

**SUMMARY OF SAFETY AND
EFFECTIVENESS DATA (SSED)**

Summary of Safety and Effectiveness

I. GENERAL INFORMATION

Device Generic Name: Total antibody to Hepatitis A virus (anti-HAV total antibody)

Device Trade Name: ADVIA Centaur® HAV Total Assay
ADVIA Centaur® HAV Total Quality Control Materials

Name and Address of Applicant: Bayer HealthCare LLC
511 Benedict Avenue
Tarrytown, NY 10591-5097

Premarket Approval Application (PMA) Number: P040017

Date of Panel Recommendation: None

Date of Notice of Approval to Applicant: March 7, 2005

II. INDICATIONS FOR USE

ADVIA Centaur® HAV Total Assay Indications for Use:

The ADVIA Centaur® HAV Total assay is an *in vitro* diagnostic immunoassay for the qualitative determination of total antibodies to hepatitis A virus (anti-HAV) in human serum or plasma (potassium EDTA, lithium or sodium heparinized) using the ADVIA Centaur System. This anti-HAV assay is indicated as an aid in the diagnosis of previous or ongoing hepatitis A viral infection or in the identification of HAV-susceptible individuals for vaccination.

ADVIA Centaur® HAV Total Quality Control Materials Indications for Use:

For monitoring the performance of the HAV Total Assay on ADVIA Centaur® Systems. The performance of the HAV Total quality control material has not been established with any other anti-HAV Total Assay.

III. CONTRAINDICATIONS: None known

IV. WARNINGS, AND PRECAUTIONS: For in vitro diagnostic use only.

Warnings and precautions for ADVIA Centaur® HAV Total ReadyPack Reagents and ADVIA Centaur® HAV Total Quality Control Materials are stated in the respective product labeling.

V. DEVICE DESCRIPTION

Kit Configuration and Components

For detection of anti-HAV total antibodies, the ADVIA Centaur® HAV Total Assay is comprised of the following:

- ADVIA Centaur HAVT ReadyPack primary reagent pack
The ADVIA Centaur HAVT ReadyPack primary reagent pack is composed of three components:
 - Lite Reagent
Monoclonal mouse anti-HAV antibody labeled with acridinium ester and biotinylated monoclonal mouse anti-HAV Fab fragment in buffer with bovine serum albumin and preservatives
 - Solid Phase
Streptavidin coated paramagnetic particles with bovine serum albumin and preservatives
 - Antigen Reagent
Inactivated purified HAV antigen (~0.06 µg/mL) in buffer with bovine serum albumin and preservatives.

- ADVIA Centaur HAVT ReadyPack ancillary reagent pack

ADVIA Centaur HAVT ReadyPack ancillary reagent pack is composed of cysteine in citrate buffer with EDTA and preservatives

In addition, the following components are required:

- ADVIA Centaur System is a dedicated random access instrumentation, which provides automated analysis of the Centaur assays
- ADVIA Centaur HAV Total quality control material which consists of a negative and positive control and an expected value card
- ADVIA Centaur Probe Wash is a solution of sodium hydroxide
- ADVIA Centaur Wash 1 is phosphate buffered saline with preservatives

Assay Principle and Format

The ADVIA Centaur HAV Total assay is a competitive immunoassay designed for the qualitative detection of total antibodies to HAV. The ADVIA Centaur HAV Total assay is performed using the ADVIA Centaur HAVT reagents, calibrators and controls on the ADVIA Centaur System. Sample is diluted in buffer then purified HAV antigen is added to allow formation of immune complexes between the purified antigen and patient total antibodies to HAV. Immune complexes are then simultaneously incubated with acridinium ester labeled mouse monoclonal anti-HAV antibodies, biotinylated mouse monoclonal anti-HAV Fab fragment and streptavidin coated paramagnetic capture microparticles. The biotinylated conjugate and acridinium labeled conjugate bind to antigen sites not occupied by sample antibodies to HAV. The microparticles are then separated by a magnet and unbound material is removed by washing. The bound acridinium ester conjugate is then measured by a chemiluminescent reaction. The amount of acridinium ester bound is inversely proportional to the concentration of HAV total antibodies present in the sample. The chemiluminescent signal is detected and quantified as relative light units (RLUs) by the photomultiplier tube (PMT) of the ADVIA Centaur Instrument. The RLUs are used to calculate an Index Value from a stored calibration curve.

Calibration

The ADVIA Centaur HAV Total assay utilizes a factory set Master Curve. The Master Curve values are contained on the Master Curve card provided with each kit. The master curve and calibration are lot specific. The barcode reader or keyboard is used to enter the Master Curve values on the system. The 2 calibrators in the kit are run when the lot is first used or after expiration of the calibration interval (14 days). When the calibrators are processed, the validity of the calibration is assessed against a quality parameter that compares the actual signal of the calibrator with the expected signal. If the calibration run is valid, the values are stored and used to “normalize” test values to the Master Curve. The system reports HAV Total results in Index Values and as reactive or non-reactive. Samples with a calculated value greater than or equal to 1.00 index value are considered reactive for antibodies to HAV.

VI. ALTERNATE PRACTICES AND PROCEDURES

Determination of the presence of anti-HAV Total antibodies in patients may be achieved by using a number of commercially available, FDA licensed/approved, serological tests. When the results of such tests are evaluated in conjunction with a physician's assessment and other biochemical test results, susceptibility to HAV can be excluded.

VII. MARKETING HISTORY

The ADVIA Centaur ® HAV Total Assay is currently being marketed internationally in accordance with Section 802 of the Food Drug & Cosmetic Act in the following countries: Colombia, Sweden, Norway, Finland, France, Germany, Italy, Spain, Portugal, United Kingdom, Belgium, Austria, South Africa, China, Hong Kong, Singapore, Malaysia, Korea, Australia, and New Zealand.

This product has not been withdrawn from any of these markets for any reason.

VIII. POTENTIAL ADVERSE EFFECTS OF THE DEVICE ON HEALTH

Since the ADVIA Centaur ® HAV Total ReadyPack Reagents and ADVIA Centaur ® HAV Total Quality Control Materials are for *in vitro* diagnostic use, there is no direct adverse effect on the health of the patient.

However, failure of the product to perform as intended, or errors in the use of the product, may lead to a false result.

Failure of HAV serological assays to perform as indicated or an error in interpretation of results may lead to improper patient management. There are no clinical features that distinguish HAV infection from infection by other etiologic agents of hepatitis such as hepatitis B virus (HBV) or hepatitis C virus (HCV). HAV serological assays are used to aid in this distinction. Therefore, false test results could contribute to improper patient management, which includes misdiagnosis.

A false negative result for HAV-specific total antibodies would result in misdiagnosis of past infection and may cause individuals to erroneously receive vaccination for HAV. This would be of minimal risk, however, since there is no contraindication for an individual immune to HAV receiving HAV vaccination.

A false positive measurement can result in incorrect diagnosis of active or past HAV infection. If HAV-specific total antibodies are detected erroneously, an individual may not receive the vaccine for HAV and could continue to be at risk for HAV infection.

IX. SUMMARY OF NON-CLINICAL STUDIES

Laboratory Studies

The following laboratory studies were conducted to determine the performance characteristics of the ADVIA Centaur HAV IgM assay. These laboratory studies included HAV performance panels, HAV seroconversion panels, potential cross reactive specimens, endogenous interferences, precision, matrix and collection tube type effects, sample handling, stability, microbial studies and instrument studies.

Assay Standardization

The ADVIA Centaur HAV Total assay cutoff (Index Value 1.00) is equivalent to 20 mIU/mL standardized to the WHO 2nd International Standard for Anti-Hepatitis A Immunoglobulin (97/646). However, assay results can not be considered quantitative and no clinical claims for immunity can be determined from the cutoff.

Detection of both anti-HAV IgM and IgG

The IgG and IgM fractions were separated from a HAV Total positive sample by Superdex 200 column chromatography. Dilutions of the fractions were made into a HAV Total negative plasma pool. The fractions were tested in the Centaur HAV Total assay and a FDA approved method. Results show that ADVIA Centaur HAV Total assay is capable of detecting anti-HAV IgM and anti-HAV IgG separately.

HAV Total Performance Panels

Two commercially available mixed titer performance panels comprised of anti-HAV total positive serum and/or plasma samples in a range of titers were tested with the ADVIA Centaur HAV Total assay and a reference assay. One panel was PHT 201 from Boston Biomedica Inc. and the other was HS C002 from Cypress Diagnostics. Results were obtained with two lots of ADVIA Centaur HAV Total reagents and calibrators. The ADVIA Centaur HAV Total assay agreed 100% with the results produced by the reference assay for both lots of ADVIA reagents tested.

HAV Seroconversion Panels

Four commercially available HAV patient seroconversion panels were tested using the ADVIA Centaur HAV Total assay and a reference assay. The table below lists the first bleed of each panel that tested reactive with the ADVIA and the reference assay as well as the difference between the two assays in identifying the first reactive panel member by number of bleeds.

Panel ID	Anti-HAV Total Positive Result From Initial Draw Date		Comparative Assay vs ADVIA Centaur Assay Difference in Bleed Numbers*
	Comparative Assay (Days)	ADVIA Centaur Assay (Days)	
RP004	7	7	0
RP013	9	9	0
PHT902	16	16	0
ProMedx 1	1	1	0

* The difference in bleed numbers is relative to the comparative assay. In all seroconversion panels both the ADVIA Centaur assay and the comparative assay detected the first reactive sample at the same day.

Potential Cross Reactive Specimens

The ADVIA Centaur HAV Total assay was evaluated for potential cross-reactivity with viral antibodies and disease state specimens. The anti-HAV Total status of each specimen was verified using a comparative anti-HAV Total assay. All specimens that were positive by the ADVIA Centaur HAV Total assay were also positive by the comparative anti-HAV Total assay. The following results were obtained using the ADVIA Centaur HAV Total assay.

<i>Clinical Category</i>	<i>Number Tested</i>	<i>Number of Positive Anti-HAV Total Results</i>	
		<i>ADVIA Centaur Assay</i>	<i>Comparative Assay</i>
Hepatitis C Infection (HCV)	10	4	4
Hepatitis B Infection (HBV)	8	2	2
Rheumatoid Arthritis	9	6	6
Systemic Lupus	2	1	1
Epstein-Barr Virus (EBV) IgG	10	3	3
Epstein-Barr Virus (EBV) IgM	10	3	3
Herpes Simplex Virus (HSV) IgG	10	5	5
Herpes Simplex Virus (HSV) IgM	10	3	3
Cytomegalovirus IgG	10	5	5
Toxoplasma IgG	10	2	2
Toxoplasma IgM	7	3	3
Human Immunodeficiency Virus (HIV1/2)	10	2	2
Varicella Zoster IgG	10	2	2
Rubeola IgG	10	2	2
Anti-Nuclear Antibody (ANA)	5	0	0
HAMA	10	1	1
Flu vaccine Recipient	10	6	6
Total Samples Tested	151	50	50

Endogenous Interferents

The Centaur HAV Total assay was tested following the guidelines described by NCCLS EP7-P for interference due to varying levels of endogenous substances. The effects were studied up to the following concentrations: conjugated bilirubin @ 60 mg/dL, unconjugated bilirubin @ 40 mg/dL, hemoglobin @ 500 mg/dL, triglycerides @ 3000 mg/dL, human serum albumin @ 12 g/dL (i.e. high total protein) and immunoglobulin G @ 60 mg/mL (i.e. hyper IgG). No interference was found. In addition, a potentially interfering effect of biotin was evaluated using 6 plasma samples spiked with several levels of biotin. Interference was observed at concentrations greater than or equal to 50 ng/mL, but not at 25 ng/mL or less. As a result, for patients receiving therapy with high doses of biotin (i.e. > 5 mg/day) no sample should be taken until at least 8 hours after the last biotin administration.

Precision Studies:

Repeatability

The repeatability component of precision was evaluated according to the National Committee for Clinical Laboratory Standards protocol EP5-A.¹ Panels of different sample types with five samples in each panel were assayed. Samples were assayed 40 times, with 3 replicates each time, over 17 days, on 1 system. The total CV had a range of 3.3 to 8.0%. The within-run CV was under 4% for all samples. The following results were obtained, using one reagent lot:

<i>Sample</i>	<i>Mean</i>	<i>With-in run</i>		<i>Between Run</i>		<i>Total</i>	
	<i>Index Value</i>	<i>SD</i>	<i>CV(%)</i>	<i>SD</i>	<i>CV(%)</i>	<i>SD</i>	<i>CV(%)</i>
Negative Control	0.21	0.03	NA*	0.08	NA	0.10	NA
Positive Control	2.01	0.04	2.0	0.06	3.0	0.10	4.7
K2 EDTA 1	0.47	0.04	NA	0.06	NA	0.09	NA
K2 EDTA 2	1.12	0.04	3.6	0.05	4.5	0.09	7.6
K2 EDTA 3	1.57	0.04	2.2	0.08	4.8	0.10	6.1
K2 EDTA 4	1.82	0.04	2.2	0.07	3.6	0.09	5.0
K2 EDTA 5	2.55	0.04	1.6	0.08	2.9	0.10	3.7
Lithium Heparin 1	0.27	0.04	NA	0.05	NA	0.08	NA
Lithium Heparin 2	1.51	0.04	2.3	0.07	4.3	0.09	6.0
Lithium Heparin 3	1.60	0.04	2.2	0.07	4.4	0.09	5.3
Lithium Heparin 4	1.75	0.04	2.0	0.07	4.0	0.09	4.9
Lithium Heparin 5	2.49	0.04	1.6	0.08	3.2	0.11	4.2
Sodium Heparin 1	0.42	0.04	NA	0.06	NA	0.08	NA
Sodium Heparin 2	1.11	0.04	3.6	0.07	5.9	0.08	7.2
Sodium Heparin 3	1.68	0.06	3.3	0.11	6.3	0.13	7.4
Sodium Heparin 4	1.93	0.04	2.1	0.13	6.8	0.14	7.3
Sodium Heparin 5	2.44	0.04	1.6	0.12	4.9	0.14	5.7
Serum 1	0.37	0.04	NA	0.05	NA	0.10	NA
Serum 2	0.94	0.03	3.2	0.05	4.8	0.08	8.0
Serum 3	1.51	0.04	2.7	0.04	2.7	0.08	5.3
Serum 4	1.71	0.04	2.3	0.06	3.2	0.08	4.7
Serum 5	2.43	0.04	1.6	0.05	1.9	0.08	3.3

* NA = Not applicable

¹ National Committee for Clinical Laboratory Standards. Evaluation of precision performance of clinical chemistry devices—second edition; approved guideline. NCCLS Document EP5-A. Wayne (PA):NCCLS;1999.

Reproducibility

The reproducibility component of precision was evaluated incorporating between site and between lot variations. The ADVIA Centaur HAV Total reproducibility study was performed at 3 testing sites utilizing 3 reagent lots per site. A 20-member panel, controls, and calibrators were assayed in replicates of 5, on a single run per day, over 6 days, for each lot. Matrices were spiked to targeted Index Value levels and are not matched donors. The study was completed with a single calibration of the assay (one calibration interval). Standard deviation and percent CV were calculated for within-run, between-day, and total.

<i>Sample</i>	<i>Mean Index Value</i>	<i>Within-run¹</i>		<i>Between Run²</i>		<i>Between Site³</i>		<i>Between Lot⁴</i>		<i>Total⁵</i>		<i>N</i>
		<i>SD</i>	<i>CV(%)</i>	<i>SD</i>	<i>CV(%)</i>	<i>SD</i>	<i>CV(%)</i>	<i>SD</i>	<i>CV(%)</i>	<i>SD</i>	<i>CV(%)</i>	
1-EDTA	0.47	0.05	NA	0.06	NA	0.06	NA	0.00	NA	0.10	NA	270
1-Li Heparin	0.28	0.05	NA	0.05	NA	0.03	NA	0.03	NA	0.09	NA	260
1-Na Heparin	0.44	0.06	NA	0.06	NA	0.07	NA	0.00	NA	0.11	NA	270
1-Serum	0.36	0.06	NA	0.04	NA	0.04	NA	0.00	NA	0.08	NA	270
2-EDTA	1.08	0.05	5.0	0.04	3.6	0.04	3.6	0.03	2.8	0.08	7.7	270
2-Li Heparin	1.50	0.05	3.1	0.05	3.3	0.04	2.6	0.04	3.0	0.09	6.0	270
2-Na Heparin	1.10	0.05	4.4	0.04	3.9	0.03	2.5	0.04	3.4	0.08	7.2	270
2-Serum	0.90	0.05	5.2	0.04	4.8	0.04	4.3	0.01	1.5	0.08	8.4	270
3-EDTA	1.52	0.05	3.1	0.05	3.0	0.03	2.1	0.05	3.1	0.09	5.7	270
3-Li Heparin	1.57	0.05	3.1	0.06	3.8	0.05	3.0	0.05	3.1	0.10	6.6	270
3-Na Heparin	1.68	0.06	3.4	0.05	2.7	0.04	2.6	0.04	2.4	0.09	5.6	270
3-Serum	1.45	0.05	3.5	0.05	3.5	0.04	3.0	0.05	3.6	0.10	6.8	270
4-EDTA	1.79	0.05	2.9	0.04	2.4	0.05	2.8	0.05	2.5	0.10	5.3	270
4-Li Heparin	1.73	0.05	2.8	0.05	3.0	0.04	2.2	0.05	2.8	0.09	5.4	270
4-Na Heparin	1.92	0.06	2.9	0.05	2.5	0.04	1.9	0.07	3.8	0.11	5.7	270
4-Serum	1.67	0.06	3.7	0.06	3.4	0.02	1.3	0.06	3.7	0.11	6.4	270
5-EDTA	2.52	0.06	2.4	0.04	1.6	0.03	1.4	0.07	2.6	0.10	4.1	270
5-Li Heparin	2.46	0.06	2.5	0.06	2.3	0.05	1.9	0.07	2.7	0.12	4.7	270
5-Na Heparin	2.42	0.06	2.5	0.05	2.0	0.04	1.5	0.07	3.0	0.11	4.7	270
5-Serum	2.36	0.06	2.5	0.05	2.2	0.04	1.7	0.06	2.5	0.11	4.5	270
Low Control	0.18	0.08	NA	0.05	NA	0.08	NA	0.00	NA	0.13	NA	270 ⁷
High Control	2.07	0.06	3.1	0.06	2.7	0.10	4.6	0.00	0.0	0.13	6.2	270

1. Variability of Index Values within day (all testing sites and reagent lots).
2. Variability of Index Values between days (all testing sites and reagent lots).
3. Variability of Index Values between sites (from testing site to testing site, across all reagent lots).
4. Variability of Index Values between reagent lots (from reagent lot to reagent lot, across all testing sites).
5. Variability of Index Values combining (root sum of squares) all four components.
6. NA = Not applicable.
7. Sixty Low Control results were below the assay reportable range. These results were not used for calculations.

Matrix, Collection Tube Type, Effects

Blood was collected during in-house blood draws from 20 healthy, normal donors in glass and plastic serum tubes (red top), serum SST, heparin (Na and Li) plasma, and K2 EDTA plasma tubes. Three of these donors were naturally positive for HAV Total and 17 were found to be HAV Total negative. Seven of the negative samples were spiked with a high titer anti-HAV positive plasma pool to form low and high positive samples (index values around 1.00 and 1.50, respectively). Ten HAV Total negative donors were not spiked. Tube type compatibility was defined as $100 \pm 20\%$ overall recovery of the “test” tube type compared to the control red top and no change in the clinical interpretation of the individual samples when testing with the ADVIA HAV Total assay.

Serum (red top; plastic), serum-SST (glass + plastic) plasma/EDTA (glass+plastic) and plasma/heparin (Na+Li) (glass+plastic) provided % recoveries within $100 \pm 20\%$ of the control condition. In addition, there was no change in the clinical interpretation of the individual samples. These tube types can all be used for collection of specimens to be assayed using the Centaur HAV Total assay.

Sample Handling Studies

Specimens were collected in each of the sample matrices claimed as suitable for use with the Centaur HAV Total assay. These specimens were then subjected to potential stresses and tested in comparison to baseline data to determine the impact of the stress on assay accuracy. The sample handling studies described here evaluate the effect of the following patient sample handling conditions on ADVIA Centaur HAV Total index values:

1. Extended time in refrigerated (2-8°C) storage.
2. Extended time at room temperature (25°C) storage.
3. Extended time in freezer (-20°C) storage.
4. Multiple freeze/ thaw (-20°C/2-8°C) cycles.

Samples were collected during in-house blood draws from healthy donors in serum and plasma with a variety of anti-coagulants. Samples were aliquotted and placed in appropriate storage/stress conditions on the day of collection. A baseline Index value for each sample was established by testing with the ADVIA Centaur HAV Total assay on the day of collection. All % recoveries are calculated against the baseline (day 0) Index value. Results from the ADVIA Centaur HAV Total sample handling studies indicate that samples can be subjected to the following conditions and still generate accurate results when tested in the ADVIA Centaur HAV Total assay:

1. Samples can be stored in primary tubes refrigerated (2-8°C) for up to 24 hours.

2. Samples can be stored in secondary tubes refrigerated (2-8°C) for up to 7 days.
3. Samples can be stored at room temperature for up to 12 hours
4. Samples can be stored frozen (-20°C) for up to one year.
5. Samples can be frozen and thawed up to 4 times.

On-The-Clot Specimen Storage

A study was done to determine if storing the processed serum or plasma sample in the original collection tube (“On the Clot”) rather than transferring the sample to a secondary container effects HAV Total measurement. Fresh samples were drawn from 10 healthy in-house donors into serum and plasma collection tube types tested. Three donors had naturally occurring anti-HAV antibody and three of the negative samples were spiked with a HAV Total positive pool. Samples remained in the primary tubes on the clot or packed red cells and were stored at 2 – 8°C. The HAV Total activity was tested at 0, 1, 5 and 7 days. HAV Total activity at day 0 was compared to the other time points. No negative samples showed any significant change. Overall the positive samples showed a change of less than 20% after 24 hours and clinical interpretation was consistent across all time points. Based on this study, samples can be stored in the primary collection tube for up to 24 hours at 2 - 8°C.

Sample Processing – Time to Centrifugation

A study was done to determine the effect of fresh sample time to centrifugation on the HAV Total Index. Blood was drawn from seven healthy in-house donors in serum (glass, plastic and SST plastic) and plasma (K₂EDTA, Li heparin and Na heparin) collection tubes. Three donors were positive and four negative for HAV Total. The HAV Total Index recovery from samples stored for 24 hours before centrifugation was compared to the results from a sample centrifuged as soon as feasible after collection. The recovery of HAV Total Index after 24 hours delay in centrifugation ranged from 98.7 to 105.2% without change in clinical interpretation for any tube type. Based on this study, the centrifugation step may occur up to 24 hours post draw.

Sample Handling – Inversion of Gel barrier Collection tubes

A study was done to determine if inversion of barrier gel blood collection tubes interferes with ADVIA Centaur HAV Total assay results. Blood was drawn from 10 healthy in-house donors using serum and plasma (Li heparin) gel barrier (separator) collection tubes. Three negative sample tubes were spiked with HAV Total at different levels, 4 were left negative and 3 had naturally occurring anti-HAV activity. The tubes were rested for 30 minutes, centrifuged, and an aliquot was taken. The tubes were then inverted 5 times and a 2nd aliquot was taken. The two aliquots were compared to determine if inversion altered sample HAV Total measurement. Inversion of the barrier gel collection tube, 5 times, had no effect on the assay results for both positive and negative samples. Recovery for HAV Total positive samples ranged from 85.9% to 102% after inversion.

Stability Studies

Real Time Stability Studies for Centaur ReadyPack reagents, Ancillary ReadyPack reagent, calibrators and controls were conducted. Three lots of HAV Total ReadyPack reagents, three lots of Ancillary ReadyPack reagents, four lots of calibrators and three lots of controls were placed on real time stability studies. All kits and reagents are stored at the recommended storage temperature of 2-8°C. Reagents were monitored at several checkpoints post manufacturing date. The shelf life studies supported a claim of 12 months expiration dating for the HAV Total ReadyPack reagents.

Reagent On-Board Stability (OBS) Studies: Three lots of reagents have undergone reagent OBS studies. Two Centaur instruments were used. OBS testing on the instruments occurred at several time points after the pack was placed on board. A fresh pack served as the control for each time-point. Dose recovery within 10% or 2SD of the fresh pack served to define acceptable performance. The calibration interval was also evaluated using these results. The on-board studies for the reagents support 41 days OBS and 14 days calibration interval for the Centaur HAV Total reagents.

The shipping study of the reagents evaluates the likelihood of aggregation and clumps occurring when the material is shipped to or handled by the customer. The recommended shipping conditions are to ship the ReadyPack reagents upright at 2-8°C and store at 2-8°C immediately after receiving.

Five lots of calibrators and three lots of controls were placed on 2-8°C long-term stability. Calibrators and controls were monitored at several time points post manufacturing date. The results supported a 12 month shelf life at 2-8°C for both calibrators and controls.

The calibrator and control open bottle stability study examined the length of time the calibrator or control is stable once the bottle is opened. Open bottles were stored at the recommended storage temperature of 2-8°C. The open bottles were sampled periodically up to 62 days post initial opening. Fresh (unopened) bottles were evaluated at each time point to serve as controls. The acceptance criteria for this study was dose recovery within 10% (or 2SD) of the fresh bottle dose. This study supported an open bottle use lifetime of up to 62 days.

A calibrator and control shipping study was also performed on one lot. The HAV Total calibrators and controls underwent 3x freeze/thaw cycles with no adverse effects.

Microbiology Studies

The ADVIA Centaur HAV Total reagents (Antigen reagent, Lite Reagent and Solid Phase Reagent) contain 0.09% sodium azide, 0.05% gentamicin sulfate and 0.02% amphotericin as preservatives to protect against adventitious contamination by microorganisms. The ADVIA Centaur HAV Total Ancillary Reagent contains 0.02% sodium benzoate as a preservative. The ADVIA Centaur HAV Total calibrators and controls contain 0.09% sodium azide as preservative. The reagents and calibrators were challenged in a study conducted according to USP requirements for Antimicrobial Effectiveness testing to assess the ability of the reagents to withstand or control microbial contamination. Calibrators and controls have the same formulation and results for calibrators therefore apply to

controls. Results indicated that the preservative systems for reagents and calibrators met the USP requirements for antimicrobial effectiveness testing. The preservative study results for calibrators and the Antigen Reagent indicated partial reduction or inhibition of growth of the USP challenge organisms. A performance microbial challenge was performed using one lot of HAV Total reagents and calibrators. Reagents were inoculated with two pools of microbes at 10E3 and 10E6 CFU/mL then run on the Centaur instrument. Calibrators were inoculated separately with seven species of microbes at 10E6 CFU/mL. Controls and QC panel all were within release ranges when tested using inoculated reagents at all time points. Inoculated Calibrator Index values were consistent with the reference material, providing a dose recovery of 100+/-10%. No clinically significant changes in Index values were observed after using inoculated reagents versus control reagents.

Additionally, Bayer routinely performs microbial load testing during the manufacturing of ADVIA Centaur HAV Total reagents, calibrators and controls. The microbial load for any batch must be < 50 CFU/ml.

Instrument Studies:

Environmental Testing

The purpose of environmental testing is to assess ADVIA Centaur HAV Total assay control recovery at the mean and extreme environmental conditions specified for the ADVIA Centaur instrument. Each assay is calibrated and run on a single ADVIA Centaur unit in an environmental chamber set at 18°C, 24°C and 30°C. The percent change in control recovery per degree is then calculated. ADVIA Centaur HAV Total assay environmental testing data meets the general specification of less than a 10% control recovery change over a 6°C ambient temperature change for samples near the cutoff. These studies therefore demonstrated acceptable performance of the HAV Total assay when performed on instruments operating at the mean and extreme environmental conditions specified for the ADVIA Centaur instrument.

Reagent Compatibility Testing

The purpose of this study was to confirm there are no primary reagent interactions for assays that share the same reagent probe, and might therefore be susceptible to reagent carryover affects on the ADVIA Centaur instrument. Mitigation of any interference identified is accomplished through Test definition (TDef) scheduling options, using multiple water washes, or a Wash Pack with a solution other than water may be required.

The ADVIA Centaur HAV Total assay was evaluated for its potential affect on all other assays using the same reagent probes and for the affect of all the other assay reagents on the HAV Total assay. To be accepted there must be <10% difference in dose between test and control, or no statistically significant change in dose, or no more than 1SD difference in dose as appropriate for the assay and the control being tested.

Conclusions Drawn from the Non Clinical Studies

The ADVIA Centaur® HAV Total assay was evaluated to demonstrate performance claims for cross-reactivity, interference, precision, matrix type, specimen handling, and reagent stability. The results of the non-clinical studies will be used in conjunction with results of the clinical trial studies to support the intended use statements of the ADVIA Centaur® HAV Total assay.

X. SUMMARY OF CLINICAL STUDIES

The objective of this clinical study was to assess the efficacy of ADVIA Centaur HAV Total assay for the qualitative determination of total antibody response to hepatitis A virus in human serum or plasma as presumptive evidence of a previous or ongoing hepatitis A viral infection.

Study Design

The safety and effectiveness of the ADVIA Centaur HAV Total assay was determined by a clinical trial consisting of the following studies:

- A prospective study consisting of patient samples from high risk for HAV population, signs and/or symptoms of HAV infection population, acute HAV infected population, HAV infected/HAV recovered population and hospitalized patient population. These samples were tested with both the ADVIA Centaur HAV Total assay and a reference HAV Total assay at the clinical trial sites.
- A retrospective study consisting of banked samples from acute HAV infected patients. These samples were tested with both the ADVIA Centaur HAV Total assay and a reference HAV Total assay at the clinical trial sites.
- A study of HAV seroconversion panels obtained retrospectively from commercial vendors. These samples were tested with both the ADVIA Centaur HAV Total assay and a reference HAV Total assay at the clinical trial sites.
- A paired matrix study in which a subset of the prospective study samples was collected in serum, EDTA plasma, and lithium heparin plasma collection tubes. The samples from all collection tube types were then compared by testing in the ADVIA Centaur HAV Total assay.

Gender Bias

1. There was no selection bias on the basis of gender identified during the review. In a prospective population of 846 patients which included both

prospectively and retrospectively obtained specimens 41.84% were women and 58.16% were men.

2. No difference in the safety and effectiveness of the Centaur HAV Total assay based on gender was identified. The distribution of reactive and non reactive results were similar for both genders. A total of 65.25% women were reactive compared to 63.41% of men.

Demographic Data and Expected Results

The prospective study population for the ADVIA Centaur HAV Total assay consisted of 846 patients. Of these 846 patients, 249 patients (29.43%) were from the high risk population, 178 patients (21.04%) were from the signs and symptoms population, 2 patients (0.24%) were from the acute HAV infected patient population, 215 patients (25.41%) were from the HAV infected/HAV recovered patient population and 202 patients (23.88%) were from the hospitalized patient population. The prospective study population was 29.20% Caucasian, 37.59% Hispanic, 28.37% Black, 1.65% Asian, and 3.2% from unknown or other ethnicity. The majority of patients were male (58.16% male and 41.84% female). The mean age was 48.42 years (range of 18 to 101 years). Patients in the prospective study population were from the following geographic regions: Florida (58.39%), Texas (29.67%), and New York (11.94%).

The ADVIA Centaur® HAV Total results for the prospective population for all sites combined by age group and gender are summarized in the following table.

ADVIA Centaur HAV Total Assay							
Distribution of Prospective Population by Age Group and Gender							
All Collection Sites							
Age (years)	Gender	Reactive (a)		Non-reactive (b)		Total	
		N	%	N	%	N	%
10-19	Female	1	50.00	1	50.00	2	40.00
	Male	1	33.33	2	66.67	3	60.00
	Overall	2	40.00	3	60.00	5	100.00
20-29	Female	16	44.44	20	55.56	36	59.02
	Male	7	28.00	18	72.00	25	40.98
	Overall	23	37.70	38	62.30	61	100.00
30-39	Female	43	60.56	28	39.44	71	47.02
	Male	39	48.75	41	51.25	80	52.98
	Overall	82	54.30	69	45.70	151	100.00
40-49	Female	52	55.91	41	44.09	93	35.63
	Male	102	60.71	66	39.29	168	64.37
	Overall	154	59.00	107	41.00	261	100.00
50-59	Female	56	74.67	19	25.33	75	36.76
	Male	84	65.12	45	34.88	129	63.24
	Overall	140	68.63	64	31.37	204	100.00
60-69	Female	34	75.56	11	24.44	45	45.92
	Male	48	90.57	5	9.43	53	54.08
	Overall	82	83.67	16	16.33	98	100.00
70+	Female	29	90.63	3	9.38	32	48.48
	Male	31	91.18	3	8.82	34	51.52
	Overall	60	90.91	6	9.09	66	100.00
Total	Female	231	65.25	123	34.75	354	41.84
	Male	312	63.41	180	36.58	492	58.16
	Overall	543	64.18	303	35.82	846	100.00

(a) Samples with ≥ 1.00 index

(b) Samples with < 1.00 index

Clinical Study Results

The results obtained using the ADVIA Centaur HAV Total assay were evaluated with results obtained using a comparative method for each population category (reactive and nonreactive). The population included 846 prospective subjects and 103 HAV acute retrospective samples. The following results were obtained:

Comparison of Results by Subject Category

ADVIA Centaur HAV Total Assay versus Comparative Anti-HAV Total Assay (All Testing Sites)

<i>Subject Category</i>	<i>Comparative Anti-HAV Total Assay Negative</i>		<i>Comparative Anti-HAV Total Assay Positive</i>		<i>Total*</i>
	<i>ADVIA Centaur HAV Total Assay</i>		<i>ADVIA Centaur HAV Total Assay</i>		
	<i>Reactive</i>	<i>Nonreactive</i>	<i>Reactive</i>	<i>Nonreactive</i>	
Acute	1	0	104	0	105
High risk	6	125	117	1	249
Signs and symptoms	12	83	83	0	178
Clinical/hospitalized	0	93	109	0	202
Infected/recovered	0	1	214	0	215
Total	19	302	627	1	949

* Total number of test results by Subject Category

Percent Agreement

The percent agreement between the ADVIA Centaur HAV Total assay and the comparative anti- HAV Total assay is summarized in the following table:

Percent Agreement and Confidence Intervals by Subject Category

ADVIA Centaur HAV Total Assay versus Comparative Anti-HAV Total Assay (All Testing Sites)

<i>Subject Category</i>	<i>Positive Percent Agreement % (x/n)¹</i>	<i>95% Exact Confidence Interval (CI)</i>	<i>Negative Percent Agreement % (x/n)²</i>	<i>95% Exact Confidence Interval (CI)</i>
Acute	100 (104/104)	96.52 to 100	0 (0/1)	0 to 97.50
High risk	99.15 (117/118)	95.37 to 99.98	95.42 (125/131)	90.30 to 98.30
Signs and symptoms	100 (83/83)	95.65 to 100	87.37 (83/95)	78.97 to 93.30
Clinical/hospitalized	100 (109/109)	96.57 to 100	100 (93/93)	96.11 to 100
Infected/recovered	100 (214/214)	98.29 to 100	100 (1/1)	2.50 to 100
Overall	99.84 (627/628)	99.12 to 100	94.08 (302/321)	90.91 to 96.40

1. x = the number of ADVIA Centaur HAV Total results that are reactive in agreement with the comparative anti-HAV Total results

n = the total number of comparative anti-HAV Total results that are reactive

2. x = the number of ADVIA Centaur HAV Total results that are nonreactive in agreement with the comparative anti-HAV Total results

n = the total number of comparative anti-HAV Total results that are nonreactive

The percent agreement between the ADVIA Centaur HAV Total assay and the reference assay for each specimen, including the upper and lower 95% confidence intervals, is presented above in a table format. The positive, negative, and overall percent agreements were also calculated. The formulas used for these calculations are presented below.

Positive percent agreement =

Number of ADVIA Centaur HAV Total reactive results in agreement with reference HAV Total X 100

Total number of reference HAV Total reactive results

Negative percent agreement =

Number of ADVIA Centaur HAV Total nonreactive results in agreement with reference HAV Total X 100

Total number of reference HAV Total nonreactive results

Overall percent agreement =

Number of ADVIA Centaur HAV Total results in agreement with reference HAV Total X 100

Total number of reference HAV Total reactive and nonreactive results

Comparison of Results for Vaccine Recipients

A population of commercially sourced HAV vaccine recipients with both pre- and post- vaccination samples was tested using both the ADVIA Centaur HAV Total assay and a HAV Total reference assay. All of the vaccine recipients received only the VAQTA[®] vaccine. The following results were obtained:

ADVIA Centaur HAV Total Assay Method Comparison in HAV Vaccine Recipients ADVIA Centaur HAV Total Assay vs. HAV Total Reference Assay All Testing Sites					
	Reference HAV Total Negative		Reference HAV Total Positive		Total ^a
	ADVIA Centaur HAV Total Assay		ADVIA Centaur HAV Total Assay		
	Reactive	Nonreactive	Reactive	Nonreactive	
	N	N	N	N	N
Pre-vaccination	0	20	0	0	20
Post-vaccination	1	0	19	0	20
Total	1	20	19	0	40

a Total number of test results by HAV categories

Seroconversion Study

Commercially available HAV patient seroconversion panels were tested using the ADVIA Centaur HAV Total assay to determine the seroconversion sensitivity of the assay. The performance of the ADVIA Centaur HAV Total assay on the seroconversion panels closely matched the performance of the comparative assay. The following results were obtained:

Panel ID	Anti-HAV Total Positive Result From Initial Draw Date		Comparative Assay vs ADVIA Centaur Assay Difference in Bleed Numbers*
	Comparative Assay (Days)	ADVIA Centaur Assay (Days)	
RP004	7	7	0
RP013	9	9	0
PHT902	16	16	0
ProMedx 1	1	1	0

* The difference in bleed numbers is relative to the comparative assay. In all seroconversion panels both the ADVIA Centaur assay and the comparative assay detected the first reactive sample at the same day.

Paired Matrix Study

Matched serum, EDTA plasma and lithium heparin plasma specimens were collected from 108 prospectively enrolled subjects. Samples were tested in replicates of 3 per sample type on the ADVIA Centaur HAV Total assay. Appropriate controls were run. Samples collected in EDTA and lithium heparin were evaluated against serum (control) to determine matrix equivalency.

Results of the Paired Matrix Study support the use of EDTA and lithium heparin plasma with the ADVIA Centaur HAV Total assay. Comparisons of the mean control values to the mean EDTA and lithium heparin plasma values are shown in the table below.

ADVIA Centaur HAV Total Assay
Paired Matrix Study
Summary (All Testing Sites)

Serum Mean 140.41	EDTA Mean 139.48	Difference -0.93	% Difference -0.70	p Value 0.14
Serum Mean 140.41	Lithium Heparin Mean 142.72	Difference 2.32	% Difference 1.60	p Value 0.00

XI. CONCLUSIONS DRAWN FROM THE STUDIES

Multi-centered clinical studies were conducted in the US. The ADVIA Centaur HAV Total assay performed with clinical sensitivity and specificity comparable to current commercially available licensed assays.

The overall positive percent agreement between the ADVIA Centaur HAV Total method and the reference assay was 99.84% (627/628) in the combined population (acute HAV, clinic/hospitalized patients, high risk for HAV, infected/recovered HAV patients, and signs / symptoms populations). The overall negative percent agreement between the ADVIA Centaur HAV Total method and the reference assay was 94.08% (302/321) in the same combined population.

The ability of the ADVIA Centaur HAV Total assay to detect HAV infections was demonstrated with the seroconversion panel evaluation. When the ADVIA Centaur HAV Total result was compared to the reference assay results, the first reactive time point for the ADVIA Centaur HAV Total assay occurred at the same time in all 4 panels.

Precision and reproducibility of the ADVIA Centaur HAV Total assay was good with minor variability from run to run, day to day, site to site and reagent lot to reagent lot.

Paired Matrix Study results support the use of human serum, EDTA plasma, and lithium heparin plasma specimens for testing in the ADVIA Centaur HAV Total assay.

The results from both the non-clinical and clinical studies indicate that the ADVIA Centaur HAV Total assay can be used safely and effectively for the qualitative *in vitro* determination of anti-HAV Total antibodies in human serum and plasma. The ADVIA Centaur HAV Total assay may be used with other HAV serological markers to define the clinical status of patients known to be infected with HAV or may be used with other HBV, HAV, and HCV assays to form a panel for the diagnosis of patients presenting with symptoms of viral hepatitis.

RISK BENEFIT ANALYSIS

As a diagnostic test, the ADVIA Centaur HAV Total Assay involves removal of blood from an individual for testing purposes. This test presents no more of a safety hazard to an individual than is presented to an individual who is having their blood drawn for any other diagnostic evaluation. The benefits to HAV-infected individuals tested by these assays outweigh any potential adverse event or risk to the patient or user due to assay malfunction or operator error.

The potential risks encountered with *in vitro* diagnostic tests are not unusual in the clinical laboratory setting. Appropriate warnings for these risks are contained in the labeling and package inserts for these devices. Standard good laboratory practices are considered sufficient to mitigate the risks to the end user.

SAFETY

Based on the results of the preclinical and clinical laboratory studies, the ADVIA Centaur HAV Total assay, when used according to the provided directions and in conjunction with other serological and clinical information, should be safe and effective and pose minimal risk to the patient due to false test results.

EFFECTIVENESS

The effectiveness of the ADVIA Centaur[®] HAV Total assay has been demonstrated for use in determining if antibodies to Hepatitis A virus are present in an individual's serum or plasma. A reasonable determination of the effectiveness of the ADVIA Centaur[®] HAV Total assay as an aid in the diagnosis of previous or ongoing hepatitis A viral infection or in the identification of HAV-susceptible individuals for vaccination has been demonstrated.

XII. PANEL RECOMMENDATIONS

In accordance with the provisions of section 515(c)(2) of the act as amended by the Safe Medical Devices Act of 1990, this PMA was not referred to the Microbiology Advisory Panel, an FDA advisory committee, for review and recommendation because the information in the PMA substantially duplicates information previously reviewed by this panel.

XIII. CDRH DECISION

FDA issued an approval order on March 7, 2005.

The applicant's manufacturing facilities were inspected on May 11, 2004 and April 28, 2004 and found to be in compliance with the Quality Systems Regulation (21 CFR 820)

XIV. APPROVAL SPECIFICATIONS

Directions for Use: See the labeling.

Hazards to Health from Use of the Device: See Indications, Contraindications, Warnings, precautions and Adverse Events in the labeling.

Postapproval Requirements and Restrictions: See approval order.