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| **PROCEDURE TITLE:** | **Alinity i Anti-Hepatitis B core antigen (anti-HBc)** | **DEPARTMENT:** | Main Laboratory |

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| EFFECTIVE DATE: | 05/20/2025 | APPROVAL: | 05/19/25 |
| APPROVED BY: | Patrice Y. Ohouo, PhD  Main Laboratory Director | **PROCEDURE NO.:** | IMM.01007 |

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| 1. *PURPOSE*    1. To provide instructions for use of the Alinity i Anti-HBc assay. The Alinity i Anti-HBc assay is used to detect the presence of IgG and IgM antibodies to hepatitis B core antigen (anti-HBc) in human serum and plasma on the Abbott Alinity i analyzer. 2. *SUMMARY AND EXPLANATION OF THE TEST*    1. The Alinity i Anti-HBc assay is a chemiluminescent microparticle immunoassay (CMIA) used for the qualitative detection of IgG and IgM antibodies to hepatitis B core antigen (anti-HBc) in human adult and pediatric serum and plasma (dipotassium EDTA, lithium heparin, sodium heparin) and neonatal serum on the Alinity i analyzer.    2. HBV is a major cause of liver disease and is endemic worldwide. The virus can be transmitted through direct contact with blood and body fluids, including sexual contact. The incubation period for HBV infection can range from 1 to 6 months, averaging around 6 to 8 weeks. Typical acute clinical symptoms of HBV hepatitis include malaise, jaundice, gastroenteritis, and fever. However, HBV infection can also result in subclinical anicteric hepatitis, fulminant hepatitis, or chronic or persistent hepatitis. Although most adult patients with HBV infection completely recover from acute illness and clear the virus, 5 to 10% of patients with HBV may become chronic carriers. It is estimated that over 300 million people worldwide are chronic carriers of the virus. Chronic HBV infection is associated with the development of hepatocellular carcinoma.    3. The Alinity i Anti-HBc assay utilizes microparticles coated with recombinant hepatitis B virus core antigen (rHBcAg) for the detection of anti-HBc antibodies. Anti-HBc antibody determinations can be used as an indicator of current or past HBV infection. Anti-HBc antibodies are found in serum shortly after the appearance of hepatitis B surface antigen (HBsAg) in acute HBV infections. They will persist after the disappearance of HBsAg and before the appearance of detectable antibodies to HBsAg (anti-HBs). In the absence of information about any other HBV markers, it must be considered that an individual with detectable levels of anti-HBc antibodies may be actively infected with HBV or that the infection may have resolved, leaving the person immune. Anti-HBc antibodies may be the only serological marker of HBV infection and potentially infectious blood.    4. The presence of anti-HBc antibodies does not differentiate between acute or chronic hepatitis B infection. 3. *BIOLOGICAL PRINCIPLES OF THE PROCEDURE*    1. This assay is a two-step immunoassay for the qualitative detection of anti-HBc antibodies in human serum and plasma using chemiluminescent microparticle immunoassay (CMIA) technology.    2. Sample, rHBcAg coated paramagnetic microparticles, specimen diluent, and assay diluent are combined and incubated. The anti-HBc antibodies present in the sample binds to the rHBcAg coated microparticles. The mixture is washed. Anti-human IgG and IgM 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 relative light units (RLUs). There is a relationship between presence of anti-HBc antibodies in the sample and the RLUs detected by the system optics.    4. The presence or absence of anti-HBc antibodies 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 Alinity i Anti-HBc assay is a chemiluminescent microparticle immunoassay (CMIA) used for the qualitative detection of IgG and IgM antibodies to hepatitis B core antigen (anti-HBc) in human adult and pediatric serum and plasma (dipotassium EDTA, lithium heparin, sodium heparin) and neonatal serum on the Alinity i analyzer.    3. The Alinity i Anti-HBc assay is to be used as an aid in the diagnosis of acute, chronic, or resolved hepatitis B virus (HBV) infection in conjunction with other laboratory results and clinical information.    4. 4.3 The Alinity i Anti-HBc assay is not intended 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. For accurate results, serum and plasma specimens should be free of fibrin, red blood cells, and other particulate matter.         6. Serum specimens from patients receiving anticoagulant or thrombolytic therapy may contain fibrin due to incomplete clot formation.         7. To prevent cross contamination, use of disposable pipettes or pipette tips is recommended.         8. Unlabeled specimens: there must be an ID link between the test order and the specimen container. Unlabeled specimens cannot be accepted.         9. All specimens are examined for correct identification when accessioned and processed and are rejected if it does not have two matching patient identifiers.         10. Leaking/improperly closed tubes cannot be accepted.         11. 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 centrifuge at > 10,000 RCF (Relative Centrifugal Force) for 10 minutes.        2. 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 Anti-HBc Reagent Kit 07P84. See table below for details. Volumes (mL) listed in the table below indicate the volume per cartridge.      * 1. Alinity i Anti-HBc Calibrator (Part# 07P8401)   2. Alinity i Anti-HBc Controls (Part# 07P8410)   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.  1. *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. 2. *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.  1. *CALIBRATIONS & CONTROL PROCEDURES*    1. Calibration       1. Cal 1 contains anti-HBc positive human plasma in recalcified anti-HBc negative human plasma. 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. Calibrator 1 is traceable to the Reference Standard of the Paul Ehrlich Institute, Langen, Germany.     6. The acceptability of the calibration is assessed against a parameter. If the calibration is acceptable, the cutoff RLU is calculated as follows:   Cutoff RLU = Calibrator 1 Mean RLU x 1.0   * + 1. The acceptable calibration is stored by the Alinity i analyzer for use with any reagent kit of that lot.     2. The calibration should be used in conjunction with control ranges to determine the validity of the calibration     3. 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.     4. Assay control must be tested to evaluate the assay calibration.     5. Once a calibration is accepted and stored, all subsequent samples may be tested.     6. For additional instructions on performing a calibration, refer to the Alinity ci-series Operations Manual, Section 5.     7. 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 Alinity i Anti-HBc Control (-) contains recalcified anti-HBc negative human.      2. The Alinity i Anti-HBc Control (+) anti-HBc positive human plasma in recalcified anti-HBc negative human plasma.      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. 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 Alinity i Anti-HBc calibrator package insert and/or Alinity i Anti-HBc control package insert 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 Anti-HBc 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 analyzer calculates results for the Alinity i Anti-HBc 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 1.0    3. The cutoff RLU is stored for each reagent lot calibration.    4. S/CO = Sample RLU/Cutoff RLU 4. *INTREPTATION OF RESULTS*    1. 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. For diagnostic purposes, results should be used in conjunction with patient history and other hepatitis markers for diagnosis of acute and chronic infection.    2. Current methods for the detection of anti-HBc antibodies may not detect all infected individuals. A nonreactive test result does not exclude the possibility of exposure to or infection with HBV.    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 Alinity i Anti-HBc that employ mouse monoclonal antibodies. Additional information may be required for diagnosis.    4. Heterophilic antibodies in human serum can react with reagent immunoglobulins, interfering within 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. 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 Anti- HBc Reagent Kit insert * Alinity i Anti- HBc calibrator insert * Alinity i Anti- HBc 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