| **Immunoglobulin A (IgA), Immunoglobulin G (IgG), Immunoglobulin M (IgM)** |
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| **Purpose** | This procedure provides instructions for performing IMMUNOGLOBULIN A (IgA), IMMUNOGLOBULIN G (IgG), and IMMUNOGLOBULIN M (IgM) ON ABBOTT INSTRUMENTATION. The Alinity c Immunoglobulin A, G and M assays are used for the quantitation of IgA, IgG, and IgM in human serum or plasma on the Alinity c analyzer.  |
| **Policy Statements** | This procedure applies to all personnel responsible for operating the Abbott Alinity c at Children’s Minnesota Laboratory. |
| **Principle** | **Methodology, IgA, IgG, IgM**: ImmunoturbidimetricThe IgA assay is an immunoturbidimetric procedure that measures increasing sample turbidity caused by the formation of insoluble immune complexes when antibody to IgA is added to the sample. Sample containing IgA is incubated with a buffer (R1) and a sample blank determination is performed prior to the addition of IgA antibody (R2). In the presence of an appropriate antibody in excess, the IgA concentration is measured as a function of turbidity.The IgG assay is an immunoturbidimetric procedure that measures increasing sample turbidity caused by the formation of insoluble immune complexes when antibody to IgG is added to the sample. Sample containing IgG is incubated with a buffer (R1) and a sample blank determination is performed prior to the addition of IgG antibody (R2). In the presence of an appropriate antibody in excess, the IgG concentration is measured as a function of turbidity.The IgM assay is an immunoturbidimetric procedure that measures increasing sample turbidity caused by the formation of insoluble immune complexes when antibody to IgM is added to the sample. Sample containing IgM is incubated with a buffer (R1) and a sample blank determination is performed prior to the addition of IgM antibody (R2). In the presence of an appropriate antibody in excess, the IgM concentration is measured as a function of turbidity. |
| **Clinical Significance** | **IgA**Approximately 10% to 15% of serum immunoglobulin is IgA. Serum IgA is predominantly in a monomeric form with 10% to 15% as a dimer. Secretory IgA, found in tears, sweat, saliva, milk, colostrum, and gastrointestinal and bronchial secretions, is synthesized mainly by plasma cells in gastrointestinal and bronchial mucous membranes and lactating breast ductules. Secretory IgA is composed of two monomers linked by a secretory molecule. This secretory component protects the IgA polymer from proteolytic enzymatic degradation. IgA can initiate complement activation by the alternative pathway. Secretory IgA plays a major role in the protection of the respiratory, genitourinary, and gastrointestinal tracts against infection. The specific role of serum IgA is still unclear; it may be important in virus neutralization.Indications for serum IgA quantitation include recurrent infections, especially of the lower respiratory or gastrointestinal tract; anaphylactic transfusion reaction; diagnosis of ataxia telangiectasia; differentiation of M-components in myeloma; and evaluation of progression of IgA myeloma.IgA does not cross the placenta and, as a result, IgA levels in infants’ sera are very low.2 Serum IgA levels do not reach adult concentrations until 12 years of age.3 Approximately one out of every 700 Caucasians is genetically IgA deficient. Of these individuals, about one-fourth develop anti-IgA antibodies and are at risk of undergoing severe anaphylactic reactions to plasma or other blood product transfusions. Inherited IgA deficiency is also seen in ataxia telangiectasia and in combined immunodeficiency disorders. Individuals with absent IgA have a higher than expected incidence of rheumatic disorders and lymphoma. Secondary IgA deficiency is seen with non-IgA multiple myeloma or macroglobulinemia, and with nephrotic syndrome.Elevated IgA levels are associated with both polyclonal (more than IgA affected) as well as monoclonal increases. Polyclonal increases include: chronic liver disease, chronic infections (especially of GI and respiratory tracts), neoplasia of lower GI tract, inflammatory bowel disease, and autoimmune diseases such as rheumatoid arthritis. Monoclonal increases include: IgA multiple myeloma and, occasionally, other lymphomas.**IgG**IgG is the major immunoglobulin in the blood and is produced in copious amounts during secondary immune responses. IgG molecules bind to specific receptors on phagocytic cells, such as macrophages and polymorphonuclear leukocytes, thereby increasing the efficiency with which the phagocytic cells can ingest and destroy infecting microorganisms that have become coated with IgG antibodies in response to the infection. Additionally, IgG molecules can bind to and thereby activate the first component of the complement system, which under these circumstances unleashes a biochemical attack that kills the microorganisms. IgG molecules are the only antibodies that can pass from mother to fetus. The ability of IgG to cross the placenta provides a major line of defense against infection for the first weeks of an infant’s life. IgG is the predominant extravascular immunoglobulin and functions to neutralize bacterial toxins and bind most types of microorganisms to facilitate phagocytosis. Additionally, IgG antibodies can bind to target cells such as tumor cells to sensitize them for destruction by killer (K) cells that have IgG-specific receptor sites.Quantitation of IgG can be used to evaluate humoral immunity; establish diagnosis and monitor therapy in IgG myeloma; and evaluate patients, especially children and those with lymphoma, with propensity to infections. Reduction of IgG, usually less than 300 mg/dL (3.0 g/L), leads to susceptibility to infection due to encapsulated bacteria.IgG deficiencies may be genetic or acquired. Conditions associated with acquired IgG deficiency include thermal burns, pemphigus, nephrotic syndrome, protein-losing enteropathies, non-IgG myelomas or macroglobulinemia, pregnancy, Wiskott-Aldrich syndrome, myotonic dystrophy, anti-immunoglobulin antibodies, immunosuppressive therapy, and monoclonal gammopathies involving non-IgG immunoglobulins. IgG values in AIDS and AIDS-related complex can span the range from severe immunodeficiency to hyperimmunoglobulinemia, depending on clinical state and disease stage.Elevated IgG levels can be polyclonal, oligoclonal, or monoclonal. Elevated polyclonal IgG levels are associated with autoimmune diseases (systemic lupus erythematosus, rheumatoid arthritis,Sjogren’s syndrome), sarcoidosis, chronic liver disease, some parasitic diseases, chronic or recurrent infections, and intrauterine contraceptive devices. Increased oligoclonal IgG levels are associated with malignancies, infections (especially in the elderly), some dysgammaglobulinemias, and autoimmune disorders. Increased monoclonal IgG levels are associated with multiple myeloma (IgG type), lymphomas, and leukemia.**IgM**IgM, primarily present as a pentamer, is the first immunoglobulin class produced during an initial immune response and antigen- IgM complexes actively fix complement. The large molecular size of the pentamer enables direct cross-linking and agglutination of particulate and cellular antigens. Because IgM is involved in primary immune response, presence of IgM is useful in assessing whether a particular infection is acute (IgM present) or chronic (IgG predominate class present). Additionally, IgM is the first immunoglobulin class to be synthesized by a fetus or newborn and IgM antibodies do not cross the placenta.Polyclonal IgM increases may indicate a viral infection, such as viral hepatitis or infectious mononucleosis, or the early response to bacterial or parasitic infection. Levels are often increased in rheumatoid arthritis, chronic hepatocellular disease, and other chronic disorders. Elevated levels are also seen with hyper-IgM dysgammaglobulinemia, active sarcoidosis, collagen vascular disease, and nephrotic syndrome. Monoclonal IgM increases are seen in Waldenstrom’s macroglobulinemia, malignant lymphoma, reticulosis, and cold agglutinin hemolysis disease. Small IgM monoclonal bands can accompany a variety of neoplasms, particularly of the GI tract.Decreased IgM levels are usually not due to primary IgM deficiency. Secondary IgM deficiency may be associated with IgA or IgG type multiple myeloma, protein-losing enteropathies, burns, or immunosuppressive therapy. IgM deficiency is associated with increased, recurrent infections. |
| **Analyzer** | **Minneapolis: Abbott Alinity ci (Sunquest method code: MACC)****St. Paul: Abbott Alinity c (Sunquest method code: SALIC)**Backup: Alinity c on the opposite campus. |
| **Sunquest Test Codes** | **IGA**: Immunoglobulin A**IGG**: Immunoglobulin G**IGM**: Immunoglobulin M |
| **Specimen** | Sample: **Preferred:** Lithium Heparin, with or without gel**Alternative:** Serum with or without gel, Sodium Heparin**Minimum sample volume:** 0.6 mL blood, 0.2 mL serum/plasma**Stability when separated from cells/gel:** **20 to 25°C** 7 Days**2 to 8°C** 7 Days**-20°C** 6 Month**Rejection criteria:** Unlabeled tube, sample type other than serum or acceptable plasma**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 to a properly labeled sendout tube. Short samples should be pipetted into an Abbott sample cup and nested on a sendout tube; any amount remaining after sampling should be pipetted into the sendout tube and tightly capped.
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.
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| **Reagents** | **Reagent Handling** **IgA**: Upon receipt, place reagent cartridges in an upright position for 8 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. **IgG:** Upon receipt, 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 8 hours before use to allow bubbles that may have formed to dissipate.**IgM:** Upon receipt, 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.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. Use a pipette to remove all bubbles prior to loading on the Alinity or Architect system.* Do not use reagents beyond the expiration date.
* Do not pool reagents within a kit or between kits.
* Do not use components from one lot with components from another lot.
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|  | **Alinity c:**

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| ***Product Description*** | ***Product Code*** | ***Stability*** |
| Abbott Alinity c Immunoglobulin A Reagent Kit | 09P6120 | Store at: 2 to 8°CUnopened: Until manufacturer’s printed expiration dateOn-board: 28 days |
| Abbott Alinity c Immunoglobulin G Reagent Kit | 09P6220 | Store at: 2 to 8°CUnopened: Until manufacturer’s printed expiration dateOn-board: 23 days |
| Abbott Alinity c Immunoglobulin M Reagent Kit | 09P6320 | Store at: 2 to 8°CUnopened: Until manufacturer’s printed expiration dateOn-board: 57 days |
| Special Proteins Multi Cal | 08P6201 | Store at: 2 to 8°CUnopened: Until manufacturer’s printed expiration dateOpen Stability: 30 days |

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| **Risk and Safety** | This product requires the handling of human specimens. It is recommended that all human-sourced materials be considered potentially infectious and handled in accordance with the OSHA Standard on Bloodborne PathogensSafety data sheets (MSDS/SDS) available on [Children’s Intranet](https://starnet.childrenshc.org/emergency-and-safety/) |
| **Calibration** |

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| Assay Range: | IgA: 6 – 3500 mg/dLIgG: 109 – 3990 mg/dLIgM: 5 – 1665 mg/dL |
| Reference Material: | Specific Proteins Multiconstituent Calibrator |
| Suggested Calibration Levels: | 5 levels; see lot-specific ranges |
| Calibration Scheme: | Spline data reduction method |
| Calibration Frequency: | IgA: 25 daysIgG: 23 daysIgM: 57 daysAlso: with every change in reagent lot, critical maintenance of the analyzer, if QC results indicate a need for recalibration |
| AMR | AMR verification meet regulatory requirements with each calibration using 5 calibrators that span the full measuring range.  |

The upper AMR for each test has been selected to be below the changes with new lots. Do not change the upper AMR of the test in the assay parameters. |
| **Quality Control** | **QC Material:** Bio-Rad Liquichek™ Immunology Control Levels 1 & 3**Frequency:** Two levels each day of use**Stability:** Stable until the expiration date when stored frozen between -20 and -40°C. Once thawed, opened, and stored tightly capped at 2 to 8°C, this product is stable for 30 days. **Preparation**: This product should be treated the same as patient specimens and run in accordance with the instructions accompanying the instrument, kit, or reagent being used. * To thaw the product, allow it to stand at room temperature (18° to 25°C) until completely thawed but no longer than one (1) hour.
* After thawing, the product **MUST** be gently swirled and inverted several times to ensure homogeneity.
* For optimal analyte stability in the thawed state, promptly return to 2 to 8°C storage after each use and minimize the time at room temperature to no more than 20 minutes daily.
* **Before each use**, gently swirl the contents until homogeneous with no visible signs of precipitate.

**Acceptable ranges:** * Non-Bio-Rad controls will utilize manufacturer ranges and 2 SD Westgard rules.
* 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](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.
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| **Interferences** | **Hemolysis, Icterus & Lipemia (HIL) Index Values apply to IgA and IgM only. IgG does not have notable interference.**

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At HIL levels at or above the specified cutoff value, append the appropriate comment AFTER visually confirming presence of interferent: -HP for “Hemolysis present, may affect results.” -BIN for “Bilirubin Interference”-LINT for “Lipid Interference”For IgA and IgM, Lipemia is a factor past 1000 mg/dL of triglyceride. Clarify sample by ultracentrifugation and repeat testing, ensuring the –LINT comment is appended.Turbidity and particles in the samples can interfere with the assay. Therefore, particulate matter should be removed by ultracentrifugation. |
| **Reference Intervals** | **IgA in mg/dL** 0 to < 1 Year: 1 - 29 1 to < 3 Years: 4 - 90 3 to < 6 Years: 26 - 147 6 to < 14 Years: 47 - 221 14 to < 19 Years: 53 - 287 Adult Male: 63 - 484 Adult Female: 65 – 421**IgG in mg/dL**0 to < 15 Days: 320 - 1407 15 Days to < 1 Year: 108 - 702 1 to < 4 Years: 316 - 1148 4 to < 10 Years: 542 - 1358 10 to < 19 Years: 658 - 1534 Adult Male: 540 - 1822 Adult Female: 552 – 1631**IgM in mg/dL** Male and Female: 0 to < 15 Days: 5 - 35 15 Days to < 13 Weeks: 12 - 71 13 Weeks to < 1 Year: 16 - 86 1 to < 19 Years: 48 - 186 Adult Male: 22 - 240Adult Female: 33 - 293 |
| **Critical Values** | None specified |
| **Limitations** | **IgA, IgG, IgM:**Results from samples containing paraproteins (abnormal monoclonal antibodies) may incorrectly fall within the reference range. Samples with elevated total protein concentrations or samples from patients with suspected paraproteinemia can be screened using other laboratory methods such as protein electrophoresis. In addition, analysis of one or more diluted samples should be performed to ensure that consistent results are obtained. Specimens collected in EDTA collection tubes are not acceptable for use. Results should be evaluated by comparing to other clinically relevant information.Turbidity and particles in the samples can interfere with the assay. Therefore, particulate matter should be removed by centrifugation prior to running the assay.Samples that are flagged with the code ">" or error code 1041 may have excess antigen. Repeat using a dilution. |
| **Dilutions** |

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| Max Auto Dilution: | **IgA:** 1:10**IgG**: 1:4**IgM**: 1:10 |
| Maximum Manual Dilution: | **IgA**: none**IgG**: 1:10**IgM:** none |
| Diluent: | Saline |
| Manual Dilution: | Follow Abbott [Alinity Operator’s Manual](https://starnet.childrenshc.org/References/labsop/chem/operator/alinity-ci-series-operations-manual.pdf) instructions for programming automated dilutions. The system will automatically calculate the concentration of the sample and report the result. IgA: The instrument performs a 1:5 on all patients. If the sample generates a less than (<) flag, the sample will run a neat repeat to generate lower values.IgG: For samples that generate a <109 value, the system auto dilutes the sample 3:1 to generate lower values.IgM: The instrument performs a 1:5 on all patients. If the sample generates a less than (<) flag, the sample will run a neat repeat to generate lower values. |

**IgA** is analyzed as follows:* The sample is run using a 1:5 dilution.
* If a patient result flag “<” is generated, the system will automatically rerun the sample undiluted.
* If a greater than (>) flag is generated, the sample should be run with the instrument 1:10 dilution. Do not dilute further.

**IgG** is analyzed as follows:* The sample runs neat. If a less than (<) result is generated, the sample repeats in a 3:1 dilution.
* If a greater than (>) flag is generated, the sample should be run with the instrument 1:4. If this yields another greater than (>) symbol, manually dilute the sample 1:10 with saline.

**IgM** is analyzed as follows:* The sample is run using a 1:5 dilution.
* If a patient result flag “<” is generated, the system will automatically rerun the sample undiluted.
* If a greater than (>) flag is generated, the sample should be run with the instrument 1:10 dilution. Do not dilute further.
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| **Result Reporting** | **IgA*** Results between 30 and 3500 without error messages are released
* Results below 30 will repeat on a neat sample. If neat sample results are below 6 without error messages, report as < 6 mg/dL
* Results > 3500 should be diluted using the onboard automated 1:10 dilution. Release results without error messages following this dilution.
* Results > 7000 following automated dilution are reported as > 7000.

**IgG*** Results between 320 and 3990 without error messages are released
* Results below 320 without error messages are repeated using the 3:1 dilution to reduce the lower AMR to 109. If resulting measurement is below 109, report as 109 mg/dL
* Results > 3990 should be diluted using the onboard automated 1:4. Release results without error messages following this dilution.
* Results > 15960 following automated dilution may be manually diluted 1:10 with saline. Results without error codes are reported. If >flag is generated after manual dilution, report as >159600 mg/dL.

**IgM*** Results between 25 and 1665 without error messages are released
* Results below 25 will repeat neat. If neat results are below 5 with no other error messages, report as <5 mg/dL.
* Results > 1665 should be diluted using the onboard automated 1:10. Release results without error messages following this dilution.
* Results > 3330 following automated 1:10 dilution are reported as > 3330.
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| **Specimen Storage** | Promptly stopper tested specimen and store upright in a specimen rack. Every 8 hours remove specimens to refrigerator/freezer storage. Samples are retained 7 days in specimen storage freezer. |
| **References** | 1. Abbott Alinity c IgA Reagent Kit Instructions for Use, Abbott Diagnostics Division, Abbott Park, IL USA. Revised June 2019.
2. Abbott Alinity c IgG Reagent Kit Instructions for Use, Abbott Diagnostics Division, Abbott Park, IL USA. Revised June 2019.
3. Abbott Alinity c IgM Reagent Kit Instructions for Use, Abbott Diagnostics Division, Abbott Park, IL USA. Revised March 2018.
4. Abbott Alinity c Specific Protein Calibrator Package Insert, Abbott Diagnostics Division, Abbott Park, IL USA. Revised February 2018.
5. Bio-Rad Liquichek Immunology Control Package Insert, Bio-Rad Laboratories, Irvine CA, USA.
6. [CALIPER Reference Range Studies.](https://caliper.research.sickkids.ca/#/)  Accessed October 27, 2020.
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| **Historical Record** | **Version** | **Written/Revised by:** | **Effective Date:** | **Summary of Revisions** |
|  | Stephen Gripentrog |  | New Procedure for Abbott analyzers |
| 1 | Erin Bartos | 10/28/2020 | Corrections made, added AMRs, added IgG and IgM to this procedure, added references and product numbers, reference intervals, etc. for new assays. |