VITROS® Immunodiagnostic Products

INSTRUCTIONS FOR USE B-hCG

VITROS Immunodiagnostic Products Total β-hCG II Reagent Pack	REF	680 2220
VITROS Immunodiagnostic Products Total β-hCG II Calibrators	REF	680 2221

Rx ONLY

Intended Use

For *in vitro* diagnostic use only.

VITROS Immunodiagnostic Products Total β-hCG II Reagent Pack

For the quantitative measurement of human chorionic gonadotropin (hCG) and its β-subunit in human serum and plasma (heparin and EDTA) using the VITROS ECi/ECiQ/3600 Immunodiagnostic Systems and the VITROS 5600/XT 7600 Integrated Systems.

VITROS Immunodiagnostic Products Total β-hCG II 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 human chorionic gonadotropin (hCG) and its β-subunit in human serum and plasma (heparin and EDTA).

Summary and Explanation of the Test

The detection of hCG in urine or blood within 3-4 weeks of the last menstrual period is the most reliable indicator for the confirmation of pregnancy. hCG is initially secreted by the trophoblast, and later by the chorion and placenta. Levels rise exponentially to a peak during the first trimester, declining to a plateau during the second and third trimesters. Measurement of hCG has also been applied in the diagnosis of ectopic pregnancy, threatened abortion, and multiple gestation. [1-4] hCG levels may also be elevated in patients with neoplasms, which may or may not be of trophoblastic origin, e.g. cancers of the small intestines, lung, testes, breast and prostate, hydatidiform mole, choriocarcinoma and cerebral metastases. [5-7] Measurement of circulating hCG levels can be useful in monitoring the treatment of these conditions.

Principles of the Procedure

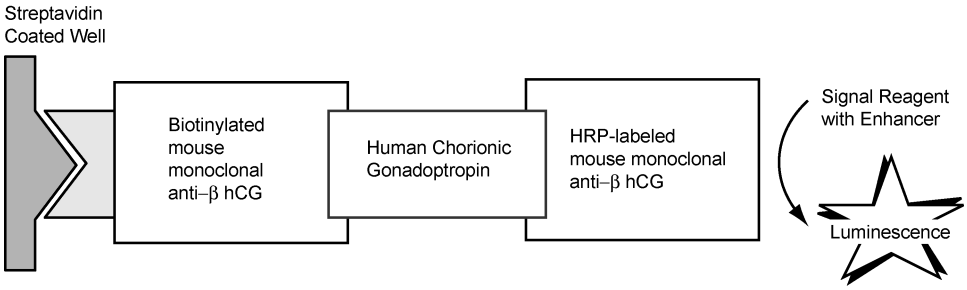
An immunometric immunoassay technique is used, which involves the reaction of hCG present in the sample with a biotinylated antibody (mouse monoclonal anti-β-hCG) and a horseradish peroxidase (HRP)-labeled antibody conjugate (mouse monoclonal anti-β-hCG). The antigen-antibody complex is captured by streptavidin on the wells. Unbound materials are removed by washing.

The bound HRP conjugate is measured by a luminescent reaction. [8] A reagent containing luminogenic substrates (a luminol 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 directly proportional to the concentration of hCG present.

Test Type	System *	Incubation Time	Time to first result	Test Temperature	Reaction Sample Volume
Immunometric	ECi/ECiQ, 3600, 5600, XT 7600	13 minutes, 20 seconds	24 minutes	37 °C	40 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 Total β-hCG II 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 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). [9]

WARNING: Contains Kathon or ProClin 200 (CAS 55965-84-9) [10]

The VITROS Total β-hCG II Reagent Pack and VITROS Total β-hCG II Calibrators contain 2.0% Kathon or ProClin 200. 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 ≥3 ng biotin/well)
- 14.4 mL biotinylated antibody reagent (biotin-mouse monoclonal anti-β-hCG, binds ≥5460 mIU hCG/mL) in buffer with mouse serum, bovine serum albumin, bovine gamma globulin and antimicrobial agent
- 19.2 mL conjugate reagent (HRP-mouse monoclonal anti-β-hCG, binds ≥4005 mIU hCG/mL) in buffer with bovine serum albumin and antimicrobial agent

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.

- Handle the reagent pack with care. Avoid the following:
  - allowing condensation to form on the pack
  - causing reagents to foam
  - agitation of the pack

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	≤12 weeks
Opened	Refrigerated	2–8 °C (36–46 °F)	≤12 weeks

- The VITROS Total β-hCG II 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 Total β-hCG II Calibrators 1, 2 and 3 (freeze-dried, recombinant hCG in human plasma with antimicrobial agent, reconstitution volume 1.0 mL), nominal values 0; 3000 and 14,000 mIU/mL (IU/L)
- Lot calibration card
- Protocol card
- 24 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)	≤13 weeks
Opened-reconstituted	Frozen	≤-20 °C (≤-4 °F)	≤13 weeks

- VITROS Total β-hCG II Calibrators are supplied freeze-dried.
- VITROS Total β-hCG II 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.
- Reconstitute with 1.0 mL distilled water.
- Opened, reconstituted calibrators may be stored frozen (with no more than 1 freeze-thaw cycle).
- The VITROS Total β-hCG II test uses 40 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.

Specimen Collection, Preparation and Storage

Patient Preparation

No special patient preparation is necessary.

Specimens Recommended

- Serum
- Heparin plasma
- EDTA plasma

Specimens Not Recommended

Do not use turbid specimens. Turbidity in specimens may affect test results.

Special Precautions

IMPORTANT:	Certain collection devices have been reported to affect other analytes and tests. [11] 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. [12- 13]
- 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 Total β-hCG II test uses 40 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.
- 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 and plasma samples may be stored for up to 5 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 Total β-hCG II Reagent Pack
- VITROS Immunodiagnostic Products Total β-hCG II Calibrators

Materials Required but Not Provided

- VITROS Immunodiagnostic Products Signal Reagent
- VITROS Immunodiagnostic Products Universal Wash Reagent
- VITROS Immunodiagnostic Products High Sample Diluent B
- Quality control materials such as VITROS Immunodiagnostic Products RE Controls
- VITROS Immunodiagnostic Products Reagent Pack Storage Box with desiccant
- Calibrated pipette, distilled water and sample containers for reconstitution of VITROS Total β-hCG II 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 with concentrations greater than the measuring range may be diluted automatically on the system up to 400-fold (1 part sample with 399 parts diluent) by the VITROS Immunodiagnostic and VITROS Integrated Systems with VITROS High Sample Diluent B Reagent Pack prior to test. Refer to the High Sample Diluent B Reagent Pack instructions for use.

Default Test Name

The default test name which will appear on patient reports is Total B-hCG II. The default short name that will appear on the test selection menus and laboratory reports is B-hCG. 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 test reagent 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 Total β-hCG II test is traceable to in-house reference calibrators, which have been value-assigned with reference to the 4th International Standard (NIBSC 75/589).

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
3600 5600 XT 7600 ECi/ECiQ	2.39*–15,000 mIU/mL (IU/L)

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

The lower limit reported by the system can be reconfigured if desired. For details on how to reconfigure the lower limit refer to the operating instructions for your system.

Quality Control

Quality Control Material Selection

VITROS RE Controls are recommended for use with the VITROS Immunodiagnostic and VITROS Integrated Systems. VITROS RE Controls contain 3 levels of hCG (low, medium and high). The performance of other 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 hCG 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 Total β-hCG II 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. [14]
- 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

hCG results are quoted in units of mIU/mL or IU/L. To configure the units, refer to the operating instructions for your system.

Conventional	Alternate
mIU/mL (IU/L× 1)	IU/L (mIU/mL× 1)

Limitations of the Procedure

Known Interferences

The VITROS Total β-hCG II test was evaluated for interference consistent with CLSI document EP7. [15] Commonly encountered substances were tested on 2 lots of reagents. Of the compounds tested, none was found to cause a bias of >10%.

Refer to "Specificity" for a list of other compounds tested that did not show interference.

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.
- Biotin levels in serum remain elevated for up to 24 hours after oral or intravenous biotin administration. [16]
- Heterophilic antibodies in serum or plasma samples may cause interference in immunoassays. [17] These antibodies may be present in blood samples from individuals regularly exposed to animals or who have been treated with animal serum products. Results which are inconsistent with clinical observations indicate the need for additional testing.
- Falsely elevated hCG results in serum and plasma [18-22] might be due to the presence of heterophilic antibodies, such as anti-mouse antibodies (HAMA), to nonspecific protein binding, and to hCG -like substances.
- A high dose hook effect was not observed in samples up to 1,300,000 mIU/mL (IU/L).
- If the hCG result is inconsistent with the clinical picture and is persistently elevated, the hCG result should be confirmed with a urine hCG test or by repeating the serum or plasma test on a different test system. [18]
- Interpretation of hCG results should be done in the context of an overall medical management protocol that includes results of other tests, clinical impressions and symptoms.
- Exogenous hCG administered within 7–10 days of sampling may give a detectable test result. When using the determination of hCG to confirm pregnancy, care should be taken to exclude the possibility of hCG secreting tumors.
- Detection of very low levels of hCG does not exclude pregnancy. A further sample should be tested after 48 hours if pregnancy is suspected.
- This test is not intended to be used for the risk evaluation of trisomy 21.

Expected Values and Interpretation of Results

It is recommended that each laboratory establish its own expected values for the population it serves. As a guide, the following ranges were determined. Concentrations of total β-hCG measured in samples from apparently healthy, non-pregnant individuals were determined to be ≤4.83 mIU/mL (IU/L) (290 samples).

Sample type	Number of samples	Units = mIU/mL (IU/L)				
		Mean	Min	Max	2.5th Percentile	97.5th Percentile
Normal Male	98	0.02	0.00	1.06	0.00	0.08
Normal Female	123	0.46	0.00	5.42	0.00	4.32
Post menopausal	69	1.52	0.00	6.66	0.00	6.46
Total	290	0.56	0.00	6.66	0.00	4.83

Concentrations of total β-hCG measured in the sera of pregnant females at defined gestational ages are summarized below.

Sample type	Number of samples	Units = mIU/mL (IU/L)				
		Mean	Min	Max	2.5th Percentile	97.5th percentile
Pregnant gestational age 1-10 weeks	112	31,142	44.71	256,740	63.7	150,854
Pregnant gestational age 11-15 weeks	43	55,425	11556	265,380	11795	151,996
Pregnant gestational age 16-22 weeks	50	27,023	7480.8	111,954	9383.8	61,410
Pregnant gestational age 23-40 weeks	45	24,031	1531.1	101,566	1737.2	98,576

Interpretation of Results

hCG results less than or equal to 5 mIU/mL (IU/L) are considered negative. hCG results greater than or equal to 25 mIU/mL (IU/L) are considered positive.

Performance Characteristics

Limit of Detection

The limit of detection (LoD) for the VITROS Total β-hCG II test is 0.70 mIU/mL (IU/L), determined consistent with NCCLS guideline EP17 [23] and with proportions of false positives (α) less than 5% and false negatives (β) less than 1%; based on 980 determinations, with 1 blank and 5 low-level samples. The Limit of Blank (LoB) is 0.05 mIU/mL (IU/L). The Limit of Quantitation (LoQ) is 2.39 mIU/mL (IU/L) as determined by the lowest concentration at which precision and accuracy design requirements are still met and within the linear range of the test.

Limit of Blank, Limit of Detection and Limit of Quantitation

LoB*	LoD**	LoQ
mIU/mL (IU/L)	mIU/mL (IU/L)	mIU/mL (IU/L)
0.05	0.70	2.39

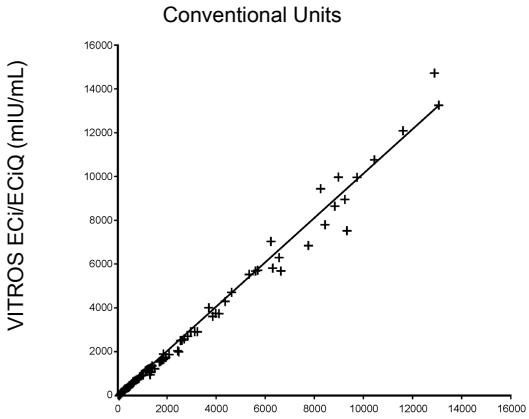
* Limit of Blank, or the highest value likely to be observed with a sample containing no analyte, replaces the term "analytical sensitivity."

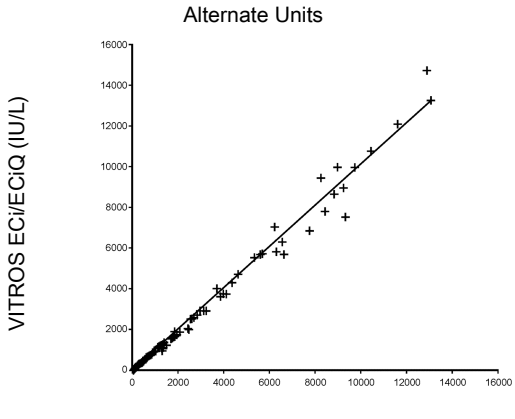
** Proportions of false positives (α) and false negatives (β) less than 5% and 1% respectively; based on 980 determinations, with 1 blank and 5 low-level samples.

Accuracy (Method Comparison)

Accuracy was evaluated consistent with NCCLS document EP9. [24] The plot and table show the results of a method comparison study using patient serum samples analyzed on the VITROS ECi/ECiQ Immunodiagnostic System compared with those analyzed using the Abbott ARCHITECT Total B-hCG test. The relationship between the 2 methods was determined by Deming regression. [25]

The table also shows the results of method comparison studies [24] using patient serum and plasma samples analyzed on the VITROS ECi/ECiQ Immunodiagnostic System compared with those analyzed using the VITROS 3600 Immunodiagnostic System and the VITROS 5600 Integrated System. The relationship between the 2 methods was determined by Passing and Bablok regression. [26]





System	n	Slope	Correlation Coefficient	Conventional Units (mIU/mL) Range of Sample	Conventional Units (mIU/mL) Intercept	Alternate Units (IU/L) Range of Samples	Alternate Units (IU/L) Intercept
ECi/ECiQ vs. Comparative Method	132	1.016	0.989	2.65–14,688	-95.04	2.65–14,688	-95.04
3600 vs. ECi/ECiQ	106	1.014	0.998	3.27–12,256	6.74	3.27–12,256	6.74
5600* vs. ECi/ECiQ	107	1.015	0.997	3.27–12,256	3.57	3.27–12,256	3.57

* 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. [27] Two replicates each of 3 freeze-dried control samples and 2 frozen patient sample pools 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. [27] Two replicates of each of 3 freeze-dried control samples were tested on 2 separate occasions per day on at least 20 different days. The experiment was performed using 1 reagent lot on each system. The data presented are representative of the product performance.

System	Units = mIU/mL (IU/L)							No. Observ.	No. Days
	Mean hCG Conc.	Within-run*		Within-calibration**		Within-lab***			
		SD	CV (%)	SD	CV (%)	SD	CV (%)		
ECi/ECiQ system 1	15.85	0.406	2.6	0.774	4.9	0.760	4.8	84	21
	48.35	0.610	1.3	1.97	4.1	1.85	3.8	80	20
	361.66	6.69	1.8	11.63	3.2	12.54	3.4	84	21
	4783.7	128.98	2.7	142.77	3.0	168.64	3.5	84	21
	9421.3	254.56	2.7	426.72	4.5	481.39	4.9	84	21
ECi/ECiQ system 2	17.03	0.235	1.4	0.396	2.3	0.349	2.0	88	22
	51.05	0.448	0.9	0.952	1.9	1.07	2.1	88	22
	376.39	3.58	1.0	7.30	1.9	8.80	2.3	88	22
	4853.0	73.91	1.5	119.66	2.5	156.29	3.2	88	22
	9459.4	203.84	2.2	290.06	3.1	336.42	3.5	88	22
3600	13.00	0.130	1.0	0.312	2.4	0.306	2.4	80	20
	42.43	0.490	1.2	1.08	2.6	1.11	2.6	80	20
	287.17	3.68	1.3	8.42	2.9	9.04	3.1	80	20
5600****	13.03	0.159	1.2	0.319	2.4	0.313	2.4	84	21
	42.26	0.439	1.0	0.828	2.0	0.789	1.8	84	21
	281.66	3.52	1.2	5.39	1.9	4.96	1.7	84	21

* 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 Total β-hCG II test was evaluated for interference consistent with CLSI document EP7. [15] Of the compounds tested, none was found to cause a bias of >10% with the test at the concentrations indicated at hCG concentrations of 0.00–62.85 mIU/mL (IU/L).

Compound	Concentration	
Bilirubin	20 mg/dL	0.342 mmol/L
Biotin	1000 ng/dL	0.040 µmol/L
Dipyrone	100 mg/dL	3.00 mmol/L
FSH	400 mIU/mL	400 IU/L
Hemoglobin*	500 mg/dL	0.310 mmol/L
LH	400 mIU/mL	400 IU/L
Sodium Azide	100 mg/dL	15.3 mmol/L
Triolein	3 g/dL	33.9 mmol/L
TSH	250 µIU/mL	250 mIU/L

* Hemoglobin was added to a series of specimens with hCG concentrations of 0.00 to 10,350 mIU/mL (IU/L).

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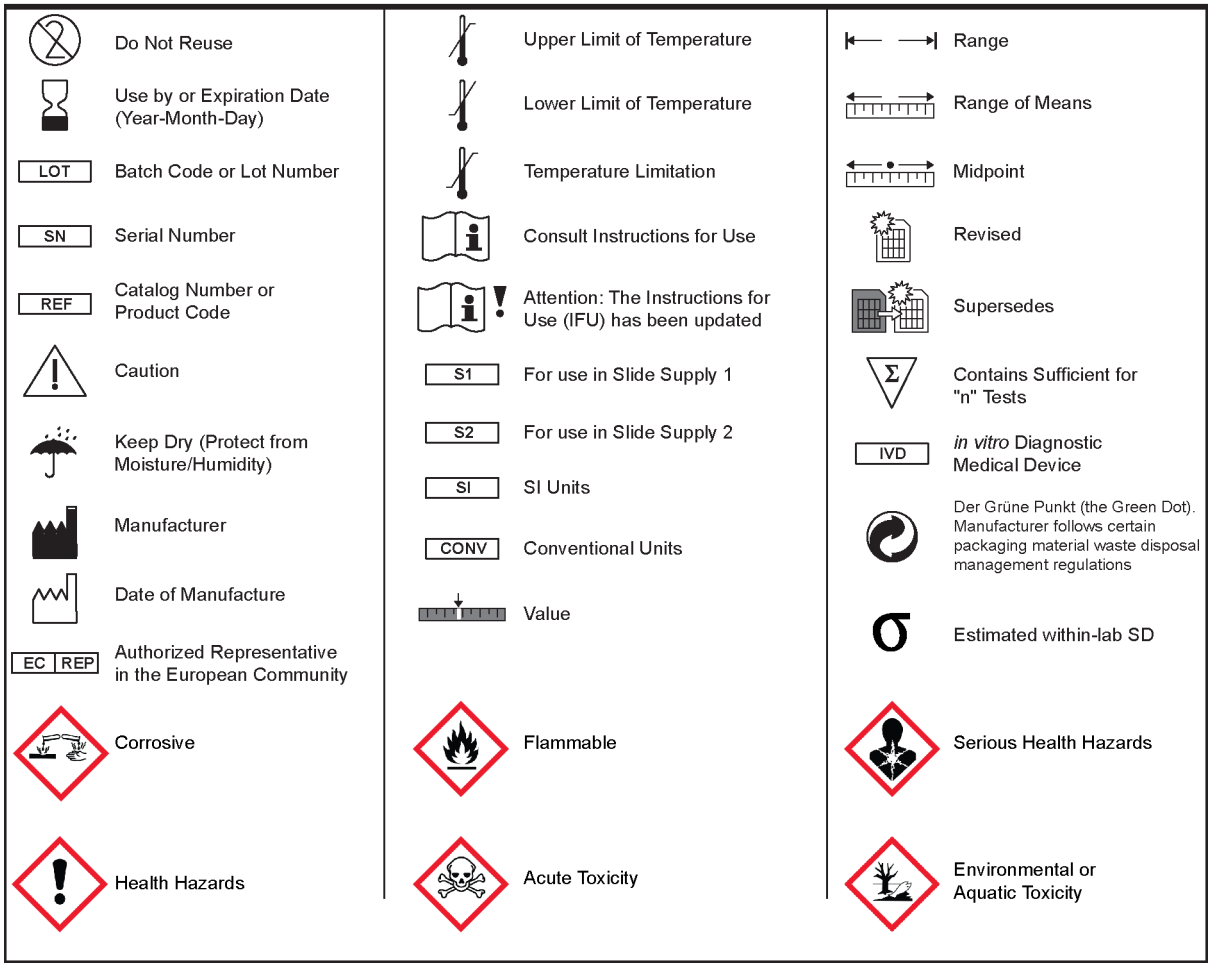
Glossary of Symbols

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



Revision History

Date of Revision	Version	Description of Technical Changes*
2019-11-18	13.0	Calibrator Contents: nominal values: changed from 13,500 mIU/mL to 14,000 mIU/mL

* 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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