## 510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
## DECISION SUMMARY

**A. 510(k) Number:**
k060770

**B. Purpose for Submission:**
New device

**C. Measurand:**
IgM antibody to hepatitis A virus (Anti-HAV IgM)

**D. Type of Test:**
Immunoassay, qualitative determination of IgM antibody to HAV in human body fluids using antibody class capture technique followed by chemiluminescence detection.

**E. Applicant:**
Ortho-Clinical Diagnostics, Inc.

**F. Proprietary and Established Names:**
VITROS Immunodiagnostic Products Anti-HAV IgM Reagent Pack
VITROS Immunodiagnostic Products Anti-HAV IgM Calibrators
VITROS Immunodiagnostic Products Anti-HAV IgM Controls

Common Name: Anti-HAV IgM Assay
              Anti-HAV IgM Controls

**G. Regulatory Information:**

1. Regulation section:
   866.3310 Hepatitis A virus (HAV) serological assays
2. Classification:
   Class II
3. Product code:
   LOL, Hepatitis A (antibody and IgM antibody)
4. Panel:
   Microbiology (83)

**H. Intended Use:**

1. Intended use(s):
   Reagent Pack:
   For the *in vitro* qualitative determination of IgM antibody to hepatitis A virus (anti-HAV IgM) in human adult and pediatric serum or plasma (EDTA, heparin or citrate) using the VITROS ECi/ECiQ Immunodiagnostic System.

The assay is indicated for testing specimens from individuals who have signs and symptoms consistent with acute hepatitis. Assay results in conjunction with other clinical information, may be used for the laboratory diagnosis of individuals with acute or recent hepatitis A.

VITROS Anti-HAV IgM Calibrator:

For *in vitro* use in the calibration of the VITROS Immunodiagnostic System for the qualitative determination of IgM antibody to hepatitis A viral antigen (HAV) in human serum and plasma (EDTA, heparin or citrate).

VITROS Anti-HAV Total Controls:

For *in vitro* use in monitoring the performance of the VITROS Immunodiagnostic System when used for the detection of anti-HAV IgM.

2. Indication(s) for use:

For the *in vitro* qualitative determination of IgM antibody to hepatitis A virus (anti-HAV IgM) in human adult and pediatric serum or plasma (EDTA, heparin or citrate) using the VITROS ECi/ECiQ Immunodiagnostic System.

The assay is indicated for testing specimens from individuals who have signs and symptoms consistent with acute hepatitis. Assay results in conjunction with other clinical information, may be used for the laboratory diagnosis of individuals with acute or recent hepatitis A.

**WARNING*: This assay is not intended for screening blood or solid or soft tissue donors.***

3. Special conditions for use statement(s):

For prescription use only

4. Special instrument requirements:

VITROS ECi/ECiQ Immunodiagnostic System: Automated Analyzer (K962919/S1)

**I. Device Description:**

The VITROS Anti-HAV IgM assay is performed using the VITROS Immunodiagnostic Products Anti-HAV IgM Reagent Pack and the VITROS Immunodiagnostic Products Anti-HAV IgM Calibrator on the VITROS ECi/ECiQ Immunodiagnostic System (VITROS Immunodiagnostic System). An antibody class capture technique is used. This involves dilution of the sample and the simultaneous reaction of human IgM in the diluted sample with biotinylated mouse monoclonal anti-human IgM antibody. The immune complex is captured by streptavidin on the wells. Unbound materials are removed by washing. Horseradish peroxidase (HRP)-labeled mouse monoclonal anti-HAV antibody which has been complexed with inactivated HAV antigen (conjugate) is then captured by anti-HAV specific IgM bound to the wells. Unbound material 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 VITROS Immunodiagnostic System. The binding of HRP conjugate is indicative of the presence of anti-HAV IgM.

The VITROS Anti-HAV IgM Reagent Pack is composed of streptavidin coated wells, biotinylated antibody reagent (biotin-mouse monoclonal anti-human IgM) in buffer with antimicrobial agent, and conjugate reagent (HRP-mouse monoclonal anti-HAV) in buffer with antimicrobial agent.  The VITROS Anti-HAV IgM Calibrator is human anti-HAV IgM positive plasma from donors tested individually and found negative for hepatitis B surface antigen (HBsAg), antibodies to human immunodeficiency virus (HIV 1+2) and hepatitis C virus (HCV).  The VITROS Anti-HAV IgM Controls are a negative control (freeze-dried normal human plasma with antimicrobial agent, obtained from HBsAg, HIV 1+2 and HCV negative donors), and a positive control (also contains human anti-HAV IgM positive plasma, from HBsAg, HIV 1+2 and HCV negative donors).

**J. Substantial Equivalence Information:**
1. Predicate device name(s):
   Abbott IMX HAVAB-M assay
2. Predicate 510(k) number(s):
   P790019
3. Comparison with predicate:

| Similarities | | |
|---|---|---|
| Item | Device | Predicate |
| Intended Use | For the qualitative determination of IgM antibody to hepatitis A virus (anti-HAV IgM) | For the qualitative determination of specific IgM antibody against hepatitis A virus (IgM Anti-HAV) |
| Basic principle | Enzyme Linked Immuno Assay | Enzyme Linked Immuno Assay |
| Antigen | Hepatitis A virus | Hepatitis A virus |
| Antibody | Monoclonal antibody: Mouse anti-HAV | Monoclonal antibody: Mouse anti-HAV |
| Instrumentation | ECi/ECiQ Immunodiagnostic System: Automated analyzer | IMx System: Automated analyzer |
| Sample type | Serum, plasma (heparin, citrate, EDTA) | Serum, plasma (heparin, citrate, EDTA) |

| Differences | | |
|---|---|---|
| Item | Device | Predicate |
| Antibody | Mouse anti-Human IgM | Goat anti-Human IgM |
| Tracer | Horseradish Peroxidase | Alkaline Phosphatase |
| Sample volume | 10μL | 150μL |

**K. Standard/Guidance Document Referenced (if applicable):**

Class II Special Control Guidance Document: Hepatitis A Virus Serological Assays, issued February 9, 2006

**L. Test Principle:**

Immunoassay, qualitative determination of IgM antibody to HAV in human body fluids using antibody class capture technique followed by chemiluminescence detection. The light signals are read by the VITROS Immunodiagnostic System. The binding of HRP conjugate is indicative of the presence of anti-HAV IgM.

| Assay Type | Assay Time and Temperature | |
|---|---|---|
| Immunometric (Antibody Class capture) | Incubation time: Time to first result: Temperature: | 32 minutes 43 minutes 37°C |

Reaction scheme:
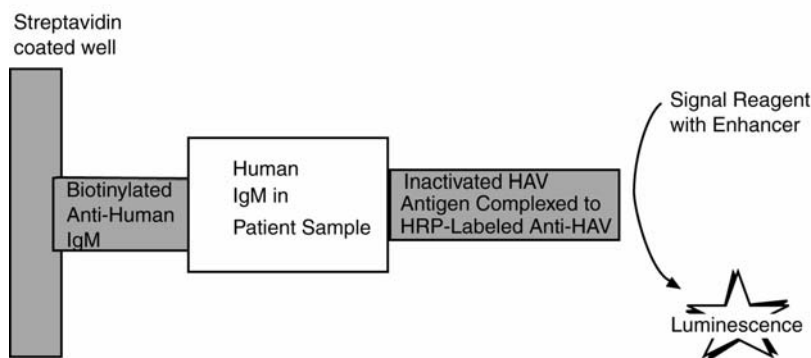


Results are calculated as a normalized signal, relative to a cut-off value (signal/cutoff, s/c). During the calibration process a lot-specific parameter, encoded on the lot calibration card, is used to determine a valid stored cut-off value for the VITROS Immunodiagnostic System. Results are automatically calculated by the VITROS Immunodiagnostic System.

$$\text{Result} = \frac{\text{Signal for test sample}}{\text{Cut-off value}}$$

Patient sample results will be displayed with a "Negative", "Borderline" or "Reactive" label. An initial result labeled "Borderline" indicates a sample that requires duplicate repeat testing for anti-HAV IgM in order to determine a final reactive or negative result relative to the cutoff.

**Interpretation of Results:**

| VITROS Anti-HAV IgM Assay Result (s/c) | Result Text (Testing Algorithm Instruction) | Clinical Interpretation |
|---|---|---|
| <0.80 | Negative (No further testing) | Indicates a non-reactive sample, "Negative" for anti-HAV IgM. A negative test result does not exclude the possibility of infection with hepatitis A virus. Levels of anti-HAV IgM may be below the cut-off in early |

| | | infection. |
|---|---|---|
| ≥0.80 and <1.20 | Borderline (Retest in duplicate) | If 2 of 3 results are ≥0.80 to <1.20 s/c, sample is "Borderline Reactive" for ant-HAV IgM. It is recommended that a new specimen be obtained in two weeks and retested. |
| ≥1.20 | Reactive (No further testing) | Indicates a "Reactive" sample and the presence of anti-HAV IgM. A reactive anti-HAV IgM result does not rule out other hepatitis infections. |

Detection of anti-HAV IgM does not necessarily imply an acute HAV infection due to the longevity of anti-HAV IgM. The detection of anti-HAV IgM can be useful for the differential diagnosis of hepatitis A from other forms of viral hepatitis. Testing with other hepatitis markers is required.

## M. Performance Characteristics (if/when applicable):
1. Analytical performance:

   a. *Precision/Reproducibility:*
   Precision was evaluated based on the Clinical and Laboratory Standards Institute (formerly NCCLS) protocol EP5. The precision panel consisting of 4 samples (a negative, a negative close to the cut-off, a positive close to the cut-off and a positive) was prepared and shipped to 3 different sites. Two replicates of each of 4 panel samples were assayed at each of the 3 different sites once per day for at least 20 different days, over two calibration intervals. The experiment was performed using 1 reagent lot on three different VITROS Immunodiagnostic Systems at three different sites. The data presented is a summary of the product performance:

| Clinical Site | Mean VITROS Anti-HAV IgM S/C (Ratio) | Within Day* | | Between Day** | | Total*** | | No. of Observ. | No. of Days |
|---|---|---|---|---|---|---|---|---|---|
| | | SD | CV (%) | SD | CV (%) | SD | CV (%) | | |
| Site 1 | 0.08 | 0.025 | 29.6 | 0.009 | 10.4 | 0.026 | 31.3 | 40 | 20 |
| | 0.64 | 0.034 | 5.3 | 0.027 | 4.3 | 0.044 | 6.8 | 40 | 20 |
| | 0.99 | 0.044 | 4.5 | 0.042 | 4.2 | 0.061 | 6.2 | 40 | 20 |
| | 2.33 | 0.112 | 4.8 | 0.121 | 5.2 | 0.164 | 7.0 | 40 | 20 |
| Site 2 | 0.07 | 0.018 | 27.1 | 0.010 | 15.9 | 0.021 | 31.4 | 40 | 20 |
| | 0.53 | 0.034 | 6.5 | 0.046 | 8.8 | 0.058 | 10.9 | 40 | 20 |
| | 0.84 | 0.035 | 4.1 | 0.072 | 8.6 | 0.080 | 9.6 | 40 | 20 |
| | 2.13 | 0.065 | 3.1 | 0.142 | 6.7 | 0.156 | 7.3 | 40 | 20 |
| Site 3 | 0.08 | 0.021 | 27.4 | 0.017 | 22.3 | 0.027 | 35.3 | 40 | 20 |
| | 0.67 | 0.039 | 5.9 | 0.063 | 9.4 | 0.074 | 11.1 | 40 | 20 |
| | 1.02 | 0.080 | 7.8 | 0.042 | 4.1 | 0.090 | 8.8 | 40 | 20 |
| | 2.58 | 0.096 | 3.7 | 0.156 | 6.0 | 0.183 | 7.1 | 40 | 20 |

* Within Day: variability of the assay performance from replicate to replicate.
** Between Day: variability of the assay performance from day to day.
*** Total: variability of the assay performance combining the effects of within day and between day.

| Mean VITROS Anti-HAV IgM S/C (Ratio) | Between Site * | | Total ** | | No. Obs. |
|---|---|---|---|---|---|
| | SD | CV (%) | SD | CV (%) | |
| 0.08 | 0.008 | 10.6 | 0.026 | 34.6 | 120 |
| 0.61 | 0.072 | 11.8 | 0.094 | 15.3 | 120 |
| 0.95 | 0.099 | 10.4 | 0.126 | 13.2 | 120 |
| 2.35 | 0.225 | 9.6 | 0.281 | 12.0 | 120 |

\* Between Site: Variability of the assay performance from site to site.

\*\* Total: Variability of the assay incorporating factors of site and day.

b. *Linearity/assay reportable range:*

The Limit of Blank (LoB) has been determined to be 0.04 s/c.

c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*
Each quoted mean value is derived from a minimum of 10 assays. The standard deviation is that which would be anticipated for singleton determinations of each control in a number of different laboratories using different reagent lots. Quoted values are lot specific.
Vitros Anti-HAV IgM Controls - Baseline Statistics:

| Control | Mean Result | SD |
|---|---|---|
| 1 (Negative) | 0.10 | 0.10 |
| 2 (Positive) | 2.14 | 0.327 |

Calibration is lot specific; reagent packs and calibrators are linked by lot number. A Master Calibration is established for each new reagent lot by performing multiple assays. This is the process by which a lot-specific parameter [a] which links the cut-off value to the calibrator signal is determined.
Cut-off value = (a x Signal of CAL1)
The lot-specific parameter, the expected calibrator signal and the data which enables a System to calculate the cut-off value, are encoded on the lot calibration card. Scanning the lot calibration card loads the encoded data onto the System. When the calibrator is processed the validity of the calibration is assessed against a quality parameter which compares the actual signal of the calibrator with the expected signal. If the calibration is acceptable the cut-off value is calculated and 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 control values to determine the validity of the calibration. Recalibration is required after a predetermined calibration interval, or when a different reagent lot is loaded.

d. *Detection limit:*
Limit of Detection (LoD) has been determined to be 0.33 s/c.

*e. Analytical specificity:*

The specificity of the VITROS Anti-HAV IgM assay was evaluated by testing 189 samples from the following potentially cross-reacting sub-groups (see table below). All initially reactive samples were tested with a reference assay for confirmation. None of these categories were found to interfere with the VITROS Anti-HAV IgM assay.

Of the 189 samples tested, two (2) were observed to be discordant.

| Summary of Data from Potentially Cross-Reacting Sub-Groups | | | | | |
|---|---|---|---|---|---|
| Sample Category | No. Samples Tested | VITROS Anti-HAV IgM Assay | | Reference Assay | |
| | | No. Negatives | No. Initial Reactives/Borderlines | No. Initial Reactives/Borderlines | No. Discordants |
| Acute Hepatitis B | 20 | 20 | 0 | 0 | 0 |
| ANA | 5 | 5 | 0 | NT* | - |
| Anti-HAV IgG | 8 | 8 | 0 | 0 | 0 |
| Anti-HCV | 12 | 12 | 0 | 0 | 0 |
| CMV IgM | 11 | 11 | 0 | 0 | 0 |
| EVB | 5 | 5 | 0 | 0 | 0 |
| HAMA | 5 | 5 | 0 | NT* | - |
| HIV | 10 | 10 | 0 | 0 | 0 |
| Mumps | 5 | 5 | 0 | NT* | - |
| Non Viral Liver | 10 | 10 | 0 | 1 | 1 |
| Parvo B-19 | 3 | 3 | 0 | 0 | 0 |
| Rheumatoid Factor | 52 | 51 | 1 | 0 | 1 |
| Rubella | 5 | 5 | 0 | NT* | - |
| Rubeola | 5 | 5 | 0 | NT* | - |
| SLE | 23 | 23 | 0 | 0 | 0 |
| Toxoplasma | 5 | 5 | 0 | NT* | - |
| VZV | 5 | 5 | 0 | NT* | - |
| **Total** | **189** | **188** | **1** | **1** | **2** |

*NT = Not Tested

<u>Substances that do not Interfere</u>

Serial dilutions were made for bilirubin, triolein, hemoglobin and biotin, and point estimates were made for sodium azide and dipyrone. The mean result of 3 determinations of a solution of each test substance was compared with that of a control pool, for both a negative and positive sample. For each substance, the highest concentration which was considered not to impact results for both positive and negative samples is shown in the table below:

| Compound | Compound Concentration | |
|---|---|---|
| Bilirubin | 0.257 mmol/L | 15 mg/dL |
| Biotin | 10 ng/mL | 1.0 µg/dL |
| Dipyrone | 1.0 mg/mL | 100 mg/dL |
| Hemoglobin | 0.31 mmol/L | 500 mg/dL |
| Sodium Azide | 1.0 g/dL | 1000 mg/dL |
| Triolein | 33.9 mmol/L | 3000 mg/dL |

*f. Assay cut-off:*

The cut-off signal was established as the light signal which gives the best discrimination between anti- HAV IgM reactive and anti-HAV IgM negative sample populations, to provide optimum specificity and sensitivity for the assay. The cut-off signal level was assigned a result of 1.00. Assay results < 0.8 s/c indicate a negative sample, negative for anti-HAV IgM. Assay results ≥ 1.20 s/c indicate a reactive sample, positive for anti-HAV IgM. A result of ≥0.8 and <1.2 s/c indicates a borderline sample.

2. Comparison studies:

a. *Method comparison with predicate device:*
   A comparison of VITROS Anti HAV IgM assay and reference anti HAV IgM assay results is presented in the following tables. Data are listed by site and population. Positive and negative percent agreement and 95% exact confidence intervals are also shown.

| VITROS Anti-HAV IgM Assay Result | Reference Anti-HAV IgM Assay Result [*] | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Site 1 | | | Site 2 | | | Site 3 | | | All Sites | | |
| | R | GZR ** | N | R | GZR ** | N | R | GZR ** | N | R | GZR ** | N |
| Reactive | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 2 | 1 | 0 |
| Borderline Reactive*** | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 2 |
| Negative | 0 | 0 | 111 | 0 | 0 | 433 | 0 | 0 | 327 | 0 | 0 | 871 |
| Total | 1 | 0 | 111 | 1 | 1 | 435 | 0 | 0 | 327 | 2 | 1 | 873 |
| Positive Percent Agreement | 100% (1/1) | | | 100% (2/2) | | | N/A | | | 100% (3/3) | | |
| 95% Exact Confidence Interval | 2.5% - 100% | | | 15.81% - 100% | | | N/A | | | 29.24% - 100% | | |
| Negative Percent Agreement | 100% (111/111) | | | 99.54% (433/435) | | | 100% (327/327) | | | 99.77% (871/873) | | |
| 95% Exact Confidence Interval | 96.73% - 100% | | | 98.35% - 99.94% | | | 98.88% - 100% | | | 99.17% - 99.97% | | |

*Table title: VITROS and Reference Anti-HAV IgM Assay Results in Population 1: Prospective Samples from the U.S. (N=876)*

   \* Reference assay result: R = Reactive; GZR = Grayzone Reactive; N = Negative

   \*\* Reference assay Grayzone Reactive (GZR) samples are interpreted as anti-HAV IgM reactive.

   \*\*\* Two subjects from Population 1 had initial results in the Borderline region. VITROS Borderline reactive results are considered anti-HAV IgM reactive in the positive and negative percent agreement calculations.

| VITROS Anti-HAV IgM Assay Result | Reference Anti-HAV IgM Assay Result [*] | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Site 1 | | | Site 2 | | | Site 3 | | | All Sites | | |
| | R | GZR ** | N | R | GZR ** | N | R | GZR ** | N | R | GZR ** | N |
| Reactive | 12 | 1 | 0 | 3 | 0 | 0 | 8 | 3 | 0 | 23 | 3 | 0 |
| Borderline Reactive*** | 0 | 1 | 0 | 0 | 0 | 5 | 0 | 2 | 3 | 0 | 3 | 8 |
| Negative | 0 | 0 | 126 | 0 | 0 | 31 | 0 | 0 | 121 | 0 | 0 | 278 |
| Total | 12 | 1 | 126 | 3 | 0 | 36 | 8 | 5 | 124 | 23 | 6 | 286 |

*Table title: VITROS and Reference Anti-HAV IgM Assay Results in Population 2: Prospective Samples from India (N=315)*

| Positive Percent Agreement | 100% (13/13) | 100% (3/3) | 100% (13/13) | 100% (29/29) |
|---|---|---|---|---|
| 95% Exact Confidence Interval | 75.29% - 100% | 29.24% - 100% | 75.29% - 100% | 88.06% - 100% |
| Negative Percent Agreement | 100% (126/126) | 86.11% (31/36) | 97.58% (121/124% | 97.20% (278/286) |
| 95% Exact Confidence Interval | 97.11% - 100% | 70.50% - 95.33% | 93.09% - 99.50% | 94.56% - 98.78% |

\* Reference assay result:  R = Reactive; GZR = Grayzone Reactive; N = Negative

\*\* Reference assay Grayzone Reactive (GZR) samples are interpreted as anti-HAV IgM reactive.

\*\*\* Eleven subjects from Population 2 had initial results in the Borderline region. VITROS Borderline reactive
       results are considered anti-HAV IgM reactive in the positive and negative percent agreement calculations

The positive percent agreement of the VITROS Anti-HAV IgM assay with the reference anti-HAV IgM assay was 100% (3/3) for Population 1 and 100% (29/29) for Population 2.  The negative percent agreement of the VITROS Anti-HAV IgM assay with the reference assay was 99.77% (871/873) for Population 1 and 97.20% (278/286) for Population 2.

The overall positive percent agreement for the VITROS Anti-HAV IgM assay with the reference assay was 100% (32/32), with a 95% exact confidence interval of 89.11% to 100% for the prospective samples in Populations 1 and 2 combined.  The overall negative percent agreement for the VITROS Anti-HAV IgM assay with the reference assay was 99.14% (1149/1159), with a 95% exact confidence interval of 98.42% to 99.59% for the prospective samples in Populations 1 and 2 combined.

b.  *Matrix comparison:*
A total of 25 donors had blood drawn which was spiked with anti-HAV IgM positive plasma to just above the assay cut-off. The spiked blood was then aliquoted into serum and plasma collection tubes and tested in the VITROS anti-HAV IgM assay. The percent difference in the plasma from serum was calculated.  Mean percent differences from serum are represented below for each plasma type tested.

Percent Difference from Serum

o Heparin   ■ EDTA   ▲ Citrate
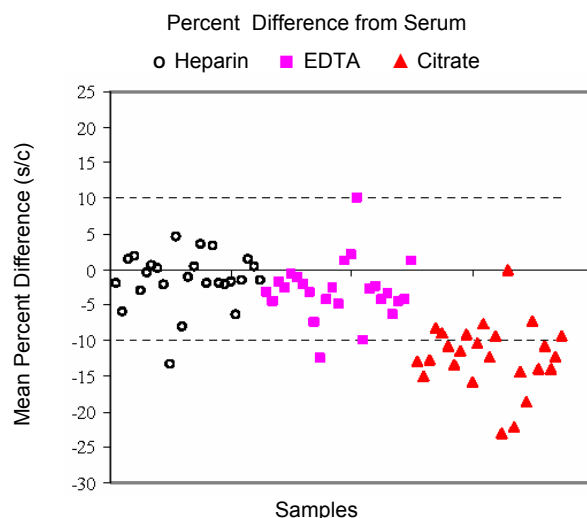


Some anti-coagulants (e.g. liquid citrate) have a dilutional effect on samples and results should be interpreted accordingly.

3. Clinical studies:
   a. *Clinical Sensitivity:*
      Not applicable.
   b. *Clinical Specificity:*
      Not applicable.
   c. *Other clinical supportive data (when a. and b. are not applicable)*:
      ***Performance of the VITROS Anti-HAV IgM Assay in Known Anti-HAV IgM Reactive Subjects:***
      The performance of the VITROS Anti-HAV IgM assay was evaluated among serum samples from subjects known to be anti-HAV IgM positive.  A total of 77 samples collected in Egypt (N=50) and India (N=27) from subjects with a medical history and laboratory results indicative of acute hepatitis A were tested concurrently with the VITROS and reference anti-HAV IgM assays. The VITROS Anti-HAV IgM assay was reactive and in 100% agreement with the reference anti-HAV IgM assay in all 77 anti-HAV IgM reactive samples. The positive percent agreement of the VITROS Anti-HAV IgM assay with the reference anti-HAV IgM assay and the 95% exact confidence interval are presented in the following table.

| Agreement of the VITROS and Reference Anti-HAV IgM Assays in Known Anti-HAV IgM Reactive Subjects | | |
| --- | --- | --- |
| **Population** | **Positive Percent Agreement** | **95% Exact Confidence Interval** |
| **Anti-HAV IgM Reactive Subjects** | 100% (77/77) | 95.32% - 100% |

***Performance of the VITROS Anti-HAV IgM Assay in Pediatric Subjects:***
The VITROS Anti-HAV IgM assay was also evaluated using residual laboratory serum samples from pediatric subjects at low risk for viral hepatitis.  The samples were unlinked to the subjects' identities, and were

included based on age, gender and available volume remaining after all testing ordered for that sample had been completed. Samples were selected such that the following age ranges (in years) were represented (2-4, 5-9, 10-14, and 15-19).

The positive and negative percent agreement of the VITROS Anti-HAV IgM assay with the reference anti-HAV IgM assay, and the 95% exact confidence intervals are presented in the following table.

| Agreement of the VITROS and Reference Anti-HAV IgM Assays in Pediatric Subjects | | |
|---|---|---|
| Population | Negative Percent Agreement | 95% Exact Confidence Interval |
| Pediatric Subjects | 100% (110/110) | 96.70% - 100% |
| | | |

The negative percent agreement for the VITROS Anti-HAV IgM assay with the reference assay was 100% (110/110), with a 95% exact confidence interval of 96.70% to 100% for the pediatric samples. None of the samples were reactive with the VITROS or reference anti-HAV IgM assays.

### *Performance of the* VITROS *Anti-HAV IgM Assay in Cord Blood:*

A total of 20 cord blood samples (as a surrogate for neonate serum) and 10 adult serum samples were tested in the VITROS Anti-HAV IgM assay. None of the samples were found to give a reactive result in the VITROS Anti-HAV IgM assay. Thirty microliters (30 μl) of anti-HAV IgM positive material was added to 270 μl of cord blood and adult serum.

| Anti-HAV IgM Cord Blood Study | | | |
|---|---|---|---|
| Sample Type | N | Mean Response  (s/c) | SD |
| Cord Blood - Neat | 20 | 0.08 | 0.018 |
| Cord Blood – Spiked | 20 | 2.24 | 0.134 |
| Adult Serum – Neat | 10 | 0.07 | 0.010 |
| Adult Serum - Spiked | 10 | 1.88 | 0.218 |

A 16 % positive bias was observed with the cord blood when compared to adult serum.

### Seroconversion Panels

Three seroconversion panels each having at least 5 individual samples with a known predetermined result were measured in the VITROS Anti-HAV IgM assay and in a reference assay. The VITROS and reference anti-HAV IgM assay results are summarized below. The VITROS Anti-HAV IgM assay gave seroconversion sensitivity equivalent to or more sensitive than a reference assay in the three panels tested, although it appears to detect IgM for a longer period than the comparator assay for qualitative determination of IgM antibody to hepatitis A.

| Panel ID | VITROS Anti-HAV IgM | | Anti-HAV IgM Reference Assay | | Difference in Days to Repeatedly Reactive Result |
|---|---|---|---|---|---|
| | Post bleed day of last non-reactive result | Post bleed day of first repeatedly | Post bleed day of last non-reactive result | Post bleed day of first repeatedly | |

| | | reactive result. | | reactive result | |
|---|---|---|---|---|---|
| **PHT902** | 3 | 16 | 3 | 16 | 0 |
| **RP-004** | 0 | 6 | 0 | 6 | 0 |

| Panel ID | VITROS Anti-HAV IgM | | Anti-HAV IgM Reference Assay | | Difference in Days from Last Repeatedly Reactive Result |
|---|---|---|---|---|---|
| | Post bleed day of earliest reactive result | Post bleed day of last positive result* | Post bleed day of earliest reactive result | Post bleed day of last positive result | |
| **RP-6201** | 0 | 42 | 0 | 28 | 14 |

*Only positive results from both VITROS and Reference assays were used. Borderline Reactive results were not used to determine a positive result.

4. Clinical cut-off:
   Not applicable

5. Expected values/Reference range:
   *HAV Prevalence Population*
   The expected results of the VITROS Immunodiagnostic Products Anti-HAV IgM assay to detect anti-HAV IgM was determined in presumably healthy individuals from both high (Western US) and low (Eastern US) HAV disease prevalence areas in the United States. The population was 50% male and 50% female, with ages that ranged from 18 to 89. The majority of the subjects were White/Caucasian (72.0%). Other ethnic groups tested were African American (12.0%), Hispanic/Latino (15.0%) and Asian (1.0%).

   The expected results for presumably healthy individuals living in either high or low prevalence areas are presented below. Two individuals of the low prevalence population produced a positive result in the VITROS Anti-HAV IgM assay

| Expected Results for the VITROS Anti-HAV IgM Assay in Subjects From Low Prevalence Areas for Hepatitis A (N=622) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Age Range | Gender | VITROS Anti-HAV IgM Result | | | | | | Total |
| | | Reactive | | Borderline | | Negative | | |
| | | N | Percent | N | Percent | N | Percent | |
| 18–20 | Female | 0 | 0.0 | 0 | 0.0 | 11 | 100.0 | 11 |
| | Male | 0 | 0.0 | 0 | 0.0 | 6 | 100.0 | 6 |
| 21–30 | Female | 0 | 0.0 | 0 | 0.0 | 27 | 100.0 | 27 |
| | Male | 0 | 0.0 | 0 | 0.0 | 39 | 100.0 | 39 |
| 31–40 | Female | 0 | 0.0 | 0 | 0.0 | 26 | 100.0 | 26 |
| | Male | 0 | 0.0 | 0 | 0.0 | 45 | 100.0 | 45 |
| 41–50 | Female | 0 | 0.0 | 0 | 0.0 | 75 | 100.0 | 75 |
| | Male | 0 | 0.0 | 0 | 0.0 | 55 | 100.0 | 55 |
| 51–60 | Female | 0 | 0.0 | 0 | 0.0 | 102 | 100.0 | 102 |
| | Male | 0 | 0.0 | 0 | 0.0 | 114 | 100.0 | 114 |
| 61–70 | Female | 1 | 4.5 | 0 | 0.0 | 21 | 95.5 | 22 |
| | Male | 0 | 0.0 | 0 | 0.0 | 33 | 100.0 | 33 |
| 71–80 | Female | 0 | 0.0 | 0 | 0.0 | 31 | 100.0 | 31 |
| | Male | 1 | 4.8 | 0 | 0.0 | 20 | 95.2 | 21 |
| 81–90 | Female | 0 | 0.0 | 0 | 0.0 | 7 | 100.0 | 7 |
| | Male | 0 | 0.0 | 0 | 0.0 | 8 | 100.0 | 8 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Total** | | **2*** | **0.3** | **0** | **0.0** | **620** | **99.7** | **622** |

*Two subjects from New Jersey giving initial results of 1.16 and 1.21 s/c, also had strong VITROS Anti-HAV Total results (s/c 0.01).

One subject gave an initial Borderline result.

| Expected Results for the VITROS Anti-HAV IgM Assay in Subjects From High Prevalence Areas for Hepatitis A (N=378) | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Age Range** | **Gender** | **VITROS Anti-HAV IgM Result** | | | | | | **Total** |
| | | **Reactive** | | **Borderline** | | **Negative** | | |
| | | **N** | **Percent** | **N** | **Percent** | **N** | **Percent** | |
| 18–20 | Female | 0 | 0.0 | 0 | 0.0 | 3 | 100.0 | 3 |
| | Male | 0 | 0.0 | 0 | 0.0 | 6 | 100.0 | 6 |
| 21–30 | Female | 0 | 0.0 | 0 | 0.0 | 40 | 100.0 | 40 |
| | Male | 0 | 0.0 | 0 | 0.0 | 39 | 100.0 | 39 |
| 31–40 | Female | 0 | 0.0 | 0 | 0.0 | 34 | 100.0 | 34 |
| | Male | 0 | 0.0 | 0 | 0.0 | 39 | 100.0 | 39 |
| 41–50 | Female | 0 | 0.0 | 0 | 0.0 | 18 | 100.0 | 18 |
| | Male | 0 | 0.0 | 0 | 0.0 | 24 | 100.0 | 24 |
| 51–60 | Female | 0 | 0.0 | 0 | 0.0 | 72 | 100.0 | 72 |
| | Male | 0 | 0.0 | 0 | 0.0 | 38 | 100.0 | 38 |
| 61–70 | Female | 0 | 0.0 | 0 | 0.0 | 20 | 100.0 | 20 |
| | Male | 0 | 0.0 | 0 | 0.0 | 15 | 100.0 | 15 |
| 71–80 | Female | 0 | 0.0 | 1* | 14.3 | 6 | 85.7 | 7 |
| | Male | 0 | 0.0 | 0 | 0.0 | 13 | 100.0 | 13 |
| 81–90 | Female | 0 | 0.0 | 0 | 0.0 | 5 | 100.0 | 5 |
| | Male | 0 | 0.0 | 0 | 0.0 | 5 | 100.0 | 5 |
| **Total** | | **0** | **0.0** | **0** | **0.0** | **378** | **100.0** | **378** |
| *One subject gave an initial Borderline result. | | | | | | | | |

### Adult Subjects at High Risk for Viral Hepatitis

Expected results of asymptomatic individuals from the multi-center study described in "Performance Characteristics" are provided below. Approximately 74.2% (650/876) of the 876 prospective subjects enrolled in the US reported no recent or current signs or symptoms of hepatitis. Of these 650 asymptomatic individuals, 8.0% were enrolled in Miami, FL, 46.2% were enrolled in Dallas, TX, and 45.8% were enrolled in Chicago, IL. The group was Caucasian (25.5%), African American (55.1%) and Hispanic (14.9%), with the remaining 4.5% represented by other ethnic groups. The group was 58.8% male and 41.2% female and ranged in age from 16 to 81 years. All were at risk for viral hepatitis due to lifestyle, behavior, occupation or known exposure event. The VITROS Anti-HAV IgM assay was reactive in 0.5% of the individuals in this group. The percent VITROS Anti-HAV IgM reactive results observed in the asymptomatic population at each collection site was 0.31% at Chicago, IL, and 0.15% at Dallas, TX. The expected results for the VITROS Anti-HAV IgM assay in subjects at high risk for viral hepatitis are presented in the following table.

| Expected Results for the VITROS Anti-HAV IgM Assay in Study Subjects at High Risk for Viral Hepatitis Without Signs or Symptoms of Hepatitis (N=650) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Age Range | Gender | VITROS Anti-HAV IgM Result | | | | | | Total |
| | | Reactive | | Borderline Reactive | | Negative | | |
| | | N | Percent | N | Percent | N | Percent | |
| 18–20 | Female | 0 | 0 | 0 | 0.0 | 11 | 100 | 11 |
| | Male | 0 | 0 | 0 | 0.0 | 7 | 100 | 7 |
| 21–30 | Female | 0 | 0 | 0 | 0.0 | 60 | 100 | 60 |
| | Male | 0 | 0 | 1 | 1.9 | 52 | 98.1 | 53 |
| 31–40 | Female | 0 | 0 | 1 | 1.3 | 78 | 98.7 | 79 |
| | Male | 0 | 0 | 0 | 0.0 | 139 | 100 | 139 |
| 41–50 | Female | 1 | 1.7 | 0 | 0.0 | 58 | 98.3 | 59 |
| | Male | 1 | 0.8 | 0 | 0.0 | 117 | 99.2 | 118 |
| 51–60 | Female | 0 | 0 | 0 | 0.0 | 35 | 100 | 35 |
| | Male | 0 | 0 | 0 | 0.0 | 34 | 100 | 34 |
| 61–70 | Female | 0 | 0 | 0 | 0.0 | 19 | 100 | 19 |
| | Male | 0 | 0 | 0 | 0.0 | 24 | 100 | 24 |
| 71–80 | Female | 0 | 0 | 0 | 0.0 | 5 | 100 | 5 |
| | Male | 0 | 0 | 0 | 0.0 | 6 | 100 | 6 |
| 81–81 | Female | 0 | 0 | 0 | 0.0 | 1 | 100 | 1 |
| Total | | 2 | 0.31 | 2* | 0.31 | 646 | 99.4 | 650 |
| *Two subjects gave an initial Borderline result. | | | | | | | | |

### Pediatric Subjects at Low Risk for Hepatitis

Expected results for the VITROS Anti-HAV IgM assay were also determined using unlinked, randomly collected samples from pediatric subjects at low risk for viral hepatitis (N=110). The group was 30.9% male and 69.1% female, and the subjects' ages ranged from 2 to 19 years. The expected results for the VITROS Anti-HAV IgM assay in pediatric subjects are presented in the following table.

| Age Range | Gender | VITROS Anti-HAV IgM Result | | | | | | Total |
|---|---|---|---|---|---|---|---|---|
| | | Reactive | | Borderline Reactive | | Negative | | |
| | | N | Percent | N | Percent | N | Percent | |
| 2-4 | Female | 0 | 0 | 0 | 0 | 7 | 100 | 7 |
| | Male | 0 | 0 | 0 | 0 | 12 | 100 | 12 |
| 5-9 | Female | 0 | 0 | 0 | 0 | 23 | 100 | 23 |
| | Male | 0 | 0 | 0 | 0 | 9 | 100 | 9 |
| 10-14 | Female | 0 | 0 | 0 | 0 | 27 | 100 | 27 |
| | Male | 0 | 0 | 0 | 0 | 5 | 100 | 5 |
| 15-19 | Female | 0 | 0 | 0 | 0 | 19 | 100 | 19 |
| | Male | 0 | 0 | 0 | 0 | 8 | 100 | 8 |
| Total | | 0 | 0 | 0 | 0 | 110 | 100 | 110 |

**N. Proposed Labeling:**

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

**O. Conclusion:**

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