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| **PROCEDURE TITLE:** | **Alinity i Anti-Hepatitis A IgG antibody (HAVAb IgG II)** | **DEPARTMENT:** | Main Laboratory |

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| EFFECTIVE DATE: | 05/22/2025 | APPROVAL: | 05/21/25 |
| APPROVED BY: | Patrice Y. Ohouo, PhD  Main Laboratory Director | **PROCEDURE NO.:** | IMM.01008 |

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| 1. *PURPOSE*    1. To provide instructions for use of the Alinity i HAVAb IgG II assay. The Alinity i HAVAb IgG II assay is used to detect the presence of IgG antibody to hepatitis A virus (IgG anti-HAV) in human serum and plasma on the Abbott Alinity i analyzer. 2. *SUMMARY AND EXPLANATION OF THE TEST*    1. The HAVAb IgG II assay is a chemiluminescent microparticle immunoassay (CMIA) used for the qualitative detection of IgG antibody to hepatitis A virus (IgG anti-HAV) in human adult and pediatric (4 through 21 years) serum (collected in serum and serum separator tubes) and plasma (collected in sodium heparin, lithium heparin, lithium heparin separator, dipotassium EDTA, and tripotassium EDTA tubes) from patients with signs and symptoms or at risk for hepatitis A on the Alinity i system.    2. HAV is a small, non-enveloped RNA virus belonging to the picornavirus family. HAV causes a self-limited illness and is almost always transmitted via the fecal-oral route. HAV infection is often mild with an acute onset but cannot be distinguished clinically from other types of acute viral hepatitis. Symptoms include fever, malaise, nausea, and abdominal pain. The presenting symptoms are often followed days later by dark urine, pale stools, and jaundice. Recent or acute infection with HAV is differentiated from other types of viral hepatitis through detection of IgM antibody to HAV (IgM anti-HAV) or HAV ribonucleic acid (RNA).    3. Early on in the illness, IgM anti-HAV is the predominant antibody. It becomes detectable within 5 to 10 days of the onset of symptoms and can persist for several (approximately 3) months. IgM anti-HAV normally becomes undetectable within 6 months after infection. IgG anti-HAV becomes detectable shortly after IgM appears. As IgM anti-HAV declines to undetectable levels, IgG anti-HAV becomes the predominant antibody. The presence of IgG anti-HAV with a nonreactive IgM anti-HAV test result implies past infection with HAV or vaccination against HAV, both scenarios indicating immunity against the virus. In acute infection, IgG anti-HAV detection is used in conjunction with IgM anti-HAV and other viral hepatitis markers (e.g., aspartate transaminase, alanine transaminase, bilirubin) for differential diagnosis. 3. *BIOLOGICAL PRINCIPLES OF THE PROCEDURE*    1. This assay is an automated, two-step immunoassay for the qualitative detection of IgG anti-HAV in human adult and pediatric serum and plasma from patients with signs and symptoms or at risk for hepatitis using chemiluminescent microparticle immunoassay (CMIA) technology.    2. Sample, HAV (human) coated paramagnetic microparticles, and assay diluent are combined and incubated. The IgG anti-HAV present in the sample binds to the HAV (human) coated microparticles. The mixture is washed. Anti-human IgG acridinium-labeled conjugate is added to create a reaction mixture and incubated. Following a wash cycle, Pre-Trigger and Trigger Solutions are added.    3. The resulting chemiluminescent reaction is measured as a relative light unit (RLU). There is a direct relationship between the amount of IgG anti-HAV in the sample and the RLU detected by the system optics.    4. The presence or absence of IgG anti-HAV in the sample is determined by comparing the chemiluminescent RLU in the reaction to the cutoff RLU determined from an active calibration.    5. For additional information on system and assay technology, refer to the Alinity ci-series Operations Manual, Section 3. 4. *INTENDED USE*    1. For In Vitro Diagnostic Use: United States Federal Law restricts this device to sale and distribution by or on the order of a physician, or to a clinical laboratory; and use is restricted to, by, or on the order of a physician.    2. The HAVAb IgG II assay is a chemiluminescent microparticle immunoassay (CMIA) used for the qualitative detection of IgG antibody to hepatitis A virus (IgG anti-HAV) in human adult and pediatric (4 through 21 years) serum (collected in serum and serum separator tubes) and plasma (collected in sodium heparin, lithium heparin, lithium heparin separator, dipotassium EDTA, and tripotassium EDTA tubes) from patients with signs and symptoms or at risk for hepatitis A on the Alinity i system.    3. The HAVAb IgG II assay is used to determine the immune status of individuals to hepatitis A virus (HAV) infection.    4. This assay has not been cleared for use in screening blood, plasma, or tissue donors. 5. *Definitions*    1. N/A 6. *Responsibilities*    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.    2. Training is documented in the training file of each qualified staff member.    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 CleanSlate’s HIPAA Policy, [https://cleanslatecenters.training.reliaslearning.com](https://cleanslatecenters.training.reliaslearning.com/) or equivalent.    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. 7. *SAFETY*    1. Standard Precautions       1. CAUTION: This product contains human-sourced and/or potentially infectious components. Refer to the REAGENTS section of assay insert for more details.       2. It is recommended that these reagents, human specimens, and all consumables contaminated with potentially infectious materials be handled in accordance with the OSHA Standard on Bloodborne Pathogens. Biosafety Level 2 or other appropriate regional, national, and institutional biosafety practices should be used for materials that contain, are suspected of containing, or are contaminated with infectious agents.       3. Care should be taken, and personal protective equipment is required when handling material of human origin. All biological specimens should be considered potentially infectious.       4. 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.    2. Safety       1. For the most current hazard information, see the product Safety Data Sheet also available at [www.corelaboratory.abbott](http://www.corelaboratory.abbott).       2. The tables below list warnings and precautions that apply to listed kit components:                * + 1. For a detailed discussion of safety precautions during system operation, refer to the Alinity ci-series Operations Manual, Section 8.   1. Computer and Web Portal      1. Passwords must be assigned only to authorized personnel.      * + 1. To ensure HIPAA compliance, it is recommended that the computer, printer and printouts be located away from the visibility and access of unauthorized individuals.  1. *SPECIMEN REQUIREMENTS,* *COLLECTION AND PREPARATION FOR ANALYSIS*    1. Specimen types:       1. The specimen types described in the table below were verified by the manufacturer for use with this assay.       2. The instrument does not provide the capability to verify specimen types. It is the responsibility of the operator to verify that the correct specimen types are used in the assay.      * 1. Specimen conditions:      1. Do not use:         1. heat-inactivated specimens         2. pooled specimens         3. grossly hemolyzed specimens         4. specimens with obvious microbial contamination         5. Specimens with fungal growth         6. For accurate results, serum and plasma specimens should be free of fibrin, red blood cells, and other particulate matter.         7. Serum specimens from patients receiving anticoagulant or thrombolytic therapy may contain fibrin due to incomplete clot formation.         8. To prevent cross contamination, use of disposable pipettes or pipette tips is recommended.         9. Unlabeled specimens: there must be an ID link between the test order and the specimen container. Unlabeled specimens cannot be accepted.         10. All specimens are examined for correct identification when accessioned and processed and are rejected if it does not have two matching patient identifiers.         11. Leaking/improperly closed tubes cannot be accepted.         12. Specimen with insufficient quantity or specimen containers that are “empty” or have improper storage cannot be accepted.   2. Preparation for analysis:      1. Follow the tube manufacturer’s processing instructions for collection tubes. Gravity separation is not sufficient for specimen preparation.      2. Specimens should be free of bubbles. Remove bubbles with an applicator stick before analysis. Use a new applicator stick for each specimen to prevent cross contamination.      3. To ensure consistency in results, recentrifuge specimens prior to testing if   they contain fibrin, red blood cells, or other particulate matter.   * + 1. NOTE: If fibrin, red blood cells, or other particulate matter are observed, mix by low-speed vortex or by inverting 10 times prior to recentrifugation.     2. Prepare frozen specimens as follows: (Avoid more than 3 freeze/thaw cycles).        1. Frozen specimens must be completely thawed before mixing.        2. Mix thawed specimens thoroughly by low speed vortex or by inverting 10 times.        3. Visually inspect the specimens. If layering or stratification is observed, mix until specimens are visibly homogeneous.        4. If specimens are not mixed thoroughly, inconsistent results may be obtained.        5. Recentrifuge specimens.     3. Recentrifugation of Specimens:        1. Transfer specimens to a centrifuge tube and a minimum of 100 000 g-minutes.        2. Examples of acceptable time and force ranges that meet this criterion are listed in the following table.        3. Centrifugation time using alternate RCF values can be calculated using the following formula:      * + - 1. Transfer clarified specimen to a sample cup or secondary tube for testing. For centrifuged specimens with a lipid layer, transfer only the clarified specimen and not the lipemic material.   1. Specimen Storage:      1. Specimen storage is as described in the table below or according to stability studies performed by the Cleanslate Centers’ Main Laboratory, where indicated.      * + 1. If testing will be delayed longer than the maximum storage time, remove serum or plasma from the clot, red blood cells, or separator gel and store frozen (-20°C or colder).     2. Avoid more than 3 freeze/thaw cycles.   1. Specimen Shipping:      1. Package and label specimens in compliance with applicable state, federal, and international regulations covering the transport of clinical specimens and infectious substances.      2. Do not exceed the storage limitations listed above or as determined based on stability studies performed by the Cleanslate Centers’ Main Laboratory, where indicated.  1. *MATERIALS & EQUIPMENT*    1. Abbott Alinity i analyzer.    2. Alinity i HAVAb IgG II Reagent Kit 06S93. See table below for details. Volumes (mL) listed in the table below indicate the volume per cartridge.      * 1. Alinity i HAVAb IgG II Calibrator (Part# 06S9301)   2. Alinity i HAVAb IgG II Controls (Part# 06S9310)   3. Alinity Trigger Solution (Part# 06P1160)   4. Alinity Pre-Trigger Solution (Part# 06P1265)   5. Alinity i-series Concentrated Wash Buffer (Part# 06P1368)   6. Alinity i Reaction Vessels (Part# 06P1401)   7. Alinity i Replacement Caps (Part# 04R4701)   8. Alinity i Sample Cups (Part# 01R3801)   9. For information on materials required for operation of the instrument, refer to the Alinity ci-series Operations Manual, Section 1.   10. For information on materials required for maintenance procedures, refer to the Alinity ci-series Operations Manual, Section 9.   *10 REAGENTS HANDLING*   * 1. Upon receipt, gently invert the unopened reagent kit by rotating it over and back for a full 180 degrees, 5 times with green label stripe facing up and then 5 times with green label stripe facing down. This ensures that liquid covers all sides of the bottles within the cartridges. During reagent shipment, microparticles can settle on the reagent septum.   2. Place a check in the square on the reagent kit to indicate to others that the inversions have been completed.   3. After mixing, place reagent cartridges in an upright position for 1 hour before use to allow bubbles that may have formed to dissipate.   4. If a reagent cartridge is dropped, place in an upright position for 1 hour before use to allow bubbles that may have formed to dissipate.   5. Reagents are susceptible to the formation of foam and bubbles. Bubbles may interfere with the detection of the reagent level in the cartridge and cause insufficient reagent aspiration that may adversely affect results.   6. For a detailed discussion of reagent handling precautions during system operation, refer to the Alinity ci-series Operations Manual, Section 7.   *11 REAGENTS STORAGE:*     * 1. Reagents may be stored on or off the system. If removed from the system, store reagents with new replacement caps in an upright position at 2 to 8°C. For reagents stored off the system, it is recommended that they be stored in their original trays or boxes to ensure they remain upright.   2. For information on unloading reagents, refer to the Alinity ci-series Operations Manual,   Section 5.   * 1. Indications of Reagent Deterioration      1. Deterioration of the reagents may be indicated when a calibration error occurs or a control value is out of the specified range. Associated test results are invalid, and samples must be retested. Assay recalibration may be necessary.      2. For troubleshooting information, refer to the Alinity ci-series Operations Manual, Section 10.   *12 CALIBRATIONS & CONTROL PROCEDURES*   * 1. Calibration      1. Cal 1 contains recalcified human plasma reactive for IgG anti-HAV. Preservatives: ProClin 950 and sodium azide.      2. The calibrator is at the following concentration:      * + 1. This product is liquid ready-to-use and may be used immediately after removal from 2 to 8°C storage.     2. Prior to each use, mix by gentle inversion.     3. The calibrator vial is placed directly on the instrument and automatically processed using the barcode on the calibrator vial. Alternatively, the calibrator can be pipetted into a sample cup. If the calibrator is pipetted into a sample cup, the calibration must be manually ordered.     4. Calibrator 1 is tested in triplicate. The Alinity i analyzer calculates the cutoff Relative Light Units (RLU) from the mean RLU of the three replicates.     5. The HAVAb IgG II Calibrator is traceable to the World Health Organization (WHO) 2nd International Standard for Anti-hepatitis A, Immunoglobulin, Human (NIBSC Code: 97/646).     6. The acceptable calibration is stored by the Alinity i analyzer for use with any reagent kit of that lot.     7. The calibration should be used in conjunction with control ranges to determine the validity of the calibration     8. The frequency of calibration is as follows:        1. A reagent kit with a new lot number is used.        2. Daily quality control results are outside of statistically-based quality control limits used to monitor and control system performance.        3. If statistically-based quality control limits are not available then the calibration should not exceed a 30-day limit for recalibration frequency.        4. This assay may also need to be recalibrated after specified service procedures have been performed or maintenance to critical part or subsystems that might influence the performance of the assay.     9. Assay control must be tested to evaluate the assay calibration.     10. Once a calibration is accepted and stored, all subsequent samples may be tested.     11. For additional instructions on performing a calibration, refer to the Alinity ci-series Operations Manual, Section 5.     12. Storage and Stability of Calibrators:         1. The analyzer will track In-use Stability, which is the time the calibrator is outside of refrigerated storage while on the analyzer.         2. The analyzer will not allow the use of the calibrator if the In-use Stability has been exceeded. Maximum In-use Stability can be found in the Assay Parameter Report. For additional information on calibrator In-use Stability, refer to the Alinity ci-series Operations Manual, Section 5.         3. Do not use past expiration date.      * 1. Quality Control Procedures      1. The Control (-) contains recalcified anti-HAV negative human plasma with protein (bovine) stabilizer.      2. The Control (+) contains recalcified human plasma reactive for IgG anti-HAV.      3. Both controls also contain preservatives (ProClin 950 and sodium azide).      4. The controls are at the following ranges and target concentrations:      * + 1. This product is liquid ready-to-use and may be used immediately after removal from 2 to 8°C storage.     2. Prior to each use, mix by gentle inversion.     3. During operation of the Alinity i analyzer, at least two levels of quality control material (one Non-reactive (Negative QC) and one Reactive (Positive QC)) will be tested at a minimum of once a day.     4. The frequency of Quality Control Procedures is as follows:        1. Once every 24 hours each day of use        2. After performing calibration        3. After instrument service procedures or maintenance that may affect assay performance have been performed.     5. Control ranges determined during method validation at the CleanSlate Centers’ Main Laboratory are used to establish basis to monitor the acceptable performance of the assay. If a control is out of its specified range, the associated sample results are invalid and the samples must be retested. Recalibration may be indicated.     6. Note: The insert ranges for the controls are not lot specific and represent the total range of values which may be generated throughout the life of the product. Means and acceptable ranges that fall within the package insert ranges were defined during validation and will be monitored and updated by the CleanSlate Centers’ Main Laboratory.     7. Once a calibration is accepted and stored, all subsequent samples may be tested.     8. To troubleshoot control values that fall outside the control range, refer to the Alinity ci-series Operations Manual, Section 10, Observed Problems.     9. Storage and Stability of QC materials:        1. This product may not be stored on the instrument.        2. Do not use past expiration date.     *13 PROCEDURE(S)*   * 1. Specimen Receipt: The test(s) have been previously ordered at the point of collection through the EMR and populated into the laboratory information system (LIS), here LabDaq or equivalent. Specimens are received into the main lab already labeled.      1. Specimens are scanned into LabDaq and received.      2. Specimens are placed into sample racks.   2. Analysis: performed as described in the “biological principles of the procedure” section above.   3. For a detailed description of how to run an assay, refer to the Alinity ci-series Operations Manual, Section 5.   4. If using primary or aliquot tubes, refer to the Alinity ci-series Operations Manual, Section 4 to ensure sufficient specimen is present.   5. To minimize the effects of evaporation, verify adequate sample cup volume is present prior to running the test.   6. Maximum number of replicates sampled from the same sample cup: 10      1. Priority:         1. Sample volume for first test: 75 µL         2. Sample volume for each additional test from same sample cup: 25 µL      2. ≤ 3 hours on the reagent and sample manager:         1. Sample volume for first test: 150 µL         2. Sample volume for each additional test from same sample cup: 25 µL      3. > 3 hours on the reagent and sample manager:         1. Replace with a fresh aliquot of sample.   7. Refer to the HAVAb IgG II calibrator package insert 06S9301 and/or HAVAb IgG II control package insert 06S9310 for preparation and usage.   8. For general operating procedures, refer to the Alinity ci-series Operations Manual, Section 5.   9. For optimal performance, it is important to perform routine maintenance as described in the Alinity ci-series Operations Manual, Section 9. Perform maintenance more frequently when required by laboratory procedures.   10. Sample Dilution Procedures       1. Samples cannot be diluted for the Alinity i HAVAb IgG II assay.  1. *REFERENCE INTERVAL OF PATIENT RESULTS*    1. Linearity       1. N/A    2. Critical Values       1. N/A 2. *ESTABLISH QC TARGET MEANS AND ACCEPTANCE CRITERIA UPON ARRIVAL OF NEW LOT*    1. Evaluate new lot to manufacturer range for updates compared to current.    2. Report any update found to laboratory leadership approval and implementation.    3. Analyze each level in 5 replicates to evaluate:       1. Need for a new mean.       2. SD range:          1. Reactive QC: 1SD set at 10% of mean.          2. Non-reactive: set according to manufacture range.       3. Mean adjustments will also be performed relative to performance trends.       4. To establish statistically-based control limits, each laboratory should establish its own concentration target and ranges for new control lots at each clinically relevant control level. This can be accomplished by assaying a minimum of 20 replicates over several (3-5) days and using the reported results to establish the expected average (target) and variability about this average (range) for the laboratory. 3. *CALCULATIONS*    1. The Alinity i system calculates results for the HAVAb IgG II assay using the ratio of the sample RLU to the cutoff RLU (S/CO) for each specimen and control.    2. Cutoff RLU = Calibrator 1 Mean RLU x 0.290    3. The cutoff RLU is stored for each reagent lot calibration.    4. S/CO = Sample RLU/Cutoff RLU 4. *INTREPTATION OF RESULTS*    1. The cutoff is 1.00 S/CO.    2. As with all analyte determinations, the IgG anti-HAV value should be used in conjunction with information available from clinical evaluation and other diagnostic procedures.    3. See Table Below For Interpretation of Results:      * 1. Flags      1. Some results may contain information in the Flags field. For a description of the flags that may appear in this field, refer to the Alinity ci-series Operations Manual, Section 5.  1. *REPORTING*    1. Report Transmission       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. 2. *LIMITATIONS*    1. Results should be used in conjunction with other data; e.g., symptoms, results of other tests, and clinical impressions.    2. Potential interference has not been evaluated for substances other than those described in the SPECIFIC PERFORMANCE CHARACTERISTICS, Analytical Specificity, Interference section of this package insert.    3. Specimens from patients who have received preparations of mouse monoclonal antibodies for diagnosis or therapy may contain human anti-mouse antibodies (HAMA). Such specimens may show either falsely elevated or depressed values when tested with assay kits such as HAVAb IgG II that employ mouse monoclonal antibodies. Additional information may be required for diagnosis.    4. 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.    5. Rheumatoid factor (RF) in human serum can react with reagent immunoglobulins, interfering with in vitro immunoassays.    6. Specimens from individuals with anti-Escherichia coli (anti-E coli), monoclonal hyper IgG antibodies, or hemodialysis patients may cross-react with this assay.    7. The percentage of previously vaccinated individuals within the intended use population in the clinical study is unknown. 3. *TROUBLESHOOTING*    1. Notify laboratory leadership or designated staff.    2. See the Abbott Alinity ci-series Operations Manual available onboard the instrument or CleanSlate Centers OneDrive.    3. Call Technical Support 1-877-422-2688, and SN # SCM28296. 4. *PERFORMANCE CHARACTERISTICS*    1. Refer to the Alinity i Anti-Hepatitis B core antigen (anti-HBc) insert for performance characteristics and validation studies completed by the CleanSlate Centers’ Main Laboratory. |

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| REFERENCES: | * Abbott Alinity ci-series Operations Manual * Alinity i HAVAb IgG II Reagent Kit insert * Alinity i HAVAb IgG II calibrator insert * Alinity i HAVAb IgG II quality control insert * Clean Slate’s HIPAA Policy * Clean Slate’s Safety Policy * CAP Laboratory General Checklist. |
| REVISION HISTORY: | N/A |

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Patrice Y. Ohouo, PhD Date

Main Laboratory Director