510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

I Background Information:

A 510(k) Number

K241534

B Applicant

Ortho Clinical Diagnostics

C Proprietary and Established Names

VITROS Immunodiagnostic Products Syphilis Reagent Pack

D Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
LIP	Class II	21 CFR 866.3830 - Treponema Pallidum Treponemal Test Reagents	MI - Microbiology

II Submission/Device Overview:

A Purpose for Submission:

To establish substantial equivalence to a predicate device and to obtain market clearance for a new assay designed to detect antibodies to *Treponema pallidum* in human serum and plasma.

B Measurand:

Antibodies to *Treponema pallidum* (IgM and IgG)

C Type of Test:

Enzyme Linked Immunoabsorption Assay, Sandwich immunoassay

III Intended Use/Indications for Use:

A Intended Use(s):

See Indications for Use below.

B Indication(s) for Use:

VITROS Immunodiagnostic Products Syphilis Reagent Pack (VITROS Syphilis test)

For the qualitative determination of total (IgG and IgM) antibodies to Treponema pallidum (TP) specific antigens in human serum and plasma using the VITROS 5600 Integrated System.

The presence of antibodies to Treponema pallidum (TP) specific antigens, in conjunction with non-treponemal laboratory tests and clinical findings may aid in the diagnosis of syphilis infection.

The VITROS Syphilis test is not intended for blood and tissue donor screening.

Special Conditions for Use Statement(s):

Rx - For Prescription Use Only

C Special Instrument Requirements:
VITROS 5600 Integrated System

IV Device/System Characteristics:

A Device Description:
The VITROS Syphilis test is performed using the VITROS Syphilis Reagent Pack and VITROS Syphilis Calibrator on the VITROS 5600 Integrated System. The reagents are provided ready to use.

B Principle of Operation:

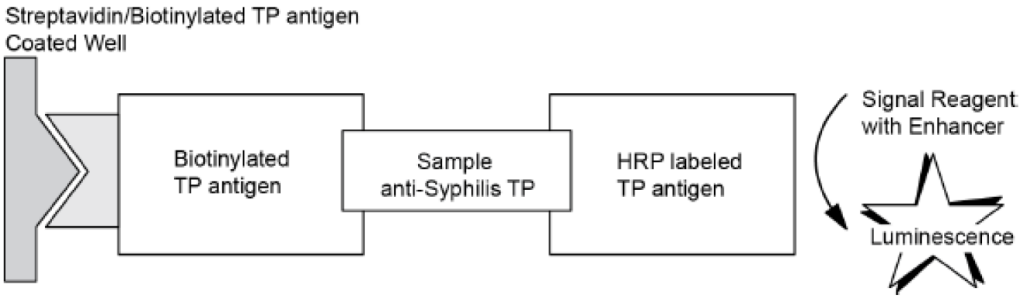
Assay Architecture
An immunometric technique is used; this involves a two-stage reaction. In the first stage, antibodies to Syphilis TP specific antigens present in the sample bind with biotinylated recombinant Syphilis TP antigens immobilized on streptavidin coated wells. Unbound sample is removed by washing. In the second stage conjugate reagent containing horseradish peroxidase (HRP)-labeled recombinant Syphilis TP antigens is added. The conjugate binds specifically to any antibody to Syphilis TP specific antigens captured on the well in the first stage. Unbound conjugate is removed by washing.

The bound HRP conjugate is measured by a luminescent reaction. 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 indicative of the amount of antibody to Syphilis TP specific antigen present.

Table 1: VITROS Syphilis test

Test Type	System	Incubation Time	Time to first result	Test Temperature	Reaction Sample Volume
Immunometric	5600	16 mins first incubation 8 mins second incubation	34 minutes	37 °C	25 µL

Figure: Reaction Scheme

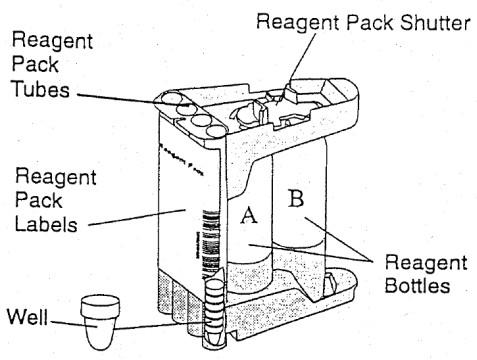


VITROS Immunodiagnostic Products Syphilis Reagent Pack

VITROS Syphilis Reagent Pack (1 pack) contains:

- 100 coated wells; biotin-recombinant Syphilis TP antigens, 0.15 µg/mL.
- 13.1 mL assay reagent (buffer with bovine gamma globulin, bovine serum albumin and antimicrobial agent).
- 20.4 mL conjugate reagent (HRP-recombinant Syphilis TP antigens, 0.15 µg/mL) in buffer with bovine serum albumin and antimicrobial agent.

The reagents required for the test are housed in a reagent pack that is depicted below:



VITROS Immunodiagnostic Products Syphilis Calibrator

VITROS Syphilis Calibrator contains:

- 1 vial of VITROS Syphilis Calibrator (human anti-Syphilis TP antigen in anti-Syphilis TP antigen negative human plasma with antimicrobial agent, 2.2 mL).

The VITROS Syphilis Calibrator is a single level liquid calibrator, containing human anti-Syphilis TP antigen in anti-Syphilis TP antigen negative human plasma with antimicrobial agent at a specified level that may be used directly on the VITROS 5600 Integrated System for calibration.

Result Calculation

Results are calculated as a normalized signal, relative to a cutoff value. During the calibration process a lot-specific parameter is used to determine a valid stored cutoff value for the VITROS Integrated System.

Result= Signal for test sample/Signal at Cutoff (Cutoff value)

Interpretation of results
Samples with results less than 1.00 will be flagged as "Non-reactive" and samples with results greater than or equal to 1.00 will be flagged as "Reactive".

Table 2: VITROS Syphilis Test Result Interpretation

VITROS Syphilis Test Result (S/C)	Status	Interpretation
< 1.00	Non-reactive	Indicates no active or previous infection with *Treponema pallidum*.
≥ 1.00	Reactive	Indicates active or previous infection with *Treponema pallidum*.

V Substantial Equivalence Information:

A Predicate Device Name(s):
Roche Elecsys Syphilis

B Predicate 510(k) Number(s):
K160910, K211302

C Comparison with Predicate(s):

Device & Predicate Device(s):	K241534	K160910/K211302
Device Trade Name	VITROS Immunodiagnostic Products Syphilis Reagent Pack	Roche Elecsys Syphilis assay
General Device Characteristic Similarities		
Intended Use/ Indications For Use	VITROS Immunodiagnostic Products Syphilis Reagent Pack (VITROS Syphilis Test) For the qualitative determination of total (IgG and IgM) antibodies to Treponema pallidum (TP) specific antigens in human serum and plasma using the VITROS 5600 Integrated System. The presence of antibodies to Treponema pallidum (TP) specific antigens, in conjunction with non-treponemal laboratory tests and clinical findings may aid in the diagnosis of syphilis infection. The VITROS Syphilis test is not intended for blood and tissue	For the in vitro qualitative detection of total antibodies (IgG and IgM) to Treponema pallidum in human serum and plasma. The test is intended as an aid in the diagnosis of syphilis infection in conjunction with clinical signs and symptoms. The Elecsys Syphilis immunoassay is not intended for use in screening blood or tissue donors. The effectiveness of this assay in testing blood or tissue donors has not been established. The electrochemiluminescence

	donor screening.	immunoassay "ECLIA" is intended for use on cobas e immunoassay analyzers.
Basic Principle	Sandwich immunoassay	Same
Analyte	Antibodies to *T. pallidum*	Same
Antigens Used	Recombinant TP15, TP17 and TP47	Same
Sample Type	Serum and Plasma	Same
Automated	Automated assay	Same
Measurement	Qualitative	Same
Interpretation of results	Samples with a sample/cutoff index < 1.00 are non-reactive (negative for anti-TP antibodies). Samples with a sample/cutoff index ≥ 1.00 are considered reactive (positive for anti-TP antibodies).	Same
General Device Characteristic Differences		
Traceability	Traceable to an in-house reference calibrator which has been value-assigned to optimize clinical sensitivity and specificity.	N/A
Sample Volume	25 µL	6 µL
Calibrator Levels	1	2
Instrument Used	VITROS 5600 Integrated System	Cobas e 411

VI Standards/Guidance Documents Referenced:

Not Applicable

VII Performance Characteristics (if/when applicable):

A Analytical Performance:

1. Precision/Reproducibility:

Internal precision

To verify the precision performance of the VITROS Syphilis test, five serum precision pools (PP) were prepared from anti-syphilis TPA negative donor serum pooled from 50 individual donors obtained from an in-house draw and by spiking with anti-syphilis-TPA reactive serum to the desired target signal cut off (S/C). All precision pools were tested on two reagent lots using two VITROS 5600 Systems. For each reagent lot, two replicates of each precision fluid were run on two occasions per day for 20 days. A nested Analysis of Variance (ANOVA) was used to estimate the Repeatability and Within lab parameters (mean, SD, and %CV) for

each precision pool/reagent lot combination. The results from the precision study are shown below.

Table 3: Within-Lab Precision

Panel Member	No. of Obs.	% Reactive	Grand Mean (S/C)	Repeatability* (Within Run)		Between-Run		Between-Day		Between-Lot		Within-Laboratory**	
				SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)
Human Serum Pool, Non-reactive	160	0% (0/160)	0.02	0.003	–	0.001	–	0.001	–	0.002	–	0.004	–
Human Serum Pool, High Non- reactive	160	0% (0/160)	0.72	0.016	2.2	0.040	5.5	0.015	2.1	0.127	17.7	0.135	18.8
Human Serum Pool, Low Reactive	160	100% (160/160)	1.27	0.026	2.0	0.053	4.2	0.038	3.0	0.199	15.7	0.211	16.6
Human Serum pool, Reactive	160	100% (160/160)	2.16	0.041	1.9	0.064	2.9	0.062	2.8	0.300	13.9	0.315	14.6
Human Serum Pool, Reactive	160	100% (160/160)	7.70	0.130	1.7	0.080	1.0	0.220	2.8	0.889	11.5	0.928	12.0

* Repeatability (formerly called within-run precision) was determined using two replicates per run.
** Within Lab precision was determined using a single reagent lot and a single calibration.

Multi-site Reproducibility

A five-level reproducibility panel was tested with one lot of reagents at three sites (2 external, 1 internal), twice a day, with two unique operators a day, three replicates per run, for a total of five testing days. Calibration was performed prior to testing and the testing was completed within one calibration cycle at each testing site, The repeatability (within day), between run/operator, between day, between site, and reproducibility (total) precision estimates (CV (%)) derived from a variance component analysis are summarized below.

Table 4: Reproducibility

Panel Description	Mean VITROS Syphilis Results (S/C)	Repeatability[a] (Within Run)		Between Run/Operator[b]		Between Day[c]		Between Site[d]		Reproducibility[e]		N
		SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	SD	CV (%)	
Human Serum Pool, Non-reactive	0.02	0.003	-	0.000	-	0.002	-	0.005	-	0.006	-	90
Human Serum Pool, High Non-reactive	0.75	0.021	2.8	0.018	2.4	0.019	2.5	0.015	2.0	0.037	4.9	90
Human Serum Pool, Low Reactive	1.29	0.036	2.8	0.024	1.9	0.038	3.0	0.024	1.8	0.062	4.9	90
Human Serum Pool, Reactive	2.14	0.048	2.2	0.030	1.4	0.047	2.2	0.047	2.2	0.088	4.1	90
Human Serum Pool, Reactive	7.42	0.145	2.0	0.000	0.0	0.080	1.1	0.246	3.3	0.297	4.0	90

[a] Repeatability: Variability of the VITROS Syphilis assay performance within run replicate, calculated using data across all sites.

b Between Run/Operator: Variability of the VITROS Syphilis assay performance from run to run, calculated using data across all sites.
c Between Day: Variability of the VITROS Syphilis assay performance from day to day, calculated using data across all sites.
d Between site: Variability of the VITROS Syphilis assay performance from site to site.
e Reproducibility (Total): Variability of the test incorporating factors of repeatability, between run/operator, between day, and between site.

2. Linearity:
   Not Applicable; this is a qualitative assay.

3. Analytical Specificity/Interference:

   Interference by Endogenous Substances
   The VITROS Syphilis test was evaluated for interference by testing syphilis non-reactive (S/C approximately 0.2) and syphilis reactive (S/C of 1.5 to 4.0) samples. Test samples were spiked with the test substance at the prescribed test levels and results were compared to matched control samples spiked with an equal volume of solvent (blank). Paired difference testing was performed using the median of four replicates of each test sample and each control sample split across two reagent lots on the VITROS System. The mean bias for the reactive samples ranged from -0.34 to 0.12. None of the 28 compounds were found to interfere with the clinical interpretation of the assay at the concentrations indicated in the table below.

Table 5: Interference by Endogenous Substances

Substance	Concentration	
	Conventional Units	SI units
Acetaminophen	15.6 mg/dL	1030 µmol/L
Acetylcysteine	15.0 mg/dL	920 µmol/L
Acetylsalicylic Acid	3.0 mg/dL	167 µmol/L
Ampicillin-Na	7.5 mg/dL	215 µmol/L
Ascorbic Acid	30.6 mg/dL	1738 µmol/L
Bilirubin (conjugated)	40.0 mg/dL	475 µmol/L
Bilirubin (unconjugated)	40.0 mg/dL	684 µmol/L
Biotin	3510 ng/mL	14.3 µmol/L
Cefoxitin	660 mg/dL	15500 µmol/L
Cholesterol	400 mg/dL	10.3 mmol/L
Cyclosporine	0.18 mg/dL	1.50 µmol/L
Doxycyclin	18 mg/L	40.5 µmol/L
Hemoglobin	1000 mg/dL	10 g/L
Heparin	330 U/dL	330 units/dL
Human anti-mouse Antibodies (HAMA)	5120 ng/mL	N/A
Ibuprofen	21.9 mg/dL	1060 µmol/L
Human IgG	2894 mg/dL	N/A
Intralipid	2000 mg/dL	N/A
Levodopa	0.75 mg/dL	38 µmol/L
Methyldopa	2.25 mg/dL	107 µmol/L
Metronidazole	123 mg/L	7190 µmol/L
Phenylbutazone	32.1 mg/dL	1040 µmol/L
Total Protein	15 g/dL	150 g/L
Rheumatoid Factor	2941 IU/mL	N/A
Rifampicin	4.8 mg/dL	58.3 µmol/L

Sodium Azide	20 mg/dL	3076 µmol/L
Theophylline	60 mg/L	333 µmol/L
Triglycerides	1500 mg/dL	16.94 mmol/L

Cross-reactivity in Specimens with Medical Conditions not Related to Syphilis
The VITROS Syphilis test was evaluated for potential cross-reactivity by testing serum samples from patients with antibodies to other microorganisms or underlying conditions which could cause false positive results. The results are summarized in the table below.

Table 6: Cross-reactivity in Specimens with other Medical Conditions

Sample Category	Number of Samples	Non-reactive	Reactive
Anti-nuclear antibodies (ANA)	10	10	0
Borrelia burgdorferi infection IgG (European and US strains)	10	10	0
Cytomegalovirus (anti-CMV IgG positive and IgM positive)	10	10	0
Epstein-Barr Virus (EBV IgG positive and IgM positive)	15	10	5*
Hepatitis A (HAV) IgG and IgM	10	10	0
Hepatitis B (HBV) IgG and IgM	10	10	0
Hepatitis C (HCV) IgG and IgM	10	10	0
Herpes Simplex Virus 1/2 (anti-HSV-1/2 IgG positive and IgM positive)	10	10	0
Human Immunodeficiency Virus (HIV 1/2) IgG and IgM	12	10	2*
Rheumatoid Arthritis/Rheumatoid Factor	10	10	0
Rubella IgG and IgM	10	10	0
Hyperglobulinemia	10	10	0
Systemic Lupus Erythematosus (SLE)	10	10	0
Toxoplasmosis IgG positive and IgM positive	10	10	0
Varicella-Zoster Virus (VZV IgG positive)	15	10	5*
E. coli antibodies	10	10	0
Leptospirosis	10	10	0
VCA (EBV Viral Capsid Antigen) IgM	10	10	0

* The 7 samples that were reactive with the VITROS Syphilis test were confirmed as coinfected and positive for anti-*Treponema pallidum* antibodies with another FDA cleared syphilis total antibody assay.

4. Assay Reportable Range:
   Not Applicable

5. Traceability, Stability, Expected Values (Controls, Calibrators, or Methods):

   Calibrator
   Calibrators are provided with the assay and are intended for the calibration of the VITROS Syphilis test on the VITROS 5600 Integrated System. The calibrator is a single level liquid calibrator, containing human anti-Syphilis TP antigen in anti-Syphilis TP antigen negative human plasma with antimicrobial agents at a specified level that may be used directly on the VITROS 5600 Integrated System for calibration.

   Calibration Traceability
   All manufactured lots of the VITROS Syphilis test are directly traceable to the VITROS Syphilis Reference Calibrator, which has been value-assigned based on clinical agreement

with a commercially available test. Calibration of Replacement Reference Calibrators (also known as Manufacturing Working Calibrators in ISO 17511) will be performed according to Ortho standard operating procedures. The current set of Reference Calibrator will be used to assign values to the new set of Reference Calibrator in a validated cross-over study, to ensure no drift in the calibration of the assay over time.

External Controls

VITROS Syphilis Controls are available separately and are recommended for use with the VITROS Integrated System. There are 2 VITROS Syphilis Controls (anti-syphilis TP negative and anti-syphilis TP positive).

Sample Stability Studies

The stability of TP antibodies in serum, serum separator tubes (SST), LiHep plasma separator tubes (PST), lithium heparin (LiHep) plasma, sodium heparin (NaHep) plasma, K2 EDTA plasma and K3 EDTA plasma was evaluated at room temperature (30°C), refrigerated (2-8°C), and frozen (-20°C) storage conditions. The test samples were targeted as non-reactive (result less than 0.50 S/C) and syphilis reactive (approximately 4.00 S/C) to assess the performance of the assay near the cutoff. Twenty (20) samples were from presumed non-reactive donors and were tested without spiking, and 23 samples were spiked to the targeted S/C.

All samples were tested at every time point for the following storage conditions:

- Room temperature stability (at 30°C)
    - Time points tested: Day 0, Day 1, Day 7
- Refrigerated stability (at 2-8°C)
    - Time points tested: Day 0, Day 3 and Day 7
- Frozen stability (at -20°C)
    - Time points tested: Day 0, Day 7 and Day 28
- Freeze-thaw stability (at -20° C)
    - Conditions tested:1 and 5 freeze thaw cycles

All tests met the acceptance criteria for all the anti-coagulants and storage conditions tested. Ortho is claiming the following storage conditions:
- Room temperature (up to 30°C) for 7 days
- Refrigerated (2-8°C) for 7 days
- Frozen (at -20°C) for 28 days
-Samples may be frozen and thawed up to 5 times

Reagent Stability

To evaluate the reagent stability and establish expiration dating of the VITROS Syphilis test, three Kit Lots were tested at the initial time-point (baseline)and then at monthly intervals up to the post expiry time-point to ensure that the stability trial performance can be accurately defined. Kit Lots were stored at 2-8°C and tested for a period of 21 weeks. Four in house quality control samples with S/C ratios (0.07, 3.15, 3.33 and 28.2) were tested in duplicates at each time-point. All assays used in testing were verified as suitable for use by assessing the appropriate Calibration Quality Parameters and by measuring the appropriate In-house Controls against the specifications. All samples tested met the acceptance criteria. The data currently supports a shelf life of 17 weeks when kept at 2 to 8°C.

On-board Reagent Stability

To assess the on-board stability of the VITROS Syphilis test reagents, three Kit Lots of the reagent pack were stored on-board of the instrument (refrigerated) for up to 13 weeks. Four in house quality controls with S/C ratios (0.07, 3.15, 3.33 and 28.2) were tested in duplicates on each Kit Lot at time-point 0, week 2, 4, 6, 8, 10, 12 and 13 using fresh and multi-use packs of QC controls. All results were acceptable and support a claim of 12 weeks on-board stability.

Calibration Stability/Interval

Calibration interval was established by monitoring test results of a set of spiked samples over time, when run on the VITROS 5600 using a single calibration over the course of 60 days. The target levels for syphilis reactivity (S/C) were approximately 0.80 (PP2), 1.30 (PP3), 2.00 (PP4) and 7.00 (PP5). The samples were aliquoted and stored frozen at ≤ -20°C until thawed and tested each test day. Each aliquot was used for one test occasion only. Four replicates of all samples were tested on two reagent lots on two VITROS 5600 Systems. The calibration interval stability was evaluated by determining the mean bias on day 21, day 28 and day 60, compared to day 0. The calibration interval, as determined by day 21 and day 28 bias, passed for both reagent lots as shown below. The calibration interval, evaluated on day 60, failed the acceptance criteria (equal or more positive than -20% for Syphilis reactive samples, and equal or less positive than 0.20 S/C for Syphilis negative samples (PP2). The calibration stability has been demonstrated for 28 days.

Carryover

The potential carryover from samples with high level of TP antibodies, when using the VITROS Syphilis assay on the VITROS 5600 Integrated System, was evaluated using a syphilis non-reactive serum pool (prepared from healthy individuals) and a syphilis reactive serum pool (prepared by spiking the non-reactive donor pool with syphilis reactive serum to a S/C of greater than 100). Each test run consisted of five (5) replicates of the non-reactive pool followed by ten (10) sets of alternating replicates between the non-reactive and reactive pools. After completion of the alternating replicates, five (5) more replicates of the non-reactive pool were tested. The 10 replicates of non-reactive pool executed at the beginning and end of the run constitute baseline results for the non-reactive pool. The 10 replicates of non-reactive pool generated by alternating with the reactive pool constitute interleaved results.

The observed range of results for non-reactive sample replicates in the baseline runs was 0.019 S/C to 0.030 S/C. The range of observed results from the interleaved runs was 0.018 S/C to 0.030 S/C. The range between highest and lowest replicate in each sample set is almost identical and spans 0.012 S/C, which is below the clinically significant bias, demonstrating that there is no observable within assay sample carryover.

High dose hook effect

Not Applicable due to a two-step indirect immunoassay with a wash cycle.

Detection of IgG and IgM Antibodies

Three test methods were utilized to demonstrate the capability of the VITROS Syphilis test to detect both IgG and IgM syphilis-specific antibodies: a) VITROS Syphilis test (Syph): to capture and detect antibodies (IgG and IgM) specific for syphilis TP antigens; b) IgM Hybrid Assay (IgM Hybrid): to illustrate the specific measurement of anti-Syphilis IgM; and c) IgG Hybrid Assay (IgG Hybrid): to illustrate the specific measurement of anti-Syphilis IgG. Syphilis reactive (16 samples) and non-reactive samples (5 samples) were selected based on known results from the VITROS Syphilis test or from vendor documentation. VITROS Syphilis Controls were tested on all three methods in singleton and all test samples were tested in duplicate. Non-reactive samples showed low signal on both hybrid assays. Reactive samples all showed signal above background in the IgG hybrid assay that increases based on the sample group (4 groups- S/C ratios 1.0-10.0, 10.0-30.0, 30.0-100.0 and above 100) and correlates with VITROS Syphilis results. Reactive specimens show variable results in the IgM hybrid assay, which is expected based on the unknown and presumed variable maturity of the immune response in these patients. After initial testing, a subset of samples was selected for IgG stripping. In all cases signal in the IgG Hybrid assay was reduced to background levels, IgM hybrid signal remained constant.

6. Detection Limit:
   Not Applicable

7. Assay Cut-Off:
   In order to define the cut-off, native human serum samples were measured with four lots of the VITROS Syphilis test and a preliminary cut-off was set. The assay cutoff position was determined using data collected during product development to optimize clinical sensitivity and specificity. The results for clinical samples tested during feasibility testing were analyzed using a receiver operator curve (ROC) to determine the optimal assay cut-off. All results were generated using the assay cut-off generated using Reference Calibrator. Results from performance validation testing (clinical trials) validate the selected assay cutoff.

B Comparison Studies:

1. Method Comparison with Predicate Device:
   See Clinical Studies section below.

2. Matrix Comparison:
   To verify the performance of the VITROS Syphilis test with different sample matrices, serum, serum separator tubes (SST), plasma separator tubes (PST), lithium heparin plasma (LiHep), sodium heparin plasma (NaHep), K_2 EDTA plasma and K_3 EDTA plasma from 63 individual whole blood donors were evaluated. Of the 63 whole blood samples, 10 were unaltered and 53 were spiked using syphilis reactive serum or plasma (endogenous serum or plasma samples previously identified or purchased as containing syphilis treponemal antigen specific antibody). Syphilis reactive sample was added to the pooled whole blood for each spiked sample and mixed thoroughly prior to distribution into the various collection devices. A total of five levels of reactivity were evaluated:
   - Negative, target S/C < 0.50
   - High Negative, target S/C 0.50 to 0.90
   - Low Positive, target S/C 1.10 to 2.00
   - Positive 1, target S/C 2.00 to 5.00

- Positive 2, target S/C 5.00 to 30.0
- Positive 3, target S/C 30.0 and above

Each sample was tested in quadruplicate using one of two reagent lots and two VITROS 5600 Systems, and the mean of the results for each matrix type was compared to the mean of the respective serum control tube. The data was tabulated for each matrix type and for each sample. Clinical equivalence between serum samples and samples from each matrix type is demonstrated if the observed mean bias is not clinically significant. A clinically significant bias is defined as a mean bias between serum and test matrix that is equal to or more negative than minus 20.0% for spiked Syphilis reactive samples and greater than or equal to +0.20 S/C for Syphilis negative samples. Table below shows results for each sample type.

Table 7: Percent and Absolute Bias of Candidate Matrices Compared to Serum

Matrix Vs Serum	Negative		High Negative		Low Positive		Positive 1		Positive 2		Positive 3	
	Mean S/C	Mean Bias	Mean S/C	Mean Bias %	Mean S/C	Mean Bias %	Mean S/C	Mean Bias %	Mean S/C	Mean Bias %	Mean S/C	Mean Bias %
Serum	0.02	-	0.61	-	1.48	-	3.37	-	14.6	-	76.8	-
SST	0.02	0.00	0.61	0	1.55	4.7%	3.45	2.4%	15.1	3.4%	80.0	4.2%
PST	0.02	0.00	0.60	-1.6%	1.47	-0.7%	3.33	-1.2%	15.1	3.4%	76.7	-0.1%
LiHep	0.02	0.00	0.61	0	1.48	0	3.37	0	14.6	0	79.0	2.9%
NaHep	0.02	0.00	0.58	-4.9%	1.44	2.7%	3.39	0.6%	14.7	0.7%	76.6	-0.3%
K_2EDTA	0.02	0.00	0.59	-3.3%	1.44	2.7%	3.42	1.5%	14.4	-1.4%	76.7	-0.1%
K_3EDTA	0.02	0.00	0.58	-4.9%	1.36	-8.1%	3.29	-2.4%	13.7	-6.1%	73.6	-4.2%

The results showed that the following blood collection tube types are acceptable for use with the VITROS Syphilis test:
• serum
• serum with separator
• K_2 EDTA plasma
• K_3 EDTA plasma
• lithium heparin plasma
• sodium heparin plasma

C Clinical Studies:

1. Clinical Performance:
   A multicenter study was conducted on the VITROS 5600 Integrated System between June 2022 to August 2023 to evaluate the ability of the VITROS Syphilis assay to detect antibodies (IgG and IgM) directed against *Treponema pallidum* (TP). A total of 1823 specimens were evaluated in the clinical study. Among these, 924 specimens were prospectively collected in the US from individuals of all ages (all comers) representing the intended use population, i.e., with signs or symptoms suggesting syphilis infection, at high risk for exposure to syphilis, or prescribed a routine laboratory test for syphilis as part of routine medical check-up, 698 specimens were purchased retrospective specimens, and 201 specimens were prospectively collected from apparently healthy individuals in the US. Of the 698 retrospective samples, 585 (83.8%) samples were collected in the US and 113 (16.2%) samples were collected outside of the US.

The 924 prospective specimens analyzed in the VITROS Syphilis clinical study were collected at clinical sites in the following geographic locations:

Table 8: Clinical Sites for prospective sample collection

Site Location	Enrolled Valid Subjects	Syphilis Positivity Rate
Fort Lauderdale, Florida	79 (8.5%)	38.0%
Cincinnati, Ohio	44 (4.8%)	2.3%
Baltimore, Maryland	183 (19.8%)	36.1%
New Haven, Connecticut	178 (19.3%)	6.2%
Tacoma, Washington	190 (20.6%)	1.1%
New Orleans, Louisiana	250 (27.1%)	43.6%
Overall	**924 (100%)**	**23.7%**

Testing with the VITROS Syphilis test was performed at three sites (two external and one internal testing sites). Comparator testing was performed at one external CLIA compliant laboratory. The clinical performance of the VITROS Syphilis test was evaluated by calculating positive percent agreement (PPA) and negative percent agreement (NPA) of the assay with the final comparator result based on an algorithm of results from three FDA-cleared Syphilis assays: a treponemal chemiluminescent immunoassay (CLIA), a non-treponemal RPR assay and a second treponemal assay- particle agglutination method. The final comparator result was determined using a two-out-of-three rule (CLIA, RPR, and TP-PA). Because the clinical diagnosis of syphilis must be supported by two reactive laboratory tests, consisting of a treponemal assay and a non-treponemal assay, or at least two treponemal assays, employing an algorithm of three syphilis assays to determine the comparator result presents the most comprehensive picture of the syphilis serological status.

The clinical performance of the VITROS Syphilis assay was evaluated against the final comparator result based on the composite algorithm, as indicated in the table below.

Table 9: Comparator Algorithm for VITROS Syphilis Test

Treponemal Assay (TP-ECLIA) (Predicate)	Non-Treponemal Assay (RPR)	2nd Treponemal Assay (TPPA)	Final Comparator Result
Non-reactive	Non-reactive	N/A*	Negative
Non-reactive	Reactive	Reactive	Positive
		Non-reactive	Negative
		Inconclusive	Negative
Reactive	Reactive	N/A	Positive
Reactive	Non-reactive	Reactive	Positive
		Non-reactive	Negative
		Inconclusive	Positive

*N/A- Not applicable: In cases where the Treponemal test and the Non-Treponamal test results agreed (non-reactive/non-reactive and reactive/reactive), TP-PA testing was not performed because results would not impact the final comparator results.

Clinical Performance in Prospectively Collected Samples

A total of 924 prospective specimens from the intended use population collected from six sites in the United States were tested at three sites using the VITROS Syphilis assay, including 615 subjects sent for routine syphilis testing, 47 pregnant women and 262 HIV-positive subjects. All samples were tested with comparator assays according to the composite

testing algorithm described above. A summary of the serological test profile for all prospectively collected specimens in the study is presented in the following table.

Table 10: VITROS Syphilis Test Serological Profile for the Prospective Intended Use Population

Anti-TP Result	RPR Result	2nd Anti-TP (TP-PA) Result	Final Comparator Result	VITROS Syphilis Result	Number of Subjects
Non-reactive	Non-reactive	N/A*	Negative	Non-reactive	684
				Reactive	2
Non-reactive	Reactive	Reactive	Positive	Non-reactive	1
				Reactive	0
		Non-reactive	Negative	Non-reactive	2
				Reactive	1
		Inconclusive	Negative	Non-reactive	0
				Reactive	0
Reactive	Reactive	N/A*	Positive	Non-reactive	0
				Reactive	84
Reactive	Non-reactive	Reactive	Positive	Non-reactive	0
				Reactive	129
		Non-reactive	Negative	Non-reactive	2
				Reactive	14
		Inconclusive	Positive	Non-reactive	0
				Reactive	5
Total					**924**

* N/A = Test not performed

VITROS Syphilis Test performance in the intended use population is summarized below.

Table 11: VITROS Syphilis Test Performance vs. Composite Algorithm Comparator Result (Prospective Samples)

Subgroup	PPA% (n/N)	95% CI* (%)	NPA% (n/N)	95% CI* (%)
Routine Syphilis**	98.8 (83/84)	93.6–99.8	99.3 (527/531)***	98.1–99.7
Pregnant Women	100 (1/1)	20.7–100	97.8 (45/46)***	88.7–99.6
HIV Positive	100 (134/134)	97.2–100	90.6 (116/128)***	84.3–94.6
Overall	**99.5 (218/219)**	**97.5-99.9**	**97.6 (688/705)**	**96.2-98.5**

* 95% Wilson Score Confidence Interval

** Does not include prospectively collected Pregnant Women and HIV Positive specimens

*** Of the 17 discordant samples that were negative by Final Comparator, 14 samples (2 routine Syphilis, 1 pregnancy, 11 HIV positive) were reactive by an FDA cleared Treponema pallidum antibody test.

Clinical Performance in Retrospective Specimens

A total of 547 retrospective purchased samples (serum samples) were tested with the VITROS Syphilis Test at 3 sites using the VITROS Syphilis assay, including 243 samples from pregnant women, 152 HIV positive samples and 152 pre-selected positive samples. All samples were tested with the comparator tests according to a composite testing algorithm using FDA-cleared tests specified above. VITROS Syphilis performance for the retrospective specimens is summarized below.

Table 12: VITROS Syphilis Test Performance vs. Composite Algorithm Comparator Result (Retrospective Samples)

Subgroup	PPA% (n/N)	95% CI* (%)	NPA% (n/N)	95% CI* (%)
Pregnant Women	100 (31/31)	89.0–100	100 (212/212)	98.2–100
HIV Positive	100 (30/30)	88.7–100	96.7 (118/122)**	91.9–98.7
Pre-selected Positive	100 (152/152)	97.5–100	N/A	N/A

* 95% Wilson Score Confidence Interval
** Four discordant samples that were negative by the Final Comparator were reactive by an FDA cleared Treponema pallidum antibody test.

Clinical Performance in Pregnant Women

Samples from 290 pregnant women were tested in the study. Of these samples, 47 were prospectively collected and 243 were retrospective samples. The percent agreement between the VITROS Syphilis results and the final comparator results is shown below stratified by pregnancy trimesters.

Table 13: VITROS Syphilis Test Performance vs. Composite Algorithm Comparator Result (Pregnant Women)

Pregnant Women	PPA% (n/N)	95% CI* (%)	NPA% (n/N)	95% CI* (%)
Prospectively Collected Specimens				
First Trimester	100 (1/1)	20.7–100	96.0 (24/25)**	80.5–99.3
Second Trimester	N/A	N/A	100 (19/19)	83.2–100
Third Trimester	N/A	N/A	100 (2/2)	34.2–100
Retrospective Specimens				
First Trimester	N/A	N/A	100 (42/42)	91.6–100
Second Trimester	100 (31/31)	89.0–100	100 (102/102)	96.4–100
Third Trimester	N/A	N/A	100 (68/68)	94.7–100

* 95% Wilson Score Confidence Interval
** One discordant sample that was negative by the Final Comparator was reactive by an FDA cleared Treponema pallidum antibody test.

Clinical Performance in HIV Positive Individuals

Samples from 414 HIV positive individuals were tested in the study. Of these samples, 262 were prospectively collected and 152 were retrospective samples. The percent agreement between the VITROS Syphilis results and the final comparator results is shown below.

Table 14: VITROS Syphilis Test Performance vs. Composite Algorithm Comparator Result (HIV-Positive Persons)

HIV Positive Specimens	PPA% (n/N)	95% CI* (%)	NPA% (n/N)	95% CI* (%)
Prospectively Collected Specimens	100 (134/134)	97.2–100.00	90.6 (116/128)**	84.3–94.6
Retrospective Specimens	100 (30/30)	88.7–100.00	96.7 (118/122)**	91.9–98.7

* 95% Wilson Score Confidence Interval.
** Of the 16 discordant samples that were negative by the Final Comparator, 15 samples (11 prospective, 4 retrospective) were reactive by an FDA cleared Treponema pallidum antibody test.

Clinical Performance in Medically Diagnosed Individuals

A total of 151 samples from medically diagnosed individuals were tested in the study. The diagnosis of syphilis and the stage of the disease were made by a licensed physician based on the patient's clinical symptoms, medical history, and laboratory test results at the time of

diagnosis. These specimens included 76 females and 75 males, 10–77 years old. Reactivity of specimens with primary, secondary, and latent syphilis by treatment status is summarized in the table below.

Table 15: VITROS Syphilis Test results for Medically Diagnosed Individuals

Syphilis Stage	Treatment Status	N	VITROS Syphilis Results
			Reactive
Primary	Treated	25	25
	Untreated	25	25
Secondary	Treated	25	25
	Untreated	25	25
Latent	Treated	25	25
	Untreated	26	26

Clinical Performance in Apparently Healthy Individuals

A total of 201 prospective specimens from apparently healthy individuals collected from 3 sites in the United States were tested at 3 sites using the VITROS Syphilis assay. Reactivity of specimens acquired from apparently healthy individuals is summarized in the table below.

Table 16: VITROS Syphilis Test results for Healthy Individuals

Category	N	VITROS Syphilis Result	
		Reactive N (%)	Non-reactive N (%)
Female	98	2 (2.0)	96 (98.0)
Male	103	2 (1.9)	101 (98.1)
Total	201	4 (2.0)*	197 (98.0)

* Three samples were positive by the Final Comparator.

Of the total number of 1823 samples tested in the clinical study, 94 samples generated exception codes on the initial testing and had to be retested. The rate of initial invalids in this study was 5.1%. with each sample producing a final valid result upon retest.

D Clinical Cut-Off:

Not applicable

E Expected Values:

A total of 924 prospectively collected specimens for the intended use population were tested with the VITROS Syphilis assay. There were 235 reactive samples for a 25.4% positivity rate for the presence of *T. pallidum* antibodies in the study population. The distribution of the VITROS Syphilis reactive and non-reactive results is summarized below by age and gender.

Table 17: VITROS Syphilis Test Expected Results for the Prospective Intended Use Population

Age Range (years)	Gender	VITROS Syphilis Results		
		Reactive N (%)	Non-reactive N (%)	Total
18-21	Female	2 (5.7)	33 (94.3)	35
	Male	5 (25.0)	15 (75.0)	20
22-29	Female	5 (3.6)	134 (96.4)	139
	Male	18 (15.7)	97 (84.3)	115
30-39	Female	8 (6.7)	112 (93.3)	120
	Male	77 (41.6)	108 (58.4)	185

40-49	Female	5 (11.6)	38 (88.4)	43
	Male	31 (50.0)	31 (50.0)	62
50-59	Female	5 (16.7)	25 (83.3)	30
	Male	30 (51.7)	28 (48.3)	58
60-69	Female	7 (41.2)	10 (58.8)	17
	Male	33 (45.2)	40 (54.8)	73
≥70	Female	0 (0.0)	4 (100.0)	4
	Male	9 (39.1)	14 (60.9)	23
Combined	**Total**	**235 (25.4)**	**689 (74.6)**	**924**

VIII Proposed Labeling:

The labeling supports the finding of substantial equivalence for this device.

IX Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.