Principle

The Primus Variant Short Program uses cation-exchange High Performance Liquid Chromatography to aid in the separation and quantitation of Hemoglobins A2 and F, and in the identification of abnormal hemoglobins present in whole blood. Hemolyzed specimens are automatically injected into a stream of buffers and pass through the analytical cartridge. Separation of hemoglobin types is done by the use of a gradient between 2 mobile phases with differing salt concentrations and pH. The absorbance (read at 413nm) at any given time is proportional to the amount of hemoglobin present. Physical characteristics, hydrophobic and hydrophilic properties will determine the migration of each hemoglobin type, which will be reported as a retention time (amount of time from injection to apex of hemoglobin peak), and relative retention time to hemoglobins F, A, S, or C.

Specimen

Whole blood specimens containing EDTA as an anticoagulant are the only acceptable specimens. Patient samples are stable for 21 days when stored at 2-8^{o}C. Frozen hemolysates may also be used, with an expiration of 1 year from preparation when stored at -70^{o}C.

Reagents, Equipment, and Supplies

- Primus Ultra2 Variant Hemoglobin Testing System with auto sampler, and Resolution software.

- Hotline Trinity Primus Ultra 2 HPLC SN 100446 1-800-325-3424

CAUTION: MOBILE PHASES 1 AND 2 CONTAIN TRACES OF CYANIDE DO NOT PIPETTE BY MOUTH; DO NOT INGEST; AVOID SKIN AND EYE CONTACT

- Mobile Phase 1 #010-03-0042: 940ml of 0.01% Potassium cyanide solution with Tris hydrochloride and Tris free base. Store at 15-30^{o}C.

- Mobile Phase 2 #010-03-0044: 940ml of 0.01% Potassium cyanide solution with Tris hydrochloride and Tris free base. Store at 15-30^{o}C.

- 2 Diluent #010-03-0059: 940ml of 0.001% sodium azide solution. Store at 15-30^{o}C.

□ System Wash reagent #010-03-0035: 940ml of ~5% alcohol blend. Store at 15-30^{o}C.

□ Reagents are ready to use.

- Store at room temperature (15-28°C). Do not use beyond the expiration date printed on the label. Unopened bottles of reagents are stable until expiration date on label.
- Reagents are stable at least 21 days at room temperature after opening.
- Keep reagents capped while in use and immediately after use.
- Reagents should be clear and colorless. Do not use if cloudy or discolored.

□ Trinity Biotech Resolution Analytical column #01-05-0017: a cation exchange cartridge capable of performing 500 analyses. Store unused columns at 2-8^{o}C. New cartridges must be validated for performance prior to use. To validate cartridge run FASC calibrator, control I and II, a previously run normal sample and two previously run variant hemoglobin samples. Values must be within limits for controls, and within 10% for patient comparison.

- Store column at 2-8^{o}C until installed on the instrument.
- If it is anticipated that the instrument will not be used for a period of 7 days or more, remove the column after shutdown. Cap each end and store at 2-8^{o}C until ready for use.
- Warm the column to room temperature prior to installing it on the instrument.
- The column should be installed initially with the direction of the arrow pointing upwards with the flow of mobile phase and run through the activation process to allow it to equilibrate properly prior to use.
- Reverse the column daily or every 250 injections prior to activation to maintain analytical column performance.

- Trinity Biotech FASC calibrator #010-04-0042: Freeze dried human whole blood controlled for hemoglobin F, A, S, and C retention times.
    - Add 1.0 ml Primus Diluent2 to lyophilized calibrator and allow to stand 10 minutes. Swirl to mix.
    - Stable 8 weeks at 2-8^{o}C and 2 years at -70^{o}C when tightly closed
- Sample shells: 500 2.5 ml glass sample vials.
- Trinity Biotech Level I and II F and A_2 Controls # 010-04-0043. Freeze dried human whole blood controlled for Hemoglobins F and A_2.
    - Add 0.3 ml Primus Diluent2 to lyophilized controls and allow to stand 10 minutes. Swirl to mix.
        - Stable 7 days at 2-8^{o}C and 1 year at -70^{o}C when tightly closed.
- Deionized water
- New Methylene Blue N Eng Scientific, Inc. Prod No. 5100 100 ml.
    - Stored at room temperature. Stable until expiration date unless growth is observed.
- SickleScreen Sickling Hemoglobin Screening Kit, Pacific Hemostasis (30 determination kit), cat#100250

Calibration

The Ultra^2 analyzes the FASC sample and generates a calibration curve relative to the 4 major hemoglobin peaks, hemoglobins F, A, S, and C. The retention times obtained from calibration are used to identify all peaks within the samples. Each peak will have a retention time and relative retention time to the 4 hemoglobin peaks.

Performance Characteristics

Column and detector performance are monitored with the response factors for FASC, as well as Level I and Level II controls. Unusual peak tailing, wide peaks or consistently erroneous control values can identify a defective or exhausted column. Such columns should be reversed and run in the reverse direction until the chromatography deteriorates. It may be reversed numerous times until the chromatography does not improve at which time it is replaced. The column should be installed in the reverse direction and allowed to equilibrate in this direction for 15 minutes at a flow of 1.5 mL/min prior to use.

Quality Control

FASC calibrator and Level I and Level II controls are run at the beginning of each run. QC must fall within departmental guidelines prior to reporting patient results. New lots of controls must be evaluated in parallel with current lot prior to use.

Procedure

- Assure that reagents lines are appropriately in place:
  - Mobile Phase 1 on pump A – Red reagent line
  - Mobile Phase 2 on pump B – Blue reagent line
  - System Wash Reagent on the clear reagent line
  - RESOLUTION^{TM} Analytical Column on the column holder
  - Reverse column if necessary
  - 2DIL to the syringe pump on the 215 sampler
  - DI Water for the Active Rinse Station on the 215 sampler
  - DI Water on the Ultra^2 for washing of the pump heads (2 clear lines)

- Using touchpad, activate system to start up analyzer

- From tool bar, select MAIN
- From MAIN menu, select ACTIVATE SYSTEM
- The instrument will undergo a series of self checks, reagent primes, and pressure charges which will take 20 minutes. There is a count-down timer located on the lower portion of screen, and when the timer reaches 0, the instrument is ready for use.
- Condition Column
  - Go to Manual-Pumps-Solvent System
  - Set flow rate to 1.5 and % Pump B to 15
  - Condition for 15 minutes

☐ Prepare calibrator and controls

- Thaw frozen reconstituted calibrator and/or controls in 37^{o}C water bath for 5-10 minutes.
- Vortex prior to use.
- For FASC calibrator:
  - Add 1580 uL of water or diluent to a glass sample vial.
  - Add 20 uL of FASC and vortex gently.
- For controls I and III:
  - Add 1780 uL of water or diluent to a glass sample vial.
  - Add 15 uL of control I or II and vortex gently.
- Diluted calibrators and controls are stable for 24 hours at room temperature or 2-8^{o}C.

☐ Sample preparation

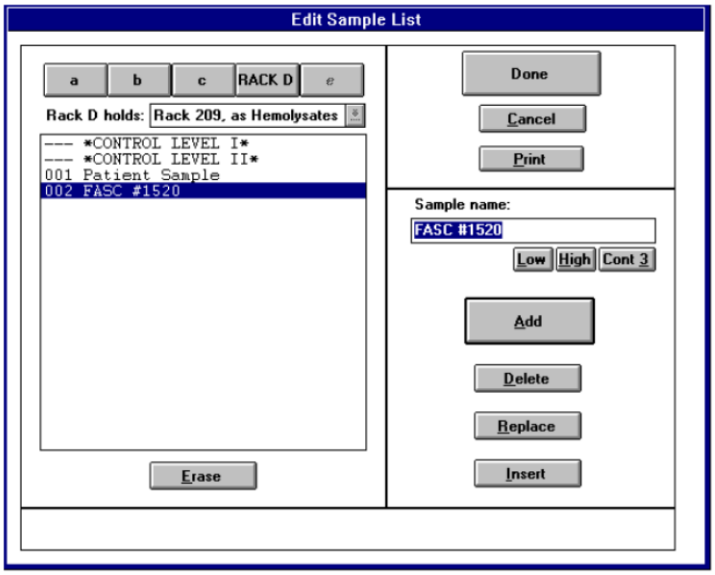
- Gently mix whole blood samples. If auto dilutor is being used, then proceed to worklist preparation.
- If auto dilutor is NOT being used, then prepare manual dilutions
  - Add 2000 uL of water or diluent to a glass sample vial (prepare one vial for each patient sample).
  - Add 15 uL of mixed whole blood to sample vial and vortex gently.
  - CAP samples and frozen whole blood samples are prepared in this same manner.

Worklist preparation

- Using touchpad, go into MAIN section of toolbar.
- Using touchpad, locate EDIT SAMPLES and hit enter on keyboard or touchpad. (See next page for example of screen that appears)
- Each rack has unique IDs and it is important to note that certain racks are designated for specific uses:
  - Use rack ID a #206, as whole blood, for all whole blood samples
  - Use rack ID D #209, as hemolysates, for all FASC, controls and prediluted samples.
- Clear any existing worklist.
- Calibrator and controls are preprogrammed. Place in glass vials on rack D #209 in the first 3 spots.
  - Place diluted FASC calibrator into rack D #209, position C1
  - Place diluted control I and III into rack D #209, position C2 and C3 respectively.
- If there are prediluted patient samples, enter them now
  - Using keypad, enter sample ID #, then “Add”.
  - Place on rack, starting with position number 1 for first pre-diluted patient sample.

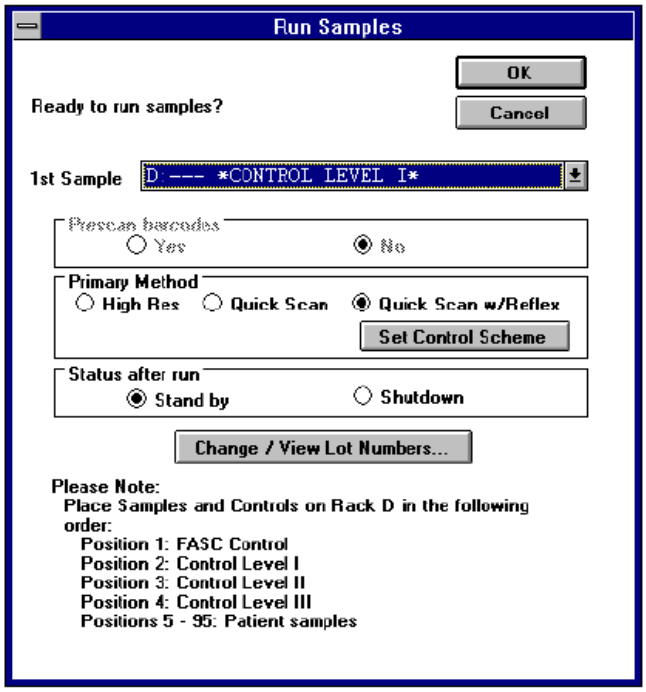
- Repeat sequence for remainder of prediluted patient samples.

- For whole blood samples (in primary EDTA tube)
  - Using keypad, select rack a, #206.
  - Begin entering patient samples ID#s using either barcode reader or manually enter sample ID# using keyboard.
  - Place samples in rack 206 in same sequence as entered into worklist.



NOTE: It is important that the appropriate rack is properly selected in the worklist function. If the worklist is not properly recorded, then sample testing may be not be correctly performed.

- Once worklist entry is completed, select DONE from screen.
- Using touchpad, go into MAIN menu from toolbar
- Select RUN SAMPLES
- Once selected, a pop-up box will appear asking the user to select operational directions. Assure that the following are implemented:



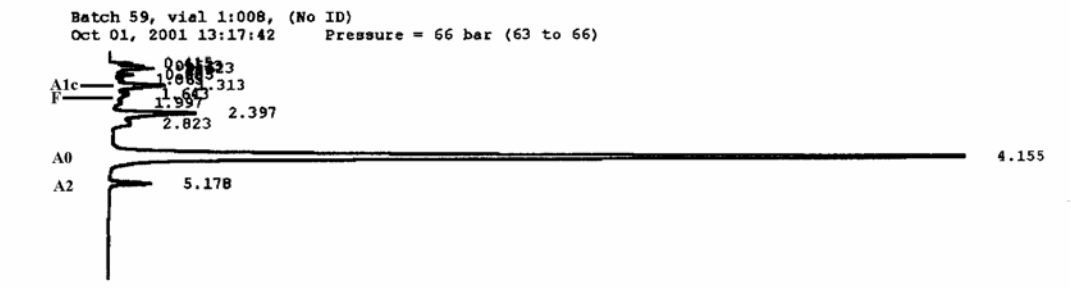
- If many samples in the run contain known and previously confirmed abnormal hemoglobins, choose the Quick Scan and Standby as after run Status. The instrument can then be quickly brought back up to perform a high resolution confirmatory run followed by shutdown.

- When starting the high resolution confirmatory run, be sure that Shutdown mode is selected. (Once the instrument has started, you can change the end of run status from the MAIN menu of the taskbar)

- In the Shutdown mode, once the "OK" icon has been selected, another pop-up box will appear instructing the use to place a fresh tube of 100% bleach into the bleach station located in the rack holder section of the instrument.

Interpretation

- The normal chromatograph will have multiple peaks, but primarily hemoglobins A0, A1c, A2, and F should be present in normal amounts



PEAK	RT	REL RT	% CONC	COMMENT
1	0.415	F 0.271	0.3%	
2	0.533	F 0.348	0.6%	
3	0.623	F 0.407	1.1%	
4	0.885	F 0.578	0.4%	
5	1.065	F 0.695	0.3%	
6	1.313	F 0.857	3.2%	A1c peak
7	1.643	F 1.073	0.5%	Consistent with F
8	1.997	F 1.304	0.4%	
9	2.397	F 1.565	4.8%	
10	2.823	A 0.691	2.0%	
11	4.155	A 1.017	84.6%	A0 peak
12	5.178	S 0.866	1.9%	A2 peak

- Criteria for acceptable chromatograms.

  - The chromatographs must have flat or nearly flat, stable baselines. Drifting baselines may be suggestive of detector problems.
  - Pressure is between mid-40s to 100bar. Pressure range at 40bar should be no more than 4bar, and pressure range at 90bar should be no more than 8bar. Pressures increase with column use. Lower pressures indicate system tubing leak or poor reagent priming.
  - Peaks must be sharp and narrow. Spreading peaks is a sign of a bad column.
  - Total area (not shown) should be between 1,500,000 and 2,500,000. Samples falling outside this range should be adjusted and reanalyzed.
  - All low hemoglobin A2 values less than 1.9% run in Quick Scan program should be repeated on Hi Res program.
  - Information contained on chromatograph

- Batch number
- Vial: position of sample on tray or rack
- Sample identification either manually or barcode entered
- Date and time of testing
- Pressure and pressure range
- A number of peaks determined from the signal output of the spectrophotometer detector, with each peak representing a unique species of hemoglobin.
- A peak report will indicate the following:
    - Number of peaks
    - Actual retention time
    - Relative retention time (to nearest calibrated peak F, Ao, S, or C)
    - % concentration which is based on the total area of each individual peak
    - Comment codes, which signify an unexpected abnormal condition
        a) There are 6 comment codes located on the bottom of each chromatograph report

1) Wide Ao peak
2) Area of Ao peak < 80%
3) Peak area greater than expected
4) Peak after A2
5) A1c > 10%
6) HbF or a variant is present

Interpretations

 Samples containing hemoglobins other than A0, A2, A1c, and F that have not been previously confirmed at UCDHS must have a High Resolution run performed for confirmatory purposes.

 Compare quick scan and high resolutions chromatographs to assure that both reports indicate same variant hemoglobin (e.g. S peak on scan and S peak on high resolution). Repeat samples with discordance between scan and high resolution.

 For samples that have abnormally occurring hemoglobins with relative retention times that are not consistent with hemoglobins S, C, or A2′:

- Use Trinity Biotech Variant Comparison Table chart to indicate possible hemoglobin variant type. The chart uses Hi Res retention times.
- Be sure to note concentration of hemoglobin variant to assist in determining hemoglobin chain variant.

 Samples with <30% of hemoglobins S or C, as seen in transfused patients, will not give a “consistent with S” or “consistent with C” comment.

 If the sample appears to have a fast moving Hemoglobin that appears to be Hgb H or Bart’s, take an aliquot of the sample and mix with equal parts of New Methylene Blue stain. Let stand at 37^{o}C for 1-2 hours and prepare wedge smears. Under high power

(100x oil immersion) examine the red cells for pale blue Hemoglobin H inclusions that cover the entire red cell giving it a typical “golf ball” appearance.

 If the sample appears to have an unexplained high level of Hemoglobin F, perform a Kleihauer Betke stain (see Technical procedure #1245) to confirm both the presence of higher levels of Hemoglobin F and distribution of Hgb F in the red cells to aid in interpretation of the sample.

 For those samples that have variant codes that are not hemoglobin S, C, E, A2′, or H may be sent to reflexively to UCSF for further identification. The hemoglobin type can then be reported as confirmed by UCSF (IEF).

 When the Scan program is not performing adequately due to deterioration of the column and testing must be performed in High Resolution mode only, those samples containing suspected Hemoglobin S that have not been previously confirmed must have solubility testing performed to confirm the presence of Hemoglobin S.

- Bring all reagents to room temperature.
- Known positive and negative controls must be run with each group of samples.
- Add 4ml of phosphate buffer to prefilled reaction vial and mix well.
- Add 50ul of whole blood or control. Cap and shake vigorously immediately.
- Incubate 10-20 minutes in Tube reading rack. If Hgb S or other sickling Hgb is present the solution will be turbid and the lines on the tube reading rack will not be clearly visible.
- Severe anemia may cause false negatives. In the severely anemic patient add 100ul of whole blood initially.
- Elevated Hgb F may cause false negative.
- Recent transfusions may cause false results due to lower levels of Hemoglobin S being present.

- Report hemoglobins A0, A2, F, and any variants noted. See Technical Procedure # 1233.t for further instruction.

Maintenance

- Daily Maintenance

  - Check number of injections and reverse column if indicated
  - Check reagents and water supply
  - Check and empty waste as needed.
  - Check paper supply in printer
  - Record system pressure on daily PM log. Change frit (membrane) if pressure >100 bar.
  - Clean probe of sampler
  - Purge air from reagent and water lines if necessary.

- Monthly Maintenance

  - Wipe interior and exterior of instrument using a cloth dampened with diluted bleach.
  - Wipe exterior of auto dilutor with diluted bleach.

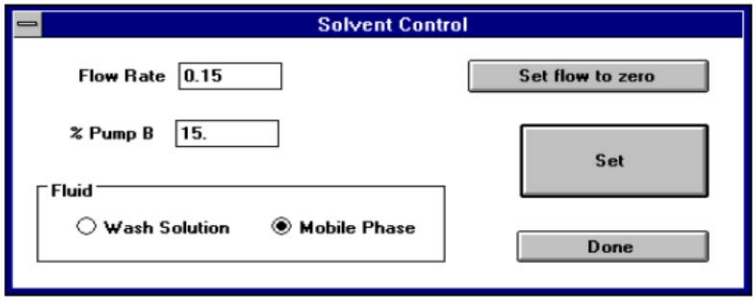
- Installing the cartridge

  - Remove the column end plugs. Save the plugs, they will need to be put back on the column after the column has been exhausted.
  - Snap the column into the two column clips on the column holder located on the left side of the pump.

- Install the tan PEEK tubing from the frit housing into the bottom of the column using the tan 1/16” fingertight fitting.
- Install the metal tubing from the detector flow cell to the top of the column using the tan 1/16” finger tight fitting.
- Change the frit when changing the column
- Activate the system by going to the Primus RESOLUTION Software, select Main and Activate System.
- Note: Do not Activate the system unless the reagents are installed and the pumps are primed.

 Installing reagents; Priming pumps A and B

- The red supply line is inserted into the Mobile Phase 1 bottle.
- The blue supply line is inserted into the Mobile Phase 2 bottle.
- The clear single supply line gone into a System Wash bottle.
- The clear double supply line goes into a System Wash bottle (this bottle is for the Piston Wash and does not need to be Wash reagent. It can be filled with DI Water).
- The clear line from the tan valve on the 215 Liquid Handler is inserted into the 2Dil bottle.
- The clear line from the Active Rinse Station (ARS) on the 215 Liquid Handler is inserted into a gallon bottle filled with DI Water.
- In the Primus RESOLUTION Software, select MANUAL and then SOLVENT SYSTEM. The SOLVENT CONTROL dialog box will be displayed as shown in Figure 9-2. For priming pump A, set %B to 0%. For priming pump B, set %B to 100%.



- Press the Set flow to zero button.
- Note: The set button or the enter key on the keyboard must be pressed to activate any changes that were made in the SOLVENT CONTROL screen with the exception of the Set flow to zero and the Done buttons, which take effect immediately.
- Confirm the mobile phase supply lines are completely submerged in the liquid of the appropriate mobile phase bottles.
- Confirm the red/blue valves located on the supply lines coming out of the mobile phase and wash bottles are turned to the open position (the arm of the valve will be inline with the valve).
- Place the 10cc syringe into the priming valve of pump A. The priming valve is located inside the front door of the pump compartment.
- Turn the arm of the priming valve down toward the pump head and pull back on the syringe plunger, filling the syringe with liquid. Repeat until the all of the air has been removed from the solvent line between the mobile phase bottle and the priming valve.
- Turn the priming valve arm toward the priming nipple in order to prevent liquid from leaking out when the syringe is removed.
- Remove the syringe from the priming valve. Remove any air in the syringe by holding the tip of the syringe straight up and slowly pushing on the plunger until all air has been removed. It may be necessary to tap the side of the syringe barrel to help any bubbles migrate to the tip of the syringe.

- Insert the syringe back into the priming valve.
- Rotate the arm of the priming valve to point up, away from the pump head.
- Loosen the inlet check valve housing (lower large nut on the pump) with a crescent wrench. Push on the plunger of the syringe and allow liquid come through the threads of the housing. When no more air bubbles are visible, tighten this check valve housing. Do not over-tighten.
- Warning!! Do not over-tighten the check valve housing or damage to the check valve can occur.
- Loosen the outlet line at the top of the outlet check valve (small nut on tubing in the upper large nut on the pump) using a 1/4" wrench. Then, loosening the check valve housing with a crescent wrench, push on the syringe plunger and allow liquid to come through the threads of the housing. Once only liquid comes out (no air bubbles), tighten the check valve housing. Do not over-tighten.
- Push on the plunger of the syringe and allow the liquid to come out around the threads and top of the nut. Once only liquid comes out (no air bubbles), tighten the nut.
- Open the purge valve by turning the black knob on top of the purge valve counter-clockwise. Push the plunger of the syringe in until the syringe has been emptied. Close the purge valve.
- Place the arm of the priming T pointing toward the nipple of the priming T (the point where the syringe is inserted).
- Remove any excess liquid around the check valves and fittings using absorbent paper towels.
- Repeat on the B pump head.

 Open the purge valve set the flow to 5mL/min at 0% B and press Set in the SOLVENT CONTROL dialog box. Allow the pump to run for 5 min.

- While running the pump, check for leaks around the check valves and any fittings. Tighten as necessary.
- Set the flow to 100% B and press Set in the SOLVENT CONTROL dialog box. Allow the pump to run for 5 min.
- While running the pump, check for leaks around the check valves and any fittings. Tighten as necessary.
- Press the Set flow to zero button.
- Close the purge valve.
- If the column is installed or the column by-pass union is installed remove the tan inlet tubing from the inlet side of the column (or bypass union). Place the tubing into a beaker.
- Set the flow to 5mL/min at 0% B and press Set in the SOLVENT CONTROL dialog box. A strong steady stream should be squirting out of the tubing.
- Repeat above step using 100% B.
- Install the column if it is not already in place.
    - Connect the tan tubing to the bottom of the column and the metal tubing from the detector flow cell to the top of the column.
- Set the flow to 1.5 mL/min at 0% B and press Set in the SOLVENT CONTROL dialog box. Allow the pressure to stabilize; this should take a few minutes. Note the pressure.
- Set the flow to 1.5 mL/min at 100% B and press Set in the SOLVENT CONTROL dialog box. Note the pressure.
- The pressure between the two pumps should be very close (within 1-2 bar difference - similar to acceptable pressure within a normal run).
- If the pressure difference is not close re-prime the pump with the lower pressure.

- NOTE: The pump with the lower pressure is usually the one that needs more priming.

☐ Priming the system wash

- In the Primus RESOLUTION Software, select MANUAL and then SOLVENT SYSTEM. The SOLVENT CONTROL dialog box will be displayed. For priming the wash lines, select a flow of 0.1 mL/min, a 50% pump B ratio, and select the radial button for Wash Solution as the fluid choice.
- Click on SET. An audible click can be heard as the solenoids switch from Mobile Phase to Wash reagent.
- Confirm the System Wash supply line is completely submerged in the liquid of the System Wash bottle. This is the Wash bottle with only one clear line in it.
- Confirm the red/blue valve located on the supply line coming out of wash bottle is turned to the open position (the arm of the valve will be inline with the valve).
- Place the 10cc syringe into the priming valve of pump A. The priming valve is located inside the front door of the pump compartment.
- Turn the arm of the priming valve down toward the pump head and pull back on the syringe plunger, filling the syringe with liquid. Repeat until the all of the air has been removed from the solvent line between the System Wash bottle and the priming valve.
- Place the arm of the priming T pointing toward the nipple of the priming T (the point where the syringe is inserted).
- Place the 10cc syringe into the priming valve of pump B. The priming valve is located inside the front door of the pump compartment.
- Turn the arm of the priming valve down toward the pump head and pull back on the syringe plunger, filling the syringe with liquid. Repeat until

the all of the air has been removed from the solvent line between the System Wash bottle and the priming valve.

- Place the arm of the priming T pointing toward the nipple of the priming T (the point where the syringe is inserted).
- Select the Mobile Phase Radial button on the Solvent Control window and press SET.

Priming the piston wash

- In the Primus RESOLUTION Software, select MANUAL and then SOLVENT SYSTEM. The SOLVENT CONTROL dialog box will be displayed. Set the flow to 1.5 mL/min, 50% B and select the Mobile Phase radial button is selected. Press SET.
- Place the priming syringe in the priming T located to the right of the pump on one of the lines coming from the Piston Wash bottle. Turn the arm of the priming T down and pull back on the plunger of the syringe. Repeat until the all of the air has been removed from the solvent line between the Piston Wash bottle and the priming valve.
- Place the priming syringe in the same priming T but turn the arm of the priming T up. Pull back on the plunger of the syringe. Repeat until the all of the air has been removed from the solvent line between the Piston Wash bottle and the other side of the priming valve.

Priming the dilutor

- Confirm the inlet line from the tan valve is completely submerged in the 2Dil reagent.
- Confirm the syringe is completely screwed into the tan valve.
- Confirm the inlet and transfer tubing are tightly attached to the tan valve.
- From the Primus RESOLUTION Software go to Manual, Injector and click on Prime Dilutor

- A Dialog Box will appear, the Autosampler will perform a homing sequence and the septum-piercing probe will move over the rinse station. The syringe will then begin to cycle at 100% of its capacity.
- When all air bubbles have been removed from the inlet and transfer lines click on the OK button to stop the cycling of the syringe.

Priming the active rinse station

- Confirm the clear tubing from the ARS is completely submerged into the gallon bottle of clean DI water.
- Insert the priming syringe into the priming T located on the tubing coming from the bottle of DI water.
- Turn the arm of the priming valve down pointing away from the bottle of DI Water and pull back on the syringe plunger, filling the syringe with liquid. Repeat until the all of the air has been removed from the solvent line between the bottle of DI water and the priming valve.
- Turn the priming valve arm toward the priming nipple in order to prevent liquid from leaking out when the syringe is removed.
- Remove the syringe from the priming valve. Remove any air in the syringe by holding the tip of the syringe straight up and slowly pushing on the plunger until all air has been removed. It may be necessary to tap the side of the syringe barrel to help any bubbles migrate to the tip of the syringe.
- Insert the syringe back into the priming valve.
- Rotate the arm of the priming valve to point up, toward the bottle of DI Water.
- Push on the syringe plunger to prime the line between the priming T and the diluter station.
- From the Primus RESOLUTION Software go to Manual, Injector and click on Prime Rinse Station Pump.

- A Dialog Box will appear and the ARS pump will turn on. A small amount of water will appear from the top of the white insert in the rinse station.
- Click on OK in the dialog box to turn the pump off.

 Troubleshooting

- Refer to Ultra2 Operators manual, Chapter 9; or Call Trinity Biotech Technical Assistance 1-800-377-4752
- Have serial number of instrument (100446) available

Reference Ranges

Normal values:	Manufacturer [mean ± 2.5 SD]	UCDHS study [mean ± 2.5 SD]
Hemoglobin A2:	0.7-3.1 %	1.4-3.6%
Hemoglobin F:	<2.0 %	0.0-1.2%
Hemoglobin A1c:		<4.6%
No hemoglobin variant detected		

Reporting Results

 See Technical Procedure #1233 for further instruction.

 Report from HPLC all levels of hemoglobins including A0, A2, F, S, C, E, and other variants as detected.

- Note that for samples with both scan and high resolution results, report the high resolution results only.
- For samples on patients with known hemoglobinopathies that are post-RBC transfusion, the variant hemoglobin is typically below (<30%) the software threshold for reporting “consistent with” and therefore use relative retention time table to verify location of hemoglobin variant.

- Note samples with elevated hemoglobin A1c (>4.6%) using canned text comment HB31.

- For those samples that have no apparent hemoglobin F, report as <2.0%.

- For those Hgb types that are not obvious, chromatograms may be faxed to Trinity/Biotech (Primus) for a presumptive identification. Primus may request the sample be sent to them for further clarification. In these circumstances only a presumptive id is given using canned text HB45 and the statement is made that further clarification requires a new sample be sent to UCSF.

- Appropriate canned text comments will also require manual entry. See attached appendix for list of HPLC canned text remarks.

Critical Values

None for this method.

References

- Primus Resolution Ultra2 Operator's Manual. Feb 2005, revision 7

- Tan, Aw, Dunstan, Lee. Evaluation of high performance liquid chromatography for routine estimation of haemoglobins A2 and F. J Clin Pathol 1993;46;852-856

- Wild and Go, A new method for the diagnosis of haemoglobinopathies and thalassemias, reprinted form European Clinical Laboratory, Sept 1993.

- Ou, and Rognerud. Rapid analysis of hemoglobin variants by cation-exchange HPLC. Clinical Chemistry.39/5, 820-824 (1993)

Procedure History

Date	Written/Revised By	Revision	Approved Date	Approved By
08/06	B. Gosselin	New procedure		
10/10	L Freeman	Revised procedure for new reporting, confirmatories and location	11/02/10	D Dwyre MD
		Biannual Review	8/24/12	D Dwyre MD
6/13	L Freeman	Reflex testing added	7/16/13	D Dwyre MD
10/13	L Gandy	New cartridge validation added-Minor		
		Biannual Review	10/1/14	D Dwyre MD
3/16	L Gandy	Minor. Added repeat low A2		