CAPILLARYS
TRAINING MANUAL

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CAPILLARYS TRAINING MANUAL

COURSE OVERVIEW

INTRODUCTION

- CAPILLARYS is a multitask instrument for automated capillary electrophoresis and is used for the separation of proteins

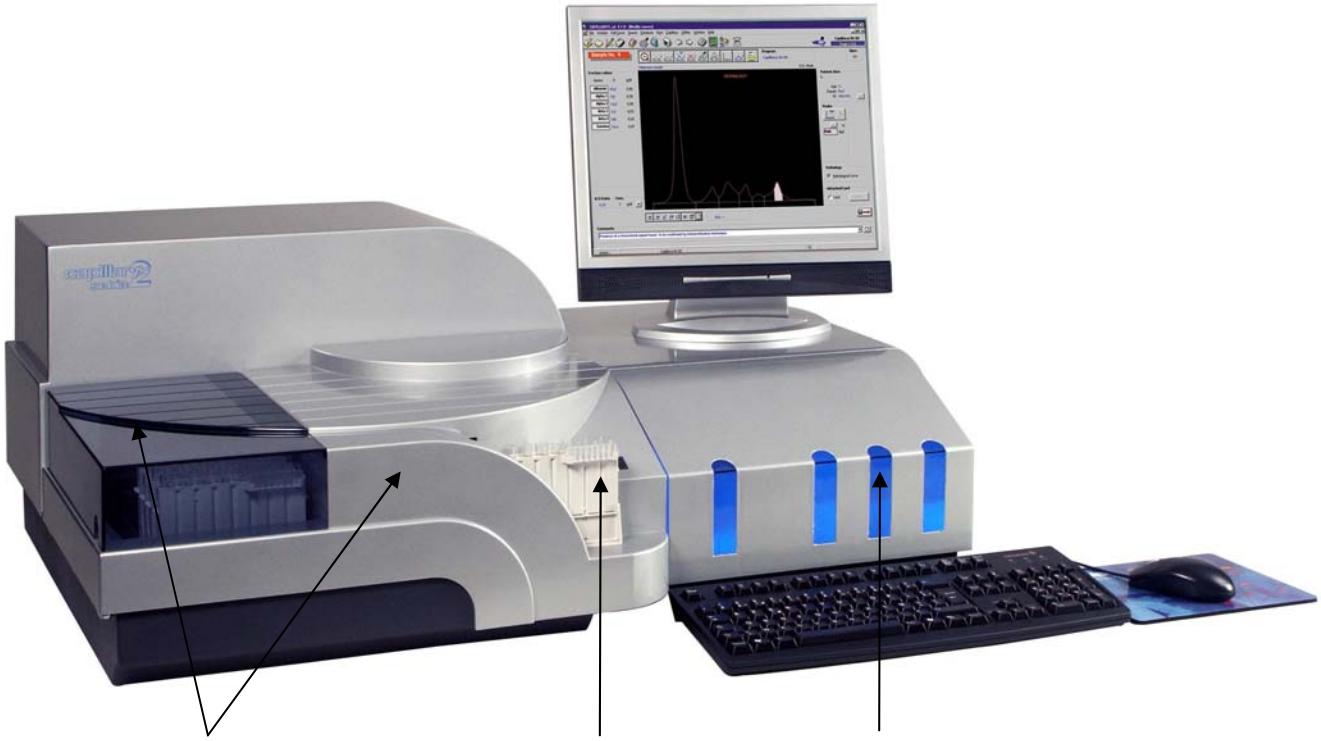
- CAPILLARYS is fitted with 8 capillaries, making it possible to carry out 8 simultaneous electrophoretic separations simultaneously

- The following processes take place during the protein separation
    - The primary tubes are placed on sample racks
    - The barcodes on each sample-rack and each tube are read with the built in barcode reader
    - The probe dilutes the sample with a dilution buffer: the dilution is made in single-use dilution segment
    - The migration is carried out at constant temperature and at constant voltage.
    - The detection of proteins is carried out directly on the capillary by a detection cell using absorbance photometry
    - Capillaries are washed with a high-pressure backflow of successive solutions coming from the reagent containers and managed by CAPILLARYS: rinsing solution, wash solution and buffer solution.

- Dialogue between the operator and the system is ensured by the PC
    - PC permanently displays the operation status of the instrument, the curves of the samples in progress and allows the operator to review and edit the results.

- The interpretation of results is carried out by using the same software as the one in charge of the analyzer: direct detection automatically gives a precise relative quantification of each fraction.

CAPILLARYS consists of three main parts: sampler, analysis unit, and reagent bay.



Analysis Unit

"Feeder belt full" indicator/Sampler

Reagent Bay

Sampler inserts sample racks placed on the feeder belt through the passage into the system and is made up of a feeder belt, a bar-code reader, a diluter, a motor-driven sample rack carrier and a discharge belt

- The feeder belt carries the samples into the instrument and can hold up to 13 sample racks
- The bar-code reader scans the bar code on the sample racks and primary sample tubes.
- The diluter dilutes each sample in the dilution segment cups.
- The motor-driven sample rack carrier moves the sample racks
- The discharge belt removes the sample racks from the unit after analysis.

The analysis unit is made up of the following:

- Eight separate heat-conducting cartridges each containing a silica glass capillary tube
- A heat-control unit for keeping the capillaries at a stable temperature using the Peltier effect
- A sensor system (a deuterium lamp, 8 optical fibres and a Coupled Charge Device array). Proteins are detected directly and simultaneously on each capillary by absorption photometry,

- A hydraulic system that circulates the reagents inside the capillaries
- A high-voltage power source connected to 2 platinum electrodes situated in each tank.

The reagents bay contains the following:

- Two reagent containers: one for the analysis buffer and one for the CAPILLARYS wash solution
- One container for the distilled or demineralized water used to flush the capillaries
- One waste container for recuperating used reagents
- A red and blue diode array showing the amount of reagents in the containers
- A pump, a pressure tank and a system of solenoid valves.

OPERATION PRINCIPLES

LEARNING OBJECTIVES

- To learn principles of operation
- To learn major factors affecting capillary electrophoresis

DEFINITIONS

- Electrophoresis is defined as the differential movement or migration of ions by attraction or repulsion in an electric field
- Capillary electrophoresis (CE) is the technique of performing electrophoresis in buffer-filled, narrow-bore capillaries.

OPERATION PRINCIPLES, Fig 1.

- A sample is diluted with buffer and it is injected by aspiration at the anodic (+) end of the capillary
    - The sample is injected into the capillary by temporarily replacing the buffer reservoir at the anode with a sample reservoir and applying either an electric potential or external pressure for a few seconds
    - The sample reservoir is removed and a buffer reservoir is replaced
- The ends of a capillary are placed in separate buffer reservoirs, each containing an electrode connected to a high-voltage power supply
- An electric potential is applied across the capillary and the separation is performed
- Optical (UV-visible) detection of separated analytes is achieved directly through the capillary wall near the opposite end, i.e., near the cathode (-)
    - For protein separation, the detection is made at 200 nm and fractions are detected in the following order: gamma globulins, beta-2 globulins, beta-1 globins, alpha-2globins, alpha-1 globulins, albumin, pre-albumin with each zone containing one or more proteins
- Capillaries are immediately washed with a Wash Solution and prepared for the next analysis with buffer.

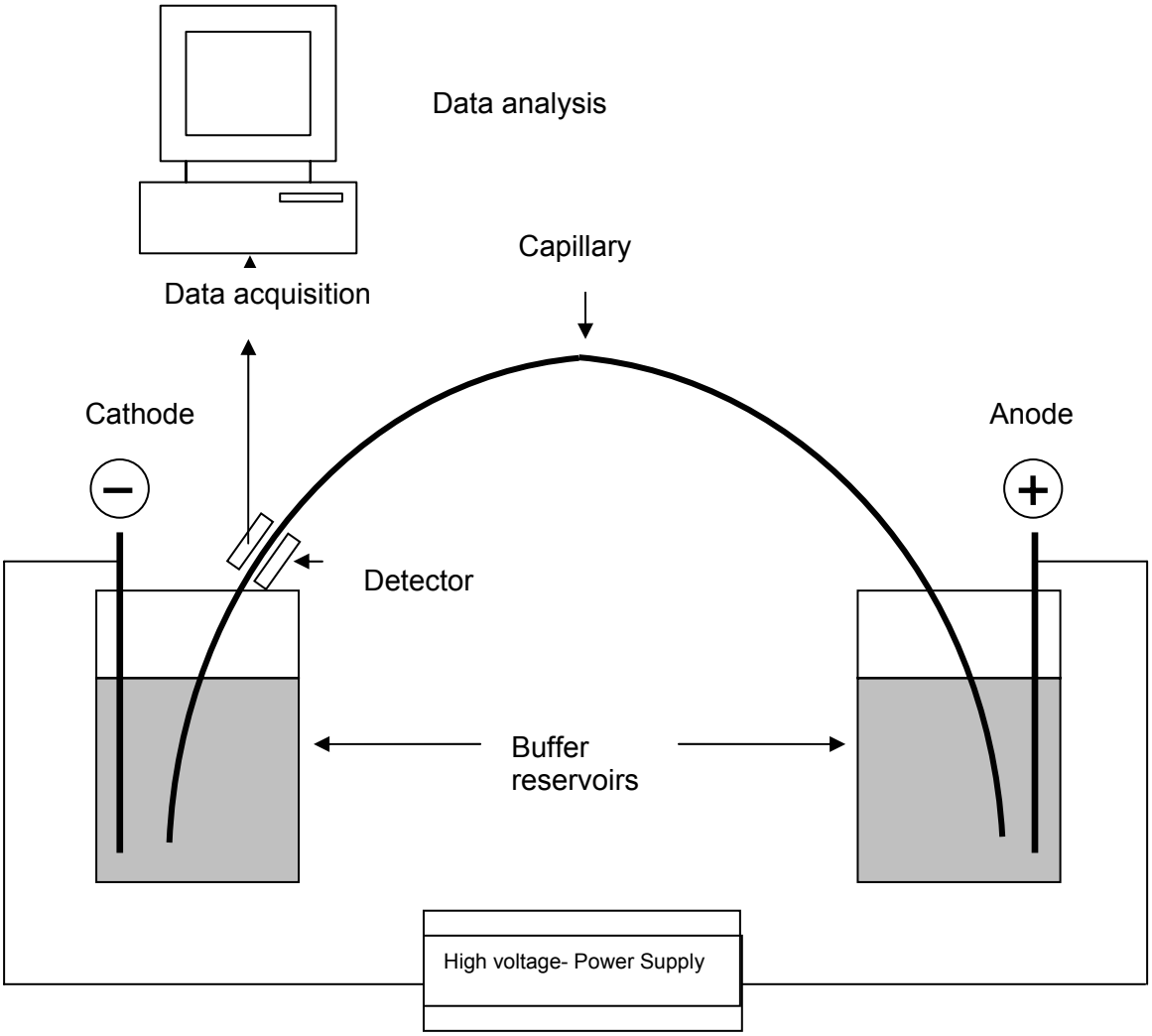


Figure 1. *The arrangement of the main components of a typical capillary electrophoresis instrument.* Positive (anode) and negative (cathode) electrodes are immersed in buffer reservoirs. When a voltage is applied across the electrodes, buffer ions of different charge, *i.e.*, anions (negative) and cations (positive), will move through the solution towards the oppositely charged electrode.

FACTORS AFFECTING CAPILLARY ELECTROPHORESIS

Electrophoretic mobility is a factor that indicates how fast a given ion or solute may move through a given medium (such as a buffer solution)

- Electrophoretic mobility is an expression of the balance of forces acting on each individual ion; the electrical force acts in favor of motion and the frictional force acts against the motion
    - Higher charge and smaller size confer greater mobility, whereas lower charge and larger size confer lower mobility.
- Since all forces are in a steady state during electrophoresis, electrophoretic mobility is a constant (for a given ion under a given set of conditions)
- Different ions and solutes have different electrophoretic mobilities, so they also have different migration velocities at the same electric field strength.
    - Because of differences in electrophoretic mobility, it is possible to separate mixtures of different ions and solutes by using electrophoresis.

Electroosmotic flow (EOF), **Fig 2**. , is the bulk flow of liquid through the capillary is important feature in capillary electrophoresis. It is caused as follows:

- An uncoated fused-silica capillary tube is typically used for CE
- The surface of the inside of the tube has silanol groups that are in contact with the buffer during CE
    - Silanol groups readily dissociate, giving the capillary wall a negative charge
    - When the capillary is filled with buffer, the negatively charged capillary wall attracts positively charged ions from the buffer solution, creating an electrical double layer
        - The first layer is rigid and consists of adsorbed ions
        - The second layer is diffused layer, in which ion diffusion may occur
- When a voltage is applied across the capillary, cations in the diffuse layer migrate towards the cathode, carrying the bulk solution with them. The result is a net flow in the direction of the cathode.

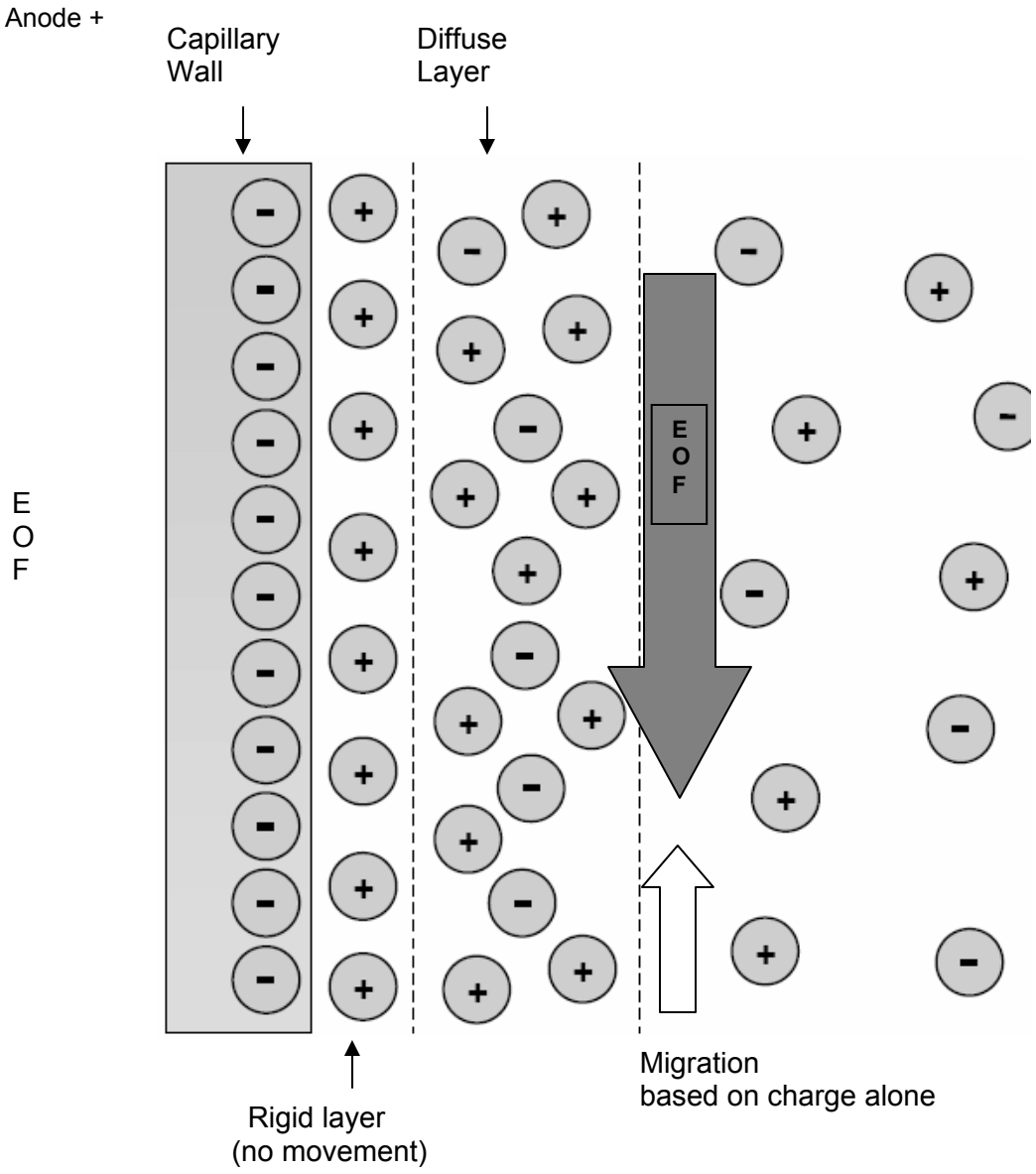


Figure 2. Formation of double layer inside of the capillary leading to electroosmotic flow towards the cathode. The grey filled block arrow symbolizes EOF, the smaller unfilled block arrow pointing towards the anode represents migration based on ion charge (i.e., negatively charged ions migrate towards anode). Since EOF is significantly stronger, the bulk of solution will be carried towards the cathode.

FACTORS AFFECTING EOF

One of the main variables affecting EOF mobility is the viscosity of the buffer.

- The use of buffer additives and/or other modifications of the buffer composition may influence the viscosity of the buffer
- Buffer viscosity will also depend on the temperature at which the CE separation is performed.

EOF mobility will also vary according to the buffer pH

- At high pH the EOF mobility will be significantly greater than at low pH
- Above pH 9, silanols are completely ionised and the EOF mobility is at its greatest
- Below pH 4, the ionisation of silanols is low and the EOF mobility is insignificant.
- At pH >7, the EOF mobility is sufficient to ensure the net migration of most ions towards the cathode, regardless of their charge. Therefore, the observed migration velocity of a solute may not be directly related to its electrophoretic mobility. Instead, it is related to a combination of both its electrophoretic mobility and the EOF mobility.

LESSON 1 **MAINTENANCE**

LEARNING OBJECTIVES

At the completion of this lesson, the operator will be able to….

- Initiate Start Up Cycle
- Perform Daily and Weekly Maintenance
- Understand Cycle Timing
- Launch Shutdown Cycle

LESSON 1 DAILY MAINTENANCE

START UP CYCLE

1. ____ Turn On Computer and Monitor.
2. ____ Turn On Capillarys Power Switch.
3. ____ Open Capillarys Software. (Enter Password)-**See Fig#1**
4. ____ Check for the Absence of Error Messages During Startup Cycle.
5. ____ Check Computer Time and Date
6. ____ Check Reagent Levels.-**See Fig #2**
7. ____ Empty Waste
8. ____ Replenish Deionized Water
9. ____ Adjust Reagent Levels. (Click ok)- **See Fig#3**
10. ____ Instrument will Perform Startup Initialization. (Wait for “ready” in the status window)-20min.-**See Fig #4**
11. _____ Record in Maintenance log

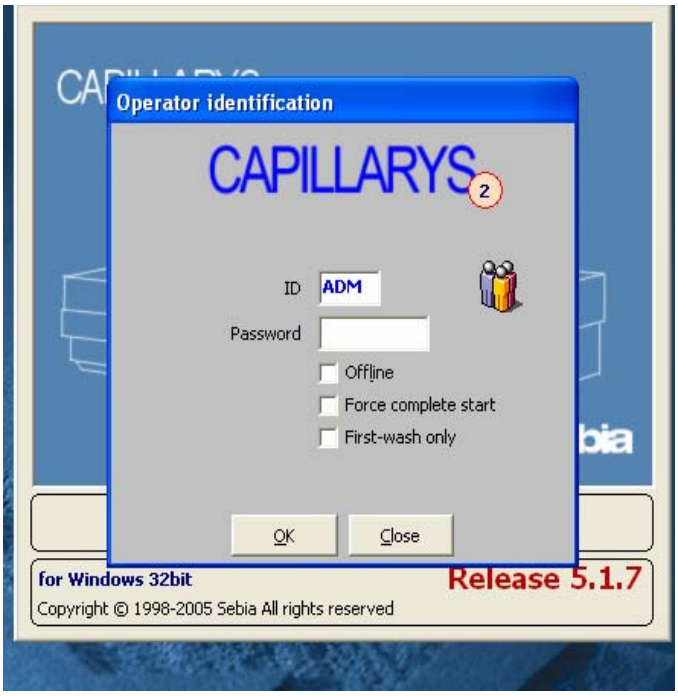

Operator identification
CAPILLARYS 2
ID
ADM
Password
Offline
Force complete start
First-wash only
OK
Close
for Windows 32bit
Release 5.1.7
Copyright © 1998-2005 Sebia All rights reserved

Fig 1

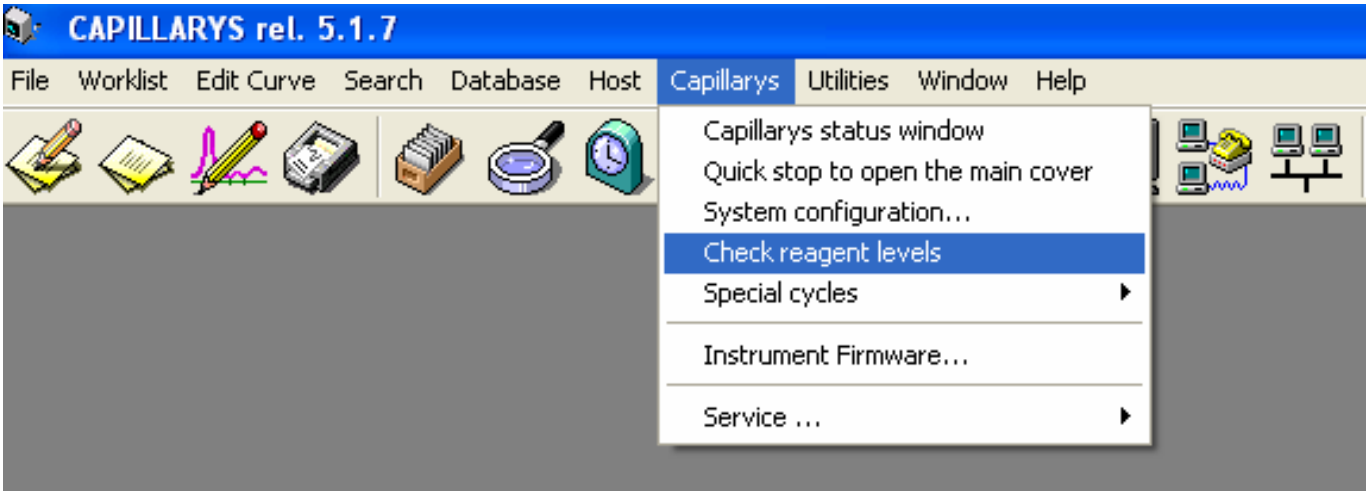

CAPILLARYS rel. 5.1.7
File
Worklist
Edit Curve
Search
Database
Host
Capillarys
Utilities
Window
Help
Capillarys status window
Quick stop to open the main cover
System configuration...
Check reagent levels
Special cycles
Instrument Firmware...
Service ...

Fig 2

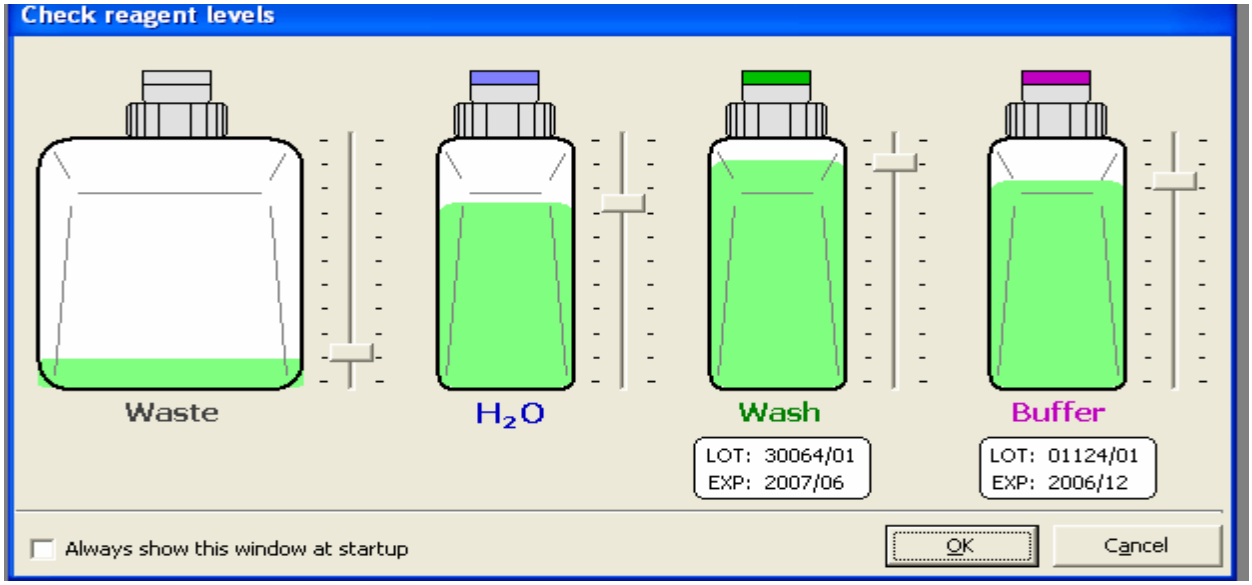

Check reagent levels
Waste
H2O
Wash
Buffer
LOT: 30064/01
EXP: 2007/06
LOT: 01124/01
EXP: 2006/12
Always show this window at startup
OK
Cancel

Fig 3


Capillarys
Ready
Ready.

Fig 4

SHUT DOWN CYCLE

1. ____ Launch shutdown cycle**. (Capillarys→ special cycles →shutdown) See Fig 6**

2. ____ Check for the Absence of Error Messages

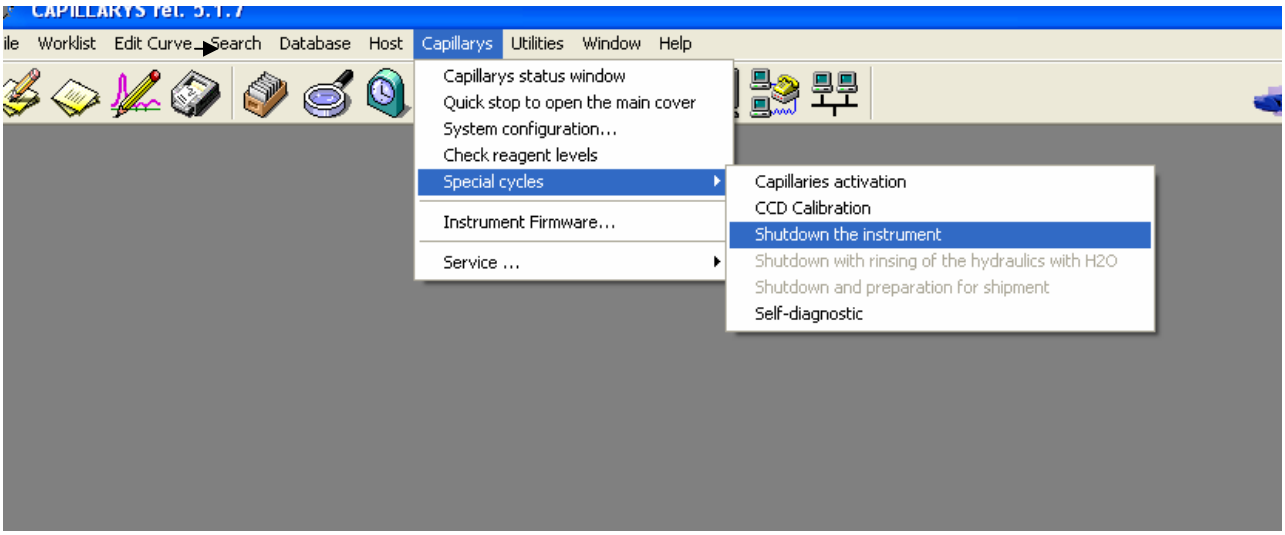


Fig 6

3. ____ Instrument will complete shutdown cycle -13min

4. ____ Wait for computer to display “Instrument Ready for Shutdown”

5. ____ Turn off CAPILLARYS.

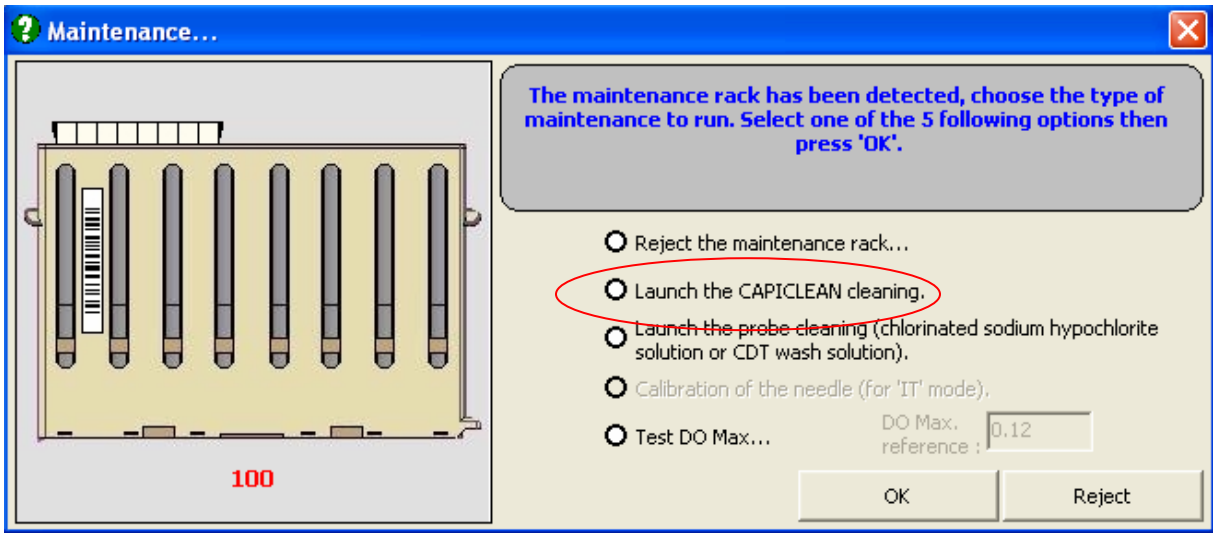
6. ____ Shutdown the Computer.

7. ____ Record in Maintenance log

Failure to properly shutdown the instrument will damage capillaries and could initiate error messages.

CAPICLEAN/PROBE WASH

1. ____ Mix 1ml of DI Water and 1ml of Capiclean in a Tube
2. ____ Dispense 200ul into each segment cup (8)
3. ____ Place the filled segment onto rack No. 100.
4. ____ Place the rest of the prepared reagent in position #1 on rack No.100.
5. ____ Slide sample rack No. 100 into the Capillarys system.
6. ____ The following screen will appear:



7. ____ Launching the Capiclean cleaning will perform the probe wash and capillary wash.
8. ____ Select “OK”
9. ____ Procedure will indicate “Ready” after completion of probe washing and capillary cleaning cycle.
10. _____ Record in Maintenance log

LESSON 1 **CYCLE TIMING**

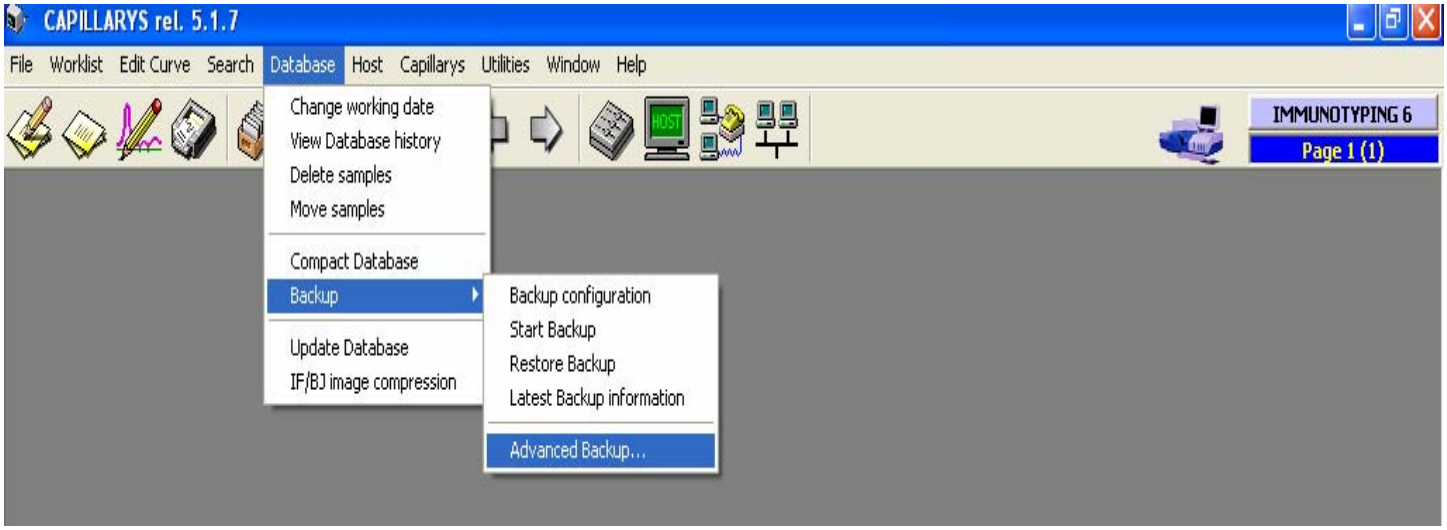
CAPILLARYS CYCLES	TIME
Normal Startup	20 min
Capillary Activation	25 min
Capiclean Cycle (capillaries + sample probe)	30 min
Sample Probe Cleaning (hypochlorite or CDT wash solution)	6 min
Normal Shutdown	13 min
Restart after a minor error (8xxx error)	8 min
Restart after a major error (0xxx error)	14 min

CYCLE TIMES WHEN CHANGING BUFFERS

CYCLE	TIMING
PROTEIN 6 ↔ CDT	45 min
PROTEIN 6 ↔ HR	45 min
PROTEIN 6 ↔ IT	1 min
PROTEIN 6 ↔ HGB	45 min

BACKUP THE SYSTEM SETTINGS/ CLEAN RACK SENSORS

1. ____ Backup the following files: "**Daily.mdb", "Phoresis.ini", "Capillarys.ini", "Modified Parameters.ini", "Host.ini" and the "Reports"** folder. (See Advance Backup QRG)



2. ____ Simply wipe off the bottom of the sample rack with an alcohol swab

3. ____ Record in Maintenance log

EXAMPLE OF CAPILLARYS MAINTENANCE LOG SHEET

Reagents Changed	1	2	3	4	5	6	7	8	9
Wash									
Buffer									
DiH20									
Empty Waste									
Wash Filter									
Buffer Filter									
DiH20 Filter									
Daily									
Check Reagent Level									
Check Waste Level									
Power instrument down									
Weekly									
EnzyClean									
Probe clean									
Monthly									
Backup Data									
Wipe Exterior of Instr.									
Service PM (6 months)									
Service PM (12 months)									
Sebia Authorized Service									
Operators Initials:									
Service:									

Reagents Changed	Lot Numbers				Date Changed				Tech

LESSON 1 CHANGING REAGENTS

LEARNING OBJECTIVES

At the completion of the lesson, the operator will be able to:

- Change reagents during startup and running
- Change filters
- Frequency of reagent changing

LESSON 1 CHANGING REAGENTS

PREPARING TO CHANGE REAGENTS

- Fresh reagents should be prepared whenever the instrument has not been in use for a one week or when a new kit is opened.
- The distilled or deionized water should be totally replaced daily, to discourage possible bacterial contamination " topping off" is discouraged.
- Check the levels of the containers before each use of the instrument.
- Filters will need to be replaced when a new kit is opened. (2 filters for each bottle of buffer in the kit, 1 filter for DI water)

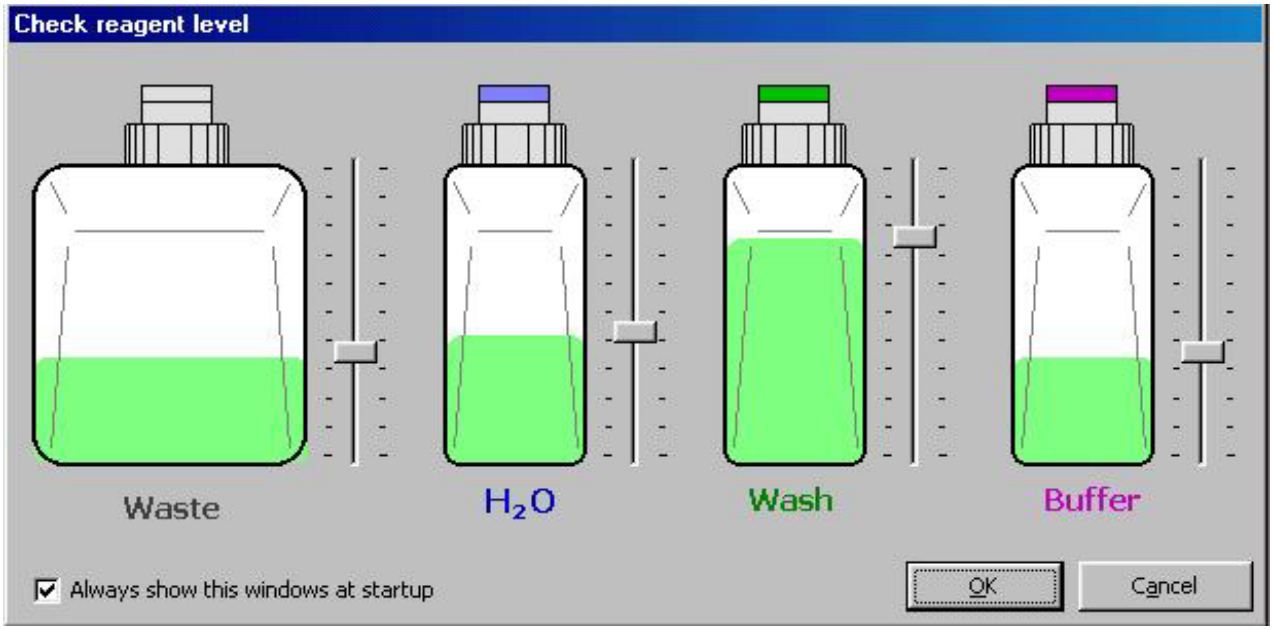
LESSON 1 CHANGING REAGENTS

INSTALLING FILTERS

- Remove the filters from their wrapping.
- The lid of the containers will have the tubing underneath the caps.
- Discard the old filter if a new kit has been opened.
- Twist the filter onto the tubing. The filter can only go one way and must be twisted on.
- The filter will now be secure on the tubing.

CHANGING REAGENTS PRIOR TO START-UP

- With two fingers squeeze the metal clips together and release the empty bottle(s) from their housing.
- Discard any remaining fluids from the container(s).
- Prepare new reagents, if necessary.
- Screw the lid back on the container but do not tighten it.
- Align the container to the nozzles and gently push. Both of the metal clips **must** lock on either side of the lid.
- Tighten the lid of the container.
- Record date/time of reagent change on bottle (buffer and wash solution)
- Reset the scroll bar on the screen, see Figure



LESSON 1 CHANGING REAGENTS

CHANGING REAGENTS DURING TESTING

1. Maximize the CAPILLARYS Status window
2. Select Replace containers. (this will only be highlighted when the system is in mode that will allow reagent replacement)
3. Wait until the screen changes to
4. Change or replenish reagent.
5. Select (√) the reagents that were changed or replenished
6. Click on OK button.
7. The unit will automatically update reagents quantity, then proceed to ready.

LESSON 2 PROCESSING CONTROLS

LEARNING OBJECTIVES

At the completion of this lesson, the operator will be able to…

- Process control samples using the designated Control Rack… **Rack 0**
- Process control samples using the designated Sebia Maintenance Rack…**Sebia Rack.**

LESSON 2 PROCESSING CONTROLS

PROTEIN CONTROLS

Sebia Normal Control……...Part Number 4785
Sebia Hypergamma Control…Part Number 4787

Both controls are packaged 5 vials per box.

Each Lab must monitor QC results in accordance with their facilities guidelines. Control results should be compared to the ± 2 SD confidence range listed on the package insert provided with each lot of controls or compared to the Laboratories established ranges to confirm acceptability of results.

1. ____ Prepare Controls:
   See Package Insert

2. ____ Pipette 400µL* of one level of control into a primary sample tube.
   (40µL x 8 capillaries = 320uL minimum volume)

3. ____ Place the primary sample tube in position 1 and the dilution segment on sample rack No. 0

4. ____ Insert rack No. 0 into the inlet conveyor located on the front center of the Capillarys. The rack should be position so that the rack barcode is facing away from the operator and towards the front of the Capillarys.

5. ____ Repeat process with second level of control.

6. ____ After control processing is completed, review the 8 results generated for each level of control. Review the results in the Capillarys software under the Edit Curve Function.

7. ____ In the Capillarys software under the Edit Curve Function, edit each result. Select the QC button located beneath the curve. When prompted, select the appropriate lot and level of QC.

LESSON 2 OPTIONAL METHOD FOR MULTIPLE INSTRUMENTS

OPTIONAL METHOD FOR PROCESSING CONTROLS ON MULTIPLE INSTRUMENTS OR FOR REPRODUCIBILITY STUDIES USING THE SEBIA RACK.

PROTEIN CONTROLS

1. ____ Run controls on one instrument using Rack No. 0
   See Processing Controls

2. ____ Remove dilution segment from Rack No. 0.

3. ____ Place segment containing diluted control material from Rack No. 0 on Sebia Maintenance Rack…Sebia Rack. No primary tube is required.

4. ____ Insert Sebia Rack into the inlet conveyor located on the front center of the 2nd Capillarys. The rack should be position so that the rack barcode is facing away from the operator and towards the front of the Capillarys.

5. ____ After control processing is completed, review control results

It is suggested that One instrument is used to run the primary sample of the Normal control and the other instrument the Abnormal control. This will allow each system to verify the instrument dilution performance. Diluted reagent segments are only stable for 24 hours.

LESSON 2 PROCESSING CONTROLS

Using Rack 0

QC is performed in parallel on each of the 8 capillaries within the CAPILLARYS system. The first result/curve will appear in 10 minutes. Thereafter, every 40 seconds an additional result/curve will appear. In the Protein 6 Assay, the throughput is 90 samples per hour.

The process begins when Rack 0 is loaded onto the Capillarys. The barcode is read on the sample rack. The Capillarys system recognizes Rack 0 as a QC rack. The QC sample in the primary sample tube is diluted 1:5 in buffer. 200µL of the diluted sample is placed in each well of the dilution segment. The dilution needle is rinsed after each dilution of the sample.

The capillaries are washed with wash solution and buffer to prepare them for analysis. Diluted samples are injected into the capillaries. Migration is carried out under constant 7000V at 35.5°C, controlled by Peltier effects for 4 minutes. Proteins are detected directly by scanning at 200nm and an electrophoretic profile appears on the screen of the system.

Once Rack 0 is offloaded into the outlet conveyor, the rack can be removed, and used to setup the 2nd level of control. The Capillary system can hold up to 13 racks at one time. A new rack can be placed on the system as soon as the rack before it is accepted into the inlet conveyor and the LCD lights turn green.

At the end of the analysis the curves/results are ready to be evaluated within the Capillarys software. Because Rack 0 was recognized as a QC rack, each sample has been labeled as QC on the Protein 6 worksheet. Samples can be evaluated in the Edit Curve Function. The samples can also be label according to the specific level of control material (low, medium, or high) and the specific lot number of QC material. Once the samples are labeled as QC in the Edit Screen, they can be evaluated within the QC section of the software. Within the QC section, the samples can be plotted on the Levey-Jennings's Chart or used to calculate various statistical data.

LESSON 2 PROCESSING CONTROLS

USING THE SEBIA RACK

When using the Sebia Rack, QC is analyzed in the same manner as when using Rack 0. The exception to the process is there is no primary tube from which the initial dilution of the QC material is made. Pre-diluted QC material in the dilution segment obtained from the original run of QC material is analyzed.

The Sebia Rack is not recognized by the Capillarys system as a QC rack. Therefore, the curves/results obtained when using the Sebia Rack will not be labeled as QC samples on the Protein 6 worksheet until they are edited in the Edit Curve Function.

Running controls in this manner allows the user to decrease the amount of QC material used. The QC material is also analyzed faster because no initial dilution of the QC material has to be made.

LESSON 2 PROCESSING CONTROLS

SEBIA'S RECOMMENDATIONS

CAP requires that 2 levels of controls be run. It is up to each Laboratory to determine which levels of QC will be run and how often they will be run. Some Labs may choose to use a Pooled Patient Sample with established ranges as part of their QC program.

The following are possible options for QC analysis:

- Sebia Normal Control and Sebia Hypergamma Control
- Sebia Normal Control and Pooled Patient Sample
- Sebia Hypergamma Control and Pooled Patient Sample

LESSON 3 LOADING RACKS

LEARNING OBJECTIVES

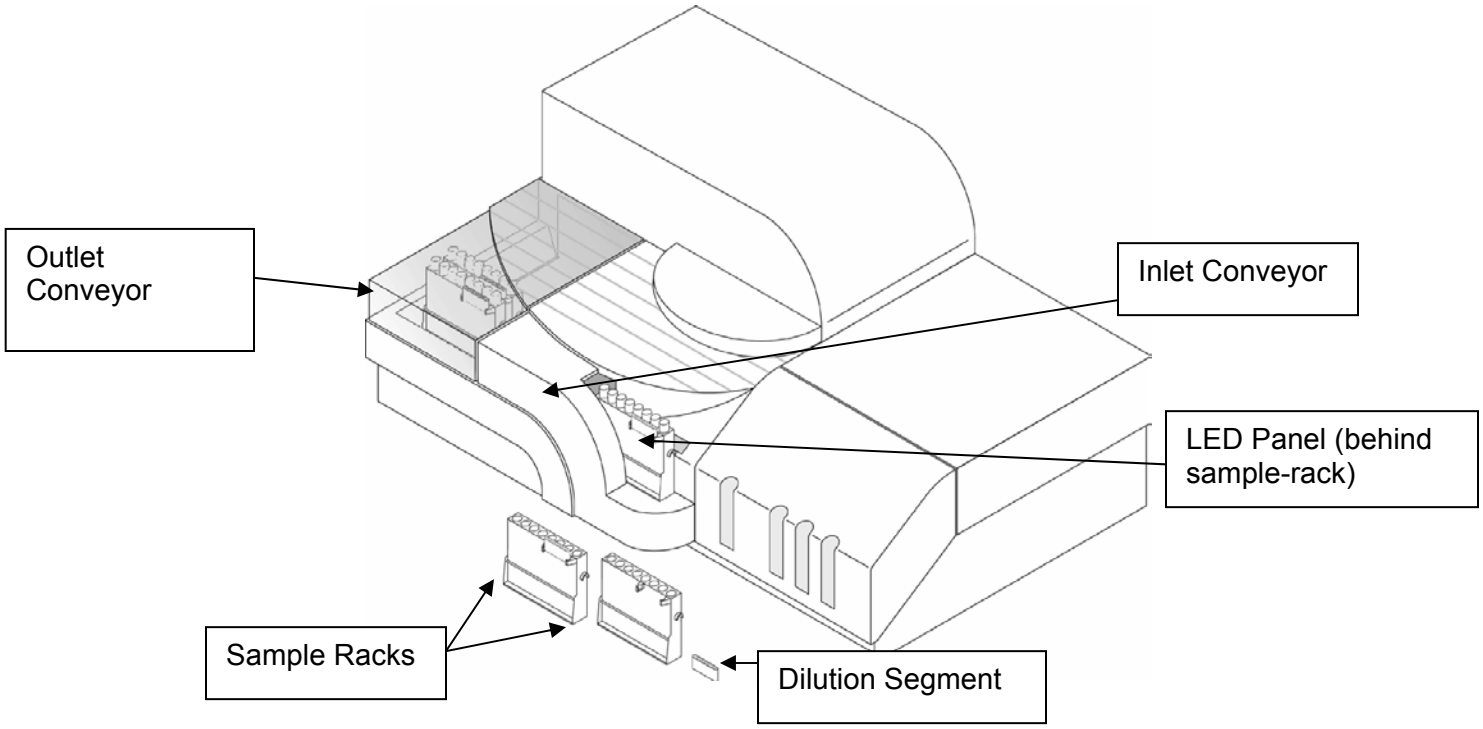
At the completion of this lesson, the operator will be able to…

- Load sample racks onto the CAPILLARYS system
- Minimum sample volumes
- Maximum rack volume

LESSON 3 **LOADING RACKS**

The Capillarys system samples directly from a primary sample tube or sample cup.
The following are approved tube sizes:

- 13X75
- 13X100
- 16X75
- 16X85
- 16X100
- 12X75
- Serum separator tubes can be used



1. ____ Place 8 primary tubes on the sample-rack. The barcodes of each tube must be positioned in front of the reading windows on the rack.
Protein= 40uL used testing, minimum recommended volume is 100uL
IT= 20 or 40uL (dilution dependant) per well, min recommended volume is 400uL

⚠ ***Warning:*** ***Do not leave empty spaces in the racks. If you are not analyzing 8 specimens, place tubes containing DI Water in the spaces that do not contain patient samples***

2. ____ Place a new dilution segment on top of the sample-rack. If no segment is placed on the rack, the system will reject the rack.

3. ____ Place the rack on the analyzer by positioning it at the opening of the inlet conveyor. The rack's barcode should be facing the Capillarys and the dilution segment should be facing the operator.

⚠ ***Warning:*** ***The LED (flashing lights at the entrance of the inlet conveyor) will flashing green when the system is ready to accept a rack. Do not introduce a rack into the system when the LED is flashing red.***

4. ____ Slide the rack into the inlet conveyor opening until you feel resistance. The system will emit an audible beep when it acknowledges a rack is in position to begin analysis.

5. ____ Up to 13 sample-racks can be loaded onto the Capillarys at one time. New sample-racks can be continuously introduced as the original 13 racks are offloaded onto the outlet conveyor.

6. ____ Remove the sample-racks when analysis is completed.

LESSON 4 INTERPRETING PROTEIN RESULTS

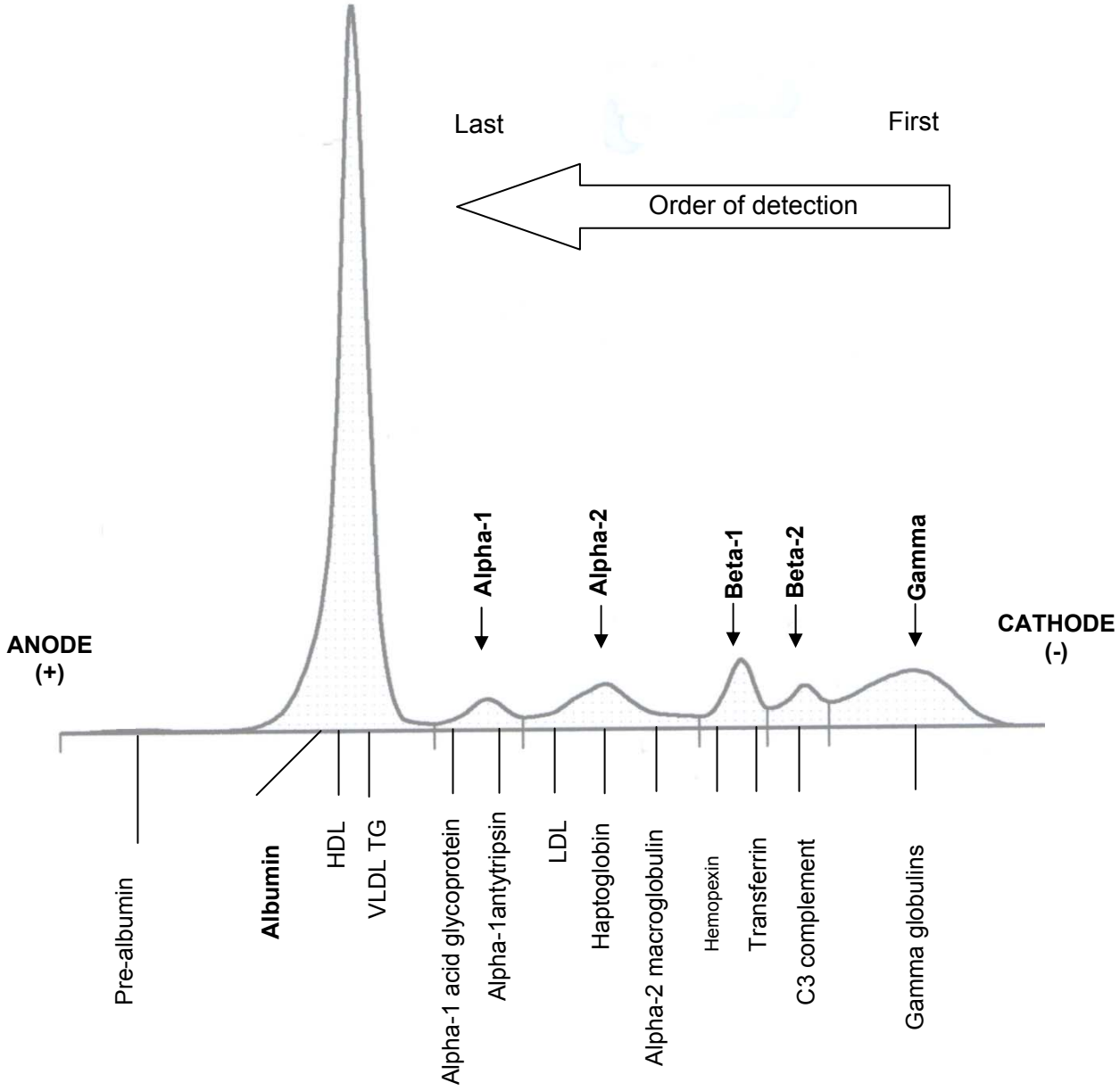
LEARNING OBJECTIVES

At the end of this lesson you should be able to:

- distinguish protein fractions and identify main components within the each fraction
- know how to treat samples presenting migration problems due to the presence of major monoclonal component
- identify samples requiring further analysis using immunofixation methods
- identify most common pattern abnormalities.

SHORT FACTS ON RESULTS OBTAINED USING PROTEIN(E) 6 BUFFER:

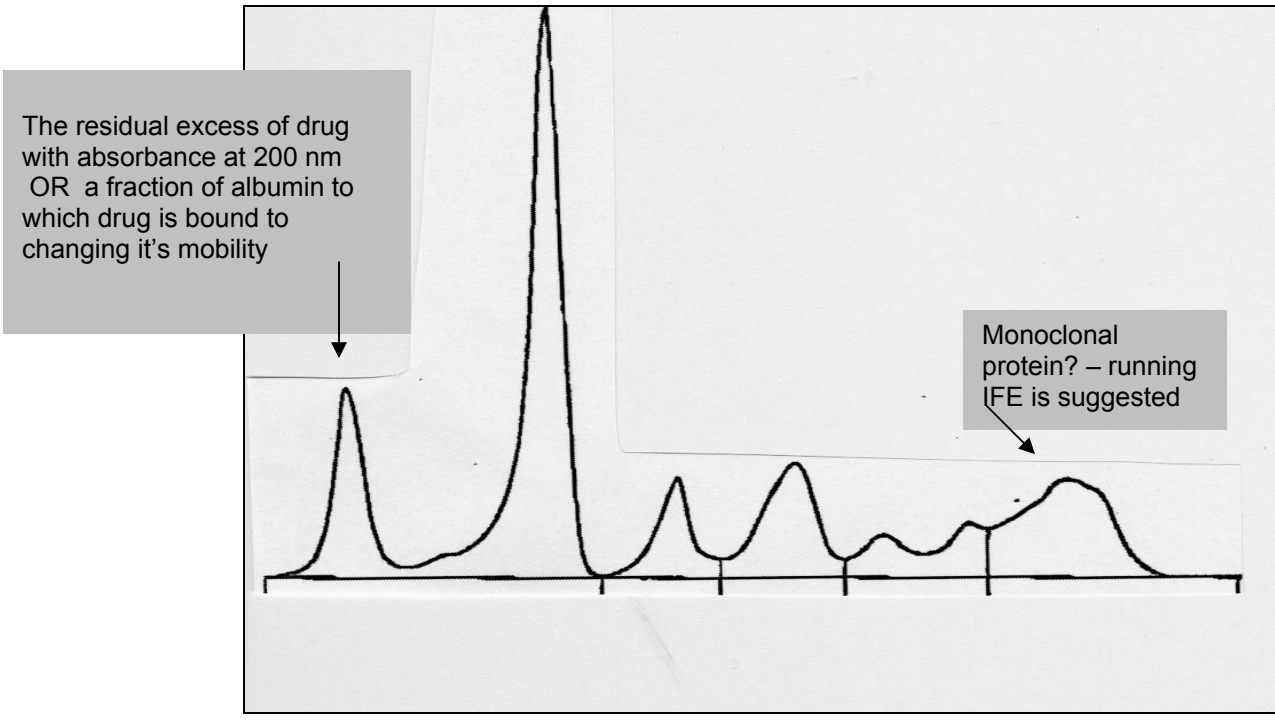
- Typical pattern has six fractions with split ß-zone
- Transferrin migrates in ß1, complement C3 in ß-2 zone
- Albumin is detected at 200nm
    - It is a direct measurement of albumin fraction versus measurement of stain affinity to this fraction by gel methods
- Alpha-1 values obtained on CAPILLARYS are closer to results from chemical analyzers than those obtained using regular gel electrophoresis
    - Alpha-1 acid glycoprotein does not have good affinity for stain regardless vendor or stain type
    - As a result, on the gel the alpha-1 fraction is underestimated because this fraction is not readily detected; not a problem with CAPILLARYS

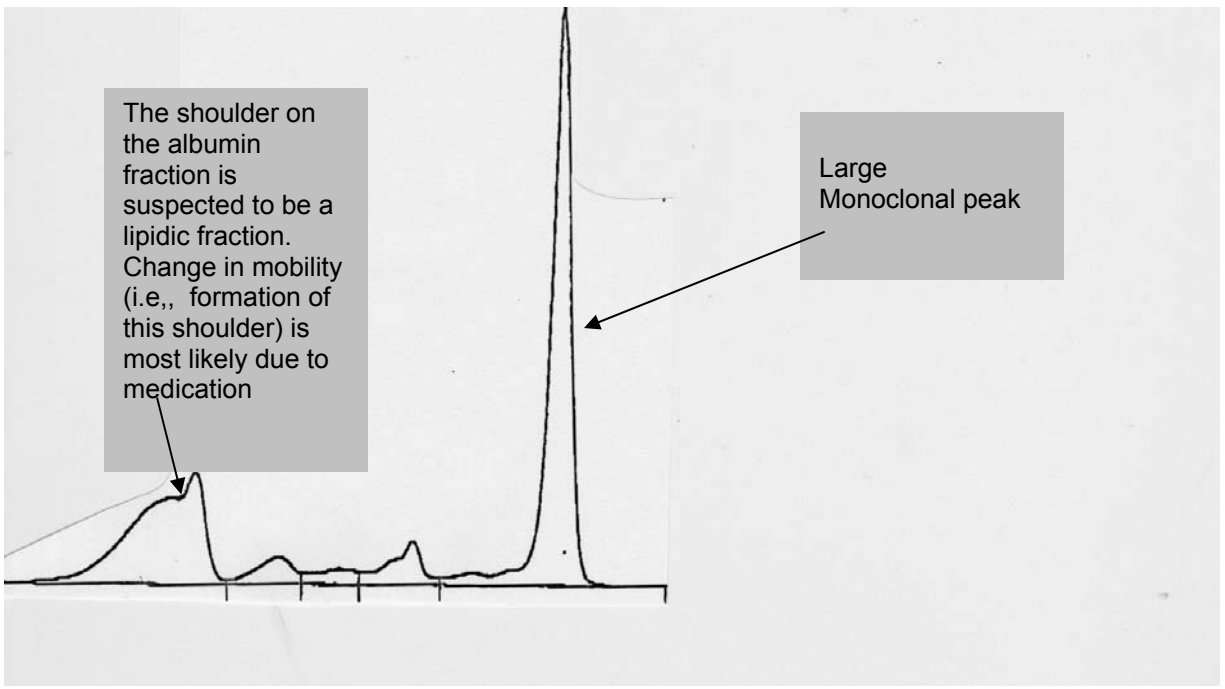


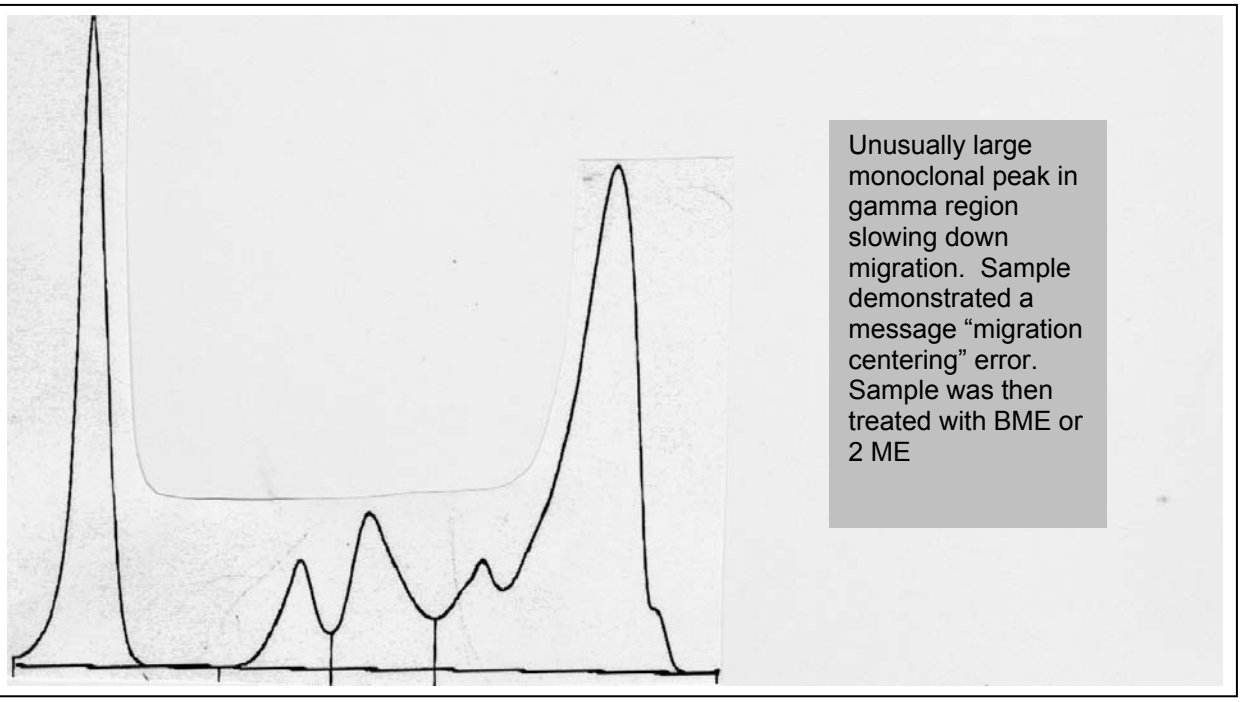
Normal Protein Pattern with CAPILLARYS PROTEIN 6 BUFFER

INDICATORS OF AN ABNORMAL PROTEIN PROFILE

- Increased/decreased fractions or Total Protein
- ß-2 fraction is smaller than ß1 in a normal serum
- If ß-2 is of equal or larger value than ß-1, the presence of monoclonal protein is suspected
- IgA usually migrates in ß-2 zone (or in ß-2—gamma bridging zone)
- Gamma zone should be rounded symmetry, without any "bumps", sharp restriction or peaks
- If any peaks are present in gamma zone, presence of monoclonal proteins is suspected
- CAPILLARYS has higher resolution than agarose gels, as a result various medication-induced abnormalities, especially in albumin fraction, can be detected which can demonstrate the appearance of what is termed " BisAlbuminemia".
    - These medication induced abnormalities are temporary and go away once medication is discontinued




The shoulder on the albumin fraction is suspected to be a lipidic fraction. Change in mobility (i.e,, formation of this shoulder) is most likely due to medication
Large Monoclonal peak


Unusually large monoclonal peak in gamma region slowing down migration. Sample demonstrated a message “migration centering” error. Sample was then treated with BME or 2 ME

Some samples may demonstrate a message “Capillary Migration Centering” due to the indicators below:

1. polymerization of immunoglobulins is suspected while running IT 6 test on CAPILLARYS and/or confirmation of a single monoclonal abnormalily is necessary .
    a. Polymerized immunoglobulins are characterized by the disappearance of several fractions of the same type of heavy chain and of the same type of the light chain.

2. migration of sample is slowed down due to the presence of a large monoclonal peak while running Protein assays on CAPILLARYS (X coordinate for Albumin peak should be around 75. If it is significantly less, i.e., at 20 to 60, the migration centering will be out of range).
    a. Note: No treatment is necessary for polymerized immunoglobulins when running Protein 6.

To resolve either of the events listed above, please follow these steps:

1. Prepare a 1% solution of beta mercaptoethanol (BME/2ME) solution using the fluidil as the diluent for a final volume of 100µL.
    a. Note 1: Sebia does not sell BME/2ME. You can purchase it from Sigma Chemicals, BASF, VWR or other chemical vendors;
    b. Note 2: Fluidil is sold by Sebia USA (PN 4587).

2. Take 300µL of the sample and mix with the 100µL(prepared fluidil and BME/2ME) solution Mix and incubate at room temp for 15minutes, then run the sample on the CAPILLARYS

LESSON 4 INTERPRETING IMMUNOTYPING RESULTS

LEARNING OBJECTIVES

At the completion of this lesson , the operator will be able to..

- Identify a " normal" immunotyping pattern
- Understand the difference between polyclonal and monoclonal deletion
- Identify an "abnormal" Immunotyping pattern
- Understand the different dilution protocols
- Recognize a pattern that requires additional testing or confirmation

LESSON 4 INTERPRETING IMMUNOTYPING RESULTS

Principle of Immunotyping

The CAPILLARYS System uses the principle of capillary electrophoresis in free solution. With this technique, charged molecules are separated by their electrophoretic mobility in an alkaline buffer with a specific pH. Separation also occurs according to the electrolyte pH and electroosmotic flow.

In capillary electrophoresis, abnormal fractions in serum protein electrophoregrams, primarily those in the beta globulin and gamma globulin zones, are always suspect of being monoclonal proteins and therefore,, an indication of monoclonal gammopathies. With CAPILLARYS IMMUNOTYPING procedure, the Immunotyping is performed with specific antibodies to identify these abnormal fractions.

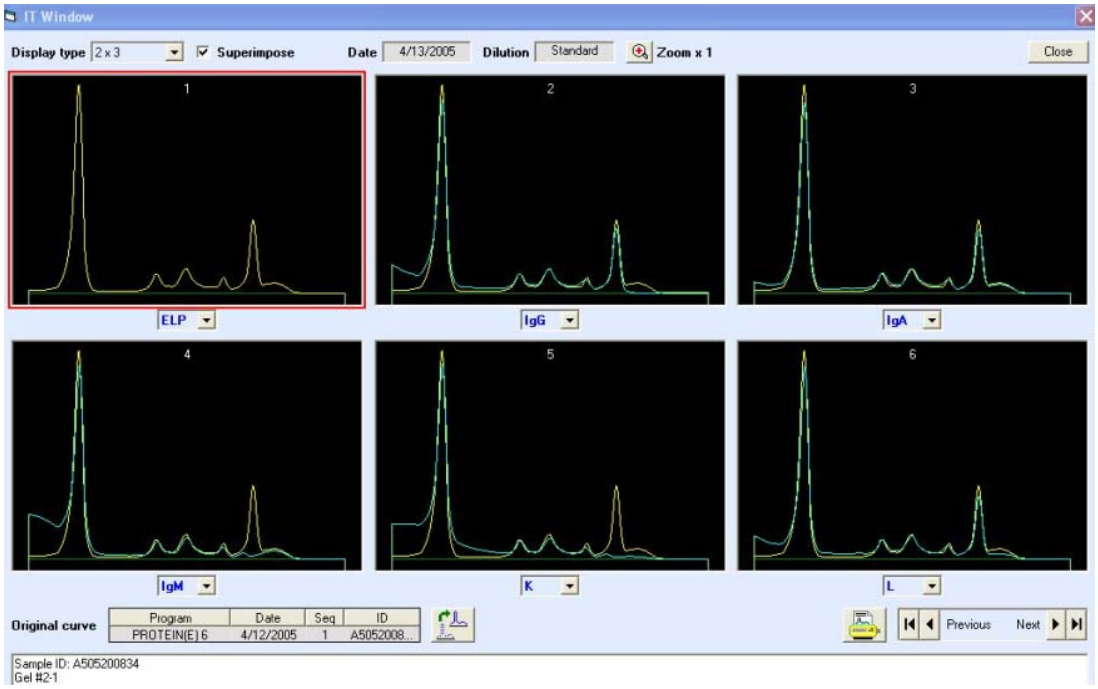
The CAPILLARYS System has 8 capillaries functioning in parallel. In this system, a sample dilution is prepared and injected simultaneously by aspiration at the anodic end of six capillaries (capillaries No. 7 and 8 are not used). The reference pattern is obtained by injection of the sample mixed with ELP solutions in capillary No. 1 providing a complete electrophoretic pattern of the sample's proteins. The antisera patterns are obtained by injection in capillaries No. 2-6 of the previously diluted samples mixed with specific antisera against IgG, IgA, IgM heavy chains, and against free and bound Kappa and Lambda light chains. The superimposition of the antisera patterns with the reference patter (ELP) permits visualization of the disappearance and /or the decrease of a monoclonal fraction on the antiserum pattern and to indicate a gammopathy.

LESSON 4 INTERPRETING IMMUNOTYPING RESULTS

Guidelines for Immunotyping Interpretation

- Within each of the antisera wells, if a monoclonal component and specific antisera react together, a large Immune complex is formed. Due to its size, the immune complex migrate more slowly out of the profile towards the Albumin/alpha 1 fractions. The area from which the complex is removed should be the primary focus for interpretation.

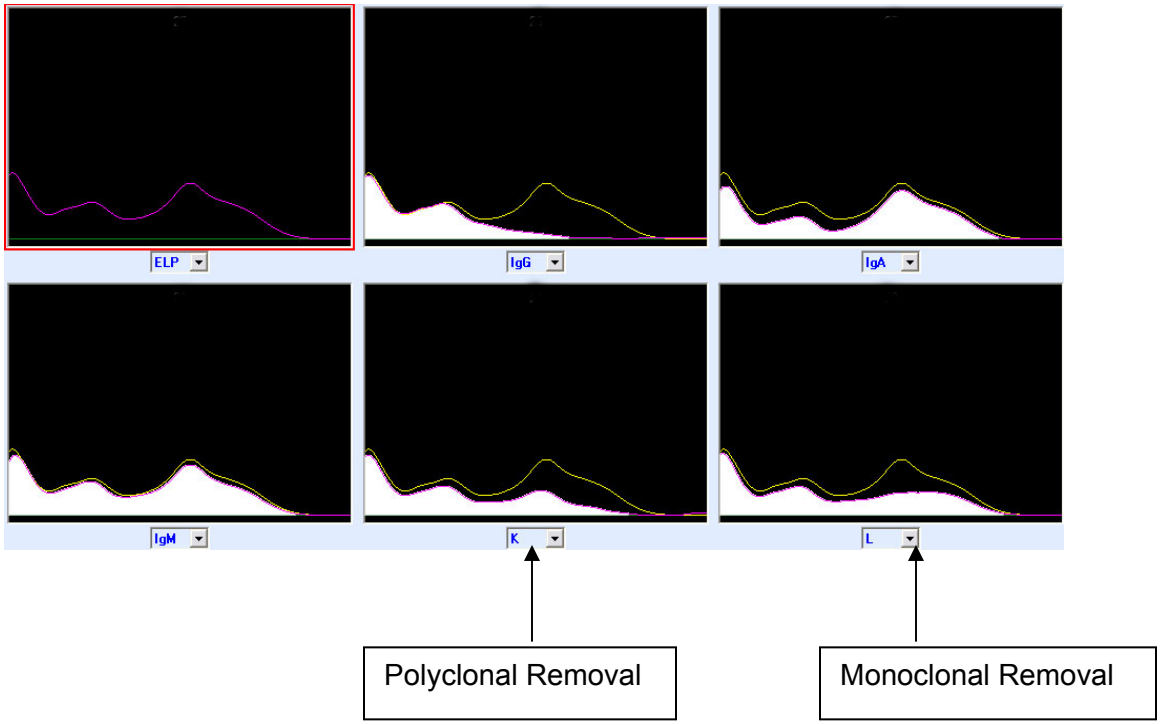
Immune Complex →



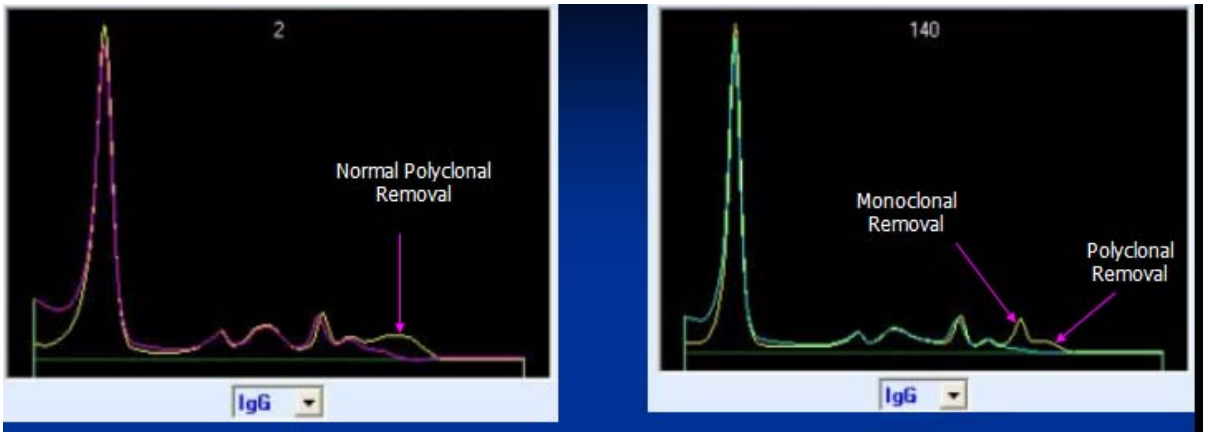
- Observe the overall appearance of the treated pattern. Examine each treated pattern comparing the reference overlay curve to the treated curve. Look for the absence or reduction of an abnormal peak.

- The beta 1 fraction, which contains transferrin, should be larger than the beta 2 fraction, which contains complement.

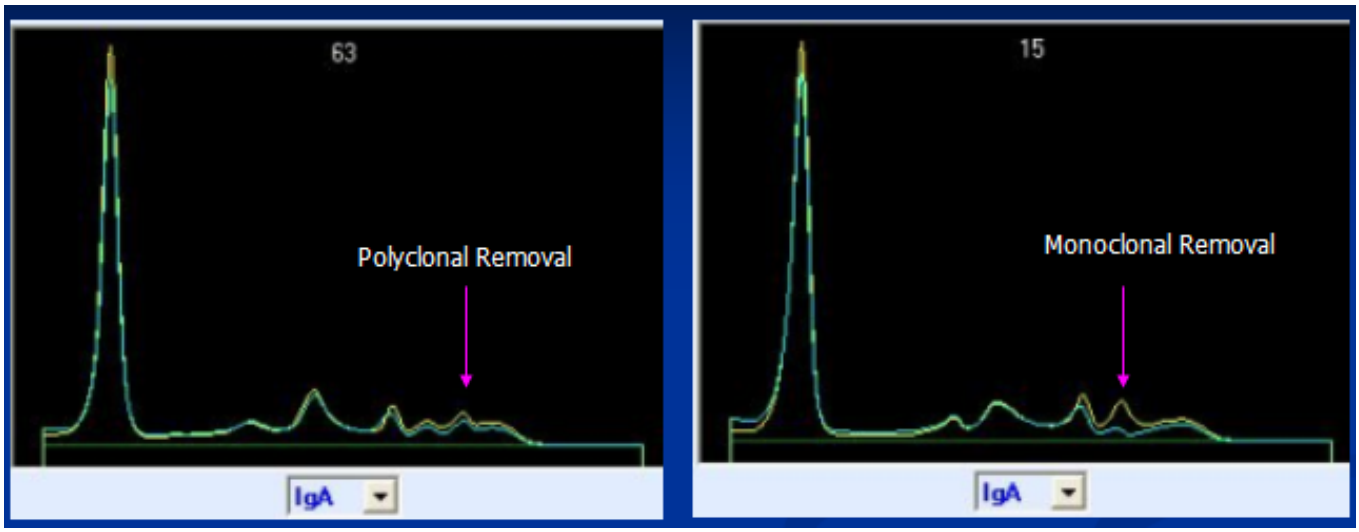
- Polyclonal removal is typically symmetrical. The shape of the reduced area mimics that of the overlaid reference pattern. Monoclonal removal is typically asymmetrical. The shape of the reduced area does not mirror that of the overlaid reference pattern.



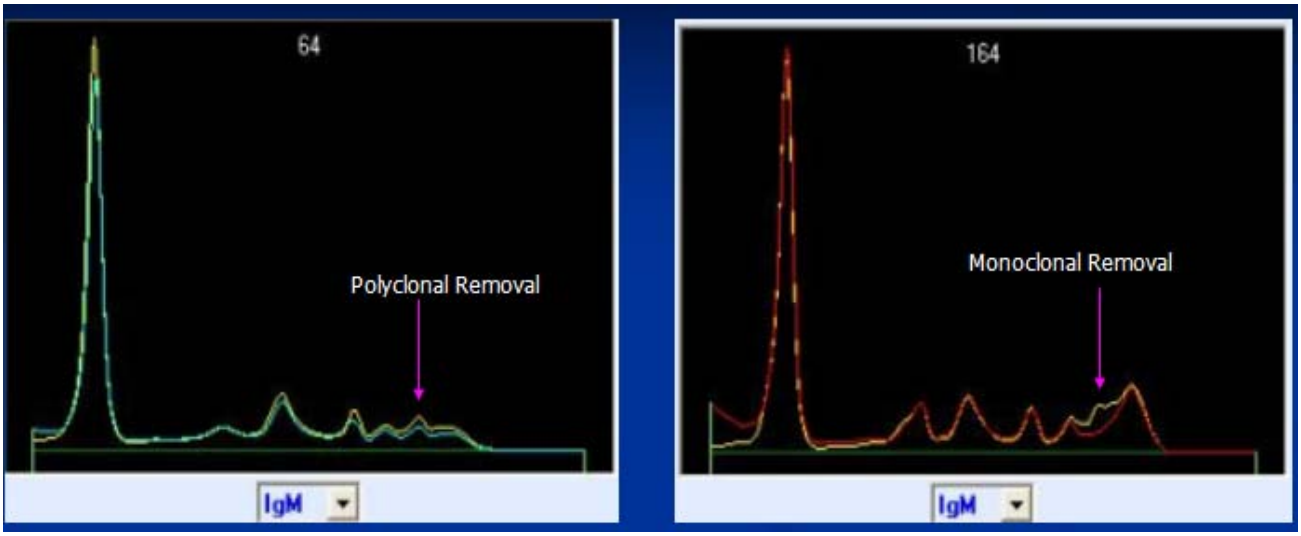
- IgG is normally the largest Ig present in the body. Some polyclonal removal of IgG is normal.



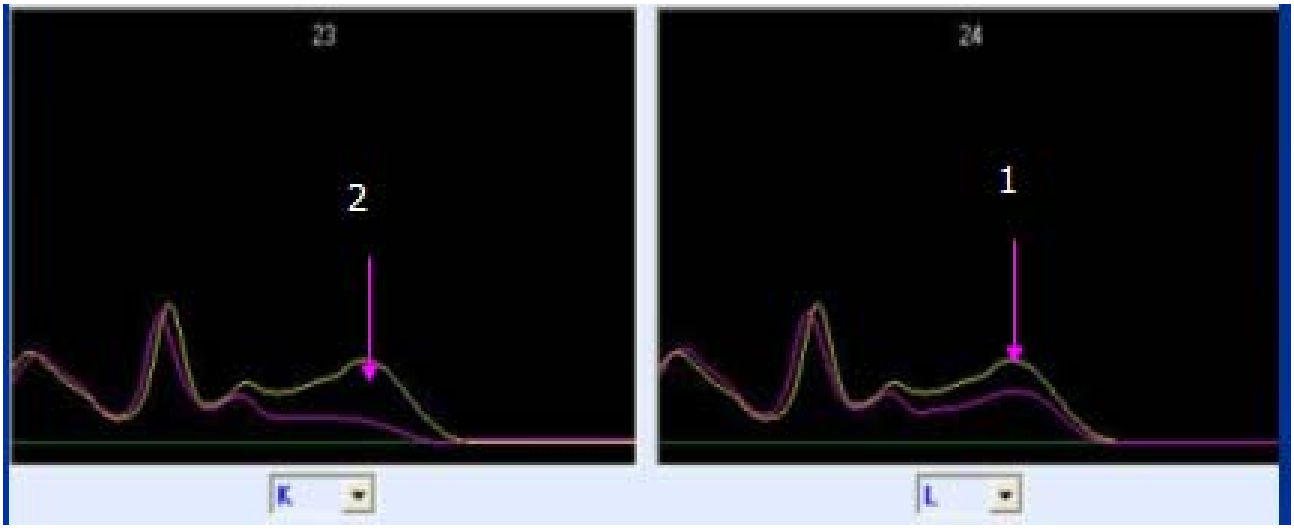
- **IgA**. Normally, IgA is in relatively small concentration compared to IgG. Look for slight reductions in the beta-early gamma area. The reference should mirror the IgA in normal samples.



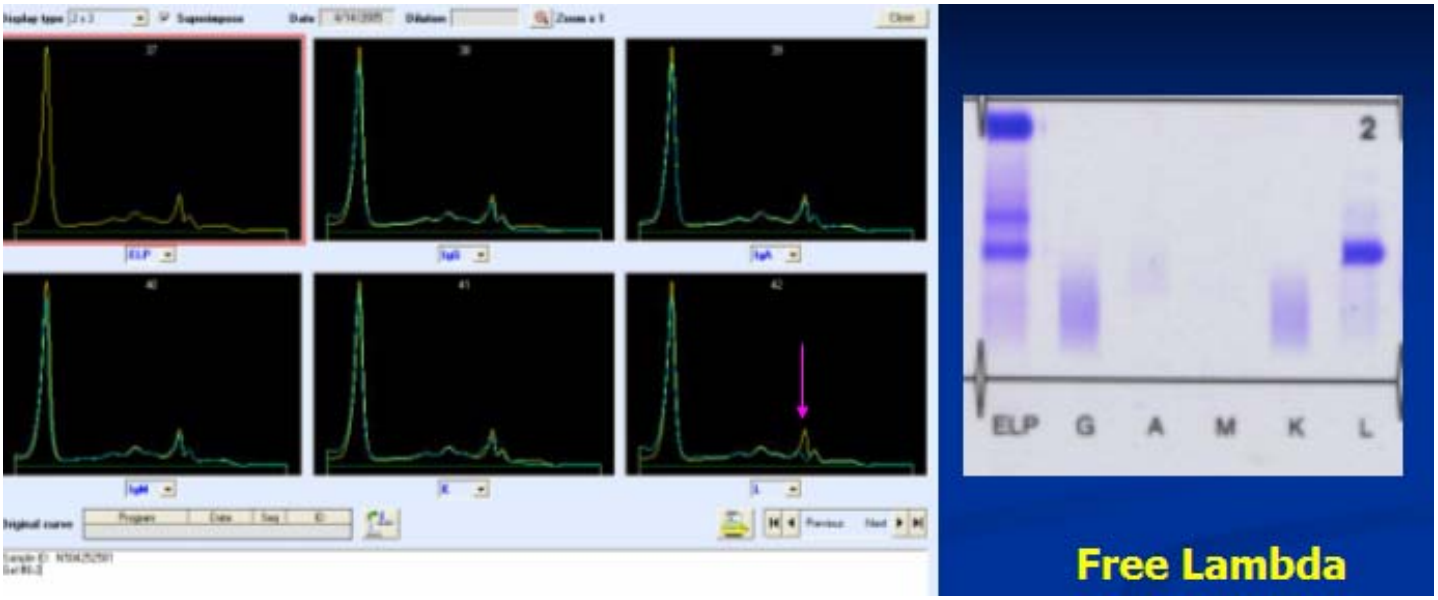
- **IgM**. Very similar to IgA except the concentration is normally even less. Normal samples will have very little reduction.



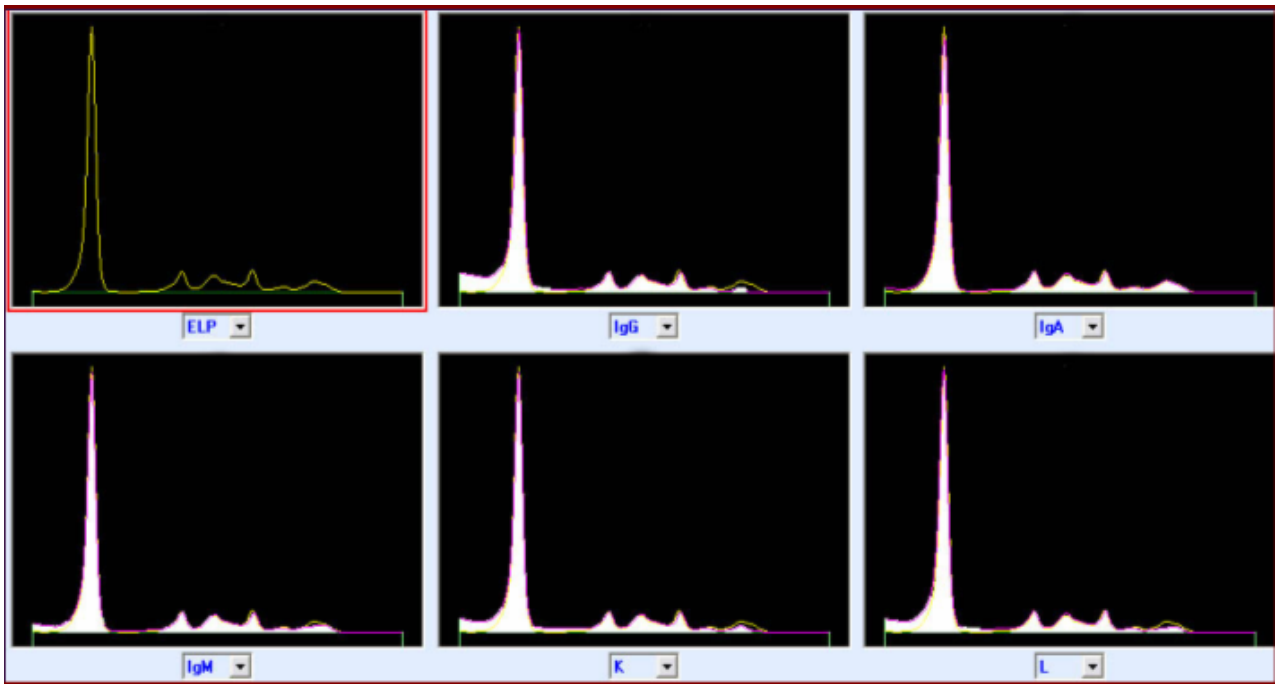
- **Kappa**. Present normally in a ratio of 2 Kappa to every 1 lambda. Should normally note a 2/3 reduction in the gamma fraction.
  Lambda. Due to 2:1 ratio of kappa to lambda, should see a 1/3 overall reduction In the gamma fraction with normal samples.



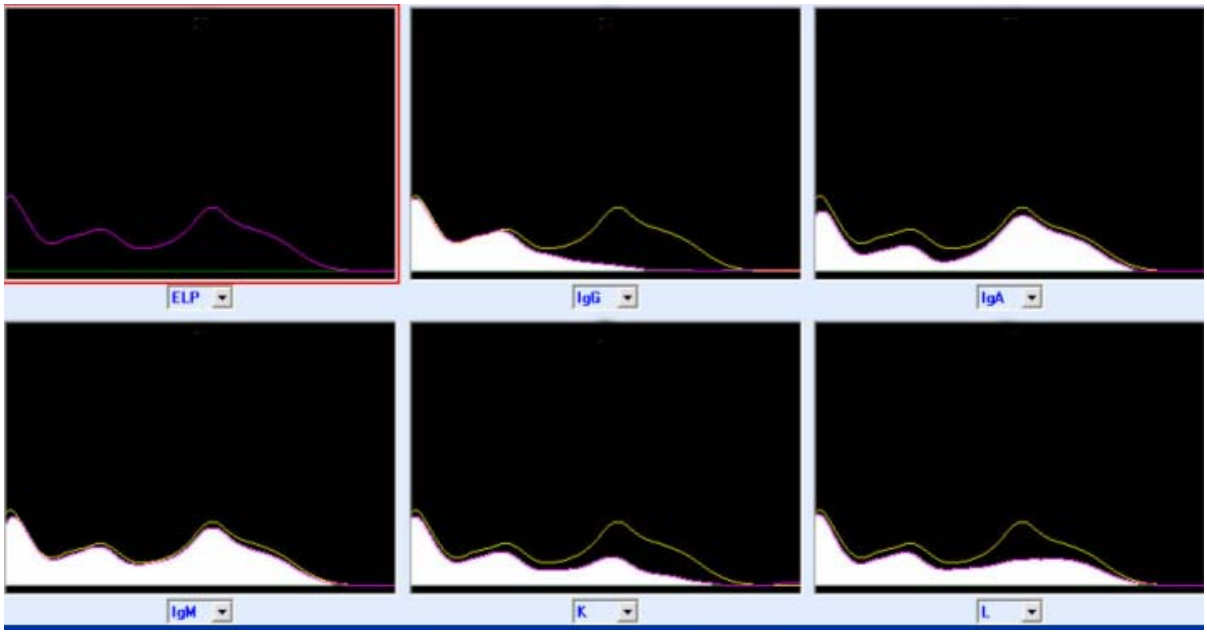
- Verify the presence of free light chains or heavy chain disease with follow-up testing on IF agarose gel utilizing free kappa, free lambda, D and/or E antisera.



- Use the Overlay Function to interpret IT Patterns



- Use the Zoom Function to enhance IT Patterns



- Choose the appropriate dilution in order to optimize the IT Assay

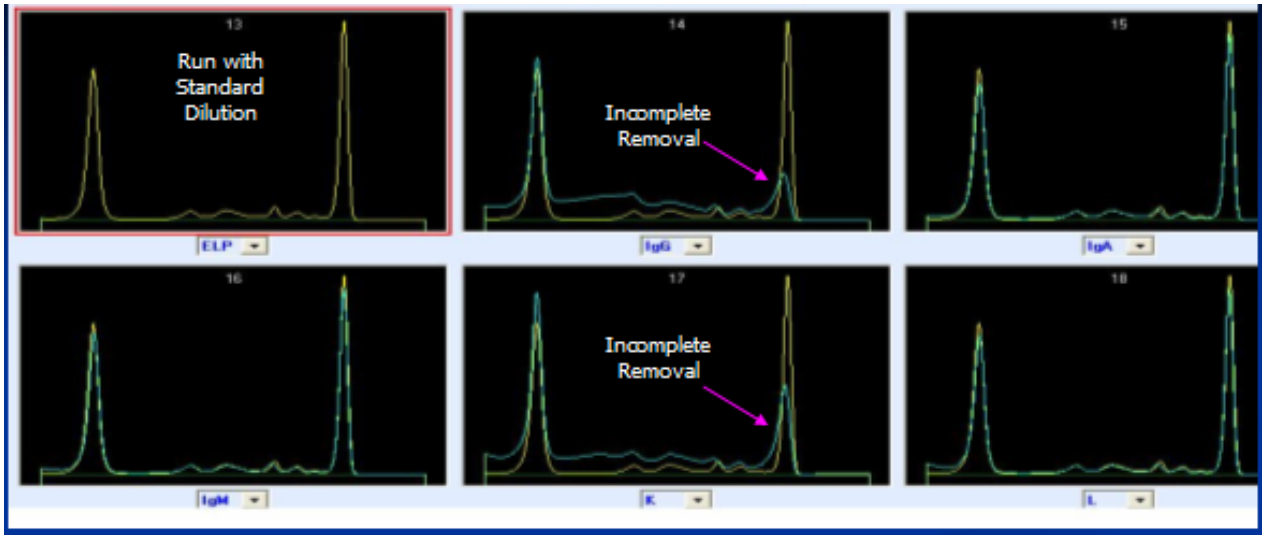
✷**Hypogamma**	**Ig <0.8 g/dL**
✷**Standard (Default)**	**Ig 0.8-2.0 g/dL**
✷**Hypergamma**	**Ig >2.0 g/dL**

✷Standard Dilution (default)
Initial 1:20 → 20 µL sample + 380 µL diluent,

✷ Hypergamma Dilution
Initial 1:20 → 20 µL sample + 380 µL diluent,
then 7 µL diluted sample + 20 µL antisera.
Total 1:40

✷ Hypogamma Dilution
Initial 1:10 → 40 µL sample + 360 µL diluent,
then 15 µL diluted sample + 20 µL antisera.

If the Ig concentration is unknown, evaluate the size of the monoclonal peak as it compares to the Albumin peak. If the monoclonal peak is roughly 2/3 the size of the Albumin peak, then a hypergamma dilution should be selected.



WARNING: Failure to select the correct dilution may lead to incomplete removal of the immune complex and make the interpretation of the pattern difficult.

Notes:

LESSON 5 TROUBLESHOOTING

REFER TO CAPILLARYS INSTRUCTION MANUAL FOR ADDITIONAL ERROR MESSAGES

LEARNING OBJECTIVES

- To identify the most common troubleshooting systematic and migration errors

1. RACK REJECTION
2. BARCODES ON TUBES
3. FLAT LINE CURVE
4. ALBUMIN FRACTION NOT DETECTED
5. ARTIFACT IN GAMMA FRACTION ON ALL CAPILLARIES
6. SPIKE AT THE END OF GAMMA , " TAIL EFFECT "
7. OLD SAMPLES – LIPOPROTEIN MOVEMENT AND COMPLIMENT DEGRADATION
8. DILUTION SEGMENTS SHOWS INCORRECT DILUTION VOLUME

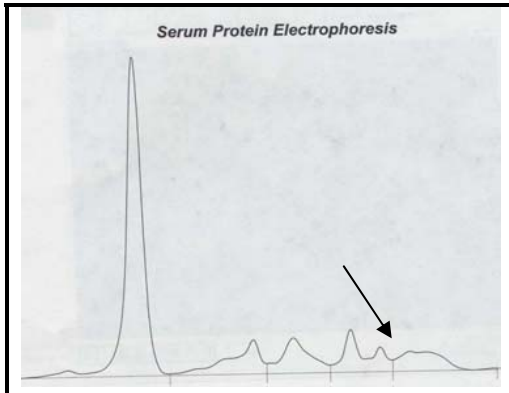
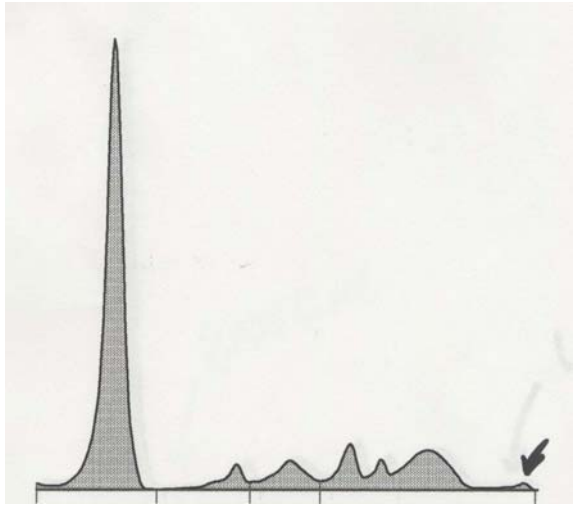
LESSON 5 TROUBLESHOOTING

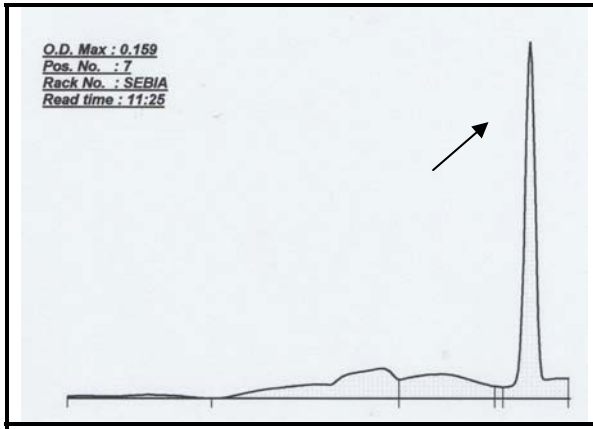
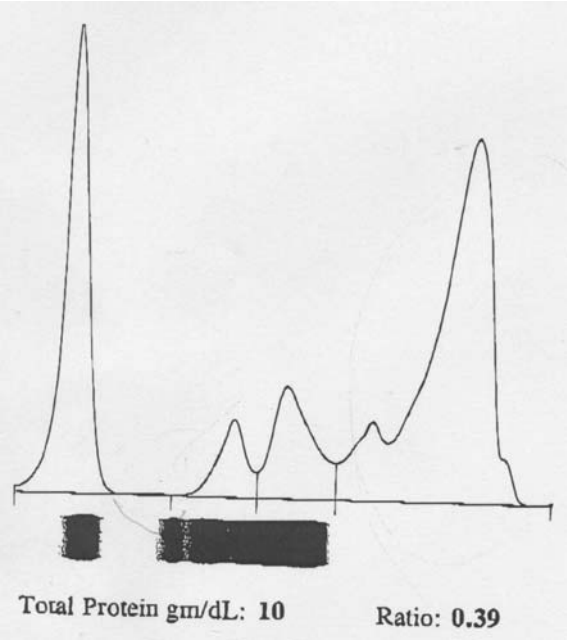
Notes :

Helpful Troubleshooting Hints for CAPILLARYS

TROUBLE	CAUSE	SOLUTION
Rack Rejection	1. Ensure that metal tab is secure on the bottom of the rack and clean	1. Metal tab required for activation of rack sensor. Prior to sample barcode reading the rack barcode is read by the system. If malfunction with rack , the sample barcoding will be skipped and rack rejected
	2. Barcode on rack not visible to barcode reader	2. Ensure rack barcode is not damaged or unreadible.
	3. Dilution segment not secured onto rack	3. Dilution segment must be present on rack for processing
	4. Running Protein but assay technique set for IMMUNOTYPING (IT)	4. Verify correct technique in upper right window of screen.
	5. Incorrect rack (0) used during IT technique	5. (0) rack is not used with IT assay.
	6. Two or more reagents at critical volume level	6. Replace critical reagents utilizing the Change Reagent program.
Barcodes on Tubes not read	1. Barcodes turned and not visible to reader	1.Place barcodes facing out of rack window
	2. Barcodes twisted and not vertically placed on the tube	2.Verify barcodes are vertical aligned
	3. Barcode reader not aligned	3. Seen when all racks are rejected; contact Sebia Service
Flat Line Curve	1.Verify accurate sample volume	1. Verify appropriate sample volume, assay specific
	2. Incorrect dilution of sample into segment.	2. Check dilution segment for adequate volume, Verify Buffer reagent bottle volume Select " Reagent Change", disconnect reagent caps and reattach. Secure reagent caps.
	3. Cap or coupling not secure	3. Verify cap and coupling secure on

	4. Capillarys not primed	bottle 4. Perform capillary activation to recharge the buffer in the capillaries. Capillarys/Special Cycles/Capillary Sanctification (30 minute) procedure
Albumin Fraction not detected Or Slow migration (Migration Centering error)	1. Incomplete washing of Capillarys To determine rate of sample, move the cursor to the Center of Albumin peak and view the electropherogram on screen. X=/ < 80 (slow) 2. Shut down the instrument and then do a complete start up of system	1. Verify reagent volumes 2. Perform a Capillary activation Capillarys/Special Cycles/Capillary Activation (30 minute) procedure 3. Shut down system and restart 4. When (1-3) steps performed and no resolution and only one capillary involved, suspect capillary and replacement of single capillary may be necessary.
Fast migration (Migration Centering error)	To determine rate of sample, move the cursor to the Center of Albumin peak and view the electropherogram on screen. X=/ < 80 (slow)	Remove the buffer and replace with water, prime system then replace water with buffer reagent.
Bumps or artifacts appearing in same location in gamma fraction on <u>all capillaries</u>	1. contaminated water source	1. Check DIH20 source Do not top of reagents Change water daily

		
"Tail" present at the end of gamma fraction 	1. Poor sample injection	1. Gamma fraction passes through the detector first . " Site of sample injection"

		
Migration centering error with large monoclonal band 	Baseline flat between albumin and alpha-1 fraction	Treat sample with 2me , See procedure Listed in " interpretation of Proteins"

Error code 606	No communication between PC and instrument- serial link was broken or interrupted .	Turn off instrument, turn off PC. Immediately turn instrument then the PC. (Firmware will loaded) approx 15min If system does not start prime mode after 15minutes, contact Sebia Service.
Motor 9 error	When running rack 100 for maintenance and wash tube not placed in location #1 in rack 100.	Verify correct procedure followed

Helpful Troubleshooting Hints for CAPILLARYS

MESSAGE	CAUSE	SOLUTION
INCONSITANT DILUTION IN SEGMENT	1. flat line curves 2. curve showing only injection peak 3. Visually improper dilution in segment	1. Verify coupling to reagents bottles 2.Change reagent filter 3.Verify tubing secure in bottle 4.Verify reagent volume in bottle

REVIEW QUESTIONS

1. What feature can determine the rate (fast or slow) of the capillary?
    a. O.D.
    b. X value on the electropherogram
    c. Not visible on the screen
    d. Overlay function

2. The CAPILLARYS reagent filters should be changed:
    a. Every week
    b. Every month
    c. With every new box of reagents
    d. With every new lot of reagents

3. Which fraction on the CAPILLARYS passes through the detector first?
    a. Albumin
    b. Pre-Albumin
    c. Gamma

4. . True or False Tap water is acceptable as a reagent on the CAPILLARYS

5.True or False The water reagent is ½ full as noted on the system startup, It is not necessary to change the water with the startup.

6. What are two major factors affecting capillary electrophoresis?
    a. Viscosity of the buffer
    b. Buffer additives
    c. Electroosmotic flow
    d. Electophoretic mobility

7. The sample flow in capillary is from
    a. Anode to cathode
    b. Cathode to anode

8. The greatest reagent mobility is achieved when buffer's
    a.pH is less than 4
    b pH 7.0
    c. pH > 9

9. Which fraction is emerging first at detector:
    a. Albumin
    b.Gamma
    c.Monoclonal protein
    d. Pre-albumin

10.Results for alpha-1 fraction obtained on CAPILLARYS have:
    a. Higher value than those from gel electrophoresis
    b. Lower value than those from gel electrophoresis.

11.IgA usually migrates in:
a.Gamma fraction
b.Beta-2 fraction
c. Beta-1 fraction
d. Alpha-2 fraction

12. The Capiclean maintenance should be preformed:
a. daily
b. bi-weekly
c. weekly
d. volume dependant

13. The rack used to run the Capiclean maintenance is rack #
a. Rack 0
b. Rack 5
c. Rack 100
d. Any Rack

14. If the protein curve patterns only show an injection peak, what should be suspected first
a. Reagent cap is not secure
b. That the sample has a problem
c. The voltage is not working correctly

15. When running the Immunotyping procedure what is the minimum volume for the assay.
a. 100uL
b. 200uL
c. 400 uL

KEY TO QUESTIONS

1.	B	X value on the electropherogram
2.	C	With every new kit of reagents
3.	B	Pre-Albumin
4.	F	False, Reagent grade/distilled/deionized water
5.	F	False, “Topping off” is discouraged
6.	C,D	Electroosmotic flow Electrophoretic mobility
7.	A	Anode to Cathode
8.	C	Ph > 9
9.	D	Pre-Albumin
10.	A	Higher alpha-1 fraction on CAPILLARYS
11.	C	Beta-1
12.	C	Weekly
13.	C	Rack 100
14.	A	Reagent cap is not secure
15.	C	400uL

SEBIA CAPILLARYS SYSTEM TRAINING CHECKLIST

Name (print): _____ Date: _____

Facility: _____

Address: _____ City & State: _____

I UNDERSTAND AND/OR HAVE PERFORMED THE FOLLOWING:

_____Basic principle of operation
_____System set up
_____Overview of components
_____Daily start up
_____QC set up
_____Sample processing
_____Result Interpretation
_____Performing system maintenance
_____Performing routine troubleshooting procedures
_____Review of Operator's Manual
_____Requesting technical and instrument assistance

Trainer's Signature: _____ Date: _____

Customer's Signature: _____ Date: _____