CleanSlate
Outpatient Addiction Medicine

PROCEDURE TITLE:	IgG Anti-Hepatitis A Virus (IgG anti-HAV) a.k.a HAVAB G	DEPARTMENT:	Main Laboratory

EFFECTIVE DATE:	02/20/2020	APPROVAL:	08/20/24
APPROVED BY:	Patrice Y. Ohouo, PhD Main Laboratory Director	PROCEDURE NO.:	IA.SOP-007

1 PURPOSE

1.1 This procedure provides instruction for performing qualitative detection of IgG antibody to hepatitis A virus (IgG anti-HAV) in human adult & pediatric serum on the Abbott Architect i1000SR analyzers.

2 BACKGROUND

2.1 Hepatitis A virus (HAV) is endemic throughout the world, occurring most commonly in areas of poor hygiene and low socioeconomic conditions. The virus is transmitted primarily by the fecal-oral route and is spread by close person-to-person contact as well as by food- and water-borne epidemics. Outbreaks frequently occur in overcrowded situations and in high-density institutions and centers, such as prisons and health care or day care centers. Viral spread by parenteral routes (ie: exposure to blood) is possible but rare because infected individuals are viremia for a short period of time (usually <3 weeks). There is little or no evidence of transplacental transmission from mother to fetus or transmission to newborn during delivery.

2.2 In most cases, antibodies to HAV (anti-HAV) are detectable by the time symptoms occur, usually 15 to 45 days after exposure. Initial antibodies consist almost entirely of the IgM subclass. HAV-specific IgM antibody level in serum usually falls to an undetectable level by 6 months after acute infection. HAV-specific IgG antibody level in serum rises quickly once the virus is cleared and may persist for many years.

3 PRINCIPLE

3.1 The ARCHITECT HAVAB-G assay is a two-step immunoassay for the qualitative detection of IgG anti-HAV in human serum using CMIA technology with flexible assay protocols, referred to as Chemiflex.

3.2 In the first step, sample, assay diluent, and hepatitis A virus (human) coated paramagnetic microparticles are combined. IgG anti-HAV present in the sample binds to the hepatitis A virus (human) coated microparticles. After washing, the anti-human IgG acridinium-labeled conjugate that is added in the second step binds to IgG anti-HAV. Following another wash cycle, pre-trigger and trigger solutions are added to the reaction mixture. The resulting chemiluminescent reaction is measured as relative light units (RLUs). The presence or absence of IgG anti-HAV in the sample is determined by comparing the chemiluminescent signal in the reaction to the cutoff signal determined from an ARCHITECT HAVAB-G calibration. Specimens with signal to cutoff (S/CO) values ≥ 1.00 are considered reactive for IgG anti-HAV. Specimens with S/CO values < 1.00 are considered nonreactive.

4 INTENDED USE

4.1 The ARCHITECT HAVAB-G assay is a chemiluminescent microparticle immunoassay (CMIA) for the qualitative detection of IgG antibody to hepatitis A virus (IgG anti-HAV) in human adult and pediatric serum from patients with signs and symptoms or at risk for hepatitis. The ARCHITECT HAVAB-G assay is used to determine the immune status of individuals to hepatitis A virus infection.

4.2 Not intended for use in screening blood, plasma, or tissue donors.

5 SCOPE

5.1 The ARCHITECT HAVAB-G assay determines the presence of IgG anti-HAV in human serum. After an acute HAV infection, IgG anti-HAV levels rise quickly and may persist for life. The presence of IgG anti-HAV implies past HAV infection (recent or distant) or vaccination against HAV. Detectable levels above the assay cut-off suggest immunity to HAV infection.

6 DEFINITIONS

6.1 N/A

7 RESPONSIBILITIES

7.1 Only trained personnel are authorized to perform this procedure. Qualified personnel are responsible for the proper execution of this procedure. Under the guidance of the Laboratory Director, it is the responsibility of the Technical Supervisor to ensure the competency of laboratory personnel performing this test.

7.2 Training is documented in the training file of each qualified staff member.

7.3 All patient information is handled in a manner that is compliant with HIPAA guidelines. Refer to http://www.hhs.gov/ocr.hipaa/. And also to Clean Slate's HIPAA Policy, https://cleanslatecenters.training.reliaslearning.com or equivalent.

7.4 Under the direction of the Laboratory Director, the Technical Supervisor is responsible for the direct review of all quality control, equipment maintenance and reporting of patient results.

8 SAFETY

8.1 Standard Precautions

8.1.1 Care should be taken and personal protective equipment is required when handling material of human origin. All biological specimens should be considered potentially infectious.

8.1.2 For up-to-date recommendations on handling biological specimens refer to the CDC website: http://cdc.gov/ncidod/dhqp/pdf/guidelines/Isolation2007.pdf or CLSI document M29-A3, Protection of Laboratory Workers from Occupationally Acquired Infections. Clinical and Laboratory Standards Institute; Approved Guidelines and or Refer to Clean Slate's safety policy, https://cleanslatecenters.training.reliaslearning.com or equivalent.

8.2 Computer and Web Portal

8.2.1 Passwords must be assigned only to authorized personnel.

8.2.2 To ensure HIPAA compliance, it is recommended that the computer, printer and printouts be located away from the visibility and access of unauthorized individuals.

9 SPECIMEN REQUIREMENTS

9.1 Serum specimens, free from hemolysis, free of fibrin, red blood cells, and other particulate matter are recommended for testing.

9.2 Only blood drawn by an acceptable medical technique into a collection tube with no anticoagulants should be used for CleanSlate.

9.3 The specimen should be allowed to clot fully and the serum separated by centrifugation.

9.4 For optimal results, inspect all specimens for bubbles. Remove bubbles with an applicator stick before analysis. Use a new applicator stick for each specimen to prevent cross contamination.

9.5 Specimens may be stored on or off the clot, red blood cells, or separator gel for up to 8 days refrigerated at 2-8·C or up to 4 days at room temperature. If will be delayed more than 8 days, remove serum or plasma from the clot, red blood cells, or separator gel and store at -20·C or colder. Avoid multiple freeze/thaw cycles.

9.6 Criteria for Unacceptable Specimens

9.6.1 Unlabeled specimens- there must be an ID link between the test order and the specimen container. Unlabeled specimens cannot be accepted.

9.6.2 All specimens are examined for correct identification when accessioned and processed or rejected if it does not have two matching patient identifiers.

9.6.3 Leaking/improperly closed tubes cannot be accepted.

9.6.4 Insufficient quantity (less than 0.3mL serum) or specimen containers that are "empty" or have improper storage cannot be accepted.

10 MATERIALS & EQUIPMENT

10.1 Abbott Architect i1000SR system.

10.2 ARCHITECT HAVAB-G Assay file.

10.3 ARCHITECT HAVAB-G Reagent Kit (Cat# 6L27)

10.4 ARCHITECT HAVAB-G Controls. (Cat # 6L27-10)

10.5 ARCHITECT HAVAB-G Calibrator (Cat# 6L27-01)

10.6 Abbott Architect i1000SR Pre-Trigger Solution

10.7 Abbott Architect i1000SR Trigger Solution

10.8 Abbott Architect i1000SR Wash Buffer

10.9 Abbott Architect i1000SR Reaction Vessels

10.10 Abbott Architect i1000SR Septum

10.11 Abbott Architect i1000SR Replacement Caps

10.12 Abbott Architect i1000SR Sample Cups

11 REAGENTS

11.1 P Preparation

11.1.1 Before loading the ARCHITECT HAVAB-G Reagent Kit on the system for the first time, the micro-particle bottle requires mixing to re-suspend micro-particles that may have settled during shipment. After the first time the micro-particles have been loaded, no further mixing is required.

11.1.2 Invert the micro-particle bottle 30 times.

11.1.3 Visually inspect the bottle to ensure micro-particles are re-suspended. If micro-particles are still adhered to the bottle, continue to invert the bottle until the micro-particles have been completely re-suspended.

11.1.4 If the micro-particles do not re-suspend, DO NOT USE. Contact your local Abbott representative.

11.1.5 Once the micro-particles have been re-suspended, place a septum on the bottle. Septum's MUST be used to prevent reagent evaporation and contamination and to ensure reagent integrity. To avoid contamination, wear gloves when placing a septum on an uncapped reagent bottle. Once a septum has been placed on an open reagent bottle, do not invert the bottle as this will result in reagent leakage and may compromise assay results. Over time, residual liquids may dry on the septum surface. These are dried salts and have no effect on assay efficacy.

11.1.6 When handling conjugate vials, change gloves that have contacted human serum, since introduction of antibody will result in a neutralized conjugate.

11.2 Storage and Stability

11.2.1 Opened HAVAB-G reagents are stable for 15 days when stored in the refrigerator compartment of the Abbott Architect i1000SR analyzer. Discard after 15 days.

11.2.2 Unopened HAVAB-G reagents are stable until the expiration date stated on the label if stored in the refrigerator at 2-8°C in an up-right position. Reagent bottles should remain closed when not in use with screw caps tightly closed.

11.2.3 Do NOT use reagents past their expiration date.

11.2.4 Do NOT pool reagents within a kit or between kits.

11.3 Indications of Reagent Deterioration

11.3.1 When a control value is out of the specified range, it may indicate deterioration of the reagents or errors in technique. Associated test results

are invalid and samples must be retested. Assay recalibration may be necessary. For troubleshooting, refer to the ARCHITECT System Operation Manual, Section 10.

12 CALIBRATIONS & CONTROLS

1.1. Calibration

1.1.1. The frequency of calibration is as needed (see section 1.1.4 below) once the reagent is on-board i1000SR system.

1.1.2. The calibrator is ready to use and does NOT require reconstitution. Mix the contents of the vial gently by inverting the vial 3-4 times.

1.1.3. To perform an ARCHITECT HAVAB-G calibration, test calibrator 1 in replicates of 3. The calibrators should be priority loaded.

1.1.4. Recalibration of this test is required when any of these conditions exist:

1.1.1.1. A change of bottle Lot number.

1.1.1.2. Controls are out of range.

1.1.2. Storage and Stability of Calibrators:

1.1.2.1. Store Calibrator at 2-8°C. DO NOT FREEZE. Store vials tightly capped when not in use.

1.1.2.2. Stability of Calibrator is the expiration date printed on each vial. DO NOT use any calibrator after its expiration date.

1.2. Controls

1.2.1. During operation of the Abbott Architect i1000SR analyzer, at least two levels of quality control material will be tested at a minimum of once a day (Nonreactive (Negative) & Reactive (Positive) QC).

1.2.2. In addition, controls should be performed:

1.2.2.1. After calibration.

1.2.2.2. With each new lot of reagent.

1.2.3. New Quality control material is poured fresh each day. The controls are ready to use and do NOT require reconstitution. Mix the contents of the vial gently by inverting the vial 3-4 times.

1.2.4. Storage and Stability of Controls:

1.2.4.1. Store Controls at 2-8°C. DO NOT FREEZE. Store control vials tightly capped when not in use.

1.2.4.2. Stability of Controls is the expiration date printed on each vial. DO NOT use any control after its expiration date.

13 PROCEDURE(S)

13.1 Specimen Receipt: The test(s) have been previously ordered at the point of collection through the EMR and populated into LabDaq. Specimens are received into the main lab already labeled.

13.1.1 Specimens are scanned into LabDaq and received.

13.1.2 Specimens are placed into sample racks.

13.2 Specimen Processing: A Two-step assay protocol is a method of sample processing in which the sample and some reagents are added prior to washing the micro-particles. The conjugate reagent is added after the micro-particles are washed. Total processing time for a Two-step assay is 22 minutes including a 15 minute incubation time. The following steps describe the *i*1000SR operation and CMIA (chemiluminescent micro-particle immunoassay) reaction that occurs during Two-step assay processing.

13.2.1 The pipettor dispenses the sample into the Reaction Vessel (RV) at position 1.

13.2.2 The pipettor dispenses the micro-particles into the RV at position.

13.2.3 Vortexer 1 mixes the sample and micro-particles at position 3.

13.2.4 The reaction mixture incubates for 11 minutes. (1 complete revolution around the process path track.)

13.2.5 The wash zone inlet diverter directs the RV with the reaction mixture to the outer track. The wash zone manifold washes the reaction mixture in the RV and then removes unbound materials.

13.2.6 The wash zone outlet diverter directs the RV back to the inner track and acridinium-labeled conjugate is added to the RV in position 2.

13.2.7 The reaction mixture incubates for 4 minutes.

13.2.8 The wash zone inlet diverter directs the RV with the reaction mixture to the outer track for a second wash.

13.2.9 Pre-trigger is dispensed into the RV and Vortexer 2 mixes the reaction mixture.

13.2.10 The CMIA optical system takes a background read and then trigger is dispensed into the RV with the reaction mixture. The CMIA optical system takes an activated read.

13.2.11 The Unloader removes the RV and disposes of it in the solid waste container.

14 REFERENCE INTERVAL OF PATIENT RESULTS

14.1 Linearity

14.1.1 N/A

14.2 Critical Values

14.2.1 N/A

15 ESTABLISH QC TARGET MEANS AND ACCEPTANCE CRITERIA UPON ARRIVAL OF NEW LOT

15.1 step 1--compare new lot to manufacturer. implement via laboratory leadership approval.

15.2 step 2--10 replicates to establish:

15.2.1 new mean

15.2.2 reactive qc 1sd set at 10%

15.2.3 non-reactive qc set at manufacture range

15.3 mean adjustments will also be performed relative to performance trends

16 CALCULATIONS

16.1 The ARCHITECT iSystem calculates the cutoff RLU from the mean RLU of 3 replicates of Calibrator 1 and stores the result. The cutoff RLU is determined by multiplying the Calibrator 1 Mean RLU by 0.29. Cutoff RLU = Calibrator 1 Mean RLU x 0.29 The ARCHITECT i System calculates the S/CO result for each specimen and control as follows: S/CO = Sample RLU / Cutoff RLU.

17 INTREPTATION OF RESULTS

17.1 See Table Below For Interpretation of Results:

ARCHITECT HAVAB-G Interpretation		
Result (S/CO)	**Instrument Interpretation**	**Final Interpretation**
< 1.00	NONREACTIVE	IgG anti-HAV not detected.
≥ 1.00	REACTIVE.	IgG anti-HAV detected.

18 REPORTING

18.1 Report Transmission

18.1.1 Patient test results uploaded into LABDAQ are reviewed by designated personnel and released for transmission into EMR chart via interface; results within the normal are transmitted to EMR via Auto-verification.

19 LIMITATIONS

19.1 If the ARCHITECT HAVAB-G results are inconsistent with clinical evidence, additional testing is suggested to confirm the result.

19.2 Specimens from patients who have received preparations of mouse monoclonal antibodies for diagnosis or therapy may contain human anti-mouse antibodies (HAMA).

19.3 Specimens containing HAMA may show either falsely elevated or depressed values when tested with assay kits such as ARCHITECT HAVAB-G that employ mouse monoclonal antibodies.

19.4 Specimens from individuals with anti-E. coli, anti-CMV, or hemodialysis patients may cross-react with this assay.

19.5 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 results may be observed.

19.6 Specimens containing low antibody concentrations (near the cutoff) assayed after a freeze/thaw may exhibit elevated values that may be false positive.

20 TROUBLESHOOTING

20.1 Notify Laboratory Manager.

20.2 See the Abbott ARCHITECT i1000SR Operators' Manual or go to link http://yeec.com/uploadimages1/forum/abbott/rchitect_c400011000.pdf.

20.3 **Abbott Architect i1000SR**: Call Technical Support 1-877-422-2688, and SN # ISR 55194.

21 PERFORMANCE CHARACTERISTICS

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REFER TO THE ARCHITECT AUSAB INSERT FOR PERFORMANCE CHARACTERISTICS AND VALIDATION STUDIES COMPLETED BY CLEANSLATE.

REFERENCES:	• Refer to Abbott Architect i1000SR Operating Procedure, IA.SOP-001 • Clean Slate's HIPAA Policy • Clean Slate's Safety Policy • Abbott ARCHITECT HAVAB-G Insert, February 2016. • CAP Laboratory General Checklist.
REVISION HISTORY:	02/20/2020

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Date