| **Complement C3, C4**  |
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| **Purpose** | This procedure provides instructions for performing COMPLEMENT C3 and COMPLEMENT C4 ON ABBOTT INSTRUMENTATION. Complement C3 and Complement C4 assays are used for the quantitation of C3 and C4 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, Minneapolis. |
| **Principle** | The **C3** assay is an immunoturbidimetric procedure that measures increasing sample turbidity caused by the formation of insoluble immune complexes when antibody to C3 is added to the sample. Sample containing C3 is incubated with a buffer (R1) and a sample blank determination is performed prior to the addition of C3 antibody (R2). In the presence of an appropriate antibody in excess, the C3 concentration is measured as a function of turbidity.The **C4** assay is an immunoturbidimetric procedure that measures increasing sample turbidity caused by the formation of insoluble immune complexes when antibody to C4 is added to the sample.Sample containing C4 is incubated with a buffer (R1) and a sample blank determination is performed prior to the addition of C4 antibody (R2). In the presence of an appropriate antibody in excess, the C4 concentration is measured as a function of turbidity.**C3 and C4 Methodology:** Immunoturbidimetric |
| **Clinical Significance** | **Complement C3:**All complement proteins are acute phase reactants and rise rapidly in concentrations during inflammatory episodes. Conversely, the rates of complement protein catabolism may greatly increase in various autoimmune diseases. Because complement component determinations represent a static measurement of the net concentrations that result from a dynamic balance between component synthesis and catabolism, serial sample quantitations are more clinically useful.In most disease states, complement functions “normally” in producing inflammation and tissue damage. When complement plays a role in the development of a disease, it is often due to activation by an “abnormal” antibody, immune complex, or foreign material. Increased C3 levels are associated with acute phase reaction, rheumatic disease, viral hepatitis, myocardial infarction, cancer, diabetes, pregnancy, sarcoidosis, amyloidosis, thyroiditis, inflammatory bowel disease, typhoid fever, and pneumococcal pneumonia. The magnitude of C3 increase is rarely more than two-fold and may mask decreases in levels due to concurrent consumption.Decreased levels of C3 occur in individuals with congenital deficiency or immunologic diseases (where complement is consumed at an increased rate). C3 and/or complement C4 (C4) levels may be decreased in cases of: systemic lupus erythematosus (SLE) (especially with lupus nephritis), acute and chronic hypocomplementemic nephritis, infective endocarditis, disseminated intravascular coagulation (DIC) (especially with hemolytic uremic syndrome form), and partial lipodystrophy (with associated nephritislike activity in serum). Cases of hereditary C3 deficiency, while rare, are characterized clinically by recurrent infection and by immune complex disease, in particular, membranoproliferative glomerulonephritis. The central role of C3 in both classical and alternate pathways, results in C3 deficient patients being at risk for especially severe infections by encapsulated bacteria such as *S. pneumoniae, H. influenzae*, and *N. meningitidis*. Bacteremia, sinopulmonary infections, meningitis, paronychia, and impetigo may occur. Deficient C3 levels have also been found in cases of uremia, chronic liver diseases, anorexia nervosa, and celiac disease. Refer to the following table for a general guide to evaluation of C3 and C4 protein levels in the presence of decreased hemolytic complement activity.**Complement C4:**All complement proteins are acute phase reactants and rise rapidly during inflammatory episodes. Conversely, the rates of complement protein catabolism may greatly increase in various autoimmune diseases. Because complement component determinations represent a static measurement of the net concentrations that result from a dynamic balance between component synthesis and catabolism, serial quantitations are more clinically useful. Complement promotes inflammation or tissue damage during the immune response, and plays an important role in the pathogenesis of some diseases. In the latter situation, complement is often activated by an abnormal antibody (autoantibody), an immune complex, or by foreign material. Increased C4 levels are associated with acute phase reactions and certain malignancies. Decreased levels of C4 occur in individuals with congenital deficiency or immunologic diseases (where complement is consumed at an increased rate). C4 levels may be decreased in hereditary and acquired angioedema, complement activation due to immune complex diseases, decreased synthesis due to liver disease, increased consumption in glomerulonephritis, systemic lupus erythematosus (SLE), rheumatoid arthritis, respiratory distress syndrome, autoimmune hemolytic anemia, cryoglobulinemia, and sepsis. Total congenital C4 deficiency is rare, but partial C4 deficiency is common. Partial and complete congenital C4 deficiencies have been associated with immune complex diseases, SLE, autoimmune thyroiditis, and juvenile dermatomyositis. Infections associated with C4 deficiency include bacterial or viral meningitis, Streptococcus and Staphylococcus sepsis, and pneumonia. Refer to the following table for a general guide to evaluation of Complement C3 (C3) and C4 protein levels in the presence of decreased hemolytic complement activity. |
| **Analyzer** | **Minneapolis: Abbott Alinity ci (Sunquest method code: MACC)****BACKUP:** Hold samples until Alinity ci (MACC) is back in service. Samples may be sent to Mayo Medical Laboratories (MML) if directed. |
| **Sunquest Test Codes** | **C3****C4** |
| **Specimen** | Sample: **Preferred**: Lithium Heparain, with or without gel.**Alternative**: SST, NaHep, or EDTA**Minimum sample volume:** 0.6mL blood, 0.2mL serum/plasma**Stability when separated from cells/gel:** **20 to 25°C:** Complete testing as soon as possible; time limit not specified. Refrigerate if testing is delayed.**2 to 8°C:** 8 days**-20°C:** 8 days. Avoid multiple freeze/thaw cycles to preserve protein viability.**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 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.
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| **Reagents** | **Reagent Handling** **C3 and C4:**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. 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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| ***Product Description*** | ***Product Code*** | ***Stability*** |
| Alinity c Complement C3 Reagent KitAbbott Alinity c Complement C4 Reagent Kit | 09P562009P5720  | **Store at:** 2 to 8°C**Unopened:** Until manufacturer’s printed expiration date**On-board**: 57 days**Opened, off the analyzer (with clean caps):** Until manufacturer’s printed expiration date |
| Abbott Alinity c Specific Proteins Multiconstituent Calibrator Kit | 08P6201 | **Store at:** 2 to 8°C**Unopened:** Until manufacturer’s printed expiration date**Opened expiration:** 30 days after opening. Store tightly capped with new replacement caps. Return to refrigerated storage after use.Lot-specific calibrator values are listed in the Alinity c Specific Proteins Multiconstituent Calibrator Kit value sheet, packaged with the calibrator. Verify that the lot number listed on each calibrator carton agrees with the lot number printed on the value sheet. The last two digits of the lot numbers can vary. Do not change the AMR upper limit with every lot number; the chosen AMR should encompass all lots of calibrator material. Ensure the upper AMR limit does not change from that which is printed in this procedure. |

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| **Risk and Safety** | Contains alcohols, C12-14-secondary, ethoxylated, tris hydroxymethyl aminomethane and sodium azide.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 Pathogens.

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| Safety data sheets (MSDS/SDS) available on [Children’s Intranet](https://starnet.childrenshc.org/emergency-and-safety/)  |

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| **Calibration** |

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| Assay Range: | **C3**: 11 to 333 mg/dL**C4**: 2.9 to 59.0 mg/dL |
| Reference Material: | **C3 and C4**: Specific Proteins Multiconstituent Calibrator |
| Suggested Calibration Levels: | **APPROXIMATE: Values vary****C3**:CAL 1: 50CAL 2: 100CAL 3: 175CAL 4: 250CAL 5: 350**C4**:CAL 1: 10CAL 2: 20CAL 3: 30CAL 4: 40CAL 5: 60 |
| Calibration Scheme: | **C3 and C4**: 5 Levels; Spline data reduction method |
| Calibration Frequency: | **C3 and C4:** 57 days, With every change in reagent lot and after maintenance to critical parts  |
| AMR | AMR is verified with every calibration. |

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| **Quality Control** | **C3 and C4:****QC Material**: Bio-Rad Liquichek Immunology Control Levels 1 and 3**Frequency:** Two levels each day of use **Stability:** Once thawed, opened, and stored tightly capped at -20 to -70°C, product will be labeled with an expiration date equal to the shortest stability of the included analytes, which is **14 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 products **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:**

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| **H** | **I** | **L** |
| **-** | **-** | **-** |

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”See Limitations section. |
| **Reference Intervals** | **C3:**0 to <15 Days: 50 - 121 mg/dL15 Days to 1 Year: 51 - 160 mg/dL1 to <19Years: 83 - 152 mg/dL19 Years+: 82 – 193 mg/dL**C4:**0 to <1 Year: 7 – 30 mg/dL1-<19 Years: 13 – 37 mg/dL19 Years+: 15 – 57 mg/dL |
| **Critical Values** | None specified. |
| **Limitations** | Samples containing paraproteins (abnormal monoclonal antibodies) may interfere with test results. Samples with elevated total protein concentrations or samples from patients with suspected paraproteinemia can be screened using other laboratory methods, such as protein electrophoresis.Elevated fibrinogen levels in EDTA plasma samples may yield a depressed result. C3 and C4 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. |
| **Dilutions** |

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| **C3 and C4:** |
| Max Auto Dilution: | 1:2 |
| Maximum Manual Dilution: | Not Specified |
| Diluent: | Onboard Diluent |
|  | 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.  |

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| **Result Reporting** | **C3:*** Results between 11 and 333 without error messages are released
* Results below 11 without error messages are reported as < 11 mg/dL.
* Results > 333 should be diluted using the onboard automated 1:2 dilution. Release results without error messages following this dilution.
* Results > 666 following automated dilution are reported as > 666 mg/dL

**C4:*** Results between 2.9 and 59.0 without error messages are released
* Results below 2.9 without error messages are reported as < 2.9 mg/dL.
* Results > 59.0 should be diluted using the onboard automated 1:2 dilution. Release results without error messages following this dilution.
* Results > 118.0 following automated dilution are reported as > 118.0 mg/dL
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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 Complement C3 Reagent Kit Instructions for Use, Abbott Diagnostics Division, Abbott Park, IL USA. Revised March 2018
2. Abbott Alinity c Complement C4 Reagent Kit Instructions for Use, Abbott Diagnostics Division, Abbott Park, IL USA. Revised March 2018
3. Abbott Alinity c Specific Proteins Multiconstituent Calibrators Package Insert, Abbott Diagnostics Division, Abbott Park, IL USA. Revised February 2018
4. Bio-Rad Liquichek Immunology Control Package Insert, Bio-Rad Laboratories, Irvine CA, USA.
5. [CALIPER Reference Range Studies](https://caliper.research.sickkids.ca/#/search). 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 | October 28, 2020 | Added All C4 information to this procedures, corrected Alinity name, added AMRs, interferences section, changed QC, reference intervals, references, reporting results, dilutions, ETC. |