YALE-NEW HAVEN HOSPITAL	TITLE: Hemoglobin A1C - Boronate Affinity HPLC SOFT codes: A1C, EAG		DEPT OF LAB MEDICINE Policy and Procedure Manual DOCUMENT # IMM 171 Page 1 of 15
WRITTEN BY: Virgilio Macalalad Penny Smith	**EFFECTIVE DATE:** September 28, 2011	**REVISION:** May 1, 2013 Penny Smith	**SUPERCEDES:** Revision from May 2, 2012

I. Introduction

Diabetes is a disease in which the body does not produce or properly use insulin. Insulin is a hormone needed to convert sugar, starches and other food into energy for daily life. The cause of diabetes continues to be a mystery, although both genetics and environmental factors, such as obesity and lack of exercise, appear to play roles. Type 1 Diabetes is characterized by destruction of the pancreatic beta cells, which produce insulin, usually leading to absolute insulin deficiency. The cause of ß-cell destruction results from a cellular-mediated autoimmune reaction. Immune-mediated diabetes commonly occurs in childhood and adolescence, but it can occur at any age, even later in adulthood. Type 2 Diabetes reflects insulin resistance, in which the body fails to use insulin properly, combined with relative (rather than absolute) insulin deficiency. Typically it occurs in those individuals over 45 years old, overweight, sedentary, and with family history of diabetes.

Hemoglobin A1C has been determined to be an important test for all individuals with all forms of diabetes, or those suspected of having diabetes. A1c directly relates the average glucose concentration (mean blood glucose) in the body over the life span of the circulating red blood cell (RBC). A1c formation is proportional to the amount of available glucose. The attachment of the glucose and hemoglobin molecule is relatively irreversible, and it remains attached on the surface of the Red Blood Cell (RBC) for the life span of the RBC.

From the HbA1C result an Estimated Average Glucose (EAG) can be calculated. Calculation of EAG is designed to estimate the expected average glucose level throughout the day from a single measurement of glycated HbA1c. The calculation is proposed by the American Diabetes Association (4). It may be less accurate in children, pregnant women and patients with certain erythrocytes disorders.

II. Principle Of The Assay

Glycated hemoglobins (GHb) differ from non-glycated hemoglobins by the attachment of a sugar moiety(s) to the former at various sites by means of ketoamine bond. GHb thus contain 1,2-cis-diol groups not found in non-glycated hemoglobins. This diol group provides the basis for separation of glycated and non-glycated hemoglobin components by boronate affinity chromatography.

This technique employs the principles of boronate affinity and high-performance liquid chromatography (HPLC). Pumps transfer reagents through an analytical column that contains aminophenylboronic acid bonded to a porous polymer support gel. Hemolysate is automatically injected onto the column during flow of Elution Reagent #1 (Buffer 2A). The glycated component binds to the boronate, while the non-glycated component passes through the column to the spectrophotometric detector, where it is detected at 413+/-2nm. After elution of the non-

glycated component, Elution Reagent #2 (Buffer 2A) displaces the glycated component from the column and then passes through the detector. In the final stage of the cycle, the column is re-equilibrated with Elution Reagent #1.

The computer processes the signal from the spectrophotometric detector and calculates the concentration of glycated hemoglobin as a percentage of the total detected. Integration is by peak area in Absorbance Units (AU)-seconds. The final HbA1c result is obtained by comparison to a reference samples using a 2 point calibration.

Major Hardware Components

Autosampler System

Consists of a 215 Liquid Handler with 819 Injection Valve Actuator, an Active Rinse Station (ARS), and an online barcode scanner that automates sample analysis. The system can automatically dilute and inject whole blood samples or can inject pre-made hemolysates. The operation of the autosampler system is controlled completely through AFFINITY Software.

UV/VIS Detector

The detector is a dual beam, microprocessor controlled variable wavelength absorbance detector. Both the visible (tungsten/halogen) and UV (deuterium) lamps are set at the appropriate wavelength and has a sensitivity range of 0.001 to 2.0 AUFS (Absorbance Units, Full Scale).

Pump

The pump is a microprocessor controlled binary system capable of delivering gradient reagent profiles. The system uses a removable solenoid box to automatically switch to Wash reagent to flush pumps, tubing, column and flowcell when the system performs an automated shutdown.

Column Heater

The column heater is placed to the left of the lamp unit and to the right of the 215 autosampler. It ensures steady boronate affinity column temperature. The inlet tubing goes in the right front tubing slot of the oven, the outlet tubing comes out the left front tubing slot of the oven. There are a series of aluminum plates (3 at the bottom, 3 on the top) that must be installed to ensure thermal contact with the analytical column.

Data Handling

The data handling system consists of a CPU, color monitor, printer and a handheld barcode scanner combined with AFFINITY software. The CPU is running with a Windows-based operating system. The handheld barcode scanner allows the barcodes on the patient tubes to be scanned and entered automatically into sample list.

III. Specimen Requirements

Whole blood sample collected in an EDTA primary collection tube (75 x 13 mm) only. **Specimen maybe stored at 2-8 °C for up to 7 days** although samples must come to room temperature prior to analysis. The minimum sample volume for direct barcode reader sampling

is 1 ml. If the height of sample is less than 25 mm, the sample can be manually lysed prior to analysis by making a 1:200 dilution with 2 Diluent (5 µl sample to 995 µl of 2 Diluent).

Requests for shared specimens from Hematology

1. Fill out Shared Sample Request form (Doc# IMM 171-D) by placing a label and the tube tracker position on the form.
2. Make a copy so the A1C tech or LA knows what has already been requested (only save copies until the end of the day).
3. Send the form to Hematology
4. Hematology will pull samples and place on the Immunology pickup bench.

IV. Materials

1. Reagents

Reagents are ready to use and stored at room temperature. Unopened bottles are stable until expiration date. Opened bottles are stable for 8 weeks at room temperature. Reagents should be clear, colorless and caps closed while in use. Do not use if cloudy or discolored.

- 2 Diluent reagent (3.8 L bottle 01-03-0056)
- Buffer 2A Elution reagent #1 (3.8 L bottle 01-03-0054)
- Buffer B Elution reagent #2 (3.8 L bottle 01-03-0012)
- System WASH reagent (940 mL bottle 01-03-0035)

2. Calibrators

Trinity Biotech Glycated Hemoglobin Calibrators Level 1 & Level 2 (01-04-0018).

1. Remove the seal and stopper from the vial.
2. Reconstitute with 400 µl of 2Diluent reagent (01-03-0056).
3. Allow to stand for ten minutes then rotate gently until completely dissolved.
4. Dilute to 1:200 with 2Diluent reagent (5 µl sample to 995 µl of 2Diluent). Transfer of a Shell Vial.

Lyophilized: Stored at 2-8 °C until the expiration date on the label.
Reconstituted: Stable 2-8 °C for 30 days.
Diluted: Stable at room temperature for 8 hours.

When To Calibrate

IMPORTANT: Before performing calibration, make sure that the calibrator values are correctly updated with the current calibrator in used. To verify, pull out the current calibrator insert to match with the Low and High calibrator values.

Click on Calibration→Enter calibrator values

1. When a column is changed.
2. If control values are out of acceptable range using Westgard rules 1-3S, 2-2S, 10X.
3. When reference material changes values or lot numbers.

How to Calibrate:

1. Prepare calibrators and controls as described above
2. Load 2Diluent in position C1 of Rack 209 (Rack D).

3. Perform a baseline calibration
   Go to Calibration →Baseline→Click Run
   After Baseline calibration is completed remove 2Diluent from position C1.
4. Load Calibrators and Controls in Rack 209 (Rack D).
   C1 – Control 1
   C2 – Control 2
   Cal 1 – Calibrator 1
   Cal 2 – Calibrator 2
4. Perform calibration, controls are run automatically.
   Go to Calibration →Run→Click Run
   Leave on default settings
      Run Calibrators – Singly
      Status After Run - Standby

3. Quality Control

Two levels of controls must be run at the beginning of each shift and again every 4 hours (twice per shift) and values must be in acceptable range before running any patient's samples. See "Immunology Laboratory Guidelines for Quality Control" (Imm 38) for control guidelines. New lots of control material are pretested to determine an in-house control range of +/- 3 standard deviations. If a new lot of control is put into use before 30 points are collected the manufacturer's range will be used until 30 data points are collected.

Materials:

Trinity Biotech Glycated Hemoglobin Controls Level 1 & Level 2.

1. Reconstitute with 0.4 mL 2Diluent.
2. Allow to stand for 10 minutes then rotate gently until completely dissolved.
3. Dilute to 1:200 with 2Diluent reagent (5 µl sample to 995 µl of 2Diluent).

Lyophilized: Stored at 2-8 ºC until the expiration date on the label.
Reconstituted: Stable 2-8 ºC for 30 days.
Diluted: Stable at room temperature for 8 hours.

When To Run Controls

1. Both Level 1& Level 2 controls should be run at the beginning of the shift and again every 4 hours.
2. Every time a calibration is performed.

Note: When looking at the manufactures recommended range refer to the NGSP percentage range.

4. Column

HPLC Boronate Affinity Column (03-02-0079)

- Stored at 2-8 °C.
- Allow column to come to room temperature before installing on system.
- **All new columns will be verified by running 5 previously tested patient samples** whose Hgb A1c results span the analytical reportable range (AMR). **The criteria for acceptability is +/- 6%.**
- Column information, including lot number, date installed, date removed, number of injections and verification data is entered onto the (Trinity Primus Ultra2 Column LogDoc# IMM 174-C) located on the L:Drive.
- If it is anticipated that the instrument will not be used for a period of 7 days or more, remove the column after deactivation. Cap each end and store at room temperature until ready to use.
- The directional arrow on the column is for initial reference only; column may be attached in either direction.
- To reduce the column pressure, improve chromatography and extend the life of the column, the column should be reversed daily as part of daily maintenance.
- To reduce the column pressure, an enzyme clean will be performed on a schedule (Mon, Wed and Fri). If the number of injections since the last enzyme clean exceeds 400, a message screen will appear indicating an enzyme clean needs to be performed. The operator can "skip" the reversal (up to 3 times, maximum) only.
- The column life is approximately 1000 injections. If at the start of the shift day the injection count is greater than 1000, the column will be changed.
- **Always change the frit when changing or reversing the column.**
- Refer to Section XII Maintenance for instructions on how to change a column.

5. Rack 209 (Rack D) for calibration and controls (hemolysates)

Must be in the last selected position on the right of 215 sampler. Uses 12x38 mm shell vials to hold the HbA1c Control 1 and 2, Calibrator 1and 2 as well as any patient sample hemolysates. Minimum volume is 1 mL.

Position	
1	Control 1
2	Control 2
3	Calibrator 1 (when calibrating)
4	Calibrator 2 (when calibrating)
5-95	Samples
96	STAT sample

6. Rack 506 (Rack A) for whole blood

Holds 96 13x75 whole blood lavender top tubes.

7. Rack 506 (Rack B) for hemolysates

Use for direct barcode reading of samples. Holds 48 13x75 whole blood lavender top tubes with the barcode facing to the right.

8. Additional Materials

- Enzyme cleaning tubes (01-12-0001, 5/pk)
- 2-micron Frits (03-11-0056, 5/pk)
- Shell vials, Glass (03-02-0112, 250/pk)
- Adjustable pipettor with tips - 20 uL and 1000 ul

V. Daily Routine Assay Procedure

1. Take out controls and samples from the refrigerator to come to room temperature.

2. Perform Daily maintenance as described in the maintenance section below.

3. Prepare quality controls level 1 and level 2, 1:200 dilutions (**10 µl sample to 1990 µl 2Diluent**) in a 13X75mm tube. Mix well and transfer to a 12X38mm glass shell vial. Load onto Rack 209 (Rack D) as hemolysates, positions C1 and C2 respectively.
   Program controls and run before any sample analysis:
   - Go to Main→Edit samples→Entering sample ID
   - Click on Rack D (should say Rack 209, as Hemolysates)
   - Click on Low then Add
   - Click on High then Add
   - Click DONE
   - Go to Main→Run samples→Run
   - When controls are finished, chromatograms will print out. Evaluate the chromatograms (see criteria below) and the A1C values for acceptability.
   - Plot all QC on QC chart, Doc# IMM 171-Q.

4. Call a Soft pending list by workstation MT22, refer to the Soft Immunology Manual, doc# IMM 120. Account for all samples. **Pending lists should be monitored throughout the shift.**

5. To run Whole Blood samples for direct barcode reading:

   1. Load primary tubes on to Rack 506 (Rack B) with the barcode facing to the right.
   2. Go to Main→Edit samples→Direct bar-code reading (see Fig 1)

- Click on **Rack A** drop down arrow and choose **Rack 506, as Whole Blood**. Enter the number of lavender top tubes loaded (patient's samples).
- Click OK
- Go to Main→Run samples→Run (leave Status after run on "Standby")

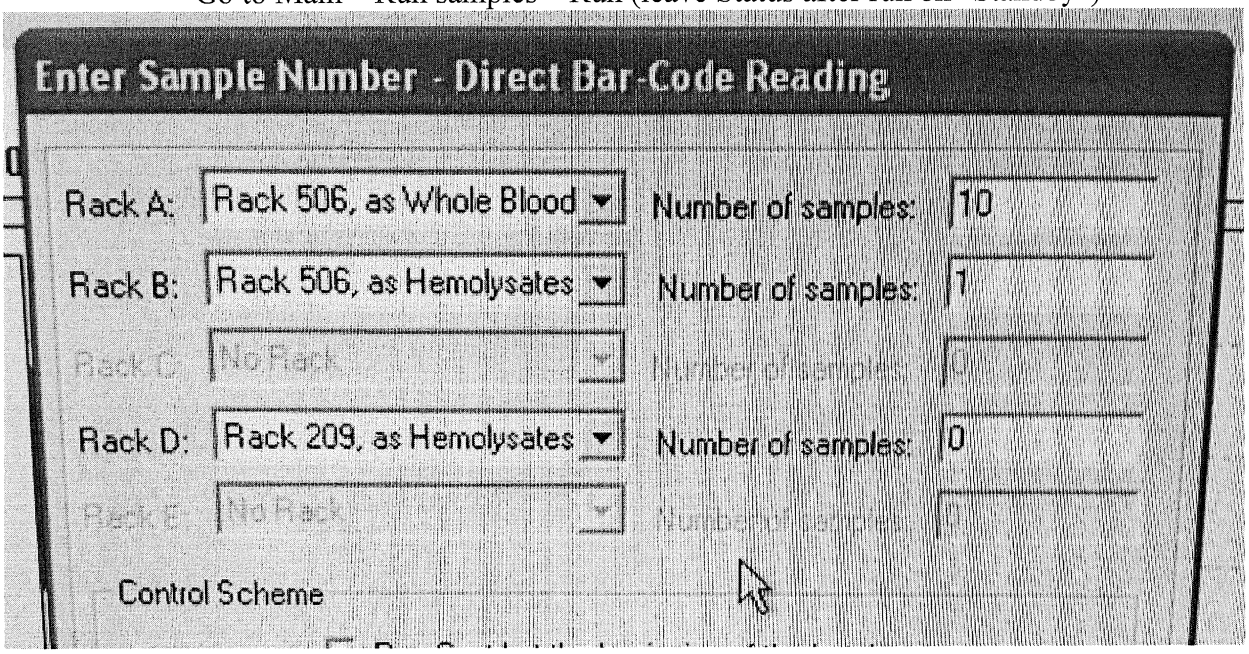


Fig 1 . Direct Barcode Reading rack choices.

Note: The first sample in any batch will be injected twice to re-equilibrate the column. The HbA1c is not calculated. These results are labeled with "ReEquiv".

8. To run Hemolysates – Used when the sample volume is less than 1ml or total peak area is below 500,000. Use Rack B.

1. Print a new barcode and label a 75 X 3mm tube.
2. Prepare a 1:200 dilution (5 µl sample to 995 µl 2Diluent). Mix well.
3. Go to Main→Edit samples→ Direct bar-code reading (see Fig. 1)

- Click on **Rack B** drop down arrow and choose **Rack 506, as Hemolysates.** Enter the number of lavender top tubes loaded (patient's samples).
- Click OK
- Go to Main→Run samples→Run (leave Status after run on "Standby")

9. To run with Shutdown – Only use if samples are being run past the time that a technologist will be present to analyze the data, for example, overnight.
   Main→Run samples→Change Status to "Shutdown" →Run

10. A batch summary report and all chromatograms are automatically printed. Review each chromatogram using the criteria listed below. The batch summary report contains a list of all samples run and their corresponding HbA1c values.

VI. Criteria for Acceptance

Chromatography (Red line)

- The baseline should be flat and parallel to the x-axis
- Peak shape should be well-defined with the glycated Hgb sharp and pointy
- Check to see that the correct peaks are identified by comparing to the retention time of that of the control
- The retention time difference between the QC and samples should be within ±0.03.
- There should a well-defined short valley between the Non and Glycated Hgb.

Peak Area

Target: 0.8 to 1.2 million

Unacceptable: ≤0.5 million or ≥2.0 million **may:**

- Fall outside of individual system's demonstrated linearity.
- May indicate abnormal RBC turnover issues.
- May simply indicate sample preparation and/or introduction error.

In case of unacceptable total peak area cases, prepare manual hemolysate dilution starting with 1:200, until target total peak area is achieved and result is within the laboratory's demonstrated linearity of instrument.

Analytical Measuring Range (AMR)

- **3.5 – 18.0 % (Rerun patient samples before posting)**
- HbA1C value of Less than 3.5% is reported as Less than 3.5%
- HbA1C value of Greater than 18.0% is reported as Greater than 18.0%

VII. Reporting of Results

LIS Interface

1. Instrument ID for Trinity Ultra 2 is MA1C in SoftLab. Interface is unidirectional without loadlist. A1C results do NOT autoverify and must be posted by technologist. Display categories are "Not Posted" and "By Sequence." Confirm QC results are in and review chromatogram before releasing patient result. Click "Post All" to verify.

2. Estimated Average Glucose (EAG) is calculated automatically by the SOFT LIS after Posting results and will autoverify.

3. It is important to note that on occasion, some barcodes will not be read by instrument. Though A1C result is transmitted to LIS, it appends to a sequence identified as "EMPTY", and this is obviously not a valid order number. Delete "EMPTY" from Order field and manually scan barcode, then Save. Before verification of result, review chromatogram and confirm that value matches sample that did not read.

3. **Rerun samples with results less than 3.5% or greater than 18.7% as hemolysates.** If results are the same "Post All" to verify. The SOFT LIS will convert the result and post it as **<3.5%** or **> 18.0%.**

4. The only paper reports that need to be saved are Batch reports containing hand written order numbers due to missed barcode reads.

HbA1c Result Comment

Hemoglobin A1c values of 5.7-6.4 % identify individuals with an increased risk for future diabetes and to whom the term pre-diabetes may be applied. Hemoglobin A1c values greater than 6.4% on more than one occasion are diagnostic of diabetes. Lowering HbA1c to below 7% is considered to reduce microvascular and neuropathic complications of diabetes.

This boronate affinity Hb A1c method provides accurate analytical results in the presence of nearly all Hb variants. Hb F higher than 10% of total Hb may yield falsely low results. Conditions that shorten red cell survival, such as the presence of unstable hemoglobins like Hb SS, Hb CC, and Hb SC, or other causes of hemolytic anemia may yield falsely low results. Iron deficiency anemia may yield falsely high results.

VIII. Calculations

The relationship between A1C and EAG is described by the formula:

$$28.7 \text{ X A1C} - 46.7 = \text{EAG}.$$

IX. Reference Range

The reference range listed below was recommended by the American Diabetes Association (3).

4.0 – 6.0%

X. Interferences and Limitations

- **Lipemic samples (Lactescence)**
  The GHb assay is not influenced by Triglycerides (6000 mg/dL). Although gross lactescence should be wash 5 volumes of isotonic saline before diluting.
- **Abnormal red cell survival**
  Patients with shortened red cell life, such as the presence of unstable hemoglobins like Hb SS, Hb CC, and Hb SC, or other causes of hemolytic anemia, will exhibit decreased GHb. Patients with extended red cell life, such as in polycythemia or after splenectomy, may exhibit increased GHb values.
- **Icteric samples (High Bilirubin and/or Fructosamine)**
  The GHb assay is not influenced by bilirubin of up to 48 mg/dL; although grossly icteric samples may be washed 5 times with isotonic saline before diluting.
- **FREE from common interferences such as:**
  1. Hemoglobin variants (HBS, HBC, HbD, etc.)
  2. Fetal hemoglobin (HbF)
  3. Non-Glycated Modifications (carbamylation, acetylation, etc.)
  4. Labile (aldimine or Schiff base)
  5. Hemichromes formed during storage

XI. Maintenance

Daily Maintenance (Startup)

1. Check all reagent levels. If possible change reagents before activation.
   - After activation (Standby mode) the pumps are active. **Before changing a reagent stop the pumps as follows:**
     Manual→Pumps→Solvent System→Set Flow rate at 0.
2. Change water with fresh CLRW.
3. Clean probe and injection port of sampler with an alcohol wipe.
4. Check waste container.
5. Check printer paper supply.
6. Reverse the Column and change the frit.
7. Activate the system.
8. Record the Column temperature; check both the computer display and the oven itself. The temperature should be 48.2 +/-0.5.
9. Record Column injection count. Click the ? icon to see the injection count.
10. Check the Flow Rate (**must be 2.0 mL/min when running**).
11. Record system pressure (**must be <35 bar when running**).
12. Check the Flow Rate (**must be 2.0 mL/min when running**).

Daily Maintenance (Shutdown)

13. Enzyme Clean only on Monday, Wednesday or Fridays
14. Deactivate system at the end of the day.

Weekly Maintenance (Performed on Sundays)

Reboot the CPU

1. Shut down the CPU computer at the end of the day.
2. The computer will remain off until Monday morning.

Periodic Maintenance

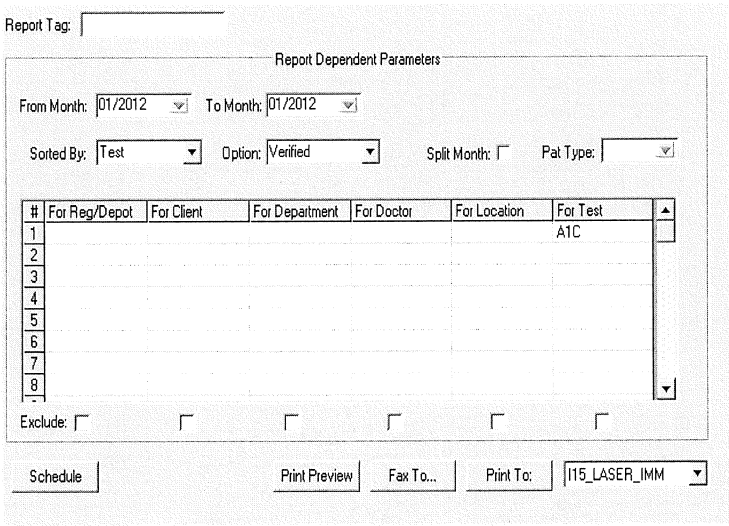
1. Change the column and the frit when:
   - The injection count at the start of the day exceeds 1000.
   - The chromatography is poor and unacceptable if all troubleshooting has been exhausted.
   - The pressure is high even after reversing the column and performing an enzyme clean
   - **All new columns will be verified by running 5 previously tested patient samples** whose HgbA1c results span the analytical reportable range (AMR). **The criteria for acceptability is +/- 6%.**
   - Column information, including lot number, date installed, date removed, number of injections and verification data is entered onto the (Trinity Primus Ultra2 Column Log Doc# IMM 174-C) located on the L:Drive.
2. **To install a new column:**
   - The instrument must be in **deactivated state** with the flow set to 0.00 mL/min.
   - The column must be at room temperature.
   - Update column lot number by going to Utilities ⟶ Edit Lot Numbers
   - Uncap the column and attached the tubing in a manner that the end of the tubing is "butted" tightly to the column fitting.
   - Replace the column and the frit.
   - Go to Manual ⟶ Pumps ⟶ Solvent System and set the Flow Rate to 2.0 and the pumps to 50%. Let flush for 15 min.
   - Check for leaks, and then activate the system.
   - Calibrate and run controls and pretest patients.

Monthly Maintenance

Monthly Inventory

1. Print the end of the month Inventory List
   View/Print → Print Inventory Checklist
2. Fill in inventory amounts.
3. **Change the injection volume** to reflect the number of tests "verified per month". (Draw a single line through the printed volume, Trinity would like to see this number as well as billable amount.
4. To call a test per month report in Soft.
   Util → Tests Per Month

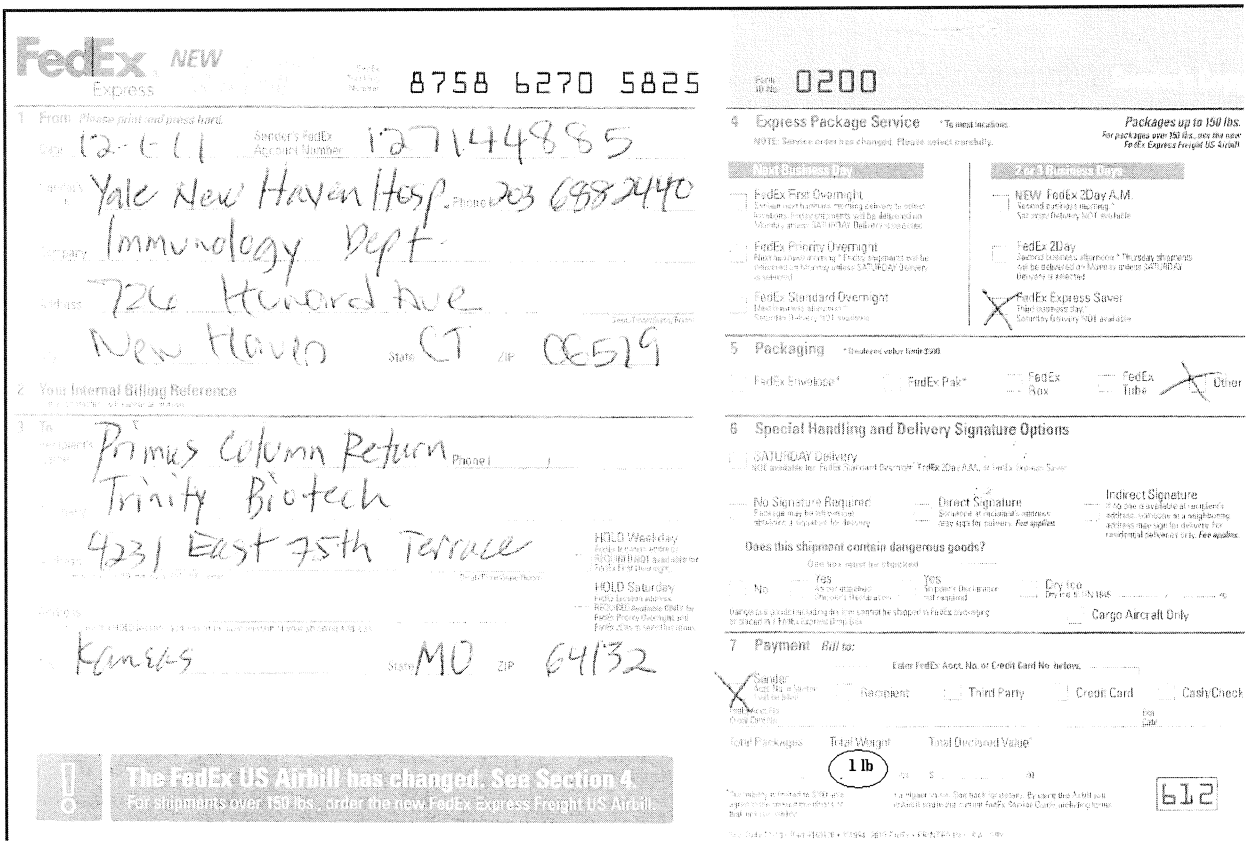
Update "From and To Month" to current month
Change "Sorted By" to Test
Change "Option" to Verified
Uncheck "Split Month"
Under "For Test" type A1C



3. Fax Inventory sheet and Test Per Month report to Trinity.
4. If inventory is needed before the end of the month, print and update inventory form. Make a note on the form that it is for inventory only and is not to be used for billing.

Column Return

1. Pack columns with usage printout in the original column box. Seal the box with tape.
2. Place column box in FedEx mailer and seal it.
3. Fill out the FedEx Airbill (see below).



4. Attach the Airbill to the mailer with the airbill envelope.
5. Place package in Chem reference area for pickup.

File Backup

1. On the desktop open the Shortcut to GHbArch folder.
2. Click on the Year folder which contains data files by date.
3. The files are list as year, month, then date (ex April 5, 2012 would be 20120405).
4. Copy the files from the previous month and transfer to a flash drive (Removable Disk E).
5. Tranfer the files from the flash drive to the O Drive folder A1C Archives. Open the current year folder and create a new folder for the current month.

XII. Method Validation and Correlation

Correlation (Method Comparison):

A correlation was performed by comparing results from the Biorad Variant II on 89 patient samples to results obtained on the Primus Ultra2. The results were analyzed using EP evaluator and showed good correlation with an R value of 0.9992.

Methods: Biorad Variant II vs Trinity Biotech Primus

# of samples: 89
R value = 0.9992

Precision:

Intrarun Precision: Intrarun-assay performance was evaluated by analyzing multiple replicates of three samples representing a low value, medium value and a high value on the same run.

	#of Replicates	Mean	%CV
Low	21	4.52	1.0
Medium	21	8.49	0.9
High	17	12.97	0.8

Interrun Precision: Intrarun-assay performance was evaluated by analyzing three samples representing a low value, medium value and a high value on 5 separate runs.

	#of Runs	Mean	%CV
Low	5	4.50	0.0
Medium	5	8.40	1.7
High	5	13.36	0.7

Linearity

A linearity study was performed using Lyphochek Hemoglobin A1C Linearity Set by Bio-Rad. Four levels of linearity standards were diluted and analyzed in triplicate. The assay was found to be linear using a 1:200 dilution with the exception of the 3.1% standard. A 1:100 dilution was then performed and the assay was found to be linear. The Analytical Measurement Range (AMR) is then determined to be 3.5 – 18.0 %.

Reference Range Verification:

The American Diabetes Association (ADA) reference range of 4.0 – 6.0% was verified by analyzing 20 random patients' samples representing the YNHH testing population. 18 of the 20 samples fell within the established range .

XIII. Troubleshooting

In some cases, error codes that describe the nature of the problem will appear in the Activity Log. The Activity Log can be accessed from the Glycated Hemoglobin Analyzes by:

View/Print→View activity log.

Please refer to Chapter 10 of the Operator's Manual for listing of error codes and remedies including any Chromatography Problems, Software Initialization Errors, General System Errors, 215 Injection Errors and 819 Injection Valve Errors.

For Help and Technical Support

Trinity Biotech Ultra2 Affinity
Serial Number: 100572

US Customers:
Trinity Biotech USA
2823 Girts Road
Jamestown, NY 14701
www.trinitybiotech.com
800-325-3424

XIV. References

1. Trinity Biotech Operator's Manual
   Revision: 11 Mar 2011
2. Diabetes Care 31:b1-6, 2008
3. Tze et al. Hemoglobin A1C an Indicator of Diabetic Control. J of Pediatrics 1978, 93:13-16.
4. Diabetes Standard of Care 2010, Volume 33, Supplement 1, January 2010
5. American Diabetes Association: Standards of medical care for patients with diabetes mellitus. Diabetes Care 2009 Jan 32:S1
6. The Diabetes Control and complications Trial (DCCT): Design and methologic considerations for the feasibility phase: the DCCT Research Group Diabetes 35: 530 – 545, May 1986

XV Appendix

IMM 171-M		A1C Primus Maintenance Chart
IMM 171-Q		A1C QC charts
IMM 171-A		A1C Primus Quiz
IMM 171-B		A1c Primus Training checklist
IMM 171-C		Trinity Primus Ultra2 Column Log
IMM 171-D		A1C Shared Sample Request Form

XVI. History

Revised by Penny Smith 1/10/2012 - Added check of cal values before calibration. Switch to from Biorad to Trinity controls. Added more info to Peak Criteria. Changed monthly maintenance to periodic maintenance.

Revised by Penny Smith 5/1/2013 - Update procedure to include SOFT LIS interface. Created a Column log and a procedure for new column verification. Made the following changes to Maintenance section: Column to be reversed daily, Enzyme clean to be performed Mon , Wed and Fri., Weekly CPU reboot to be done on Sun., monthly to include inventory, file backup and column return.

Revised by Penny Smith 5/3/2013 – Added a more detailed procedure for changing the column, including changing the column if the injection count is greater 1000 at the start of the day. Corrected the AMR from 18.7% to 18.0%. Changed the requirement for running Quality Control to every 4 hours. Added information on obtaining shared specimens. Updated the acceptability limit to 6% per new CAP guidelines.

Trinity Biotech Ultra2 Maintenance Checklist

Month:_____Year:_____

Daily AM	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
Check reagent levels																															
Change CLRW water																															
Clean probe/injection port																															
Check waste container																															
Check printer paper																															
Reverse Column / Change Frit																															
Activate system																															
Column temperature (48.2C +/-0.5)																															
Column injection count																															
Check flow rate (2.0 mL/min)																															
System pressure (<35 bar)																															
Tech initials																															
Daily PM																															
Enzyme Clean (Mon, Wed, Fri)																															
Deactivate system																															
Tech initials																															

Weekly (Sunday)

Reboot CPU					
Date/Tech initials					

Monthly (end of the month)

Print and fax monthly inventory	
Return Columns	
Back up files	
Date/Tech initials	

Periodically

Change column (Fill out column log on L:Drive)
Dates/Initials

Comments:

_____

_____

Supervisor Review _____

Yale New Haven Hospital
Department of Laboratory Medicine
Immunology

Document Number: 171-B

Trinity Biotech Primus Hemoglobin A1c
Training checklist

Principle of the Assay
Specimen requirements
- Minimum volume
- Run sample as whole blood
- Storage and stability
- Manual dilution
- Run sample as hemolysates

Instrument
- Activation and deactivation
- Shutdown

Familiarization with major parts and components
- Autosampler System
  - 215 Liquid Handler with 819 Injection Valve Actuator
  - Active Rinse Station (ARS)
  - Barcode Scanner (Handheld and Automatic)
- UV/VIS Detector
- Pump
- Column Heater
- Data Handling

Reagents
- 2 Diluent reagent
- Buffer 2A Elution reagent
- Buffer B Elution reagent
- System Wash reagent

Calibrator
- Reconstitution
- Dilution
- Expiration/Storage
- How to calibrate
- When to calibrate

Quality Controls
- Reconstitution
- Dilution
- Expiration/Storage
- How to run QC
- When to run QC
- Westgard rules

Column
- Storage
- When to change the column
- How to change the column
- When and how to reverse the column
- When and how to enzyme-clean the column
- Changing frit

Results Acceptance
- Total peak area
- Reference range
- Linearity
- Interpretation of reports
- Clinical reportable range

Maintenance
- Daily
- Weekly
- Monthly
- Periodic

Trainer/Date:_____ Trainee/Date:_____

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SHARED SAMPLE REQUEST FORM

ATTENTION HEMATOLOGY

Immunology requests a lavender top tube. Please place lavender in Immunology bucket in the processing area. Thanks!

Date/ Requesting Technologist_____

LABEL	TUBE TRACKER POSITION

Document Author
Virgilio Macalalad
September 28, 2011

Signature Approval for Annual Review
Name: Hemoglobin A1C - Boronate Affinity HPLC
Document #: Imm 171

Name (Print)	Title	Signature	Date of Review	Effective Date for Use	Issue Date for Training if Applicable	
TEODORICO LEE	LAB MANAGER	Teodorico Lee	9/28/11			9/28/11
BRIAN SMITH	LAB DIRECTOR	Brian R. Smith MD	9/28/11			9/28/11
TEODORICO LEE	LAB MANAGER	Teodorico Lee	1/10/12	Added check of cal values before calibration. Switch to from Biorad to Trinity controls. Added more info to Peak Criteria. Changed monthly maintenance to periodic maintenance.		1/10/12
BRIAN SMITH	LAB DIRECTOR	Brian R. Smith MD	1/10/12			1/10/12
TEODORICO LEE	LAB MANAGER	Teodorico Lee	5/7/12	Update procedure to include SOFT LIS interface. Created a Column log and a procedure for new column verification. Made the following changes to Maintenance section: Column to be reversed daily, Enzyme clean to be performed Mon , Wed and Fri., Weekly CPU reboot to be done on Sun., monthly to include inventory, file backup and column return.		5/2/12
BRIAN SMITH	LAB DIRECTOR	Brian R. Smith MD	5/2/12			5/2/12
TEODORICO LEE	LAB MANAGER	Teodorico Lee	5/3/13	Added a more detailed procedure for changing the column, including changing the column if the injection count is greater 1000 at the start of the day. Corrected the AMR from 18.7% to 18.0%. Changed the requirement for running Quality Control to every 4 hours. Added information on obtaining shared specimens. Changed acceptability limit to 6% per new CAP guidelines.		5/3/13
BRIAN SMITH	LAB DIRECTOR		5/3/13			5/3/13