| **HIV Ag/Ab Screen with Reflex**  |
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| **Purpose** | This procedure provides instructions for performing ARCHITECT HIV ANTIGEN/ANTIBODY SCREEN WITH REFLEX on the Abbott Architect i1000SR |
| **Policy Statements** | This procedure applies to all personnel responsible for performing testing on the Abbott Architect i1000SR |
| **Principle** | The ARCHITECT HIV Ag/Ab Combo assay for use on the ARCHITECT i System is a two-step immunoassay to determine the presence of human immunodeficiency (HIV)-1 p24 antigen, antibodies to HIV-1 (group M and group O), and antibodies to HIV-2 in human serum or plasma using Chemiluminescent Microparticle Immunoassay (CMIA) technology with flexible assay protocols, referred to as Chemiflex.In the first step, sample, ARCHITECT i Wash Buffer, assay diluent, and paramagnetic microparticles are combined. HIV-1 p24 antigen and HIV-1/HIV‑2 antibodies present in the sample bind to the HIV‑1/HIV-2 antigen and HIV-1 p24 monoclonal (mouse) antibody coated microparticles. After washing, the bound HIV-1 p24 antigen and HIV-1/HIV‑2 antibodies bind to the acridinium-labeled conjugates. 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 (RLU). A relationship exists between the amount of HIV antigen and antibodies in the sample and the RLU detected by the ARCHITECT i System optics. The presence or absence of HIV-1 p24 antigen or HIV-1/HIV‑2 antibodies in the specimen is determined by comparing the chemiluminescent signal in the reaction to the cutoff signal determined from an ARCHITECT HIV Ag/Ab Combo calibration. Specimens with signal to cutoff (S/CO) values greater than or equal to 1.00 are considered reactive for HIV-1 p24 antigen or HIV-1/HIV-2 antibodies. Specimens with S/CO values less than 1.00 are considered nonreactive for HIV-1 p24 antigen and HIV-1/ HIV‑2 antibodies.Specimens that are initially reactive in the ARCHITECT HIV Ag/Ab Combo assay should be retested in duplicate. Repeat reactivity is highly predictive of the presence of HIV-1 p24 antigen and/or HIV-1/HIV-2 antibodies. However, as with all immunoassays, the ARCHITECT HIV Ag/Ab Combo assay may yield nonspecific reactions due to other causes, particularly when testing in low prevalence populations. A repeatedly reactive specimen should be investigated further with supplemental confirmatory HIV-specific tests, such as immunoblots, antigen tests, and HIV nucleic acid tests. Supplemental testing of repeatedly reactive specimens obtained from individuals with HIV infection usually confirms the presence of HIV antibodies, HIV antigen, or HIV Nucleic acid. A full differential diagnostic work-up for the diagnosis of AIDS and AIDS-related conditions includes an examination of the patient’s immune status and a clinical history.For additional information on system and assay technology, refer to the ARCHITECT System Operations Manual, Section 3. |
| **Clinical Significance** | Acquired immunodeficiency syndrome (AIDS) is caused by two types of human immunodeficiency viruses, collectively designated HIV. HIV is transmitted by sexual contact, exposure to blood or blood products, and prenatal or perinatal infection of a fetus or newborn. Antibodies against HIV are nearly always detected in AIDS patients and HIV‑infected asymptomatic individuals.Phylogenetic analysis classifies HIV type 1 (HIV-1) into groups M (major), N (non-M, non-O), O (outlier), and P. HIV‑1 group M is composed of genetic subtypes (A-D, F-H, J, and K) and circulating recombinant forms (CRFs). Group M viruses have spread throughout the world to cause the global AIDS pandemic. However, the geographic distribution and regional predominance of HIV‑1 subtypes and CRFs vary. HIV‑1 subtype B is the predominant subtype in North America, South America, Europe, Japan, and Australia, although other subtypes and CRFs are present in these regions as well. A significant percentage of new HIV-1 infections in Europe are caused by non-B subtype strains. All subtypes and many recombinant strains exist in Africa. In Asia, subtypes B and C, and CRF01\_AE (formerly called subtype E) are found. HIV-1 groups N, O, and P are endemic to west central Africa and are relatively rare. However, group O infections have been identified in Europe and the USA.HIV type 2 (HIV-2) is similar to HIV-1 in its structural morphology, genomic organization, cell tropism, in vitro cytopathogenicity, transmission routes, and ability to cause AIDS. HIV-2 is endemic to West Africa, but HIV-2 infections have been identified in North America and Europe at a low frequency compared to HIV-1. Early after infection with HIV-1, but prior to seroconversion, HIV-1 core protein, p24 antigen, may be detected in HIV-1‑infected individuals. ARCHITECT HIV Ag/Ab Combo uses anti-HIV-1 p24 antibodies as reagents to detect HIV-1 p24 antigen, thereby decreasing the window period and improving early detection of HIV infection.The key immunogenic protein for serodetection of HIV infection is the viral transmembrane protein (TMP). Antibodies against the TMP are consistently among the first to appear during seroconversion of HIV-infected individuals and remain relatively strong throughout the asymptomatic and symptomatic stages of HIV infection. ARCHITECT HIV Ag/Ab Combo detects antibodies to HIV-1 groups M and O, and HIV-2 through the use of five recombinant proteins and two synthetic peptides derived from native TMP sequences of HIV-1 groups M and O, and HIV-2. |
| **Instrument** | **PRIMARY METHOD: Abbott Alinity ci (Sunquest method code MACI)****PRIMARY METHOD:** Abbott Architect i1000SR (Sunquest method code: AI1)Confirmatory: Mayo Medical Laboratories  |
| **Sunquest Test Code** | **PRIMARY METHOD: HIVI**Confirmatory**: HIVDI** |
| **Specimen** | **Sample type:** EDTA plasma **Alternative tubes:** Lithium heparin (gel or no gel), Sodium heparin, SST. **Please note, using an alternative tube type does not allow for confirmatory testing. Patient will need to be re-drawn to perform confirmatory testing.****Preferred Sample Draw Volume**: 3.0 mL  **Minimum Processed Volume:** 0.5 mL(does not permit confirmation testing and is not recommended)Maximum number of replicates sampled from the same sample cup: 10Priority Loaded:◦Sample volume for first test: 150 μL◦Sample volume for each additional test from same sample cup: 100 μLRoutinely Loaded:◦Sample volume for first test: 150 μL◦Sample volume for each additional test from same sample cup: 100 μL **Sample Stability**:  Up to 3 days (separated) @ room temperature  Up to 7 days refrigerated @ 2-8°C Up to 2 years frozen @ -20°C **Processing:** Centrifuge specimen within one hour of collection. Remove EDTA plasma into a pilot tube.**Transport**: Ship refrigerated @ 2-8°C to Minneapolis lab. **Confirmation testing to Mayo must be EDTA plasma and sent frozen.****Rejection Criteria:** * Specimens not separated within one hour of collection.
* Mislabeled or unlabeled specimen
* grossly hemolyzed
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| **Reagents** |

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| ***Product Description*** | ***Product Code*** | ***Stability*** |
| Architect HIV Combo Reagent | 02P3625 | **Store at:** 2 – 8 °C**Unopened/Opened:** Manufacturer expiration date.**On-board:** 30 Days |
| Architect HIV Combo Calibrator | 02P3601 | **Store at:**  2-8°C**Unopened**: Manufacturer expiration date.**Opened**: Store at 2 – 8 °C, stable until expiration date when stored and handled as directed.  |
| Architect Abbott Architect HIV Combo Controls, 5 levels | 02P3610 | **Store at:** 2-8°C**Unopened**: Manufacturer expiration date.**Opened**: Store at 2 – 8 °C, stable until expiration date when stored and handled as directed. Must be stored upright. May be used directly from the refrigerator. |
| Abbott Alinity i HIV Ag/Ab Combo Reagent Kit | 08P0721 | **Store at:** 2 – 8 °C**Unopened/Opened:** Manufacturer expiration date.**On-board:** 30 Days**Opened**: 2 to 8°C Until expiration dateStore 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 potential to compromise reagent performance.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. |
| Abbott Alinity i HIV Ag/Ab Combo Calibrators | 08P0702 | **Store at:**  2-8°C**Unopened**: Manufacturer expiration date.**Opened**: Store at 2 – 8 °C, stable until expiration date when stored and handled as directed. |
| Abbott Alinity i HIV Ag/Ab Combo Controls | 08P0712 | **Store at:** 2-8°C**Unopened**: Manufacturer expiration date.**Opened**: Store at 2 – 8 °C, stable until expiration date when stored and handled as directed. Must be stored upright. May be used directly from the refrigerator. |

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| **Risk and Safety:** | Contains sodium azide. Avoid contact with skin and eyes. Causes serious eye irritation. Wear gloves. Contact with acids liberates very toxic gas. Recap and dispose of in appropriate Hazardous Waste Container. Dispose of empty HIV reagent bottles in the red biohazard waste.Safety data sheets (MSDS/SDS) available on [Children’s Intranet](https://starnet.childrenshc.org/emergency-and-safety/) |
| Calibration/ Verification/AMR |

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| Analytical Measuring Range: | 0.0 – 99999.9 (Qualitative 1 Pt Cal) |
| Reference Material: | Abbott Architect HIV Ag/Ab Combo CalibratorAbbott Alinity i HIV Ag/Ab Combo Calibrator |
| Suggested Calibration Levels | 1. 4000 RLU
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| Calibration Frequency: | * For each new lot of reagent
* After major maintenance or service, if indicated by quality control results
* As indicated in laboratory quality control procedures
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| Quality Control | **Abbott Architect HIV Controls or Abbott Alinity i HIV Controls****Frequency:** Five levels each day of use.**Stability:** Until manufacturer expiration date when stored upright at 2-8°C**Preparation**: None, ready to use. Invert several times prior to aliquoting,and keep bottles tightly sealed.**Acceptable ranges:** * Non-Bio-Rad controls will utilize manufacturer ranges and 2 SD Westgard rules for quantitativetests. This assay is a qualitative assay. The negative control should be non-reactive, and the 4 other levels should be reactive.
* New lots of Bio-Rad controls should be run for 20 days in parallel with the current lot whenever possible prior to switching to the new lot.
* 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 and navigate to the QC Troubleshooting section.
* Do not load or release patients until QC is acceptable in Unity Real Time.
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| Interpretation and Result Reporting |  Results that cross the Sunquest interface in OEM will be Reactive (S/CO greater than or equal to 1) or Nonreactive (S/CO <1.0). Each result will have the comment “Check procedure for repeat and interpretation protocol.” If the result is reactive, follow the detailed instructions below. If the result is nonreactive, accept the Nonreactive Sunquest result in OEM If retesting is required: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.
3. Manually order the specimen **in duplicate** (two replicates from the same sample) with an “R” (for “repeat”) in front of the accession number. For example, H111 would be manually ordered on the Architect as “RH111”.
4. When testing is complete, both results will cross into Sunquest in two different cups.
* 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.

**Go to the analyzer and check results** to properly determine the interpretation:* If both repeat tests are <1 non reactive, then manually accept the Sunquest result of nonreactive. **Do not free-text “non-reactive.”** If you wish to manually enter your result in MEM, you must enter the numeric value of one of the non-reactive results so that the appropriate comment will append.
* If one of the repeat tests is <1 and the other test is-≥1reactive, then (M) modify the result in Sunquest. When modifying results you must enter the reactive **numeric** result from the analyzer and Sunquest will change the numerical result to the correct interpretation and append the correct comment.
* If both repeat tests are ≥1 reactive, accept one of the Sunquest results of reactive. Mayo Medical Laboratories test HIVDI 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.

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| **Initial Result** | **Retest Result** | **Result** | **Interpretation** | **What to enter into Sunquest** |
| <1 S/CO: | No Retest Required | Nonreactive | HIV Ag/Ab not detected | Accept Result |
| ≥1 S/CO:CHECK SAMPLE and Retest in duplicate | Both of the duplicate retests are <1 S/CO | Nonreactive | HIV Ag/Ab not detected | Accept Result of Nonreactive |
| One result <1 S/CO and the other result is ≥1.0 S/CO | Reactive | “Presumptive evidence of HIV-1 p24 Ag and Or HIV-1/HIV-2 AB. Confirmatory testing reflexed.” Send to MML (HIVDI)**Freeze Minimum 0.8 mL EDTA plasma** | Accept Result of Reactive or manually enter the NUMERICAL result value. |
| Both of the duplicate retests are ≥1.0 S/CO | Reactive | “Presumptive evidence of HIV-1 p24 Ag and Or HIV-1/HIV-2 AB. Confirmatory testing reflexed.” Send to MML (HIVDI)**Freeze Minimum 0.8 mL EDTA plasma**  | Accept Result of Reactive. |

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| **Interferences** | **For accurate results, serum and plasma specimens should be free of fibrin, red blood cells, and other particulate matter.** Serum specimensfrom patients receiving anticoagulant or thrombolytic therapy may contain fibrin due to incomplete clot formation. Reactive samples must be checked prior to retesting and recentrifuged if red cells or fibrin are visible or suspected.No other known interfering substances detected in testing done by Abbott Laboratories.  |
| **Reference Range** | <1 S/CO = Nonreactive |
| **Critical Values** | None specified |
| **Limitations** | Technical range 0-99,999,999 S/COThe instrument reporting system contains error messages to warn the operator of specific malfunctions. Refer to Operator’s Manual for troubleshooting specific error messages.An individual who has antibodies to HIV is presumed to be infected with the virus; however, an individual who has participated in an HIV vaccine study may develop antibodies to the vaccine and may or may not be infected with HIV. Clinical correlation is indicated with appropriate counseling, medical evaluation, and possibly additional testing to determine whether a diagnosis of HIV infection is accurateThe interpretation of specimens with a final result of reactive by the Alinity i HIV Ag/Ab Combo assay and indeterminate by supplemental testing is not definitive; further clarification may be obtained by testing another specimen taken at least 1 month later.The performance of these assays have not been established for individuals younger than 2 years of age and is not recommended. Nearly all infants born to HIV‑infected mothers passively acquire maternal antibody and, in some cases, will test antibody positive until age 18 months regardless of whether they are infected. Definitive diagnosis of HIV infection in early infancy requires other assays, including HIV nucleic acid tests or viral culture. |
| **Dilutions** | None specified |
| **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. Reactive samples will reflex and should be sent frozen immediately to Mayo Medical Laboratories for confirmatory testing. |
| **References** | 1. Abbott Architect HIV Ag/Ab Combo Calibrator Package Insert, Abbott Laboratories, Abbott Park, IL 60064 USA. Revised April 2012.
2. Abbott Architect HIV Ag/Ab Combo Controls Package Insert, Abbott Laboratories, Abbott Park, IL 60064 USA. Revised January 2014.
3. Abbott Architect HIV Ag/Ab Combo Reagent Package Insert, Abbott Laboratories, Abbott Park, IL 60064 USA. Revised October 2017.
4. Abbott Alinity HIV Ag/Ab Combo Calibrator Package Insert, Abbott Laboratories, Abbott Park, IL 60064 USA. Revised September 2019.
5. Abbott Alinity HIV Ag/Ab Combo Controls Package Insert, Abbott Laboratories, Abbott Park, IL 60064 USA. Revised September 2019.
6. Abbott Alinity HIV Ag/Ab Combo Reagent Package Insert, Abbott Laboratories, Abbott Park, IL 60064 USA. Revised September 2019.
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| **Historical Record** |

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| **Version** | **Written/Revised By** | **Effective Date** | **Summary of Revisions** |
| 1 | Stephen Gripentrog/ Erin Bartos | May 15, 2018 | New Procedure |
| 2 | Kelsi Brown | June 5, 2018 | Added that plasma samples are acceptable for the screen, but will need serum for confirmatiory test.  |
| 3 | Erin Bartos | August 14, 2018 | Changed sample type due to reference lab change in confirmatory testing. EDTA plasma required. |
| 4 | Elauteria Earnhardt | April 13, 2020 | Reviewed for new Medical Director  |
|  | Elauteria Earnhardt | May 6, 2020 | Analyzer site moved to St. Paul |
| 5 | Erin Bartos | October 28, 2020 | Added Alinity i analyzer and updated all sections requiring changes. |

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