Laboratory Procedure Manual

Analyte: **Tina-quant α1-Acid Glycoprotein**

Matrix: **Serum**

Method: **Immunoturbidimetric**

Method No: **4049.04**

Revised: January 2023

as performed by: Nutritional Biomarkers Branch (NBB)
Division of Laboratory Sciences (DLS)
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Important Information for Users

CDC periodically refines these laboratory methods. It is the responsibility of the user to contact the person listed on the title page of each write-up before using the analytical method to find out whether any changes have been made and what revisions, if any, have been incorporated.

Images are included in this document as visual aids for certain topics. They are intended to be representative images only and should not be construed as absolute references. Discrepancies between the images in this document and the actual application design are not a cause for revisions to this document.

Public Release Data Set Information

This document details the Lab Protocol for testing the items listed in the following table:

Data File Name	Variable Name	SAS Label
AGP_L	LBXAGP	alpha-1-acid glycoprotein

1. Summary of Clinical Relevance and Principle

A. Clinical Relevance

α_1-Acid Glycoprotein (AGP) is synthesized in the liver and structurally belongs to the lipocalin superfamily of secretory proteins such as retinol-binding protein and α_1-microglobulin. It is made up of a polypeptide chain having 5 carbohydrate chains bonded through N-glycosidic linkage (molar mass of 41,000 Daltons) **[1]**.

AGP is a sensitive acute phase reactant whose concentration can increase by a factor of 3 within 24-48 hours when inflammation occurs. It can also be used to differentiate between acute phase reactions (elevated serum level) and estrogen effects (normal or decreased serum level) whereas the serum level of other positive reactants such as ceruloplasmin and haptoglobin increases during such reactions. Moderate and isolated increases occur when glomerular filtration is inhibited in the early stages of uremia. The determination is used in the assessment of the activity of acute and recurring inflammations as well as of tumors with cell necrosis **[2,3]**.

B. Test Principle

The Tina-quant Roche AGP assay is based on the principle of immunological agglutination. Anti- α_1-acid glycoprotein antibodies react with antigen in the sample to form an antigen/antibody complex. Following agglutination, this is measured turbidimetrically.

2. Safety Precautions

Consider all serum/plasma specimens potentially positive for infectious agents including HIV and the hepatitis B virus. We recommend the hepatitis B vaccination series for all analysts working with whole blood and serum/plasma. Observe universal precautions; wear protective gloves, laboratory coats, and safety glasses during all steps of this method. Discard any residual sample material by autoclaving after analysis is completed. Place disposable plastic, glass, and paper (pipet tips, autosampler vials, gloves, etc.) that contact serum/plasma in a biohazard autoclave bag and keep these bags in appropriate containers until sealed and autoclaved. Wipe down all work surfaces with 10% bleach or similar disinfectant solution when work is finished.

Handle acids and bases with extreme care; they are caustic and toxic. Handle organic solvents only in a well-ventilated area or, as required, under a chemical fume hood. Reagents and solvents used in this study include those listed in Section 6. Safety data sheets (SDSs) for these chemicals are readily accessible as hard copies in the lab. If needed, SDSs for other chemicals can be viewed at http://intranet.cdc.gov/ossam/workplace-safety/safety-practices/chemical-safety/index.html or at http://www.ilpi.com/msds/index.html. Additional information on hazard identification, risk evaluation and risk mitigation for this method can be found in the method risk assessment document.

3. Computerization; Data System Management

(A) During sample preparation and analysis, samples are identified by their sample ID. The sample ID is a number that is unique to each sample that links the laboratory information to demographic data recorded by those who collected the sample.

(B) Calculation of AGP concentration is accomplished with the software on the *Cobas® 6000* and data files are transferred and saved on DLS network. The results file is imported into a STARLIMS database for review of the patient data, statistical evaluation of the QC data, and approval of the results. See **Appendix B_C "JA-4049-DR-01-Computerization and Data System Management"** for a step-by-step description of data transfer, review, and approval.

(C) The data files from the instrument workstation are routinely backed up to a USB for long-term storage. Files stored on the DLS network are automatically backed up nightly by ITSO support staff.

4. Specimen Collection, Storage, and Handling Procedures; Criteria for Specimen Rejection

(A) Centrifuge samples containing precipitate before performing the assay.

(B) Specimens for AGP analysis may be fresh or frozen serum or plasma. Use serum or plasma collected by standard venipuncture technique. Plasma specimens may be collected with Li-Heparin or K2-EDTA as an anticoagulant.

(C) The appropriate amount of serum or plasma is dispensed into a Nalgene cryovial or other plastic screw-capped vial labeled with the participant's ID. A 500-µL sample of serum or plasma is preferable to allow for repeat analyses; a minimum volume of 150 µL is required for pipetting into the sample cup.

(D) Specimens collected in the field should be kept refrigerated (+2°C to +8°C) and are protected from light. After processing, frozen and then shipped on dry ice by overnight mail. Once received, samples should be stored at ≤-20°C during the "in-processing" which is typically completed within less than 4 hours and then stored frozen at ≤-50°C for up to 15 business days until samples are transferred to the testing laboratory for longer storage at deep frozen condition (-50°C to -90°C). Serum samples are stable <72 hours when stored refrigerated at about 4°C (+2°C to +8°C) and 6 months at ≤-20°C **[4]**. Multi-year storage under deep frozen conditions showed excellent sample stability (≤3% change; Appendix A - stability data). Multiple freeze/thaw cycles are generally not recommended; however, samples can withstand up to 3 freeze/thaw cycles (≤2% change; Appendix A - stability data).

(E) Ensure patient samples, calibrators and QC are at ambient temperature (+15°C to +30°C) before measurement. Once the samples, calibrators, and controls are loaded on the analyzers, they should be measured within 2 hours to avoid possible evaporation effects.

(F) Specimen handling conditions are outlined in the DLS Policies and Procedures Manual. The protocol discusses collection and transport of specimens and the special equipment required. If there is more than one test of interest in the specimen and it needs to be divided, the appropriate amount of blood, serum or plasma should be transferred into a sterile Nalgene cryovial labeled with the participant's ID; avoid cross contamination.

(G) The criteria for unacceptable specimens are insufficient sample volume (<150 µL) for at least one analysis, suspected contamination such as leaking, or damaged sample container. These samples are assigned an appropriate comment code and/or description and are set "no reportable (code 98).

(H) A series of standard comment codes are available in the STARLIMS database to identify any issues related to sample quality. These codes can be used, along with text descriptions, to document why a result was not reported (specimen rejection) or that a result should be interpreted with caution based on the sample quality.

5. Procedures for Microscopic Examinations; Criteria for Rejection of Inadequately Prepared Slides

Not applicable for this procedure

6. Preparation of Reagents, Calibration (Standards), Controls, and All Other Materials; Equipment and Instrumentation

A. Reagent Preparation

All reagents are supplied by Roche Diagnostics in liquid form ready for use in a Cobas c pack. If the entire reagent pack is not used in one run, store the kit under refrigerated conditions (+2°C to+8°C) on the Analyzer until the expiration date of the reagent is reached or the reagent has been on the Analyzer for up to 12 weeks, whichever comes first.

B. Standards Preparation

C.f.a.s. Protein, supplied by Roche Diagnostics, is a liquid ready-for-use calibrator based on human serum (used for **STD2 - STD6** calibrators). Mix carefully before use. Deionized water (diH_2O) is used as the zero standard (**STD1**). The lot specific standard concentrations are encoded in electronic files sent via the Cobas link to the analyzers and these are installed before use. Unopened calibrators are stored under refrigerated conditions at (+2°C to + 8°C) until the expiration date of the kit. After opening, the calibrator is stable for up to 4 weeks when kept cold (+2°C to + 8°C), provided that dispensing of the calibrator takes place without microbial contamination.

C. Preparation of Quality Control Materials

1) Roche QC Materials

Commercially prepared quality control material purchased from Roche Diagnostics at two levels, Precinorm Protein and Precipath Protein (liquid forms ready to use) are used for the daily quality control checks of α1-Acid Glycoprotein (AGP) an immunoturbidimetric assay on c501 module. Unopened controls are stable under refrigerated conditions (+2°C to +8°C) until the expiration date on the package insert. Once opened, controls should be refrigerated (+2°C to +8°C) and are stable for up to 4 weeks. Controls should be allowed to come to ambient temperature (+15°C to +30°C); mixed carefully avoiding foam formation before analyzing Control values are lot specific, and values are to be entered into the Cobas® 6000 before analyzing a new lot number of control.

2) CDC QC pools

Additional QC materials for this assay are prepared in-house from blood products acquired from blood banks or from other volunteer blood donors. After screening the pools for AGP, the serum is pooled to obtain the desired QC levels. All pools are filtered through gauze to remove debris before being dispensed. Serum (usually 750 µL) is aliquoted into labeled 2.0-mL Nalgene cryovials, capped, and stored deep frozen, typically around-70°C (-50°C to -90°C). The QC pools are stable for at least 3 years (based on in-house QC data). The CDC QC pools are analyzed a few times per month to assess long-term trends; they are not used on a daily basis as quality control checks.

For more detailed information on the preparation of QC materials, homogeneity testing, and characterization refer to **SOP "NBB-OC-LABOP.01.01 QC Materials"**.

3) QC Set-up on the Analyzer

QC information must be installed on the instrument system before use when a new lot number of QC is needed. Roche QC can be added manually or by using the Cobas link. Non-Roche QC are added by going to **"QC/Install/Add".** After adding new QC these need to be activated for the test and assigned to a white QC rack and position.

D. Other Materials

The following materials are available from the manufacturer (Roche Diagnostics):

(1) Sample racks

(2) Sample cups (standard and micro)

(3) Reaction cells

(4) Wash solutions

(5) 2% Eco-Tergent

(6) Halogen lamp

(7) Diluent, NaCl 9%

(8) Elecsys SysClean

(9) C.f.a.s. Protein calibrators (STD2 - STD6)

(10) Controls (Precinorm Protein and Precipath Protein)

(11) Reagent c-packs (R1 and R2)

(12) Controls (Precinorm Protein and Precipath Protein)

E. Instrumentation

In the case of simple laboratory instrumentation (e.g., pipettes, vortex mixer, analytical balance, etc.) a product listed herein may be substituted with equivalent product from a different manufacturer provided it meets or exceeds the specifications of the product listed.

(1) Roche Diagnostics Cobas® 6000 system; c501 Module (Roche Diagnostics, Indianapolis, IN)

(2) Daigger Vortex Genie 2 (VWR, Suwanee, GA)

(3) Eppendorf micropipette and tips (Brinkmann Instruments Co., Westbury, NY)

7. Calibration and Calibration Verification Procedures

For commercial kit assays, calibration procedures recommended by the manufacturer are followed.

This method has been standardized against the reference preparation of the IRMM (Institute for Reference Materials and Measurements) BCR470/CRM470 (RPPHS – Reference Preparation for Proteins in Human Serum) **[5]**.

A six-point calibration curve is used for AGP calibration, C.f.a.s. Proteins (**STD2 - STD6**) and diH_2O (**STD1**) as zero standard. Allow the calibrators to reach ambient temperature (+15°C to +30°C) before use and mix carefully to ensure homogeneity. Avoid the formation of foam.

The concentrations of the components are lot-specific and must be entered in the analyzer before use. The exact calibrator values are encoded in electronic files sent via the Cobas® link to the analyzers.

C.f.a.s. Proteins is stable when unopened and refrigerated (+°2C to+8°C) until the expiration date on the package. After opening, the calibrator is stable for up to 4 weeks under refrigerated conditions (+2°Cto +8°C), provided that dispensing of the calibrator occurs without microbial contamination.

Calibration is recommended as follows:

- Full calibration after reagent lot change
- Full calibration following any dispense system component replacement or any major maintenance performed on the instrument, i.e., lamp, reaction cells.

Calibration verification is conducted at least twice a year using international reference materials. For details, refer to **Appendix B_A "JA-4049-G-01-Calibration and Calibration Verification".**

There is an IFCC (distributed by IRMM) international reference material available for AGP and Albumin (**ERM-DA470k; formerly CRM470**). This material, when reconstituted, has a certified value of 0.617 g/L. Dilutions can be made to check accuracy and linearity as part of calibration verification. Unopened ampoule should be stored frozen (-20°C±2°C). The material is reconstituted according to the package insert instructions. The solution stored in its original vial can be used for up to one week under refrigerated conditions (+2°C to +8°C) provided any microbial contamination has been excluded. Reference material is stored deep frozen (-50°C to -90°C).

Details about our proficiency testing (PT) activities can be found in the proficiency testing form. An external proficiency testing program is not available for the analysis of α1-Acid Glycoprotein (AGP). An in-house proficiency testing program has been developed and is conducted at least twice a year, details of which can be found in **Appendix B_A "JA-4049-G-02-Alternative In-house Proficiency Testing**."

As this assay must be performed according to the manufacturer's specifications, none of the parameters can be altered. Therefore, ruggedness testing cannot be performed for this assay.

8. Procedure Operating Instructions; Calculations; Interpretation of Results

A. Preliminaries

(1) Allow Calibrators, QCs, and patient samples to reach ambient temperature (+15°C to +30°C).

(2) Ensure that the amount, of reagents, diluent, and wash solutions are adequate for the number of samples to be run. You may place more than one bottle of reagent at a time on the analyzer; however, avoid using more than one lot number of reagent for a single run.

(3) Make sure the analyzer and/or tests required are not masked.

(4) Check to see if calibration is required for the tests that will be run.

(5) If running the same tests on all samples, go to the **"Start"** global button and set the **"default profile".**

(6) Be sure to clear all previously programmed samples from the Data Review screen after backing up the data.

(7) Perform the required maintenance on the c501 system.

B. Instrument Maintenance & Function Checks

(1) The c501 system maintenance (Cobas 6000 Clinical Analyzer) consists of daily, weekly, monthly, quarterly, and as needed maintenance. Daily maintenance should be performed at the start of each 8-hour shift, or more frequently, if necessary.

- Daily maintenance and function checks include monitoring photometer reading at 340 nm (reading should be <14000); running the pre-programmed daily maintenance (***Daily pipe***); checking the deionized (di H_2O) water supply to instrument water tank; checking reagent levels; manually cleaning the sample probe & shield pipe using gauze pads moistened with alcohol followed by diH_2O soaked swabs; cleaning reagent probes, ISE probes, sipper probes, pre-wash probes using alcohol moistened swabs followed by diH_2O soaked swabs; cleaning cell rinse nozzles and the drain port with diH_2O; emptying the concentrated waste tank; maintaining printer paper supply. After sample analysis is completed for the day the **"green rack"** is processed before the **"Sleep pipe"** is run to bring the module in standby mode.

- Weekly maintenance consists of cleaning reservoirs, mixers, separation stations, incubator, rinse stations, fill nozzles, IS bath, including cell wash and cell blank. The entire Cobas® 6000 should be powered "Off/On" typically once per week.

- Monthly checks include cleaning the diH_2O tank, all air filters (4), water bath and KCl and detergent aspiration filters.

- As needed maintenance also includes changing the reaction cells and lamp on the c501. Change is required when the photometer readings (>14000 nm) and/or cell blank readings are out of spec limits.

(2) For additional maintenance requirements, refer to **Appendix B_B "JA-4049-I-01-Instrument Maintenance & Function Checks"**.

C. Preparing a Run

A run is defined as 100 patient samples or less and 2 sets of AGP QC pools analyzed with patient samples at the beginning and at the end of each run.

(1) Allow reagents, calibrators, QC, and patient samples to reach ambient temperature (+15°C to +30°C) prior to use and mix thoroughly before pipetting. Visually check for any unusual sample volume, specimen color or debris/precipitate. Ensure barcodes are facing the open slot in the racks.

(2) Prior to loading samples on the instrument, ensure that no air bubbles are present in the sample cups. Break a wood applicator into pieces and use them to pop the bubbles if necessary.

(3) Verify photometer readings (<14000) and update maintenance log sheet; load reagents onto the instrument and allow module to perform reagent registration.

(4) For a calibration run, use "**black"** calibrator racks. Nonbarcoded calibrators must be pipetted (150 µL) into sample cups and placed in their assigned positions in "**black**" calibrator racks. When calibration is completed, the results will be printed

(5) Calibration must be performed at least once per reagent lot for all analytes done on the Roche c501 analyzer. Use class A glassware as required.

(6) To run QC, use the "**white"** QC racks. If using Roche barcoded QCs, open the QCs and place those in a "**white"** unassigned rack or assigned positions in white QC racks and pipette (150 µL) into sample cups. For CDC QC, pipette (150 µL) of each non-barcoded QC into a sample cup and place in the assigned control position of the "**white"** QC racks. When the instrument is started, it will automatically run the correct tests on the preprogrammed QC and print the results. It is important to verify that calibration and QC results are valid before measuring the routine samples.

(7) To run patient samples, use the "**gray"** sample racks. Place empty sample cups in barcode labeled 13 X 75 tubes in "**gray"** sample racks and pipette 150 µL of the serum samples into the sample cup.

(8) Pipette 20-25 samples at a time and place the racks on the input buffer tray. Patient results do not print until requested.

(9) Avoid loading racks on the input tray if the instrument green light is flashing or turned off.

D. Initiating a Run

Note: Backup all previous data from the instrument before starting a new run. Check the default profile before starting a run.

(1) Instrument starts with running default profile on all samples unless programmed differently prior to loading.

(2) Once the calibrator, control or sample racks are loaded on the input tray, they should be measured within 2 hours because of possible evaporation effects.

(3) Calibration and QC checks must be completed before pipetting patient samples. Verify calibration and QC results print outs for run acceptability criteria.

(4) To run patient samples, load the "**gray"** (serum) sample racks onto the sample input buffer tray and click on the **"Start"** global button.

(5) On the Start Screen, click **"Start"** for sampling on the instrument.

(6) Instrument completes tube barcode scanning; next click data review on "Workplace" screen.

(7) Highlight the line with tube barcode number and click the **"demographics"** to match the tube barcode position to the correct sample vial. Place the cursor in the vial ID box to scan patient vial barcode.

(8) Click **"OK"** and continue until all patient ID's are entered, print results and back-up the data.

(9) Repeat all samples that require confirmation or dilutions.

E. Processing and Reporting a Run

The Roche Cobas® 6000 Control Module is used to review data and check for samples that need to be diluted or repeated for confirmation.

(1) Once results are complete, review all results from the **"Data Review"** screen.

(2) Highlight all results to be printed and click the **"print"** global button.

(3) Check **"both"** to print original results and all repeat results and click the "print" to print the data.

(4) To back-up the data, the instrument must be in *Stand By.* In case instrument is not in *Stand By*, select the global "Start" button; in Rack Reception mode box select "Change" then deselect the Rack Reception Mode box and click **"OK".** The instrument will finish all samples and washes before going into Stand By.

(5) Upon instrument return to Stand-by mode, highlight samples that need to be backed-up to the instrument USB drive. Click "**Backup data";** a box appears for **"Save the data"** (*Floppy or Backup Media; ASCII or binary*) with appropriate file name. If more than one run is done/day, file names as "a", "b" "c", etc. with appropriate dates are assigned.

(6) From instrument USB drive import data on network folder for transfer into STARLIMS database for further data review (analyst, project lead), approval and reporting (QA officer and supervisor). For details, refer to **Appendix B_C "JA-4049-DR-01-Computerization and Data System Management"**.

(7) The data files can be backed up or printed from the Print View screen while system is in Stand-by or Operation mode (these files auto delete when the analyzer is put into Sleep mode after back-up/print but can still be accessed in Data view before next run).

F. Special Method Notes

The system can be completely turned off for the weekend or extended holidays or when indicated by maintenance procedure or error code. Refer to **Appendix B_B "JA-4049-I-01-Instrument Maintenance & Function Checks"**.

G. Calculations

All calculations are performed by the c501 Software system using a machine-stored calibration curve.

H. CDC Modifications

The method is run exactly as stipulated by Roche Diagnostics; CDC has introduced no modifications.

9. Reportable Range of Results (AMR – Analytical Measurement Range)

The reportable range for AGP is 0.1 – 4.0 g/L. Samples with AGP values less than 0.1 g/L are reported as <0.1 g/L. Samples with AGP values above 4.0 g/L are automatically diluted 1:1.5 and reanalyzed via the rerun function. Results from samples diluted by the rerun function are automatically multiplied by a factor of 1.5. There is no known maximum acceptable dilution. When possible, avoid small volume pipetting and minimize use of serial dilutions when generating diluted sample results.

10. Quality Control (QC) Procedures

As part of each analytical run two levels of manufacturer QC are analyzed in duplicate and the manufacturer limits are used as run judge for this assay. However, twice a month in-house bench QC pools are analyzed in duplicate to assess potential assay shifts or trends.

A. Blind Quality Controls

Blind QC specimens can be inserted into the mix of patient specimens. These QC specimens are generally prepared at two levels that would be encountered in patient samples; the labels used are identical to those used for patient samples. One blind QC specimen randomly selected for concentration is included at a randomly selected location in every 20 specimens analyzed.

Alternatively, open label blind QC specimens can be used where the analyst knows that the sample is a blind QC, but they do not know what pool the sample is from. Open label blind QCs are only used if one can choose from at least 6 different pools and the analyte concentrations are similar to those found in patient samples.

After a run is completed, used blind QC are removed from the run, marked with a black dot on the cap to indicate that the vial has been thawed, and returned to the blind QC box. This helps to identify which vials have been used. If a run needs to be repeated, the same blind QC can be inserted as in the initial run.

The use of blind QCs is optional but encouraged. Blind QCs are used in this method as a supplementary tool to assist in monitoring accuracy, precision, and aid in detecting errors; these are not used as part of the primary control procedures to determine if a run is out of control.

B. Bench Quality Controls

Bench QC specimens are prepared from a minimum of 2 pools that represent low and high levels AGP. This assay typically uses three serum pools, which represent low, medium, and high levels of AGP. These pools are prepared in the same manner as patient samples and analyzed in duplicate as part of each run.

The QC results are checked after each run using of a multi-rule quality control program **[6]** based their characterization data, namely: the pool mean; the pooled within-run standard deviation associated with individual QC results measured in the same run (S_w); the standard deviation associated with individual QC results (S_i); and the standard deviation associated with run mean QC results (S_m). QC rules have been designed to accommodate the use of 1–3 different QC pools during a run, the use of 1–2 measurements of each pool per run, and as many instruments as needed. These QC rules are described in the DLS Policies and Procedures Manual and a relevant selection applicable to this assay is shown below. The system is declared “in control” if all individual QC results are within 2S limits; the run is accepted. If not, then the rules shown below are applied and the run is rejected if any condition is met; the run is declared “out of control”

Three QC pools per run with two or more QC results (replicates) per pool:

(1) If all three QC run means are within 2 S_m limits and individual results are within 2 S_i limits, accept the run

(2) If 1 of the 3 QC run means is outside a 2 S_m limit – reject run if:

   a) 1 3S Rule—Run mean is outside a 3 S_m limit or

   b) 2 2S Rule—Two or more of the three run means are outside the same 2 S_m limit or

   c) 10 X-bar Rule—Current and previous nine run means are on the same side of the characterization mean

(3) If one of the six QC individual results is outside a 2 S_i limit – reject run if:

   a) Outlier – One individual result is beyond the characterization mean ± 4 S_i or

   b) R 4S Rule—Two or more of the within-run ranges in the same run exceed 4 S_w (i.e., 95 percent range limit)

Abbreviations:

S_i = Standard deviation of individual results (the limits are not shown on the chart unless run results are actually single measurements)

S_m = Standard deviation of the run means (the limits are shown on the chart)

S_w = Within-run standard deviation (the limits are not shown on the chart)

A QC program written in SAS is available from the DLS Quality Assurance Officer and should be used to apply these rules to QC data and generate Shewhart QC charts. No results for a given analyte are to be reported from an analytical run that has been declared "out of control" for that analyte as assessed by internal (bench) QC. The initial limits are established by analyzing pool material in 20 consecutive runs. The SAS QC program is used to monitor the QC performance over time for potential shifts, trending, or changes in assay precision. For assays performed routinely, quarterly statistics (mean, SD, CV) are calculated for each pool and compared to the characterization target values. For assays performed infrequently, statistics are calculated at least annually. As more QC data become available (covering multiple lots of reagents, multiple analysts, etc.), the initial QC limits can be reevaluated and updated. QC limits can also be reevaluated and updated as a result of a non-conforming event when the assay shows a higher than expected out of control rate and the root cause investigation does not reveal a correctable course of action to bring the assay back into control. This needs to be documented by a CAPA in STARLIMS.

While a study is in progress, QC results are stored in STARLIMS database. For runs that are not imported into the database (i.e., R&D, troubleshooting, research-type runs), QC results are stored electronically in the analyte-specific folder on the DLS network. At the conclusion of studies complete QC records are prepared and submitted as a study QC report in STARLIMS for review by the laboratory chief, branch chief, and a DLS statistician.

C. Sample QC Criteria

Sample QC is set of criteria used to evaluate the quality of individual test result within run, and to evaluate the quality of the calibrators associated with the run. In addition to the sample QC criteria set forth in the DLS Policies and Procedures Manual that pertain to the reportable range of concentration results and calibration curves, sample QC criteria are also established for method-specific concentration and non-concentration data associated with an individual result.

The method-specific concentration and non-concentration parameters identified for sample QC evaluation, along with their associated thresholds and flagging protocols ('Pass', 'Check', 'Warn', 'Fail') are maintained and updated in the STARLIMS database, and sample QC assessment is performed and documented as part of run review process. A sample QC result flagged as 'Fail' should not be reported. A sample QC result flagged as 'Warn' or 'Check' should be reviewed both by the analyst and supervisor to determine if the quality of the result is suitable for reporting. Results that are flagged during sample QC evaluation may also be assigned one of a series of standard comment codes available in the STARLIMS database to identify the nature of the sample QC flag.

To assess the sample specific data quality, the following parameters are subject to sample QC evaluation in this method.

- On Cobas c501 instrument, check calibration for pass/fail.
- In STARLIMS database, check runs for:

- Measurable range (≥LOD to ≤4.0 g/L) (pass, code 0); no action needed
- Results > measurable range (>4.0 g/L) (fail; code 26); repeated after either auto or manual dilution (pass; code 97)
- Results <LOD (LOD: 0.1 g/L) (incomplete; code 37); repeat/confirm (warn; code 37)
- Results null or 0 (fail; code 26); repeat/confirm (code 37 or 33)
- Delta difference for repeat results should be ≤15%; otherwise, repeat/confirm
- Specimen volume less than expected for test (code 21); set no reportable (code 98)
- Check and update results for appropriate comment codes, e.g., not enough specimen for repeat analysis (code 22; set no reportable; code 98); lab error, spills, contamination etc. (code 23; set no reportable); or instrument error/failure (code 24; repeat analysis)

11. Remedial Action if Calibration or QC Systems Fail to Meet Acceptable Criteria

For initial steps to investigate QC failures see **Appendix B_C "JA-4049-DR-02-Out-of-Control Corrective Action"**. Additional steps are provided as a general guideline for identifying possible problems resulting in "out of control" values for QC materials. The troubleshooting process should be done in consultation with the supervisor and may involve additional experiments beyond what is indicated below.

(A) Check to make sure the instrument is working properly.

(B) Recalibrate the instrument.

(C) Rerun Bench QC; run Roche QC.

(D) Analyze reference material.

(E) Call the Roche "hotline" or service engineer.

(F) Do not report analytical results for runs not in statistical control.

(G) If the steps outlined above do not result in correction of the "out of control" values for QC materials, consult the supervisor for other appropriate corrective actions.

12. Limitations of Method; Interfering Substances and Conditions

(A) Samples containing particulate matter should be centrifuged and the material removed before analysis.

(B) Results are not known to be affected by icteric, hemolyzed or lipemic specimens **[7]**. In very rare cases, gammopathy, in particular type IgM (Waldenstrom's macroglobulinemia), may cause unreliable results **[8]**.

(C) Assay needs to be performed within 2 hours of the samples being placed on board the instrument to minimize the effect of evaporation.

13. Reference Ranges (Normal Values)

The reference range is 0.5 – 1.2 g/L **[9]**. Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary, determine its own reference ranges.

14. Critical Call Results ("Panic Values")

There are no critical call results for this analyte.

15. Specimen Storage and Handling during Testing

Specimens are allowed to reach ambient temperature (+15°C to +30°C) during preparation. After analysis, the unused portion of the patient specimen is then returned to deep frozen storage (-50°C to -90°C) as soon as possible.

16. Alternate Methods for Performing Test of Storing Specimens if Test System Fails

If the analytical system fails, we recommend that the specimens be stored at ≤-20°C until the analytical system is restored to functionality.

17. Test Result Reporting System; Protocol for Reporting Critical Calls (If Applicable)

NHANES data is transmitted electronically on a regular basis (approximately weekly for 3-week turnaround analytes). Abnormal values are confirmed by the analyst, and codes for missing data are entered by the analyst and are transmitted as part of the data file. NCHS makes arrangements for the abnormal report notifications to the NCHS Survey Physician. For smaller studies, hard copies of a data report are sent, as well as the results in electronic format.

Test results are reported to the collaborating agency at a frequency and by a method determined by the study coordinator. Generally, data from this analysis are compiled with results from other analyses and sent to the responsible person at the collaborating agency as an Excel file, generally through electronic mail or via ftp site.

Data are transmitted via the CLIA Director, Division of Laboratory Sciences, NCEH, CDC after review by the Laboratory Supervisor, Branch Chief, and a CDC Statistician.

18. Transfer or Referral of Specimens; Procedures for Specimen Accountability and Tracking

This protocol does not involve referral of specimens for testing the analytes of this method at another laboratory.

A STARLIMS database is used to keep records and track specimens for NHANES 1999+. If analyses are performed for smaller, non-NHANES studies, records may be kept in Excel files on the DLS network.

We recommend that records, including related QA/QC data, be maintained for 10 years after completion of the NHANES study. Only numerical identifiers should be used (e.g., case ID numbers). All personal identifiers should be available only to the medical supervisor or project coordinator. Residual serum from these analyses for non-NHANES studies are retained for at least 1 year after results have been reported and may then be returned or discarded at the request of the principal investigator. Very little residual material will be available after NHANES analyses are completed, however residual serum is retained for at least 2 years after results have been publicly released; at that point, samples with sufficient volume (>0.2 mL) are returned to NHANES and samples with insufficient volume may be autoclaved.

The exact procedure used to track specimens varies with each study and is specified in the study protocol or the interagency agreement for the study. Copies of these documents are kept by the supervisor. In general, when specimens are received, the specimen ID number is entered into a database and the specimens stored deep frozen, typically around -70°C (-50°C to -90°C). The specimen ID on the vial is scanned by a barcode reader and is used to prepare the electronic specimen table for the analytical system. When the analyses are

completed, the result file is loaded into the database, and the analytical results are linked to the database by ID number. The analyst is responsible for keeping records of specimens prepared incorrectly, those with labeling problems, and those with abnormal results, together with information about these discrepancies. In general, these are documented using codes in the STARLIMS.

19. Method Performance Documentation

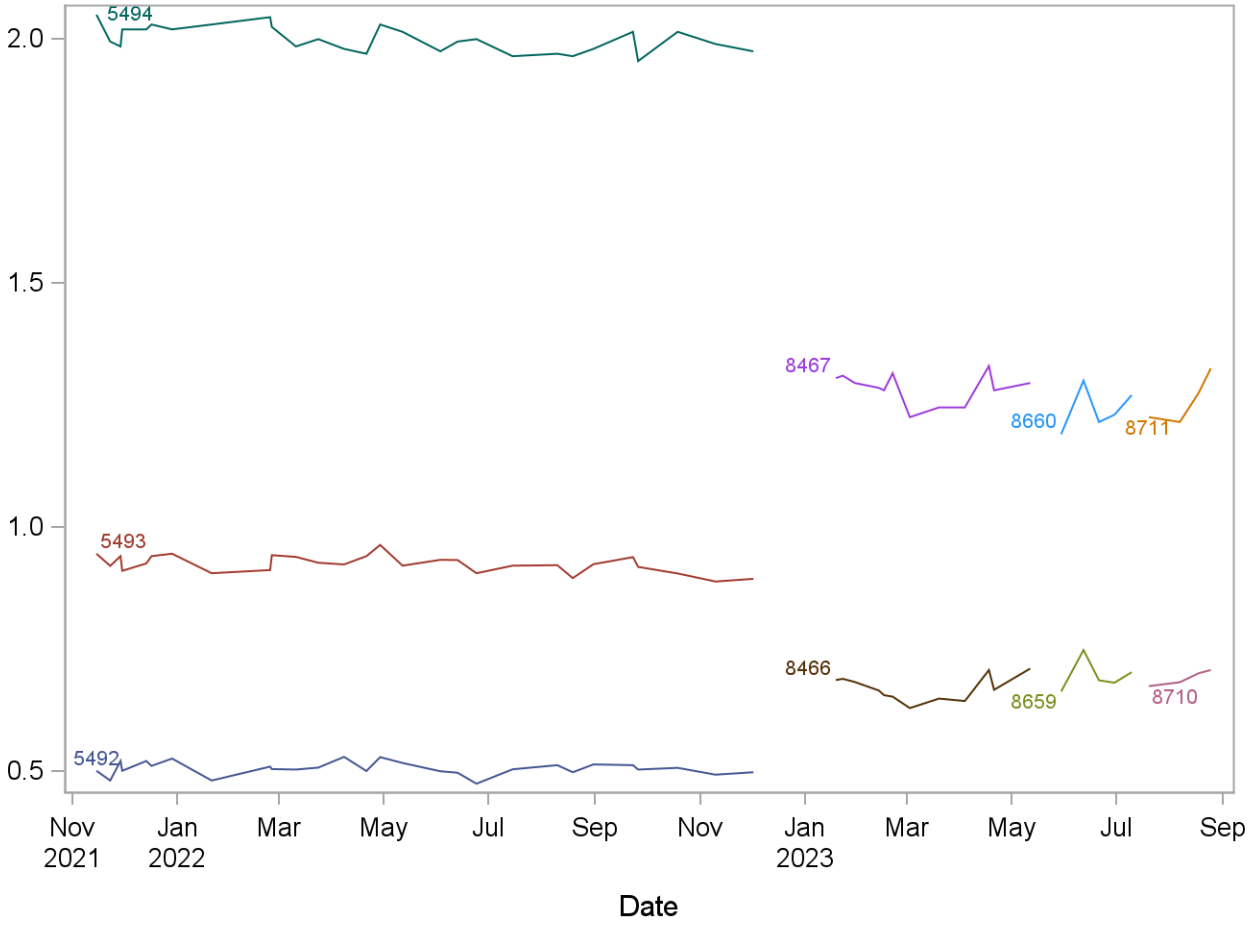
Method performance documentation for this method including accuracy, precision, sensitivity, specificity, and stability is provided in **Appendix A** of this method documentation. The approval of this procedure by the Branch chief and CLIA Director denote that the method performance is fit for the intended use of the method.

20. Summary Statistics and QC Graphs

Please see following page

August 2021-August 2023 Summary Statistics and QC Chart
LBXAGP (alpha-1-acid glycoprotein (g/L))

Lot	N	Start Date	End Date	MEAN	Standard Deviation	Coefficient of Variation
5492	28	15NOV21	02DEC22	0.5046	0.0136	2.7
5493	28	15NOV21	02DEC22	0.9238	0.0179	1.9
5494	28	15NOV21	02DEC22	2.0000	0.0263	1.3
8466	12	19JAN23	12MAY23	0.6691	0.0255	3.8
8467	12	19JAN23	12MAY23	1.2842	0.0316	2.5
8659	5	30MAY23	10JUL23	0.6956	0.0322	4.6
8660	5	30MAY23	10JUL23	1.2410	0.0439	3.5
8710	4	20JUL23	25AUG23	0.6904	0.0155	2.2
8711	4	20JUL23	25AUG23	1.2600	0.0507	4.0



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Acknowledgements

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Appendix A: Method Performance Documentation

Accuracy compared to Reference Material - fill in yellow shaded cells

Mean concentration should be within ±15% of the nominal value except at 3*LOD, where it should be within ± 20%

Method name:	α1-Acid Glycoprotein
Method #:	4049
Matrix:	Serum
Units:	g/L
Reference material:	**ERM-DA470k**
Analyte:	α1-Acid Glycoprotein (AGP)

Reference material	Replicate	Nominal value	Measured concentration								Difference from nominal value (%)
			Day 1	Day 2	Day 3	Day 4	Day 5	Mean	SD	CV (%)	
Level 1	1	0.617	0.59	0.62	0.63	0.65	0.65	0.64	0.02	2.95	2.9
ERM-DA 470K-A	2		0.63	0.65	0.65	0.64	0.64				
Level 2	1	0.450	0.45	0.45	0.47	0.47	0.46	0.46	0.01	2.70	2.9
ERM-DA 470K-B	2		0.47	0.45	0.48	0.48	0.45				
Level 3	1	0.300	0.29	0.28	0.31	0.31	0.27	0.30	0.01	4.76	-1.0
ERM-DA 470K-C	2		0.29	0.28	0.31	0.32	0.30				

Precision - fill in yellow shaded cells

Total relative standard deviation should be ≤ 15% (CV ≤ 15%)

Method name:	α1-Acid Glycoprotein
Method #:	4049
Matrix:	Serum
Units:	g/L
Analyte:	α1-Acid Glycoprotein (AGP)

ROCHE Precinorm Protein LOT # 186515

Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2
1	0.83	0.79	0.81	0.0004	0.0004	1.3122
2	0.79	0.8	0.80	0.000025	0.000025	1.26405
3	0.83	0.8	0.82	0.000225	0.000225	1.32845
4	0.77	0.75	0.76	0.0001	0.0001	1.1552
5	0.79	0.79	0.79	0	0	1.2482
6	0.82	0.81	0.82	0.000025	2.5E-05	1.32845
7	0.75	0.78	0.77	0.000225	0.000225	1.17045
8	0.76	0.8	0.78	0.0004	0.0004	1.2168
9	0.81	0.79	0.80	0.0001	0.0001	1.28
10	0.8	0.78	0.79	0.0001	0.0001	1.2482
Grand sum	15.84	Grand mean	0.792			

	Sum squares	Mean Sq Error	Std Dev	Rel Std Dev (%)
Within Run	0.0032	0.00032	0.017888544	2.26
Between Run	0.00672	0.000746667	0.014605935	1.84
Total	0.00992		0.023094011	**2.92**

ROCHE Precipath Protein LOT # 129504

Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2
1	1.51	1.52	1.52	2.5E-05	2.5E-05	4.59045
2	1.51	1.52	1.52	2.5E-05	2.5E-05	4.59045
3	1.59	1.54	1.57	0.000625	0.000625	4.89845
4	1.54	1.47	1.51	0.001225	0.001225	4.53005
5	1.52	1.53	1.53	2.5E-05	2.5E-05	4.65125
6	1.52	1.54	1.53	0.0001	0.0001	4.6818
7	1.50	1.48	1.49	0.0001	0.0001	4.4402
8	1.50	1.51	1.51	2.5E-05	2.5E-05	4.53005
9	1.54	1.54	1.54	0	0	4.7432
10	1.50	1.46	1.48	0.0004	0.0004	4.3808
Grand sum	30.34	Grand mean	1.517			

	Sum squares	Mean Sq Error	Std Dev	Rel Std Dev (%)
Within Run	0.0051	0.00051	0.02258318	1.49
Between Run	0.01092	0.001213333	0.018752778	1.24
Total	0.01602		0.029354159	**1.94**

Stability - fill in yellow shaded cells

The initial measurement can be from the same day for all stability experiments.

Freeze and thaw stability = Assess for a minimum of 3 freeze-thaw cycles; conditions should mimic intended sample handling conditions

Describe condition: three times frozen at -70°C and three times thawed (4 hrs) at room temperature (3 freeze-thaw cycles)

Bench-top stability = Assess short-term stability for length of time needed to handle study samples (typically at room temperature)

Describe condition: QC material stored at room temperature for 6 hrs, refrozen and thawed

Processed sample stability = Assess short-term stability of processed samples, including resident time in autosampler

Describe condition: QC material aliquoted into sample cups and stored on instrument for 2 hrs at room temperature before analysis

Long-term stability = Assess long-term stability that equals or exceeds time between date of first sample collection and date of last sample analysis

Describe condition: QC material stored at -70°C for 2 year

All stability sample results should be within ±15% of nominal concentration

Method name:	**α1-Acid Glycoprotein**
Method #:	4049
Matrix:	Serum
Units:	g/L
Analyte:	**AGP**

LOW Bench QC LS17523

10/23/2017	**Initial measurement**	**Three freeze-thaw cycles**	**Initial measurement**	**Bench-top stability**	**Initial measurement**	**Processed sample stability**	**Initial measurement**	**Long-term stability**
Replicate 1	0.596	0.587	0.596	0.575	0.596	0.630	0.486	0.500
Replicate 2	0.587	0.589	0.587	0.581	0.587	0.641	0.519	0.540
Replicate 3	0.587	0.590	0.587	0.577	0.587	0.640	0.489	0.490
Mean	**0.590**	**0.589**	**0.590**	**0.578**	**0.590**	**0.637**	**0.498**	**0.510**
% difference from initial measurement	--	**-0.2**	--	**-2.1**	--	**8.0**	--	**2.4**

MEDIUM Bench QC MS17524

10/23/2017	**Initial measurement**	**Three freeze-thaw cycles**	**Initial measurement**	**Bench-top stability**	**Initial measurement**	**Processed sample stability**	**Initial measurement**	**Long-term stability**
Replicate 1	1.034	1.051	1.034	1.019	1.034	1.119	0.917	0.910
Replicate 2	1.030	1.059	1.030	1.010	1.030	1.147	0.939	0.910
Replicate 3	1.025	1.042	1.025	1.039	1.025	1.147	0.920	0.960
Mean	**1.030**	**1.051**	**1.030**	**1.023**	**1.030**	**1.138**	**0.925**	**0.927**
% difference from initial measurement	--	**2.0**	--	**-0.7**	--	**10.5**	--	**0.1**

LOD, specificity and fit for intended use - fill in yellow shaded cells

Method name:	α1-Acid Glycoprotein
Method #:	4049
Matrix:	Serum
Units:	g/L

Analytes	Limit of Detection (LOD)	Interferences successfully checked in at least 50 human samples	Accuracy, precision, LOD, specificity and stability meet performance specifications for intended use
α1-Acid Glycoprotein	0.1	no significant interferences	yes

Kit assay is FDA approved - LOD and interference information are provided by the manufacturer

Appendix B: APM Job Aids

A. General:

JA-4049-G-01-Calibration and Calibration Verification

Roche standardized this method against the reference preparation of the IRMM (Institute for Reference Materials and Measurements) BCR470/CRM-DA 470 (RPPHS- Reference Preparation for Proteins in Human Serum).

Calibration

After entering new calibrator values via the Cobas Link, a calibration must be performed prior to measurement of patient samples and controls. Manufacturer calibrators are used for automatic calibration if the calibrators are placed on-board on the analyzer and calibration is requested by the analyst.

STD 1: **diH_2O (g/L):** Zero calibrator

STD 2 - STD 6: **C.f.a.s. Proteins (g/L):** Calibrator for automated systems (liquid form & ready to use)

A full six -point calibration is recommended:

- After reagent lot change
- As required following quality control problems

After lamp and/or cell changes; any major repairs or maintenance to the instrument. The Cobas c pack containing reagents (R1 and R2) is ready for use and stable for up to 12 weeks when opened and stored refrigerated (+2°C to +8 ^{0}C) on the analyzer or until the expiration date on the c pack of reagents is reached, whichever comes first. Unopened c packs stored refrigerated (+2°C to +8 ^{0}C) are stable until the expiration date on the package. **C.f.a.s. Proteins** is stable when unopened and refrigerated (+2°C to +8^{o}C) until the expiration date on the package. After opening, the calibrator is stable for up to 4 weeks under refrigerated conditions (+2°C to +8^{o}C), provided that dispensing of the calibrator occurs without microbial contamination.

Note: Allow the calibrators, QC, and patient samples to reach ambient temperature (+15°C to +30°C); before use mix carefully to avoid the formation of foam and ensure homogeneity.

Update calibrator information in the system when a new lot number of calibrators is used for calibration, review calibrator information after update. Check calibrator status in the system before running QC and patient samples. Calibration status can be checked by going to the **"Calibration**" tab; next go to **"Calibration Status"** tab. Any test highlighted gray requires calibration. Calibration status can also be checked in the system overview page by selecting **"Calibration and QC Select"** and if calibration update is required **"Calibration and QC Select"** will be yellow in the status window. Touch under calibration on "**Recommended"** and under QC on **"Routine QC"** to confirm if calibration and/or QC status is required. Ensure rack position is assigned to non-barcoded calibrators.

Analytical Measurement Range

Reportable Range: 0.1 g/L up to 4.0 g/L

Determine samples having higher concentrations via the rerun function. Dilution of samples via the rerun function is a 1:1.5 dilution. Results from samples diluted by the rerun function are automatically multiplied by a factor of 1.5.

If the result is still >4 g/L, repeat by diluting the sample manually 1:4 using 50 µL of patient sample and 150 µL of 0.9% NaCl (commercially available or Roche c pack**, 9% NaCl** that has been diluted by instrument to 0.9%). Multiply the result by 4. There is no known maximum acceptable dilution.

Calibration verification

Calibration verification is **not required by the manufacturer**.

According to the updated CLIA regulations from 2003 (see also statement of the Joint Commission on Accreditation of Healthcare Organizations), the requirement for calibration verification is met if the test system's calibration procedure includes three or more levels of calibration materials, **and** includes a low, mid, and high value, **and** is performed at least once every six months. All these conditions are met with the calibration procedure of this assay, and therefore no additional calibration verification is required by CLIA.

Perform Calibration verification whenever any of the following occur:

- All of the reagents used for a test procedure are changed to new lot numbers, unless the laboratory can demonstrate that changing reagent lot numbers does not affect the range used to report patient test results, and control values are not adversely affected by reagent lot number changes.
- There is major preventative maintenance or replacement of critical parts that may influence the test's performance. This includes when the laboratory sends a test system to the manufacturer for repairs. The laboratory must check the calibration of a repaired test system before resuming patient testing and reporting results.
- Control materials reflect an unusual trend or shift, or are outside of the laboratory's acceptable limits, and other means of assessing and correcting unacceptable control values fail to identify and correct the problem.
- The laboratory has determined that the test system's reportable range for patient test results should be checked more frequently.

For calibration verification or for troubleshooting, international standard reference material can be analyzed throughout the year. However, it should be noted that the concentration of the reference material is much lower than the upper range of the assay.

European Community Institute for Reference Materials and Measurements (IRMM, https://ec.europa.eu/jrc/en/reference-materials) **ERM-DA470k (formerly CRM470 – May 2004)**.

The AGP certified mass concentration of 0.617 + 0.013 g/L (concentration of reconstituted vial) is traceable to ERM-DA470. Reference material stored deep frozen (-50°C to -90°C).

The material must be reconstituted according to the following procedure:

1. Remove the vial from the freezer or refrigerator during the afternoon of the day before use and place the vial for 1 hour on work bench at ambient temperature (+15°C to +30°C).
2. After 1 hour tap the bottom of the vial gently on the surface of the work bench. Make sure that all the material has settled down on the bottom of the vial. Remove the screw cap.
3. Weigh the vial together with the rubber stopper. Note down the mass or press the "TARE" knob on the balance. Lift the rubber stopper with care until air is allowed to enter the vial and the groove in the rubber stopper becomes accessible.
4. Add exactly 1.00 mL deionized water through the groove and press the rubber stopper back into place. Weigh the vial and note down the mass. If you have used the "TARE" function, the value can be used directly for the **"mass *m*"**. Otherwise, the first mass must be subtracted from the second to obtain '***m***'.
5. The concentration of a particular protein in the solution, corrected for the reconstitution mass, can be obtained by multiplying the certified value for that protein with $m_{intended}$ / m, with m_{intended} the mass intended to be added (1.000 g).
6. Leave the vial at ambient temperature (+15°C to +30°C) for one hour, then invert it carefully at least five times (do not shake it) during the next hour.
7. Leave the vial at ambient temperature (+15°C to +30°C) overnight. On the day of use invert the vial carefully five times within one hour.

Dilutions of the reference material with Roche diluent, saline or diH2O appears to under-recover AGP, likely due to a matrix effect. This is also supported by the fact that the Roche calibrator is in a protein solution. A simple calibration verification experiment will be done using the Roche CFAS Protein calibrator to make dilutions of reference material.

	Volume (uL)	
	ERMDA470k	CFAS Protein
Soln A	0	200
Soln B	200	200 of Soln A
Soln C	200	200 of Soln B
Soln D	200	200 of Soln C

Assay these solutions at least in duplicate as an unknown. Results should be within ±20% of the target values according to Roche Diagnostics product specification for low AGP concentrations:
Roche linearity specifications: for values <1.2 g/L accept a deviation of the absolute value of < ± 0.12 g/L.

AGP Gen2 kits were validated for use on the new Cobas c501 in April 2017.

JA-4049-G-01-Alternative In-house Proficiency Testing

Background

An external proficiency testing program is not available for the analysis of α1-Acid Glycoprotein (AGP). Because of this situation, the Audit-Sample Procedure alternative proficiency testing program, as described in the guideline of the **Clinical Laboratory Standards Institute** (CLSI) QMS24 [1]. Because of the lack of other laboratories performing the same type of testing, this procedure was considered the most appropriate among those described in this guideline.

Principle

Aliquots of a pooled specimen are stored deep frozen (-50°C to -90°C) by the laboratory and analyzed periodically across time. Periodic analysis of aliquots of the audit samples assesses imprecision of the assay. The pooled specimens are blinded in a manner that the analysts do not recognize the type of the pool based on the pool ID. The Audit-Sample Procedure does not evaluate accuracy (i.e., bias), nor provide inter-laboratory comparison.

Procedure

Generation of pools

A set of proficiency testing pools were generated by using serum ordered from Bioreclamation-IVT and Tennessee Blood Services. Serum specimens were screened for AGP concentrations; pools were made by combining multiple serum samples to achieve appropriate AGP levels and pool volume. This coincides with the Audit-Sample Procedure, which states that the minimum number of pools must be at least, but not limited to 3.

Labeling/aliquoting of pools

Labels for proficiency testing aliquots were generated by a QA officer within NBB. The QA officer wrote a code, not available to the analyst, capable of randomly generating 7-digit, non-repeating numbers so that each vial has a unique identifier. Each label contains the randomly generated 7-digit sample ID number, and the corresponding barcode. The 7-digit sample ID number will be decoded by the QA officer or supervisor into the pool ID at the time of result verification. The decoding program is only available to the QA officer and supervisor. All pools were aliquoted in 1-mL increments (approximately 800–900 aliquots per pool) into 2-mL Nalgene cryogenic vials and stored deep frozen (-50°C to -90°C).

Characterization of pools

To generate the target values for open label blind QC pools (OLB), the analysts must first characterize the pools by measuring 10 vials from each pool in duplicate across a minimum of 10 different runs to obtain the means and standard deviations.

Receipt of Samples

Two times per year a proficiency testing challenge will be performed as requested by the project lead or supervisor. For that, QA officer will randomly select 5 vials for the analyst to run.

Documentation, Review, and Reporting

Twice per year a proficiency testing challenge will be performed using 5 in-house prepared PT vials. If the run with the PT results has been approved, the TL uploads the PT results in the STARLISM's PT application and then

checks the data to see if at least 4 of the 5 results are within limits (80% is considered passing for CLIA purposes), (manually until Dec 2019, performed in STARLIMS starting January 2020). The limits are determined using the characterization mean ± 3 SD (for OLB QC pools) or the COA assigned target ± 15% (for SRM materials). The TL then passes the data to the QA officer, who grades the PT challenge with a report, which automatically attaches to the PT challenge (Graded Challenge). Starting the 2nd half of 2021, the QA officer and the supervisor sign the report to acknowledge its review.

If the results meet the above criteria, no further action is required. If less than 4 of the 5 proficiency testing samples are within the limits for a given analyte, the challenge is considered as failed and appropriate corrective action needs to be initiated to correct this problem. After correcting the problem, another set of 5 proficiency testing samples will be requested and the proficiency testing challenge must be repeated. Patient samples will be analyzed only after the proficiency testing challenge was passed.

Data Handling and Storage

The analyst formally acknowledges on the results sheet that the PT samples have been handled the same way as patient samples.

The Lab Chief reviews the results and signs the results sheet to attest to the routine integration of the samples into the patient workload using the routine method. Data and results from the proficiency testing challenges should be stored on the network.

The characterization data for the OLBQC pools is saved on network and In-house PT challenge data is saved on STARLIMS database for documentation

Current Status

The procedure (alternative in-house PT) as described above has been implemented for 4049 method starting November 2017.

Reference

[1] Clinical and laboratory Standards Institute (CLSI). Using Proficiency Testing and Alternative Assessment to Improve Medical Laboratory Quality. 3ed ed. CLSI guideline QMS24. Wayne, PA: CLSI, 2016.

B. Instrumentation:

JA-4049-I-01-Instrument Maintenance & Function Checks

Ensure the analyzer is in shutdown status or in an appropriate maintenance mode prior to maintenance actions. For most routine maintenance actions (e.g., daily, weekly, monthly, and 3-monthly) the c501 module can be in standby mode.

Daily Maintenance: Daily maintenance includes processing of green wash rack, cleaning sample probe, reagent probes, ISE probe & ISE sipper nozzle; cleaning cell rinse nozzles; cleaning the drain port for high concentrated waste. Materials required for daily maintenance of c501 module are green rack (wash rack), sample cleaner I; ISE cleaning solution (SysClean), ISE conditioning solution (activator), lint-free gauze squares, Alcohol (e.g., isopropyl alcohol or ethanol), deionized water (diH_2O) & paper towel.

Cleaning green wash rack: A green wash rack containing detergents (SysClean) and activator must be processed once every 24 h or at the end of the day after sample analysis is completed. A sufficient volume of Activator and SysClean must be filled into the respective sample cups (otherwise alarm comes on) and then placing these in the correct position of the green wash rack.
P-mask the ISE before running the green wash. From masking window click **Start (**global button) select **Masking**. Place sample cups at position 1 to 3 on a green rack (wash rack) and fill with suitable amounts of sample cleaner I, SysClean and activator. Check number of cycles (5 to 15 cycles recommended for photometric & ISE unit) for detergent pipetting by choosing **Utility** > **System;** save changes and start maintenance**.** Next request a full calibration for all ISE tests on the **Calibration Status** screen. Place the racks in the respective sequence on the rack loader: green wash rack > Calibrator rack containing the ISE calibrators > QC rack containing the QCs for ISE tests. Next choose **Start >** (global button) > **Start Conditions** from screen. Check the results for calibration and QCs and if the analyzer has generated a valid calibration and the QC results unmask the ISE tests. The analyzer is ready to process routine samples.

> **Note:** *Note: If the processing of the green wash rack is interrupted for some reason, the maintenance of (1)* ***Wash Reaction Parts*** *(2)* ***Reagent Prime*** *should be performed.*

Cleaning sample probe, reagent probes, ISE probe and ISE sipper nozzle: Impurities on the sample probe may cause problems and affect results. Prior to sample analysis clean the outside of pipettor probes (sample probe, reagent probes, ISE probe) and ISE sipper nozzle to remove residual solution and precipitation. This is a combined maintenance procedure for both ISE and photometric unit.

1. Put Analyzer in shutdown status or standby mode and unlock/open module top cover.
2. Move pipettor probes and sipper nozzle by hand to an accessible position.
3. Wipe (from top to bottom) the outsides of the sample, reagent and ISE probes and the sipper nozzle with gauze pads moistened with alcohol.
4. Also wipe the inside of the shield pipe with a gauze pad moistened with alcohol.
5. Close the top cover of the module and lock it.
6. Switch "**ON"** Analyzer if in Shutdown mode and test probe operation.
7. Proceed with **Cell blank measurement:** Choose **Utility** > choose **Maintenance & from maintenance list** >Choose **Cell Blank Measurement >** Choose **Select** to open the **Cell Blank Measurement** window>Select a module**>**Choose **Execute** to complete the task**.** Print & review the cell blank measurement results and add to the calibration binder.

> **Notes:** *After cleaning the probe, its discharge and operation should be checked. When cleaning, take care not to bend or damage the probes or sippers.*

Cleaning cell rinse nozzles: Prior to sample analysis, cell rinse nozzles are cleaned. Regular cleaning prevents contamination, crystal formation, and blockages.

1. Put Analyzer in shutdown status or standby mode and unlock/open module top cover.
2. Loosen the retaining screw of the cell rinse unit and lift off the entire unit.
3. Moisten a lint-free gauze pad with diH_2O and gently wipe all tips of the cell rinse unit nozzles in a downward motion.
4. If a nozzle is clogged, insert the probe cleaning wire (stainless steel wire, 0.5 mm diameter) into the tip of the nozzle and eliminate the blockages.
5. Align the pin holes of the cell rinse unit with the guide pins and attach the rinse unit.
6. Fix the tube retainer below the screw and then tighten the retaining screw. Close the top cover of the module and lock it.
7. Switch "**ON"** Analyzer if in shutdown mode.

Cleaning the drain port for high concentrated waste: Prior to sample analysis the outlet of the drain port for high concentrated waste is cleaned. Regular cleaning prevents contamination, crystal formation, and blockages. Analyzer is put in shutdown status/standby mode. diH_2O is applied to the outlet of the drain port at the rear of the module and crystals rinsed off using cotton swabs. Switch "**ON"** Analyzer if in shutdown mode.

Weekly Maintenance: Weekly maintenance includes cleaning of reaction system, cell covers, rinse stations and manual removing & cleaning of IS bath. Materials required for weekly maintenance are detergents for sample probe, reagent probe & reaction cell probe; reagent (sample cleaner 1 & NaOH-D cassettes), wash bottle, cotton swabs, 2% Eco-Tergent solution, diH_2O, and alcohol.

Rinsing the reaction system: Choose **Utility** > **Maintenance** >from **Maintenance Types** >Select **Wash Reaction Parts** on the **Maintenance** Items list> Choose **Select** to open the **Wash Reaction Parts** window & Select a module > & choose **Execute** to complete the task. When procedure finishes the module returns to standby mode. Next proceed with the cell blank measurement (as described above), print/review cell blank measurement results & add to the calibration binder. If cell blank measurement is not completed an error would be indicated & cell blank measurement must be performed again.

Cleaning the cell covers: Analyzer is put in shutdown status or standby mode. Unlock and open the top cover of the module and loosen the screws; remove the cell cover above the ultrasonic mixers. Wipe the front and rear faces of the cell covers using a gauze pad moistened with alcohol. Wipe the openings of cell covers using a cotton swab moistened with alcohol (carefully so that alcohol doesn't splash in the reaction cells). Return the cell cover above the ultrasonic mixers. Close the top cover of the module and lock it. Switch **"ON**" Analyzer if in shutdown status.

Cleaning the rinse stations: To prevent bacterial growth or precipitation that may clog the rinse stations cleaning the rinse stations of the sample probe, reagent probes, ISE probe and ISE sipper nozzle at least once a week is recommended.

Analyzer is put in shutdown status or standby mode. Unlock/open the top cover of the module and move the sample probe, reagent probes, ISE probe and ISE sipper nozzle to the positions that leave the rinse stations easily accessible. Using cotton swabs moistened with 2% Eco-Tergent solution, clean the inside of each rinse station. Inject about 10 mL of 2% Eco-Tergent solution into each of the rinse stations (Be careful not to splash water on the drying cylinder [for vacuum suction]). Inject about 100 mL of diH_2O to each of the rinse stations to thoroughly rinse those. Wipe the drying cylinder (used for vacuum suction in the sample probe rinse station) with cotton swabs moistened with alcohol. Close the top cover of the module and lock it. Switch **"ON"** Analyzer if in shutdown status.

Removing and manually cleaning the IS bath: Crystals may remain on the upper part of the IS bath even after daily automatic rinsing. Therefore, removing and manually cleaning the IS bath once a week is recommended. Analyzer is put in shutdown status or standby mode. Unlock/open the top cover and loosen the screws to remove the IS bath. Wash away any crystals or contaminations in the IS bath with diH_2O. After washing IS bath drain the

water from it and attach in the Analyzer. Close the top cover of the module and lock it. Switch **"ON"** the Analyzer, if in shutdown status.

Monthly Maintenance: Incudes, cleaning the water tank, replacing reaction cells, cleaning incubator bath, detergent aspiration filters, the ISE ref (KCl) aspiration filter, and filters behind the front doors & filters of the rack sampler unit. Materials needed for monthly maintenance are 0.5% sodium hypochlorite solution, diH_2O, lint-free gauze pads, paper towels & brush.

Cleaning the water tank: Instrument is shut down and water tank is disconnected, taps are closed at the outlet of the water tank. A paper towel is placed under the hose unit to absorb extra water. Disconnect the quick release connector by rotating the release collar until it lines up with its key. Pull back the spring-loaded collar to separate the eater hose from the tank. Pulling the water tank sideways, remove the liquid level sensor assembly from the tank and place it on a paper towel, then empty the water from the tank. Wipe the liquid level sensor assembly with gauze pads soaked with diH_2O. Rinse (x3) the water tank thoroughly with diH_2O (x3). Fill the tank (at least 1/3 full) with fresh diH_2O, reattach the liquid level sensor assembly; place the tank to its original position and reconnect the water hose to the water tank and open the taps. Ensure main water supply is "ON" and next turn Analyzer **"ON".**

Notes: *A thorough cleaning of water tank is done using 0.5% sodium hypochlorite solution for rinsing and a brush is used to clean the interior surface followed with thorough washing with tap water and* diH_2O *(x3 each). If the fittings of the water tank are not connected properly, water may leak. Ensure that all joints are connected properly.*

Replacing reaction cells: Replacing the reaction cells and cleaning the incubator bath (with its drain filter) can be done simultaneously. Put the c501module in shutdown status/or standby mode; unlock and open top cover of the module; by removing thumbscrew loosen & remove reaction cells & dispose of these in appropriate waste container. Remount new reaction cells & perform maintenance for "**Wash Reaction Parts"**> next proceed with **Maintenance** for Cell Blank Measurement and to verify the integrity of the reaction cells. Print/review cell blank results & add to the calibration binder.

Cleaning the incubator bath: Contamination inside the incubator bath or on the photometric window will reduce the reproducibility of measurement results. At least once a month cleaning of incubator bath and photometric window is recommended.

Notes: *Roche recommends that this maintenance be combined with the weekly cleaning of the IS bath, with the monthly replacement of reaction cells and with the quarterly cleaning of the ultrasonic mixers.*

Put the c501 module in shutdown status (or in incubator bath cleaning mode) **>** choose **Utilit**y>**Maintenance**> Select **Maintenance (1)** from the maintenance list**> select Incubator Bath Cleaning** & choose **Execute** to complete the task. Wait until the message for the incubation cleaning is displayed. Unlock and open top cover of the module; detach cell rinse unit and reaction cells. Clean incubator bath, carefully wipe the photometer windows using lint-free gauze pads soaked in diH_2O. Reattach reaction cells and cell rinse unit and refill the bath (~500 mL diH_2O); start up the analyzer performing maintenance item **(5).** If Incubation Water Exchange or/Incubator Bath Cleaning is used >choose **Continue** on the Utility to release the incubator bath cleaning mode. Perform maintenance item **(7**) for Wash Reaction Parts and item **(4**) for Cell Blank Measurement; print/review cell blank results & add to the calibration binder.

Cleaning the detergent aspiration filters: Cleaning these filters (attached to the tube ends) each time when a reagent bottle is replaced or at least once a month is recommended. Remove the filter from the tube end; clean and rinse the filter with diH_2O. Perform maintenance item **(9)** for Cell Detergent Prime.

Cleaning the ISE Ref. (KCl) aspiration filter: Cleaning the filter each time when ISE Ref. bottle is replaced or at least once a month is recommended. Remove the filter from the tube end, clean and rinse the filter with diH_2O. Perform maintenance item (**8**) for Reagent Prime with the Ref. option selected.

Additionally, filters behind the front doors and the filter at the rear end of the rack sampler unit are cleaned at least once a month using a vacuum cleaner to remove any dust.

3-months Maintenance: Cleaning the ultrasonic mixers every 3 months is recommended. Contamination and precipitation on the surface of the ultrasonic mixers may cause inadequate mixing and lead to inaccurate results. If the ultrasonic mixer cleaning coincides with the monthly incubator bath cleaning, the procedure can be performed together.

Notes: *The ultrasonic output intensity is continually monitored during measurement. If the data alarm <Mix occurs frequently, replacement of the ultrasonic mixer is required (service call is logged for replacement).*

Cleaning the ultrasonic mixers comprises of 2 maintenance items (1) cleaning the surface of the ultrasonic mixer and (2) checking the intensity of the ultrasonic output. Materials required for cleaning are: 2% Eco-Tergent solution, diH_2O, and cotton swabs. Analyzer is put in incubator bath cleaning mode next > choose **Utility** > **Maintenance** >Select (1); from the **Maintenance Type** list select **(10)** for **Incubator Bath Cleaning > Select** to open the **Incubator Bath Cleaning** window > choose **Execute** to complete the task. The Analyzer turns to incubator bath cleaning mode and water drains out from the incubator bath. Choose **Monitor (**to confirm message**) >** choose **OK** to open the **Maintenance Monitor** window & wait for the message about the Incubator bath cleaning mode. Unlock and open the top cover of the module; remove the cell cover, loosen, and remove the thumbscrews of the reaction cell near the ultrasonic mixers. Lift the reaction cells out of the reaction disk (be careful not to touch the optical surfaces); gently wipe the surface of the ultrasonic mixers with cotton swabs moistened with 2% Eco Tergent solution. Next wipe off the detergent with cotton swabs moistened with diH_2O; return the removed sections of reaction cells; return the cell cover; close the top cover of the module and lock it. Choose **Start** (>global button) > **Start** to release the incubator bath cleaning mode and fill in water into the incubator bath.

To check the intensity of the ultrasonic output: Choose **Utility** > **Maintenance >** select **Check (2);** from the **Maintenance Type** list select **(7) for Cuvette Mixing >**choose **Select**, to open the **Cuvette Mixing** window > select a module >verify the **Cell Wash** check box is not selected (*selecting the **Cell Wash** check box would rinse all reaction cells prior to the actual intensity check*) > choose **Execute** to complete the task.

Photometer lamp is replaced every 6 months as the reproducibility of measurement will decrease if the photometer lamp deteriorates. Replace the photometer lamp if the lamp has been used for more than six months, for more than 750 hours of continuous powered-on time or if the photometer reading exceeds 14000 (*whichever comes first*). Roche recommends combining this maintenance with the monthly cleaning of incubator bath if the readings have exceeded 14000.

Notes: *Every six months preventive maintenance of the entire instrument unit is done by Roche service engineer as part of the service plan agreement*

C. Data Review:

JA-4049-DR-01-Computerization & Data System Management

1. **Sample Identification**

During sample preparation and analysis, samples are identified by their sample ID. The sample ID is a number that is unique to each sample that links the laboratory information to demographic data recorded by sample collectors.

2. **Data Collection & Data Back-up**

Roche Cobas® 6000 result files are collected and stored using the software on the Roche Cobas® 6000 workstation. After the data from each run is carefully reviewed, the result files containing patient data as well as QC data are transferred to the NBB Instrument drive on the network via a CDC encrypted USB drive.

3. **Data Import**

The data files are imported from the NBB Instrument drive into the STARLIMS database for QC and statistical evaluation.

4. **STARLIMS Data Review**

Level I – Analyst

- Double click the STARLIMS icon on desktop
- Under 'Run-based Tasks', select 'Pending Runs Assigned to My Labs'
- Choose 'Show Pending Tests' and select test from drop down menu
- Click on 'Add' and select the Instrument
- Run# and Equipment ID will be populated
- [0] Run Instrument Macro – select the excel result file to run macro for STARLIMS import
- [1] Upload Instrument File – import the post-macro result file to STARLIMS
- [2] Mark Null Results – click this button which replaces the null with "|" (a pipe tab)
- [3] Evaluate Sample QC – check the sample QC flags according to the defined criteria
- [4] Evaluate Run QC – evaluate bench QC via the DLS SAS Multi-Rule System QC program to determine QC pass/fail
- [5] Set Run QC Statuses – *set analytes pass/fail based on SAS out-of-control assessment*
- [6] Attach SAS QC file – upload both the SAS input file (.csv) and output file (.pdf)
- Enter run bench QC (SAS) information in Run Comments column
- Click on 'Manage Attachments' and upload the data review checklist for the run
- *Click 'Finish Results' located under the test workflow steps and notify Project Lead*

Level II – Project Lead

- Double click the STARLIMS icon on desktop
- Under 'Run-based Tasks', select 'Run Approval'
- Choose 'Show Pending Tests' and select test from drop down menu
- Review analyst data review checklist and Sample QC evaluation
- Review and confirm Run QC evaluation
- Assess blind QC results - click on 'Blind QC Results Only' tab, 'Assess Blind QC', 'Final Result' and 'Use Default Characterization Sets', 'Proceed to Next Step', 'OK'
- Print blind QC report - click on 'BQC Reports', 'All data displayed in the data-grid', 'A paper-based report from template', 'NBB Blind QC Report v2', 'OK', 'Proceed to the SSRS Report', Save PDF
- Enter bench QC (SAS) and blind QC evaluation status in the Run Comments column
- Set results final - in 'All Results (S)' tab, click on 'Set Final' Wizard, select 'Process all samples displayed in the 'data-grid' and 'Run the Set Final Wizard' and click 'Proceed'
- Choose Set final criteria - check 'Required Sample QC Passed' and 'Required Run QC Passed'; check 'Pass' and 'Warn' for 'Allowable Results Statuses for Set Final'; choose date range to cover runs that may include the previous analysis of these samples; click 'Proceed'
- Resolve samples with retest results and set final
- Submit sample IDs and repeat instructions to the analyst to schedlue the repeats
- Click on 'Manage Attachments' and upload the blind QC report and Team lead data review checklist for the run
- In Run Approval tab click Release Run and notify QA Officer (for level III review)

General Supervisor (Lab Chief)

- Conduct random “spot checks” to verify proper handling of lab results
- Discuss with Team Lead or QA Officer course of action on difficult questions
- Results set reportable, released, and reported by QA officer

JA-4049-DR-02-Out-of-Control Corrective Action

1. QC performance is evaluated by SAS – run comments updated appropriately (pass/fail)

2. SAS QC failure is investigated, and appropriate corrective action measures as indicated are applied.

    - Verify that the proper QC was used.
    - If QCs are failing, Check flags to see if there was problem during sample preparation/analysis.
        - If a QC failed due to sample preparation/analysis issue, apply the appropriate code, flag this QC as rejected & fail (update comment section); exclude failed QC and re-run SAS to check if the remaining QC pass.
        - If run passes notify team lead/supervisor for approval.
    - If the QC failure is true (potential statistical issue); apply code 61 and repeat samples in next run.
    - Enter appropriate comments in STARLIMS database (User fields 1&2).
    - In case QCs failed due to changes in calibrator lot, instrument issues etc. troubleshoot, recalibrate, and re-evaluate otherwise login for service.

JA-4049-DR-03-STARLIMS Data Review Flowchart