Range of Samples	Alternate Units (nmol/L) Intercept
3600 vs. LC-MS/MS	154	1.09	0.90	8.48–141	0.49	21.2–353	1.23
5600* vs. 3600	152	1.01	0.97	8.48–108	1.49	21.2–270	3.73
ECi/ECiQ vs. 3600	155	0.94	0.96	8.48–141	1.35	21.2–353	3.38

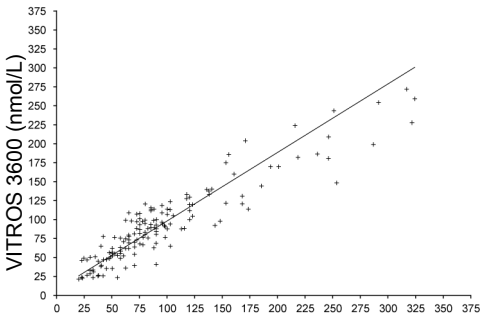
* Performance characteristics for the VITROS 5600 System are applicable to the VITROS XT 7600 System.

Conventional Units



Comparative Method: DiaSorin LIAISON test (ng/mL)

Alternate Units



Comparative Method: Diasorin LIAISON test (nmol/L)

System	n	Slope	Correlation Coefficient	Conventional Units (ng/mL) Range of Samples	Conventional Units (ng/mL) Intercept	Alternate Units (nmol/L) Range of Samples	Alternate Units (nmol/L) Intercept
3600 vs. DiaSorin LIAISON	152	0.90	0.92	8.79–108	3.09	22.0–270	7.73
5600* vs. 3600	152	1.01	0.97	8.48–108	1.49	21.2–270	3.73
ECi/ECiQ vs. 3600	155	0.94	0.96	8.48–141	1.35	21.2–353	3.38

* Performance characteristics for the VITROS 5600 System are applicable to the VITROS XT 7600 System.

Precision

VITROS ECi/ECiQ Immunodiagnostic System

Precision was evaluated consistent with NCCLS document EP5. [19] Two replicates each of 4 patient samples and 1 commercial control sample were tested on 2 separate occasions per day on at least 20 different days. The experiment was performed using 2 reagent lots on 2 different systems. The data presented are a representation of the product performance.

VITROS 3600 Immunodiagnostic System and VITROS 5600 Integrated System

Precision was evaluated consistent with NCCLS document EP5. [19] Two replicates each of 4 patient samples and 1 commerical control sample were tested on 2 separate occasions per day on at least 20 different days. The experiment was performed using 3 reagent lots on 2 different systems. The data presented are a representation of the product performance.

System	Units = ng/mL							No. Observ.	No. Days
	Mean 25-OH Vitamin D Total Conc.	Within-run*		Within-calibration**		Within-lab***			
		SD	CV (%)	SD	CV (%)	SD	CV (%)		
ECi/ECiQ System 1 Lot 1	22.5	1.66	7.4	3.14	14.0	3.43	15.3	80	20
	31.1	2.25	7.2	3.86	12.4	4.13	13.3	80	20
	70.0	3.86	5.5	5.86	8.4	6.24	8.9	80	20
	121	4.1	3.4	6.1	5.1	6.7	5.5	80	20
ECi/ECiQ System 2 Lot 2	20.7	2.46	12.0	3.32	16.2	3.43	16.4	80	20
	28.1	3.06	11.0	3.34	12.0	3.43	12.1	80	20
	65.0	5.20	8.1	5.66	8.8	5.94	9.1	80	20
	108	4.1	3.8	5.5	5.2	5.9	5.4	80	20
3600 System 1 Lot 1	22.9	2.26	10.5	2.90	13.5	4.04	16.5	80	20
	31.6	2.66	8.9	3.36	11.2	4.65	14.0	80	20
	72.2	4.30	6.1	5.73	8.1	6.80	9.2	80	20
	123	4.8	3.9	6.5	5.3	7.4	6.0	80	20
3600 System 1 Lot 3	21.0	3.22	15.3	3.29	15.6	3.32	15.8	80	20
	29.5	3.35	11.3	3.43	11.6	3.62	12.3	80	20
	71.1	5.93	8.3	6.07	8.5	5.92	8.4	80	20
	120	5.8	4.7	5.9	4.9	5.8	4.8	80	20
5600**** System 1 Lot 2	23.5	2.43	10.1	2.95	12.2	2.93	12.8	80	20
	31.9	2.52	7.7	3.22	9.9	3.13	10.1	80	20
	69.4	3.75	5.3	4.82	6.8	4.39	6.5	80	20
	117	6.1	5.1	6.5	5.4	6.4	5.6	80	20

* Within-run (repeatability). Between Duplicate precision averaged over all runs

** Within-calibration. Total precision with weighted components of within-run, between–run and between-day variation

*** Within-lab. A measure of the effect of recalibration on total precision, calculated within reagent lot, using data from at least 4 calibrations

**** Performance characteristics for the VITROS 5600 System are applicable to the VITROS XT 7600 System.

System	Units = nmol/L							No. Observ.	No. Days
	Mean 25-OH Vitamin D Total Conc.	Within-run*		Within-calibration**		Within-lab***			
		SD	CV (%)	SD	CV (%)	SD	CV (%)		
ECi/ECiQ System 1 Lot 1	56.3	4.15	7.4	7.85	14.0	8.58	15.3	80	20
	77.8	5.63	7.2	9.65	12.4	10.30	13.3	80	20
	175	9.7	5.5	14.7	8.4	15.6	8.9	80	20
	303	10.3	3.4	15.3	5.1	16.8	5.5	80	20
ECi/ECiQ System 2 Lot 2	51.8	6.15	12.0	8.30	16.2	8.58	16.4	80	20
	70.3	7.65	11.0	8.35	12.0	8.58	12.1	80	20
	163	13.0	8.1	14.2	8.8	14.9	9.1	80	20
	270	10.3	3.8	13.8	5.2	14.8	5.4	80	20
3600 System 1 Lot 1	57.3	5.65	10.5	7.25	13.5	10.10	16.5	80	20
	79.0	6.65	8.9	8.40	11.2	11.60	14.0	80	20
	181	10.8	6.1	14.3	8.1	17.0	9.2	80	20
	308	12.0	3.9	16.3	5.3	18.5	6.0	80	20
3600 System 1 Lot 3	52.5	8.05	15.3	8.23	15.6	8.30	15.8	80	20
	73.8	8.38	11.3	8.58	11.6	9.05	12.3	80	20
	178	14.8	8.3	15.2	8.5	14.8	8.4	80	20
	300	14.5	4.7	14.8	4.9	14.5	4.8	80	20

System	Units = nmol/L							No. Observ.	No. Days
	Mean 25-OH Vitamin D Total Conc.	Within-run*		Within-calibration**		Within-lab***			
		SD	CV (%)	SD	CV (%)	SD	CV (%)		
5600**** System 1 Lot 2	58.8	6.08	10.1	7.38	12.2	7.33	12.8	80	20
	79.8	6.30	7.7	8.05	9.9	7.83	10.1	80	20
	174	9.4	5.3	12.1	6.8	11.0	6.5	80	20
	293	15.3	5.1	16.3	5.4	16.0	5.6	80	20

* Within-run (repeatability). Between Duplicate precision averaged over all runs

** Within-calibration. Total precision with weighted components of within-run, between–run and between-day variation

*** Within-lab. A measure of the effect of recalibration on total precision, calculated within reagent lot, using data from at least 4 calibrations

**** Performance characteristics for the VITROS 5600 System are applicable to the VITROS XT 7600 System.

Specificity

Substances that do not Interfere

The VITROS 25-OH Vitamin D Total test was evaluated for interference consistent with CLSI document EP7. [10] Of the compounds tested, none was found to cause a bias of >10% with the test at the concentrations indicated at 25-OH Vitamin D concentrations of 30–80 ng/mL (75–200 nmol/L).

Compound	Concentration	
Acetaminophen	1324 µmol/L	200 µg/mL
Acetylsalicylic Acid	3.62 mmol/L	65.16 mg/dL
Bilirubin (unconjugated)	513 µmol/L	30 mg/dL
Bilirubin (conjugated)	356 µmol/L	30 mg/dL
Biotin	61.35 nmol/L	1.5 µg/dL
Hemoglobin (hemolysate)	0.124 mmol/L	200 mg/dL
Ibuprofen	0.576 mmol/L	12 mg/dL
Triolein	33.0 mmol/L	3000 mg/dL

Endogenous Substances that do not interfere

The VITROS 25-OH Vitamin D Total test was evaluated for interference consistent with CLSI document EP7 A2-Section 8. [10] Samples were collected from a normal cohort and a 'disease' or 'clinical sub-group' cohort and tested for 25-OH Vitamin D Total levels. Samples were tested on a comparative test: LC-MS/MS, the distribution of bias to the comparative test of normal vs. disease was evaluated. The confidence interval shows no statistical difference in the results.

Clinical Sub-Group	High Level Tested
Cholesterol	306 mg/dL
HAMA	160,000 IU/mL
Rheumatoid Factor	3800 IU/mL
Total Protein	12.5 g/dL
Triglycerides	491 mg/dL

Cross-Reactivity

The cross-reactivity of the VITROS 25-OH Vitamin D Total test was evaluated by adding the following substances to samples containing 25-OH Vitamin D.

Compound	Concentration	Sample 25-OH Vitamin D Concentration		Mean 25-OH Vitamin D Result of Cross-reactant Pool		% Cross-reactivity
		ng/mL	nmol/L	ng/mL	nmol/L	
Vitamin D_2(Ergocalciferol)	100 ng/mL	8.81	22.0	9.77	24.4	1.0
Vitamin D_3(Cholecalciferol)	100 ng/mL	8.81	22.0	9.66	24.2	0.9
25-OH Vitamin D_2	100 ng/mL	8.10	20.3	113	283	104.9
25-OH Vitamin D_3	100 ng/mL	8.10	20.3	107	268	98.9
1,25 $(OH)_2$Vitamin D_2	0.2 ng/mL*	8.81	22.0	10.1	25.3	>100
1,25 $(OH)_2$Vitamin D_3	0.2 ng/mL*	8.10	20.3	8.09	20.2	-5.0
24,25 $(OH)_2$Vitamin D_3	10 ng/mL*	7.92	19.8	11.4	28.5	34.8
3-epi 25-OH Vitamin D_3	100 ng/mL	7.92	19.8	45.3	113	37.4

* Levels tested were 2x to 4x the typical endogenous levels of analyte. [20, 21]

Cross-reactivity was expressed as the mean result obtained for the cross-reactant pool divided by the cross-reactant concentration in percentage term.

$$\% \text{ Cross-reactivity} = \frac{\text{Mean Value spiked (ng/mL)-Mean Value un-spiked (ng/mL)}}{\text{Concentration of Cross-reactant (ng/mL)}} \times 100$$

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Glossary of Symbols

The following symbols may have been used in the labeling of this product.

Do Not Reuse	Upper Limit of Temperature	Range
Use by or Expiration Date (Year-Month-Day)	Lower Limit of Temperature	Range of Means
LOT Batch Code or Lot Number	Temperature Limitation	Midpoint
SN Serial Number	Consult Instructions for Use	Revised
REF Catalog Number or Product Code	Attention: The Instructions for Use (IFU) has been updated	Supersedes
Caution	S1 For use in Slide Supply 1	Σ Contains Sufficient for "n" Tests
Keep Dry (Protect from Moisture/Humidity)	S2 For use in Slide Supply 2	IVD *in vitro* Diagnostic Medical Device
Manufacturer	SI SI Units	Der Grüne Punkt (the Green Dot). Manufacturer follows certain packaging material waste disposal management regulations
Date of Manufacture	CONV Conventional Units	σ Estimated within-lab SD
EC REP Authorized Representative in the European Community	Value	
Corrosive	Flammable	Serious Health Hazards
Health Hazards	Acute Toxicity	Environmental or Aquatic Toxicity

Revision History

Date of Revision	Version	Description of Technical Changes*
2019-09-06	9.1	• Glossary of Symbols: updated • Added EC Representative address
2017-09-27	9.0	• Added information for the VITROS XT 7600 Integrated System • Minor formatting and wording updates • References: updated • Glossary of Symbols: updated

* The change bars indicate the position of a technical amendment to the text with respect to the previous version of the document.

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