Title: Diabetes Diagnostic Lab- C-peptide II (C-PEPII) Assay - Standard

MU Health Care	**Document Owner:** Rhonda Rojas	**Last Approved Date:** 05/02/2022
	Content Expert: Rhonda Rojas	

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I. Purpose Statement

a. This assay is designed for IN VITRO DIAGNOSTIC USE ONLY for the quantitative measurement of C-peptide in human serum and EDTA Plasma (for Clinical Research Testing Use) on Tosoh AIA System Analyzers.
b. Scope: Technical Staff competent to perform the C-PEPII assay on the AIA 900.

II. Definitions

a. Not Applicable

III. Content

a. **Summary of Test Principle and Clinical Relevance**

The ST AIA-PACK C-Peptide II is a two-site immunoenzymometric assay which is performed entirely in the ST AIA-PACK C-Peptide II test cups. This assay is designed for IN VITRO DIAGNOSTIC USE ONLY for the quantitative measurement of C-peptide in human serum and EDTA plasma on Tosoh AIA System analyzers. C-peptide present in the test sample is bound with monoclonal antibody immobilized on a magnetic solid phase and enzyme-labeled monoclonal antibody in the test cups. The magnetic beads are washed to remove unbound enzyme-labeled antibody and are then incubated with a fluorogenic substrate, 4-methylumbelliferyl phosphate (4MUP). The amount of enzyme-labeled monoclonal antibody that binds to the beads is directly proportional to the C-peptide concentration in the test sample. A standard curve is constructed, and unknown sample concentrations are calculated using this curve.

C-peptide, a polypeptide 31 amino acids in length, originates in pancreatic B-cells as a metabolically inert by-product in the synthesis of insulin from proinsulin. Insulin and C-peptide are released from proinsulin in equimolar concentrations into the portal circulation. Therefore, C-peptide levels can serve as an index to insulin secretion. Where insulin secretion is diminished, as in insulin-dependent diabetes, low C-peptide levels are to be expected. Elevated C-peptide levels may result from increased B-cell activity associated with insulinomas.

Anti-insulin antibodies are commonly found in patients who have undergone insulin therapy. These circulating anti-insulin antibodies may interfere with certain immunoassays for insulin, making it difficult to use as a measure of residual B-cell activity. C-peptide measurement is, therefore, used as an alternative measurement index in this context. C-peptide measurement is also used as an additional means of evaluating glucose tolerance tests.

b. **Specimen Collection and Handling**

i. Patient Preparation:
Per manufacturer's instructions, no special patient preparation is necessary. However, the patient may fast at the request of the physician.

ii. Specimen type and stability:

i. For serum collection, a venous blood sample collected aseptically without additives (Red Top Tube) is preferred sample type for the assay.

ii. For plasma collections, venous blood samples collected aseptically using EDTA vacutainers, can also be used (applicable for Clinical Research Testing Use).

iii. Heparinized and citrated plasmas SHOULD NOT BE USED.

iv. At the draw station – for serum collection, allow specimen to clot completely at room temperature for 45 min, then centrifuge. Separate serum from cells ASAP, transfer serum to a plastic vial/tube then freeze serum. For plasma collection, centrifuge and separate plasma from the packed cells as soon as possible.

v. As per manufacturer's instructions, samples may be stored at 2 – 8 ° C for up to 24 hr prior to analysis. If analysis cannot be done within 24 hr, samples should be stored frozen at – 20 °C or below for up to 60 ays.

vi. For QA purposes and for long-term clinical trials (i.e., not for patient care): a stability study performed in-house and approved by the Lab Director shows that c-peptide in serum (only) is stable at:

a. Room temperature: 6 hr
b. Frozen (-70 °C or below): 10 years
c. Liquid Nitrogen: 15 years

vii. Repeated freeze-thaw cycles should be avoided. Turbid serum samples or samples containing particulate matter should be centrifuged (return to IR processing for additional sample processing) prior to testing. Prior to assay, slowly bring frozen samples to room temperature and mix gently.

viii. The minimum volume required for analysis directly from the plastic vial/tube (primary tube) is ~600 µL (ideally 1 ml, if repeat testing is necessary). Sample volumes less than 500 µL should be transferred to a pre-labeled sample cups (shorter vials). Refer to the Diabetes Diagnostic Laboratory-Quality Management Program-Standard

for guidance on labeling Secondary Specimen Containers/test tubes.

ix. Specimens should be transported and maintained under frozen conditions until testing.

x. Specimens are received in room M764 after being sorted by IR Processing. Each c-peptide sample must arrive in the lab labeled with a unique accession number generated by the Cerner Pathnet system, unless downtime procedures are in effect.

xi. Each patient aliquot should be labeled with a pathnet label and verified within the pathnet system (once logged in) using the below identifiers:

a. Unique accession ID and patient's name.
b. MRN.
c. Collection Date and Time (verified within the LIS).
d. Container type (e.g. Red Top Tube), sample type (e.g. serum).
e. Lab name (DDL).
f. Test name (c-peptide).

xii. Each non-pathology sample, (e.g. a clinical research study sample) should be labeled with at least two specific identifiers. Example of acceptable identifiers include but are not limited to; patient/subject's name, date of birth, accession number. Identifiers may be in a machine readable format, such as a barcode. In limited situation, a single identifier may be used if it can uniquely identify the specimen. Examples include coded or de-identified research specimens. Each labeled non-pathology aliquot once received, should be verified against a provided sample list (Sample Manifest).

iii. **Unacceptable specimen (for testing) criteria:**

1. Samples with bubbles should not be used. All bubbles should be removed prior to analysis.
2. Clotted samples (unable to obtain an aliquot from tube for testing). These should not be placed directly onto the analyzer.
3. Gross Lipemia, as indicated by triglyceride concentration (**refer to Interference section below**).
4. Specimen types and stability not listed above.
5. Sample analysis volume < 200 µL.
6. Unlabeled patient samples (specimens are to be labeled with patient name, rec. number, accession, test order, and transferring lab name).
7. Incorrectly labeled specimens not following the above criteria.
8. Indication of a sample leakage (unable to obtain a sample for testing).

9. Refer to the 'Limitation to Procedure, Interference and Appendix' section below for additional details on handling hemolyzed specimens.
10. Samples not suitable for testing should be rejected. Refer to the Laboratory - Specimen Rejection - Guideline and the Laboratory- Test Cancellation- Guideline for additional details. The ordering clinician should be communicated to with a reason for cancellation. Refer to the 'Limitations to the Procedure, Interference and Appendix' section below for additional details and the Diabetes Diagnostic Laboratory-Quality Management Program-Standard
11. Document each rejected specimen by filling out the test name, date received/collected, patient name, accession ID, reason for rejection, primary contact, form of contact, and tech's initials using the Diabetes Diagnostic Laboratory - Preanalytical Problem Resolution and Specimen Rejection Log . Other pre-analytical problems, such as duplicate orders or processing errors, should also be documented using this log.
12. The QAQR database should also be used to electronically document unacceptable specimens and/or other specimen occurrences relating to pre-analytical issues.
13. Samples not belonging to DDL should be returned to IR processing.

iv. Handling Conditions:

1. Samples are to be kept frozen.
2. Transport (ideally) under frozen conditions.
3. Upon receipt by the Diabetes Lab, the specimen will be logged in and stored at – 70°C until analysis. Analyzed specimens will be archived at -70°C for two months, after which they should be discarded (unless required for Alternative Assessment studies, competency assessment testing or Quality Assurance purposes for which samples should be re-labeled as blinded samples).

c. **Equipment and Materials**

i. **Equipment:**
Tosoh Automated Enzyme Immunoassay Analyzer (AIA - 900); Refer to the Diabetes Diagnostic Laboratory - Tosoh Automated Enzyme Immunoassay Analyzer (AIA - 900) Start Up, Operation, Shut Down, and Maintenance Procedure for additional guidance on instrument Start up, Operation, and Shut Down. If the assay specifications for this test are not ready in the system software, the specifications must be entered under the test code **040.**

ii. **Equipment Maintenance:**

i. Routine, Daily, Weekly Maintenance, Monthly, and Preventative should be performed according the attached AIA-900 Operator's

Manual and the Diabetes Diagnostic Laboratory - Tosoh Automated Enzyme Immunoassay Analyzer (AIA - 900) Start Up, Operation, Shut Down, and Maintenance Procedure

ii. Instrument Start-up, Operation, and Shut Down:
The AIA-900 Operator's Manual is attached in this procedure located at the bench for emergency start-up, operation, and shut down. At the workbench, the AIA-900 manual is tagged for start-up, operation, and shutdown using sticky notes for immediate access. Refer also to the Diabetes Diagnostic Laboratory - Tosoh Automated Enzyme Immunoassay Analyzer (AIA - 900) Start Up, Operation, Shut Down, and Maintenance Procedure

ii. Perform the Daily maintenance on the day of assay by following the DAILY MAINTENANCE schedule form. Discard the used sample cups when the analysis is complete. Turn off the instrument once every 24 hours.

iv. Clean the B/F Wash Probe tip weekly with a cotton ball moistened with 70% ethanol.

v. Wash the Substrate line weekly.

vi. Replace the wash probe tip on the end of the B/F wash probe monthly.

vii. Clean diluents and wash tanks tri-monthly with 1:100 dilution of hypochlorous acid solution then rinse reservoirs with DI water.

viii. AIA-900 performs a substrate background measurement each time daily maintenance is run and the results are automatically printed out. If the substrate background measurement is within specifications, an OK will be displayed next to 4MU Background. If the substrate background is too high a "BH" (blank high) error flag will be printed and Substrate Replacement will be incomplete. Prime or replace the substrate and repeat daily maintenance. If the lamp intensity level is within specifications an OK will be displayed next to Lamp Intensity Level. If the lamp intensity level is too low an "LL" (lamp low) error flag will be printed. The "LL" is warning that the lamp will need to be replaced soon.

ix. Refer to the Diabetes Diagnostic Laboratory - Tosoh Automated Enzyme Immunoassay Analyzer (AIA - 900) Start Up, Operation, Shut Down, and Maintenance Procedure and the Operator's Manual for additional maintenance guidance.

iii. **Materials:**

i. Reagents – Supplied by Tosoh Bioscience (South San Francisco CA). Part Numbers are subject to change. Any questions or concerns about the materials used for the assay please refer to your supervisor/delegate or call Tosoh Scientific hotline at 1-800-248-6764, customer #2425. The following components are available for the AIA-900 analyzer.

MATERIALS	**Part #**
AIA-900	022930
AIA-900 9tray Sorter	022931
AIA-900 19tray Sorter	022932
ST. AIA-PACK C-Peptide II	025283
AIA-PACK Substrate Set II	020968
AIA-PACK Substrate/Reconstituent	
AIA-PACK C-Peptide II Calibrator Set (approx.): Calibrator # 1 ~ 0 ng/mL (0 nmol/L) Calibrator # 2 ~ 0.5 ng/mL (0.16 nmol/L) Calibrator # 3 ~ 2.0 ng/mL (0.66 nmol/L) Calibrator # 4 ~ 6.0 ng/mL (2.00 nmol/L) Calibrator # 5 ~ 15.0 ng/mL (5.00 nmol/L) Calibrator # 6 ~ 33.0 ng/mL (11.00 nmol/L)	025383
AIA-PACK C-Peptide II Sample Diluting Solution	025583
AIA-PACK Wash Concentrate Set	020955
AIA-PACK Diluents Concentrate	020956
AIA-PACK Detector Standardization Test Cups	020970
AIA-PACK Sample Treatment Cup	020971
Sample Cups	018581
Pipette Tips	019215
Tip Rack	019216

Warnings and Precautions

i. The AIA-PACK C-Peptide II is intended for in vitro diagnostic use only.
ii. Inspect the packaging and the exterior of the aluminum pouch or the vials for any sign of damage before use. If any damage is visible, contact Tosoh for a replacement package.
iii. Test cups from different lots or different assays should not be mixed within a tray.
iv. After measurement, the ST AIA-PACK C-peptide II contains sodium azide, which may react with lead or copper plumbing to form potentially explosive metal azides. When disposing of such reagents, always flush with large volumes of water to prevent azide build-up.

v. Do not use beyond the expiration date.
vi. The AIA-PACK C-Peptide II has been designed so that the high dose "hook effect" is not a problem for the vast majority of samples. Serum and EDTA plasma with C-peptide concentration between 30 and 90 ng/mL (10 and 30 nmol/L) will read > 30 ng/mL (10 nmol/L). The "hook effect" phenomenon may occur at C-peptide concentrations > 90 ng/ml (30 nmol/L) in serum and EDTA plasma.
vi. For safe waste disposal, it is recommended that each laboratory complies with established laboratory procedures and local, state, and federal regulations.
vii. After opening, the bottle of ST AIA-PACK C-Peptide II Sample Diluting Solution should be kept tightly sealed with a clean cap. Sealing with dirty material may cause deterioration of the reagent.
viii. The remaining sample diluting solution after use should not be mixed with another bottle but be discarded to avoid contamination.
ix. Serum, dust, metal, or microorganism contamination may cause degradation of reconstituted substrate solution. Store in a clean environment, away from direct sunlight and ultraviolet light.
x. Although material derived from human origin is not used for these calibrators, it is recommended that this product be handled with the sample precautions. Because no test method can offer complete assurance that products derived from human blood will not transmit infectious agents, it is recommended that this product be handled with the same precautions as used for patient samples.
xi. Tosoh Automated Immunoassays utilizing alkaline phosphatase-based technologies should not be used with samples from patients under Asfotase Alfa treatment.
xii. Glass volumetric pipettes are discarded after use.

v. **Storage Requirements and Stability:**
   i. All unopened materials are stable until the expiration date on the label when stored at the specified temperature;
      a. Refrigerator Temperature:
      ST AIA-PACK C-Peptide II Test Cups
      AIA-PACK CPEP II Calibrator Set
      AIA-PACK CPEP II Sample Diluting Solution
      AIA-PACK Substrate Set II
      AIA-PACK Wash Concentrate
      AIA-PACK Diluents Concentrate
      b. Room Temperature
      AIA-PACK Detector Standardization Test Cups
      AIA-PACK Sample Treatment Cups

ii. The ST AIA-PACK C-Peptide II test cup is stable until the expiration date on the label when stored unopened and refrigerated at 2-8°C. After opening the aluminum pouch, if the test cups are stored in the refrigerator, they are stable for up to 30 days. As per package insert, the test cups can be left on-board of the Tosoh AIA-900 analyzer under room temperature conditions for a maximum of 7 days (7 x 24 hours) and when stored overnight under refrigerated conditions they can be stored for up to 21 days (refer to the PI for additional details). However, an adequate amount of test cups (in use and needed for the day of testing) should be pulled from the refrigerator and brought to room temperature prior to use. Plastic test cups contain lyophilized twelve magnetic beads coated with anti-C-peptide mouse monoclonal antibody and 100 µL of anti-C-peptide mouse monoclonal antibody conjugated to bovine alkaline phosphatase.

iii. Bio-rad controls are stable for 7 days under refrigerated conditions after reconstitution. These controls are also stable for 30 days under frozen conditions of – 70 ° C (or below) after reconstitution. Controls should be obtained from the freezer at the time of testing. Once controls are brought to room temperature, they should be immediately used for testing and returned to the refrigerator after use. Controls should be discarded after daily use.

iv. In-House controls (for QA purposes only) are provided ready for use. Once controls are brought to room temperature, they should be immediately used for testing and returned to the refrigerator after use. Controls should be discarded after daily use. See above stability requirement for in-house controls.

v. **Reagent labeling –** reagents (e.g. washer), diluent, calibrators, controls, and solutions should be traceably identified to indicate the following:

1. Content, concentration, and expiration date.
2. Storage requirements (see above stability requirements for serum).
3. The below should be followed for working reagents (open reagents in use for testing):
   a. Preparation date or opened date and the identity of the preparer.
   b. For example: diluent, substrate solution, diluting solution, (etc. from the listed reagents above), should be labeled with the name of the reagent and source (name of vendor e.g. Tosoh), preparation or opened date, expiration date (from open or preparation date), and tech's initials.
   c. Each control aliquot in use should be traceably identified with the control name, preparation date, tech initials, expiration date, and discard date. Expired controls should not be utilized. Refer to the Diabetes Diagnostic Laboratory Insulin & C-peptide Quality Control Recording Lab Note Book and the Quality Control and Calibration Manual for current control details.

vi. **Preparation of Reagents, Solutions, Calibrators (Standards) and Stability: Refer to package insert for additional details**

Reagent Preparation – Bring all reagents to room temperature before use. Mix all reagents thoroughly before use. When new reagents are received, they need to be initialed and dated by technician who received them. Opened and working reagents should be labeled with the opened/ expiration date, and tech's initials. Refer to the package insert for additional details.

a. Substrate Solution
    1. Bring all reagents to room temperature before preparing the working reagent. Add the entire contents of the AIA-PACK Substrate Reconstituent II (100 mL) to the lyophilized AIA-PACK Substrate Reagent II and mix thoroughly to dissolve the solid material.
    2. Mix thoroughly. Let stand for 20 minutes to dissolve contents completely.
    3. Label the bottle with the preparation date, tech's initials, and expiration.
    4. Reconstituted substrate solution is stable for 3 days at room temperature or 30 days refrigerated at 2-8°C. Refer to Package Insert for additional details.

b. Wash Concentrate Solution
    1. Add the entire contents of the AIA-PACK Wash Concentrate (100 mL) to approximately 2.0 L of CAP Class I water or clinical laboratory reagent water.
    2. Mix well thoroughly and adjust the final volume to 2.5 L.
    3. Label the bottle with the preparation date, tech's initials, and expiration date.
    4. Once prepared the wash solution is stable for 30 days at room temperature.
    5. Reagents should not be used if they appear cloudy or discolored.
    6. Refer to Package Insert for additional details.

c. Diluent Concentrate
    1. Add the entire contents of the AIA-PACK Diluent Concentrate (100 mL) to approximately 4.0 L of CAP Class I water or clinical laboratory reagent water.
    2. Mix well, and adjust the final volume to 5.0 L.
    3. Label the bottle with the preparation date, tech's initials, and expiration.
    4. Once prepared the working diluent wash solution is stable for 30 days at room temperature.

d. AIA-PACK C-Peptide II Sample Diluting Solution
    1. The AIA-PACK C-Peptide II Sample Diluting Solution is provided ready to use.

2. The sample diluting solution should be used after equilibrating at room temperature for about 30 minutes.
3. Always store the AIA-PACK C-Peptide II Sample Diluting Solution in an upright position at 2-8°C when not in use.
4. When stored unopened at refrigerated conditions, this reagent is stable until the expiration date on the label.
5. Adequate amount of dilution solution (in use) should be obtain from the refrigerator and brought to room temperature prior to use.
6. After opening, the Sample Diluting Solution is stable for up to 90 days when refrigerated.
7. Refer to package insert for additional details.

e. AIA-PACK C-Peptide II test cup
   1. The AIA-PACK C-Peptide II test cup is provided ready to use.

f. C-PEP II Calibration Set – The ST AIA-PACK C-Peptide II Calibrator Set contains protein matrix with assigned levels of C-peptide.
   1. Inspect the packaging and the exterior of the vials for any sign of damage before use. If any damage is visible, contact your local TOSOH sales representative.
   2. The calibrators must be kept tightly sealed, in an upright position, and refrigerated when not in use.
   3. Refer to the AIA-PACK IRI Calibrator Set package insert for additional procedural instructions regarding calibrator handling details.
   4. The ST AIA-PACK C-Peptide II Calibrator (1) is provided ready for use.
   5. The ST AIA-PACK C-Peptide II Calibrator (2) – (6) are lyophilized and should be reconstituted.
   6. Bring the calibrators to room temperature prior to use.
   7. Using a volumetric pipette or a calibrated adjustable pipette, reconstitute the lyophilized calibrators accurately with 1.0 mL of CAP Class I water or clinical laboratory reagent water.
   8. Allow the lyophilized material to fully dissolve, and then mix the calibrators gently but thoroughly prior to performing the calibration.
   9. Always store the calibrators in an upright position at 2-8°C when not in use.
   10. When stored unopened and refrigerated, calibrator set is stable until the expiration date on the label. The calibrators should be used within 1 day of opening or reconstitution, provided the vials are kept sealed and refrigerated at 2-8 °C.
   11. Do not use calibrators beyond the expiration date.

g. Reagent lot to lot comparisons should be performed after;
1. A new lot of calibrators.
2. New test cup lot.
3. Refer to the Diabetes Diagnostic Lab-New Reagent Lot Confirmation of Acceptability-Standard for additional details.

d. **Calibration**

i. **Calibration Procedure:** Refer to section above for calibration preparation and stability guidance, and the attached AIA System Operator's Manual for additional guidance.

a. The calibrators for use have been standardized against WHO 1st IRP 84/510 (1986). The Calibrator Set contains assigned concentrations of c-peptide.

b. The assigned value is determined on a lot-to-lot basis. Verify that both the calibrator lot and the concentration have been correctly entered into the analyzer software.

c. When using new calibrator lots, enter the calibrator concentration values and lot number into the software test file (Refer to the Tosoh AIA System Operator's Manual for details).

d. Before starting the calibration, be sure to confirm that the quantities of the Diluent, the Wash, and the Substrate are sufficient. Add the appropriate amounts of each calibrator to the samples cups. Calibrators should be run in triplicate.

e. Load the appropriate amount of ST AIA-PACK C-peptide test cups on to the instrument.

f. Add the appropriate amount of each calibrator to sample cups;
1. Press the ORDER (NON-BAR) button on the HOME screen.
2. Press the CALIB button to order calibration.
3. Select the c-peptide analyte to be calibrated
4. Enter the calibrator lot number and concentrations.
5. Press OK.
6. Print a Worklist: press FUNCTION, WORKLIST, OK.
7. Pour Calibrators into labeled samples cups and load into a rack with cup adaptors according to the worklist.
8. Place loaded racks on the instrument.
9. Press ASSAY START (NON) and START.

ii. **Frequency; Calibration/Recalibration is to be performed:**

i. The calibration curve for the AIA-PACK C-peptide is stable for up to 90 days. Calibration verification is performed, at a minimum, when a system is first placed in service and every 90 days (and as specified by the manufacturer).

ii. Recalibration may be necessary (regardless of the length of time since last performed) immediately if any of the following occurs:
1. If controls are out of the established ranges for this assay, QC reflect

an unusual trend or shift, and if other means of assessing and correcting unacceptable control values fail to identify and correct the problem.

2. Test cup lot and calibration lot changes.
3. After major preventative maintenance, a change of a critical instrument component or if certain service procedures are performed (e.g. sample mechanism changes, or detector lamp adjustment or changes).
4. If the calibration has failed, the test system should be recalibrated. Refer to the Calibration Review and Acceptance section below for additional details.
5. Refer to the Operator's Manual for additional guidance.

iii. **Calibration and Calibration Acceptability Criteria**:

a. Calibration Curve:

1. After checking if the calibration curve is acceptable, carry out the finalization operation in order to make the calibration curve valid.
2. Calibration stability is monitored by quality control performance and is dependent upon proper reagent handling and AIA System maintenance according to the manufacturer's instructions.

b. Calibration Acceptability Criteria:

1. The mean rate for the ST AIA-PACK C-Peptide II Calibrator (1) should be </= 1.1 nmol/L•s
2. Since there is a direct relationship between concentration and rate, the rate should increase as concentration increases.
3. The replicate values (precision) should be within a 10 % of each other.
4. Two curves for the same test cup lot cannot be accepted.

c. Calibration Review and Acceptance

1. Upon completion of the assay, review the calibration curve carefully using the criteria listed above.
2. Edit the calibration if necessary, then accept the calibration curve by following the below steps:
   a) Press SUB MENU, CALIBRATION.
   b) The first pending calibration will be displayed.
   c) Review the rates according to the acceptance criteria.
   d) To omit any points, use the 'arrow up and down' keys to select the point (s).
   e) Press DECISION to remove the point (s) from the curve.
   f) Once the assay results have been confirmed, press the (CALCULATE) button, to display the calibration curve. The Calibration Curve Graph will appear on the screen.
   g) When all the data is acceptable, press ACCEPT.
   h) Press the (PRINT) button to print the calibration curve.

i) If the calibration curve of the same lot already exists, the previous curve will be overwritten. If the calibration is not acceptable immediately trouble shoot the system to locate the probable cause of the problem. Patient testing should not be performed until the calibration curve is acceptable.

j) QC should also be considered acceptable prior to running patient samples and reporting results. Refer to the QC section below for additional details.

3. If the calibrator curve is unacceptable per manufacturer's instructions do not ACCEPT the curve. Recalibrate using the same materials following the above calibrator procedure. If the calibrator curve is still unacceptable, perform another calibration using new calibration materials. Immediately call the Tosoh hotline at 1-800-248-6764 (account #2425) for assistance if calibration continues to fail. Records of recalibration are noted on the Diary Sheet, if calibration or calibration verification has failed. Records are maintained for at least 2 years.

e. **Quality Control**

i. At least two levels of controls (normal and abnormal) are purchased from Bio-Rad Laboratories; Lyphochek® Immunoassay Plus Controls (Refer to QC Binder for additional details and the manufacturer's package insert guidelines for processing and handling instructions).

ii. Preparation and Stability –

a. Using a volumetric pipet or equivalent, reconstitute each vial with 5.0 mL distilled or deionized water. Replace the stopper and allow this product to stand for approximately 15 minutes swirling occasionally. Gently swirl the vial several times to ensure homogeneity.

b. Label each control level with techs name, prep and expiration date. Transfer 500 µL aliquots each into polypropylene storage tubes (and cap tightly) and freeze at -70° C. Enter this information into the AIA-900 Insulin & C-peptide Controls' Log Book.

c. Controls are stable for 30 days under frozen conditions after reconstitution. Controls are stable for 1 day under refrigerated conditions after reconstitution. Once controls are brought to room temperature, they should be immediately used for testing and returned to the refrigerator after use. Controls should be discarded after daily use.

d. Each control aliquot in use should be traceably identified with the control name, preparation date, discard date, expiration date, and tech initials.

iii. In-house Control—Donors are recruited and compensated for their donation of blood. The In-House control is prepared from serum of three - four subjects, 500 µL serum aliquots are transferred into to polypropylene storage tubes.
Aliquots are cap tightly and stored under frozen conditions at -70 °C or below. See Specimen Collection and Handling section above for serum stability guidance.

a. These controls are assigned a lot number, labeled with the preparation date (the date the controls are collected), and stored at under frozen conditions on the same day. Remaining aliquots are assigned the same lot number/date and placed in LN2 in freezer boxes on the same day (long term storage). **Note:** In-House controls are utilized and assayed for Quality Assurance purposes only; results from these controls do not affect the reporting of patient results.

b. Reconstitution is not required for the In-House control. Thaw one aliquot prior to testing. Once controls are brought to room temperature, they should be immediately used for testing and returned to the refrigerator after use. Controls should be discarded after daily use.

iv. Quality Control (QC) Procedures:

a. Batch QC: At least two levels of controls, which cover the spectrum of c-peptide ranges for both normal and diabetic populations are utilized for patient testing and reporting. Matrix appropriate controls are prepared in-house. One vial of each level of control is thawed and used in each assay.

b. If the stock of these controls becomes low, another batch is ordered or prepared in time to analyze it concurrently with the current quality control materials. The new controls are used only after their means and SDs are established with the approval of the Lab Director.

c. The bias limit is set at 1 SD or the 68% limit; the warning limit (WL) is the 2 SD or the 95% limit and the control limit (CL) is the 3 SD or the 99% limit.

d. QC Guidelines—Controls means and acceptability limits are established using at least 20 inter-assay observations. For manufacturer controls, the established acceptable limits should fall within manufacturer's defined limits for each level of control.

e. Any corrective actions observed for QC results outside of the acceptability limits should be documented in the C-Peptide Diary Sheet. The QC policy found in the Quality Management Program should be used as a guide for additional details.

f. Refer to the Quality Controls' Manual for the current QC materials used with acceptable ranges. QC acceptable ranges are also located at the bench where the assay is being performed. Please refer to the current QC acceptable ranges if there are concerns prior to releasing patient results. Consult with the lab supervisor (or delegate) if you have questions.

v. Tolerance Limits – The analysis is either accepted or rejected following the guidelines below guidelines.

a. The quality control limits for this assay are established from calculating the mean, SD, and CV (for each level of control) from repetitive analysis of at least 20 inter- assay observations performed in duplicate measurements. For the assayed controls, acceptable limits must fall within the acceptability ranges supplied by the manufacturer.

b. Beginning of the day QC is rejected if any of the following events occur for an individual quality control level;

1. A single control level falls outside the 99% (3SD) confidence limits (1 3s rule);
2. Two or more controls fall outside the 95% (2SD) confidence limits (2 2 s rule); or
3. Eight sequential values for a control fall either all above or all below the mean, not including values that fall within 1 SD.
4. QC results should be acceptable prior to reporting patient results.
5. For QA verification purposes – In-House controls (matrix appropriate control used to test the precision of the analytical performance of the AIA- 900) should fall within ± 3 SD. If the results are outside of ± 3 SD, immediately reach out to the Lab Director or Lab Manager (or delegate) for immediate corrective actions.

c. End of the day QC should be performed when the number of samples on the batch run exceeds five samples (n > 5 samples). Results from end of day QC should all fall within ± 3SD of the established acceptable ranges.

d. If QC falls outside of the established acceptable limits, patient results should not be reported and a corrective action should be performed. Refer to the QC Handling, Frequency and Troubleshooting guidance section below for additional details on performing QC corrective actions.

e. Levey-Jennings Plots – Monthly

1. Quality controls Levy-Jennings plots are prepared monthly using imprecision statistics (e.g. mean, SD, CV, trend) to monitor analytical performance of the test system.

2. The Laboratory Director or delegate reviews these on a monthly basis.

f. QC Handling, troubleshooting guidance, and Frequency:

i. In the event of damage to packing, contact Bio-Rad Technical Services at 210-748-2199.

ii. In order to monitor and evaluate the precision of the assay's analytical performance, QC is tested in the same manner as patient samples and by the same personnel performing patient testing.

iii. If the controls have been frozen, allow to stand at room temperature until completely thawed.

iv. Beginning of the day QC should be assayed and acceptable prior to testing patient samples.

v. QC results must be acceptable prior to releasing patient results. If beginning control results fall outside the established acceptability limits, patient results should not be reported. Patient samples should be returned to optimal storage conditions (see above stability guidelines) until all QC issues are resolved. All QC Corrective actions should be documented (refer to Quality Control Policy within the Diabetes Diagnostic Laboratory-Quality Management Program-Standard for additional details).

vi. Once all analyzer issues are resolved and QC is acceptable, all patient samples from the beginning of the day run should be reanalyzed.

vii. QC should also be performed at the end of the run (end of day) as a quality assurance measure to verify the acceptable system performance of the AIA-system. End of day (closing) QC should fall within ± 3SD of the established acceptable limits. If QC result for each level is not within ± 3SD an immediate corrective action should be performed. QC corrective action could include retrieving new controls of the same lot, reconstituting of new QC, performing a re-calibration, or performing analytical precision studies to access the integrity of the QC in use against the acceptable limits.

viii. Once Closing QC are within acceptable limits, selected samples (3 – 5 samples) from the 'beginning of the day' run should be reassayed and results should be within ± 20 % or 0.2 nmol/L (whichever is greater) of the previous results. If it is determined that there is a systemic issue with the AIA-system it is the responsibility of the tech to immediately call the technical service hot line at 1-800-248-6764 for additional troubleshooting advice.

ix. After each assay run, all control data are recorded on the Tosoh C-peptide Diary Sheet.

x. QC should also be performed after calibration; at least two levels of controls are run in order to accept the calibration curve.
xi. At least two levels of controls should also be repeated after calibration when service procedures are performed (e.g. temperature adjustment, sample mechanism changes, maintenance of the wash probe or detector lamp adjustment or change)
xii. Controls are also performed after a PM.
xiii. Controls should be performed after daily maintenance to verify the overall performance of the analyzer.
xiv. If one or more control sample values are out of the acceptable ranges, immediately investigate the validity of the calibration curve before reporting patient results.

f. **Proficiency Testing**
College of American Pathologist (CAP) survey: Refer to the Diabetes Diagnostic Laboratory-Quality Management Program-Standard for additional guidance of receiving, handling, processing, testing, and reporting PT and Alternative Performance Assessment samples.

g. **Carryover Studies**- Not applicable (N/A) since this platform uses plastic disposable tips.

h. **Procedure –Stepwise**

i. Special Safety Precautions:

i. While working in the lab and handling specimens, proper PPE is enforced. This includes wearing of gloves, face masks/shields, lab coats, protective eye wear such as goggles, and closed toe shoes.
Once gloves are removed, wash your hands or use hand sanitizer to ensure your hands are clean before leaving the lab.
ii. All plastic tips, sample cups, gloves, etc. that contact blood are considered contaminated and are to be placed in a biohazard waste container.
iii. All telephones, doorknobs and work surfaces are wiped down with Oxivir disinfectant or 10% bleach at least one time during each work shift. Any area in which blood is spilled is also to be cleaned and disinfected immediately with Oxivir disinfectant or 10% bleach. Refer to the Lab Safety Manual located in room M764 (for additional details).

	All healthcare personnel shall routinely use appropriate barrier precautions to prevent skin and mucous membrane exposure when contact with blood or other body fluids of any patient is anticipated. All products or objects that come in contact with human or animal body fluids should be handled, before and after cleaning, as if capable of transmitting infectious diseases. Wear appropriate Personal Protective Equipment (PPE), including facial protection, gloves, and protective clothing. Dispose of all biological samples and diluted specimens in a biohazard waste container at the end of analysis. Dispose of all liquid hazardous waste in a properly labeled hazardous waste container.

ii. **Initial processing of specimens:**
   i. All pathology specimens must be verified as received by the laboratory on the Cerner Pathnet computer system prior to testing, using the below procedure.
   ii. Login to Pathnet.
   iii. Open Specimen Log-In.
   iv. Select the Accession Radio Button.
   v. Click on the Retrieve button.
   vi. Select UH Diagnostic Diabetes as the Location (after 1st use, this will default to this location upon subsequent log-ins).
   vii. Scan the barcode on each tube to be logged in.
   viii. After scanning all tubes to be logged in, verify only tubes with c-peptide as orders are selected, and click the log-in button.
   ix. After specimens are logged in, close the window.
   x. Place specimens in the rack designated for samples to be analyzed in numeric order and into the refrigerator immediately after completing initial processing. Refer to the Specimen Collection and Handling section above for sample handling after results are reported.
   xi. Clinical trials specimens (e.g., ITN) should be verified against a Sample Manifest (or a Test Request Form that lists the incoming samples) prior to testing.

iii. **Start Up, Operation and Shut Down of the AIA-900 Analyzer System:**
   i. Refer to the Diabetes Diagnostic Laboratory - Tosoh Automated Enzyme Immunoassay Analyzer (AIA - 900) Start Up, Operation, Shut Down, and Maintenance Procedure for additional details.
   ii. Place Substrate in substrate compartment. Power on. Wait for Log On screen.
   iii. Log on by pressing OK to retain current operator ID. For new operator, press (OPERATER) button then (MODIFY) button to enter your ID. After enter your ID, press OK. Your ID will be saved on user list. Press OK again and the following screen will appear with your user ID.
   iv. Press OK again and daily check screen will appear. Following the screen to

do the daily, weekly and monthly maintenance.

v. Place a cup adapter rack with a Standardization Cup (STD) in position 2 to the instrument. Press OK. Automated maintenance will begin.

vi. Record results of the Substrate Background Measurement on the Substrate Background form and keep the printed result for troubleshooting.

vii. Press (RT.OPEN) button (orange color) to refill tip rack. Then press OK.

iv. **Controls and Sample Preparation** – Refer to the calibration section above for guidance on calibrator preparation, handling, and testing.

i. Preliminaries

a. Allow frozen samples and controls to reach room temperature. Invert gently to mix.

b. Ensure there is sufficient quantity of ST AIA-PACK C-peptide test cups for the number of samples to be run.

c. Remove any bubbles. Load patient samples, controls, and corresponding test cups according to the Operator's Manual and the Diabetes Diagnostic Laboratory - Tosoh Automated Enzyme Immunoassay Analyzer (AIA - 900) Start Up, Operation, Shut Down, and Maintenance Procedure

i. Put sample cups into the sample rack with test cups. Place the rack on the instrument using a maximum of eight racks at a time. Put end marker tube in the last hole of the last rack so that the instrument will stop.

ii. Press ASSAY START (NON) button on HOME screen. The number 0 will be displayed in the box of the END OF REQUEST.

iii. Press START to start the assay.

iv. To run more samples repeat step ii and iii.

v. If a number other than 0 is displayed in box of the END OF REQUEST, press the cell to display the NUMERIC KEYPAD screen. Enter 0 than press OK.

vi. Printing after Calibration

i. The Tosoh AIA System Analyzers perform all sample and reagent handling operations automatically. The Tosoh AIA System Analyzers read the rate of fluorescence produced by the reaction and automatically convert the rate to C-peptide concentration in nmol/L.

ii. If the calibration curve is undetermined before the analysis, use the steps below to recalculate results after a calibration curve is determined.

a. Press RESULTS button in HOME screen.
b. Place the cursor line under the first sequence number desired.
c. Press SELECT button
d. Use the down arrow key to move the cursor to the last sequence number desired.
e. Press SELECT again. A green > sign will be on left of the result selected.

f. Press FUNCTION button, the screen will appear with 4 options. Select option RECALC then press OK.

g. Repeat step 3 to 6 but select option PRINT instead of RECALC then press OK. The result will be printed on the printer tape.

vii. **Recording of Data and Reporting Results**

Procedure:

i. Quality Control data and patient results— QC must be considered acceptable prior to reporting patient results. Enter the assay date, tech initials, and relevant reagent details (e.g. test cup number #, calibration lot #, etc.) in the C-peptide Diary Sheet located on the network drive. Verify that the assay information, QC data, and sample details listed on the diary sheet are correct prior to printing.

ii. Reporting Results – Test instrument printouts are placed in the Insulin and C-peptide Test Instrument lab notebook. Results are then entered using Cerner Pathnet following the below procedure;

a. Manual Result Entry:

i. Open MyApps and double-click on the Cerner. Log into the Pathnet Appbar (Refer to Section h above).

ii. Click the Batch Result Entry icon on the tool bar.

iii. In the Procedure box, Type the test name (c-peptide) then click the icon beside the box. A group test name will be show on the screen. Choose the exact test listed on the specimen order.

iv. In the test site box, type “uh dd man” then click OK.

v. Enter the accession ID and corresponding sample result. Click the TASK to choose Print screen.

vi. Ensure the test result and patient accession number from the instrument print out matches the entered result.

vii. Click ‘Perform’ to finish result entry. Print the Batch Result Entry Screen.

viii. Submit Batch Result Entry Screen and instrument printout with completed diary sheet to supervisor (or delegate) for verification.

b. Refer to section k. below ‘Supervisor (or delegate) Responsibility’ for additional details on Supervisor’s Quality Assurance role.

i. Reference Range (Reference Intervals):

Reference Ranges (Non-Diabetic Values):

i. Reference ranges for c-peptide were updated at the Diabetes Diagnostic Laboratory in January 2013 by combining results from two volunteer groups. A 360 caloric standard meal (Boost™) challenge was performed in February 2009 for fasting and 120 minutes on non-overweight, nondiabetic subjects (n=15, mean age=34, M:F=8:7). A 360 caloric standard meal (Boost™) challenge was performed in October 2012 for fasting, and 120 minutes on non-obese, non-diabetic subjects (n=29, mean age =39,

M:F=20:9). All participants fasted overnight for at least 10 but no more than 15 hours. Any subject with a BMI greater than 25 kg/m2 and fasting glucose greater than 100mg/dl were excluded from the calculations.

Refer to validation study binder. The means and observed ranges are:

	C-peptide Reference Range nmol/L	
	Fasting	**120 min**
N	44	44
Mean	0.52	1.20
Range	0.23-0.86	0.34-2.33

ii. Interpreting c-peptide results should be in conjunction with other data (e.g. symptoms, results from other tests, clinical impressions, therapy, etc.). Values that are outside of these reference ranges do not necessarily mean the abnormal test result is of clinical significance. This should only be determined by a physician after careful evaluation of the individual person's health record.

Note: Each c-peptide order should indicate the patient 'Time Point' status '(C-peptide Time PP)' for example as either Fasting, 120 min or Random sample. If no 'C-peptide Time PP' is indicated, select "Random Sample" as the time point for this patient with the added below comment, "Selected as a 'Random Sample'. Time point not indicated. Clinical correlation is recommended."

iii. **Panic Results**: Since test values vary depending on the individual's health record and overall 'clinical presentation', all values are reported to the physician with no further action taken. Specimens may be repeated for verification upon request of the physician.

iv. **Calculations;** The AIA Systems performs all sample reagent handling operation automatically. The AIA Systems reads the rate of fluorescence produced by the reaction and automatically converts the rate of c-peptide concentration to nmol/L.

v. **Conversion Factors**: 1 ng/mL = 0.333 nmol/L

j. Linearity/AMR/Calibration Verification

a. The ST AIA-PACK C-Peptide II Calibrator Set contains assigned concentrations of C-peptide. The assigned value is determined on a lot-by-lot basis and is designed to provide an assay calibration range of 0.04 to 30 ng/mL (0.013 to 10 nmol/L) of C-peptide.

    a. AMR verification is performed every 3 months when the assay is calibrated. The **Reportable Range** for this assay is 0.015 to 10 nmol/L.
    b. Results ≤ 0.015 nmol/L should be repeated for verification and resulted at "< 0.015 nmol/L." C-peptide results that exceed 10 nmol/L should be 1:10 diluted with the ST AIA-PACK C-peptide II Sample Diluting Solution, and reassayed so the diluted specimen reads between 0.015 to 10 nmol/L. C-peptide results that exceed 10 nmol/L are reported as ">10.0" (greater than 10.0) with the added comment, "c-peptide concentration above the linear range for this assay, sample was re-analyzed as a 1:10 dilution and result was multiplied by 10 to yield a final result of (add final result) nmol/L."

k. Supervisor (or delegate) Responsibility:

i. The supervisor or delegate ensures quality control passes within the acceptable ranges prior to releasing patient results.
ii. The supervisor or delegate ensure that the test results from the instrument printout match the 'Perform' manual entry results.
iii. All results requiring further evaluation are noted and are not uploaded into pathnet (observed typos).
iv. The Result Report is checked against the Laboratory Worksheet and is verified (acceptable results) in Pathnet by the testing personnel.

l. Procedural Notes

i. The AIA-900 is provided with a camera which reads the analytic name and lot number printed on the test cup. It enables the Instrument to automatically distinguish one specimen from another and also to recognize the assay to be done for each specimen.
ii. Any changes to procedure must be documented. Major changes to the SOP may include the way a procedure is performed or calculations and require the approval from the Medical Director. Minor changes include typo graphical errors or other minor corrections that do not change the way the procedure or calculation is performed and do not require approval of the laboratory director. Major SOP changes must be reviewed by the Medical Director prior to SOP approval.
iii. Any changes to the SOP will be communicated to technical staff via verbal communication and email notification. Technical Staff after reading the changes made to the SOP will sign and date their SOP review.
iv. If the analytical system fails (or becomes inoperable), all specimens are returned to storage at -70^{o}C. The specimens are re-analyzed when the system is back in control (repeat freeze/thaws should be avoided). All Corrective Actions and Preventative Actions should be documented.

v. Refer to the ST AIA-PACK C-Peptide Package Insert for details on performance characteristics for this assay.

m. Limitations of the Procedure:

i. For diagnostic purposes, the results obtained from this assay should be used in conjunction with other data (e.g., symptoms, results of the other tests, clinical impressions, therapy, etc.).

ii. Using ST AIA-PACK C-Peptide II, the highest measurable concentration of C-peptide in serum, heparinized plasma or EDTA plasma specimens without dilution is 30 ng/mL (10 nmol/L). The lowest measurable concentration in serum, heparinized plasma or EDTA plasma specimens is 0.04 ng/mL (0.013 nmol/L, assay sensitivity). Per Package Insert, the LoB was determined to be 0.001665 nmol/L. The LoD was determined to be 0.002664 nmol/L and the LoQ was determined to be 0.012987 nmol/L.

iii. Although the approximate value of the highest calibrator is 33 ng/mL (10.99 nmol/L) , the exact concentration may be slightly different from lot to lot. The assay specification, ASSAY RANGE HIGH, should be defined as the upper limit of the assay range, 30 ng/mL (10.0 nmol/L).

v. Although hemolysis has an insignificant effect on the assay, hemolyzed samples may indicate mistreatment of a specimen prior to assay.

Hemolysis Note: if a specimen is observed to be gross hemolyzed, the ordering physician should be contacted. The ordering physician should be advised that the test could be cancelled or performed with a note advising to interpret the result with caution. If the ordering physician requests the c-peptide result from a grossly hemolyzed sample, the c-peptide result should be reported with the comment “LR Specimen Hemolyzed, results should be interpreted with caution.” If you are unable to communicate with the physician (by the end of your shift), the result should be reported with the above comment.

v. Lipemia has an insignificant effect on the assay except in the case of gross lipemia where spatial interference may occur.

vi. Certain medications may interfere with assay performance. Specimens from patients taking medicines and/or medical treatment may show erroneous results. All results should be interpreted with respect to the clinical picture of the patient.

vii. Samples containing fibrin may exhibit either falsely elevated or falsely decreased results.

viii. C-peptide concentrations in serum typically increase post-prandially.

ix. Specimens from patients who have received preparations of mouse monoclonal antibodies for diagnosis or therapy may contain human anti-mouse antibodies (HAMA). Such specimens may show falsely elevated or decreased C-peptide values.

x. For a more complete understanding of the limitations of this procedure, please refer to the SPECIMEN COLLECTION AND HANDLING, WARNINGS AND PRECAUTIONS, STORAGE AND STABILITY, and PROCEDURAL NOTES sections in the insert sheet.

n. Interference:

i. Hemoglobin (up to 440 mg/dL), free bilirubin (up to 16 mg/dL), and conjugated bilirubin (up to 18 mg/dL) do not interfere with the assay.

ii. *Lipemia, as indicated by triglyceride concentration (up to 1,600 mg/dL), does not interfere with the assay. In the case of gross lipemia the test should be cancelled. The appearance of milky or cloudy samples should be verified by the lab manager or delegate.

iii. Grossly Lipemic samples should be rejected, and the test cancelled with the comment "LR Specimen Grossly Lipemic". The ordering physician should be notified with a reason for the cancelled test. All communications regarding cancelled/rejected tests should be documented with a reason for why the test was canceled or rejected within Pathnet and tracked using the Specimen Rejection Log and QAQR data.

All relevant information regarding cancelled/rejected tests should be properly communicated to the ordering clinician (as applicable) and documented with a reason for why the test was canceled or rejected within Pathnet, and tracked using the Specimen Rejection Log and QAQR database. Refer to the Diabetes Diagnostic Laboratory-Quality Management Program-Standard for additional details.

iv. Ascorbic acid (up to 20 mg/dL) does not interfere with the assay.

v. Protein, as indicated by human albumin concentration (up to 5 g/dL added to samples from apparently healthy subjects), does not interfere with the assay.

vi. Heparin (up to 100 U/mL) does not interfere with the assay.

viii. EDTA•2K (up to 10 mg/mL) does not interfere with the assay.

ix. Tosoh Automated Immunoassays utilizing alkaline phosphatase-based technologies and should not be used with samples from patients under Asfotase Alfa treatment.

. For patients that are being treated with Asfotase Alfa (Strensiq®), elevated test results are likely to occur using this method. Results should be not be used for patients receiving Asfotase Alfatreatment. Asfotase Alfa is a prescription medicine used for treatment of patients with hypophosphatasia (HPP), a rare genetic metabolic disorder characterized by the abnormal development of bones and teeth because of defective mineralization. About HPP, from NORD (National Organization for Rare Disorders)
ref: http://raredisease.org/rare

An alternative test method should be used that does not utilize alkaline phosphatase technology. All results should be interpreted with respect to the clinical 'picture' of the patient.

ix. Refer to Product Insert for additional details.

IV. **Attachments: Please review attachments**

a. AIA 900 Operator's Manual.
b. Recall Notice AIA Substrate Monitoring 2020.
c. Attached AIA-PACK C-peptide Package Inserts.

V. **References:**

1. C-peptide ST AIA-PACK C-peptide Product Insert.
2. AIA C-peptide and Insulin Validation Study Binder.
3. https://www.sciencedirect.com/science/article/pii/S0099176705002059
4. https://www.mlo-online.com

VI. **Appendix**

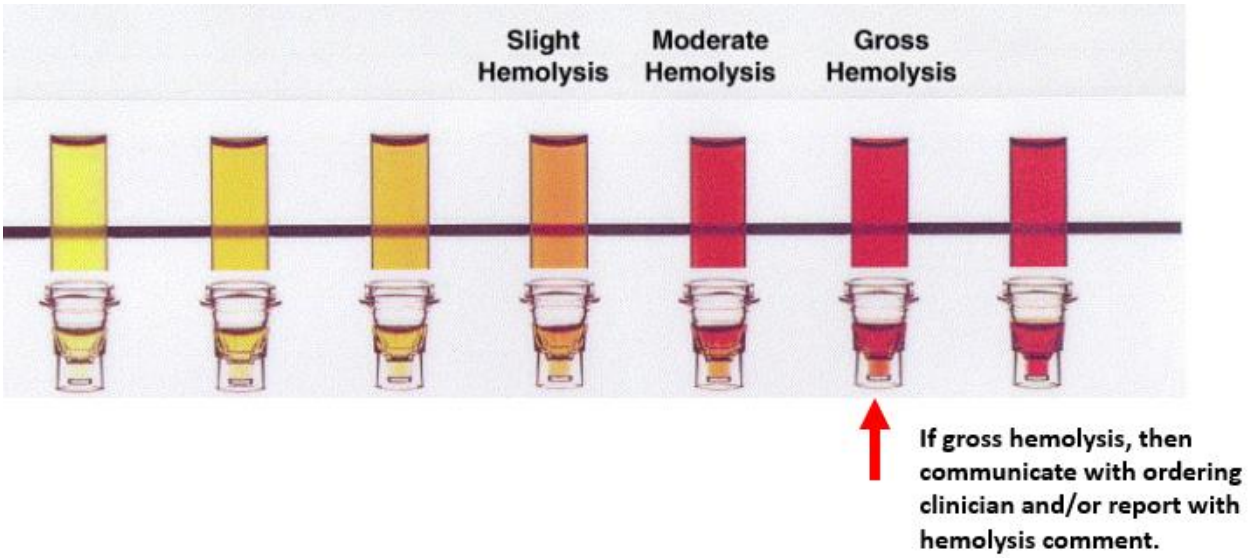

Fig.1 Degree of hemolysis guide.