SIEMENS

ADVIA Centaur® CP
Immunoassay System

HAV Total (HAVT)

Assay for the Detection of Total Antibodies to Hepatitis A Virus

Assay Summary

Sample Type	**Serum, potassium EDTA plasma, lithium or sodium heparinized plasma**
Sample Volume	**20 µL**
Calibrator	**HAVT**

Contents

REF	*Contents*	*Number of Tests*
07720961	1 ReadyPack® primary reagent pack containing ADVIA Centaur® HAVT Lite Reagent, Solid Phase, and Antigen Reagent	100
	1 Ancillary pack containing ADVIA Centaur HAVT Ancillary reagent ANC	
	ADVIA Centaur and ADVIA Centaur CP HAVT Master Curve card	
	1 vial HAVT Low Calibrator CAL L	
	1 vial HAVT High Calibrator CAL H	
	ADVIA Centaur and ADVIA Centaur CP HAVT Calibrator Assigned Value Cards	

Intended Use

The ADVIA Centaur HAV Total (HAVT) assay is an *in vitro* diagnostic immunoassay for the qualitative determination of total antibodies to hepatitis A virus (anti-HAV) in human neonatal, pediatric, and adult serum or plasma (potassium EDTA, lithium or sodium heparinized) using the ADVIA Centaur CP 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.

Assay performance characteristics have not been established for immunocompromised or immunosuppressed patients.

WARNING: This assay has not been FDA cleared or approved for the screening of blood or plasma donors.

United States federal law restricts this device to sale by or on the order of a physician.

Materials Required but Not Provided

REF	*Description*	*Contents*
03395373	ADVIA Centaur Ancillary Probe Wash 1 APW 1	2 ReadyPack ancillary reagent packs containing 25 mL per pack
01313752	ADVIA Centaur HAVT quality control material	2 x 7.0 mL Negative Control CONTROL -
		2 x 7.0 mL Positive Control CONTROL +
		Expected Value card

Summary and Explanation of the Test

The ADVIA Centaur HAV Total assay is a competitive chemiluminometric immunoassay used for the detection of total antibody to hepatitis A virus in human serum or plasma.

Hepatitis A is caused by infection with the hepatitis A virus. HAV is a 27-nanometer single-stranded, non enveloped, RNA virus that is classified as a picornavirus. Transmission of hepatitis A is via the fecal-oral route, and infection occurs mainly due to contaminated food or poor sanitary conditions.[1,2]

Hepatitis A virus replicates in the liver. The virus is excreted in the bile and shed in the stool. Only one serotype has been observed among HAV isolates collected from various parts of the world. The average incubation period for HAV infection is 30 days with a range of 15 to 40 days. Chronic infection has not been reported to occur following HAV infection. Symptoms last approximately 2 weeks and include hepatomegaly, jaundice, dark urine, fatigue, and gastrointestinal distress such as anorexia, nausea, vomiting, and abdominal pain. At the onset of symptoms resulting from HAV infection, antibody to HAV is detectable. The early antibody response is largely comprised of the IgM antibody subclass. Anti-HAV IgM is detectable for 3 to 6 months after the onset of illness, whereas anti-HAV IgG can persist indefinitely. The specific determination of anti-HAV IgM is the most useful serological marker for diagnosing acute HAV infection. Total anti-HAV is used primarily for determination of previous exposure to Hepatitis A virus.[1-4]

The ADVIA Centaur HAV Total assay detects all classes of antibodies against hepatitis A virus. The measurement of anti-HAV total activity is used to identify HAV susceptible individuals for vaccination.[5,6]

Assay Principle

The ADVIA Centaur HAV Total assay is a fully automated, competitive immunoassay using direct, chemiluminescent technology. The assay consists of three reagent addition and incubation steps. First, the sample is pretreated with Ancillary Reagent containing cysteine. Next, HAV antigen is added from the ancillary well (Antigen Reagent). Lite Reagent and Solid Phase are then added. The Lite Reagent contains monoclonal mouse antibody to HAV antigen labeled with acridinium ester and biotinylated Fab fragment of a monoclonal mouse antibody to HAV antigen. The Solid Phase contains streptavidin covalently coupled to paramagnetic particles. After the final incubation, the immuno-complex formed is washed with Wash 1 prior to initiation of the chemiluminescent reaction.

The system automatically performs the following steps:

1. Dispenses 20 µL of sample and 50 µL of Ancillary Reagent into a cuvette and incubates for 4 minutes at 37°C.
2. Dispenses 100 µL of Antigen Reagent and incubates for 26.67 minutes at 37°C.
3. Dispenses 100 µL of Lite Reagent and 175 µL of Solid Phase and incubates for 16.67 minutes at 37°C.
4. Separates, aspirates, and washes the cuvettes with Wash 1.
5. Dispenses 300 µL each of Acid Reagent (R1) and Base Reagent (R2) to initiate the chemiluminescent reaction.
6. Reports results according to the selected option, as described in the system operating instructions or in the online help system.

The relative light units (RLUs) detected by the ADVIA Centaur CP System are used to calculate the Index Value from the Master Curve. Assay results above the cutoff of the assay are not indicative of antibody level.

Specimen Collection and Handling

Serum, potassium EDTA plasma, and sodium or lithium heparinized plasma are the recommended sample types for this assay. Do not use specimens with obvious microbial contamination. The performance of the ADVIA Centaur HAV Total assay has not been established with cadaver specimens, heat-inactivated specimens, or body fluids other than serum or plasma such as saliva, urine, amniotic fluid, or pleural fluid.

The following recommendations for handling and storing blood samples are furnished by the Clinical and Laboratory Standards Institute (CLSI, formerly NCCLS),[7] and augmented with additional sample handling studies using the ADVIA Centaur HAV Total assay:

- Handle all samples as if capable of transmitting disease.
- Samples are processed by centrifugation, typically followed by physical separation of the serum or plasma from the red cells. The centrifugation step may occur up to 24 hours post draw.
- Test samples as soon as possible after collecting. Store samples at 2° to 8°C if not tested within 12 hours of collection.
- Store samples in secondary tubes, stoppered and upright at all times at 2° to 8°C up to 7 days.
- Store primary tube samples at 2 to 8°C up to 24 hours. Keep samples stoppered and upright at all times. Primary tube samples include serum stored on the clot, plasma stored on packed red cells, and samples processed and stored in gel-barrier blood collection tubes.
- Freeze samples, devoid of red blood cells, at or below -20°C for longer storage. Samples may be stored at or below -20°C for up to 365 days. Do not store in a frost-free freezer. When specimens are subjected to up to 4 freeze/thaw cycles, no clinically significant differences were observed. Thoroughly mix thawed samples and centrifuge at 10,000 g for 2 minutes before using. Collect the supernatant into a clean vial.
- Package and label samples for shipment in compliance with applicable federal and international regulations covering the transport of clinical samples and etiological agents. Store samples stoppered and upright at 2° to 8°C upon arrival. If shipment is expected to exceed 2 days, ship specimens frozen.

Before placing samples on the system, ensure that samples have the following characteristics:

- Samples are free of fibrin or other particulate matter. Remove particulates by centrifugation. (example: 1500 x g for 10 minutes; follow tube manufacturer’s recommendations).
- Samples are free of bubbles or foam.

Reagents

Store the reagents upright at 2–8°C.
Mix all primary reagent packs by hand before loading them onto the system. Visually inspect the bottom of the reagent pack to ensure that all particles are dispersed and resuspended. For detailed information about preparing the reagents for use, see the system operator's guide.

Protect from sunlight
Protect reagent packs from all light sources. Reagent packs loaded on the system are protected from light. Store unused reagent packs at 2–8°C away from light sources.

Reagent Pack	Reagent	Volume	Ingredients	Storage	Stability
ADVIA Centaur HAVT ReadyPack primary reagent pack	Lite Reagent	10.0 mL/ reagent pack	monoclonal mouse anti-HAV antibody[1] (~1.0 µg/mL) labeled with acridinium ester and biotinylated monoclonal mouse anti-HAV Fab fragment (~0.08 µg/mL) in phosphate buffer with bovine serum albumin, sodium azide (< 0.1%) and preservatives	2–8°C	Until the expiration date on the pack label. For onboard stability, refer to Onboard Stability and Calibration Interval.
	Solid Phase	17.5 mL/ reagent pack	streptavidin coupled to paramagnetic particles in phosphate buffer with bovine serum albumin, sodium azide (< 0.1%) and preservatives	2–8°C	Until the expiration date on the pack label. For onboard stability, refer to Onboard Stability and Calibration Interval.
	Antigen Reagent	10.0 mL/ reagent pack	HAV antigen[2] (~0.06 µg/mL) in tricine buffer with bovine serum albumin, stabilizers, sodium azide (< 0.1%) and preservatives.	2–8°C	Until the expiration date on the pack label. For onboard stability, refer to Onboard Stability and Calibration Interval.
ADVIA Centaur HAVT ANC ReadyPack ancillary reagent pack	Ancillary Reagent	25.0 mL/ reagent pack	cysteine in citrate buffer with EDTA and preservatives	2–8°C	Until the expiration date on the pack label. For onboard stability, refer to Onboard Stability and Calibration Interval.
HAVT calibrator vials	Calibrators	2.0 mL/ vial	processed human plasma positive for anti-HAV antibodies with sodium azide (< 0.1%)	2–8°C	Until the expiration date on the vial, 60 days after opening vial, or onboard 8 hours.
HAVT quality control material vials[3]	Controls	7.0 mL/ vial	processed human plasma negative and positive for anti-HAV antibodies with sodium azide (< 0.1%)	2–8°C	Until the expiration date on the vial, or 60 days after opening vial, or onboard 8 hours.
ADVIA Centaur APW 1 ReadyPack ancillary reagent pack[3]	Probe Wash	25 mL/ pack	0.4 N sodium hydroxide	2–8°C	Until the expiration date on the pack label or 14 consecutive days after accessing the ancillary reagent pack.

1 The antibody recognizes a conformational epitope on the assembled hepatitis A virus.

2 HAV Antigen is an inactivated partially purified hepatitis A virus preparation.

3 See Materials Required But Not Provided.

Precautions and Warnings

Safety data sheets (MSDS/SDS) available on www.siemens.com/diagnostics.

H319, H315, H290
P280, P264, P305 + P351 + P338

Warning!
Causes serious eye irritation. Causes skin irritation. May be corrosive to metals. Wear protective gloves/protective clothing/eye protection/face protection. Wash hands thoroughly after handling. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
Contains: Sodium hydroxide; ADVIA Centaur Probe Wash 1

CAUTION! POTENTIAL BIOHAZARD: Some components of this product contain human source material. No known test method can offer complete assurance that products derived from human blood will not transmit infectious agents. All products manufactured using human source material should be handled as potentially infectious. Handle this product according to established good laboratory practices and universal precautions.[8-10]
The calibrators and controls have been assayed by FDA-approved methods and found nonreactive for hepatitis B surface antigen (HBsAg), antibody to hepatitis C (HCV) and antibody to HIV 1/2 and HIV antigen. The positive control and calibrators contain human plasma that is reactive for anti-HAV total but negative for anti-HAV IgM.

CAUTION: This device contains material of animal origin and should be handled as a potential carrier and transmitter of disease.

CAUTION: Sodium azide can react with copper and lead plumbing to form explosive metal azides. On disposal, flush reagents with a large volume of water to prevent the buildup of azides, if disposal into a drain is in compliance with federal, state, and local requirements.

Dispose of hazardous or biologically contaminated materials according to the practices of your institution. Discard all materials in a safe and acceptable manner and in compliance with prevailing regulatory requirements.

For *in vitro* diagnostic use.

Loading Reagents

Ensure that the system has sufficient primary and ancillary reagent packs. For detailed information about preparing the system, refer to the system operating instructions or to the online help system.

CAUTION: Mix all primary reagent packs by hand before loading them onto the system. Visually inspect the bottom of the reagent pack to ensure that all particles are dispersed and resuspended. For detailed information about preparing the reagents for use, see the system operator's guide.

Load the primary reagent packs in the primary reagent area. You can use the arrows on the end label as a placement guide. However left, center, and right placement of the primary reagent packs is not required, because the ADVIA Centaur CP System has only one reagent probe. The system automatically mixes the primary reagent packs to maintain homogeneous suspension of the reagents. For detailed information about loading reagents, refer to the system operating instructions or to the online help system.

CAUTION: The Low and High Calibrators provided in this kit are matched to the ReadyPack primary reagent pack. Do not mix calibrator lots with different lots of reagent packs.

CAUTION: The Ancillary Reagent provided in this kit is matched to the ReadyPack primary reagent pack. Do not mix reagents from different kit lot numbers.

NOTE: The Ancillary Reagent pack contains more volume than required to perform 100 tests. Since the Ancillary Reagent is matched to the Lite Reagent, Solid Phase, and Antigen Reagent in the ReadyPack primary reagent pack, discard the Ancillary Reagent pack when the ReadyPack primary reagent pack is discarded. Do not use beyond the onboard stability.

Onboard Stability and Calibration Interval

Onboard Stability	Calibration Interval
41 days	14 days

Additionally, the ADVIA Centaur HAV Total assay requires a two-point calibration:

- When changing lot numbers of primary reagent packs.
- When replacing system components.
- When quality control results are repeatedly out of range.
- When loading a fresh primary reagent pack and the current calibration was established with a reagent pack that was in use on the system for 14 days.

CAUTION:

- Discard reagent packs at the end of the onboard stability interval.
- Do not use reagents beyond the expiration date.

Master Curve Calibration

The ADVIA Centaur HAV Total assay requires a Master Curve calibration when using a new lot number of Lite Reagent, Solid Phase, and Antigen Reagent. For each new lot number of Lite Reagent, Solid Phase, and Antigen Reagent use the bar-code reader or keyboard to enter the Master Curve values on the system. The Master Curve card contains the Master Curve values. For detailed information about entering calibration values, refer to the system operating instructions or to the online help system.

Calibration

For calibration of the ADVIA Centaur HAV Total assay, use ADVIA Centaur HAV Total Calibrators provided with each kit. The calibrators provided in this kit are matched to the ReadyPack primary reagent pack.

Using Bar-code Labels

NOTE: Calibrator bar-code labels are lot-number specific. Do not use bar-code labels from one lot of calibrators with any other lot of calibrators.

Use the ADVIA Centaur HAV Total Calibrator bar-code labels to identify the Low and High Calibrator sample cups when performing the ADVIA Centaur HAV Total assay. Place the bar-code label on the sample cup so that the readable characters on the side of the label are vertical on the sample cup.

Performing a Calibration

Each calibrator is packaged with a lot-specific Calibrator Assigned Value card to facilitate entering the calibration values on the system. Enter the values using the bar-code scanner or the keyboard. For detailed information about entering calibrator values, refer to the system operating instructions or to the online help system.

Perform the calibration procedure using the following steps:

NOTE: This procedure uses calibrator volumes sufficient to measure each calibrator in duplicate.

1. Schedule the calibrators to the worklist.
2. Label 2 sample cups with calibrator bar-code labels: one cup for the low calibrator and another cup for the high calibrator.

NOTE: Each drop from the calibrator vial is approximately 50 µL.

3. Gently mix the low and high calibrators and dispense at least 4–5 drops of each calibrator into the appropriate sample cups. Avoid bubbles.
4. Load the sample cups in a rack.
5. Place the rack in the sample compartment.
6. Ensure that the assay reagents are loaded.
7. At the main menu, open the Reagent Compartment Screen.
8. Select the ReadyPack test to calibrate.
9. Select **Calibrate**.

NOTE: Dispose of any calibrator remaining in the sample cups after 8 hours. Do not refill sample cups when the contents are depleted; if required, dispense fresh calibrators.

Quality Control

Follow government regulations or accreditation requirements for quality control frequency.

For quality control of the ADVIA Centaur HAV Total assay, use ADVIA Centaur HAV Total quality control materials. Refer to the Expected Value card for the suggested expected values specific for the lot number of the positive and negative controls. Additional controls may be tested according to guidelines or requirements of local, state, and/or federal regulations or accrediting organizations.

NOTE: The quality control material furnished is intended to monitor substantial reagent failure. If additional controls are desired, run a negative control and a positive control close to the clinically relevant point.

Using Bar-code Labels

NOTE: Control bar-code labels are lot-number specific. Do not use bar-code labels from one lot of controls with any other lot of controls.

Use the ADVIA Centaur HAV Total quality control bar-code labels to identify the positive and negative sample cups when performing the ADVIA Centaur HAV Total assay. Place the bar-code label on the sample cup so that the readable characters on the side of the label are vertical on the sample cup.

Performing Quality Control

For detailed information about entering quality control values, refer to the system operating instructions or to the online help system.

To monitor system performance and chart trends, as a minimum requirement, assay quality control material on each workshift that samples are analyzed. Assay quality control samples when performing a two-point calibration. Treat all quality control samples the same as patient samples.

Perform the quality control procedure using the following steps:

NOTE: This procedure uses control volumes sufficient to measure each control in duplicate.

1. Schedule the quality control samples to the worklist.
2. Label 2 sample cups with quality control bar-code labels: one cup for the positive control, and another cup for the negative control.

NOTE: Each drop from the control vial is approximately 50 µL.

3. Gently mix the quality control materials and dispense at least 5–6 drops of each control into the appropriate sample cups. Avoid bubbles.
4. Load the sample cups in a rack.

5. Place the rack in the sample compartment.
6. Ensure that the assay reagents are loaded.
7. Start the run, if required.

NOTE: Dispose of any quality control materials remaining in the sample cups after 8 hours. Do not refill sample cups when the contents are depleted; if required, dispense fresh quality control materials.

Taking Corrective Action

If the quality control results do not fall within the Expected Values or within the laboratory's established values, do not report results. Take the following actions:

- Verify that the materials are not expired.
- Verify that required maintenance was performed.
- Verify that the assay was performed according to the instructions for use.
- Rerun the assay with fresh quality control samples.
- Investigate and determine the cause for the unacceptable control results. When the condition is corrected, retest the controls and confirm that results are within acceptable limits. Repeat patient specimens before reporting results for this run.
- If necessary, contact your local technical support provider or distributor for assistance.

Sample Volume

This assay requires 20 µL of sample for a single determination. This volume does not include the unusable volume in the sample container or the additional volume required when performing duplicates or other tests on the same sample. For detailed information about determining the minimum required volume, refer to the system operating instructions or to the online help system.

Assay Procedure

For detailed procedural information, refer to the system operating instructions or to the online help system.

Interpretation of Results

For detailed information about how the system calculates results, refer to the system operating instructions or to the online help system.

- The system reports anti-HAV Total results in Index Values.
- Samples with a calculated value of less than 1.00 Index Value are considered nonreactive for antibodies to hepatitis A virus.
- Samples with a calculated value greater than or equal to 1.00 Index Value are considered reactive for antibodies to hepatitis A virus.
- The cutoff for the ADVIA Centaur HAV Total assay was verified based on results of Receiver Operator Characteristics (ROC) Curve and clinical agreement generated from the clinical studies.[11]
- The cutoff for the ADVIA Centaur HAV Total assay on the ADVIA Centaur CP system was verified based on results of clinical agreement generated from clinical studies.
- The magnitude of the measured result above the cutoff is not indicative of the total amount of antibody present.
- Sample results are invalid and must be repeated if the controls are out of range.

Interpretation of results was determined for this assay using the ADVIA Centaur system.

Limitations

The following information pertains to limitations of the assay:

- The ADVIA Centaur HAV Total assay is limited to the detection of total antibodies to hepatitis A virus in human serum or plasma (potassium EDTA plasma, lithium or sodium heparinized plasma).
- The results from this or any other diagnostic kit should be used and interpreted only in the context of the overall clinical picture. A nonreactive test result does not exclude the possibility of exposure to hepatitis A virus.
- The ADVIA Centaur HAV Total assay does not distinguish among different classes of antibodies. The assay cannot be used to determine if a reactive sample is due to an acute infection or is the result of a previous infection. The sample should be tested in a specific HAV IgM assay to determine if there is an ongoing or recent infection.
- The performance of the ADVIA Centaur HAV Total assay has not been established with cadaver specimens, heat-inactivated specimens, or body fluids other than serum or plasma, such as saliva, urine, amniotic fluid, or pleural fluid.
- The ADVIA Centaur HAV Total assay cutoff is equivalent to 20 mIU/mL standardized to the WHO Second International Reference Standard for Anti-Hepatitis Immunoglobulin (97/646). However, assay results cannot be considered quantitative and no clinical claims for immunity can be determined from the cutoff.
- The performance of the assay has not been established for populations of immunocompromised or immunosuppressed patients.
- Do not use specimens with obvious microbial contamination.
- Heterophilic antibodies in human serum can react with reagent immunoglobulins, interfering with in vitro immunoassays.[12] Patients routinely exposed to animals or to animal serum products may develop heterophilic antibodies which may cause interference in immunoassays and, thus, anomalous values may be observed.
- A reactive HAV Total result does not exclude co-infection by another hepatitis virus.

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% White, 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

		Reactive [a]		*Non-reactive [b]*		*Total*	
Age (years)	***Gender***	***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

As with all *in vitro* diagnostic assays, each laboratory should determine its own reference ranges for the diagnostic evaluation of patient results.[13] The expected results were determined for this assay using the ADVIA Centaur system. Assay performance was confirmed for the ADVIA Centaur HAV Total assay on the ADVIA Centaur CP system. Refer to *Percent Agreement*.

Performance Characteristics

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% White, 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%).

Comparison of 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 acute retrospective specimens were characterized as acute if a commercially available anti-HAV IgM test result was reactive. 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)

	Comparative Anti-HAV Total Assay Negative		*Comparative Anti-HAV Total Assay Positive*		
Subject Category	*ADVIA Centaur HAV Total Assay*		*ADVIA Centaur HAV Total Assay*		
	Reactive	*Nonreactive*	*Reactive*	*Nonreactive*	*Total **
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

The comparison of results was determined with the ADVIA Centaur HAV Total assay. Assay performance was confirmed for the ADVIA Centaur HAV Total assay on the ADVIA Centaur CP system. Refer to *Percent Agreement*.

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)

Subject Category	Positive Percent Agreement % (x/n) [1]	95% Exact Confidence Interval (CI)	Negative Percent Agreement % (x/n) [2]	95% Exact Confidence Interval (CI)
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 same 100 negative and 101 positive samples were tested at each of the three sites. The percent agreement between the ADVIA Centaur HAV Total assay on the ADVIA Centaur CP system and the ADVIA Centaur HAV Total assay across three testing sites is summarized in the following table:

		ADVIA Centaur System HAV Total Assay		
		Positive	Negative	Total
ADVIA Centaur CP System HAV Total Assay	Positive	100	0	100
	Negative	1	100	101
	Total	101	100	201

Positive Agreement = 99.01%
95% Confidence Interval = 94.61 to 99.97%
Negative Agreement = 100%
95% Confidence Interval > 96.38%

Comparison of Results for Vaccine Recipients

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

Comparison of Results in HAV Vaccinated Population
ADVIA Centaur HAV Total Assay versus Comparative Anti-HAV Total Assay (All Testing Sites)

HAV Classification	Comparative Anti-HAV Total Assay Negative		Comparative Anti-HAV Total Assay Positive		
	ADVIA Centaur HAV Total Assay		ADVIA Centaur HAV Total Assay		
	Reactive	Nonreactive	Reactive	Nonreactive	Total *
Pre-vaccination	0	20	0	0	20
Post- vaccination	1	0	19	0	20
Total	1	20	19	0	40

* Total number of test results by HAV classification

The vaccine study was determined with the ADVIA Centaur HAV Total assay. Assay performance was confirmed for the ADVIA Centaur HAV Total assay on the ADVIA Centaur CP system. Refer to Percent Agreement.

Seroconversion Panels

Four commercially available HAV patient seroconversion panels were tested using the ADVIA Centaur HAV Total assay on the AVDVIA Centaur CP system 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 ADVIA Centaur HAV Total assay. The following results were obtained:

	HAV Total Positive Result From Initial Draw Date		*ADVIA Centaur vs ADVIA Centaur CP Systems HAV Total Assay*
Panel ID	*ADVIA Centaur System (Days)*	*ADVIA Centaur CP System (Days)*	*Difference in number of bleeds*
RP004	7	7	0
RP013	9	9	0
PHT902	16	16	0
ProMedx HAV-01	1	1	0

Neonate vs Adult Comparison

A study was conducted to evaluate the results observed when neonatal samples are tested with the ADVIA Centaur HAVT assay. Cord blood serum was used as a surrogate for neonatal serum. A total of thirty (30) cord blood and 30 adult serum samples were spiked with anti-HAV positive stock to yield samples at different analyte levels. The distribution of percent bias (+/-) between the index values of the cord blood serum samples and the mean index values of the adult serum samples are summarized in the following table:

Distribution of % Bias (Neonatal Cord Blood vs. Adult Serum)

Adult Spiked Observed Mean (Index)	**Number Tested (n)**	**Distribution of % Bias**			
		≤ 10%	**> 10% to ≤ 20%**	**> 20% to ≤ 30%**	**> 30%**
Negative (0.6)	6	0.0% (0/6)	33.3% (2/6)	33.3% (2/6)	33.3% (2/6)
Cut-off (1.0)	6	16.7% (1/6)	33.3% (2/6)	50.0% (3/6)	0.0% (0/6)
Low Pos. (1.7)	12	100.0% (12/12)	0.0% (0/12)	0.0% (0/12)	0.0% (0/12)
High Pos. (5.8)	6	83.3% (5/6)	16.7% (1/6)	0.0% (0/6)	0.0% (0/6)
Total:	30	60.0% (18/30)	16.7% (5/30)	16.7% (5/30)	6.7% (2/30)

Pediatric vs Adult Comparison (Analytical)

A study was conducted to evaluate the results observed when pediatric samples are tested with the ADVIA Centaur HAVT assay. A total of thirty (30) pediatric (ages 3 - 20) and 30 adult serum samples were spiked at different analyte levels. The distribution of percent bias (+/-) between the index values of the spiked pediatric serum samples and the mean index values of the adult serum samples are summarized in the following table:

Distribution of % Bias (Pediatric vs. Adult Serum)

Adult Spiked Observed Mean (Index)	Number Tested (n)	Distribution of % Bias			
		≤ 10%	> 10% to ≤ 20%	> 20% to ≤ 30%	> 30%
Negative (0.6)	6	0.0% (0/6)	0.0% (0/6)	0.0% (0/6)	100.0% (6/6)
Cut-off (1.0)	6	66.7% (4/6)	16.7% (1/6)	16.7% (1/6)	0.0% (0/6)
Low Pos. (1.7)	12	50.0% (6/12)	41.7% (5/12)	0.0% (0/12)	8.3% (1/12)
High Pos. (5.8)	6	83.3% (5/6)	16.7% (1/6)	0.0% (0/6)	0.0% (0/6)
Total:	30	50.0% (15/30)	23.3% (7/30)	3.3% (1/30)	23.3% (7/30)

Pediatric Testing (Clinical)

Fifty-five (55) pediatric serum samples (male and female, age range from 2 to 21 years), including samples from high risk population, were evaluated with the ADVIA Centaur HAVT assay and another commercially available assay.

The percent agreement (including 95% confidence intervals) of results for reactive and nonreactive samples between the ADVIA Centaur HAVT and comparative assay for the pediatric population age groups is shown in the following table:

Results of Pediatric Population (2 to 21 years) Comparison Study

		Comparative anti-HAV Total Assay			
		Positive	Borderline	Negative	Totals
ADVIA Centaur anti-HAV Total Assay	Reactive	11	0	1	12
	Nonreactive	0	2	41	43
	Total	11	2	42	55

% Positive Agreement = 84.62% (11/13*)

95% Confidence Interval = 54.55 to 98.08%

% Negative Agreement = 97.62% (41/42)

95% Confidence Interval = 87.43 to 99.94%

* The 2 borderline results from the comparative assay are scored as discordant results in the % Positive Agreement calculation. The ADVIA Centaur HAVT assay does not have a borderline or an equivocal zone.

Precision

Precision was evaluated according to the CLSI protocol EP5-A2.[14] Samples were assayed in 2 replicates 2 times a day for 20 days. The following results were obtained from testing performed on 2 ADVIA Centaur CP systems:

Sample	Mean Index Value	Within run		Between run		Total	
		SD	CV%	SD	CV%	SD	CV%
Negative Control	< 0.05	< 0.01	NA*	< 0.01	NA	< 0.01	NA
Positive Control	1.99	0.05	2.6	0.08	4.0	0.09	4.8
Serum 1	< 0.05	0.02	NA	0.02	NA	0.03	NA
Serum 2	0.79	0.06	7.1	0.05	7.0	0.08	9.9
Serum 3	1.17	0.05	4.1	0.06	4.9	0.07	6.4
Serum 4	2.39	0.05	2.2	0.07	3.0	0.09	3.7
Serum 5	4.45	0.06	1.3	0.12	2.6	0.13	2.9

* NA = Not Applicable

System Reproducibility

System reproducibility was determined by testing a five-member panel and controls, using 1 reagent lot on 3 ADVIA Centaur CP systems at 3 sites over 5 days. Panel members were run in replicates of 4 twice a day. The following results were obtained:

Sample	Mean Index	Within Run		Within Site		Among Site		Total	
		SD	%CV	SD	%CV	SD	%CV	SD	%CV
Negative Control	0.17	0.08	NA*	0.08	NA	0.04	NA	0.09	52.4
Positive Control	2.04	0.07	3.3	0.08	3.8	0.07	3.6	0.11	5.3
Serum 1	0.14	0.06	NA	0.06	NA	0.04	NA	0.07	51.0
Serum 2	1.00	0.05	4.8	0.07	7.1	0.10	9.5	0.12	11.8
Serum 3	1.38	0.05	3.9	0.06	4.5	0.08	5.9	0.10	7.4
Serum 4	2.61	0.06	2.2	0.14	5.5	0.10	3.7	0.17	6.6
Serum 5	3.03	0.07	2.3	0.20	6.5	0.13	4.3	0.24	7.8

* NA = Not Applicable

Cross-Reactivity

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.

Clinical Category	Number Tested	Number of Positive Anti-HAV Total Results	
		ADVIA Centaur Assay	Comparative Assay
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

Clinical Category	Number Tested	Number of Positive Anti-HAV Total Results	
		ADVIA Centaur Assay	Comparative Assay
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

Cross-reactivity was determined with the ADVIA Centaur HAV Total assay.

Endogenous Interferents

The potentially interfering effects of hemoglobin, triglycerides, conjugated bilirubin, unconjugated bilirubin, high protein, low protein, and high human IgG were evaluated. Interference testing was determined according to CLSI Document EP7-A2.[15]

Serum specimens that are...	Demonstrate a ≤ 10% change in results or have an insignificant effect on the assay up to...
icteric	60 mg/dL of conjugated bilirubin
icteric	40 mg/dL of unconjugated bilirubin
lipemic	3000 mg/dL of triglycerides
hemolyzed	500 mg/dL of hemoglobin
hypoproteinemic	3.5 g/dL of protein
hyperproteinemic	12.0 g/dL of protein
hyper IgG	60 mg/mL of immunoglobulin G

In addition, a potentially interfering effect of biotin was evaluated using 6 plasma samples spiked with several levels of biotin.

	Biotin Test Level (ng/mL)								
	0	9	19	38	75	150	300	600	1200
Negative Sample									
Index Value	0.82	0.71	0.63	0.61	0.88	1.32	2.73	4.79	>AR[a]
% Bias	NA[b]	-13	-23	-26	7	61	234	485	NA
Interpretation	NR[c]	NR	NR	NR	NR	FR[d]	FR	FR	NA
Positive Sample									
Index Value	2.03	1.93	1.84	1.88	2.26	2.79	4.37	>AR	>AR
% Bias	NA	-5	-9	-7	12	37	116	NA	NA
Interpretation	R[e]	R	R	R	R	R	R	R	R

a AR = Assay Range
b NR = Not Applicable
c NR = Nonreactive
d FR = False Reactive
e R = Reactive

Specimens that contain biotin at a concentration of 38 ng/mL demonstrate no change in interpretation. Biotin concentrations greater than this may lead to a change in interpretation.

The recommended adult daily dietary intake for biotin is 30 µg/day. Over the counter dietary supplements promoted for use in hair, skin and nail health may contain 5–100 mg of biotin, with recommendations to take multiple pills per day. Pharmacokinetic studies in healthy adults have shown that, in subjects ingesting 5 mg, 10 mg, and 20 mg of biotin, serum concentrations of biotin can reach up to 73 ng/mL, 141 ng/mL, and 355 ng/mL, respectively.[16] Subjects who take up to 300 mg of biotin per day may have plasma biotin levels as high as 1160 ng/mL.[17]

Interference was determined with the ADVIA Centaur HAV Total assay.

Technical Assistance

For customer support, contact your local technical support provider or distributor.

siemens.com/healthcare

References

1. Hollinger FB, Ticehurst JR. Hepatitis A virus. In: Fields BN, Knipe DM, Howley PM, Fields virology, 3rd ed. Philadelphia, PA: Lippincott-Raven Publishers, 1996:735–782.Stapleton JT. Hepatitis A virus: biology, pathogenesis, epidemiology, clinical description, and diagnosis. In: Specter S, Viral hepatitis: diagnosis, therapy, and prevention; Totowa, NJ: Humana Press Inc., 1999:7–33.
2. Cuthbert JA. Hepatitis A: old and new. *Clin Microbiol Rev* 2001;14(1):38–58.
3. Bower WA, Nainan OV, Han X, Margolis HS. Duration of viremia in hepatitis A virus infection. *J Infect Dis* 2000;182:12–17.
4. Siegl G, Lemon SM. Recent advances in hepatitis A vaccine development. *Virus Res* 1990:17:75-92.
5. Maiwald H, Jilg W, Bock HL, Loscher T, Sonnenburg F. Long-term persistence of anti-HAV antibodies following active immunization with hepatitis A vaccine. *Vaccine* 1997:15:346-348.
6. Recommendations of the of the Advisory Committee on Immunization Practices (ACIP). Prevention of hepatitis A through active or passive immunization. MMWR 1996:45:(RR-15).
7. Clinical and Laboratory Standards Institute (formerly NCCLS). Procedures for the Handling and Processing of Blood Specimens; Approved Guideline - Third Edition. Wayne, PA: Clinical and Laboratory Standards Institute; 2004. NCCLS Document H18-A3.
8. Centers for Disease Control. Update: Universal precautions for prevention of transmission of human immunodeficiency virus, hepatitis B virus and other bloodborne pathogens in healthcare settings. MMWR 1988;37:377-82, 387-8.
9. Clinical and Laboratory Standards Institute (formerly NCCLS). Protection of Laboratory Workers From Occupationally Acquired Infections; Approved Guideline - Third Edition. Wayne, PA: Clinical and Laboratory Standards Institute; 2005. CLSI Document M29-A3.
10. Federal Occupational Safety and Health Administration, Bloodborne Pathogens Standard, 29 CFR 1910.1030.
11. Clinical and Laboratory Standards Institute (formerly NCCLS). Assessment of the Clinical Accuracy of Laboratory Tests Using Receiver Operating Characteristic (ROC) Plots; Approved Guideline. Wayne, PA: Clinical and Laboratory Standards Institute; 1995. NCCLS Document GP10-A.
12. Boscato LM, Stuart MC. Heterophilic antibodies: a problem for all immunoassays. *Clin Chem* 1988;34:27–33.
13. Clinical and Laboratory Standards Institute (formerly NCCLS). *How to Define and Determine Reference Intervals in the Clinical Laboratory; Approved Guideline - Second Edition.* Wayne, PA: Clinical and Laboratory Standards Institute; 2000. NCCLS Document C28-A2.
14. Clinical and Laboratory Standards Institute (formerly NCCLS). *Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline - Second Edition.* Wayne, PA: Clinical and Laboratory Standards Institute; 2004. NCCLS Document EP5-A2.
15. Clinical and Laboratory Standards Institute (formerly NCCLS). *Interference Testing in Clinical Chemistry;* Approved Guideline - Second Edition. Wayne, PA: Clinical and Laboratory Standards Institute; 2005. CLSI document EP7-A2.
16. Grimsey P, Frey N, Bendig G, et al. Population pharmacokinetics of exogenous biotin and the relationship between biotin serum levels and *in vitro* immunoassay interference. *Int. J. Pharmacokinet.* 2017;2(4):247–256.
17. Piketty ML, Prie D, Sedel F, et al. High-dose biotin therapy leading to false biochemical endocrine profiles: validation of a simple method to overcome biotin interference. *Clin Chem Lab Med.* 2017;55(6):817-825.

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Definition of Symbols

The following symbols may appear on the product labeling:

Symbol	Definition	Symbol	Definition
IVD	*In vitro* diagnostic medical device	REF	Catalog number
	Legal manufacturer	EC REP	Authorized Representative in the European Community
CE	CE Mark	CE 0088	CE Mark with identification number of notified body
	Consult instructions for use		Biological risk
0°C	Do not freeze (> 0°C)		Temperature limitation
	Lower limit of temperature		Upper limit of temperature
	Keep away from sunlight and heat		Up
	Use by	Σ (n)	Contains sufficient for (n) tests
LOT	Batch code		Shake the reagent pack vigorously. Refer to *Preparing Reagents* in the assay-specific ADVIA Centaur product instructions for detailed information.
YYYY-MM-DD	Date format (year-month-day)	Rev.	Revision
MC DEF	Master Curve Definition	CHECKSUM	Variable hexadecimal number that ensures the Master Curve and Calibrator definition values entered are valid.
LOT DTL	Lot Details	DER GRÜNE PUNKT	Green dot
	Recycle	PRINTED WITH SOY INK	Printed with soy ink