Manual 7- Addendum
Central Laboratory Procedures

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Study website - http://www.cscc.unc.edu/hchs/

Central Laboratory Procedures
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SECTION 1 – ANALYTICAL METHODS FOR CLINICAL ASSAYS

Alanine Aminotransferase

Minimal Description for Publication: Alanine aminotransferase is measured in serum on a Roche Modular P Chemistry Analyzer (Roche Diagnostics Corporation) using an alpha-ketoglutaratic enzymatic method (Roche Diagnostics, Indianapolis, IN 46250).

Principle: ALT activity is determined by a modification of the method recommended by the International Federation of Clinical Chemistry (IFCC). ALT catalyzes the reaction of alpha-ketoglutarate with L-alanine to form L-glutamate and pyruvate. Under the action of LDH, pyruvate converts to lactate, and NADH is converted to NAD. The activator pyridoxal phosphate is not included in this reagent system. The decrease in absorbance of NADH, measured at 340 nm (secondary wavelength is 700 nm), is directly proportional to the serum activity of ALT. It is a kinetic rate reaction.

Specimen: Serum from a serum separator tube (biospecimen collection tube #1) is used for analysis. The serum is separated from the cells within 2 hours of collection and stored at -70° C until assayed.

Interferences: Bilirubin does not interfere up to an I index of 60. Hemolysis interferes due to the presence of ALT in erythrocytes. Therefore hemolyzed specimens should not be analyzed for ALT activity. Lipemia does not interfere up to an L index 500.

Equipment: Roche Modular P chemistry analyzer (Roche Diagnostics, 9115 Hague Road, Indianapolis, IN 46250).

Reagent: Roche product #11876805, ALT (ALAT/GPT) reagent kit (Roche Diagnostics, 9115 Hague Road, Indianapolis, IN 46250).

Calibration: Roche Calibrator for Automated Systems (C.F.A.S.), catalog #759350. The ALT value assigned to the C.F.A.S. calibrator is traceable to the 1985 IFCC reference method. Calibration of the ALT method is typically performed only at the time of instrument installation. At that time a K factor is assigned to the test, and re-calibration is usually not necessary. Monitor control values to determine stability of the current calibration.

Quality Control: Two levels of control are assayed each time the ALT method is performed. It is acceptable to run each control at the start of the day, and again at the end of the day. The operator may run them more frequently, if desired. One control is prepared from pooled, normal human serum. The other is an elevated, abnormal commercial control. Consult the quality control detail table for current ranges and lots in use.

Expected Values:

- Reference range: female 0-44 U/L male 0-66 U/L
- Linear range of the method: 4-600 U/L. Specimens exceeding the high limit are automatically diluted (1:11) by the instrument, and reported accordingly. If a manual dilution is required, dilute the specimen in normal saline, and multiply the result by the dilution factor. Report values less than 4 as <4 U/L.
- Analytical Measurement Range: 4-600 U/L
- Clinically Reportable Range: 4-2000 U/L

Reference:

1. Roche/Hitachi System Application Sheet for ALT, 2005.
2. Package insert for C.F.A.S., 2005.
3. Roche/Hitachi Modular Analytics Operator's Manual, version 2.0, October 2006.

Albumin, Urine and Albumin/Creatinine Ratio

Minimal Description for Publication: Albumin is measured in urine using an immunoturbidometric method on the ProSpec nephelometric analyzer (Dade Behring GMBH. Marburg, Germany D-35041).
Principle: A solution of rabbit-derived anti-human albumin is incubated with the urine specimen. An immunocomplex forms between the antibody and the albumin in the specimen, resulting in an increase in light scatter. The higher the concentration of albumin, the more intense the degree of light scatter. The albumin concentration of the test specimen is determined by comparing its light scatter to that observed using known standards in a calibration curve.
Specimen: A random urine specimen not treated with any stabilizer or additive is used for analysis. Specimens are centrifuged for at least 10 minutes at 1,500 x g prior to analysis. This removes are particulate matter that could affect the light scatter measurements.
Equipment: ProSpec nephelometer (Dade Behring GMBH. Marburg, Germany D-35041).
Reagent: Product #OSAL 15. (Dade Behring GMBH. Marburg, Germany D-35041).
Calibration: Dade Behring product #OQIM 13 (3 x 1.0 mL). Albumin concentration will vary with lot. Dade Behring provides periodic calibrator lot and concentration updates on compact disk. When these parameters are read into the system, it is only necessary for the instrument to read the calibrator's barcode to determine its albumin concentration. The reference line is valid until controls demonstrate drift, the reagent lot changes, or the calibrator lot changes. After re-calibration, assay at least five specimens on the old lot and on the new lot. Each of their differences must be within the current posted QC duplicate limit.
Quality Control: There are two levels of controls: one is pooled from routine urinalysis specimens and the other is a dilution of a serum pool. Both controls are assayed with each batch of samples. Consult the quality control detail table for current ranges and pools in use.
In addition to pools, at least one specimen as a within-batch duplicate. The difference in the results must be within the current posted QC duplicate limit.
Expected Values:
Reference range: 0 – 20 mg/g creatinine (see Creatinine method, pg 9 for details)
References:
1. Dade Behring BN ProSpec Nephelometer Instruction Manual. Dade Behring Diagnostics GmbH, Postbox 1149, D35001 Marburg 1, Germany.

Aspartate Aminotransferase

Minimal Description for Publication: Aspartate aminotransferase is measured in serum on a Roche Modular P Chemistry Analyzer (Roche Diagnostics Corporation) using an alpha-ketoglutaratic enzymatic method (Roche Diagnostics, Indianapolis, IN 46250).
Principle: AST activity is determined by a modification of the method recommended by the International Federation of Clinical Chemistry (IFCC). AST catalyzes the reaction of alpha-ketoglutarate with L-aspartate to form L-glutamate and oxaloacetate. Under the action of malate dehydrogenase (MDH), oxaloacetate converts to malate, and NADH is oxidized to NAD. The decrease in absorbance of NADH, measured at 340 nm (secondary wavelength = 700 nm), is directly proportional to the serum activity of AST. It is a kinetic rate reaction.
Specimen: Serum from a serum separator tube (biospecimen collection tube #1) is used for analysis. The serum is separated from the cells within 2 hours of collection and stored at -70° C until assayed.
Interferences: Bilirubin does not interfere up to an I index of 60. Hemolysis interferes due to the presence of AST in erythrocytes. Therefore hemolyzed specimens should not be analyzed for AST activity. Lipemia does not interfere up to an L index 500.
Equipment: Roche Modular P chemistry analyzer (Roche Diagnostics, 9115 Hague Road, Indianapolis, IN 46250).
Reagent: Roche product #11876848, AST (ASAT/GOT) reagent kit (Roche Diagnostics, 9115 Hague Road, Indianapolis, IN 46250).
Calibration: Roche Calibrator for Automated Systems (C.F.A.S.), catalog #759350. The ALT value assigned to the C.F.A.S. calibrator is traceable to the 1985 IFCC reference method. Calibration of the ALT method is typically performed only at the time of instrument installation. At that time a K factor is assigned to the test, and re-calibration is usually not necessary. Monitor control values to determine stability of the current calibration.
Quality Control: Two levels of control are assayed each time the ALT method is performed. It is acceptable to run each control at the start of the day, and again at the end of the day. The operator may run them more frequently, if desired. One control is prepared from pooled, normal human serum. The other is an elevated, abnormal commercial control. Consult the quality control detail table for current ranges and lots in use.
Expected Values:

- Reference range: female 0-42 U/L male 0-52 U/L
- Linear range of the method: 4-800 U/L. Specimens exceeding the high limit are automatically diluted (1:11) by the instrument, and reported accordingly. If a manual dilution is required, dilute the specimen in normal saline, and multiply the result by the dilution factor. Report values less than 4 as <4 U/L.
- Analytical Measurement Range: 4-800 U/L
- Clinically Reportable Range: 4-3000 U/L

References:

1. Roche/Hitachi System Application Sheet for AST, 2005.
2. Package insert for C.F.A.S., 2005.
3. Roche/Hitachi Modular Analytics Operator's Manual, version 2.0, October 2006.

Cholesterol, Total

Minimal Description for Publication: Total cholesterol is measured in serum on a Roche Modular P Chemistry Analyzer (Roche Diagnostics Corporation) using a cholesterol oxidase enzymatic method (Roche Diagnostics, Indianapolis, IN 46250).
Principle: In this enzymatic method esterified cholesterol is converted to cholesterol by cholesterol esterase. The resulting cholesterol is then acted upon by cholesterol oxidase to produce cholest-4-en-3-one and hydrogen peroxide. The hydrogen peroxide then reacts with 4-aminophenazone in the presence of peroxidase to produce a colored product that is measured at 505 nm (secondary wavelength = 700 nm). The final step is also known the Trinder reaction. This method is a single reagent, endpoint reaction that is specific for cholesterol.
Specimen: Serum from a serum separator tube (biospecimen collection tube #1) is used for analysis. The serum is separated from the cells within 2 hours of collection and stored at -70° C until assayed.
Interferences: Bilirubin does not interfere up to an I index of 25. Hemolysis does not interfere up to an H index of 700. Lipemia does not interfere up to an L index 1250.
Equipment: Roche Modular P chemistry analyzer (Roche Diagnostics, 9115 Hague Road, Indianapolis, IN 46250).
Reagent: Roche product #1491458, CHOL reagent kit (Roche Diagnostics, 9115 Hague Road, Indianapolis, IN 46250).
Calibration: The calibrator used for this assay is obtained from a unit of whole blood collected from a single donor. The unit of blood is collected at the UMMC donor center, then it is allowed to clot overnight at room temperature. There are no additives in the collection bag. Cholesterol concentration will vary with each donor selected. The calibrator is stored at -70° C. The new calibrator is first assayed in duplicate for 20 consecutive days. The new calibrator is also assayed in duplicate on two consecutive days using the reference Abell-Kendall method. The values obtained from the two different methods should agree within two percent of each other. The ModP will automatically calibrate (2-point) cholesterol when there is a reagent lot number change, and it will perform a blank (1-point) calibration when there is a bottle change. There is no automatic time-dependent calibration. Monitor control values to determine stability of the current calibration.
Quality Control: Two levels of control are assayed each time the ALT method is performed. It is acceptable to run each control at the start of the day, and again at the end of the day. The operator may run them more frequently, if desired. One control is prepared from pooled, normal human serum. The other is an elevated, abnormal commercial control. Consult the quality control detail table for current ranges and lots in use. The Roche cholesterol assay meets the 1992 National Institutes of Health (NIH) goal of less than or equal to 3 per cent for both precision and bias.
Expected Values:

- Reference range: <200 mg/dL is considered “desirable”
- Linear range of the method: 0-800 mg/dL (serum). Specimens exceeding the high limit are automatically diluted (1:5.5) by the instrument, and reported accordingly. If a manual dilution is required, dilute the specimen in normal saline, and multiply the result by the dilution factor.
- Analytical Measurement Range: 0-800 mg/dL
- Clinically Reportable Range: 10-1000 mg/dL

References:

1. Roche/Hitachi System Application Sheet for CHOL, 2005.
2. Roche/Hitachi Modular Analytics Operator’s Manual, version 2.0, October 2006.

C-Reactive Protein, High Sensitivity

Minimal Description for Publication: High sensitivity C-reactive protein is measured in serum on a Roche Modular P Chemistry Analyzer (Roche Diagnostics Corporation) using an immunoturbidimetric method (Roche Diagnostics, Indianapolis, IN 46250).
Principle: This is a two-reagent, immunoturbidimetric system. The specimen is first combined with a Tris buffer, then incubated. The second reagent (latex particles coated with mouse anti-human CRP antibodies) is then added. In the presence of circulating CRP, the latex particles aggregate and form immune complexes. These complexes cause an increase in light scattering that is proportional to the CRP concentration. The light absorbance resulting from this light scatter is read against a stored CRP standard curve. The concentration of CRP is determined from this line. Turbidity is measured at a primary wavelength of 546 nm (secondary wavelength 800 nm).
Specimen: Serum from a serum separator tube (biospecimen collection tube #1) is used for analysis. The serum is separated from the cells within 2 hours of collection and stored at -70° C until assayed.
Interferences: Bilirubin does not interfere up to an I index of 60. Hemolysis does not interfere up to an H index of 1000. Lipemia does not interfere up to an L index 500.
Equipment: Roche Modular P chemistry analyzer (Roche Diagnostics, 9115 Hague Road, Indianapolis, IN 46250).
Reagent: Roche product #1972855, CRP(Latex)HS reagent kit.
Calibration: Roche Protein Calibrator for Automated Systems (C.F.A.S. Protein), catalog #11355279. The C.F.A.S. Protein calibrator CRP setpoint value is traceable to reference material CRM 470 (RPPHS-Reference Preparation for Proteins in Human Serum). The Mod P will automatically perform a two-point calibration (saline + C.F.A.S. Protein) when there is a reagent lot number change. The Mod P will not allow testing to proceed until a successful calibration has been completed. Monitor control values to determine stability of the current calibration.
Quality Control: Two levels of control are assayed each time the CRP method is performed. It is acceptable to run each control at the start of the day, and again at the end of the day. The operator may run them more frequently, if desired. One control is prepared from pooled, normal human serum. The other is an abnormal commercial control. Consult quality control charts for current ranges and lots in use.
Expected Values:

- Reference range, plasma: adults: <5.0 mg/L
- Linear range of the method: 0.1-20 mg/L (serum). Specimens exceeding the high limit are automatically diluted (1:15) by the instrument, and reported accordingly. If a manual dilution is required, dilute the specimen in normal saline, and multiply the result by the dilution factor. Specimens reading below the linear range of the assay should be reported as <0.1 mg/L. Results are reported to two decimal places.
- Analytical Measurement Range: 0.1-20 mg/L
- Clinically Reportable Range: 0.1-500 mg/L

References:

1. Roche/Hitachi System Application Sheet for CRP(Latex)HS, 2005
2. Package insert for C.F.A.S. Protein, 2005.
3. Roche/Hitachi Modular Analytics Operator's Manual, version 2.0, October 2006.

Creatinine

Minimal Description for Publication: Creatinine is measured in serum or urine on a Roche Modular P Chemistry Analyzer (Roche Diagnostics Corporation) using a creatinase enzymatic method (Roche Diagnostics, Indianapolis, IN 46250).
Principle: In this enzymatic method creatinine is converted to creatine under the activity of creatininase. Creatine is then acted upon by creatinase to form sarcosine and urea. Sarcosine oxidase converts sarcosine to glycine and hydrogen peroxide, and the hydrogen peroxide reacts with a chromophore in the presence of peroxidase to produce a colored product that is measured at 546 nm (secondary wavelength = 700 nm). This is an endpoint reaction that agrees well with recognized HPLC methods, and it has the advantage over Jaffe picric acid-based methods that are susceptible to interferences from non-creatinine chromogens.
Specimen: Serum from a serum separator tube (biospecimen collection tube #1) or a random urine specimen not treated with any stabilizer or additive is used for analysis. Serum is separated from the cells within 2 hours of collection; both serum and urine are stored at -70° C until assayed.
Interferences: Bilirubin does not interfere up to an I index of 25. Hemolysis does not interfere up to an H index of 1000. Lipemia does not interfere up to an L index 1000. Cephalosporin antibiotics do not interfere.
Equipment: Roche Modular P chemistry analyzer (Roche Diagnostics, 9115 Hague Road, Indianapolis, IN 46250).
Reagent: Roche product #1775685, CREA plus reagent kit (Roche Diagnostics, 9115 Hague Road, Indianapolis, IN 46250).
Calibration: Roche Calibrator for Automated Systems (C.F.A.S.), catalog #759350. The C.F.A.S. calibrator is traceable to reference material SRM 909b (Isotope Dilution Mass Spectroscopy--IDMS). This is a reference material provided by the National Institute of Standards and Technology. This traceability means that this creatinine method yields results that are routinely lower (5-10%) than those creatinine methods using a "traditional" calibrator. The Mod P will automatically perform a two-point calibration when there is a reagent lot number change. It will also perform a two-point calibration every seven days thereafter. The Mod P will not allow testing to proceed until a successful calibration has been completed. Monitor control values to determine stability of the current calibration.
Quality Control: Two levels of control are assayed each time the ALT method is performed. It is acceptable to run each control at the start of the day, and again at the end of the day. The operator may run them more frequently, if desired. One control is prepared from pooled, normal human serum. The other is an elevated, abnormal commercial control. Consult the quality control detail table for current ranges and lots in use.
Expected Values:

- Reference range, serum: female: 0.4 – 1.1 mg/dL male: 0.5 – 1.2 mg/dL
- Linear range of the method: 0-30 mg/dL (serum), 0-600 mg/dL (urine). Specimens exceeding the high limit are automatically diluted (1:2) by the instrument, and reported accordingly. If a manual dilution is required, dilute the specimen in normal saline, and multiply the result by the dilution factor.
- Analytical Measurement Range: 0-30 mg/dL
- Clinically Reportable Range: 0.1-50 mg/dL

References:

1. Roche/Hitachi System Application Sheet for CREA plus, 2006.
2. Package insert for C.F.A.S., 2005.
3. Roche/Hitachi Modular Analytics Operator's Manual, version 2.0, October 2006.
4.. NKDEP Suggestions for Laboratories (Revised December 2005). Internet website: www.nkdep.nih.gov/resources/laboratory_reporting.htm
5. "NKDEP Launches Creatinine Standardization Program", by Richard Pizzi, Clinical Laboratory News, April 2006.
6. "Recommendations for Improving Serum Creatinine Measurement: A Report from the Laboratory Working Group of the National Kidney Disease Education Program", by Gary L. Myers, et. al., Clinical Chemistry, Vol. 52, No. 1, pages 5-18 (2006).

Notes:

1. The MDRD equation for estimating GFR based upon a creatinine value derived from a NIST-traceable calibration is as follows:

Estimated GFR (ml/min/1.73m2)

=175 x (SCr)-1.154 x (Age)-0.203 x (0.742 if female) x (1.210 if African-American)

= exp[5.228 - (1.154 x ln(SCr)) – (0.203 x ln(Age)) – (0.299 if female) + (0.192 if African-American)]

SCr = serum creatinine in mg/dL
exp = e raised to the power of a given number
ln = natural logarithm of a number

2. Note: The eGFR will be calculated by the HCHS/SOL Coordinating Center using this formula.

DNA Isolation

Minimal Description for Publication: DNA is extracted from the white blood cells (WBC) of frozen packed cells from EDTA anticoagulated whole blood using a salt precipitation method (Puregene reagents, Qiagen Inc., Germantown, MD 20874).
Principle: DNA is extracted from the white blood cells (WBC) of frozen packed cells. The red cells that lack DNA are lysed and the sample centrifuged to separate the RBCs from the WBCs. The isolated WBCs are then lysed with an anionic detergent which solubilizes the cellular components. The DNA preservative in this detergent limits the activity of enzymes that can digest DNA (DNases) which are also present. Contaminating RNA is removed by treatment with RNase. Contaminating proteins are removed by salt precipitation. DNA is then isolated by precipitation with alcohol and dissolved in a buffered solution.
Specimen: Frozen packed cells from EDTA anticoagulated whole blood (Tube #4 and #5).
Interferences: None
Equipment: AUTOPURE LS™ (Qiagen Inc., Germantown, MD 20874).

Reagent: Purchased from Qiagen Inc., 19300 Germantown Road, Germantown, MD 20874:
1. RBC Lysis Solution Reagent 1 (9.5 L), catalog #949004
2. Cell Lysis Solution Reagent 2 (3.8 L), catalog #949006
3. Protein Precipitation Solution Reagent 3 (3.8 L), catalog #949008
4. 100% Isopropanol Reagent 4 (3.8 L), catalog #949016
5. 70% Ethanol Reagent 5 (3.8 L), catalog #949018
6. DNA Hydration Solution Reagent 6 (3.8L), catalog #949010
7. RNase A Solution 4 mg/ml (19 mL), catalog #949014

Calibration: None
Quality Control: See DNA Quantitation (7) and DNA Visualization (8) procedures.
Expected Values: Values vary with the number of lymphocytes; average yield is 200 ug/mL of whole blood.
References:
1. AUTOPURE LS™ Operator's Manual Catalog#OM-0001 9/04 Rev D.
2. Puregene DNA Isolation Kit, instructions. Qiagen, Inc., 19300 Germantown Rd, Germantown, MD 20874.
3. Extraction of DNA From Whole Blood, procedure.

DNA Quantitation

Minimal Description for Publication: DNA is quantitated spectrophotometrically on the NanoDrop 1000 Spectrophotometer (NanoDrop Products, Wilmington, DE 19810).
Principle: A 1:20 dilution is made from each DNA extract prior to quantitation. The optical density (OD) of each is then measured spectrophotometrically at 260 nm and 280 nm. Measurement at 260 nm permits calculation of the DNA concentration in the sample. Extraneous protein absorbs at 280 nm. An OD of 1.0 corresponds to approximately 50 ug/mL of double-stranded DNA. The ratio between readings at 260 nm and 280 nm (OD 260/OD 280) provides an estimate of the purity of the nucleic acid. Pure preparations of DNA have an OD 260/OD 280 ratio of 1.7 to 1.9. If there is contamination with protein, the OD 260/OD 280 ratio will be significantly less than 1.7.
Specimen: Extracts of DNA in Tris-EDTA (TE) buffer: genomic DNA extracted from frozen packed cells collected from EDTA-anticoagulated whole blood. (See DNA extraction procedure.)
Interferences: NA
Equipment: NanoDrop 1000 Spectrophotometer (NanoDrop Products, Wilmington, DE 19810)
Reagent: Milli-Q water. Milli-Q is the trade name of the water system purchased from Millipore Corporation (Continental Water System). Milli-Q is deionized water treated with activated carbon and deionization cartridges and filtered to remove microorganisms larger than 0.22 um. This meets CAP Class I water requirements.
Calibration: Spectrophotometer wavelength calibration is performed quarterly.
Quality Control: None
Calculations: An OD of 1.0 corresponds to approximately 50 ug/mL double-stranded DNA.
DNA Concentration (ug DNA/uL TE buffer):

ug DNA/uL TE = A260 x dil factor x 50 ug/mL x 1 mL/1000 uL

Total ug of DNA:

ug DNA = Conc (ug/uL) x vol TE (mL) x 1000 uL/mL

Expected Values:

- **Concentration:** Target range for DNA quantitation at 260 nm based on a 1:20 dilution of extract in Milli-Q water is 0.1 – 0.9 ug/uL. This concentration range will be used to make dilutions for future procedures which analyze DNA. Results falling below this concentration range are acceptable if intact DNA is observable on electrophoresis. If the concentration is greater than 1.0 ug/uL, add sufficient volume of TE buffer to the DNA extract vial to dilute and retip vial overnight as this is above the dynamic range of the spectrophotometer. Make a new 1:20 dilution and repeat quantitation. Notes recorded on the DNA Extraction worksheet may serve to explain unusual results or discrepancies.
- **Purity Index:** Target range of OD 260/OD 280 ratio is 1.7 – 1.9. If results fall outside the range, make a new 1:20 dilution and repeat quantitation to confirm the results. Notes recorded on the DNA Extraction worksheet may service to explain unusual results or discrepancies. In general, ratios falling outside this range will present no problem for most methodologies and the extract may be used as is. In any case, the DNA extract may be re-extracted at a future date if desired. If the ratio is very low or the extract is highly colored resulting in a poor ratio, the specimen should be re-extracted in the next batch. If the results after re-extraction are still poor, notify the clinic for re-collection.

References:

1. Sambrook, et al. Molecular Cloning: A Laboratory Manual. Cold Springs Harbor Laboratory, 1986.

DNA Visualization for Quality

Minimal Description for Publication: DNA is checked for high molecular weight quality by electrophoresis and visual inspection.

Principle: Protein molecules acquire a net negative charge when placed in a solution at a pH alkaline to their isoelectric points. When an electric current is applied, the molecules are free to move to the electrode of opposite polarity. Distance and rate of migration is affected by fragment size. Intact DNA migrates slowly relative to fragmented DNA, which appears as a smear moving anodally across the gel. Submarine electrophoresis is carried out under buffer after a loading dye with high density (ficoll) is added to the samples to weight them into the application wells. The loading dye also contains tracking dyes (xylene cyanole and bromphenol blue) that act as electrophoretic markers. Ethidium bromide incorporated into the gel intercalates between the base pairs of double-stranded DNA and fluoresces under UV light permitting visualization of the DNA. Lambda DNA/Hind III Fragment marker is applied to the gel as a control for migration.

Specimen: Extracts of DNA in Tris-EDTA (TE) buffer: genomic DNA extracted from frozen packed cells collected from EDTA-anticoagulated whole blood. (See DNA extraction procedure.)

Interferences: NA

Equipment:

1. FB 105 Power Supply (Fisher Biotech Electrophoresis Systems, Fisher Scientific, Pittsburgh, PA.).
2. DNA Electrophoresis assembly, Model 156 EC-H with 156-CB20 comb (1.0 mm) (DanKar Corporation, Reading, MA 01867).
3. Polaroid 667 High Speed Coaterless Professional Instant Pack Film.

Reagent:

1. UltraPURE Agarose, electrophoresis grade. Cat. No. 5510UB. Gibco BRL Life Technologies, Inc., P.O. Box 68, Grand Island, NY 14072-0068.

Calibration: NA

Quality Control: Lambda DNA/Hind III Fragments (Digest), aqueous solution, 500 ug (molecular biology reagent), Cat. No. 5612 Staphylococcus aureus (Gibco BRL Life Technologies, Inc). Verify that the Lambda DNA/Hind III marker in the first well has migrated anodally and that visible bands appear on the gel.

Expected Values: Intact DNA migrates slowly relative to fragmented DNA, which appears as a smear moving anodally across the gel. Some degree of shearing (smear) is acceptable as long as a fair amount of intact DNA is also present.

References:

1. Sambrook, et al. Molecular Cloning: A Laboratory Manual. Cold Springs Harbor Laboratory, 1986.

Glucose

Minimal Description for Publication: Glucose is measured in EDTA plasma on a Roche Modular P Chemistry Analyzer (Roche Diagnostics Corporation) using a hexokinase enzymatic method (Roche Diagnostics, Indianapolis, IN 46250).
Principle: In this enzymatic method glucose is converted to glucose-6-phosphate (G-6-P) by hexokinase in the presence of ATP, a phosphate donor. Glucose-6-phosphate dehydrogenase then converts the G-6-P to gluconate-6-P in the presence of NADP+. As the NADP+ is reduced to NADPH during this reaction, the resulting increase in absorbance at 340 nm (secondary wavelength = 700 nm) is measured. This is an endpoint reaction that is specific for glucose.
Specimen: Plasma from EDTA-anticoagulated whole blood tube (biospecimen collection tube #4 or #10 for the OGTT) is used for analysis. Per HCHS/SOL Biospecimen Collection and Processing Manual, specimens must be centrifuged and separated within 30-45 minutes following collection. Red blood cells will metabolize glucose via glycolysis, and the measurable glucose will decrease if the cells are left in contact with the cells for a prolonged period of time. This decrease in concentration can be as much as 7 per cent per hour.
Interferences: Bilirubin does not interfere up to an I index of 60. Hemolysis does not interfere up to an H index of 1000. Lipemia does not interfere up to an L index 1000.
Equipment: Roche Modular P chemistry analyzer (Roche Diagnostics, 9115 Hague Road, Indianapolis, IN 46250).
Reagent: Roche product #1876899, GLU reagent kit (Roche Diagnostics, 9115 Hague Road, Indianapolis, IN 46250).
Calibration: Roche Calibrator for Automated Systems (C.F.A.S.), catalog #759350. The C.F.A.S. calibrator is traceable to reference material SRM 965 (IDMS). This is a reference material provided by the National Institute of Standards and Technology. Calibration frequency: The Mod P will automatically perform a two-point calibration when there is a reagent lot number change. No other auto-calibrations are defined for the glucose assay. The Mod P will not allow testing to proceed until a successful calibration has been completed. Monitor control values to determine stability of the current calibration.
Quality Control: Two levels of control are assayed each time the glucose method is performed. It is acceptable to run each control at the start of the day, and again at the end of the day. The operator may run them more frequently, if desired. One control is prepared from pooled, normal human serum. The other is an elevated, abnormal commercial control. Consult the quality control detail table for current ranges and lots in use.
Expected Values:

- Reference range: fasting, 60-99 mg/dL;

post OGTT 0-139 mg/dL

- Linear range of the method: 0-750 mg/dL (serum). Specimens exceeding the high limit are automatically diluted (1:2) by the instrument, and reported accordingly. If a manual dilution is required, dilute the specimen in normal saline, and multiply the result by the dilution factor.
- Analytical Measurement Range: 0-750 mg/dL
- Clinically Reportable Range: 2-2000 mg/dL

References:

1. Roche/Hitachi System Application Sheet for Glucose/HK, 2004.
2. Package insert for C.F.A.S., 2005.
3. Roche/Hitachi Modular Analytics Operator's Manual, version 2.0, October 2006.

Glycosylated Hemoglobin

Minimal Description for Publication: Glycosylated hemoglobin is measured in EDTA whole blood using a Tosoh G7 Automated HPLC Analyzer, (Tosoh Bioscience, Inc, South San Francisco, CA 94080).
Principle: The G7 Automated HPLC Analyzer – HbA1c Variant Analysis Mode uses non-porous ion-exchange high performance liquid chromatography (HPLC) for rapid, accurate, and precise separation of the stable form of HbA1c from other hemoglobin fractions. Analysis is carried out without off-line specimen pretreatment or interference from Schiff base. The analyzer dilutes the whole blood specimen with Hemolysis & Wash Solution, and then injects a small volume of this specimen onto the TSKgel G7 HSi Variant Column. Separation is achieved by utilizing differences in ionic interactions between the cation exchange group on the column resin surface and the hemoglobin components. The hemoglobin fractions (designated as A1a, A1b, F, LA1c+, SA1c, A0, and H-V0, H-V1, H-V2) are subsequently removed from the column by performing a step-wise elution using the varied salt concentrations in the Elution Buffers HSi Variant 1, 2, and 3. The separated hemoglobin components pass through the LED photometer flow cell where the analyzer measures changes in absorbance at 415 nm. The analyzer integrates and reduces the raw data, and then calculates the relative percentages of each hemoglobin fraction. The Total Area of the SA1c is divided by the sum of the total areas of all peaks up to and including the A0 to obtain a raw SA1c percentage. This uncorrected result is substituted as the "x" value in the linear regression formula determined during calibration. The analyzer prints the final numerical results and plots a chromatogram showing changes in absorbance versus retention time for each peak fraction. The Tosoh G7 Automated HPLC Analyzer – HbA1c Variant Analysis Mode is certified by the National Glycohemoglobin Standardization Program (NGSP). The final reportable result is traceable to the Diabetes Control and Complications Trial (DCCT).
Specimen: Whole blood from EDTA anticoagulated tube (biospecimen collection tube #3).
Interferences: Icterus, as indicated by free and conjugated bilirubin concentrations up to 18.0 and 20.0 mg/dL, respectively, does not interfere with the assay. Lipemia, as indicated by triglyceride concentrations up to 2000 mg/dL, does not interfere with the assay. Concentrations of up to 20 mg/dL of sodium cyanate and acetaldehyde do not interfere with the assay.
Equipment: Tosoh G7 Automated HPLC Analyzer, (Tosoh Bioscience, Inc, South San Francisco, CA 94080).
Reagent:

1. DIAMAT HbA1c Sample Preparation Kit, Cat. No. 196-1026 (Bio-Rad Laboratories, Clinical Division, 4000 Alfred Nobel Drive, Hercules, CA 94547).
2. TSKgel G7 HSi Variant Column, Cat. No. 019680 (Tosoh Bioscience, Inc, So. San Francisco, CA 94080).
3. G7 His Variant Elution Buffer 1, Cat. No. 021446 (Tosoh Bioscience, Inc, So. San Francisco, CA 94080).
4. G7 HSi Variant Elution Buffer 1, (S) Cat. No. 019552 (Tosoh Bioscience, Inc, So. San Francisco, CA 94080).
5. G7 Hsi Variant Elution Buffer 2, (S) Cat.No. 019553 (Tosoh Bioscience, Inc, So. San Francisco, CA 94080).
6. G7 Hsi Variant Elution Buffer 3 (S), Cat. No. 019554 (Tosoh Bioscience, Inc, So. San Francisco, CA 94080).
7. Hemolysis & Wash Solution, Cat. No. 018431 (Tosoh Bioscience, Inc, So. San Francisco, CA 94080).

Calibration: The analyzer has a two-point automatic calibration function. Studies have shown the calibration to be stable for at least seven days. Weekly calibration of the instrument is performed prior to analysis of controls and patient samples. Calibration must also be performed after repeated control failure, major maintenance or service has been performed or whenever a new column is installed.

Quality Control: Two levels of glycated hemoglobin control (Normal and Elevated) are analyzed in duplicate (or more) with each batch. Controls are prepared from whole blood drawn from a normal (Normal) and a diabetic (Elevated) individual. Stable indefinitely stored at –70° C.

Expected Values:

- Reference Range: 4.3 – 6.0 %
- Linear Range: 3.0 – 19.0 % Results falling outside this range are reported as <3.0 or >19.0 %.
- Clinically Reportable Range: 3.0 – 19.0 % Report results falling outside this range as <3.0 or >19.0 %.
- The American Diabetes Association recommends that a primary goal of therapy should be HbA1c < 7%, and that physicians should reevaluate the treatment regimen in patients with HbA1c values consistently above 8%.

References:

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4. Nathan DM, Francis TB, Palmer JL. Effect of aspirin on determinations of glycosylated hemoglobin. Clin Chem 1983;29:466-9.
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6. Tze et al. Hemoglobin A1c – An Indicator of Diabetic Control. J of Pediatrics 1978, 93:1316.
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Hemogram, Differential, and Platelet Count

Minimal Description for Publication: Hemogram, differential and platelet count are measured in EDTA whole blood using a Sysmex XE-2100 instrument, (Sysmex America, Inc., Mundelein, IL 60060).
Principle: The Sysmex XE-2100 is a quantitative automated hematology analyzer for in vitro diagnostic use in determining 25 hematological parameters. Examination of the numerical and/or morphologic findings of the complete blood count are useful in diagnosis of such disease states as anemias, leukemias, allergic reactions, viral, bacterial, and parasitic infections. The Sysmex XE analyzer directly measures the WBC, RBC, HGB, MCV, PLT, PLT-O, RDW-CV, LYMPH%, MONO%, EO%, BASO%, NRBC#, RET%. The remaining parameters calculated or derived are HCT, MCH, MCHC, NEUT%, RDW-SD, NRBC%, RET# and differential absolute counts. WBC count, differential, reticulocytes (RET) and nucleated red blood cells (NRBC) are all evaluated using flow cytometry with a semiconductor laser exploiting the differences in cell size, complexity and RNA/DNA content. WBC and basophils (BASO) are treated with an acidic lyse, that lyses RBC and WBC, but not BASO. The remaining WBC nuclei and intact BASO are differentiated by cell size. The WBC differential channel classifies lymphocytes (LYMPH), monocytes (MONO), eosinophils (EO), and granulocytes by cellular complexity and nucleic acid content. Reticulocytes are separated from mature RBC and PLT by size and RNA content. NRBC are separated from WBC based on nuclear size after lysing and DNA/RNA staining. The Immature Information channel, (IMI) cytochemically differentiates immature myeloid cells from mature granulocytes based on membrane lipid content, and are identified using Direct Current and Radio Frequency technologies.
Specimen: Whole blood from a 4 mL lavender-stoppered tube containing EDTA anticoagulant is used for analysis (biospecimen collection tube #3).
Interferences: Specimens must be free of clots and fibrin strands; marked changes in plasma constituents (e.g., low sodium, extremely elevated glucose) may cause cells to swell or shrink; specimens must have the correct blood to anticoagulant ratio; red cell fragments, microcytic RBC's or white cell cytoplasmic fragments may interfere with automated platelet counts - an optical platelet may be performed to avoid this interference; both cold agglutinins and rare warm agglutinins produce spurious macrocytosis (increased MCV), elevated MCH's and MCHC's, falsely decreased RBC counts and HCT's; extremely elevated WBCs may cause turbidity and increase the hemoglobin - WBC >320x109/L require a dilution to be performed; severely hemolyzed samples (in vitro) falsely decrease RBC and hematocrit - recollect hemolyzed specimens; giant platelets and clumped platelets may falsely elevate the WBC count and falsely decrease the platelet count; platelet clumping and/or "platelet satellitism" can occur in specimens collected in EDTA and may falsely elevate the WBC and falsely decrease the platelet count - recollect the specimen in Sodium Citrate anticoagulant and multiply the result by 1.1 to correct for anticoagulant dilution; abnormal paraproteins found in Multiple Myeloma patients can falsely increase the HGB and MCHC - consult a supervisor if MCHC is greater than 37.5; lipemia falsely elevates the HGB and MCHC; severely icteric samples may falsely elevate the HGB value and related indices; rocking specimen excessively, may affect the WBC differential; megakaryocytes may falsely increase WBC counts; abnormal proteins as seen in Multiple Myeloma and Waldenstrom's Macroglobulinemia may falsely increase the WBC count.
Equipment: Sysmex XE-2100 instrument

Reagents: Sysmex reagents and bleach used on the Sysmex XE-2100. Reagents are supplied by Cardinal Health. Reagents are stored at room temperature and stable until manufacturer's expiration date on each container if not opened.

REAGENT	ABBREVIATION	OPEN EXPIRATION
CELLPACK	EPK	60 days
CELLSHEATH	ESE	60 days
STROMATOLYSER-4DL	FFD	60 days
STROMATOLYSER-4DS	FFS	60 days
STROMATOLYSER-FB	FBA	60 days
STROMATOLYSER-IM	SIM	60 days
RET-SEARCH (II) diluent & dye	RED	60 days
STROMATOLYSER-NR lyse & dye	SNR	60 days
SULFOLYSER	SLS	90 days

Calibration: SCS-1000 is a secondary whole blood calibrator for use with the Sysmex XE-2100 hematology analyzer. Assay values for primary parameters are traceable to reference methods. Initial calibration is performed during installation and verified bi-annually during preventive maintenance (PM) by the Sysmex Field Service Representative. Calibration compensates for any bias inherent to the pneumatic, hydraulic, and electrical system that may affect the accuracy of results. Calibrators traceable to reference methods are used in the calibration of the instrument. WBC differential parameters are calibrated in the factory prior to shipment, and verified by the field service representative upon installation.

Sysmex service will verify calibration every six months or if one or more of the following occur:
- Critical parts are replaced such as manometers, apertures or detector circuit boards.
- Controls show an unusual trend or are outside of acceptable limits and cannot be corrected by maintenance or troubleshooting.
- When advised by Sysmex Field Service Representative.

Accuracy and precision is checked every 6 months when the instrument is calibrated by service. Calibration verification is performed by review and documentation of all three levels of commercial. The operator may calibrate the following parameters: WBC, RBC, HGB, HCT, PLT and PLT-O. Before calibration, ensure that the XE-2100 is both clean and precise. Calibration verification is performed by analyzing three levels of commercial controls. All controls must be within limits prescribed in each of their files.

Quality Control:

1. e-CHECK manufactured by Streck is a tri-level whole blood commercial control used with the Sysmex XE-2100 hematology analyzer. (Product # 199-4004-1). e-CHECK control levels: 1, 2, 3 are analyzed on the night shift in the closed mode (which is the mode most used for patient analysis). For each parameter of each level of control, an acceptable range around the mean must be established. This range, called the LIMIT % is based on historical performance of the commercial control material when the instrument is in good working condition. Historical LIMIT %'s are established using three different lots of e-CHECK (over a 6 month period for the 56 day-dated lot). Interim Limit %'s, suggested by Sysmex, are used prior to establishing the analyzer-specific limits during the evaluation period. Once three lots of QC data are collected, the CV %'s for each parameter is averaged. To establish a 3CV% limit, multiply the average CV's x 3. These historical limits are manually entered for the LIMIT % in each file, for each level of control and are used for all subsequent lots of controls. These limits should provide acceptable error detection with a low probability of false rejection, and need not be reestablished.
2. Five patient specimens (one with WBC >12.0, one with WBC <5.0, one with HGB <10.0, one normal and one with HGB >15.0) are analyzed on the two Sysmex instruments weekly. Specimens are also analyzed in the secondary mode (Sysmex 1 on the first and third week, Sysmex 2 on the second and fourth week). The percent difference between the instruments is obtained. Limits are 5% for WBC, 2.5%

for RBC, HGB, MCV and 7% for PLT. If the percent difference is not within limits, check for data entry error and then consult a supervisor.

3. Results are reviewed by the supervisor and kept in the instrument comparison book at the supervisor's desk. The supervisor or lead tech reviews commercial, patient, and patient moving averages (Xm) charts every week. Monthly peer review values outside of +/- 2 SDI range will be evaluated for instrument problems or possible recalibration needs in consultation with Sysmex technical service.

Expected Values:

Hemogram:

Age	WBC (x 10^9/L)	RBC (x 10^{12}/L)	HGB (g/dL)	HCT (%)	MCV (fL)
18 y &older, F	4.0-11.0	3.8-5.2	11.7-15.7	35.0-47.0	78-100
18y &older, M	4.0-11.0	4.4-5.9	13.3-17.7	40.0-53.0	78-100

Age	MCH (pg)	MCHC (g/dL)	RDW (%)	PLT (x 10^9/L)	MPV (fL)
1 y & older	26.5-33.0	31.5-36.5	10.0-15.0	150-450	6.5-10.0

Leukocyte Differential Count:
Relative Frequency (%)

Age	Neut	Lymph	Mono	Eos	Baso
18 y & older	40-75	20-48	0-12	0-6	0-2

Absolute Diff Value (x 10^9/L)

Age	Neut	Lymph	Mono	Eos	Baso
18 y & older	1.6-8.3	0.8-5.3	0-1.3	0-0.7	0-0.2

References:

1. Sysmex XE-2100 Operator's Manual, Sysmex Corporation, Kobe, Japan, May, 2000.
2. NE-Series User's Guide, Sysmex Corporation (USA), Inc., Clinical Applications Division, Los Alamitos, CA, 1991 pg. 39.
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Hepatitis A Total Antibody

Minimal Description for Publication: Total hepatitis A antibody is measured in serum using direct chemiluminescent technology on the ADVIA Centaur System (Siemens Healthcare Diagnostics, Deerfield, IL 60015-0778).
Principle: The ADVIA Centaur HAV Total assay is a fully automated, competitive immunoassay using direct, chemiluminescent technology. The assay consists of three reagent additions and incubation steps. First, the sample is pretreated with Ancillary Reagent containing cysteine. Next, HAV antigen is added from the ancillary well (Antigen Reagent). Lite Reagent and Solid Phase are then added. The Lite Reagent contains monoclonal mouse antibody to HAV antigen labeled with acridinium ester and biotinylated Fab fragment of a monoclonal mouse antibody to HAV antigen. The Solid Phase contains streptavidin covalently coupled to paramagnetic particles. After the final incubation, the immuno-complex formed is washed with Wash 1 prior to initiation of the chemiluminescent reaction. The relative light units detected by the ADVIA Centaur System are used to calculate the Index Value from the Master curve. Assay results above the cutoff of the assay are not indicative of antibody level.
Specimen: Serum from a serum separator tube (biospecimen collection tube #1) is used for analysis. The serum is separated from the cells within 2 hours of collection and stored at -70° C until assayed.
Interferences: Less than 10% change in results was found with bilirubin up to 60 mg/dL conjugated, 40 mg/dL unconfugated, triglycerides up to 3000 mg/dL, hemoglobin up to 500 mg/dL.
Equipment: ADVIA Centaur System (Siemens Healthcare Diagnostics, Deerfield, IL 60015-0778).
Reagent:
1. ADVIA Centaur HAVT ReadyPack primary reagent pack (Cat # 07720961)
2. ADVIA Centaur HAVT ancillary reagent

Calibration: HAVT calibrators are used to establish calibration every 14 days. Additionally, the ADVIA Centaur HAV Total assay requires a two-point calibration: 1) when changing lot numbers of primary reagent packs, 2) when replacing system components, or 3) when quality control results are repeatedly out of range.
Quality Control: To monitor system performance and chart trends, two levels of quality control material are assayed each day. Controls are also assayed when performing a two-point calibration. Consult quality control charts for controls currently in use and established ranges.
Expected Values:
- The system reports anti-HAV Total results in Index Values.
- Samples with a calculated value of less than 1.00 Index Value are considered nonreactive for antibodies to hepatitis A virus.
- Samples with a calculated value greater than or equal to 1.00 Index Value are considered reactive for antibodies to hepatitis A virus.
- The magnitude of the measured result above the cutoff is not indicative of the total amount of antibody present.
- Sample results are invalid and must be repeated if the controls are out of range.

References:
1. ADVIA Centaur Assay Manual, 07716220, Rev. A, 2005-02.
2. General Laboratory Procedure Manual

Hepatitis B Core Antibody

(If this test is positive, hepatitis B surface antigen testing is performed.)
Minimal Description for Publication: Hepatitis B core antibody is measured in serum using sandwich immunoassay method on the ADVIA Centaur System (Siemens Healthcare Diagnostics, Deerfield, IL 60015-0778).
Principle: The ADVIA Centaur HBc Total assay is a two wash antigen sandwich immunoassay in which antigens are bridged by antibody present in the patient sample. The Solid Phase contains a preformed complex of streptavidin coated microparticles and biotinylated recombinant HBc antigen and is used to capture anti-HBc in the patient sample. The Lite Reagent contains recombinant HBc antigen labeled with acridinium ester and is used to detect anti-HBc in the sample. Solid Phase and Chaotrope Reagent are added to the sample, followed by Ancillary Reagent and Lite Reagent. Antibody-antigen complexes will form if anti-HBc antibodies (IgM and IgG) are present in the sample. The relative light units (RLUs) detected by the ADVIA Centaur system are used to calculate the Index Value from the Master Curve. Assay results above the cutoff of the assay are not indicative of antibody level.
Specimen: Serum from a serum separator tube (biospecimen collection tube #1) is used for analysis. The serum is separated from the cells within 2 hours of collection and stored at -70° C until assayed.
Interferences: Less than 10% change in results was found with bilirubin up to 60 mg/dL conjugated, 40 mg/dL unconfugated, triglycerides up to 1000 mg/dL, hemoglobin up to 500 mg/dL.
Equipment: ADVIA Centaur System (Siemens Healthcare Diagnostics, Deerfield, IL 60015-0778).
Reagent:
1. ADVIA Centaur HBcT ReadyPack primary reagent pack (Cat # 07569996)
2. ADVIA Centaur HBcT Ancillary Reagent Readypack (20.0 mL/pack) Buffer

Calibration: ADVIA Centaur HBc Total Calibrators are used to establish calibration every 14 days. Additionally, the ADVIA Centaur HAV Total assay requires a two-point calibration: 1) when changing lot numbers of primary reagent packs, 2) when replacing system components, or 3) when quality control results are repeatedly out of range.
Quality Control: To monitor system performance and chart trends, two levels of quality control material are assayed each day. Controls are also assayed when performing a two-point calibration. Consult quality control charts for controls currently in use and established ranges.
Expected Values:
- The system reports anti-HBc total results in Index Values and as reactive or nonreactive.
- Samples with a calculated value of less than 0.50 Index Value are considered non reactive for total antibodies to hepatitis B core antigen.
- Samples with initial results in the range from 0.50 to 0.99 Index Value require retest. Repeat the testing in duplicate. Samples which are repeatedly ≥ 0.50 Index Value (by at least two of the three results) will be considered reactive for HBc Total. Samples which are repeatedly < 0.50 Index Value (by at least two of the three results) will be considered nonreactive for HBc Total.
- The cutoff for the ADVIA Centaur HBc Total assay was verified based on results of clinical agreement generated from clinical studies.
- Sample results are invalid and must be repeated if the controls are out of range.

References:
1. ADVIA Centaur Assay Manual, 07716220, Rev. A, 2005-02.
2. General Laboratory Procedure Manual

Hepatitis B Surface Antibody

Minimal Description for Publication: Hepatitis B surface antibody is measured in serum using direct chemiluminometric technology on the ADVIA Centaur System (Siemens Healthcare Diagnostics, Deerfield, IL 60015).
Principle: The ADVIA Centaur Anti-HBs assay is a sandwich immunoassay using direct, chemiluminometric technology. Purified human sourced HBsAg (subtypes Ad and Ay) are covalently coupled to magnetic latex particles in the Solid Phase. In the Lite reagent, the purified HBsAg (subtypes Ad and Ay) is labeled with acridinium ester. Non-magnetic latex particles are added from the ancillary well. The sample is incubated simultaneously with Lite Reagent, Solid Phase and Ancillary Reagent. Antibody-antigen complexes form if anti-HBs is present in the sample. The relative light units (RLUs) detected by the ADVIA Centaur system are used to calculate the Index Value from the Master Curve. Assay results above the cutoff of the assay are not indicative of antibody level.
Specimen: Serum from a serum separator tube (biospecimen collection tube #1) is used for analysis. The serum is separated from the cells within 2 hours of collection and stored at -70° C until assayed.
Interferences: Less than 15% change in results was found with bilirubin up to 20 mg/dL conjugated, 40 mg/dL unconfugated, triglycerides up to 1000 mg/dL, hemoglobin up to 500 mg/dL.
Equipment: ADVIA Centaur System (Siemens Healthcare Diagnostics, Deerfield, IL 60015-0778).
Reagent: ADVIA Centaur aHBs ReadyPack primary reagent pack (Cat # 01463789)
Calibration: ADVIA Centaur Anti-HBs Calibrators are used to establish calibration every 28 days. Additionally, the ADVIA Centaur HAV Total assay requires a two-point calibration: 1) when changing lot numbers of primary reagent packs, 2) when replacing system components, or 3) when quality control results are repeatedly out of range.
Quality Control: To monitor system performance and chart trends, two levels of quality control material are assayed each day. Controls are also assayed when performing a two-point calibration. Consult quality control charts for controls currently in use and established ranges.
Expected Values:

- The ADVIA Centaur Anti-HBs assay is traceable to the World Health Organization (WHO) Hepatitis B Immunoglobulin 1st International Reference Preparation (1977). An Index Value of 1.00 is equivalent to 10 mIU/mL. Samples with an Index Value of 1.00 or greater are considered reactive (protective) in accordance with the CDC guidelines. The accepted criteria for immunity to HBV is anti-HBs activity ≥10 mIU/mL, as defined by the WHO International Reference Preparation.
- The system reports anti-HBs antibody results in Index Values and as reactive (positive), nonreactive (negative) or needing retest.
- Samples with an initial Index Value < 0.75 are considered nonreactive. Anti-HBs is below 10 mIU/mL and the patient is considered not to have protective immunity to HBV infection.
- Samples with an initial Index Value ≥ 1.25 are considered reactive. Anti-HBs is detected at ≥10 mIU/mL and the patient is considered to have protective immunity to HBV infection.
- Samples with an initial Index Value ≥ 0.75 and <1.25 are in the retest zone. If results are within the retest zone after initial testing, samples are to be retested. After retesting, if 3 results are available and 2 results are ≥ 1.00, then the sample is considered to be reactive. If 3 results are available and 2 results are < 1.00, then the sample is considered to be nonreactive.
- The magnitude of the measured result above the cutoff is not indicative of the total amount of antibody present.

References:

1. ADVIA Centaur Assay Manual, 07716220, Rev. A, 2005-02.
2. General Laboratory Procedure Manual

Hepatitis B Surface Antigen

(This test is only performed on samples that are positive for hepatitis B core antibody.)
Minimal Description for Publication: Hepatitis B surface antigen is measured in serum using direct chemiluminometric technology on the ADVIA Centaur System (Siemens Healthcare Diagnostics, Deerfield, IL 60015-0778).
Principle: The ADVIA Centaur HBsAg assay is a sandwich immunoassay using direct, chemiluminometric technology. Non-magnetic latex particles are added from the ancillary well. The Lite Reagent, packaged in a ReadyPack ancillary reagent pack, contains a biotinylated anti-HBs mouse monoclonal capture antibody and an acridinium-ester labeled anti-HBs mouse monoclonal antibody. HBsAg in the sample complexes with the antibodies and streptavidin-coated magnetic latex particles in the Solid Phase capture the HBsAg-antibody complexes. The sample is incubated simultaneously with Solid Phase, Lite Reagent and Ancillary Reagent. Antibody-antigen complexes will form if hepatitis B surface antigen is present. The relative light units (RLUs) detected by the ADVIA Centaur system are used to calculate the Index Value from the Master Curve. A result of reactive or non-reactive is determined according to a cutoff of 1.0 Index Value established with the calibrators.
Specimen: Serum from a serum separator tube (biospecimen collection tube #1) is used for analysis. The serum is separated from the cells within 2 hours of collection and stored at -70° C until assayed.
Interferences: Less than 10% change in results was found with bilirubin up to 40 mg/dL conjugated, 40 mg/dL unconfugated, triglycerides up to 1000 mg/dL, hemoglobin up to 500 mg/dL.
Equipment: ADVIA Centaur System (Siemens Healthcare Diagnostics, Deerfield, IL 60015-0778).
Reagent:
1. ADVIA Centaur HBsAg ReadyPack primary reagent pack (Cat # 03393362)
2. ADVIA Centaur HBsAg ReadyPack ancillary reagent pack

Calibration: ADVIA Centaur HBsAg Calibrators are used to establish calibration every 21 days. Additionally, the ADVIA Centaur HAV Total assay requires a two-point calibration: 1) when changing lot numbers of primary reagent packs, 2) when replacing system components, or 3) when quality control results are repeatedly out of range.
Quality Control: To monitor system performance and chart trends, two levels of quality control material are assayed each day. Controls are also assayed when performing a two-point calibration. Consult quality control charts for controls currently in use and established ranges.
Expected Values:
- The system reports HBsAg results in Index Values and as reactive or nonreactive.
- Samples with an Index Value of less than 1.00 are considered nonreactive (negative) for HBsAg.
- Samples with an Index Value of greater than or equal to 1.00 but less than or equal to 50 are considered initially reactive for HBsAg. Perform repeat testing in duplicate and / or supplemental testing on these samples. Repeat the test two times and if two of three results are nonreactive (<1.00), the sample is considered negative for HBsAg. If at least two of three results are reactive (>50), the sample is considered repeat reactive for the presence of HBsAg. If results remain <1.00 and >50.0 proceed to the HBsAg confirmatory assay.
- If the sample is greater than 50, the specimen is reactive (positive) for HBsAg.
- The magnitude of the ADVIA Centaur HBsAg assay result does not correlate to a quantitative amount of HBsAg present in the sample.

References:
1. ADVIA Centaur Assay Manual, 07716220, Rev. A, 2005-02.
2. General Laboratory Procedure Manual

Hepatitis B Surface Antigen Confirmation

Minimal Description for Publication: Hepatitis B surface antigen (HBsAg) confirmation is measured in serum using the principle of antibody neutralization to confirm the presence of HBsAg that is repeatedly reactive for HBsAg on the ADVIA Centaur System (Siemens Healthcare Diagnostics, Deerfield, IL 60015-0778).

Principle: The ADVIA Centaur HBsAg Confirmatory assay uses the principle of specific antibody neutralization to confirm the presence of HBsAg in a sample that is repeatedly reactive for HBsAg. The sample is pretreated and tested in parallel; one sample aliquot is dispensed and incubated with a neutralizing reagent containing high titers of anti-HBs (Reagent A); the second sample aliquot is incubated with a non-neutralizing control reagent (Reagent B). HBsAg in the patient sample is bound by the anti-HBs in reagent A and not allowed to react in the ADVIA Centaur HBsAg assay. When both aliquots are run in the ADVIA Centaur HBsAg assay, the inhibition of the RLU signal in the aliquot with Reagent A is compared to the RLU signal in the aliquot with Reagent B. The relative percent neutralization is calculated and an interpretation of the sample is generated. The non-neutralizing control reagent serves as a control for the neutralization as well as the 0 percent baseline for calculation of the amount of reduction in signal as percent neutralization. A sample is considered positive for HBsAg if the percent neutralization is 50% or greater after treatment with the antibody (neutralizing reagent). Percent neutralization is calculated by comparing the RLU values obtained with Reagent A to those obtained with the control reagent, Reagent B. If the RLU value with Reagent B is below the cutoff, the assay is invalid.

Specimen: Serum from a serum separator tube (biospecimen collection tube #1) is used for analysis. The serum is separated from the cells within 2 hours of collection and stored at -70° C until assayed.

Interferences: Less than 10% change in results was found with bilirubin up to 40 mg/dL conjugated, 40 mg/dL unconfugated, triglycerides up to 1000 mg/dL, hemoglobin up to 500 mg/dL.

Equipment: ADVIA Centaur System (Siemens Healthcare Diagnostics, Deerfield, IL 60015-0778).

Reagent:

1. ADVIA Centaur Conf Reagent ReadyPack ancillary reagent(Cat #03393818)
2. ADVIA Centaur HBsAg ReadyPack primary reagent pack (Cat # 03393362)

Calibration: ADVIA Centaur HBsAg Calibrators are used to establish calibration every 21 days. Additionally, the ADVIA Centaur HAV Total assay requires a two-point calibration: 1) when changing lot numbers of primary reagent packs, 2) when replacing system components, or 3) when quality control results are repeatedly out of range.

Quality Control: For quality control of the ADVIA Centaur HBsAg Confirmatory assay, use ADVIA Centaur HBsAg positive quality control material. The positive control should be scheduled in the same manner as a repeatable reactive sample. The ADVIA Centaur will run one aliquot of Positive Control with Reagent A and one aliquot of Positive Control with Reagent B. For the run to be valid, the % neutralization for the Positive Control must be greater than or equal to 50% and a result of confirmed must be obtained. Consult quality control charts for controls currently in use and established ranges.

Expected Values: The system calculates the cutoff and percent neutralization automatically based on the calibration of the ADVIA Centaur HBsAg Confirmatory assay and the values obtained for the sample run with Reagent A and Reagent B. The cutoff is based on RLU values of the calibrators and not on the ADVIA Centaur HBsAg Index Value (Index Value = 1.0). This is so that the cutoff can be adjusted to allow for dilution of the sample with Reagent A and Reagent B.

- Samples are reported as Invalid if the sample run with Reagent B is below the cutoff value of the ADVIA Centaur HBsAg Confirmatory assay. The assay is invalid and should be repeated. If the interpretation is Invalid after repeat testing, it means a valid result cannot be obtained with this sample and a new sample should be obtained.
- Samples reported as Redilute require further dilution for confirmation.
- Samples reported as Not Confirmed are HBsAg negative.
- Samples reported as Confirmed are HBsAg positive.

Heterophilic antibodies in human serum can react with reagent immunoglobulins, interfering with in vitro immunoassays. Patients routinely exposed to animals or to animal serum products can be prone to this

interference and anomalous values may be observed. Additional information may be required for diagnosis. Samples containing heterophilic antibodies should not neutralize using the ADVIA Centaur HBsAg Confirmatory assay.

For diagnostic purposes and to differentiate between acute and chronic HBV infection, the detection of HBsAg should be correlated with patient clinical information and other HBV serological markers. It is recognized that current methods for detection of hepatitis B surface antigen may not detect all potentially infected individuals. A false reactive HBsAg test result or invalid Confirmatory result does not exclude the possibility of exposure to or infection with hepatitis B.

It has been reported that certain assays will not detect all HBV mutants. If acute or chronic HBV infection is suspected and the HBsAg result is nonreactive it is recommended that other HBV serological markers be tested to confirm the HBsAg nonreactivity.

References:

1. ADVIA Centaur Assay Manual, 07716220, Rev. A, 2005-02.
2. General Laboratory Procedure Manual

Hepatitis C Antibody

(If this test is positive or indeterminate, hepatitis C virus (HCV) quantitation by PCR is performed.)
Minimal Description for Publication: Hepatitis C antibody is measured in serum using an indirect, two wash immunoassay on the ADVIA Centaur System (Siemens Healthcare Diagnostics, Deerfield, IL 60015-0778).
Principle: The ADVIA Centaur HCV assay is an indirect two wash sandwich immunoassay. The sample is incubated with Solid Phase containing recombinant and synthetic peptide HCV antigens. Antigen-antibody complexes will form if anti-HCV antibody is present in the sample. Lite Reagent containing monoclonal anti-human IgG labeled with acridinium ester is used to detect anti-HCV IgG in the sample. The relative light units (RLUs) detected by the ADVIA Centaur system are used to calculate the Index Value from the Master Curve. Assay results above the cutoff of the assay are not indicative of antibody level.
Specimen: Serum from a serum separator tube (biospecimen collection tube #1) is used for analysis. The serum is separated from the cells within 2 hours of collection and stored at -70° C until assayed.
Interferences: Less than 10% change in results was found with bilirubin up to 60 mg/dL conjugated, 40 mg/dL unconfugated, triglycerides up to 1000 mg/dL, hemoglobin up to 500 mg/dL.
Equipment: ADVIA Centaur System (Siemens Healthcare Diagnostics, Deerfield, IL 60015-0778).
Reagent:
1. ADVIA Centaur HCV ReadyPack primary reagent pack (Cat. # 03438099)
2. ADVIA Centaur HCV Ancillary Reagent

Calibration: ADVIA Centaur HCV Calibrators are used to establish calibration every 28 days. Additionally, the ADVIA Centaur HAV Total assay requires a two-point calibration: 1) when changing lot numbers of primary reagent packs, 2) when replacing system components, or 3) when quality control results are repeatedly out of range.
Quality Control: To monitor system performance and chart trends, two levels of quality control material are assayed each day. Controls are also assayed when performing a two-point calibration. Consult quality control charts for controls currently in use and established ranges.
Expected Values:
The system reports anti-HCV antibody results in Index Values and as reactive, nonreactive or equivocal. Index values above the cutoff of the assay are not indicative of the antibody level present in the sample.
- Samples with a calculated value of less than 0.80 Index Value are considered nonreactive (negative) for IgG antibodies to HCV.
- Samples with a calculated value greater than or equal to 0.80 Index Value and less than 1.00 Index Value are considered equivocal. It is recommended that the sample be repeated in duplicate. If 2 of the 3 sample results are less than 0.80 Index Value, the sample is considered nonreactive. If 2 of 3 sample results are greater than or equal to 1.00 Index Value, the sample is considered reactive and supplemental testing of the sample is recommended. If 2 of 3 sample results are greater than or equal to 0.80 Index Value and less than 1.00 Index Value supplemental testing of the sample is recommended.
- Samples with a calculated value greater than or equal to 1.00 Index Value are considered reactive for IgG antibodies to HCV. Supplemental testing of the sample is recommended.
- The supplemental testing algorithm is described in the Guidelines for Laboratory Testing and Result Reporting of Antibody to Hepatitis C Virus, MMWR 2003: 52(RR03) from the Centers for Disease Control.

References:
1. ADVIA Centaur Assay Manual, 07716220, Rev. A, 2005-02.
2. General Laboratory Procedure Manual

Hepatitis C Virus Quantitation, RNA Extraction

Minimal Description for Publication: In order to quantitate hepatitis C virus, RNA is extracted from serum using QIAamp Viral RNA Mini Kit (Qiagen Inc., Germantown, MD 20874).
Principle: QIAamp Viral RNA Mini Kit provides a fast and easy method to purify viral RNA for amplification. The purified RNA is free of protein, nucleases, and other contaminants and inhibitors. This procedure includes the addition of HCVRNA ASR which serves as an internal control and is used to calculate the HCV quantitation.
Specimen: Serum from a serum separator tube (biospecimen collection tube #1) is used for analysis. The serum is separated from the cells within 2 hours of collection and stored at -70° C until assayed.
Interferences: None
Equipment: Microcentrifuge with fixed angle rotor, capable of spinning up to 13,200 rpm.
Reagent: QIAmp Viral RNA Mini Kit (Qiagen PN 52906) 250 kit
Calibration: NA
Quality Control: ASR for HCV Template (Internal Control), HCV Genoytpe positive control specimen, HCV negative control specimen, BBI positive controls 1.7 x 10^5, 10^4 and 10^3
Expected Values: NA
References:
1. QIA amp Viral RNA mini Kit, Qiagen, Package insert #1030454, 12/2005
2. Abbott-Celera hand-outs, 2002

Hepatitis C Virus Quantitation

(This test is only performed on samples with positive or indeterminate Hepatitis C Antibody test)
Minimal Description for Publication: Hepatitis C virus is quantitated in serum using real-time polymerase chain reaction (PCR) on the ABI 7000 Analyzer, (Applied BioSystems, Foster City, CA 94404).
Principle: The Abbott-Celera platform utilizes the Applied BioSystem 7000 Real-Time polymerase chain reaction technology incorporating the 5' exonuclease (Taqman) probe chemistry utilizing Celera Diagnostic analyte specific reagents manufactured by Abbott Laboratories and uses the Qiagen Viral RNA kit to isolate the RNA from plasma or serum. For the quantitative PCR assay an internal control was added to the patient samples prior to extraction to provide an internal quantitation control and control for potential inhibitors or loss of sample during extraction. The real time assays are performed in 100 microliter volumes on an ABI 7000 sequence detection system using 50 microliters of the extracted RNA sample and 50 microliters of freshly prepared master mix in a one step RT-PCR reaction. After 50 cycles of PCR the data is extracted through an EXCEL program to provide a calculation of the viral load.
Specimen: Refer to the "HCV Quantification ASR, RNA Extraction" procedure above.
Interferences: None
Equipment: ABI 7000 Analyzer, (Applied BioSystems, Foster City, CA 94404).
Reagent: HCV Oligonucleotide reagent PN: 5000072, Zo5 DNA polymerase PN: 5000063, Manganese reagent PN: 5000062 (Celera Diagnostic analyte specific reagents manufactured for Abbott laboratories, Abbott Park, IL 60064-3500).
Calibration: NA
Quality Control: Positive and Negative controls are to be extracted with each run. A high (1.7 x 10^5), moderate (1.7 x 10^4) and low (1.7 x 10^3) control will be analyzed with each run of patients. The controls consist of a dilution of BBI quantitated HCV control. Each new lot of controls should be run in duplicate a minimum of 20 times and the mean standard deviation calculated. The control charts will be set based upon these calculations. A 1:10 dilution of the BBI positive control (concentration 1.7 x 10^5) with Base Matrix is made from the original vial to make a 10^4 and 10^3 concentration. This control is purchased from BBI Diagnostics, a Boston Biomedica Company. An HCV negative and an HCV positive of known genotype will be extracted and analyzed with each run. A No Target Control (NTC) will be added to each plate. The NTC is Molecular grade RNASE and DNASE free water. If controls are invalid or if they fall outside of the determined range, patient results should not be reported and the entire run must be repeated.
Expected Values:

- Dynamic range 25 to 50,000,000 (1.4 to 7.7 Log 10 IU/mL).
- If the results are below 25 they are reported as <25 and a log of <1.4.
- If results are greater than 25, they are reported as follows: Results are rounded to the nearest hundred and as the log value
- If the results are greater than 50,000,000 they are reported as >50,000,000 and a log of >7.7.

References:

1. ABI Prism 7000 Sequence Detection System User Guide, 2001,2002. Applied BioSystems.
2. Widen, R.H., Cummins, C.A. (2004) Tampa General Hospital, Tampa, FL. Evaluation of the Abbott Molecular Diagnostics Real Time PCR Assays for HCV Quantitative Viral load and HCV Genotyping. Clinical Virology Symposium Poster S30.
3. BBI Diagnostics, A Boston Biomedica Company. A Boston Biomedica Company, 375 West Street, West Bridgewater, MA 02379. Telephone Number 508-580-1900.
4. Package Inserts 2002-Manufactured by Celera Diagnostics, Alameda, CA 94502. Manufactured for Abbott Laboratories.

Hepatitis C Antibody Confirmation, RIBA for anti-HCV

(This test is only preformed if a sample has a hepatitis C antibody signal to cutoff ratio great than 1.0 and less than 11.0 and the hepatitis C virus by PCR result is <25. ARUP Laboratories performed this assay from the start of the study until May 10, 2011. On May 11, 2011 samples were sent to Mayo Medical Laboratories for this assay.)

Minimal Description for Publication: Hepatitis C antibody recombinant immunoblot assay (RIBA) is performed at ARUP Laboratories, Salt Lake City, UT 84108 prior to May 10, 2011 after which the test was performed at Mayo Medical Laboratory, Rochester, MN 55901.

Principle: This is a recombinant immunoblot assay (RIBA) that is performed to confirm samples that are positive for the hepatitis C antibody screening assays. Samples are sent to a commercial reference laboratory for this test. ARUP Laboratories performed this assay from the start of the study until May 10, 2011. On May 11, 2011 samples were sent to Mayo Medical Laboratories for this assay.

The CHIRON RIBA HCV 3.0 SIA is a 3-stage test that utilizes individual recombinant hepatitis C virus (HCV) antigens and synthetic peptides immobilized as individual bands onto the test strip. In the first stage, the specimen or assay control is diluted and incubated with the strip. Antibodies specific to HCV, if present, will bind to the corresponding recombinant antigen and/or synthetic peptide bands on the strip. Removal of unbound serum components is accomplished by aspiration and washing. In the second stage, the strip is incubated in the presence of a peroxidase-labeled goat-antihuman IgG conjugate. The conjugate binds to the human IgG portion of the antigen-antibody complexes. Removal of unbound conjugate is accomplished by decantation and subsequent wash steps. In the third stage, a colorimetric enzyme detection system composed of hydrogen peroxide and 4-chloro-1-naphthol is added. If bound conjugate is present, the enzymatic reaction will produce an insoluble blue-black colored reaction product at each specific HCV antigen, peptide, or control band. The color reaction involves the initial divalent oxidation of the peroxidase enzyme by hydrogen peroxide. Subsequent reduction of peroxidase to its initial state by 2 successive univalent interactions with soluble 4-chloro-1-naphthol results in the insoluble blue-black colored reaction product. After the development of color on the strip, the reaction is stopped by removal of the reactants and final wash steps. The visual band patterns that develop on each individual strip are the result of specific antibody being bound to each of the individual recombinant antigens and/or synthetic peptides on that strip. The reactivity of specimens towards each antigen band is determined by visually comparing the intensity of the individual antigen band with that of the low and high human IgG internal control bands blotted onto each strip. (Package insert: CHIRON RIBA HCV 3.0 SIA. CHIRON Corporation, Emeryville, CA)

Specimen: Serum from a serum separator tube (biospecimen collection tube #1) is used for analysis. The serum is separated from the cells within 2 hours of collection and stored at -70° C until assayed.

Interferences:

- This assay is not useful for detection of early or acute hepatitis C virus (HCV) (<2 months from exposure). Immunocompromised patients may not develop detectable anti-HCV until 6 months after infection.
- This assay is not useful for differentiating between past (resolved) and chronic hepatitis C. Such differentiation is best determined by detection of HCV RNA (#83142 "Hepatitis C Virus [HCV] RNA Detection and Quantification by Real-Time Reverse Transcription-PCR [RT-PCR], Serum."
- Performance characteristics have not been established for the following types of specimen:

- Grossly icteric (total bilirubin level of >60 mg/dL)
- Grossly lipemic (triglyceride level of >1,600 mg/dL)
- Grossly hemolyzed (hemoglobin level of >80 mg/dL)
- Cadaveric specimens
- Presence of particulate matter

Expected Values: Negative

References:

1. Carithers RL, Marquardt A, Gretch DR: Diagnostic testing for hepatitis C. Semin Liver Dis 2000;20(2):159-171

2. Germer JJ, Zein NN: Advances in the molecular diagnosis of hepatitis C and their clinical implications. Mayo Clin Proc 2001;76(9):911-920

3. Pawlotsky JM: Use and interpretation of virological tests for hepatitis C. Hepatology 2002;36:S65-S73

4. Alter MJ, Kuhnert WL, Finelli L: Centers for Disease Control and Prevention: guidelines for laboratory testing and result reporting of antibody to hepatitis C virus. MMWR Morb Mortal Wkly Rep 2003;52(No. RR-3):1-14

HDL-Cholesterol

Minimal Description for Publication: HDL-Cholesterol is measured in serum on a Roche Modular P Chemistry Analyzer (Roche Diagnostics Corporation) using a direct magnesium/dextran sulfate method (Roche Diagnostics, Indianapolis, IN 46250).
Principle: In this a 3rd generation, direct-method in which a magnesium/dextran sulfate solution is first added to the specimen to form water-soluble complexes with non-HDL cholesterol fractions. These complexes are not reactive with the measuring reagents added in the second step. With addition of reagent 2, HDL-cholesterol esters are converted to HDL-cholesterol by PEG-cholesterol esterase. The HDL-cholesterol is acted upon by PEG-cholesterol oxidase, and the hydrogen peroxide produced from this reaction combines with 4-amino-antipyrine and HSDA under the action of peroxidase to form a purple/blue pigment that is measured photmetrically at 600 nm (secondary wavelength = 700 nm). When the cholesterol measuring enzymes are modified with PEG, they are preferentially more reactive with HDL-cholesterol than the other cholesterol fractions. This is an endpoint reaction that is specific for HDL-cholesterol. This 3rd generation method differs from 2nd generation assays in the type of buffer used in the reagents, and the concentration of the reagent components. The basic reaction principle is unchanged.
Specimen: Serum from a serum separator tube (biospecimen collection tube #1) is used for analysis. The serum is separated from the cells within 2 hours of collection and stored at -70° C until assayed.
Interferences: Bilirubin does not interfere up to an I index of 30. Hemolysis does not interfere up to an H index of 1200. Lipemia does not interfere up to an L index 1000.
Equipment: Roche Modular P chemistry analyzer (Roche Diagnostics, Indianapolis, IN 46250).
Reagent: Roche product #04713214, HDL-C plus 3rd generation reagent kit (Roche Diagnostics, IN 46250).
Calibration: Roche Calibrator for Automated Systems (C.F.A.S.) Lipids, catalog #2172623, 3 x 1 mL. The Mod P will automatically perform a two-point calibration when there is a reagent lot number change. No other auto-calibrations are defined for the direct HDL-C assay. The Mod P will not allow testing to proceed until a successful calibration has been completed. Monitor control values to determine stability of the current calibration.
Quality Control: Two levels of control are assayed each time the direct HDL-C method is performed. It is acceptable to run each control at the start of the day, and again at the end of the day. The operator may run them more frequently, if desired. Both controls are prepared from pooled, normal human serum. One control is the pooled serum run without pre-dilution, the other is a 1:2 dilution of the pool. Consult the quality control detail table for current ranges and lots in use.
Expected Values:

- National Cholesterol Education Program (NCEP) guidelines (mg/dL): Major risk factor for CHD (<40), Negative risk factor for CHD (>60)
- Linear range of the method: 0-120 mg/dL. Specimens exceeding the high limit are automatically diluted (net 1:2) by the instrument, and reported accordingly. If a manual dilution is required, dilute the specimen in normal saline, and multiply the result by the dilution factor.
- Ananlytical Measurement Range: 0-120 mg/dL
- Clinically Reportable Range: 3-200 mg/dL

References:

1. Roche/Hitachi System Application Sheet for HDL-C plus 3rd generation, 2007.
2. Roche/Hitachi System Application Sheet for HDL-C plus 2rd generation, 2005.
3. Package insert for C.F.A.S. Lipids, 2005.
4. Roche/Hitachi Modular Analytics Operator's Manual, version 2.0, October 2006

Insulin Mercodia Assay - Used from Study beginning through Oct 28, 2009

Minimal Description for Publication: Insulin is measured in serum using a commercial ELISA assay (Mercodia AB, Uppsala, Sweden).

Principle: The Merocodia Insulin ELISA is a two-site enzyme immunoassay utilizing the direct sandwich technique with two monoclonal antibodies directed against separate antigenic determinants of the insulin molecule. Specimen, control, or standard is pipetted into the sample well, then followed by the addition of peroxidase-conjugated anti-insulin antibodies. Insulin present in the sample will bind to anti-insulin antibodies bound to the sample well, while the peroxidase-conjugated anti-insulin antibodies will also bind to the insulin at the same time. After washing to remove unbound enzyme-labeled antibodies, TMB-labelled substrate is added and binds to the conjugated antibodies. Acid is added to the sample well to stop the reaction, and the colorimetric endpoint is read on a microplate spectrophotometer set to the appropriate light wavelength.

Specimen: Plasma from EDTA-anticoagulated whole blood tube (biospecimen collection tube #4 or #10 for the OGTT) is used for analysis.

Interferences: Grossly lipemic, icteric, or hemolysed samples do not interfere in the assay.

Equipment:

1. SpectraMax 250 microplate reader capable of measuring absorbance at 450 nm. (Molecular Devices, Sunnyvale, CA).
2. Beckman Coulter Biomek 2000 Workstation, Beckman Coulter Brea, CA)

Reagent: Mercodia Insulin ELISA Kit. Catalog number 10-1113-10 (Mercodia AB, Uppsala, Sweden)

Calibration: Recombinant human insulin calibrators in concentrations of 2, 3, 10, 30, 100, and 200 mU/L and a blank calibrator (0 mU/L) are assayed on each tray with the samples.

Quality Control: Three levels of controls are assayed on each tray with the samples: Control Low (Catalog #10-1134-01), Control High (Catalog #10-1164-01), and the pooled in-house control. Consult quality control charts for current ranges and lots in use.

Expected Values:

- Reference Range: Fasting levels for 137 tested, apparently healthy individuals, yielded a mean of 9.2mU/L, a median of 6.9mU/L and a range of 2-25mU/L. The clinical reportable range (CRR) is 2 - 300 mU/L.
- Linearity The CSCL laboratory has determined that the Mercodia Insulin Elisa Kit is linear up to 135mU/L. Thus the analytical measurement range (AMR) is 2 - 135 mU/L. Specimens above 135 mU/L should be diluted with 0 calibrator and re-assayed. Values less than 2 mU/L are repeated. If the duplicates agree, the sample is reported as <2mU/L unless if otherwise directed by a study.
- Sensitivity: The detection limit is <1mU/L calculated as two standard deviations above the Calibrator 0.

References:

1. Mercodia AB. Mercodia Insulin ELISA kit insert. Mercodia AB, Sylveniusgatan 8A, SE-754 50 Uppsala, Sweden.

Insulin (ElecSys) - Used from Oct 29, 2009 through Study End

Minimal Description for Publication: Insulin is measured in serum on a Roche Elecsys 2010 Analyzer (Roche Diagnostics Corporation) using a sandwich immunoassay method (Roche Diagnostics, Indianapolis, IN 46250).

Principle: Insulin is measured in serum or plasma on a Roche Elecsys 2010 Analyzer (Roche Diagnostics Corporation) using a sandwich immunoassay method (Roche Diagnostics, Indianapolis, IN 46250). In the first incubation, the patient sample reacts with a biotinylated monoclonal insulin-specific antibody and a monoclonal insulin-specific antibody labeled with a ruthenium complex to form a sandwich complex. During the second incubation, streptavidin-coated microparticles are added and the complex becomes bound to the solid phase via interaction of biotin and streptavidin. The microparticles are then captured magnetically and unbound substance is removed. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier. The amount of light produced is directly proportional to the amount of insulin in the sample.

Specimen: Plasma from EDTA-anticoagulated whole blood tube (biospecimen collection tube #4 or #10 for the OGTT) is used for analysis.

Interferences: Hemolysis interferes, as insulin-degrading peptidases are released from erythrocytes. The assay is unaffected by icterus (bilirubin < 1539 µmol/L or < 90 mg/dL), lipemia (Intralipid < 1800 mg/dL), and biotin < 246 nmol/L or < 60 ng/mL.

Equipment: Elecsys 1010/2010, MODULAR ANALYTICS E170 or cobas e analyzer, (Roche Diagnostics Corporation, Indianapolis, IN 46250).

Reagent: Insulin Kit, Cat. No. 12017547 122, (Roche Diagnostics Corporation, Indianapolis, IN 46250).

Calibration: Traceability: This method has been standardized using the 1st IRP WHO Reference Standard 66/304 (NIBSC). Every Elecsys Insulin reagent set has a barcoded label containing the specific information for calibration of the particular reagent lot. The predefined master curve is adapted to the analyzer by the use of Elecsys Insulin CalSet. Calibration must be performed once per reagent lot using fresh reagent (i.e. not more than 24 hours since the reagent kit was registered on the analyzer). Renewed calibration is recommended as follows: after 1 month (28 days) when using the same reagent lot, after 7 days (when using the same reagent kit on the analyzer), as required: e.g. quality control findings outside the specified limits.

Quality Control: One commercial control (high range) and a pooled serum control (normal range) are run at the start of the day and then throughout the testing day along with test samples. The range of these controls is established within our laboratory. The values of the controls need to be evaluated as they are run on the instrument. The controls are plotted daily in the spreadsheet 'Elecsys 2010' within the CSCL Q drive, 'Daily QC tally' folder. Intra-assay precision is monitored by running one specimen in duplicate within the day, and inter-assay precision is monitored by running one specimen in duplicate between days.

Expected Values:

- Reference range, fasting: 12-150 pmol/L (2-25 mU/L).
- Linear range: 1.20-6000 pmol/L (0.200-1000 mU/L). Values below the detection limit are reported as <1.20 pmol/L (< 0.200 mU/L). Values above the measuring range are reported as >6000 pmol/L (> 1000 mU/L).
- Analytical measurement range: 1.2 – 4368 pmol/L (0.2 - 728 mU/L).
- Clinical reportable range:12 – 1800 pmol/L (2-300 mU/L) for specimens not associated with an OGTT.

References:

1. Sapin R. Review: Insulin Assays: Previously Known and New Analytical Features. Clin Chem 2003;49(3+4):113-121.

2. Roche Diagnostics. Insulin immunoassay package insert. Roche Diagnostics 9115 Hague Road Indianapolis, IN 46250-0457.

3. Marcovina, S., Bowsher, R., Miller, W.G., Staten, M., Myers, G., Caudill, S.P., et al. Standardization of Insulin Immunoassays: Report of the American Diabetes Association Workgroup. Clinical Chemistry 2007; 53:4:1-6.

Iron and Total Iron Binding Capacity (TIBC)

Minimal Description for Publication: Iron is measured in serum on a Roche Modular P chemistry analyzer, (Roche Diagnostics, Indianapolis, IN 46250) using a ferrozine reagent (Roche Diagnostics, Indianapolis, IN 46250). When UIBC is also assayed on the same sample, total iron binding capacity is calculated (TIBC = FE + UIBC).

Principle: This method for iron measurement utilizes the FerroZine® reagent without deproteinization. In an acidic environment (pH <2) iron is liberated from transferrin (binding protein). Lipemic specimens are also cleared during this step. Ascorbate reduces these released Fe3+ ions to Fe2+ ions which then react with FerroZine® to form a colored complex. The color intensity of this complex is directly proportional to the iron concentration, and this final product is measured as an endpoint reaction at 570 nm (secondary wavelength = 700 nm).

Specimen: Serum from a serum separator tube (biospecimen collection tube #1) is used for analysis. The serum is separated from the cells within 2 hours of collection and stored at -70° C until assayed.

Interferences: Bilirubin does not interfere up to an index of 60. Hemolysis does not interfere up to an H index of 80. Higher hemoglobin concentrations lead to false-positive values due to contamination of the specimen with hemoglobin bound iron. Lipemia does not interfere up to an L index 1000.

Equipment: Roche Modular P chemistry analyzer, (Roche Diagnostics, Indianapolis, IN 46250).

Reagent: Roche product #1876996, Fe reagent kit, (Roche Diagnostics, Indianapolis, IN 46250).

Calibration: Roche Calibrator for Automated Systems (C.F.A.S.), catalog #759350. The C.F.A.S. calibrator is traceable to reference material SRM 937. This is a reference material provided by the National Institute of Standards and Technology. The Mod P will automatically perform a two-point calibration when there is a reagent lot number change. No other auto-calibrations are defined for the iron assay. The Mod P will not allow testing to proceed until a successful calibration has been completed. Monitor control values to determine stability of the current calibration.

Quality Control: Two levels of control are assayed each time the iron method is performed. It is acceptable to run each control at the start of the day, and again at the end of the day. The operator may run them more frequently, if desired. One control is prepared from pooled, normal human serum. The other is an elevated, abnormal commercial control. Consult quality control charts for current ranges and lots in use.

Expected Values:

- Reference range: Serum, female: 30-160 ug/dL; male: 45-160 ug/dL
- Linear range of the method: 5-1000 ug/dL (serum). Specimens exceeding the high limit are automatically diluted (7:15) by the instrument, and reported accordingly. If a manual dilution is required, dilute the specimen in normal saline, and multiply the result by the dilution factor. Report values <5 as <5 ug/dL. If the iron result is <5, then the iron binding calculations (see below) cannot be performed. Enter ;NA-C7014 for test code FEB (iron binding capacity) and ;NA-C7014 for test code ISI (iron saturation index).
- If UIBC (unbound iron binding capacity) is also performed with the iron test, the Misys host computer system will automatically calculate total iron binding capacity (TIBC = FE + UIBC) and iron saturation index ((FE/(FE + UIBC))*100. The Mod P is also programmed to perform this calculation, but the ModP value is not transmitted to Misys.
- Analytical Measurement Range: 5-1000 ug/dL
- Clinically Reportable Range: 5-2000 mg/dL

References:

1. Roche/Hitachi System Application Sheet for Iron, 2004.
2. Package insert for C.F.A.S., 2005.
3. Roche/Hitachi Modular Analytics Operator's Manual, version 2.0, October 2006.

Triglycerides

Minimal Description for Publication: Triglycerides are measured in serum on a Roche Modular P chemistry analyzer, (Roche Diagnostics, Indianapolis, IN 46250) using a glycerol blanking enzymatic method (Roche Diagnostics, Indianapolis, IN 46250).
Principle: In this enzymatic method reagent 1 (glycerol blanking) is added first. Free glycerol is converted to glycerol-3-phosphate (G3P) by glycerol kinase. G3P is acted upon by glycerol phosphate oxidase to produce dihydroxyacetone phosphate and hydrogen peroxide. The hydrogen peroxide combines with 4-chlorophenol under the action of peroxidase to produce an oxidation product that that does not react with the colorometric component of reagent 2. After this initial reaction sequence is completed, the Mod P records a blank absorbance reading. Then reagent 2 is added. The second reaction is driven by the reagents from bottle 1, with lipase added in reagent 2 to convert triglycerides to glycerol, and 4-aminophenzone added to react with the hydrogen peroxide produced in the last reaction. The reaction is measured at 505 nm (secondary wavelength = 700 nm). This method is a two-reagent, endpoint reaction that is specific for triglycerides.
Specimen: Serum from a serum separator tube (biospecimen collection tube #1) is used for analysis. The serum is separated from the cells within 2 hours of collection and stored at -70° C until assayed.
Interferences: Bilirubin does not interfere up to an I index of 25. Hemolysis does not interfere up to an H index of 400. There is a poor correlation between the triglyceride concentration and visible lipemia. Specimens with an exceptionally high triglyceride concentration (>3000 mg/dL) may produce a normal result. Therefore, very lipemic specimens should be manually pre-diluted 1:5 or assayed on decreased sample volume.
Equipment: Roche Modular P chemistry analyzer, (Roche Diagnostics, Indianapolis, IN 46250).
Reagent: Roche product #1877771, Trig/GB reagent kit, (Roche Diagnostics, Indianapolis, IN 46250).
Calibration: The calibrator used for this assay is obtained from a unit of whole blood collected from a single donor. The unit of blood is collected at the UMMC donor center, then it is allowed to clot overnight at room temperature. There are no additives in the collection bag. Triglyceride concentration will vary with each donor selected. The calibrator is stored at -70° C. The new calibrator is assayed in duplicate for 20 consecutive days. The new set point is derived from the mean of these analyses. The ModP will automatically calibrate (2-point) triglycerides when there is a reagent lot number change. There is no automatic time-dependent calibration. Monitor control values to determine stability of the current calibration.
Quality Control: Two levels of control are assayed each time the triglycerides method is performed. It is acceptable to run each control at the start of the day, and again at the end of the day. The operator may run them more frequently, if desired. One control is prepared from pooled, normal human serum. The other is an elevated, abnormal commercial control. Consult quality control charts for current ranges and lots in use.
Expected Values:

- Reference range: Serum, adult: <200 mg/dL
- Linear range of the method: 0-1000 mg/dL (serum). Specimens exceeding the high limit are automatically diluted (1:5.5) by the instrument, and reported accordingly. If a manual dilution is required, dilute the specimen in normal saline, and multiply the result by the dilution factor.
- Analytical Measurement Range: 0-1000 mg/dL
- Clinically Reportable Range: 10-4000 mg/dL

References:

1. Roche/Hitachi System Application Sheet for Triglycerides/GB, 2005-06.
2. Roche/Hitachi Modular Analytics Operator's Manual, version 2.0, October 2006.

Unbound Iron Binding Capacity (UIBC)

Minimal Description for Publication: Unbound iron binding capacity (UIBC) is measured in serum on a Roche Modular P chemistry analyzer, (Roche Diagnostics, Indianapolis, IN 46250) using a ferrozine reagent without deproteinization (Roche Diagnostics, Indianapolis, IN 46250). When iron is also assayed on the same sample, total iron binding capacity is calculated (TIBC = FE + UIBC).
Principle: This method for UIBC measurement is a modification of the method of Goodwin, utilizing the FerroZine® reagent without deproteinization. Serum is added to an alkaline buffer/reductant solution containing a known concentration of iron to saturate the available transferrin binding sites in the specimen. Reduced excess iron then reacts with the FerroZine® reagent. The UIBC is equal to the difference measured in the concentrations of the added iron and the excess unbound iron. This is an endpoint reaction with sample blank, measured at 570 nm (secondary wavelength = 700 nm).
Specimen: Serum from a serum separator tube (biospecimen collection tube #1) is used for analysis. The serum is separated from the cells within 2 hours of collection and stored at -70° C until assayed.
Interferences: Bilirubin does not interfere up to an I index of 60. Hemolysis does not interfere up to an H index of 125. Lipemia does not interfere up to an L index 100.
Equipment: Roche Modular P chemistry analyzer, (Roche Diagnostics, Indianapolis, IN 46250).
Reagent: Roche product #1815156, UIBC reagent kit, (Roche Diagnostics, Indianapolis, IN 46250).
Calibration: Roche Iron Standard, 500 ug/dL, catalog #1985922. The Mod P will automatically perform a two-point calibration every 24 hours, when there is a reagent bottle change, and when there is a reagent lot change. The Mod P will not allow testing to proceed until a successful calibration has been completed. Monitor control values to determine stability of the current calibration.
Quality Control: Two levels of control are assayed each time the UIBC method is performed. It is acceptable to run each control at the start of the day, and again at the end of the day. The operator may run them more frequently, if desired. One control is prepared from pooled, normal human serum. The other is an elevated, abnormal commercial control. Consult quality control charts for current ranges and lots in use.
Expected Values:

- Reference ranges: UIBC: 110-370 ug/dL
  TIBC: 228-428 ug/dL (TIBC = Iron + UIBC)
- Linear range of the method: 10-500 ug/dL (serum). Specimens exceeding the high limit must be manually diluted in Milli-Q water. Assay the dilution on normal sample volume, and multiply the result by the dilution factor. Values falling below the lower technical limit may still be used in calculation of the TIBC. If the iron result is <5, then the iron binding calculations (see below) cannot be performed. Enter ;NA-C7014 for test code FEB (iron binding capacity) and ;NA-C7014 for test code ISI (iron saturation index).
- If iron is also performed with the UIBC test, the Misys host computer system will automatically calculate total iron binding capacity (TIBC = FE + UIBC) and iron saturation index ((FE/(FE + UIBC))*100.
- Analytical Measurement Range: 10-500 ug/dL
- Clinically Reportable Range: 1-1000 mg/dL

References:

1. Roche/Hitachi System Application Sheet for UIBC, 2005.
2. Roche/Hitachi Modular Analytics Operator's Manual, version 2.0, October 2006.

Quality Control and Quality Assurance for Clinical Chemistry

A. QUALITY CONTROL CALCULATIONS

Internal quality control procedures monitor analytical performance relative to medical goals and alert analysts to unsatisfactory analytical performance. Quality control statistics are used to make judgments about the quality of analytical results, whether system correction is necessary, whether patient data should be accepted or rejected, and for estimating performance parameters which can be compared to analytical and medical goals.

NEW METHOD OR INSTRUMENTATION:

For every analyte, use the standard deviation overall (SDo) to establish limits for quality control assays. Calculate a new permanent SDo and duplicate range whenever a new method or instrument is put into use. Additional data may be needed to calculate an appropriate duplicate range for patient samples.

1. Analyze 50-100 control values on 50-100 different days. (Values obtained on different analyzers on the same day may be considered to be "different days".) Fewer values may be used for procedures that are not performed daily (at least 20 values), or for procedures using highly automated systems with minimal operator influence (at least 30 values). If procedures are performed less than weekly, fewer values (at least 10) may be used.
2. Within each day, randomly select the control value used in the calculation to establish the ranges. Do not exclude values unless the control currently in use is unacceptable.
3. Calculate mean, SDo, and coefficient of variation (CV). (Recommended method is StatView.) After calculating, inspect data for outliers; do not exclude values from the data unless outside the 3SDo limits.
4. Refer to the "Duplicate Range" section.
5. Enter the required information on the Cumulative Control Tabulation Sheet. Submit to the laboratory manager or laboratory director for approval.

NEW CONTROL

1. For a new lot of control, calculate a mean using 20 control values analyzed on 20 different days. (Values obtained on different analyzers on the same day may be considered to be "different days".) If necessary, establish a temporary mean using fewer than 20 values. Recalculate when 20 values are available. If procedures are performed less than weekly, 10 values may be used.
2. Calculate SDo and CV using 20 control values. The calculated SDo and CV serve to monitor the permanent SDo and CV, and should not be used to establish permanent confidence limits routinely. If there is a question as to whether new permanent ranges need to be established, additional values must be collected (see previous section).
3. Enter the required information on the Cumulative Control Tabulation Sheet. Submit to the laboratory manager or laboratory director for approval.

CONTROL CHARTS

1. Design control charts including mean, 2SDo and 3SDo confidence limits. Choose the scale of the y-axis to provide a concentration range from mean minus 4SDo to mean plus 4SDo.
2. When possible record control values to one more significant digit than patient values are reported. Calculate significant digits for QC limits using the following guidelines:

	Preferred Method	Instrument reports QC and patients to same number of digits:
Report patients	XX.	XX.
Measure control	XX.X	XX.
Calculate control mean	XX.XX, round to XX.X	XX.X
Calculate SD	X.XX, round to X.X	X.X
Calculate ranges	XX.X ± X.X	XX.X ± X.X
Design control charts	Plot mean XX.X; plot SD limits to XX.X	Plot mean XX.X; round SD limits to XX

3. Plot all control values daily. If more than 10 controls are analyzed daily, plot only the mean, high value, and low value; note the number of controls analyzed and the number of controls out of range.

Record the initials of the person performing the analysis on the control chart; when this is not possible, record the initials of the individual plotting the data.

4. Use three Westgard rules to determine whether data is acceptable (i.e., in statistical control) to be reported.

RULES	BATCHED TESTING	NON-BATCHED TESTING (continuously reported testing):
Warning Rule: 1-2s	If one control observation falls outside the 2SDo limits, additional inspection of control data and application of the rules below are required before the analytical run is accepted or rejected.	
Action Rule: 1-3s	Reject the analytical run if one control observation falls outside the 3SDo limits.	Consult the charge technologist and spot-check an appropriate number of patient samples analyzed since the last acceptable control or since the problem detected occurred if one control observation falls outside the 3SDo limits. Complete result correction in the computer as necessary.
Action rule: 2-2s	Reject the analytical run if two consecutive control observations exceed the same 2SDo limit (mean plus 2SDo or mean minus 2SDo). (This rule applies to two different control materials in the same run or two consecutive observations on the same material in two consecutive runs.)	Consult the charge technologist if two consecutive control observations exceed the same 2SDo limit (mean plus 2SDo or mean minus 2SDo). (This rule applies to two different control materials in the same run or two consecutive observations on the same material in two consecutive runs.)
Accept/ Reject:	Accept the run if the rules indicate that the run is in statistical control.	
Any exception in reporting results from a run which violates rules a, b, or c requires the approval of the Charge Technologist, Technical Supervisor, Laboratory Manager, or Laboratory Director. See attached exceptions to QC policy.		

5. Document action taken when control values are unacceptable, reagent changes, and other pertinent information on the control chart.
6. The faculty advisor for the section reviews and initials control charts monthly. Review occurs at Quality Control meetings attended by the appropriate Laboratory Manager and Technical Supervisors.
7. Exceptions to quality control policies which are to apply to a given analyte or instrument on an ongoing basis require the approval of the Chemistry Quality Assurance Committee. File exceptions with the quality control charts for the instrument/analyte, with the Quality Control procedure master in B203, and General Procedure Books located in lab section involved and in C215 Mayo.

DUPLICATE RANGE:

Calculate the statistical confidence limits for duplicates (used to check precision) by one of several methods.

For each analyte determine whether the within day duplicate range, the between day duplicate range, or both will be utilized. In all cases it is important that the appropriate within or between batch SD, CV, or average R for duplicates be used. It is not possible to predict how within and between batch SD (or CV) relate to each other.

	WITHIN DAY DUPLICATES	BETWEEN DAY DUPLICATES
WHEN TO USE:	To evaluate duplicate determinations within an analytical run.	To evaluate duplicate determinations between days and in combination with intraindividual biological variability information, to help determine whether a change in a patient value is statistically significant.
METHOD 1	Use the SDw for the control material	Use the SDo for the control material
METHOD 2	Use the CV for the control material calculated from an SDw	Use the CV for the control material calculated from an SDo
METHOD 3	Use the average R from a series of 20 or more control material within day duplicates analyzed over a 1-20 day period	N/A
METHOD 4	Use the average R from a series of 50 or more patient within day duplicates analyzed over a 1-50 day period	Use the average R from a series of 50 or more patient duplicates analyzed between day
NOTE	Theoretically, all four methods should give identical answers if: 1) the control materials behave identically to patient samples, and 2) the SD is independent of analyte concentration. However, these assumptions are often not correct. To determine whether control materials behave similarly to patient samples, compare the duplicate range calculated by method 1, 2, or 3 with that calculated by method 4. For some assays, neither SD nor CV are constant over the range of analyte concentrations, and it may be difficult to obtain patient samples for duplicate determinations over the potential range of patient values. In these instances, use the SD for the control material at various levels to calculate duplicate ranges using method 1. (This method assumes control materials behave like patient specimens.)	

Select the correct method(s) to calculate appropriate duplicate ranges (95% confidence limits).

1. If the SD is constant over the entire range of analyte concentrations and patient specimens behave like the control material:

$Duplicate_w$ range (absolute value) = 2.77 x SD_w

$Duplicate_o$ range (absolute value) = 2.77 x SD_o

Calculate SD_w using 10-25 control values analyzed the same day. (The estimated calculation ,SD_w = R/1.128 is no longer used.)

2. If the CV is constant over the entire range of analyte concentrations and patient specimens behave like the control material:

$Duplicate_w$ range (% value) = 2.77 x CV_w

$Duplicate_o$ range (% value) = 2.77 x CV_o

3. If patient specimens behave like the control material and a within day duplicate range is needed (but an SDw is not available), use the average R of 20 or more control material duplicates:

$Duplicate_w$ range (absolute value) = 2.46 x $\bar{R}_w$

4. When quality control materials behave significantly differently from patient samples, for example with blood gas analysis, use the average R of 50 or more patient specimen duplicates representing the reportable range:

$Duplicate_w$ range (absolute value) = 2.46 x $\bar{R}_w$

$\bar{Duplicate}_o$ range (absolute value) = 2.46 x R_o

Plot the absolute difference between duplicate determinations vs. the average of the two duplicate values to determine whether the same duplicate range can be applied to patient values over the entire instrument analytical range. If the duplicate range appears to be independent of analyte concentration, the duplicate range can be applied at all concentrations.

If the reproducibility of duplicates seems to change as analyte concentration changes, estimate the duplicate range for two or more ranges of patient values by collecting 50 or more duplicates within each range of values or decision points to determine an individual R for each range.

Use of this method to approximate duplicate range at a concentration very different from that of the 50 duplicates is not recommended, because: 1) the formula used in method 4 assumes the SD is constant over analyte concentrations, and 2) for many assays (e.g., most immunoassays) there is no reason to expect the CV to remain constant over the entire range of analyte concentrations.

Alternatively, estimate the duplicate range at one concentration from the duplicate ranges at another concentration by calculating the duplicate range as a percentage, rather than absolute value. Fundamentally, this approximation assumes the CV, rather than the SD, is constant over the analytical range of the instrument.

SECTION 2 - QUALITIY ASSURANCE

Quality Assurance Systems for Clinical Chemistry

REFERENCE STANDARDS:

Analyze aqueous standards or protein-based calibrators with all analytical runs whenever appropriate. Check permanent calibrations at least every six months.

Where applicable, use NIST standard reference material to prepare the standard or to check the material used as the standard. Prepare stock standards at least yearly. Check new stock standards against current stock standards to a stated tolerance, usually +1% of the nominal value, before introduction into use. Dilute working standards from a stock standard which has been checked. Check working standards according to the requirements of the method, most commonly by assaying against the current standards to ensure they read within marker range, as defined in the individual analytical procedure.

CONTROLS:

Analyze two or more levels of controls daily, whenever possible. Evaluate quality control using ranges established in the FUMC laboratory or manufacturers' stated ranges..

The following control materials may be used:

1. Commercial liquid bovine or human based serum
2. Commercial lyophilized bovine or human based serum control
3. Commercial lyophilized urine control
4. Frozen human donor pools (tested to be negative for HIV and hepatitis B and hepatitis C) prepared by the laboratory

EXTERNAL PROFICIENCY SURVEYS:

The Chemistry Laboratory participates in a number of proficiency testing surveys provided by such organizations as CAP and CDC. The reports submitted are signed by the staff performing the assays and Laboratory Manager when appropriate. Deficiencies are documented and discussed at Lab Administration Meeting.

REPORTING OF RESULTS:

Define the lowest and highest concentration for each analyte which is reported, based on the linearity, sensitivity, precision and clinical utility of the method.

Define technical limits in the laboratory computer which represent "impossible" values, whenever possible.

Report results to no more than three significant figures, e.g., report 1286 U/L as 1290 U/L or pH 7.386 as 7.39. An exception to this policy is instruments which are on-line to the Laboratory computer.

QUALITY ASSURANCE SYSTEMS IN OPERATION TO DETECT ERRORS OR UNUSUAL LABORATORY RESULTS:

In order to minimize the possibility of clerical and analytical errors, the Chemistry Laboratory utilizes a laboratory computer system for the entry and verification of test results before the results are released to the hospital information system (STAR/Unisys) or to the patient's permanent record.

1. Review all results against raw instrument data to ensure the proper calculation and interpretation of results.
2. Perform result entry via computer interface for instruments with high volume tests to avoid errors in manual entry.

3. After computer entry and prior to reporting results, review results on the CRT or computer-generated printout against the original protocol book. Whenever possible, review results against the patient's previous result (delta check value) to detect possible discrepancies.
4. Analyze daily or periodic duplicate specimens to check the analytical performance in laboratory areas where duplicate instrumentation performs the same analytical tests. Take corrective action if necessary.
6. Refer unusual or questionable laboratory results to the supervisor. If appropriate, the supervisor will refer to a faculty member or Laboratory Medicine resident via an Action Report.
7. Consult with supervisor about remedial action to be taken when calibration or controls fail to meet criteria for acceptability.
8. Call results which exceed critical limits to the immediate attention of the patient care unit or clinic.
9. Use backup equipment or consult a supervisor if a test system becomes inoperable.

GENERAL QUALITY ASSURANCE SYSTEMS

1. Record temperature and humidity as necessary. Tolerance limits are defined in the Temperature and Humidity Procedure in the General Procedure Book.
2. Reagent labels must include storage requirements, name of reagent, concentration, date prepared, date received, date of expiration, and special safety information. Purchased prepared reagents must be labeled appropriately.
3. New or revised methods must be validated before being put into use. New or revised test report information from LIS must be checked before use.
4. All procedures must be reviewed annually.
5. Complete Incident Reports when appropriate.
6. Document complaints, problems and other feedback on "Customer Feedback Log" located near the telephones. These items are reviewed at Laboratory Administration Meeting, and appropriate follow-up is initiated.
7. Evaluate quality assurance data and reports at Laboratory Administration Meeting. Reports may include turn-around time, proficiency surveys, Feedback Logs, and special projects.

REFERENCES:

1. Youden, WJ. Statistical methods for chemists. New York: John Wiley & Sons, 1951: 8-23.
2. Natrella, MG. Experimental statistics. Washington, D.C.: U.S. Government Printing Office, 1963: 2-6, 2-7, T-18, T-19.
3. Westgard JO, Barry PL, Hunt MR. A multi-rule shewhard chart for quality control in clinical chemistry. Clin Chem 1981; 27:493-501.
4. Westgard, JO, Quam EF, Barry PL. Selection grids for planning quality control procedures. Clin Lab Science 1990; 3:271-8.
5. National Committee for Clinical Laboratory Standards. Internal quality control: Principles and definitions; Approved Guidelines, NCCLS document C24-1 (ISBN 1-56238-112-1). NCCLS, 771 East Lancaster Avenue, Villanova, PA 19085, 1991.

Appendix - Laboratory Testing Quality Control

		Internal Lab QC Data				Blind Replicate Data			
Test Name	Units	N	Mean±SD	CV		N	Mean±SD	Reliability	CV
*Hemogram (CBC):									
White Blood Count (WBC)	x 10^9/L	83	6.794±0.12	1.8		892	6.5±0.38	0.96	5.8
Red Blood Count (RBC)	x 10^{12}/L	83	4.348±0.04	0.9		972	4.7±0.08	0.96	1.7
Hemoglobin	g/dL	83	12.49±0.10	0.8		970	13.7±0.25	0.97	1.8
Hematocrit	%	83	35.99±0.45	1.2		971	41.8±0.79	0.96	1.9
Mean Corpuscular Volume (MCV)	fL	83	82.78±0.64	0.8		969	88.9±0.58	0.99	0.7
Mean Corpuscular Hemoglobin (MCH)	pg	83	28.72±0.23	0.8		973	29.1±0.28	0.98	1.0
Mean Corpuscular Hemoglobin Concentration (MCHC)	g/dL	83	34.68±0.34	1.0		976	32.8±0.35	0.94	1.1
Red Cell Distribution Width (RDW)	%	83	14.63±0.11	0.7		974	13.7±0.10	0.99	0.7
Platelet Count	x 10^9/L	83	212±5.18	2.4		968	251.5±9.54	0.98	3.8
WBC Differential:									
Neutrophils	%	83	48.56±0.84	1.7		881	54.7±3.55	0.90	6.5
Lymphocytes	%	83	31.34±0.75	2.4		877	33.4±2.87	0.90	8.6
Monocytes	%	83	9.57±0.47	4.9		890	8.0±1.06	0.81	13.2
Eosiniphils	%	83	10.53±0.71	6.7		891	3.0±0.55	0.96	18.4
Basophils	%	83	69.18±0.76	1.1		889	0.5±0.38	0.53	70.1
Absolute Neutrophils	x 10^9/L	83	3.301±0.09	2.7		886	3.6±0.37	0.94	10.2
Absolute Lymphocytes	x 10^9/L	83	2.129±0.06	2.8		881	2.1±0.18	0.92	8.3
Absolute Monocytes	x 10^9/L	83	0.650±0.04	6.2		894	0.5±0.07	0.84	14.1
Absolute Eosiniphils	x 10^9/L	83	0.716±0.05	7.0		893	0.2±0.04	0.97	19.0
Absolute Basophils	x 10^9/L	83	4.701±0.10	2.1		891	0.0±0.03	0.58	124.8
Total cholesterol	mg/dL	100	174.3±3.8	2.2		815	198.2±5.93	0.98	3.0
Triglycerides	mg/dL	100	115.6±3.2	2.8		815	133.8±5.16	1.00	3.9
HDL-cholesterol	mg/dL	100	50.7±1.3	2.6		818	49.7±1.56	0.99	3.1
LDL-cholesterol*	mg/dL	21	99.2±3.6	3.6		795	121.3±4.56	0.98	3.8
Glucose, fasting	mg/dL	100	50.3±1.33	2.6		809	105.6±2.91	0.99	2.8
Glucose, post OGTT	mg/dL	100	240.2±3.9	1.6		966	122.3±3.67	0.99	3.0
Glycosylated Hemoglobin	%	100	5.36±0.03	0.6		969	5.8±0.05	1.00	0.8
Insulin, fasting	mU/L	100	19.81±1.18	6.0		767	13.5±1.55	0.98	11.3
Insulin, post OGTT	mU/L	100	57.81±3.39	5.9		934	88.0±7.55	0.99	8.6
Alanine aminotransferase (ALT)	U/L	100	23.8±1.2	5.0		820	25.9±1.63	0.99	6.3
Aspartate aminotransferase (AST)	U/L	100	25.0±1.5	6.0		822	23.8±1.83	0.98	7.7
hsCRP	mg/L	100	2.43±0.06	2.5		813	3.7±0.17	1.00	4.7
Creatinine, serum	mg/dL	100	0.76±0.031	4.1		823	0.8±0.06	0.97	6.6
Creatinine, urine	mg/dL	100	96.57±1.40	1.4		990	147.0±14.53	0.97	9.9
Albumin, urine	Mg/dL	100	13.81±0.67	4.8		929	28.5±3.96	1.00	13.9
Albumin/creatinine ratio*	mg/g creat	12	139.5±6.5	4.7		929	22.0±3.61	1.00	16.4
Iron	ug/dL	100	91.1±3.1	3.4		819	88.6±2.91	0.99	3.3
Total Iron Binding Capacity (TIBC)*	ug/dL	13	311.5±8.5	2.7		818	323.4±10.57	0.95	3.3
Transferin saturation*	%	13	30.4±0.72	2.4		815	28.2±0.76	1.00	2.7

* These laboratory analytes were not measured analytically but were calculated arithmetically using values from other analytes. The Internal lab QC data for the calculated analytes is based on control values from one month of the measured analytes.