| **Hepatitis C Antibody Screen (Anti-HCV)** | |
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| **Purpose** | This procedure provides instructions for performing Anti-HCV on the Abbott Alinity ci analyzer. The Alinity i Anti-HCV assay is a chemiluminescent microparticle immunoassay (CMIA) used for the qualitative detection of immunoglobulin G (IgG) and immunoglobulin M (IgM) antibodies to hepatitis C virus (anti-HCV) in human adult serum and plasma (potassium EDTA, lithium heparin, and sodium heparin) on the Alinity i analyzer. **This is a screening test.** Equivocal and positive results will automatically reflex to confirmation by Mayo Medical Laboratories. |
| **Policy Statements** | This procedure applies to all personnel responsible for performing testing on the Abbott Alinity ci analyzer in Children’s Minnesota (Minneapolis) Laboratory. |
| **Principle** | This assay is a two-step immunoassay for the qualitative detection of anti-HCV in human serum and plasma using chemiluminescent microparticle immunoassay (CMIA) technology. Sample, recombinant HCV antigen coated paramagnetic microparticles, and assay diluent are combined and incubated.  The anti-HCV present in the sample binds to the HCV coated microparticles. The mixture is washed. Anti-human IgG/IgM acridinium-labeled conjugate is added to create a reaction mixture  and incubated. Following a wash cycle, Pre-Trigger and Trigger Solutions are added. The resulting chemiluminescent reaction is measured as relative light units (RLUs). There is a relationship between the amount of anti-HCV in the sample and the RLUs detected by the system optics. The presence or absence of anti-HCV in the sample is determined by comparing the chemiluminescent RLU in the reaction to the cutoff RLU determined from an active calibration. If the chemiluminescent signal of the sample is greater than or equal to the cutoff signal, the sample is considered reactive for anti-HCV.  For additional information on system and assay technology, refer to the Alinity ci-series Operations Manual, Section 3. |
| **Clinical Significance** | The Alinity i Anti-HCV assay is for the detection of antibodies to the hepatitis C virus (HCV). Chemiluminescent immunoassays are a variation of the enzyme immunoassay (EIA) principle. Solid phase EIAs, first described in the early 1970s, use antigens and/or antibodies coated on a surface to bind complementary analytes. The bound analyte is detected by a series of antigen-antibody reactions. EIAs are available to identify antigens and antibodies related to viral hepatitis infection. In the Alinity i Anti-HCV final reaction, bound acridinylated conjugates are used to generate a chemiluminescent signal.  HCV is a bloodborne virus. Serological studies employing EIAs for detection of antibodies to recombinant antigens of HCV have established HCV as the cause of most bloodborne as well as  community-acquired non-A, non-B hepatitis. The presence of anti- HCV indicates that an individual may have been infected with HCV, may harbor infectious HCV, and/or may be capable of transmitting HCV infection.  Although the majority of infected individuals may be asymptomatic, HCV infection may develop into chronic hepatitis, cirrhosis, and/or increased risk of hepatocellular carcinoma. The implementation of blood donation screening for anti-HCV by EIAs has led to a marked decline in the risk of transfusion-transmitted hepatitis. Alinity i Anti-HCV has been designed to detect antibodies to putative structural and nonstructural proteins of the HCV genome. The relationship between the recombinant HCV proteins in Alinity i Anti- HCV and the putative structural and nonstructural proteins of the HCV genome is depicted below.  • HCr43: The HCr43 protein is expressed in Escherichia coli (E. coli) and is composed of two noncontiguous coding regions of the HCV genome sequence. The first region represents  amino acids 1192 to 1457 (33c) of the HCV sequence. The second of the two regions represents amino acids 1 to 150 (core) of the HCV sequence. Because of the similarity of the genomic organization of the flaviviruses, it is suggested that the first sequence is from the NS3 coding region and the second sequence is from the core coding region of HCV.  • c100-3: The c100-3 antigen is a recombinant HCV protein expressed in Saccharomyces cerevisiae (yeast). The genomic organization of flaviviruses suggests that the cloned sequence  is contained within the putative nonstructural (NS3 and NS4) regions of HCV. The c100-3 protein is a chimeric fusion protein with 154 amino acids of human superoxide dismutase (hSOD),  five linker amino acids, amino acids number 1569 to 1931 of the HCV polyprotein, and the additional five amino acid linker at the carboxyl terminus. |
| **Instrument** | **PRIMARY METHOD:** Abbott Alinity i (Sunquest code: MACI)  **Backup Method:** Hold samples until instrument is back in service. Refer samples to Mayo Medical Laboratories if urgent. |
| **Sunquest Test Code** | **AHCV** |
| **Specimen** | **Preferred Container:** SST (gold, marble) or Red no gel  **Preferred Draw Volume:** 4.5 mL blood  **Minimum Processed Volume:** 1.1 mL serum  • Maximum number of replicates sampled from the same sample cup: 10  **Priority Loaded**:  Sample volume for first test: 70 μL  Sample volume for each additional test from same sample cup: 20 μL  **Sample Loaded Routinely**:  Sample volume for first test: 150 μL  Sample volume for each additional test from same sample cup: 20 μL  Note: Any sample volume below the minimum volume does not permit confirmatory testing.  **Stability:** 3 days at Room Temperature**,** stored on or off the clot/cells/gel (not acceptable for confirmatory testing requirements. See Mayo Medical Laboratories Test Catalog for more information.)  7 days refrigerated at 2-8°C  2 years frozen at -20°C. Avoid more than 3 freeze/thaw cycles to maintain viability  **Transport:** Ship refrigerated to Minneapolis laboratory.  **Rejection:**Specimens not removed from red cells within two hours of collection, mislabeled or unlabeled specimens**,** or grossly hemolyzed specimens.  **Preparation:**   1. Whole blood specimens should be centrifuged following complete clot formation, according to Specimen Processing procedures, prior to analysis. 2. Serum or plasma should be physically separated from cells as soon as possible with a maximum limit of two hours from the time of collection. 3. Specimens should be free of particulate matter. 4. Transfer serum or plasma directly to a properly labeled pilot tube. 5. Architect and Alinity systems utilize a specimen level detect mechanism, so special racks specific to tube-type are not required. 6. Minimum labeling includes sample accession ID, and/ or patient name, medical record number, collection date and time. |
| **Reagents** | |  |  |  |  | | --- | --- | --- | --- | | ***Product Description*** | ***Product Code*** | ***Stability*** | | | Abbott Alinity i Anti-HCV reagent kit | 08P0521 | **Store at:** 2 – 8 °C  **Unopened:** Store in upright position. If cartridge does not remain upright, gently invert the cartridge 10 times and place in an upright position for 1 hour before use.  Prior to loading on the analyzer for the first time, gently invert cartridges 30 times. May be used immediately after removal from 2-8°C storage.  **Opened:** UntilManufacturer expiration date. Store in upright position. If cartridge does not remain upright during storage, discard the cartridge. Do not reuse original reagent caps or replacement caps due to the risk of contamination and the potential to compromise reagent performance. May be used immediately after removal from 2-8°C storage.  **On-board:** 30 Days | | Abbott Alinity i Anti-HCV Calibrator, 1 x 3.0 mL  (Green color) | 08P0501 | **Store at:**  2-8°C  **Unopened**: Manufacturer expiration date.  **Opened**: Store tightly capped with new replacement cap.  Return to refrigerated storage 2 - 8°C after use.  The analyzer will track In-use Stability, which is the time the calibrator is outside of refrigerated storage while on the analyzer.  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. |   **Reagent Handling:**  • 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.  –– **Place a check in the square on the reagent kit to indicate to others that the inversions have been completed.**  After mixing, place reagent cartridges in an upright position for 1 hour before use to allow bubbles that may have formed to dissipate.  • 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.  **• Prior to loading on the analyzer for the first time, gently invert cartridges 30 times.**  **• Reagent cartridges cannot be inverted after the septum has been pierced by the analyzer.**  • 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. |
| **Risk and Safety:** | Contains sodium azide at a concentration that does not require special disposal.  Avoid contact with skin and eye. Causes serious eye irritation. Wear gloves. Contact with acids liberates very toxic gas.  Safety data sheets (MSDS/SDS) available on [Children’s Intranet](https://starnet.childrenshc.org/emergency-and-safety/) |
| Calibration/ Verification/AMR | |  |  | | --- | --- | | Analytical Measuring Range: | 0-999999.0 S/CO | | Reference Material: | Abbott Alinity i Anti-HCV Calibrator | | Suggested Calibration Levels | See Calibrator Package insert | | Verification Scheme: | n=1 | | Verification Frequency: | * For each new lot of reagent * After major maintenance or service, if indicated by quality control results, technical support, or a field service engineer * As indicated in laboratory quality control procedures | | AMR: | Verification of AMR is not required for qualitative tests. | |  | | | |
| **Quality Control** | **Bio-Rad Viroclear and Bio-Rad Virotrol 1 Controls**  **Frequency:** Run both controls each day of use.  **Stability:** 60 Days at 2-8°C.  **Acceptable ranges:**   * Refer to the Westgard Rules in Chemistry procedure for current Westgard rules in place for each analyte. * **Acceptable ranges are current in Unity Real Time only.** Quality Control results must be rejected in Sunquest when the results cross the interface. * In the event of a QC failure, refer to the [Unity Real Time QC Review, General User](https://starnet.childrenshc.org/References/labsop/chem/quality/ch-2.17-unity-real-time-qc-review-general-user.pdf) and navigate to the QC Troubleshooting section. * Do not load or release patients until QC is acceptable in Unity Real Time. * Because Anti-HCV is a qualitative test, when the results of Viroclear (negative) and Virotrol 1 (positive) recover appropriate positive and negative values, new lots of controls are considered acceptable for use. |
| **Reference Range** | 0.00-0.79 S/CO Nonreactive (No retest required)  0.80-0.99 S/CO Equivocal (Retest in duplicate)  >1.00 S/CO Reactive (No retest required) |
| **Critical Values** | None specified |
| **Limitations** | * If antibodies to HCV are not detected this does not exclude the possibility of exposure to HCV. * A reactive Anti-HCV does not exclude co-infection by another hepatitis virus. * Antibody levels below the detection of this assay or antibodies not reacting to the recombinant antigens used in this assay will yield nonreactive results. * Immunocompromised patients who have HCV may produce levels of antibodies below the sensitivity of this assay and may not be detected as reactive. * No common interferents were recognized in testing by Abbott Laboratories. * The affinity or avidity differences of anti-human IgG/IgM for anti-HCV have not been determined with this assay. Therefore, there may not be a demonstration of a significant increase in antibody level between acute and convalescent specimens for a patient in the late acute stage of infection when IgM antibodies are decreasing. * Results obtained with the Alinity i Anti-HCV assay may not be used interchangeably with values obtained with different manufacturers’ assay methods. * Assay performance characteristics have not been established for newborns, infants, children, or populations of immunocompromised or immunosuppressed patients; therefore, every equivocal or positive test will be reflexed to Mayo Medical Laboratories for confirmation. **This is a screening test**. For diagnostic purposes, results should be used in conjunction with patient history and other hepatitis markers for diagnosis of acute or chronic infection. * 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. * The magnitude of an Alinity i Anti-HCV assay result cannot be correlated to an end point titer. |
| **Dilutions** | Do not dilute |
| **Result Reporting**  **Result Reporting (cont.)** | The result that will cross over in OEM will be Reactive, Nonreactive or Equivocal. Each result will have the comment “Check procedure for repeat and interpretation protocol.” Use the interpretation chart on page 7 of this procedure to determine if a retest is required. If a repeat test is not needed (specimen is nonreactive), manually accept the Sunquest result.  **If retesting is required (sample is in the Equivocal/Grayzone range of 0.80 to 0.99)**:   1. Reject the cup in OEM. 2. Take specimen off the analyzer and check for clots, red cells, or other particulate matter. **Recentrifuge if any are seen or suspected.** 3. Manually order the specimen **in duplicate** (two replicates) with an ‘R’ in front of the accession number to signify the accession number that crosses OEM is a retest. For example, accession number “H111” would be manually ordered in the Architect as “RH111” with 2 replicates. 4. When testing is complete, both results will cross into Sunquest in two different cups. 5. To accept results in Sunquest, you will have to manually retype the correct accession number without the (R) for the result you wish to report in OEM. Sunquest will ask CHANGE EXISTING ACCESSION NUMBER (Y/<N>). Type Y then press ENTER to enter the accession number for the cup you wish to report. 6. **\*Go to the analyzer and check results**\* 7. If both repeat tests are <0.8 (non reactive), then manually accept the Sunquest result of nonreactive. 8. If both of the repeat tests are ≥0.8 to <0.99 (equivocal), then manually accept the Sunquest result of equivocal. 9. If one of the repeat tests are <0.8 **or** >1 and the other test is ≥0.8 or < 1 equivocal, then you must (M) modify the result in Sunquest. When modifying results, you must enter the equivocal **numeric result** from the analyzer. Sunquest will append an equivocal comment. **Do not free text the result in Sunquest because the correct interpretation will not append**. 10. If both repeat tests are >1 (reactive), accept one of the numeric reactive results. Mayo Medical Laboratories test HCVQN will automatically reflex for confirmation and a label will print. Place label on sample and place in the Send outs freezer for transport to MML.      |  |  |  |  |  | | --- | --- | --- | --- | --- | | **Initial Result** | **Retest Result** | **Result** | **Interpretation** | **What to enter into Sunquest** | | <0.79 S/CO | No Retest Required | Nonreactive | Antibodies to HCV are not detected: does not exclude the possibility of exposure to HCV | Accept Result | | ≥0.8 S/CO to < 1 S/CO | Both of the duplicate retests are <0.8 S/CO | Nonreactive | Antibodies to HCV are not detected: does not exclude the possibility of exposure to HCV | Accept Result of Nonreactive | | One or both of the duplicate retest results are ≥0.8 S/CO to <1 S/CO | Equivocal | “Antibodies to HCV may or may not be present: recommend repeat testing with another specimen.” | Modify the Result and **enter the Numerical Value** from the Architect **(result should be ≥0.80 and <1)** | | Both of the duplicate retests are >1.0 S/CO | Reactive | “Presumptive evidence of antibodies to HCV. Confirmatory testing reflexed.” Send to MML (HCVQN)  **Freeze at least 0.8mL serum immediately for reflex to MML** | Accept Result of Reactive. | | ≥1.0 S/CO | No Retest Required | Reactive | “Presumptive evidence of antibodies to HCV. Confirmatory testing reflexed.” Send to MML (HCVQN)  **Freeze at least 0.8mL serum immediately for reflex to MML** | Accept Result of Reactive. | |
| **Specimen Storage** | Promptly stopper tested specimen and store upright in specimen rack. Every 8 hours remove specimens to refrigerator/freezer storage. Samples are retained 14 days in specimen storage freezer. |
| **References** | 1. Abbott Alinity i Anti-HCV Package insert, Abbott Diagnostics, Abbott Park, IL 60064. Revised July 2019 2. Abbott Alinity i Anti-HCV Calibrator Insert, Abbott Diagnostics, Abbott Park, IL, 60064. Revised July 2019. 3. Bio-Rad Viroclear Control Product Insert, Bio-Rad Laboratories, Irvine, CA 92618 July 2017. 4. Bio-Rad Virotrol II Control Product Insert, Bio-Rad Laboratories, Irvine, CA 92618 October 2017. |
| **Historical Record** | |  |  |  |  | | --- | --- | --- | --- | | **Version** | **Written/Revised By** | **Effective Date** | **Summary of Revisions** | | 1 | Stephen Gripentrog/Erin Bartos | May 15, 2018 | New Procedure | |  | Erin Bartos | May 1, 2019 | Updated Positive QC to Virotrol I | | 2 | Erin Bartos | October 28, 2020 | Changed analyzer from Architect to Alinity, updated some verbiage. Limitations noted and added to Lab Directory page. Added “this is a screening test.” | |