VITROS Products Chemistry

INSTRUCTIONS FOR USE

ASO

VITROS Chemistry Products ASO Reagent

Antistreptolysin O

REF **680 2218**

Rx ONLY

Intended Use

For *in vitro* diagnostic use only.

VITROS Chemistry Products ASO Reagent is used on the VITROS 5,1 FS Chemistry System, the VITROS 4600 Chemistry System and the VITROS 5600 Integrated System for the quantitative determination of antibodies to streptolysin O in human serum and plasma. Determination of antibodies to streptolysin O can aid in the diagnosis of individuals infected with group A streptococcus bacteria.

Summary and Explanation of the Test

Group A β-hemolytic streptococci (*Streptococcus pyogenes*) infections can lead to development of post-streptococcal complications, including rheumatic fever, acute glomerulonephitis and reactive arthritis. [1] Because these sequelae usually do not present until 2-3 weeks after infection when tests for streptococci are no longer positive, clinical diagnosis is aided by measuring antibody response against extracellular products of Group A streptococcus, including streptolysin O, DNAase B, hyaluronidase, NADase, and streptokinase. [1, 2]

Antistreptolysin O (ASO) is the antibody response most often measured to provide serological evidence of recent infection of Group A streptococcus in patients suspected of having acute rheumatic fever or acute glomerulonephritis. ASO titers are elevated in the sera of 80% to 85% of patients with rheumatic fever and in 95% of patients with acute glomerulonephritis. [3] ASO titers begin to rise about 1 week after infection, peak 2–4 weeks later, and then usually fall to pre-infection levels within 6–12 months [3]. Streptococcal infections of the upper respiratory tract generally result in strong ASO response, whereas the ASO response is attenuated following infections of the skin. [2, 4] Confirmation of antecedent streptococcal infection can be improved by measuring other antibody responses such as anti-DNAase B. [2]

Principles of the Procedure

The quantitative measurement of antistreptolysin O is performed using the VITROS Chemistry Products ASO Reagent in conjunction with the VITROS Chemistry Products Calibrator Kit 28 and VITROS Chemistry Products FS Calibrator 1 on the VITROS 5,1 FS/4600 Chemistry System and the VITROS 5600 Integrated System.

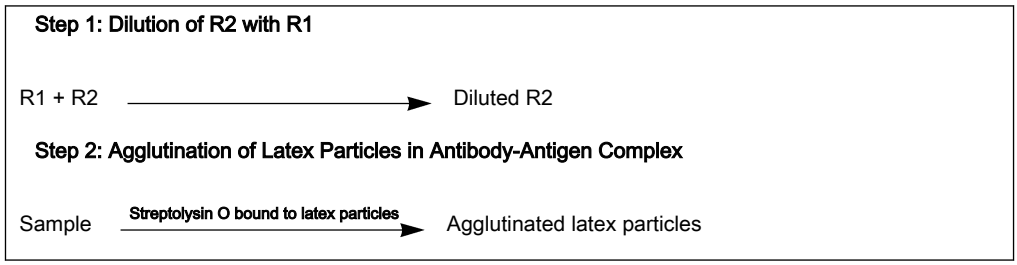
The VITROS ASO Reagent is a dual-chambered package containing stable, ready-to-use liquid reagents for use in a two-step reaction to quantitatively measure antistreptolysin O (ASO). In the first step, buffer contained in Reagent 1 is used to dilute streptolysin O bound to latex particles in Reagent 2. In the second step, test sample is added and an antigen-antibody reaction occurs between the antistreptolysin O in the sample and the streptolysin O bound to latex particles, resulting in agglutination. The agglutination is detected as an absorbance change at 575 nm, with the magnitude of the change being proportional to the quantity of ASO in the sample. After a calibration has been performed for each reagent lot, the ASO concentration in each unknown sample can be determined using the stored calibration curve and the measured absorbances obtained in the assay of the sample.

Test Type and Conditions

Test Type	VITROS System	Approximate Incubation Time	Temperature	Wavelength	Reaction Sample Volume
Two-point Rate	5600, 4600, 5,1 FS	Incubation 1: 5.2 minutes Incubation 2: 4.4 minutes	37 °C (98.6 °F)	575 nm	2.7 µL

Not all products and systems are available in all countries.

Reaction Scheme



Warnings and Precautions

For *in vitro* diagnostic use only.

This product contains bovine blood components and should be handled using the same precautions as with any other blood or blood-derived product.

WARNING:	***This product contains sodium azide. Disposal of reagents into sinks with copper or lead plumbing should be followed with plenty of water to prevent formation of potentially explosive metallic azides.***
WARNING:	***Take care when handling materials and samples of human origin. Since no test method can offer complete assurance that infectious agents are absent, consider all clinical specimens, controls, and calibrators potentially infectious. Handle specimens, solid and liquid waste, and test components in accordance with local regulations and CLSI Guideline M29[5] or other published biohazard safety guidelines.***

For specific warnings and precautions for calibrators, quality control materials, and other components, refer to the Instructions for Use for the appropriate VITROS product, or to other manufacturer's product literature.

Reagents

Reactive Ingredients

Reagent 1 (R1): None

Reagent 2 (R2): Latex particles coated with streptolysin O antigen, 0.17% (w/v)

Other Ingredients

Reagent 1 (R1): Preservatives, buffer, inorganic salt, protein

Reagent 2 (R2): Preservative, buffer, inorganic salt, protein

Reagent Handling

Caution:	**Do not use reagent packs with damaged or incompletely sealed packaging.**

- Inspect the packaging for signs of damage.
- Be careful when opening the outer packaging with a sharp instrument so as to avoid damage to the individual product packaging.
- The reagents are supplied ready for use.
- Avoid agitation, which may cause foaming or the formation of bubbles.

Reagent Preparation

1. Remove from refrigerated storage.
2. Immediately load into Supply 3.

IMPORTANT:	*Do not loosen or remove caps prior to loading.*

Reagent Storage and Stability

VITROS Chemistry Products ASO Reagent is stable until the expiration date on the carton when it is stored and handled as specified. Do not use beyond the expiration date.

IMPORTANT:	*Do not freeze.*

Reagent	Storage Condition		Stability
Unopened	Refrigerated	2–8 °C (36–46 °F)	Until expiration date
Opened	On-analyzer	System turned on	≤ 21 days*
	On-analyzer	System turned off	≤ 30 minutes

*Once a reagent pack is loaded, the system automatically determines the appropriate on analyzer stability time. The on analyzer stability time is continuously adjusted based upon the number of days the reagent pack is on the analyzer and the number of tests remaining in the pack. Refer to Reagent Management for the actual on analyzer stability time remaining for each reagent pack.

Verify performance with quality control material after reloading reagents that have been removed from Supply 3 and stored for later use.

Specimen Collection, Preparation and Storage

Specimens Recommended

- Serum
- Plasma:
  - Heparin
  - EDTA

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.*

Specimens Not Recommended

None identified.

Serum and Plasma

Specimen Collection and Preparation

Collect specimens using standard laboratory procedures.[7, 8]

Note: For details on minimum fill volume requirements, refer to the operating instructions for your system.

Patient Preparation

No special patient preparation is necessary.

Special Precautions

- EDTA plasma specimens collected in partially filled sample tubes may result in negative prediction biases.
- Centrifuge specimens and remove the serum or plasma from the cellular material within two hours of collection.[9]

Specimen Handling and Storage

- Handle and store specimens in stoppered containers to avoid contamination and evaporation.
- Mix samples by gentle inversion and bring to room temperature, 18–28 °C (64–82 °F), prior to analysis.

Specimen Storage and Stability

Storage	Temperature	Stability
Room Temperature	18–28 °C (64–82 °F)	≤ 4 days
Refrigerated	2–8 °C (36–46 °F)	≤ 7 days
Frozen	≤-20 °C (≤-4 °F)	2 months

IMPORTANT: *Avoid repeated freeze-thaw cycles.*

Testing Procedure

Materials Provided

VITROS Chemistry Products ASO Reagent

Materials Required but Not Provided

- VITROS Chemistry Products FS Calibrator 1

- VITROS Chemistry Products Calibrator Kit 28
- Quality control materials, such as VITROS Chemistry Products ASO/RF Performance Verifiers I and II
- Isotonic saline

Operating Instructions

- Check reagent inventories at least daily to ensure that quantities are sufficient for the planned workload.
- For additional information, refer to the operating instructions for your system.

IMPORTANT: *Bring all fluids and samples to room temperature, 18–28 °C (64–82 °F), prior to analysis.*

Sample Dilution

On-Analyzer Sample Dilution:

On-analyzer dilution is not recommended.

Manual Sample Dilution:

If antistreptolysin O concentrations exceed the system's measuring (reportable) range:

1. Dilute 1 part sample with 1 part saline.
2. Re-analyze.
3. Multiply the results by 2 to obtain an estimate of the original sample's antistreptolysin O concentration.

Calibration

Required Calibrators

- VITROS Chemistry Products Calibrator Kit 28
- VITROS Chemistry Products FS Calibrator 1

Calibrator Preparation, Handling, and Storage

Refer to the Instructions for Use for VITROS Chemistry Products Calibrator Kit 28 and VITROS Chemistry Products FS Calibrator 1.

Calibration Procedure

Refer to the operating instructions for your system.

When to Calibrate

Calibrate:

- When the reagent lot number changes.
- When critical system parts are replaced due to service or maintenance.
- When government regulations require.
  For example, in the USA, CLIA regulations require calibration or calibration verification at least once every six months.

The VITROS ASO assay may also need to be calibrated:

- If quality control results are consistently outside acceptable range.
- After certain service procedures have been performed.

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

Calculations

Absorbance is measured at 575 nm at two fixed time points during the incubation period, and the change between these two readings is calculated. After a calibration has been performed for each reagent lot, antistreptolysin O concentration in the unknown samples can be determined using the stored calibration curve and the measured rate of absorbance obtained in the assay of each sample.

Validity of a Calibration

Calibration parameters are automatically assessed by the system against a set of quality parameters detailed in the Review Assay Data screen (found via Options → Review/Edit Calibrations → Review Assay Data). Failure to meet any of the pre-defined quality parameters results in a failed calibration. The calibration report should be used in conjunction with quality control results to determine the validity of a calibration.

Measuring (Reportable) Range

Conventional and SI Units (IU/mL)	Alternate Units (kIU/L)
25–900	25–900

Traceability of Calibration

The values assigned to the VITROS Chemistry Products Calibrator Kit 28 and VITROS Chemistry Products FS Calibrator 1 for antistreptolysin O (ASO) are traceable to the Antistreptolysin O, NIBSC Reagent 97/662. [10]

Quality Control

Quality Control Material Selection

IMPORTANT: *VITROS Chemistry Products ASO/RF Performance Verifiers are recommended for use with the VITROS 5,1 FS/4600 Chemistry and VITROS Integrated Systems. Evaluate the performance of other commercial control fluids for compatibility with this assay before using for quality control.*

Control materials other than VITROS Chemistry Products ASO/RF Performance Verifiers may show a difference when compared with other ASO methods if they:

- Depart from a true human matrix.
- Contain high concentrations of preservatives, stabilizers, or other nonphysiological additives.

Quality Control Procedure Recommendations

- Choose control levels that check the clinically relevant range.
- Analyze quality control materials in the same manner as patient samples, before or during patient sample processing.
- To verify system performance, analyze control materials:
  - After calibration.
  - According to local regulations or at least once each day that the assay is being performed.
  - After specified service procedures are performed. Refer to the operating instructions for your system.
- If control results fall outside your acceptable range, investigate the cause before deciding whether to report patient results.
- For general quality control recommendations, refer to CLSI, *Statistical Quality Control for Quantitative Measurements: Principles and Definitions; Approved Guideline-Third Edition* [11] or other published guidelines.
- For additional information, refer to the operating instructions for your system.

Quality Control Material Preparation, Handling, and Storage

Refer to the Instructions for Use for VITROS Chemistry Products ASO/RF Performance Verifiers I and II or to other manufacturer's product literature.

Results

Reporting Units and Unit Conversion

The VITROS 5,1 FS/4600 Chemistry and VITROS Integrated Systems may be programmed to report ASO results in conventional, SI, or alternate units.

Conventional Units	SI Units	Alternative Units
IU/mL	IU/mL (IU/mL × 1)	kIU/L (IU/mL x 1)

Limitations of the Procedure

Known Interferences

None identified.

Other Limitations

- No antigen excess effect was observed for samples with ASO concentrations up to 4000 IU/mL.
- Certain drugs and clinical conditions are known to alter antistreptolysin O concentrations *in vivo* or *in vitro*. [19]

Expected Values

Population	Conventional Units and SI Units (IU/mL)	Alternative Units (kIU/L)
Adults [12, 13]	≤200	≤200
Children [14]	≤240	≤240

Values above the indicated cutoffs for each population are consistent with recent infection with Group A streptococcus. These expected values are provided only as a guideline. Because ASO levels are dependent on factors such as age, geographical location of the patient, site of infection, season of the year and the incidence of streptococcal infections [13, 14, 15, 16, 17, 18] it is recommended that each laboratory establish expected values appropriate for the population it serves.

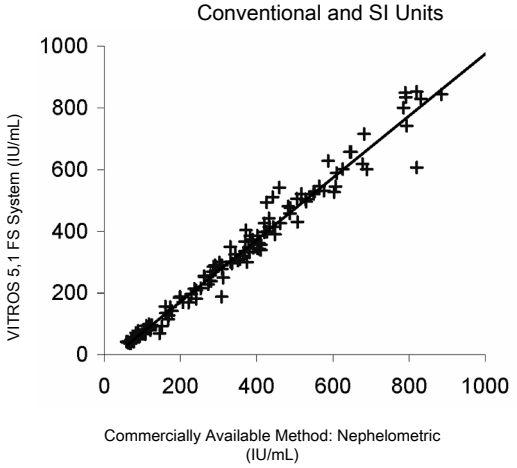
Performance Characteristics

Method Comparison

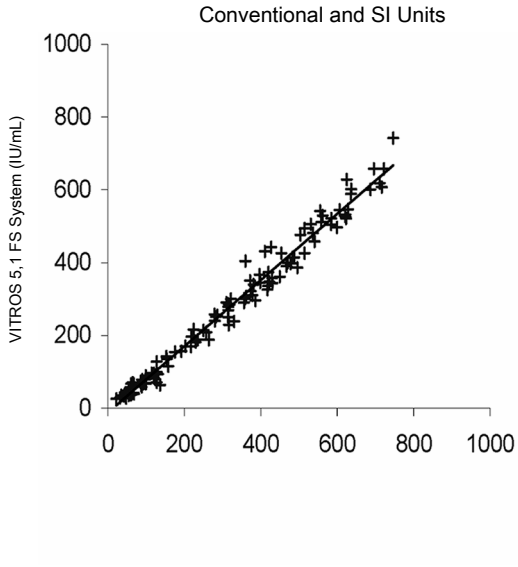
The plots and data below show the results of a method comparison study with serum samples analyzed on the VITROS 5,1 FS Chemistry System using the Dade Behring N Latex ASL Assay nephelometric method and using the COBAS INTEGRA Antistreptolysin O turbidimetric method, based on NCCLS Protocol EP9. [20]

The table also shows the results of comparisons with serum and plasma samples on the VITROS 5600 Integrated System and the VITROS 5,1 FS Chemistry System. The testing followed NCCLS Protocol EP9. [20]

Method Comparison: Nephelometric



Method Comparison: Turbidimetric



				Conventional/SI Units (IU/mL)		
	n	Slope	Correlation Coefficient	Range of Sample Conc.	Intercept	Sy.x
5,1 FS† vs. Commercially available nephelometric method*	127	1.00	0.987	37–876	-27.93	36.21
5,1 FS† vs. Commercially available turbidimetric method**	126	0.91	0.991	26–742	-12.24	25.49
5600 vs. 5,1 FS†	106	0.97	0.998	34–890	4.31	14.70

* Dade Behring N Latex ASL Assay

** COBAS INTEGRA Antistreptolysin O assay

† Analytical processing hardware and software algorithms on the VITROS 4600 Chemistry System are designed to the same specifications as those applied to the VITROS 5,1 FS Chemistry System. Assay performance on the VITROS 4600 System has been demonstrated to be comparable to that on the VITROS 5,1 FS System. All performance characteristics for VITROS 5,1 FS System are therefore applicable to the VITROS 4600 System.

Precision

Precision was evaluated with quality-control materials on the VITROS 5,1 FS System following NCCLS Protocol EP5. [21]
Precision was also evaluated with quality-control materials on the VITROS 5600 Integrated System following NCCLS Protocol EP5. [21]
These results are guidelines. Variables such as instrument maintenance, environment, reagent storage/handling, control material reconstitution, and sample handling can affect the reproducibility of test results.

System	Conventional and SI Units (IU/mL)			Within Lab CV %**	No. Observ.	No. Days
	Mean Conc.	Within Day SD*	Within Lab SD**			
5,1 FS†	146	4.8	5.5	3.8	86	22
	289	4.7	7.0	2.4	88	22
	495	7.1	10.6	2.2	88	22
	709	12.4	16.9	2.4	88	22
5600	135	3.8	5.0	3.7	88	22
	275	4.4	6.0	2.2	88	22

* Within Day precision was determined using two runs per day with two replications per run.

** Within Lab precision was determined using a single lot of reagent and at least four calibrations.

† Analytical processing hardware and software algorithms on the VITROS 4600 Chemistry System are designed to the same specifications as those applied to the VITROS 5,1 FS Chemistry System. Assay performance on the VITROS 4600 System has been demonstrated to be comparable to that on the VITROS 5,1 FS System. All performance characteristics for VITROS 5,1 FS System are therefore applicable to the VITROS 4600 System.

Specificity

Substances that Do Not Interfere

The substances listed in this table were tested on the VITROS Chemistry Products ASO Reagent at a nominal ASO concentration of 250 IU/mL using protocols based on NCCLS Protocol EP7 [22], and found not to interfere, bias <12.1% at the concentration shown.

Compound	Concentration	
Acetominophen	20 mg/dL	1.3 mmol/L
Acetylsalicylic acid (Aspirin)	50 mg/dL	2.8 mmol/L
Amoxicillin	2 mg/dL	1.15 mmol/L
Ampicillin	5 mg/dL	143 µmol/L
Ascorbic acid	3 mg/dL	170 µmol/L
Atorvastatin	1.6 mg/dL	13.2 µmol/L
Bilirubin	60 mg/dL	1.03 mmol/L
Caffeine	10 mg/dL	515 µmol/L
Carbamazepine	12 mg/dL	508 µmol/L
Creatinine	30 mg/dL	2.6 mmol/L
Diazepam	2 mg/dL	70 µmol/L
Dipyrone	30 mg/dL	854 µmol/L
Ethamsylate	3 mg/dL	114 µmol/L
Gentamicin sulfate	12 mg/dL	0.2 U/L
Hemoglobin	1000 mg/dL	10 g/L
Ibuprofen	40 mg/mL	1.9 mmol/L
Intralipid	1000 mg/dL	N/A*
Methotrexate	454 mg/dL	10 mmol/L
Nicotine	2 mg/dL	123 µmol/L
Procainamide	10 mg/dL	368 µmol/L
ProClin 300	140 mg/dL	N/A*
Propranolol	500 µg/dL	19 µmol/L
Protein	9 g/dL	90 g/L
Rantidine	20 mg/dL	570 µmol/L
Rheumatoid Factor	300 IU/mL	300 IU/mL
Sodium Azide	200 mg/dL	30.8 mmol/L
Sodium Naproxen	90 mg/dL	3.6 mmol/L
Theophylline	25 mg/dL	1.4 mmol/L
Triglyceride	1200 mg/dL	13.4 mmol/L

Compound	Concentration	
Urea	50 mg/dL	8.3 mmol/L
Valproic acid	50 mg/dL	3.5 mmol/L

* N/A: Not Applicable; compound is a mixture.

References

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2. Ayoub, EM, Harden E. Immune response to streptococcal antigens: diagnostics methods. *Manual of Clinical Laboratory Immunology, 6th ed*. Rose NR, et al, eds.; 2002.
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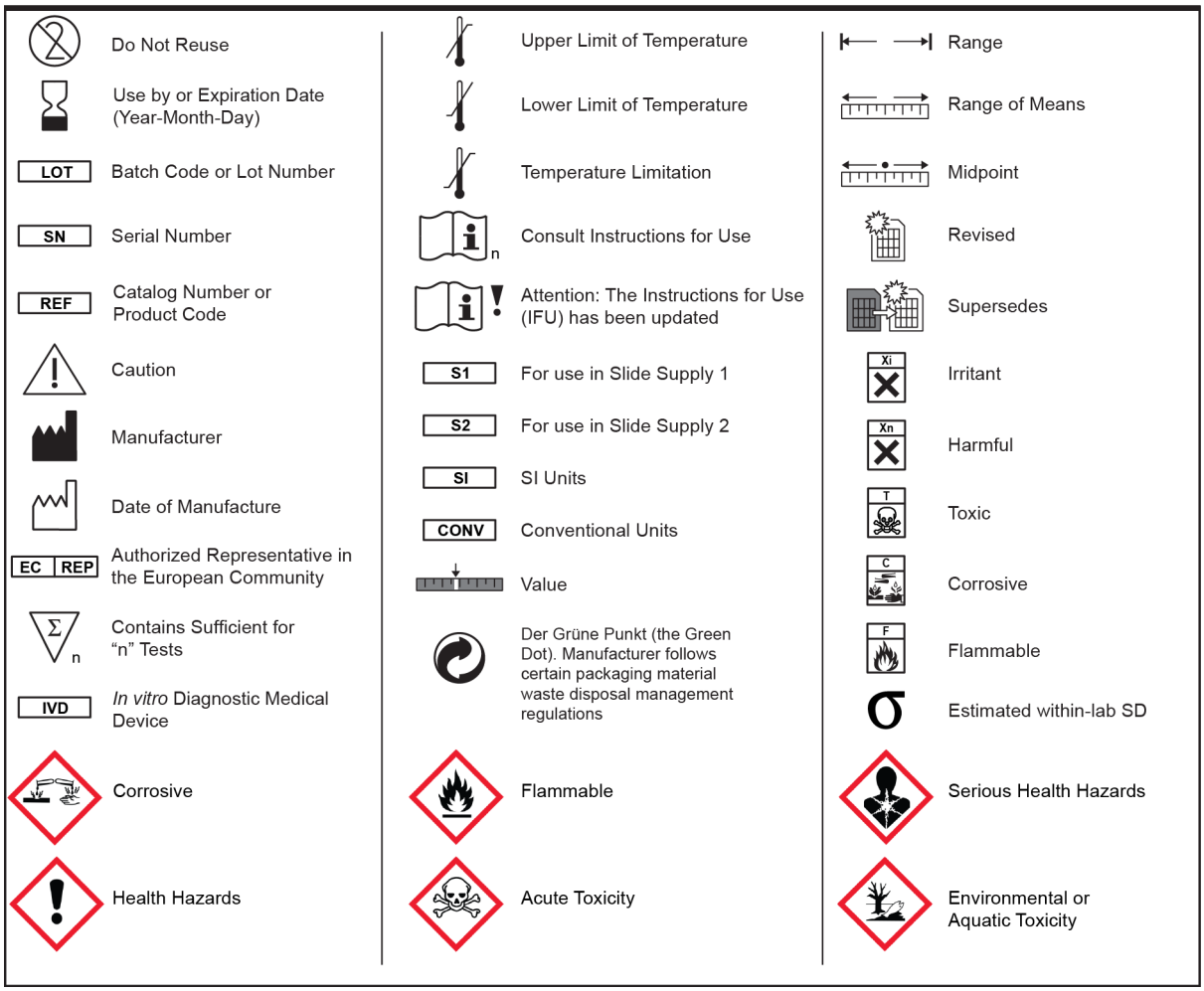
Glossary of Symbols

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



Revision History

Date of Revision	Version	Description of Technical Changes*
2015-05-13	6.0	• Prescription Use Statement added • Warnings and Precautions: updated to align with the new Safety Data Sheets • References: updated M29 • Glossary of Symbols: added Globally Harmonized Symbols to comply with the Classification. Labelling and Packaging (CLP) Regulations
2014-09-05	5.0	Glossary of Symbols: added Date of Manufacture
2012-02-28	4.0	Glossary of Symbols: updated
2010-11-01	3.0	Added information for the VITROS 4600 Chemistry System
2009-04-22	2.1	Precision – Corrected data
2009-03-12	2.0	• Added information for the VITROS 5600 Integrated System • Test Type and Conditions – Added statement • Method Comparison – Added information on sample types • References – Updated • Glossary of Symbols – Updated • Minor wording and formatting changes

Date of Revision	Version	Description of Technical Changes*
2005-12-15	1.0	First release of document

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

When this Instructions For Use is replaced, sign and date below and retain as specified by local regulations or laboratory policies, as appropriate.

Signature

Obsolete Date

CE

EC REP

Ortho-Clinical Diagnostics
50-100 Holmers Farm Way
High Wycombe
Buckinghamshire
HP12 4DP
United Kingdom

Ortho-Clinical Diagnostics, Inc.
100 Indigo Creek Drive
Rochester, NY 14626

Ortho Clinical Diagnostics