Policy Statement	Core Laboratory personnel are responsible for insuring the specimen submitted for testing is acceptable and the procedure for performing the test is adhered to.
Purpose	This procedure provides technical instruction for the performance of HAV IgM on the ADVIA Centaur System.
Scope	This procedure applies to testing Personnel authorized to perform testing on the ADVIA Centaur System. This group includes, but is not limited to Medical Laboratory Technicians/Technologists, as well as leads and supervisory personnel.
Responsibility	All above personnel are responsible for following the ADVIA Centaur System procedure without exception. In addition, testing personnel are also responsible for evaluating the results and taking proper remedial action.
Related Documents	CORE 6910 R ADVIA Centaur System Operation CORE 6950 R Infectious Disease Reportable Tests CORE 6035 R Daily Quality Control Procedure Daily Handling, Processing and Reporting Results

Title: Detection of IgM Antibodies to Hepatitis A Virus in Serum on the ADVIA Centaur System

Reporting Results

Reference Interval

- Nonreactive: less than 0.80 S/CO Value
- Equivocal: greater than or equal to 0.80 S/CO Value and less than 1.20 S/CO Value.
- Reactive: greater than or equal to 1.20 S/CO Value

Note: For equivocal results attach the following comment. “Results in the equivocal range are repeated in duplicate. It is recommended that patients testing in the equivocal range receive supplemental testing as clinically indicated.”

Alert Values

N/A

Reporting Protocol for Alert Values

Reactive results are called to a licensed care provider and reported to the. See CORE 6950 R Infectious Disease Reportable Tests for detailed instructions.

Units for Reporting Results

The system reports HAV IgM results in S/CO Values and as reactive, equivocal, or nonreactive.

Interpretation of Results

1. The system reports HAV IgM results in S/CO Values and as reactive, equivocal, or nonreactive.
2. Samples with a calculated value of less than 0.80 S/CO Value are considered **nonreactive** for IgM antibodies to hepatitis A virus.
3. Samples with a calculated value greater than or equal to 0.80 S/CO Value and less than 1.20 S/CO Value are considered **equivocal** and must be repeated. It is recommended that the test be repeated in duplicate and the results be reported based on the repeat results. If the results are still equivocal after repeat testing, it is recommended to obtain a new specimen and retest using the ADVIA Centaur HAV IgM assay.
4. Samples with a calculated value greater than or equal to 1.20 S/CO Value are considered **reactive** for IgM antibodies to hepatitis A virus.
5. The cutoff for the ADVIA Centaur HAV IgM assay was verified based on results of Receiver-Operator characteristics (ROC) Curve and clinical agreement generated from the clinical studies.
6. The magnitude of the measured result above the cutoff is not indicative of the total amount of antibody present.
7. Sample results are invalid and must be repeated if the controls are out of range.

CAUTION: Do not use Heparin Plasma samples.

Procedure Notes

Dilutions

Do not dilute patient samples.

Equivocal Values

Equivocal values must be repeated. It is recommended that the test be repeated in duplicate and that the results be reported based on the repeat results. If the results are still equivocal after repeat testing, obtain a new specimen and retest using the ADVIA Centaur HAV IgM assay.

Interchangeability of Hepatitis A Values

The calculated values for hepatitis A in a given specimen as determined by assays from different manufacturers can vary due to differences in assay methods and reagent specificity. Do not use values obtained with different assay methods interchangeably.

Disposal

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 all federal, state, and local requirements.

QC Materials

Use ADVIA Centaur HAV IgM quality control material to monitor assay performance.

QC Frequency

Analyze at least two levels of quality control material on each shift that samples are analyzed.

Analyze all levels of quality control material each time a two-point calibration is performed.

Troubleshooting Out-of-Range QC Values

A QC run is acceptable when all values fall within the expected ranges.

If the quality control results do not fall within defined ranges technologists are expected to follow procedure CORE 6035 R

Specimen Collection and Handling

Specimen Collection

- Serum is are the recommended sample type for this assay.
- Test samples as soon as possible after collecting.
- Do not use specimens with obvious microbial contamination.
- This assay requires 20 *u*L of sample for a single determination.
- Samples are processed by centrifugation, typically followed by physical separation of the serum from the red cells.
- Centrifuge samples within 2 hours post draw.
- Samples are free of fibrin or other particulate matter. Remove particulates by centrifugation.
- Samples are free of bubbles or foam.

Specimen Storage and Stability

- Store samples at 2 – 8°C if not tested within 8 hours of collection.
- Store specimens covered and upright at all times at 2 – 8°C up to 2 days.
- Freeze samples, devoid of red blood cells, at or below -20°C for longer storage up to 180 days.
- When specimens are subjected to up to 4 freeze/thaw cycles, no qualitative differences are observed. Thoroughly mix thawed samples and centrifuge before using. Collect the supernatant into a clean vial.

Reagents

Storage and Stability

- Store the reagents upright at 2 – 8 °C and protected from light.
- Store Wash 1 upright at 2 – 25 °C.
- Primary and ancillary reagents stable until the expiration date on the pack label, or for 41 days onboard the system.
- Calibrators and controls stable until the expiration date on the vial label, or for 8 hours onboard the system.
- Diluents are stable until the expiration date on the pack label, or for 28 consecutive days after accessing the ancillary reagent pack.
- Wash stable until the expiration date on the pack label, or for 14 days onboard the system.

CAUTION:

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

Reagents Special Preparation

No special preparation of reagents is required.

Calibration

For detailed procedural information about scheduling a calibration, refer to the ADVIA Centaur Reference Manual or to the online help system.

Two-point Calibration Interval

Use ADVIA Centaur HAV IgM Calibrators to perform two-point calibrations.

Perform a two-point calibration every 28 days. Additionally, calibrate when the following conditions occur:

- the lot numbers of the primary reagent packs have changed
- system components are repaired or replaced
- quality control results do not fall within established ranges

Method Limitations

1. The ADVIA Centaur HAV IgM assay is limited to the detection of IgM antibodies to hepatitis A virus in human serum.
2. The results from this or any other diagnostic kit should be used and interpreted only in the context of the overall clinical picture. A negative test result does not exclude the possibility of exposure to hepatitis A virus.
3. The ADVIA Centaur HAV IgM assay can be used to determine if a patient has or recently had an acute or asymptomatic hepatitis A infection. This test does not measure anti-HAV IgG and therefore cannot be used to determine a patient's immune status to hepatitis A.
4. The calculated values for hepatitis A in a given specimen as determined by assays from different manufacturers can vary due to differences in assay methods and reagent specificity. The results reported by the laboratory to the physician must include the identity of the assay used. Values obtained with different assay methods cannot be used interchangeably.
5. Assay performance characteristics have not been established for immunocompromised, immunosuppressed, infants, children, or adolescent patients.
6. The performance of the ADVIA Centaur HAV IgM assay has not been established with cord blood, neonatal specimens, cadaver specimens, heat-inactivated specimens, or body fluids other than serum, such as saliva, urine, amniotic fluid, or pleural fluid.
7. Do not use specimens with obvious microbial contamination.
8. Heterophilic antibodies in human serum can react with reagent immunoglobulins, interfering with *in vitro* immunoassays. Patients routinely exposed to animals or to animal serum products can be prone to this interference and anomalous values may be observed. Additional information may be required for diagnosis.
9. In 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.

10. A reactive HAV IgM result does not exclude co-infection by another hepatitis virus.

For additional information on performance characteristics including cross reactivity, see the product information in the ADVIA Centaur Assay Manual.

Principle of the Test

The ADVIA Centaur® HAV IgM assay is an IgM capture immunoassay using a 2-pass format. In the first pass the sample is diluted using Multi-Diluent 2. After sample dilution biotinylated anti-human IgM monoclonal antibody is added to the cuvette binding IgM from the diluted patient sample. The IgM complex is then captured by the addition of streptavidin coated magnetic latex particles (MLP). The IgM-MLP is washed and resuspended.

In the second pass the anti-HAV IgM captured on the Solid Phase is detected by the sequential addition of HAV antigen and acridinium ester-labeled mouse anti-HAV antibody.

The relative light units (RLUs) detected by the ADVIA Centaur System are used to calculate the Signal-to-Cutoff (S/CO) Value from the Master Curve. A result of reactive or nonreactive is determined according to the (S/CO) Value established with the calibrators. Refer to *Interpretation of Results* for a description of the Cutoff Value calculation.

Clinical Application and Usefulness

The ADVIA Centaur HAV IgM assay is an *in vitro* diagnostic immunoassay for the qualitative determination of IgM response to the hepatitis A virus (HAV) in human serum using the ADVIA Centaur System. This assay is intended for use as an aid in the diagnosis of acute or recent infection (usually 6 months or less) with hepatitis A virus.

Assay performance characteristics have not been established for immunocompromised or immunosuppressed patients, cord blood, neonatal specimens, infants, or children.

Equipment and Supplies

- ADVIA Centaur HAV IgM Primary ReadyPack
- ADVIA Centaur HAV IgM Ancillary Reagent
- ADVIA Centaur HAV IgM Calibrators
- ADVIA Centaur HAV IgM Quality Control Materials
- ADVIA Centaur Multi-Diluent 2
- ADVIA Centaur Wash 1

- ADVIA Centaur Sample Cups and Caps
- ADVIA Centaur Cuvettes
- ADVIA Centaur Tips
- ADVIA Centaur Cleaning Solution Concentrate
- ADVIA Centaur Acid Reagent (0.5% H_20_2, 0.1N HNO_3)
- ADVIA Centaur Base Reagent (0.25N NaOH and surfactant)
- Reagent Water

References

1. Siemens HealthCare ADVIA Centaur HAV IgM (aHAVM) product insert, Rev G.
2. Siemens Diagnostics ADVIA Centaur Operator's Guide, Revision A
3. National Committee for Clinical Laboratory Standards (NCCLS). Clinical Laboratory Procedure Manuals—Third Edition (GP2-A3), 1996.

Technical Assistance

Siemens Technical Care Center: 1-877-229-3711

Customer Service: 1-800-255-3232

Serial Number: 21251109