

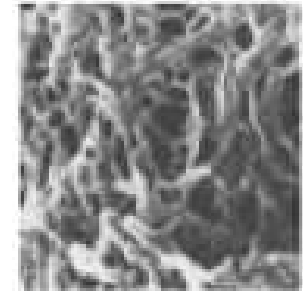
SERUM PROTEIN ELECTROPHORESIS AND IMMUNOTYPING ON CAPILLARYS

RESULTS AND INTERPRETATION

GEL ELECTROPHORESIS REVISITED

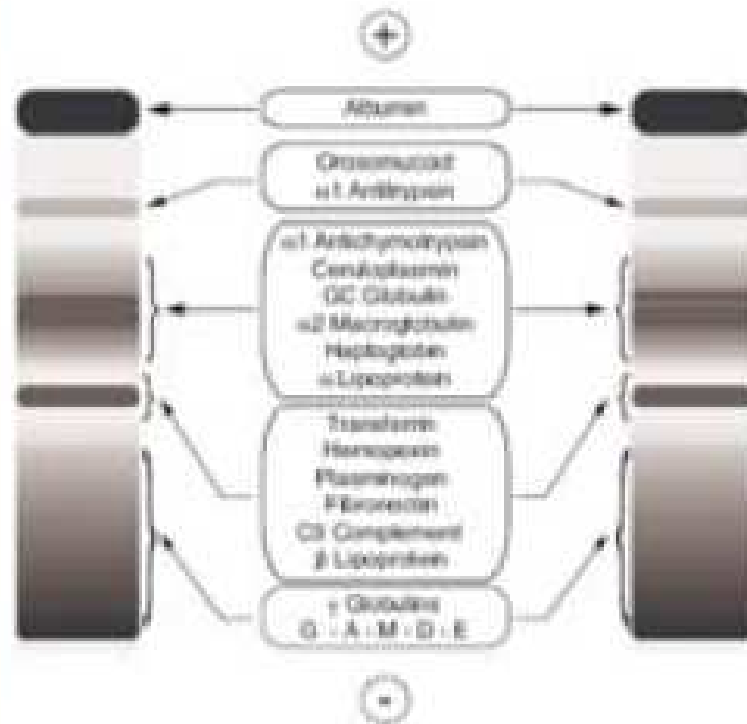


- On gels, proteins are separated in **solid** matrix (something similar to fishing net)
- In electrical field, proteins are separated according to their size and charge
 - Smaller proteins will migrate faster than larger proteins if charge is the same
- After separation, proteins are visualized by staining
 - Higher staining intensity indicates higher protein concentration

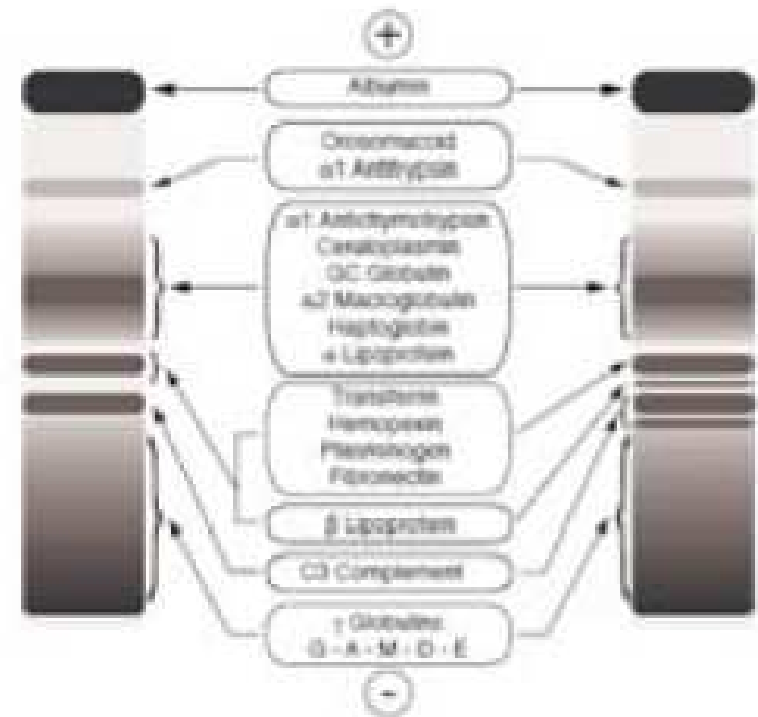


GEL MIGRATION PATTERNS

HYDRAGEL PROTEIN(E)



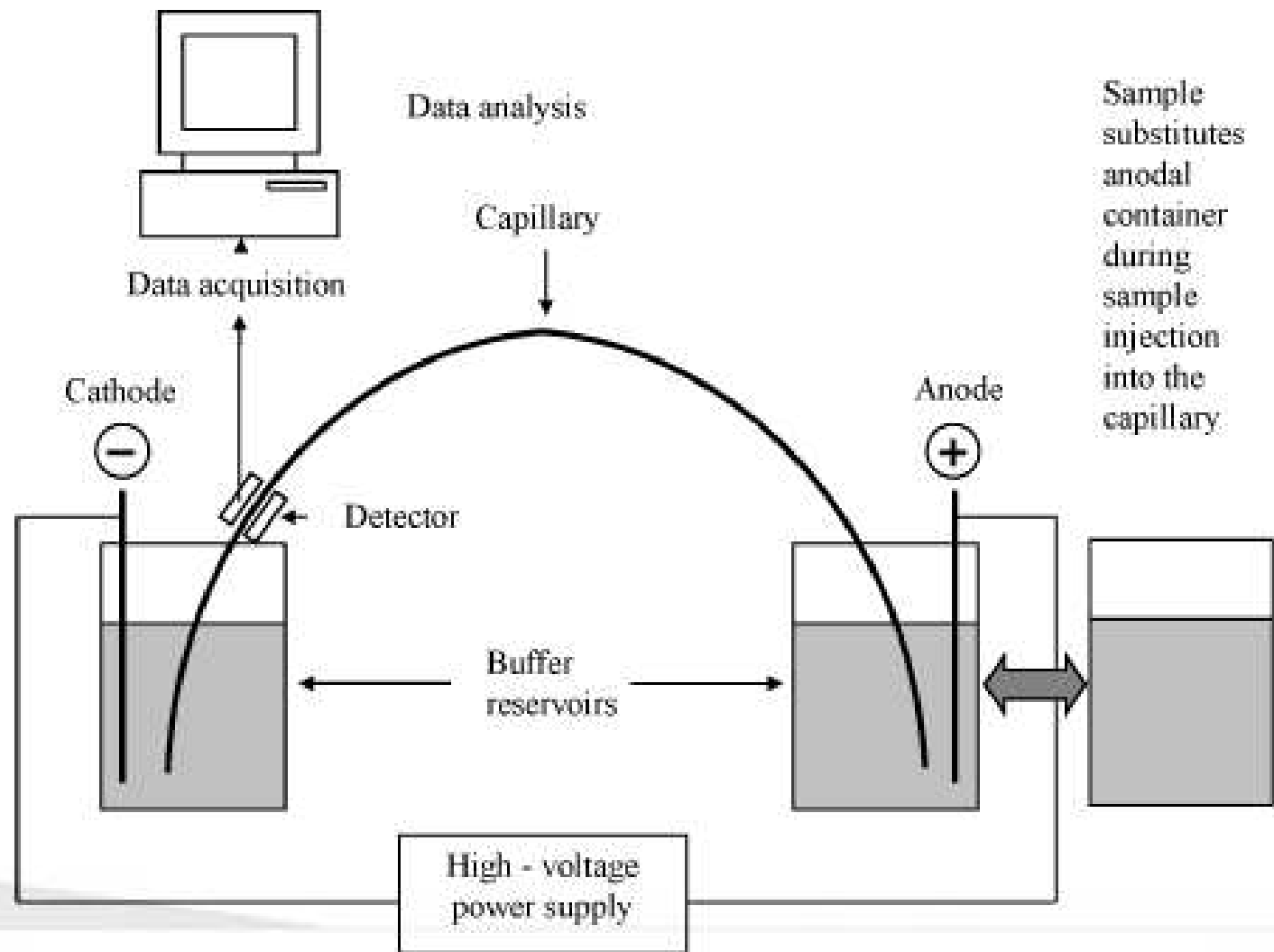
HYDRAGEL $\beta_1\beta_2$



CAPILLARY ELECTROPHORESIS

- Capillary electrophoresis is based on a sample separation in electrical field
 - Separation is achieved in a small glass tube - capillary, filled with buffer
 - Instead of staining, OD changes in UV light at 200 nm are measured (direct detection)
 - Absorbance of peptide bonds is measured
 - No staining is involved

CAPILLARY SYSTEM DIAGRAM

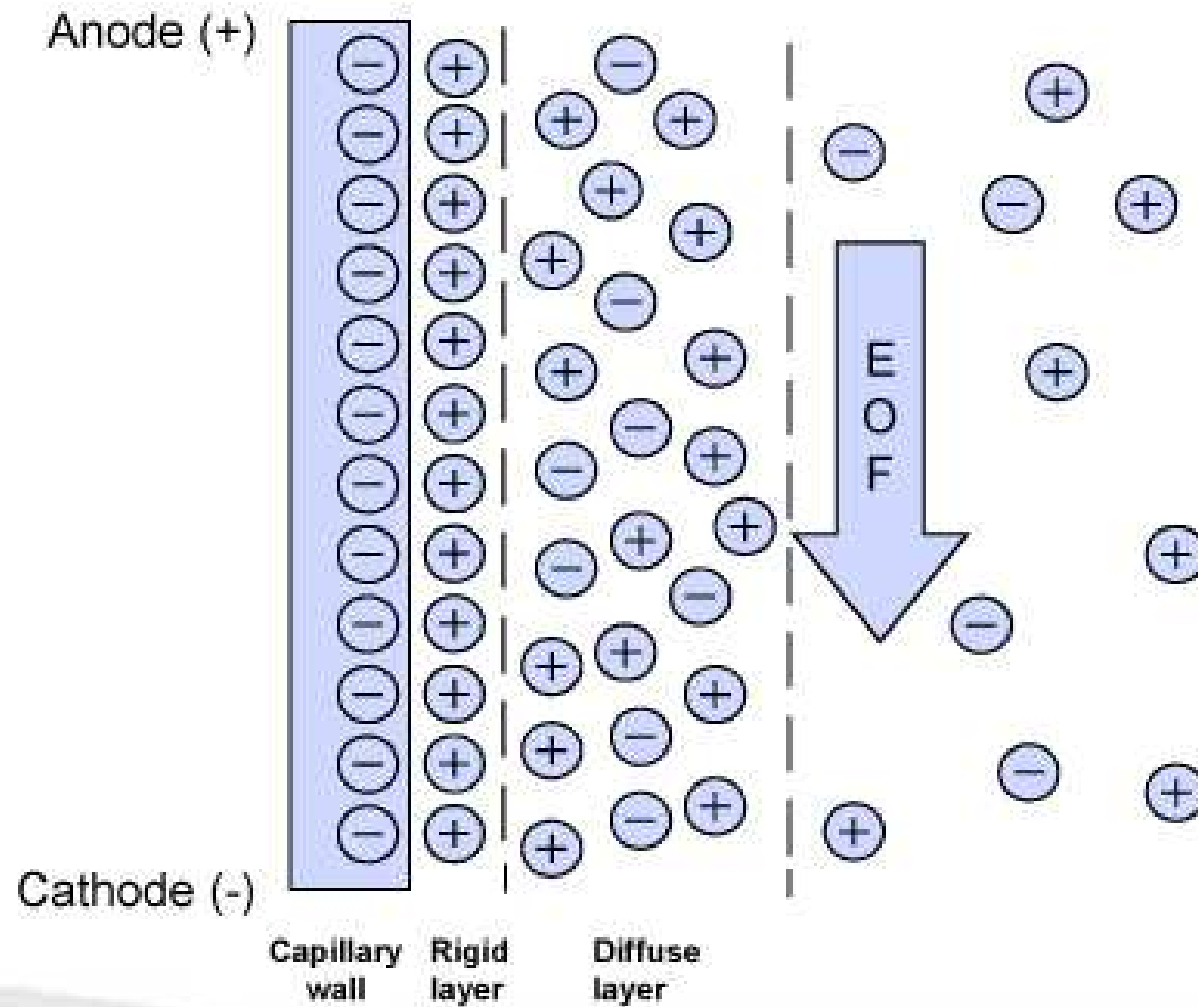


CAPILLARY PARAMETERS

- Internal Diameter: 25 μm
- Up to 12,000 V
 - 7,000 V for protein
 - 10,000V for Hb
- Length 175 mm
- Injection: a few nL
- Bubble Cap = 100 μm

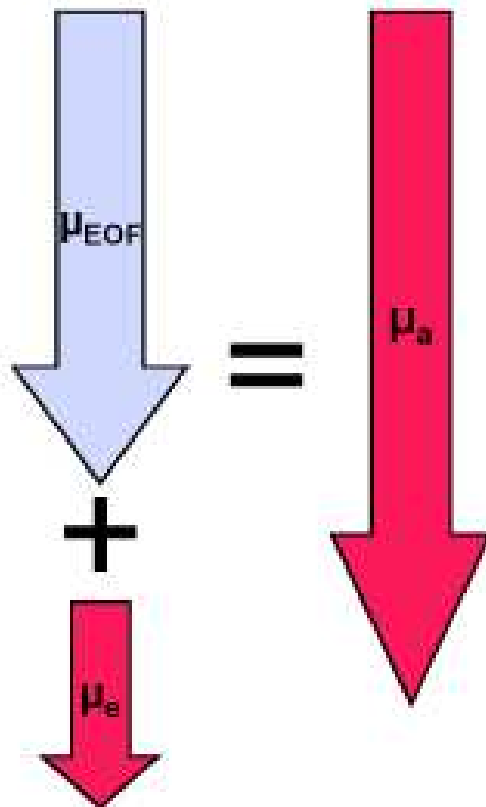
But what happens inside of the capillary?

NET FLOW OF BUFFER IS TOWARDS THE NEGATIVE ELECTRODE (CATHODE)



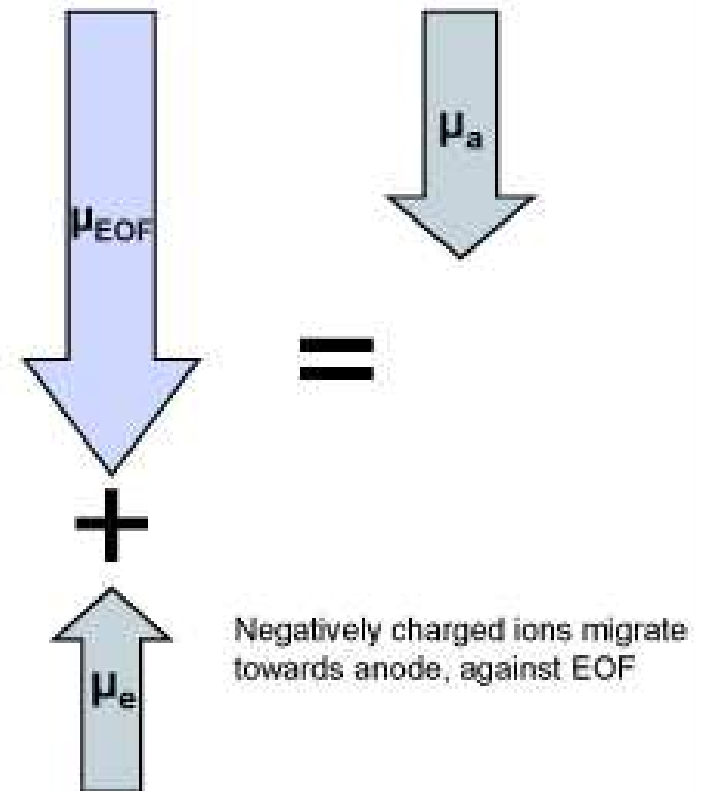
POSITIVELY CHARGED IONS WILL MOVE FASTER THAN NEGATIVELY CHARGED ONES

(+)



Positively charged ion mobility is a sum of two vectors facing the same direction; as a result, positive ions inside of the capillary will migrate very fast

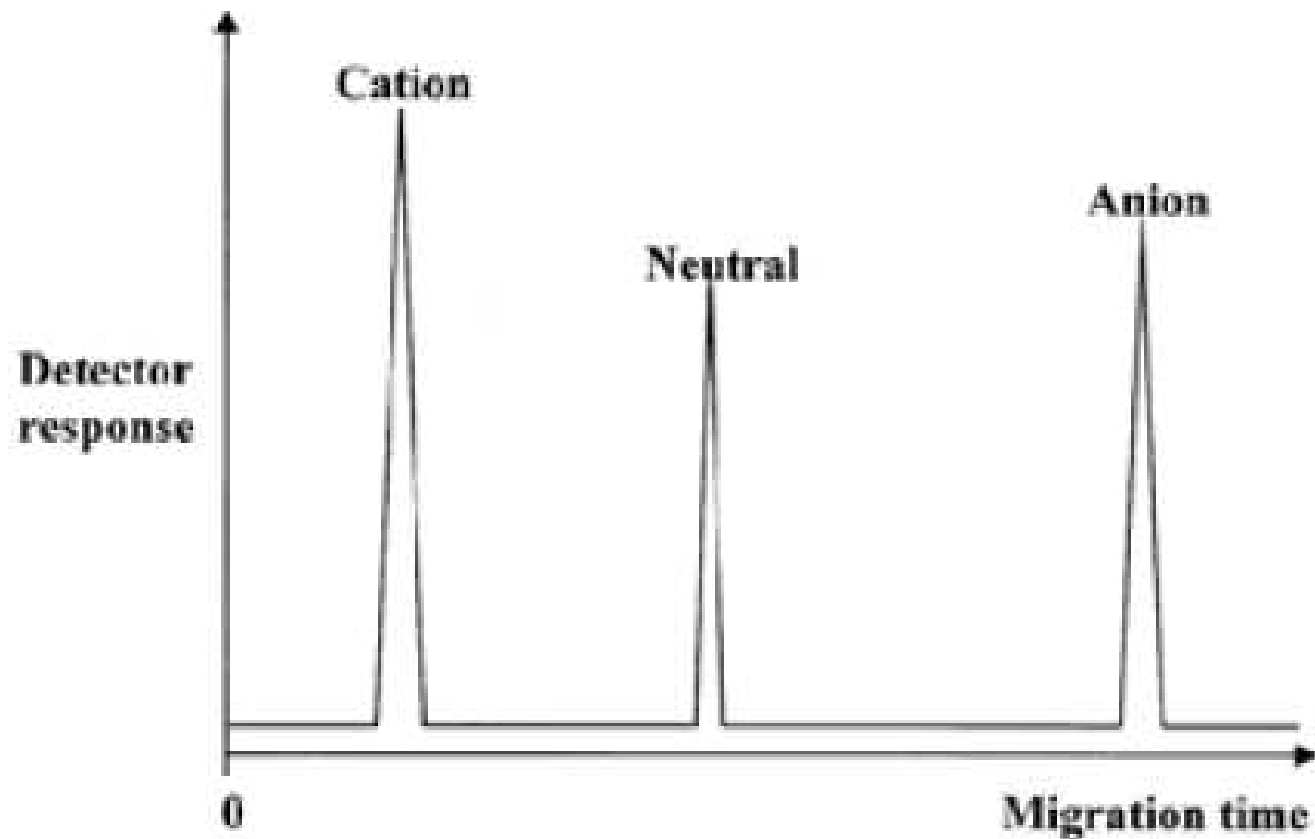
(-)



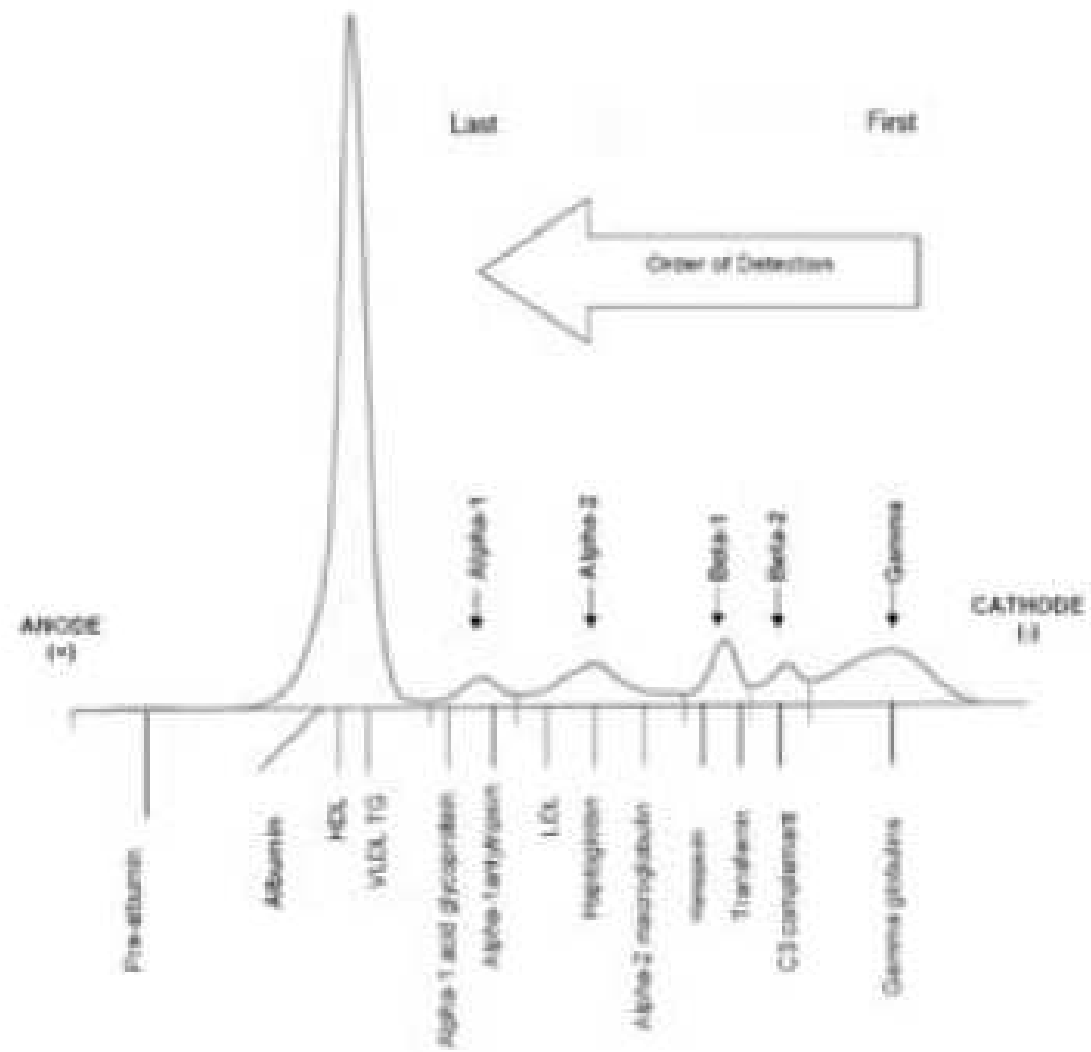
Negatively charged ion migration mobility is a sum of two vectors facing opposite directions; as a result, negative ions inside of the capillary will migrate slower than the positive ions. They will still migrate the same direction – towards the cathode

Negatively charged ions migrate towards anode, against EOF

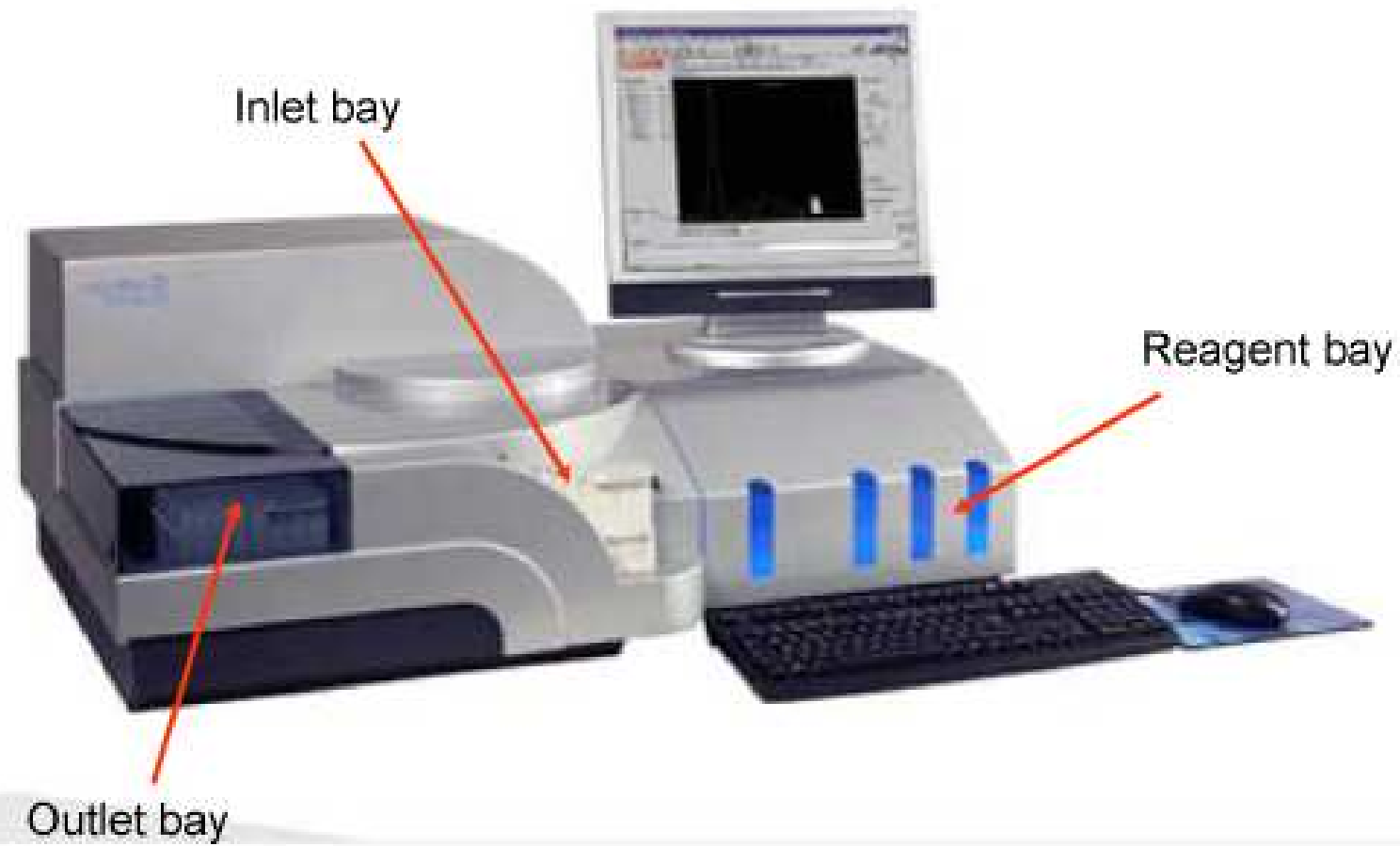
CATIONS WILL EMERGE AT THE DETECTOR FIRST,
FOLLOWED BY NEUTRAL IONS AND ANIONS



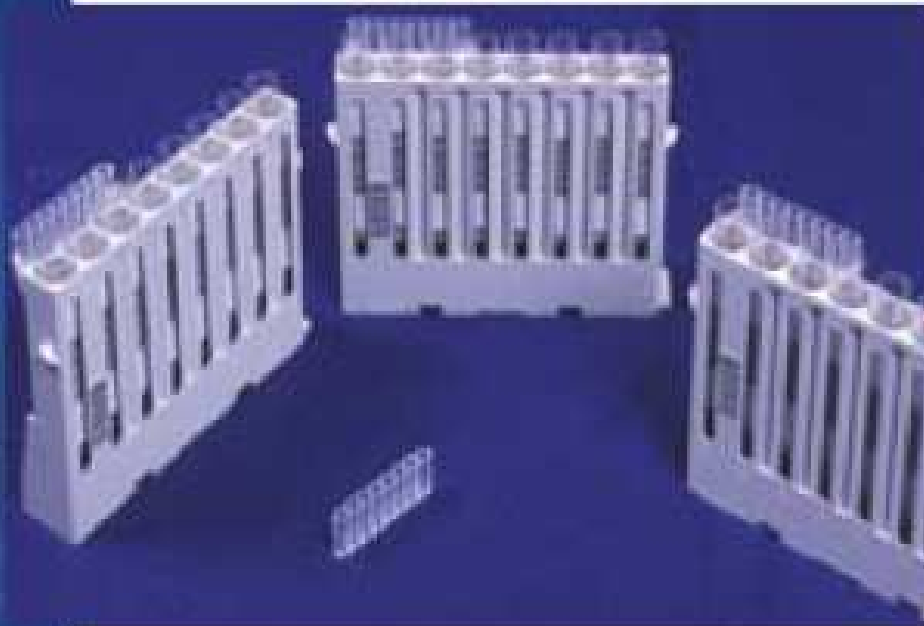
CAPILLARYS ELECTROPHORETIC PATTERN



CAPILLARYS 2

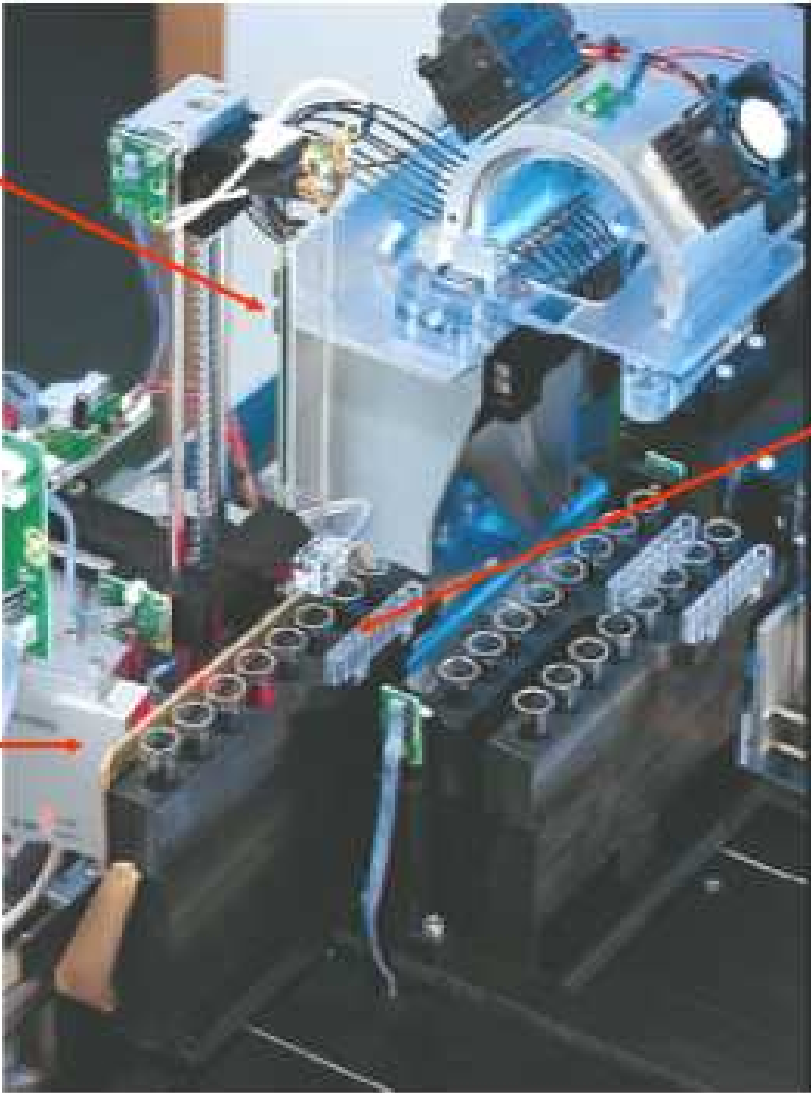


CAPILLARYS SAMPLE RACKS



SAMPLE PROCESSING

Dilution
probe



Dilution
segment

Barcode
reader

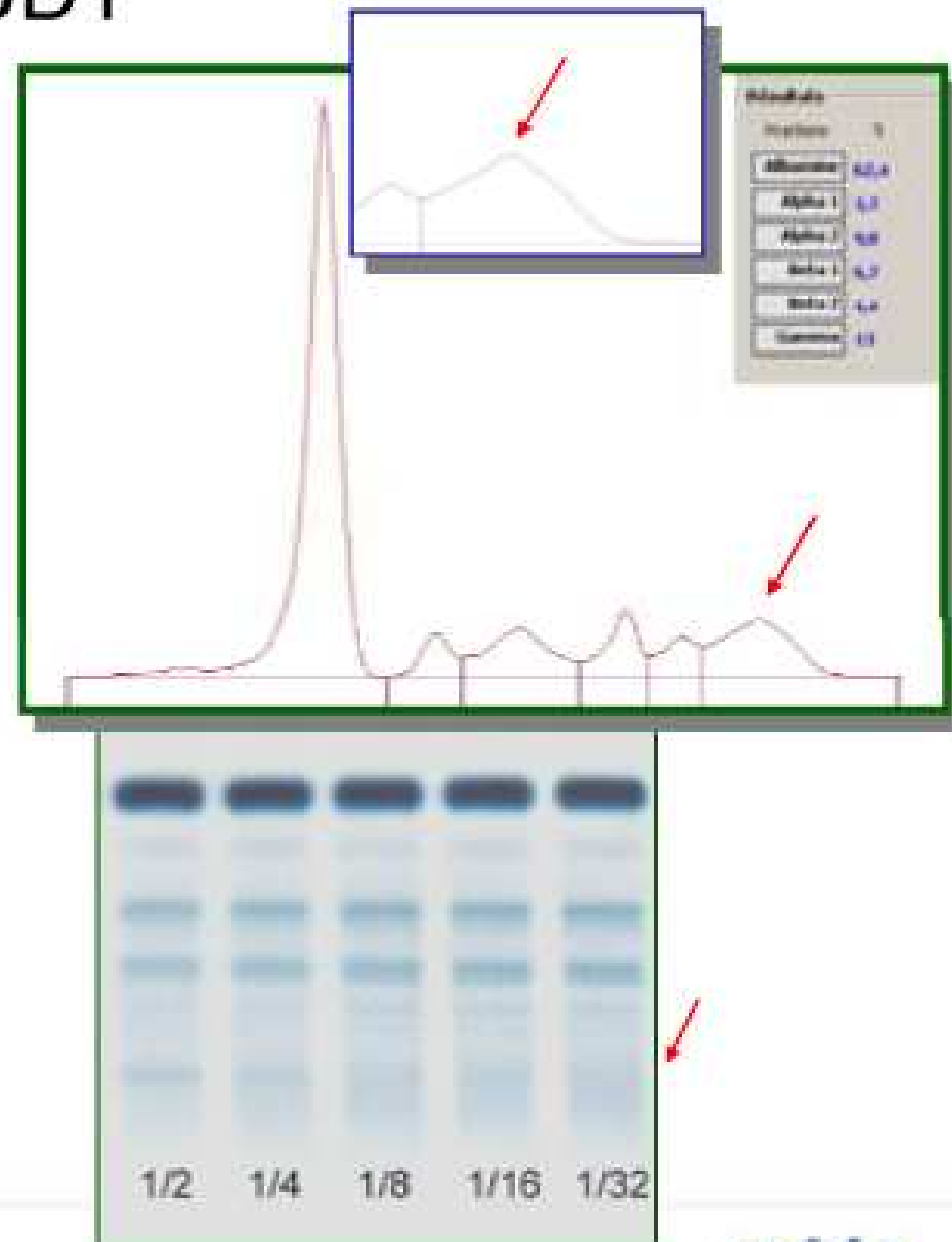
SENSITIVITY STUDY

A monoclonal component was diluted with normal serum and analyzed on both HYDRAGEL and CAPILLARYS.

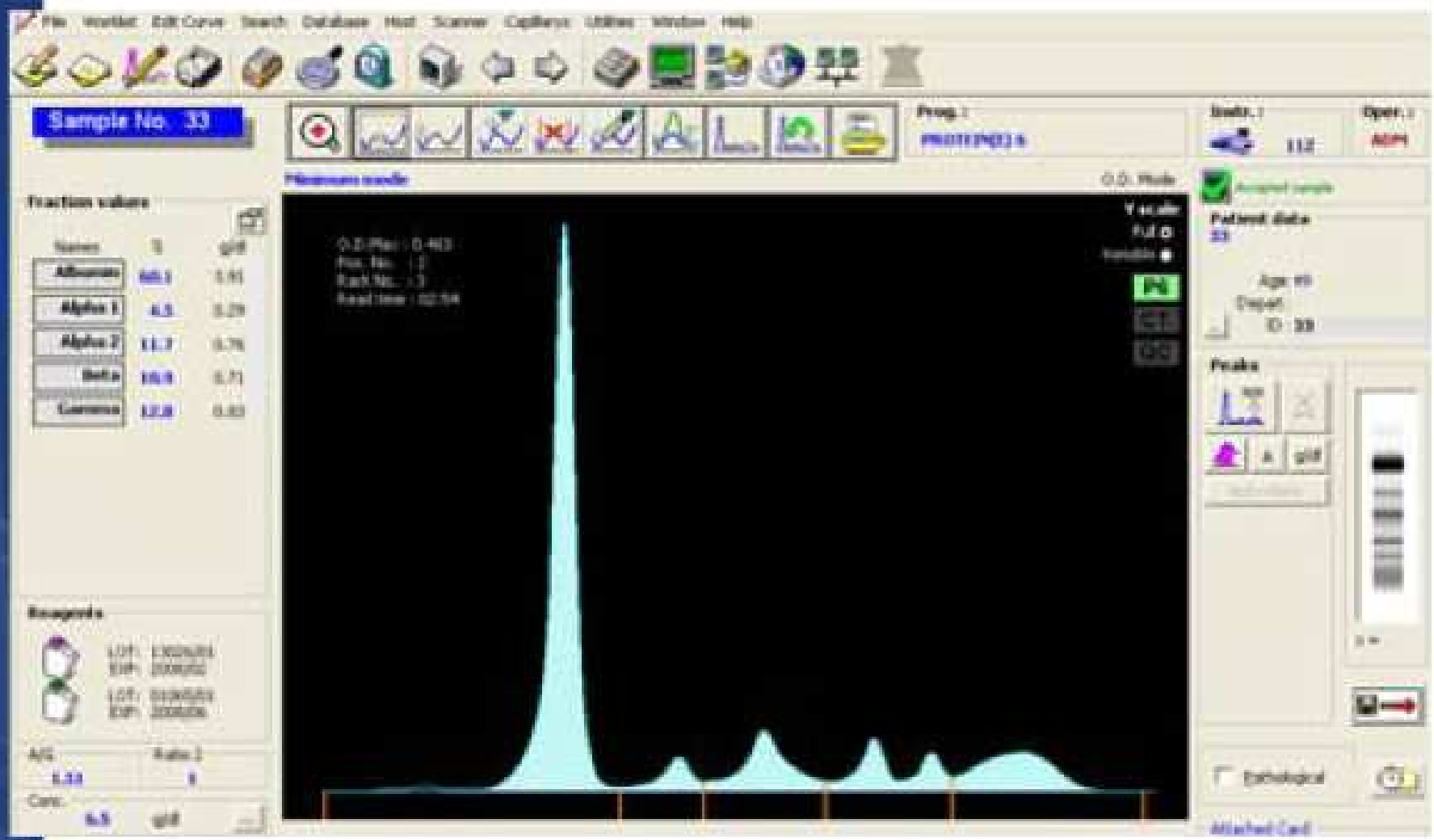
The lowest concentration detected was 0.18 g/L on both systems.

Sensitivity is affected by:

- *monoclonal component mobility*
- *polyclonal background*

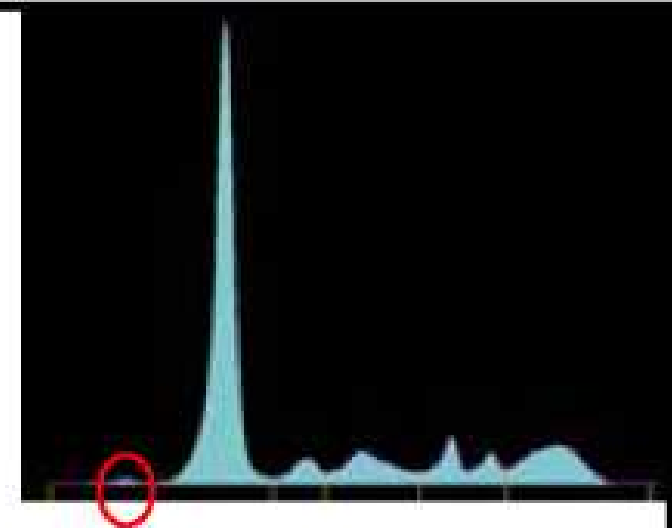


NORMAL SAMPLE RUN ON CAPILLARYS

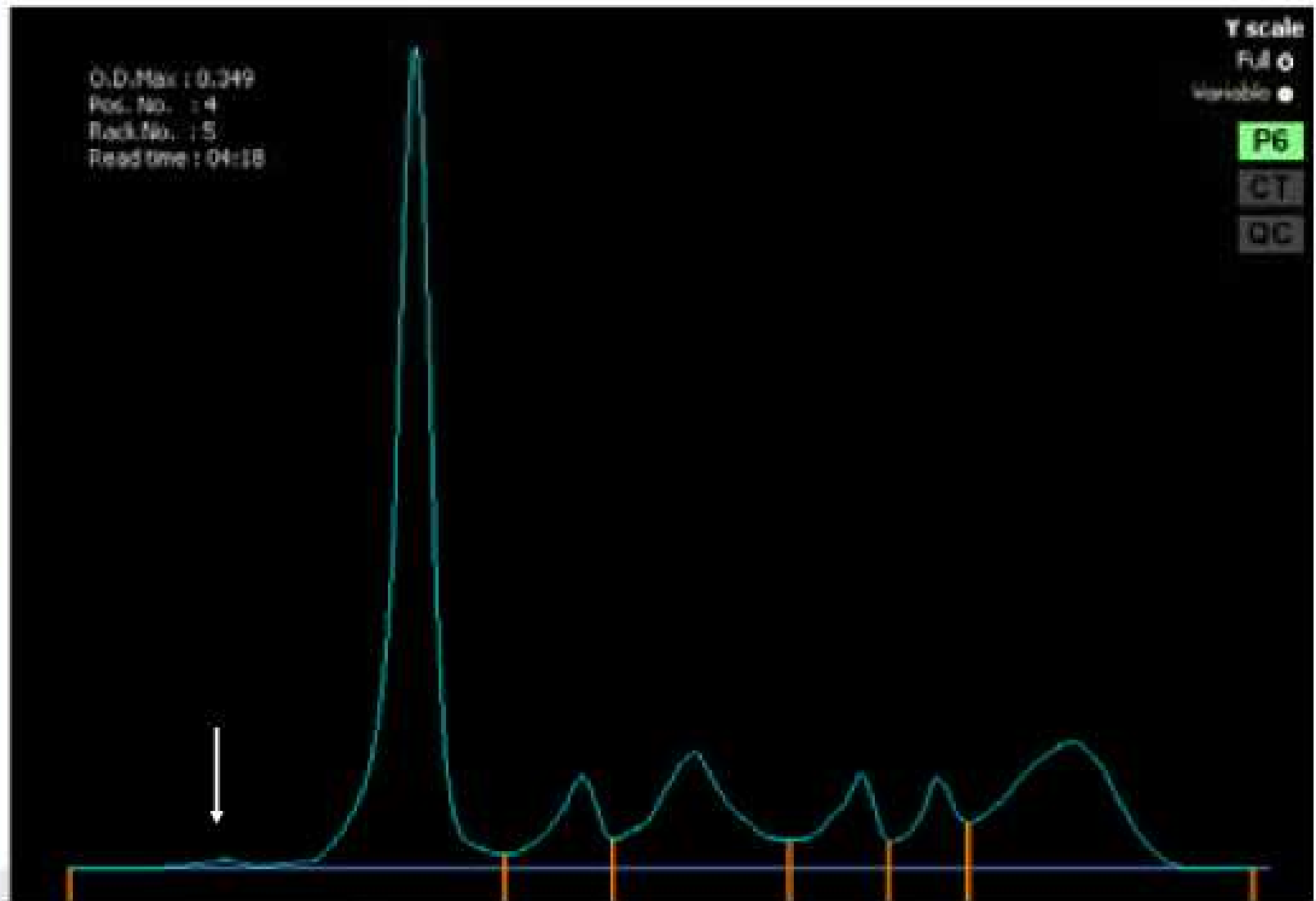


TRANSTHYRETIN (PREALBUMIN)

- The first peak encountered on anodal end of electropherogram
- Synthesized mainly in the liver
- Molecular mass: 50 kDa
- Functions:
 - Binds and transports ~20% of thyroid hormones
 - Binds and transports retinol binding protein
 - Hence the name: *transporting thyroxin and retinol-binding protein*
- Normal range: 20 - 40 mg/dl
 - Peak is too small for useful monitoring of any clinical condition
- Useful in assessing the nutritional status of patients
 - Due to 2-day half-life, compared with the 3-week half life of albumin
- Decreased levels can be caused by liver disease, sieving protein loss, malnutrition
- Levels can be increased as a response to anti-inflammatory drugs, stress and depression, or due to inflammatory bowel disease

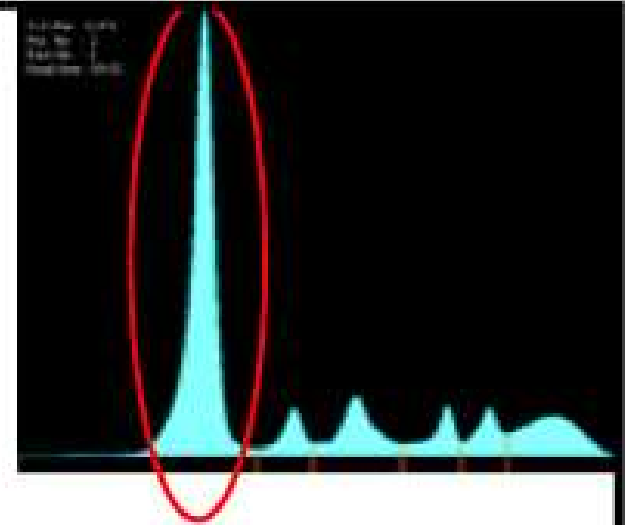


TRANSTHYRETIN (PREALBUMIN)



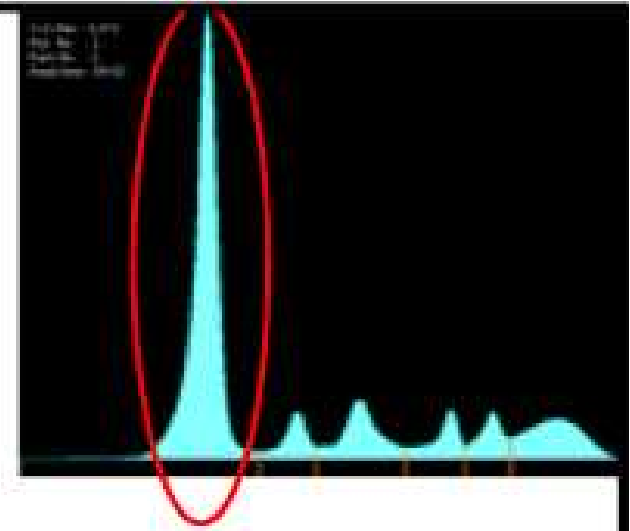
ALBUMIN (I)

- The most prominent protein in normal serum
- Molecular mass: 69 kDa
- Synthesized in liver
- Functions:
 - Maintains osmotic pressure within the body
 - General transport protein
 - Transports metal ions, fatty acids, amino acids, drugs, hormones, bilirubin etc.)
 - Source of amino acids
- Normal range: 3.5 – 5.0 g/dL

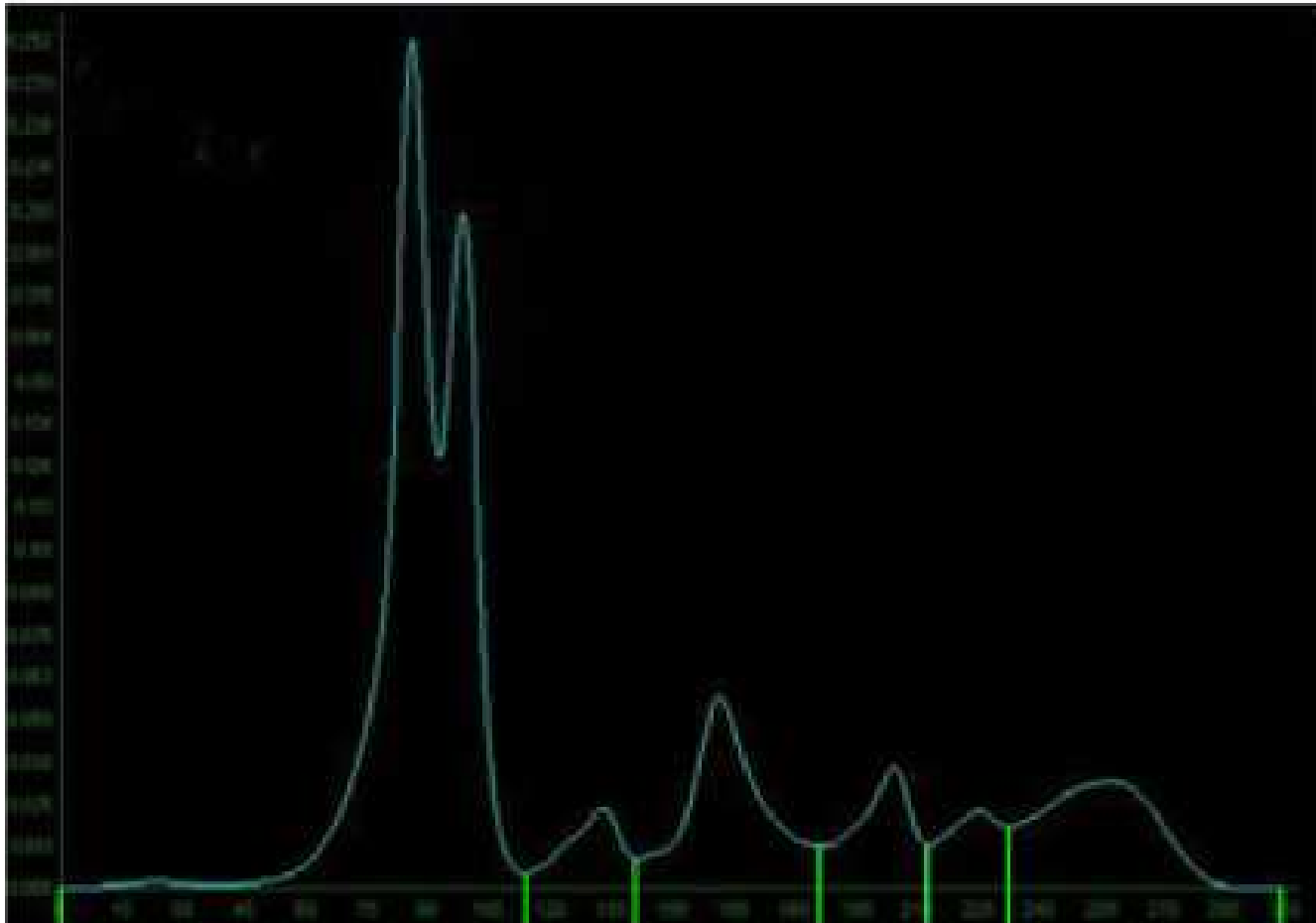


ALBUMIN (II)

- Hypoalbuminemia may be caused by:
 - liver disease
 - response to disease (albumin is negative acute phase reactant)
 - protein loss (urine, GI tract, burns, neoplasia)
 - malnutrition
- Hyperalbuminemia can be caused by:
 - Dehydration
 - Accompanied by an increase in the other serum proteins
- Bisalbuminemia
 - Genetic
 - Two types of albumin with slightly different electrophoretic mobility are produced
 - No pathological consequences
 - Incidence: up to 1:10,000 in Caucasians and Japanese, but as high as 1% in some native American tribes
 - Induced by drugs
 - In acute pancreatitis

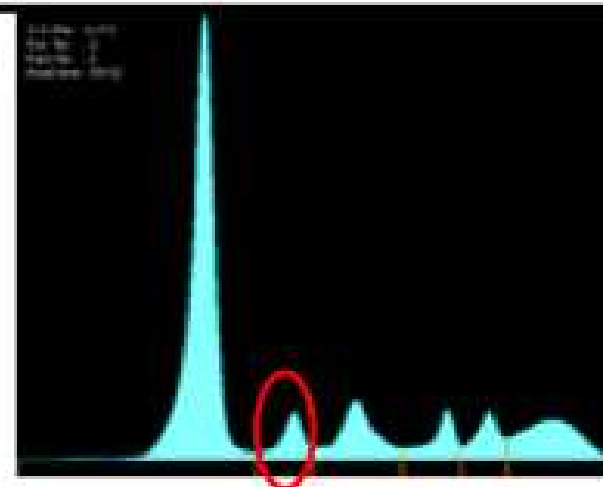


BISALBUMINEMIA



ALPHA-1 FRACTION:

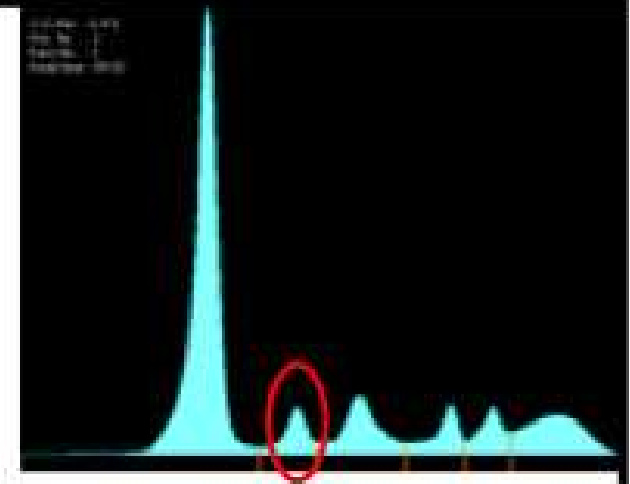
α -1 ACID GLYCOPROTEIN (OROSOMUCOID)



- Reference range: 50 – 250 mg/dl
- Size: ~40 kDa
- Does not stain well; underestimated in gel electrophoresis
- AGP is a positive acute phase reactant
 - Concentration increases in response to systemic tissue injury, inflammation or infection
- Increased AGP is also associated with an increased risk for myocardial infarction
- Decreased in pregnancy or due to sieving protein loss

ALPHA-1 FRACTION:

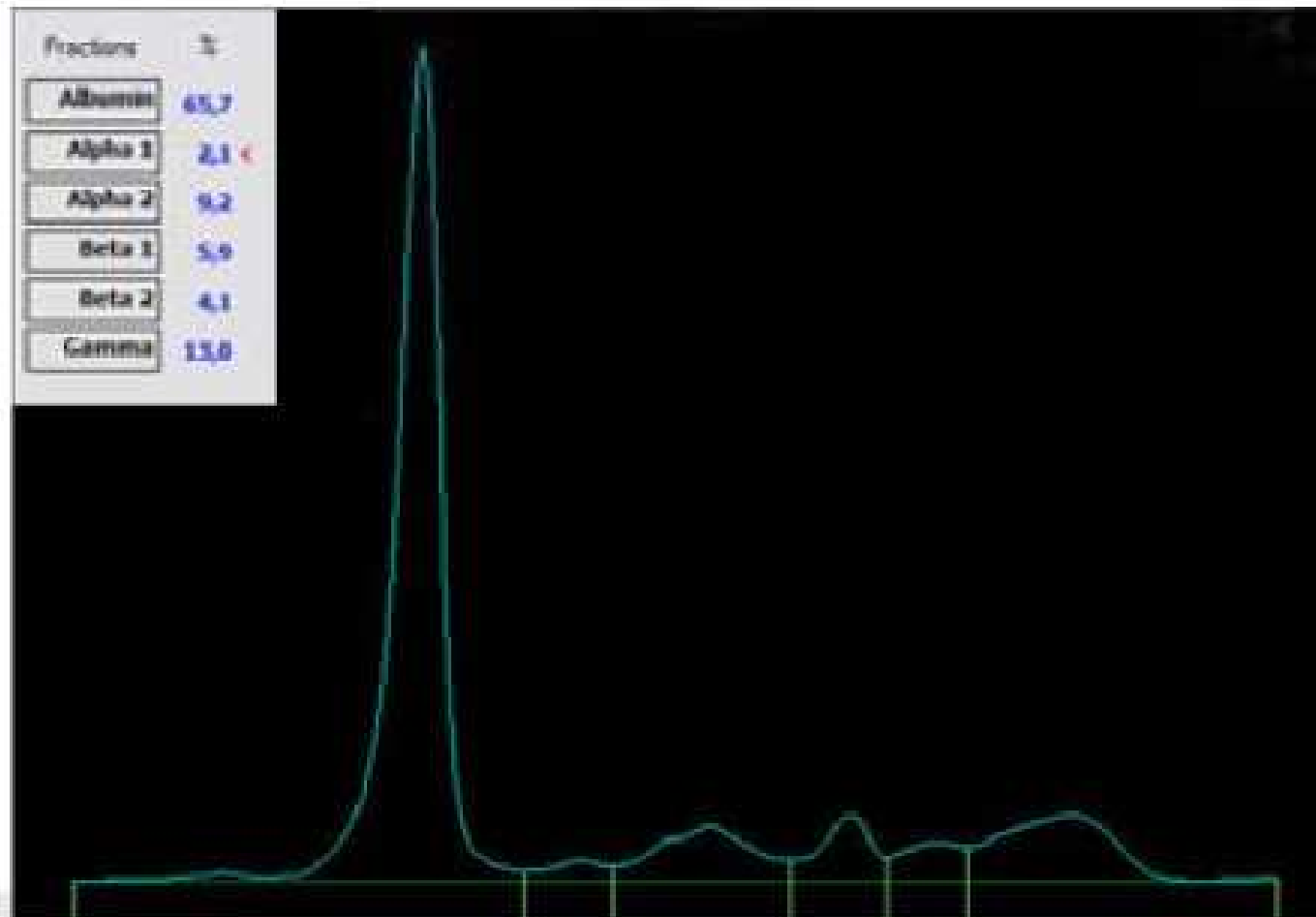
α -1 ANTITRYPSIN (A1AT)



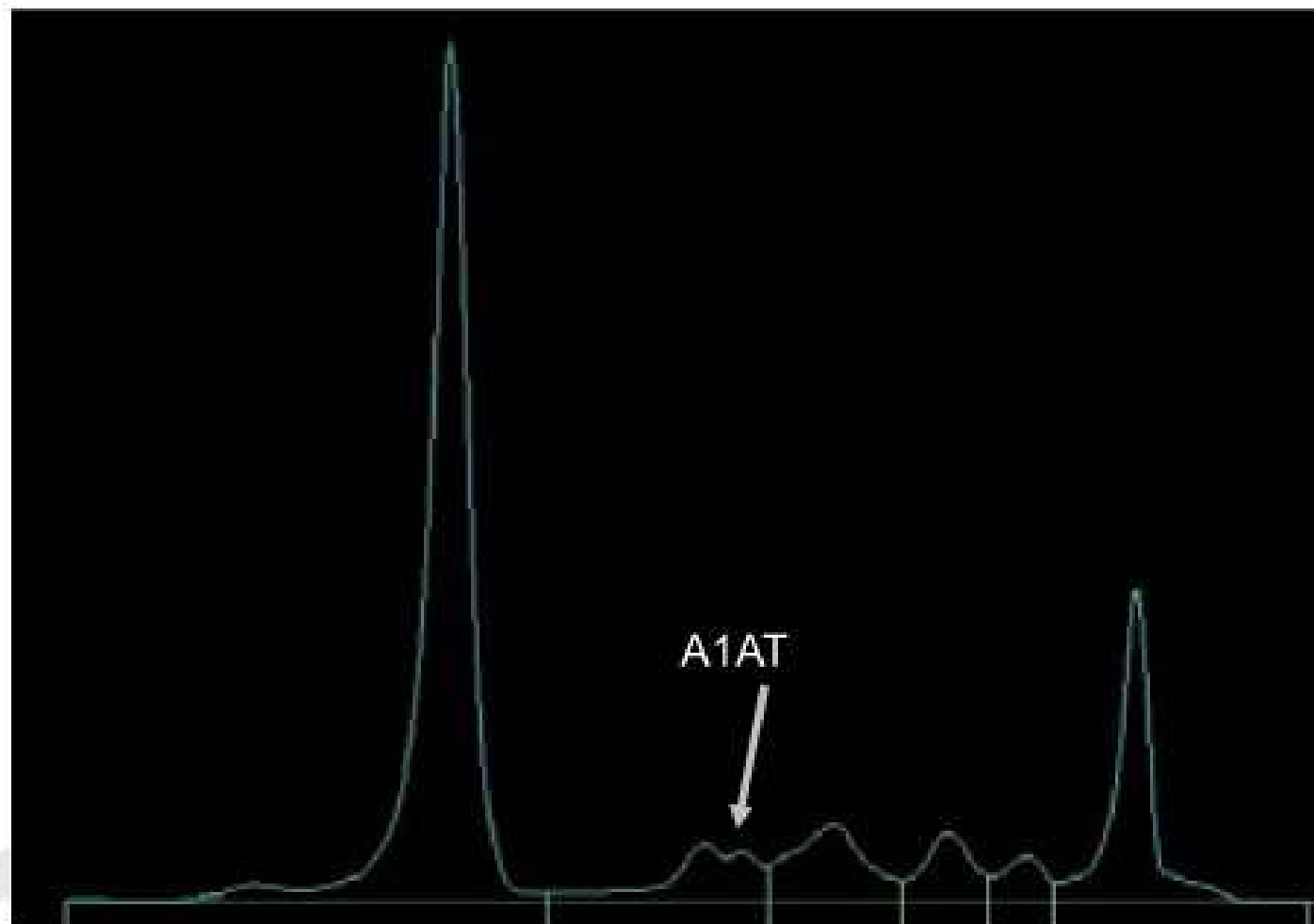
- Alpha-1 antitrypsin (or alpha-1 protease inhibitor):
 - Produced in the liver
 - Molecular mass: 52 kDa
 - Reference range: 90 – 200 mg/dl
 - Inhibits the activity of proteolytic enzymes
 - deficiency of this protein is associated with development of emphysema
 - Increased in acute-phase response, in patients with hyperestrogenemia (pregnancy, oral contraceptives), tumors or with liver disease
 - Increase in alpha-1 antitrypsin may help to predict the onset of labor
 - Decreased due to genetic deficiency, in severe pancreatitis and in sieving protein loss

ALPHA-1 FRACTION: A1AT DEFICIENCY

A1AT = 17 mg/dL (normal range 97 – 193 mg/dL)

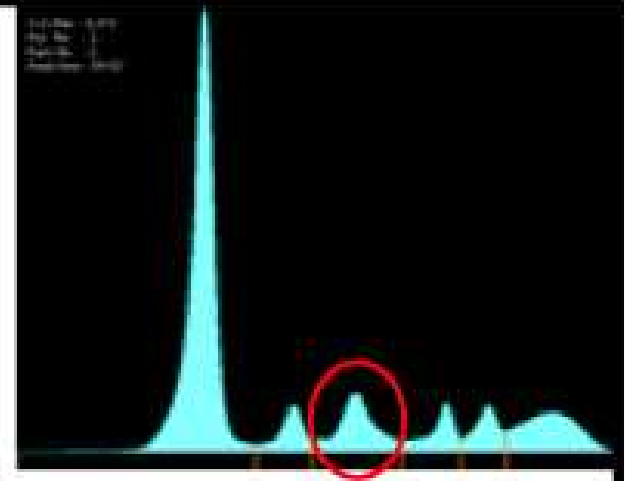


ALPHA-1 FRACTION:
HETEROZYGOUS A1AT PHENOTYPE



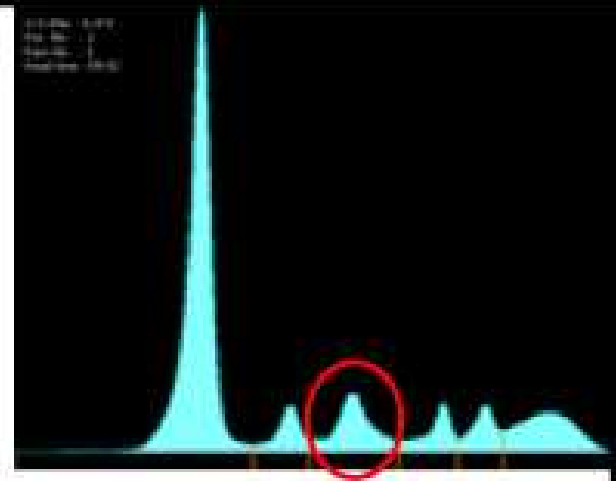
ALPHA-2 FRACTION

- Normally appears as single peak cathodic to (before) alpha-1 fraction
- Several proteins migrate together:
 - Alpha-2 macroglobulin
 - Haptoglobin (double band may be indicator of phenotype)
- Decreased alpha-2 values are due to intravascular hemolysis
- Increased alpha-2 values are due to:
 - Acute inflammation
 - Nephrotic syndrome
 - Hemolysed samples (in vitro)



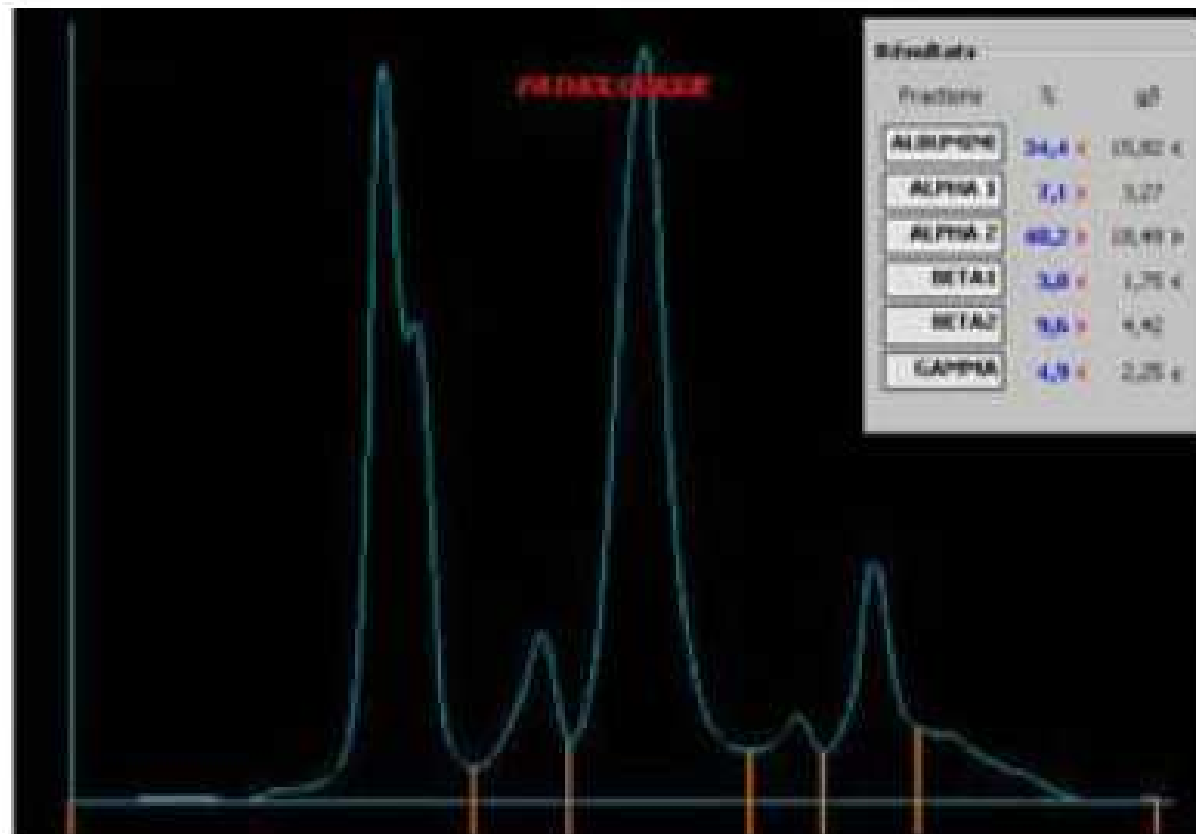
ALPHA-2 FRACTION:

α -2 MACROGLOBULIN



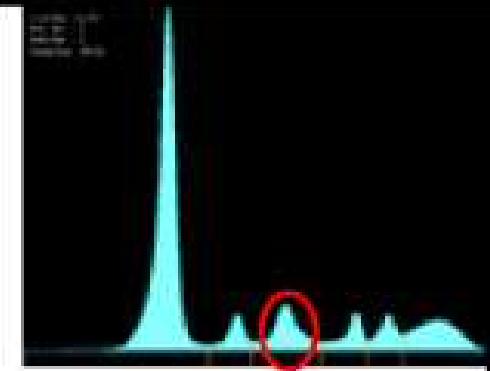
- Protease inhibitor; synthesized mainly by liver
- Concentration range: 150 - 350 mg/dl
- Alpha-2 macroglobulin is not eliminated through kidney because of its huge size (720 kDa); it may increase 10 fold in nephrotic syndrome (secondary to synthesis)
- Elevated in neonates, patients with elevated estrogen levels, diabetics or in patients with alpha-1 antitrypsin deficiency and emphysema
- No specific disease states are associated with low levels
- Not an acute-phase reactant; does not increase with inflammation

ALPHA-2 FRACTION: INCREASED α -2 MACROGLOBULIN (NEPHROTIC PATTERN)



ALPHA-2 FRACTION

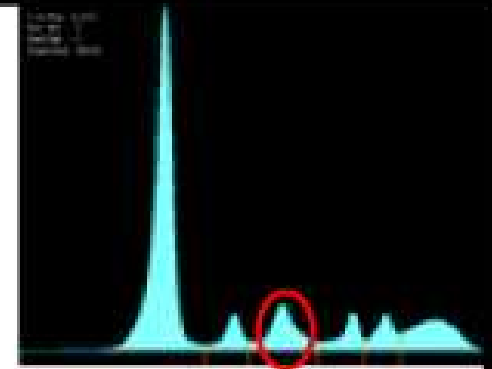
HAPTOGLOBIN (I)



- Binds free hemoglobin liberated during intravascular hemolysis, thereby preserving iron and preventing renal damage
- Synthesized by the liver
 - Molecular mass: 85 – 840 kDa
 - Reference range: 30 – 280 mg/dl
 - Size and concentration is phenotype dependent
 - Three phenotypes: 1-1, 2-1 and 2-2

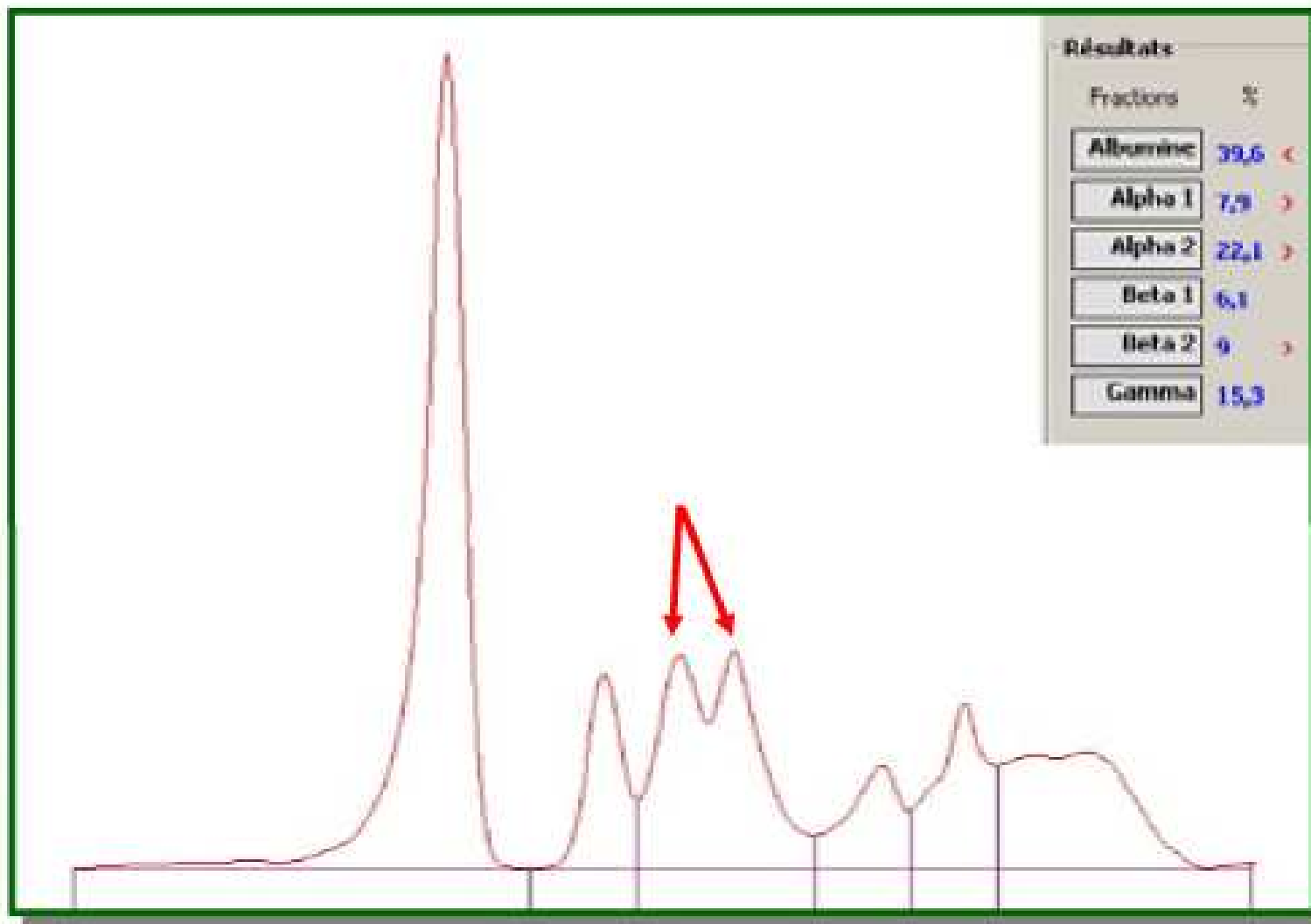
ALPHA-2 FRACTION

HAPTOGLOBIN (II)



- Increased in:
 - acute inflammatory response
 - in patients with increased corticosteroid and estrogen stimulation (pregnancy, contraceptive drugs, neoplasms, cirrhosis)
 - In renal disease (Hp 2-1 and Hp 2-2); due to the large size
- Decreased in:
 - Hemolysis and hemolytic anemia
 - incompatible transfusion reaction
 - In malarial and clostridial infections
 - severe liver disease
 - genetic deficiency (Hp 0-0)

ALPHA-2 ZONE (I)

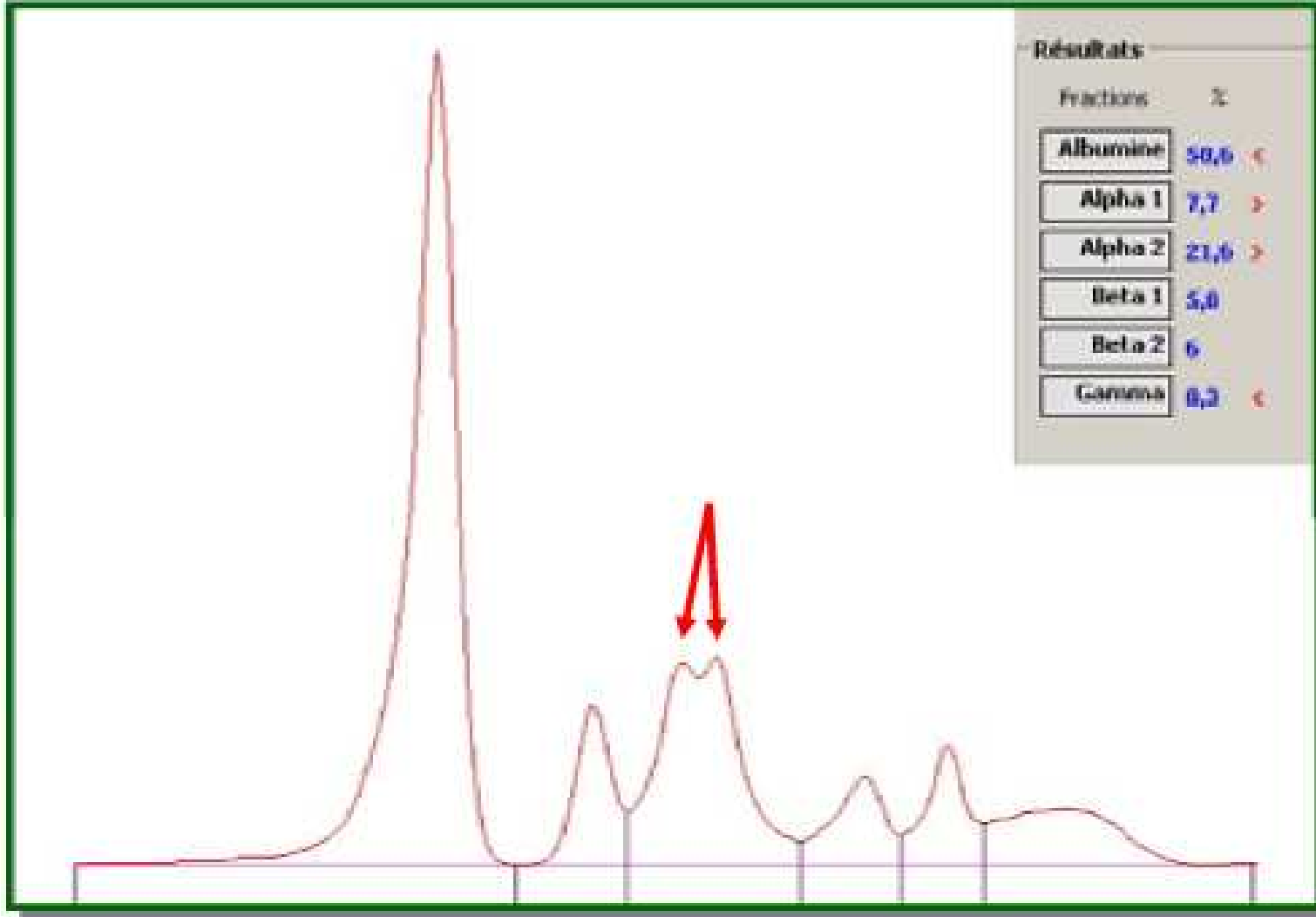


Haptoglobin phenotype 1-1

2 distinctive bands in Alpha-2

Inflammatory profile

ALPHA-2 ZONE (II)

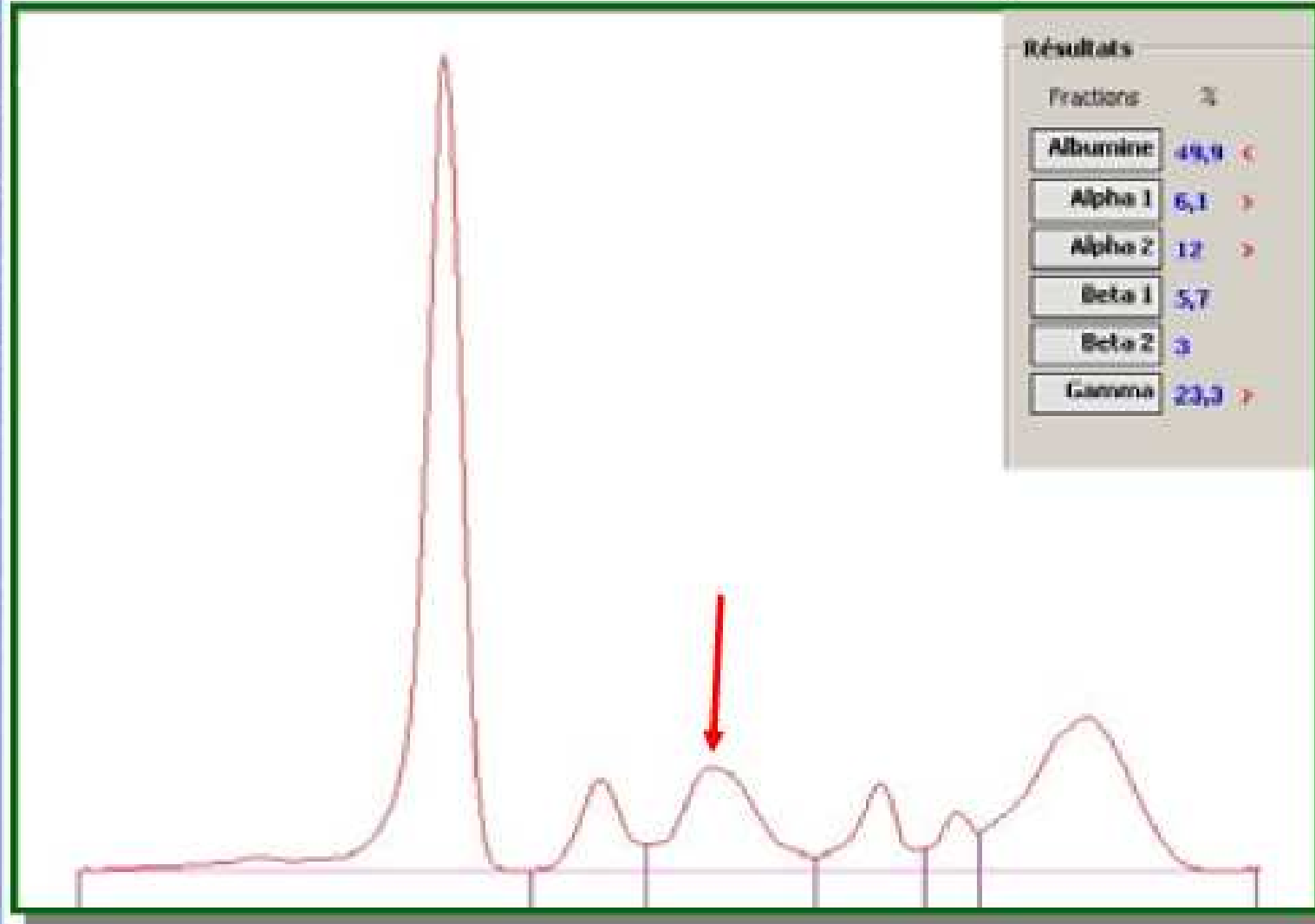


Haptoglobin Phenotype 2-1

2 close bands in Alpha 2

Accute inflammatory profile

ALPHA-2 ZONE (III)

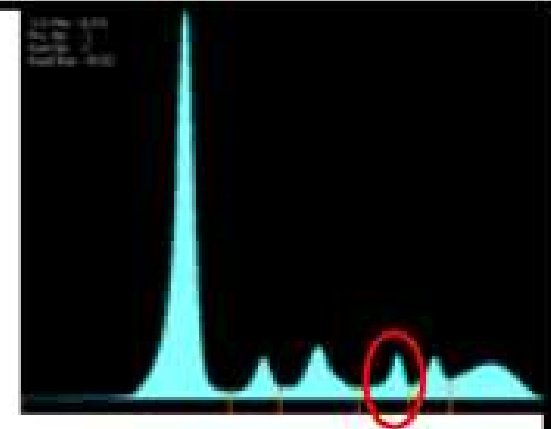


Haptoglobine Phenotype 2-2

Only 1 band in Alpha-2

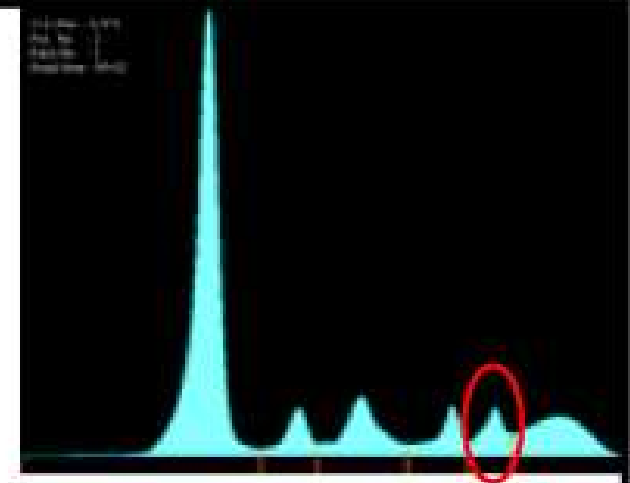
BETA-1 FRACTION: TRANSFERRIN

- Transferrin (TRF) is the principle protein for iron transport from gastrointestinal tract and from the Hb breakdown to the bone marrow
 - Synthesized in the liver
 - Molecular mass: 76 kDa
 - Reference range: 240 – 480 mg/dl
- Genetic variants exist
 - Both fast and slow transferrin variants have been identified (heterozygotes can present two closely migrating bands in beta-1 region)
- Increased in:
 - Iron deficiency anemia
 - Patients that are pregnant or in patients receiving estrogens
- Decreased in:
 - Alcoholic cirrhosis
 - Renal disease and thermal injuries
 - Iron overload
 - Acute or chronic inflammation



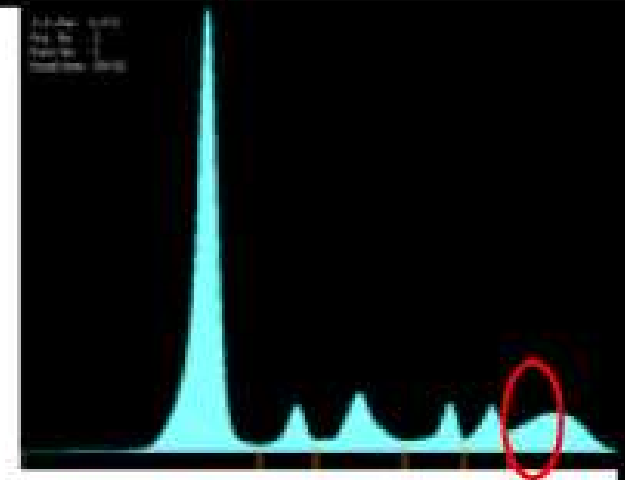
BETA-2 FRACTION:

C3 COMPLEMENT



- The only component of complement present in sufficient concentration to allow detection by electrophoresis
 - Complement = system of ~30 proteins activated in inflammatory response
 - Molecular mass: 185 kDa
 - Reference range: 90 – 180 mg/dl
- Genetic variants of C3 have been described in literature, but are uncommon
- Decreased:
 - > 3 day old sample
 - Breakdown products migrate in early gamma zone
- Increased:
 - Late during the acute phase response
 - Due to co-migrating monoclonal protein

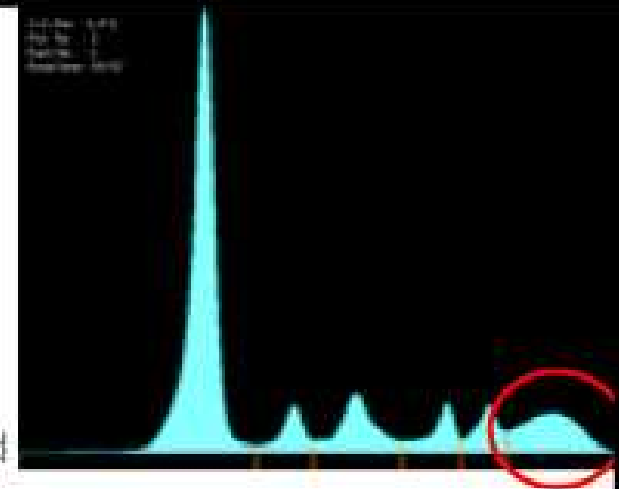
C-REACTIVE PROTEIN (CRP)



- Non-immunoglobulin protein with MW 135 kDa
- Normal concentration: <2 mg/dl
 - Higher in individuals at increased risk of stroke or coronary artery disease
- Non-specific binding to bacterial cell walls, activating complement and opsonization
 - Particularly strong indicator of bacterial infections; usually no increase in viral infections (influenza A is an exception)
 - Considerable increase during acute inflammatory responses to >100 mg/dl
 - Levels decrease as a result of anti-inflammatory therapy
- May be mistaken for a monoclonal band

GAMMA FRACTION

- Dogma: Immunoglobulins migrate within the gamma fraction
 - IgA migrates closer to the Beta-2 fraction
 - IgM very often tends to migrate between beta and gamma fractions
 - Light chains may migrate anywhere from alpha-2 to gamma region



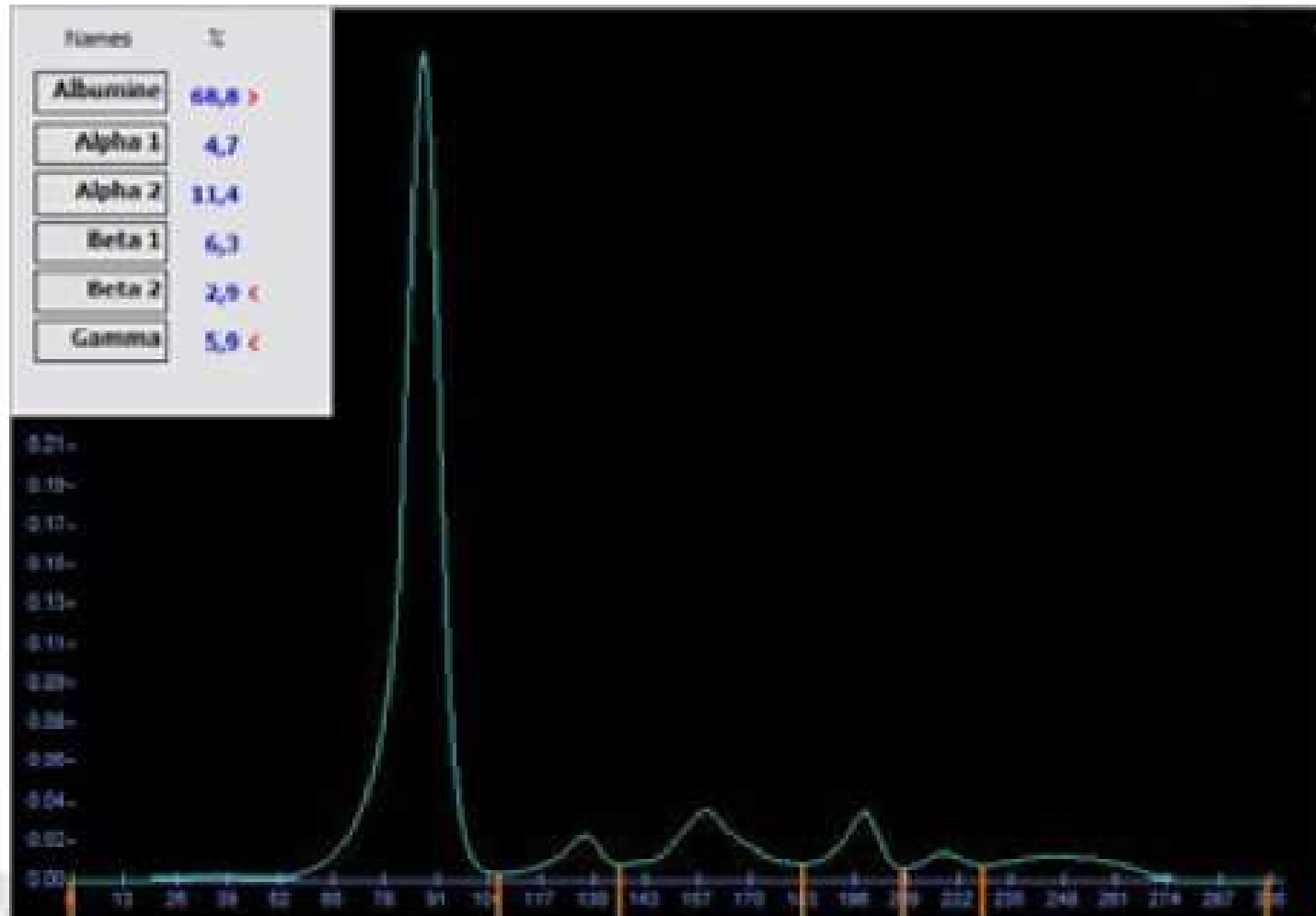
Decreased:

- In elderly
- Congenital immunodeficiency
- Immunosuppressive treatment (HIV, Transplant etc..)

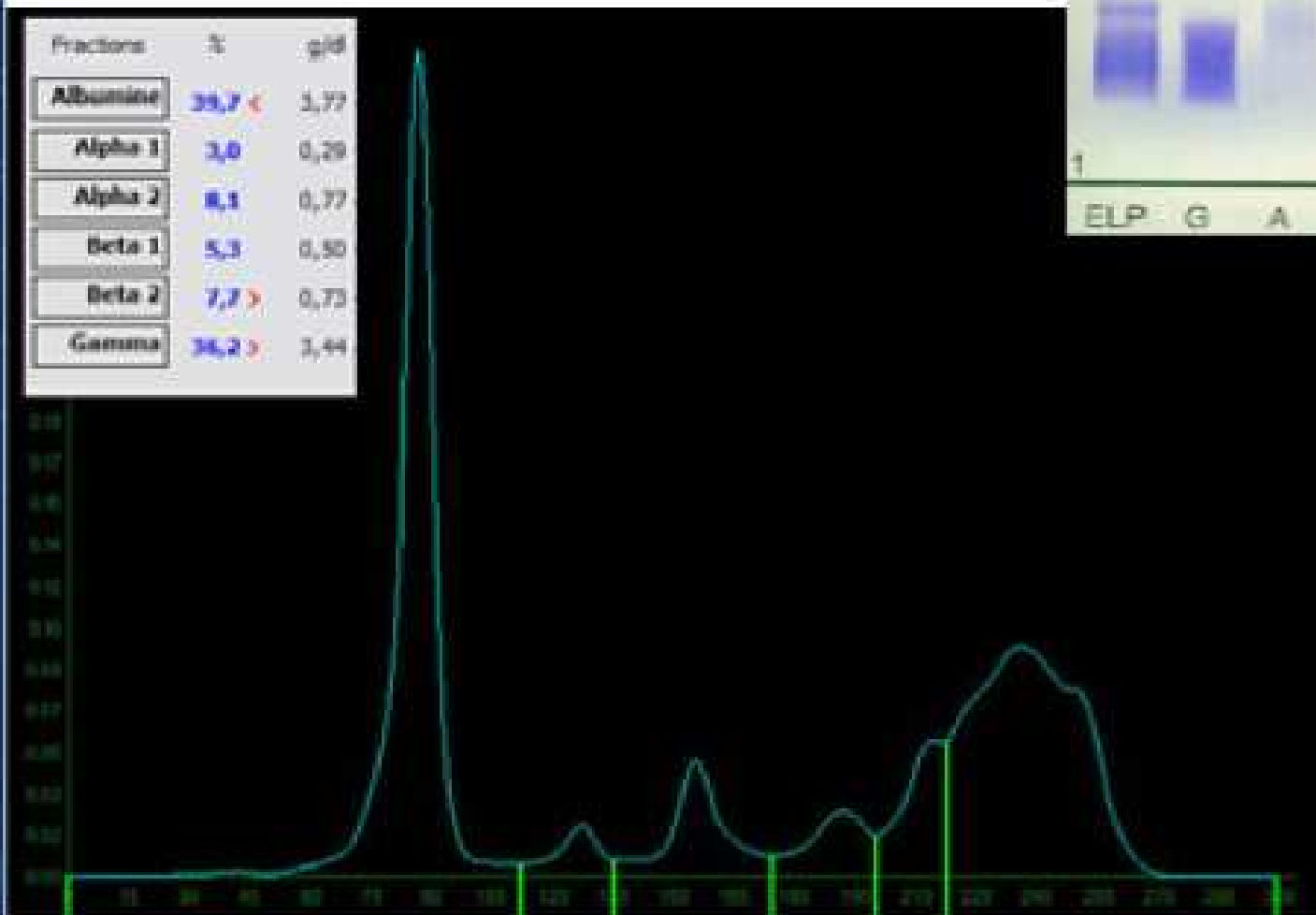
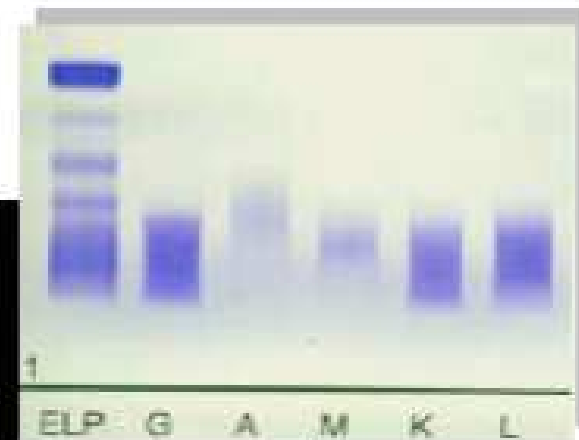
Increased:

- In lymphoplasmocytic syndromes such as Multiple Myeloma
- Hyperimmunization
- Acute infections (HIV...)
- Chronic liver disease
- Chronic infections

GAMMA FRACTION: HYPOGAMMAGLOBULINEMIA



GAMMA FRACTION: OLIGOCLONAL PATTERN



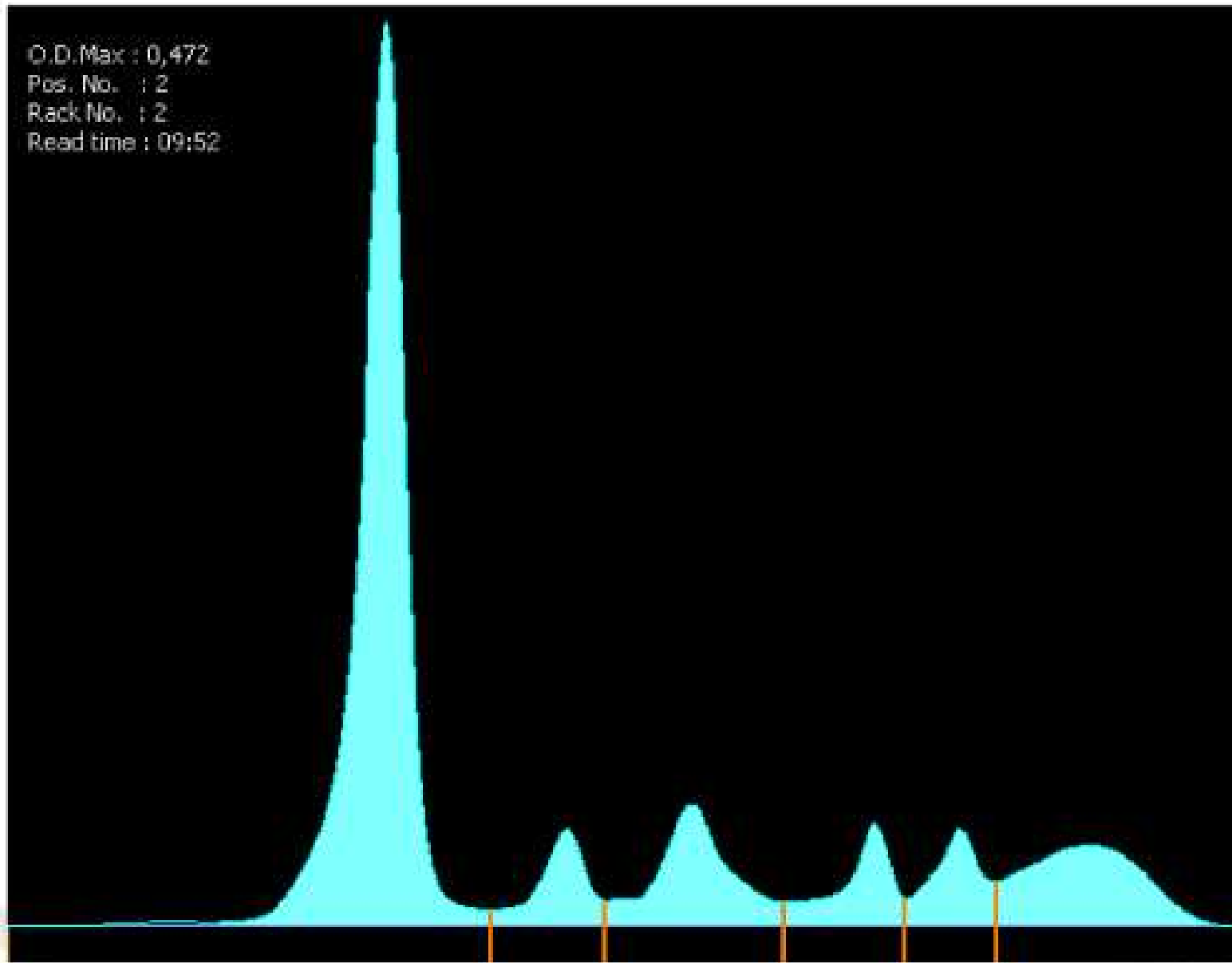
WHAT DO FRACTION VALUES MEAN?

	TP, g/dL	Alb	α -1	α -2	β	Split β	β - γ bridge	γ
Acute inflammatory pattern and Hyper-estrogenism pattern		↓	↑	↑				
Chronic inflammatory disorders		↓	↑	↑				↑
Monoclonal or polyclonal gammopathy (IF recommended)				+/-	+/-			+/-
Liver disease/Cirrhosis		↓					+/-	↑
Nephrotic syndrome	↓	↓	+/-	↑	↑	+/-		↓
Protein-losing pattern/malnutrition	↓	↓	↓	↓	↓			↓
Autoimmune reactions			↑	↓				↑
Alpha-1-antitrypsin deficiency			↓↓					
Hypogammaglobulinemia (UPE recommended)	↓							↓↓
Hemolytic anemias/congenital hemoglobin defects				↓				

↓ decreased; ↑ increased; ↓↓ markedly decreased; +/- increased or decreased

NORMAL SAMPLE RUN ON CAPILLARYS

O.D. Max : 0,472
Pos. No. : 2
Rack No. : 2
Read time : 09:52

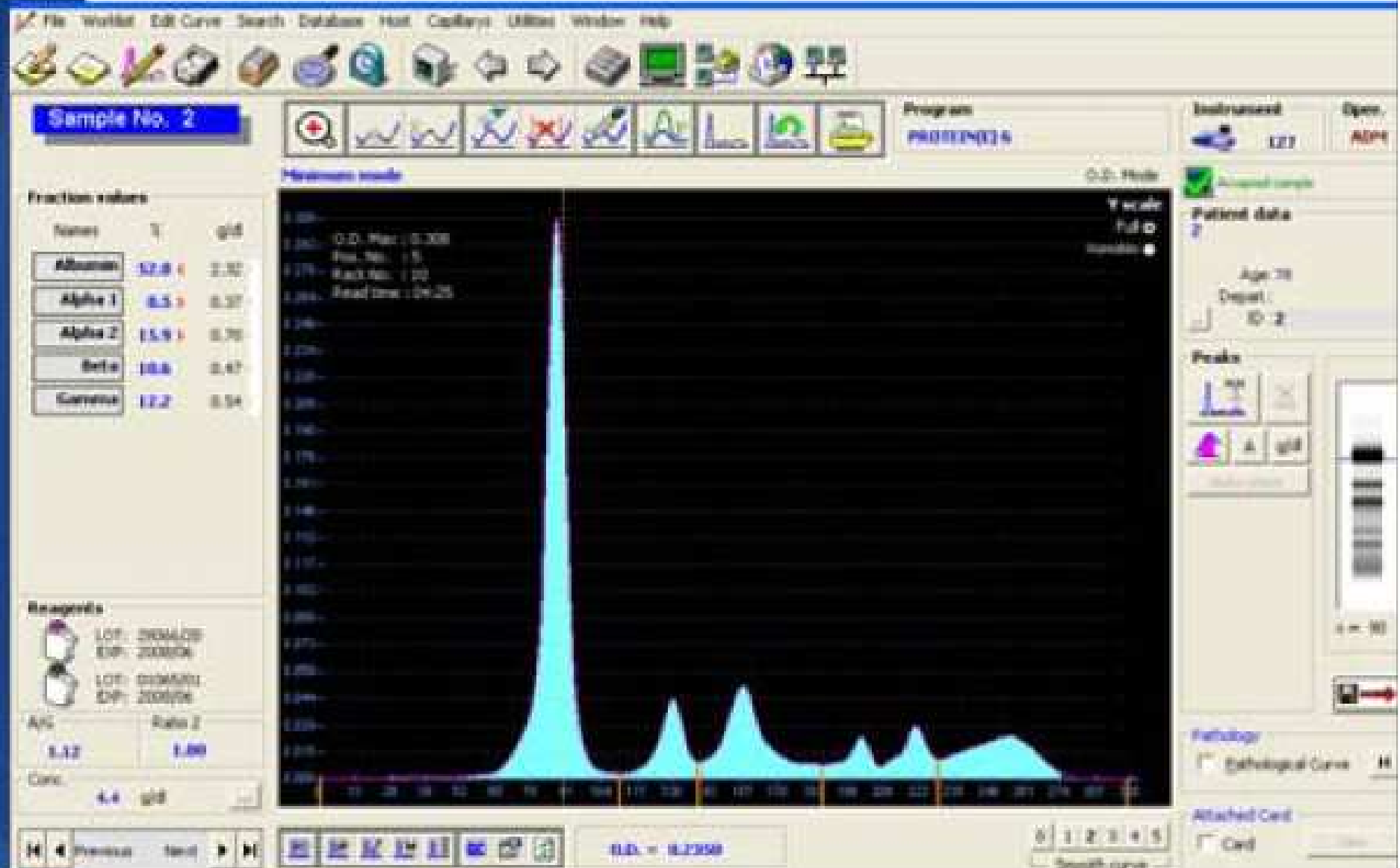


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SOME “TECHNICALITIES”

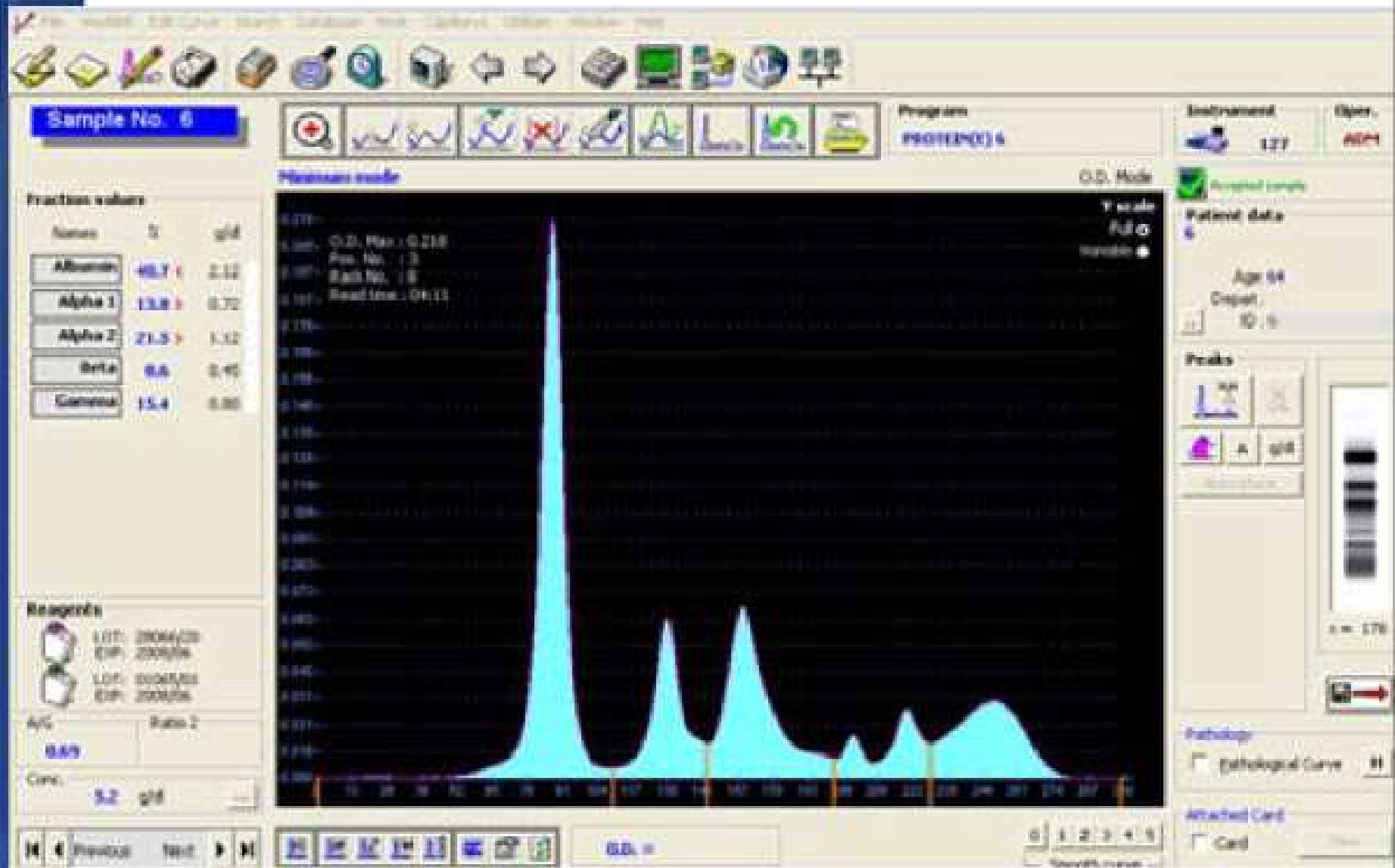
- Albumin is “anchored” at a constant position
- Grid on X axis is divided into 300 units (0-300) that are used as reference points
- Migration length is affected by distribution of gamma globulins
 - such as the presence of monoclonal component
- Maximum Optical Density is measured on albumin fraction

PROTEIN LOSS PATTERN



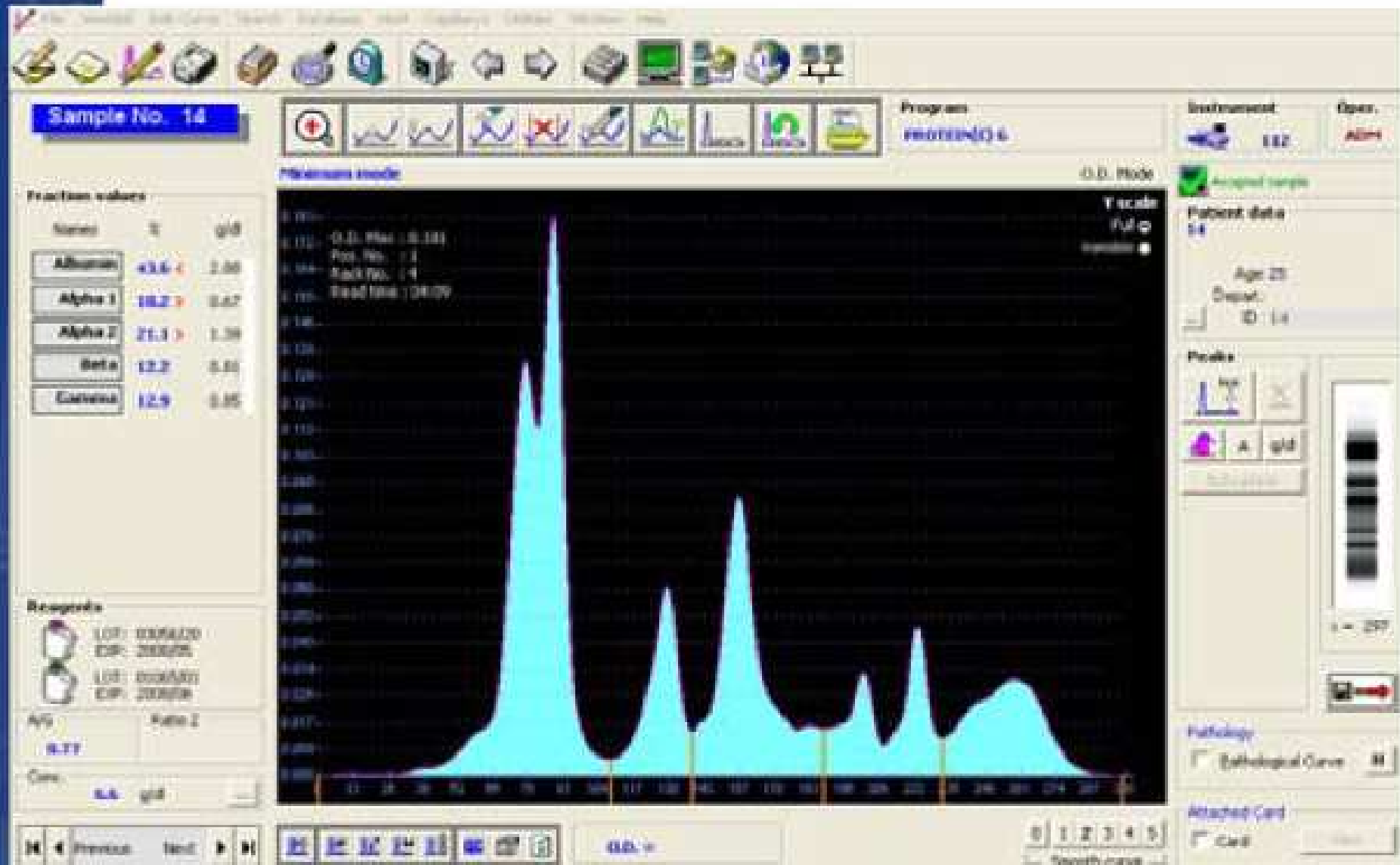
Relative concentration of alpha 1 and alpha 2 are elevated . Albumin and transferrin are decreased. IF?... Gamma questionable

ACUTE INFLAMMATION



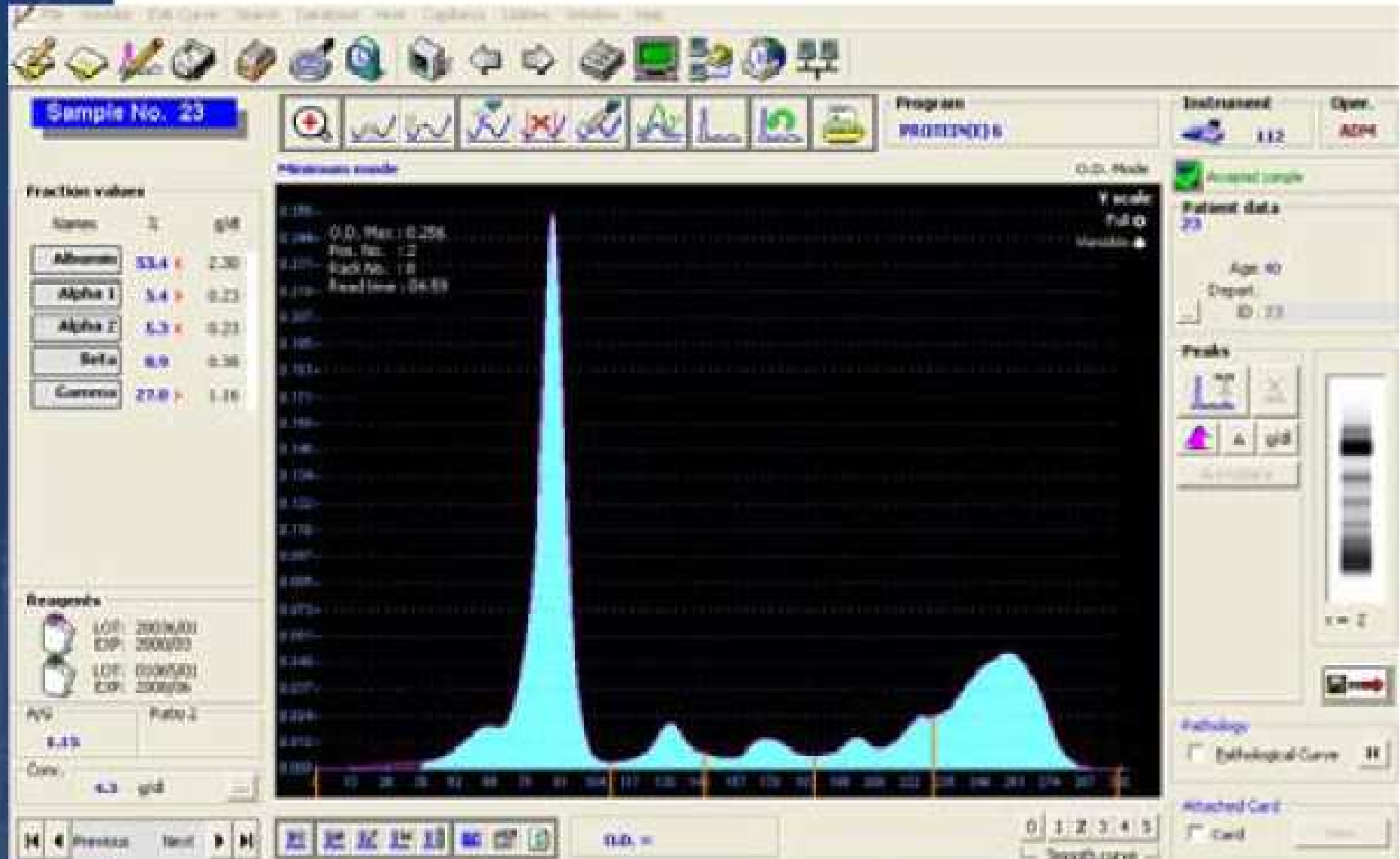
Acute phase proteins are relatively increased. Renal problem? Patient follow up appropriate. IF not suggested

WHAT CAN WE SEE HERE?



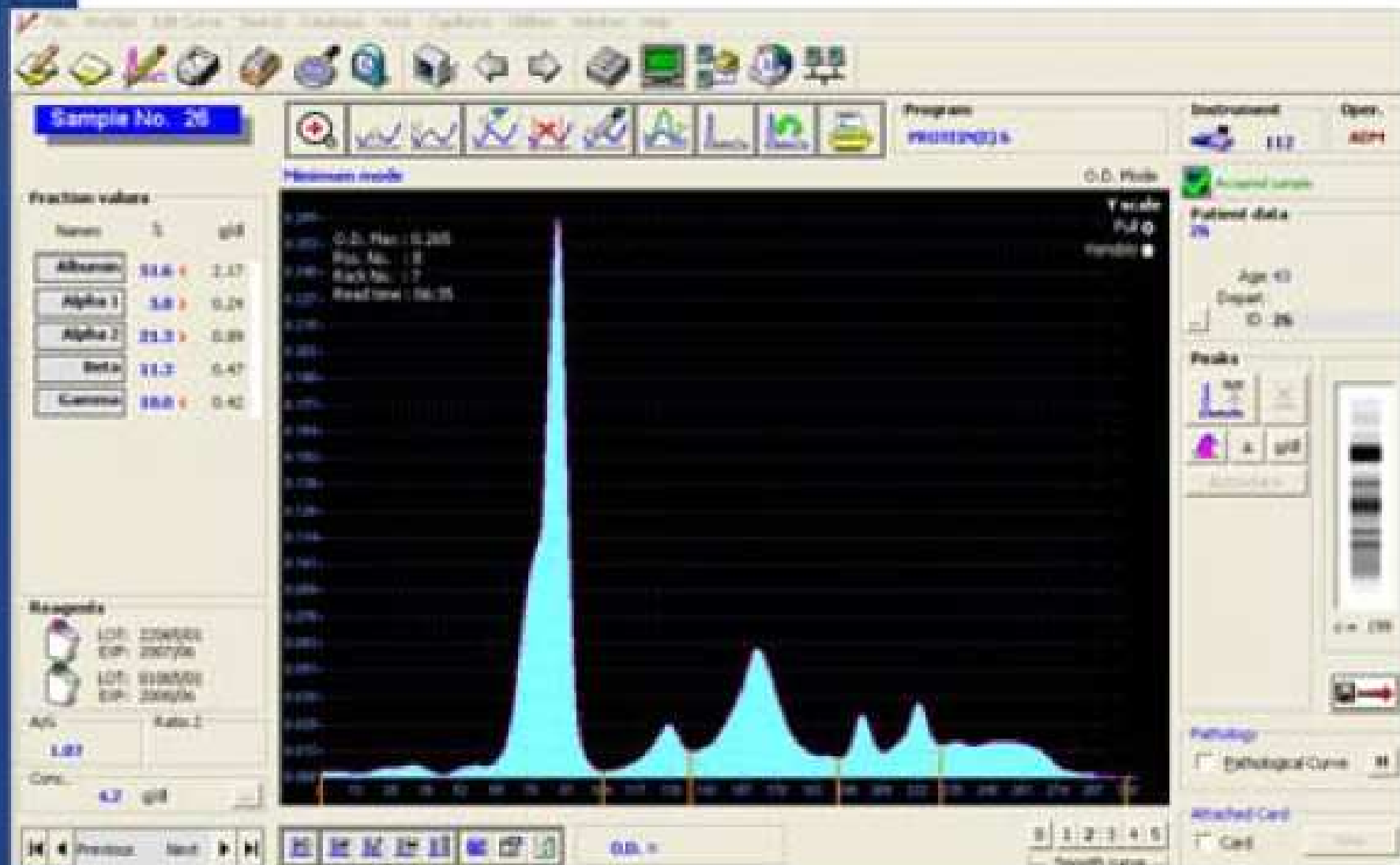
Bisalbuminemia is present. Acute phase proteins are increased, gamma may contain CRP. IF? No (age of patient)

CIRRHOSIS



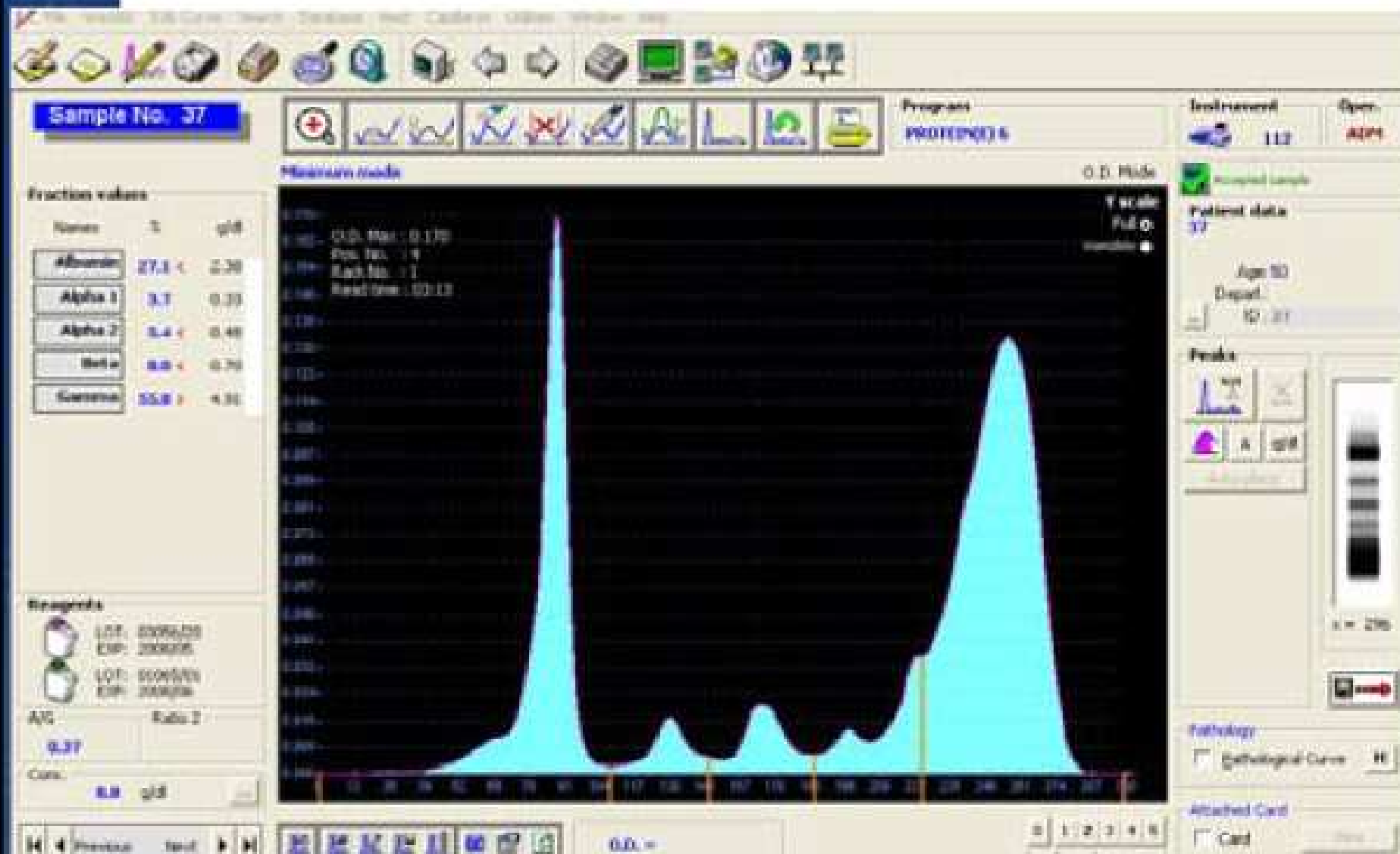
Shoulder on the left (anodal) side of the albumin peak which may be due to either drug binding (i.e., antibiotics) or lipoproteins. Beta-gamma bridging suggestive of increase in IgA due to cirrhosis, infections. NO IF

VARIOUS ISSUES



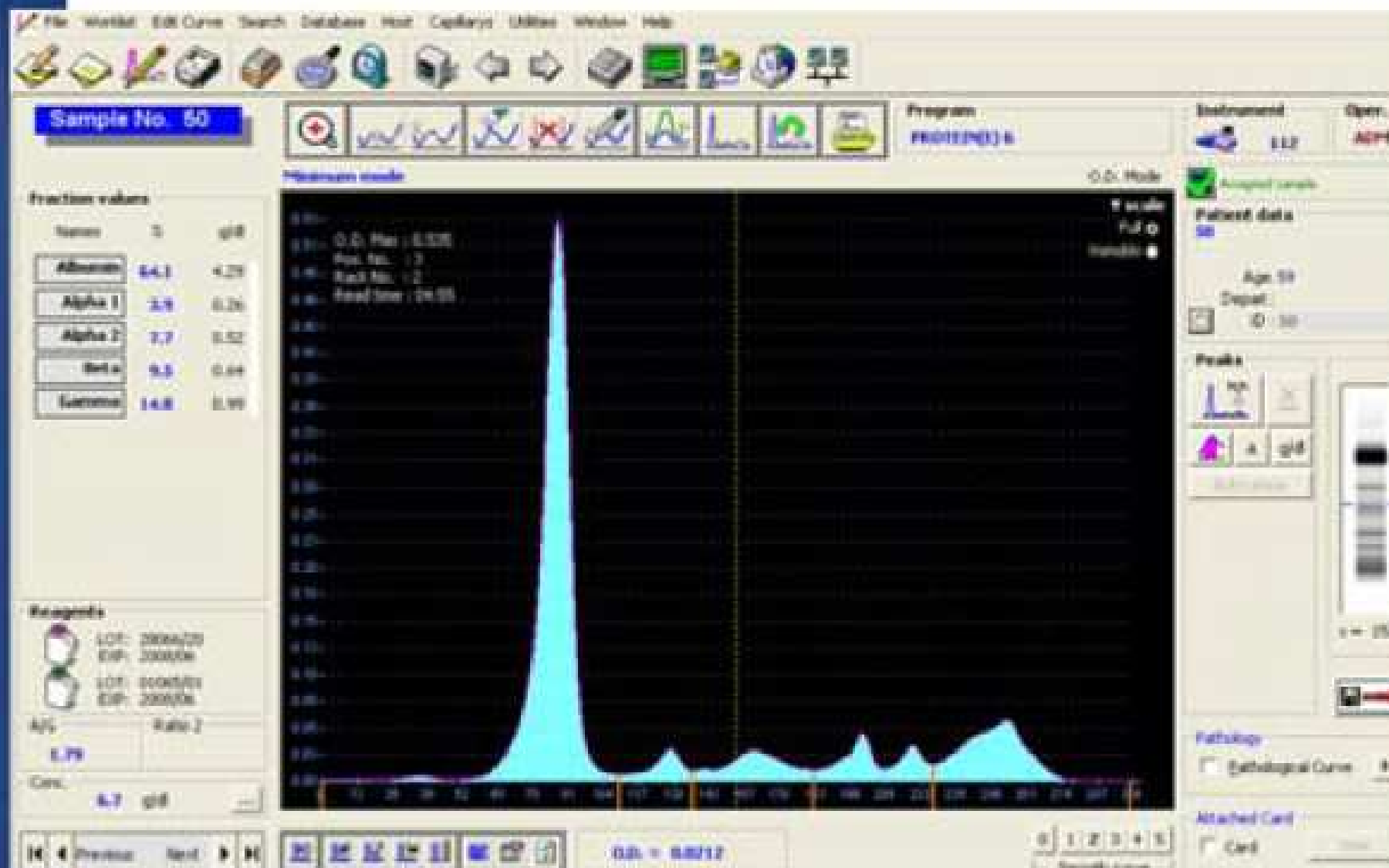
Shoulder on the left of Alb may be lipoproteins, more visible because of the Alb decrease. Relative increase of Alpha1 and Alpha2. Gamma irregular due to low concentration (loss of Igs through the kidney). IF (age of patient?) and urine analysis is suggested.

BETA-GAMMA BRIDGING



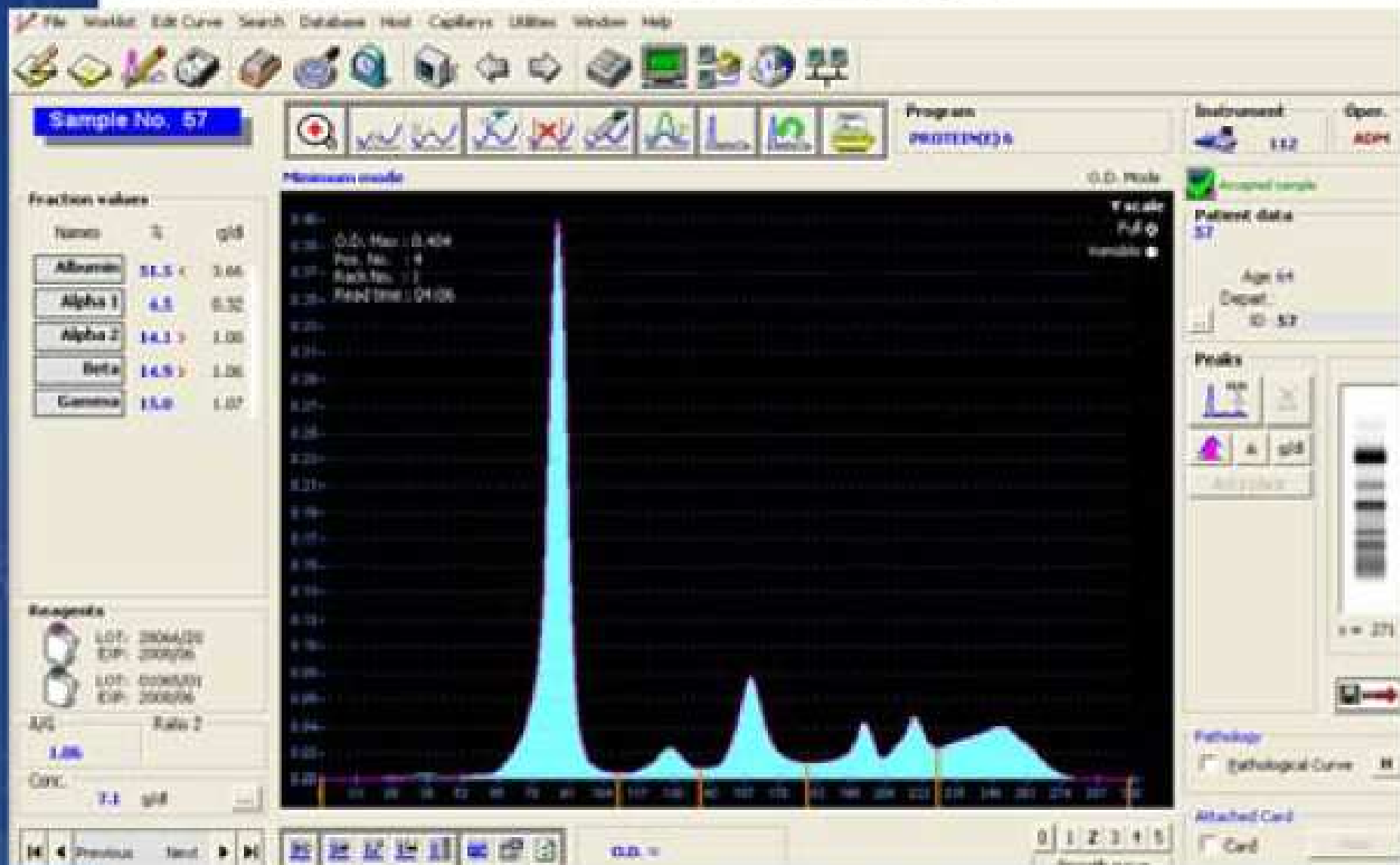
Shoulder on the left side of the Alb. Beta-gamma bridging. Suggestive of increase in IgA due to cirrhosis and some infections. Huge polyclonal increase in gamma. IF?... According to clinical context.

MONOCLONAL PROTEIN PRESENT



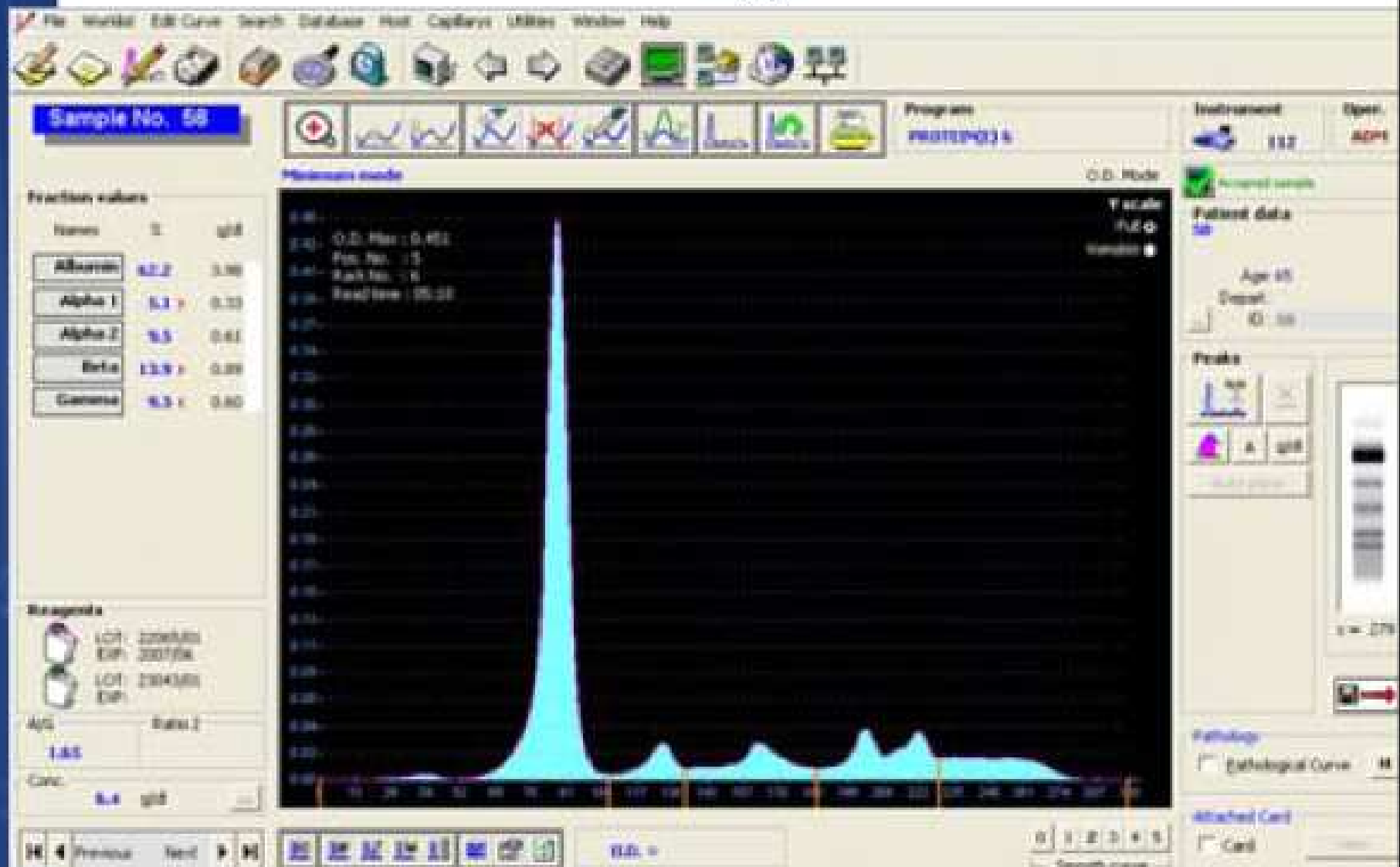
Morphological abnormality in Gamma zone. Monoclonal protein suspected. IF?... Yes

ABNORMAL GAMMA REGION



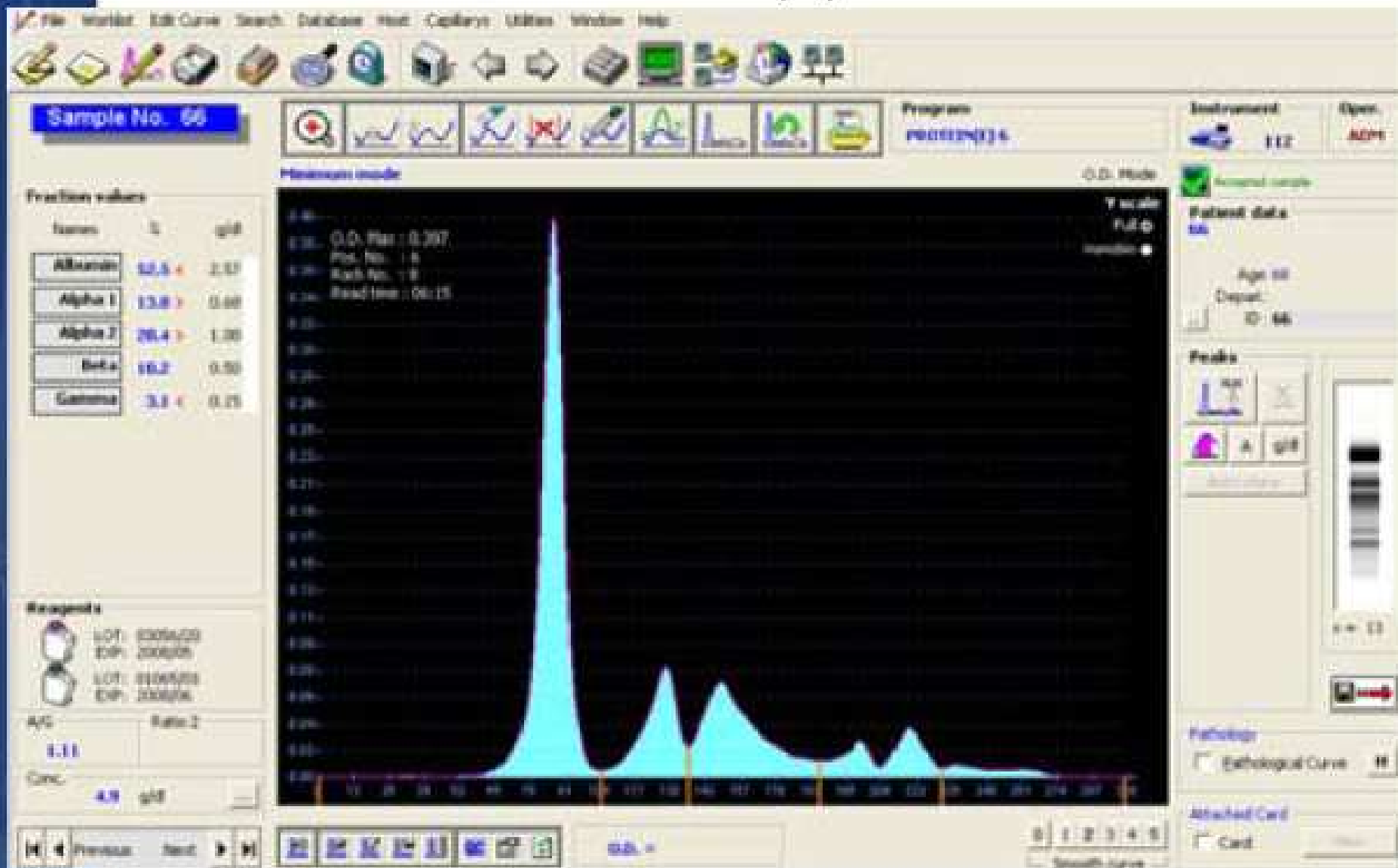
Morphological abnormality in gamma zone. Monoclonal protein suspected.
IF?... Yes

IS IT NECESSARY? (I)



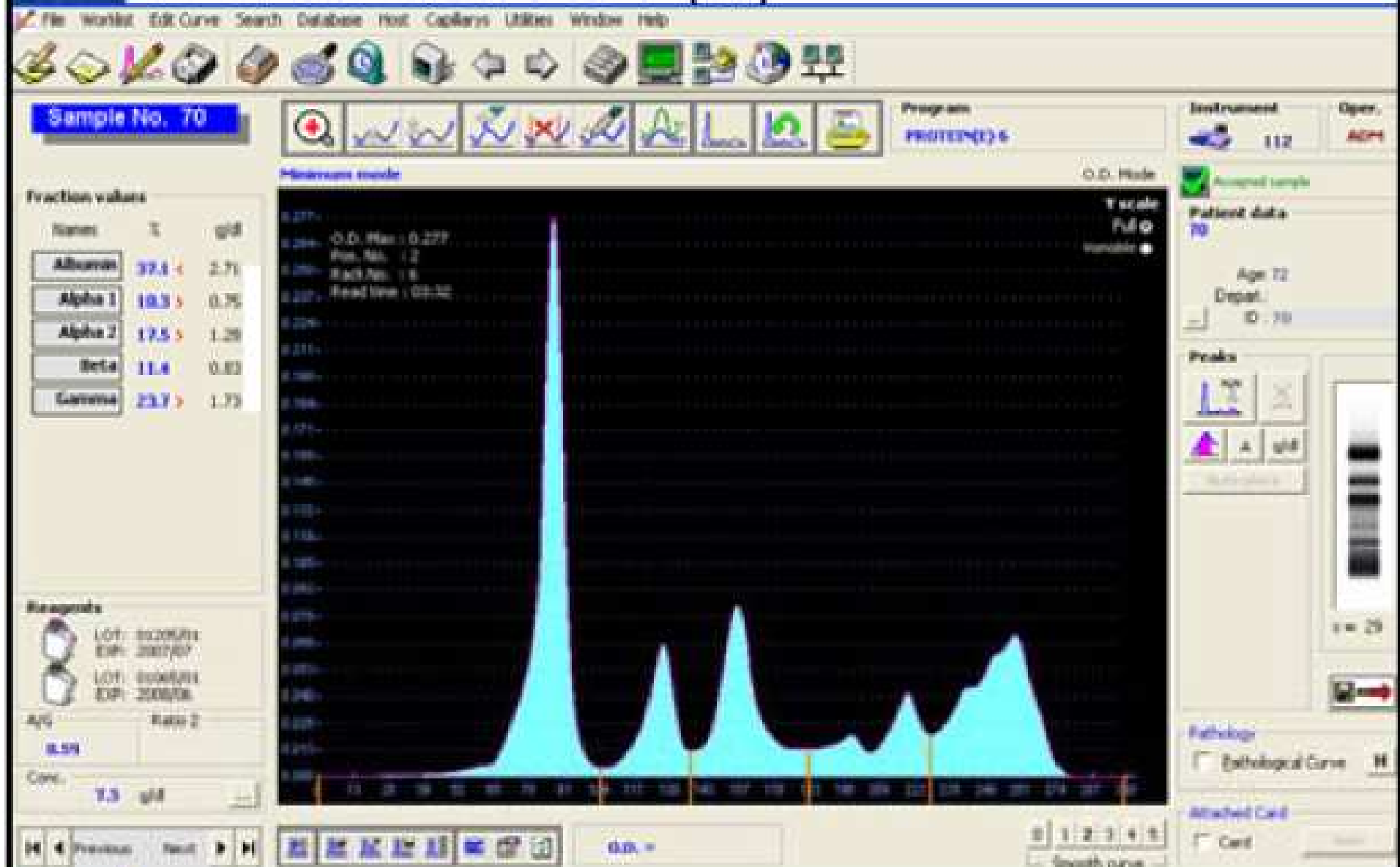
Hypogammaglobulinemia and Beta 2 > Beta 1. Perform IF.

IS IT NECESSARY? (II)



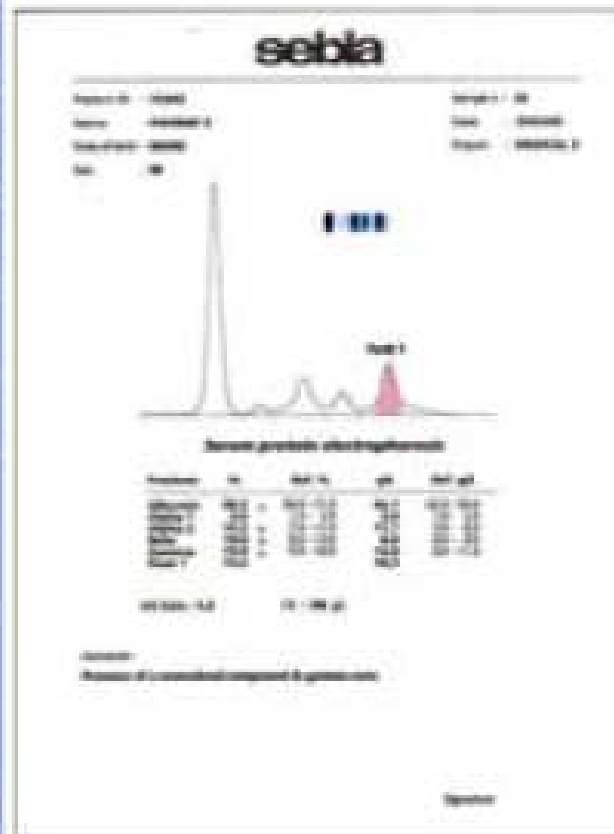
Severe hypogamma, beta 2 irregular and increased. Perform IF

IS IT REQUIRED? (III)



Inflammatory profile with oligoclonal banding. IF suggested

FINAL RESULT IS THE SAME FOR BOTH GEL AND CAPILLARY ELECTROPHORESIS



- Proteins are separated into fractions and quantitated
- Results from both methods are extremely close and correlate well
- Differences:
 - Protein fractions are quantitated in real time on CAPILLARYS
 - no "material" proof on results (such as film, gel etc.) is available
 - Direct fraction measurement on CAPILLARYS vs. indirect (stain based) on gels

CAPILLARYS IMMUNOTYPING (IT) INTERPRETATION

Immunofixation (IFE)

Separation of proteins by electrophoresis



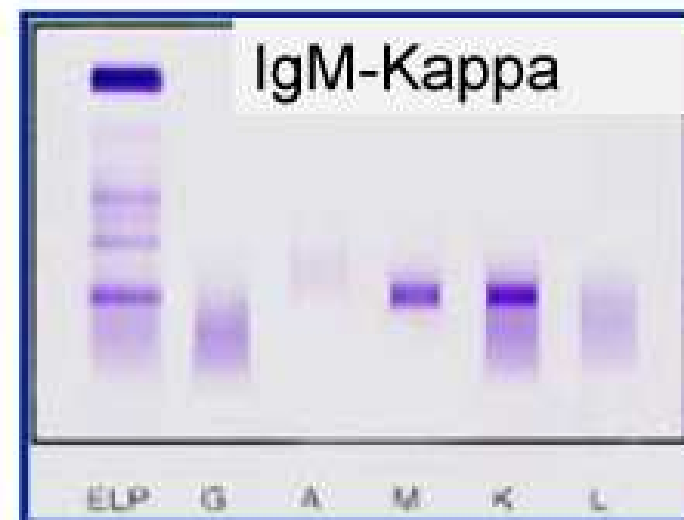
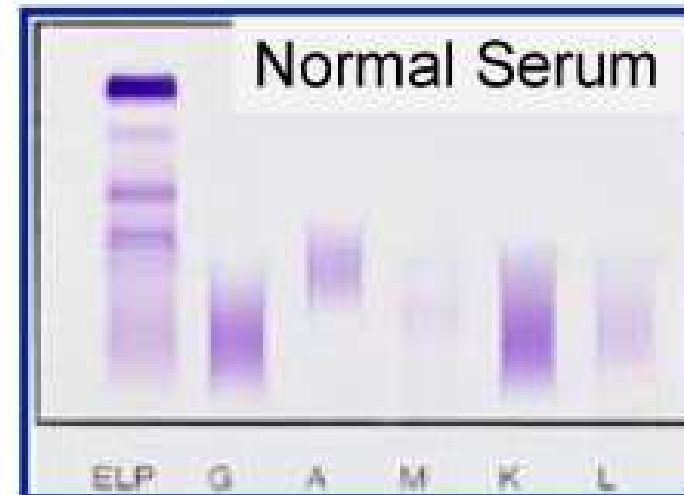
Immunoprecipitation of electrophoresed proteins



Removal of non-precipitated proteins



Acid violet staining of precipitated proteins



IMMUNOTYPING

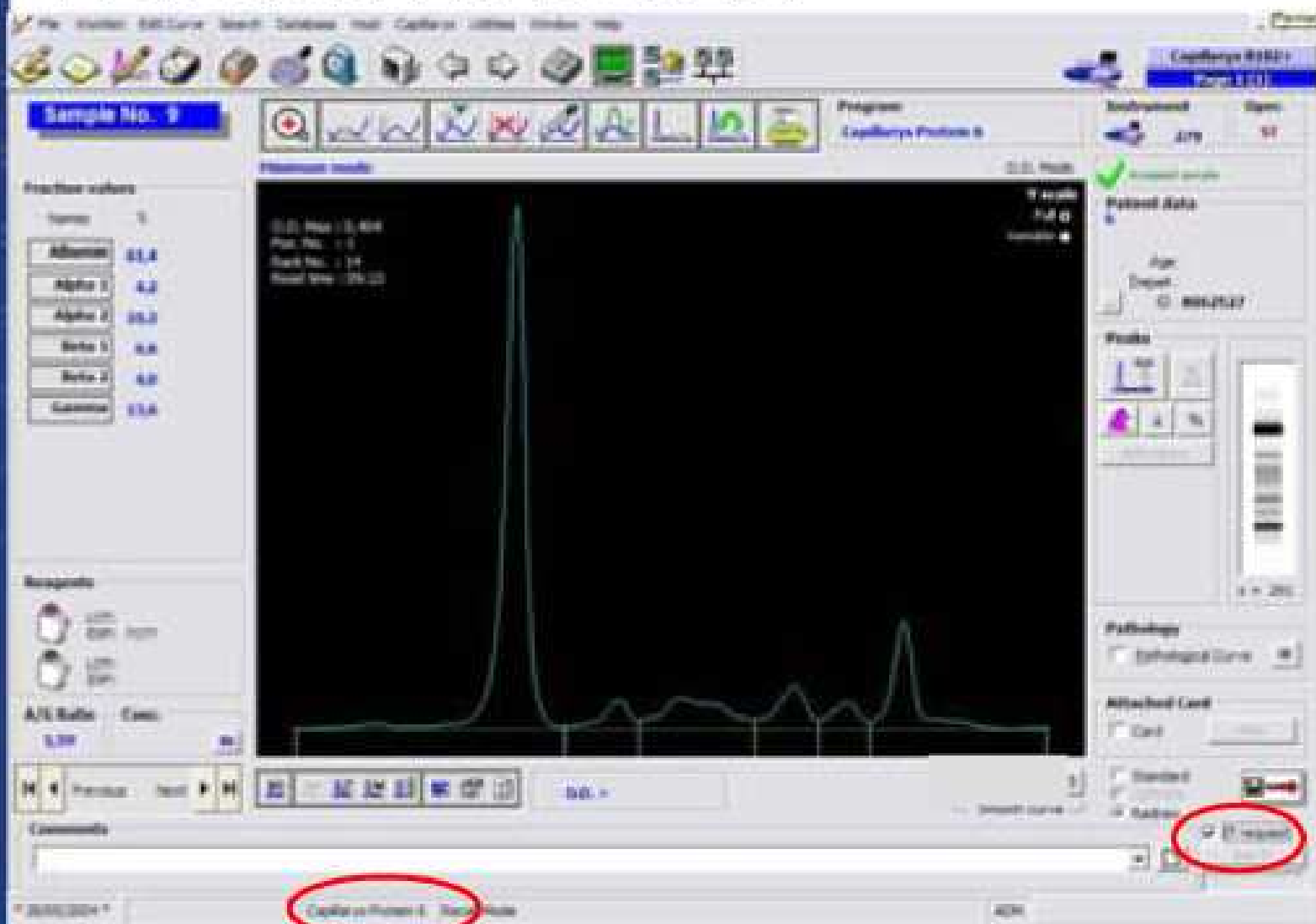
SEBIA'S Automated Alternative to Immunofixation




Immunotyping: The “New” IFE








- Immunotyping is the capillary electrophoresis equivalent of an IFE
 - No gel – migration takes place in a buffer medium, rather than on agarose gel
- Uses mono-specific antisera
- Immunotyping is a reduction technique
 - The antiserum combines with the immunoglobulin, creating a large molecule which migrates slowly and is pulled out of the viewing area
- A positive reaction in one of the antisera treated frames will result in the Ig component being removed from the sample curve

ABNORMAL PROTEIN




Capillary 00001
April 11, 2012

Sample No. 4








Program
Capillary Protein 4

Peak list values

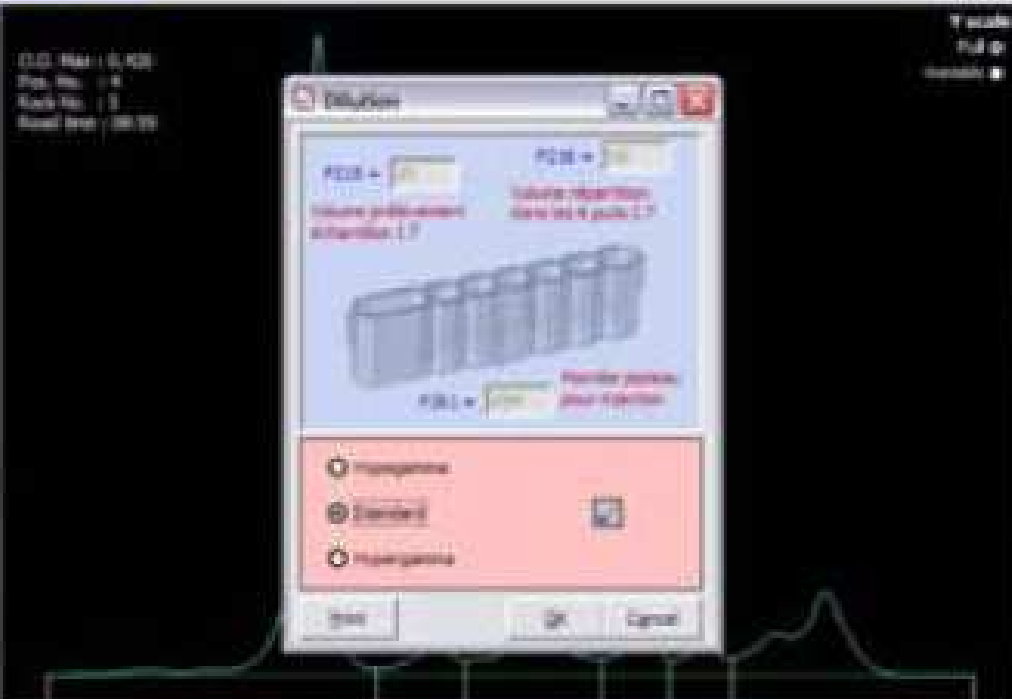
Time	Area
Alpha 1	10.0
Alpha 2	10.0
Beta 1	10.0
Beta 2	10.0
Gamma	10.0

Settings

100%
 50%
 25%

All Peaks
 Cont.

Minimum width



Original
 Diluted
 Magnified

Peak List
 Peak List
 Peak List
 Peak List

Contour
 Contour
 Contour
 Contour

Attached Card
 Card

Standard
 A Series

21 Report

Previous
 Next

 1
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Comments

 Print
 Save
 Close

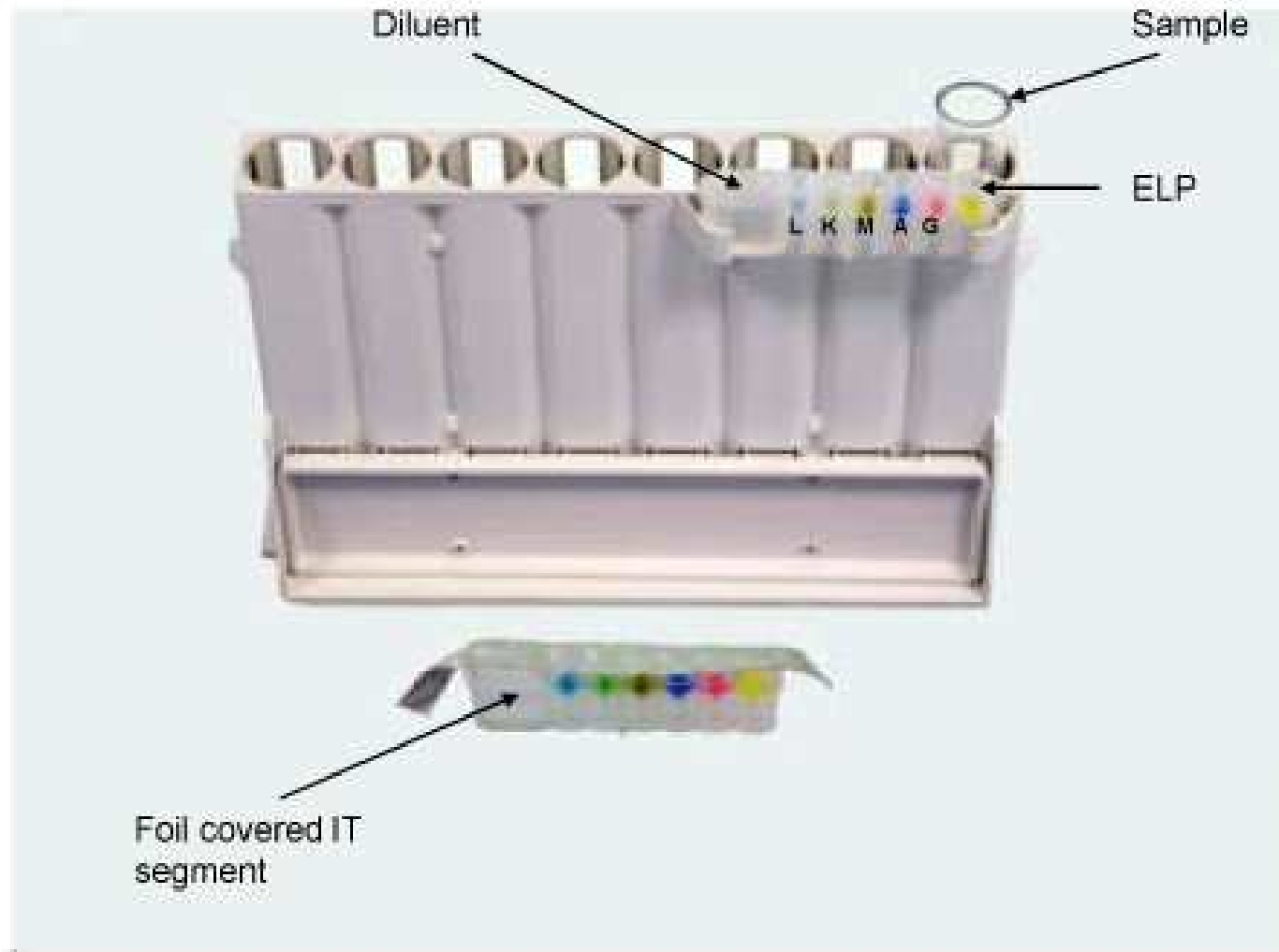
* 0000001 *
Capillary Protein 4
APL

The screenshot displays the Sebia software interface. At the top, there is a menu bar with options like 'File', 'Edit', 'View', 'Tools', 'Help', and 'Windows'. Below the menu bar is a toolbar with various icons. The main window is divided into two sections. The upper section contains a table with columns: 'No.', 'Sex', 'Age', 'Date of Birth', 'Age Report', 'ID', 'Sample Date', and 'Time'. The lower section contains a larger table with columns: 'No.', 'Name', 'Sex', 'Date of Birth', 'Age Report', 'ID', 'Sample Date', and 'Time'. To the right of the upper table, there is a chromatogram showing a single prominent peak. The status bar at the bottom of the window reads 'Sebia-Protein 6 - Sample Data'.

No.	Sex	Age	Date of Birth	Age Report	ID	Sample Date	Time
1		21			000001		
2		20			000002		
3		20			000003		
4		20			000004		
5		21			000005		
6		21			000006		
7		24			000007		
8		20			000008		
9		4			000009		
10		20			000010		
11		20			000011		
12		20			000012		
13		20			000013		
14		20			000014		
15		20			000015		
16		20			000016		
17		20			000017		
18		20			000018		
19		20			000019		
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IT PROCESS CAN BE DESCRIBED AS A THREE-STEP PROCESS

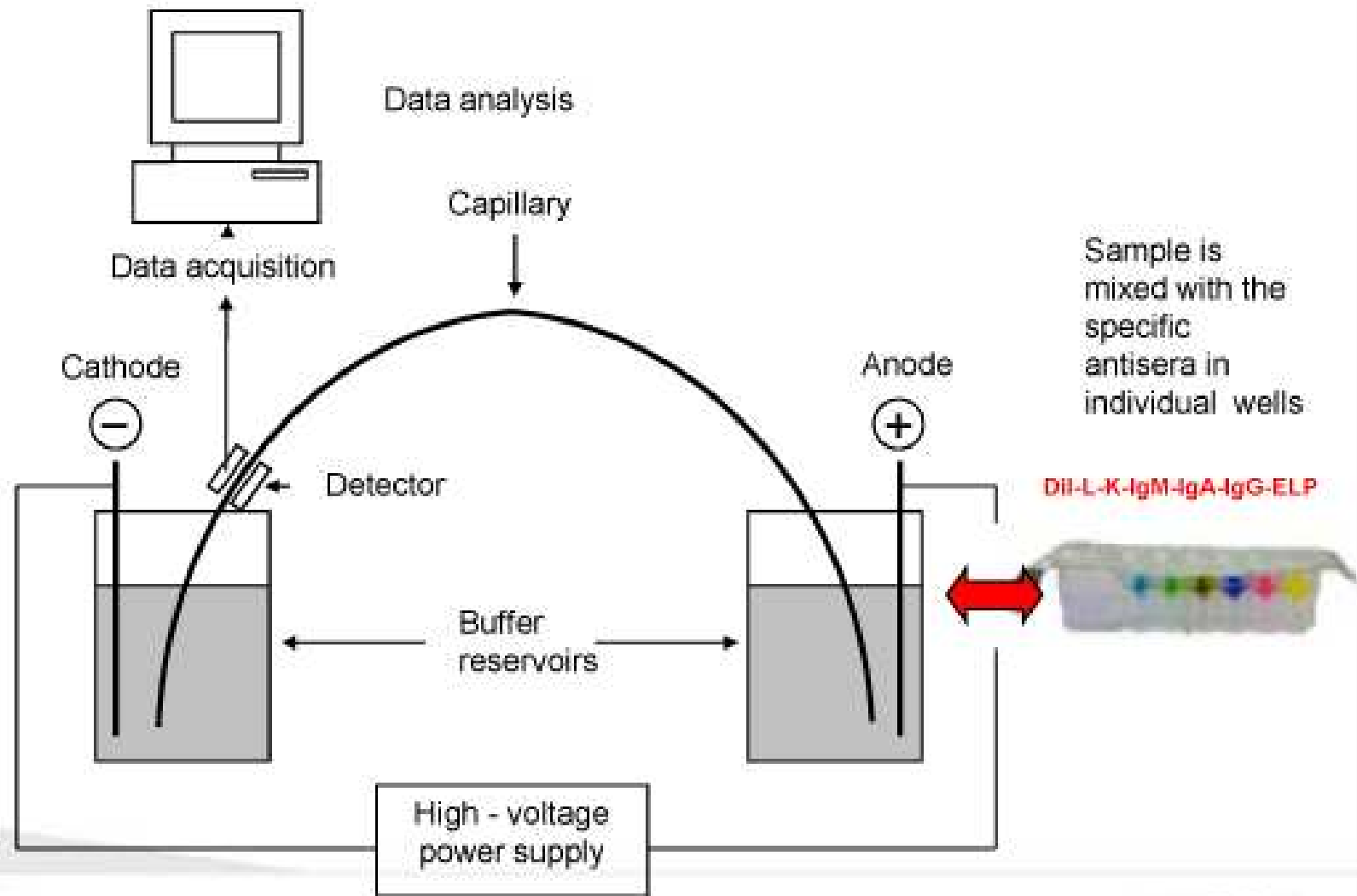
SAMPLE IS MIXED WITH ANTISERA



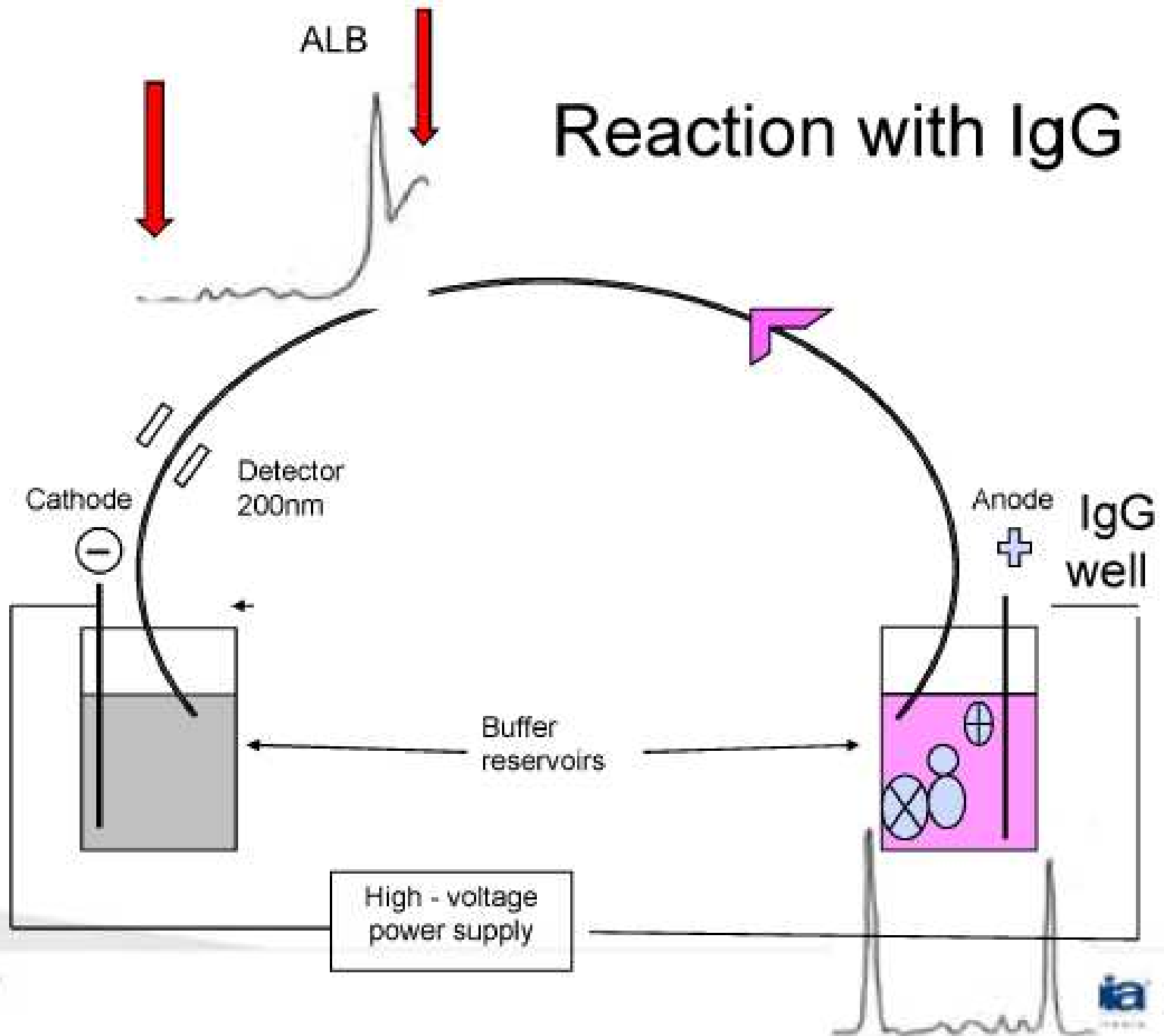
Diluted serum aliquots are mixed with individual specific antisera and an ELP reagent which will be the reference pattern

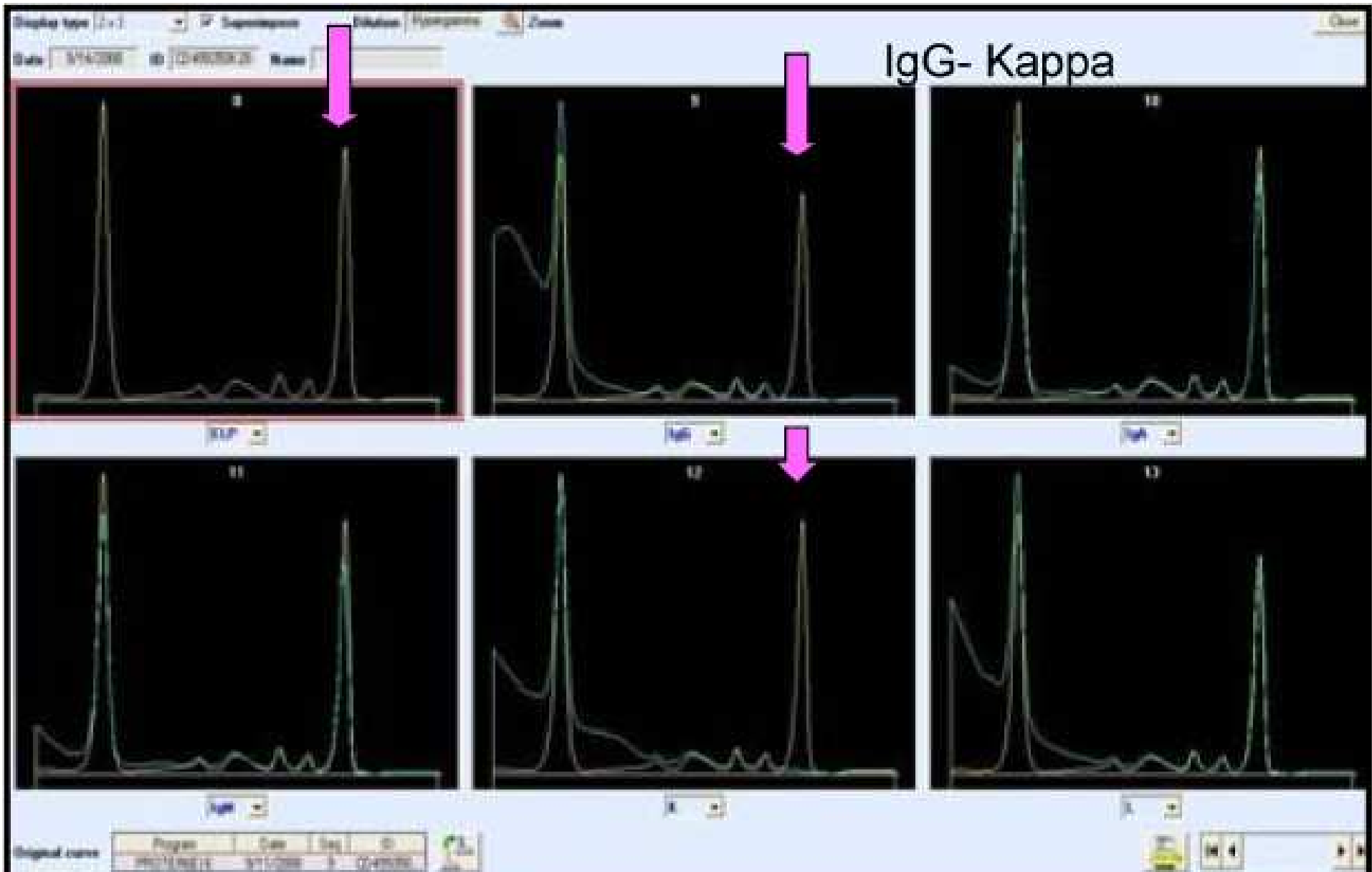
- Large soluble immune complexes are quickly formed. These migrate slower than other proteins

CAPILLARY SYSTEM DIAGRAM



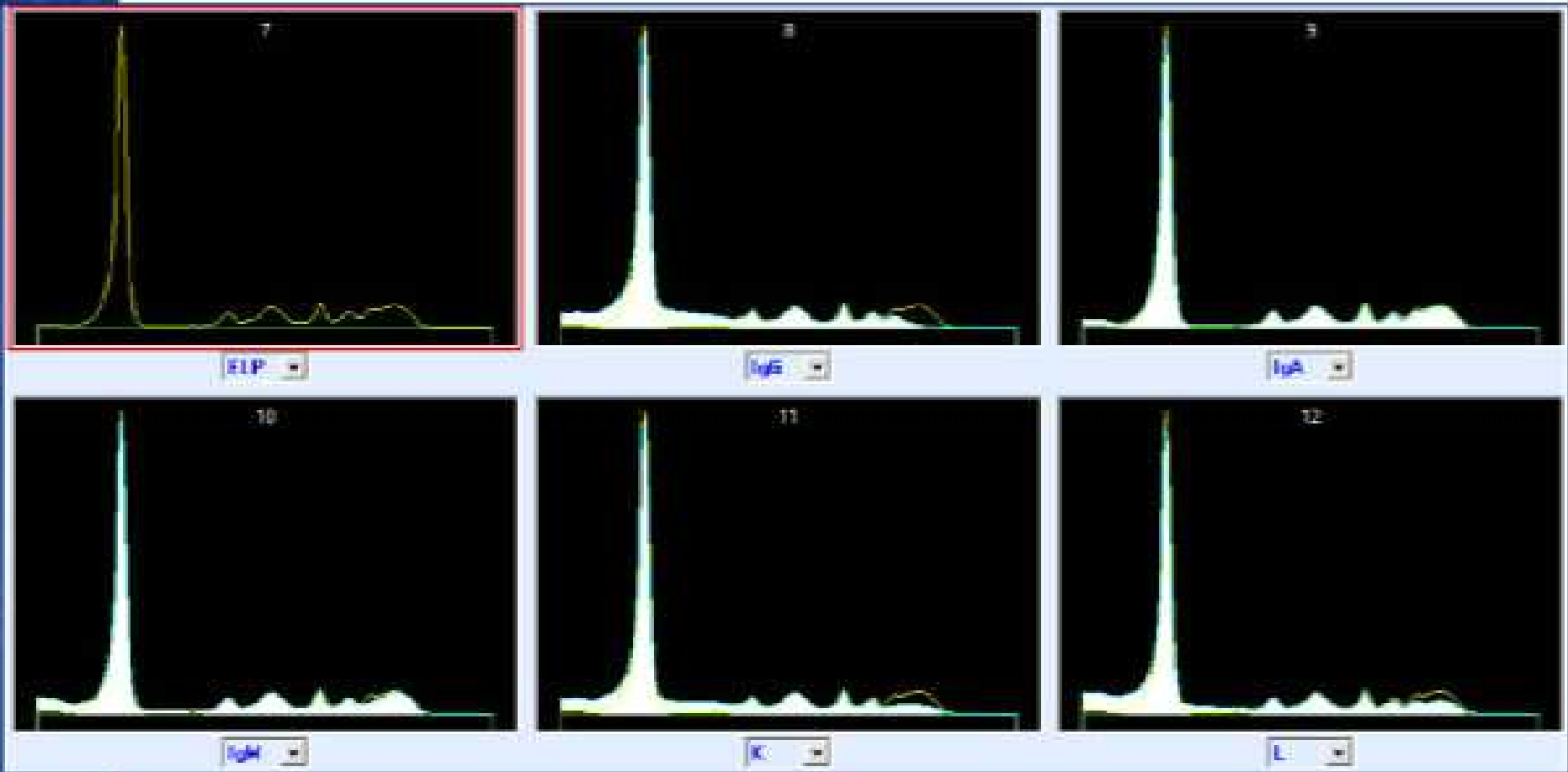
Reaction with IgG





Treated- mixed with antisera (green) and untreated (yellow) profiles are overlaid for easy interpretation.

WHAT IS IT ABOUT?



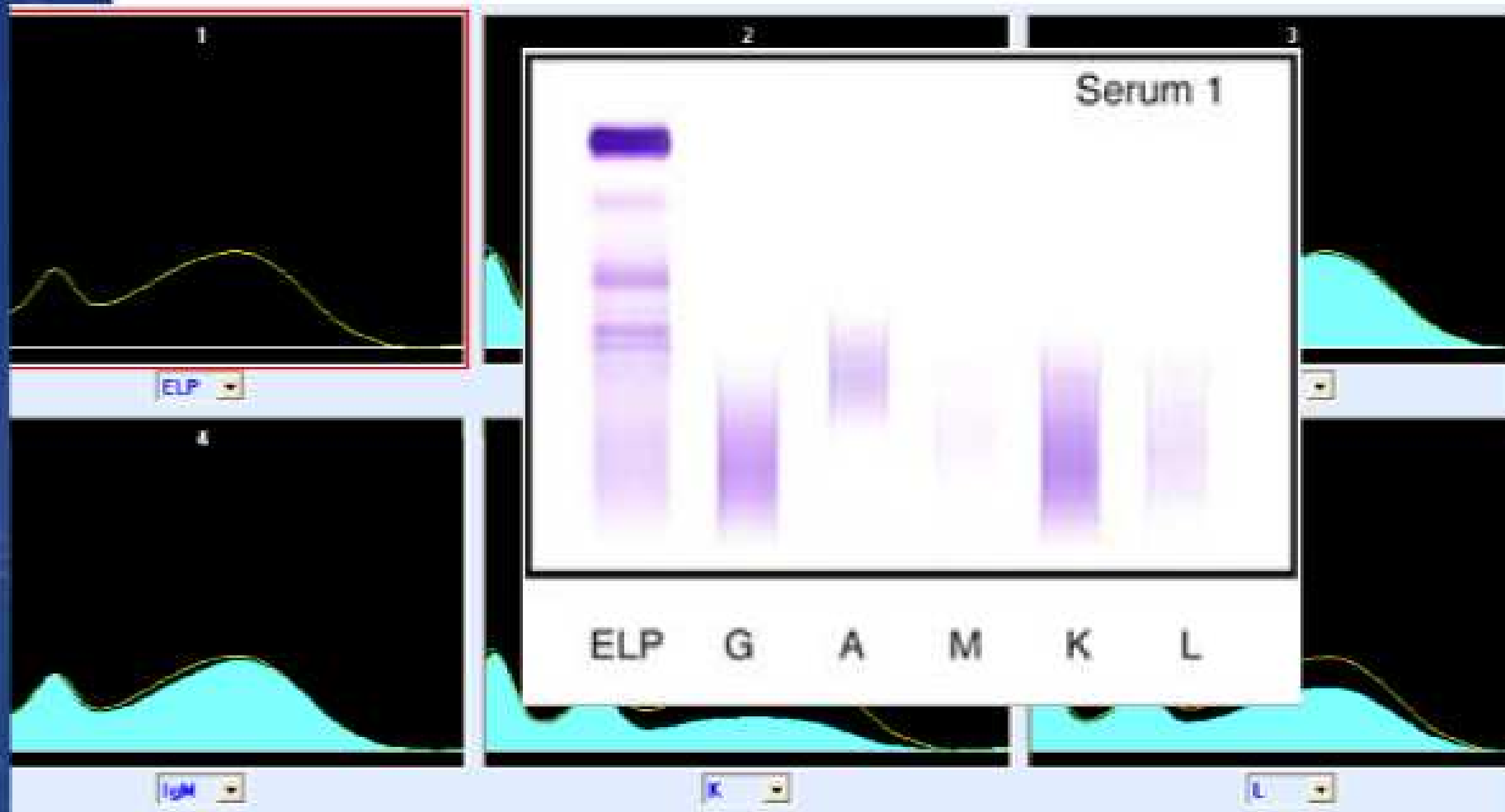
Treated and untreated profiles/patterns are overlaid for easy interpretation. Ig complexes migrate slower, out of the profile and away from the area of interpretation



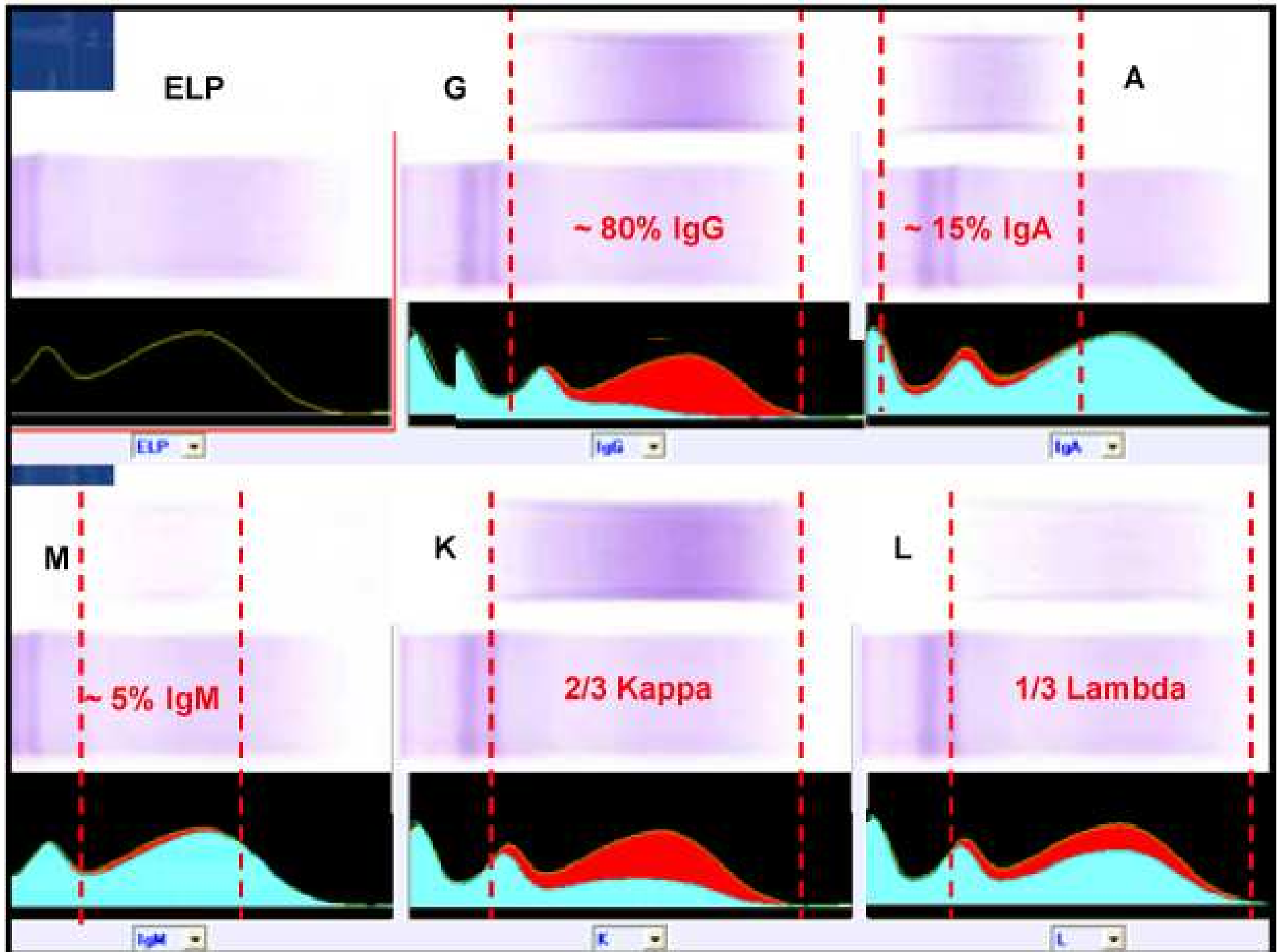
GENERAL GUIDELINES

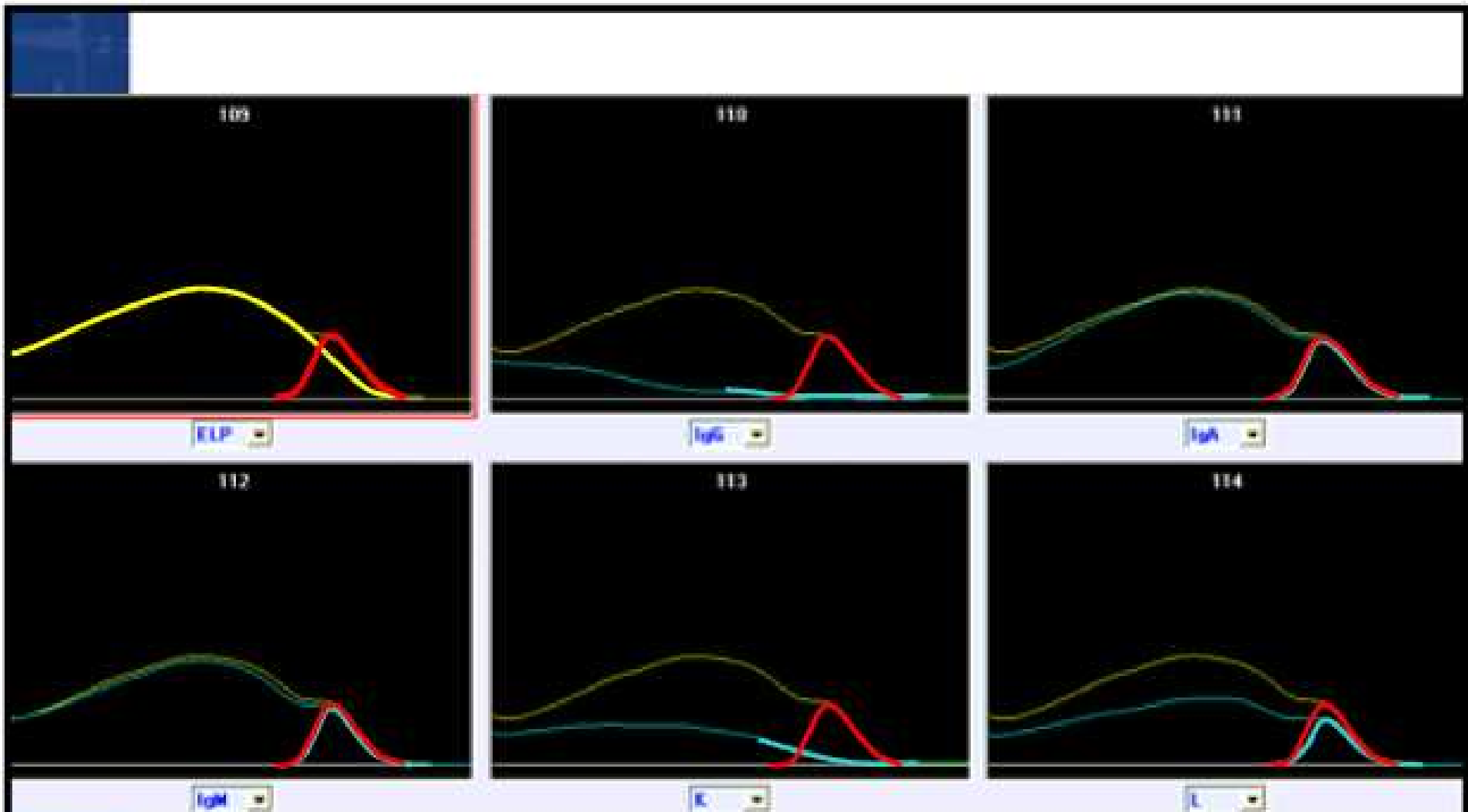
1. Look at the ELP track and closely observe all zones: Albumin, Alpha-1, Alpha-2, Beta-1, Beta-2 and Gamma. Locate area of interest and concentrate on it
2. If necessary, use zoom and smooth curve functions to better observe suspected monoclonal component

INTERPRETING IT IS AS EASY AS INTERPRETING IFE



IT is the negative of the IFE





3. On the ELP track, locate the monoclonal component (MC)
4. Locate the MC in each window, and look for the disappearance of the peak in heavy chain and light chain windows

Ig G

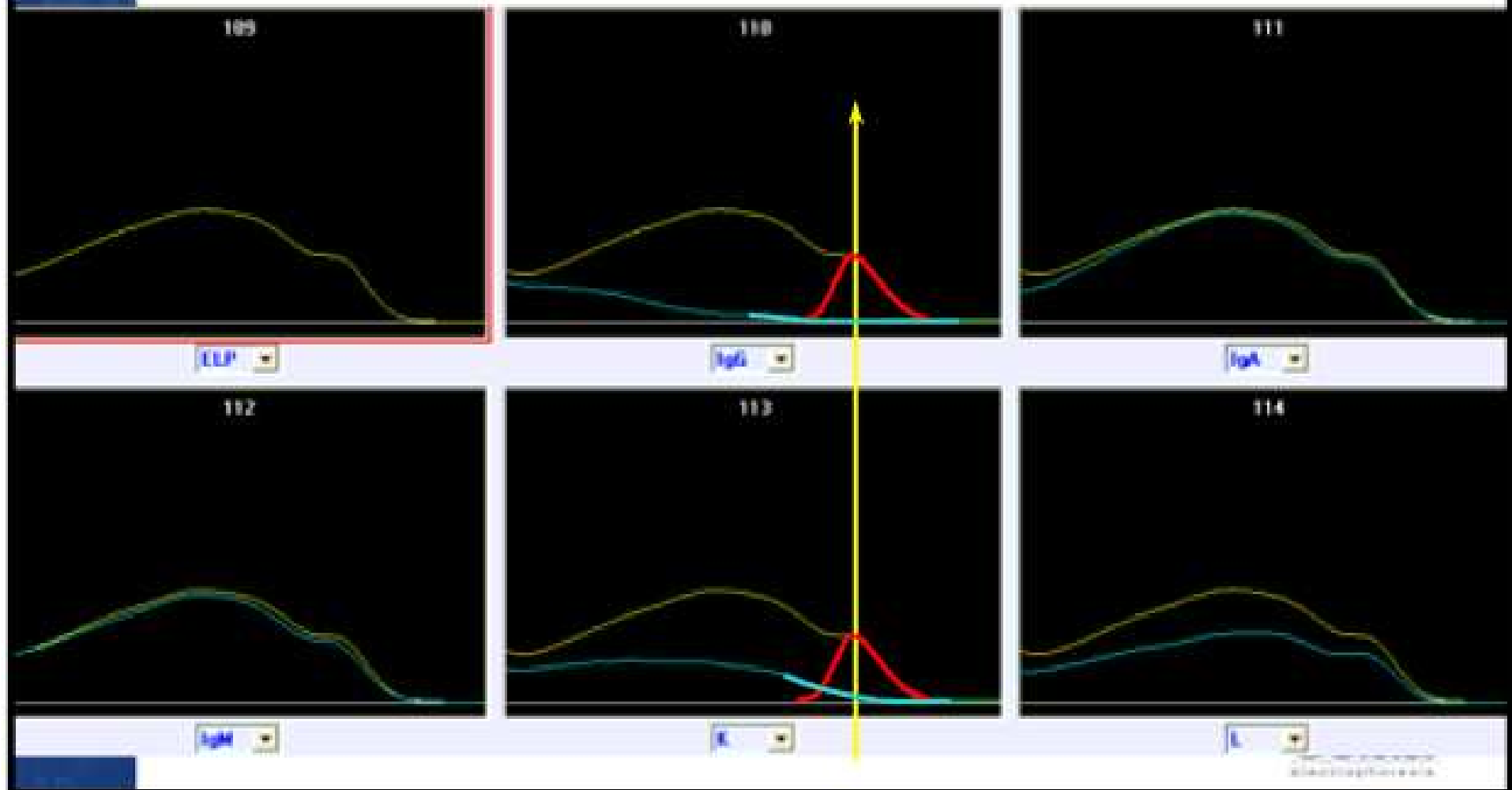
Ig A

Ig M

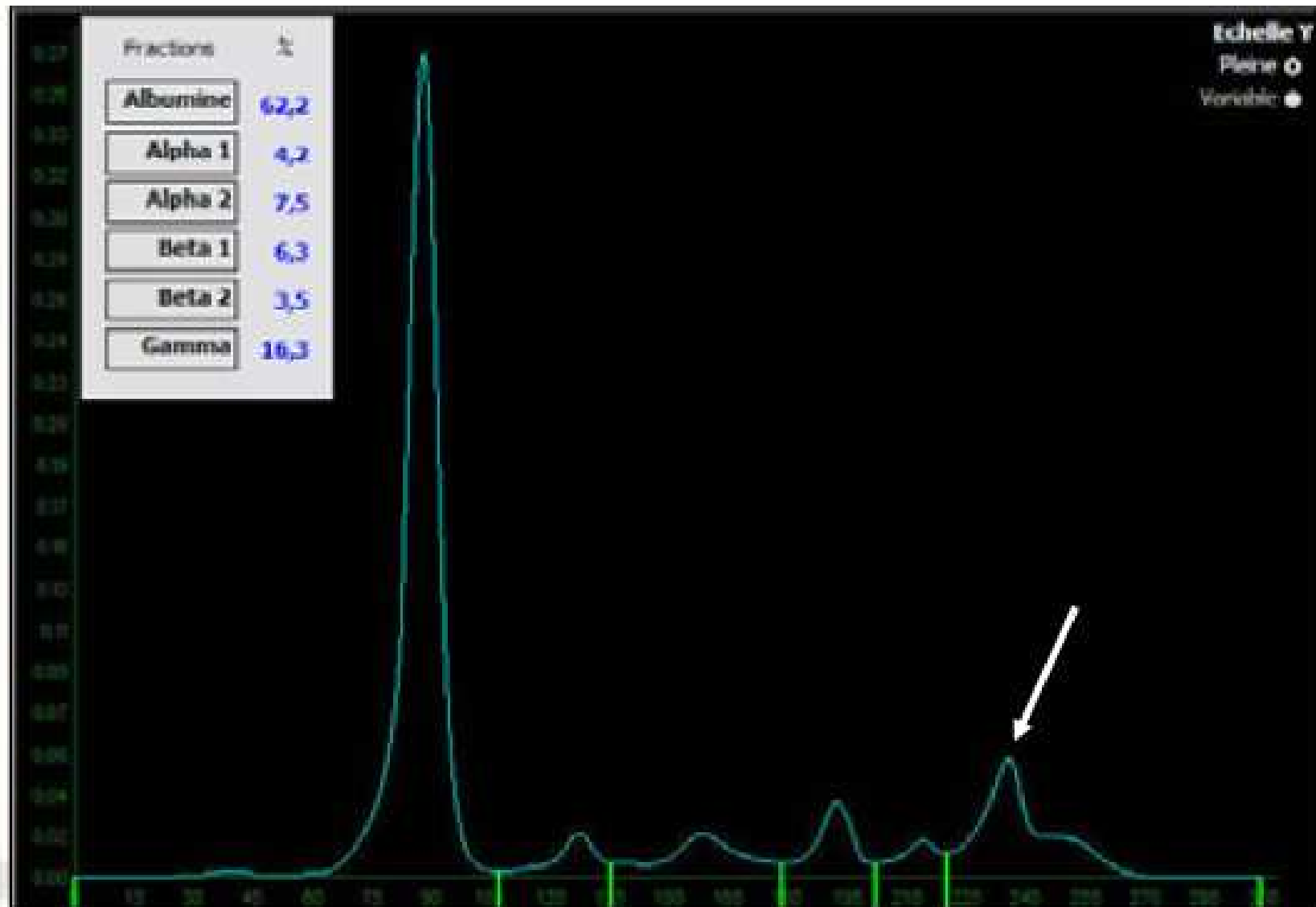
Kappa

Lambda

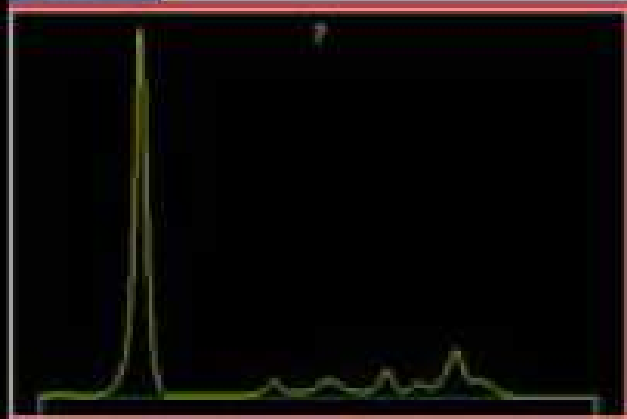
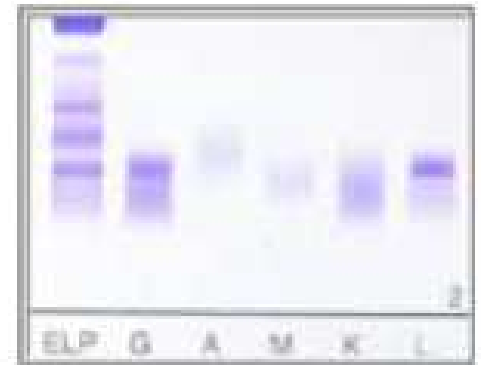
5. The MC has two heavy chains combined with two light chains. The MC must be in the same position on the heavy and the light chain track.



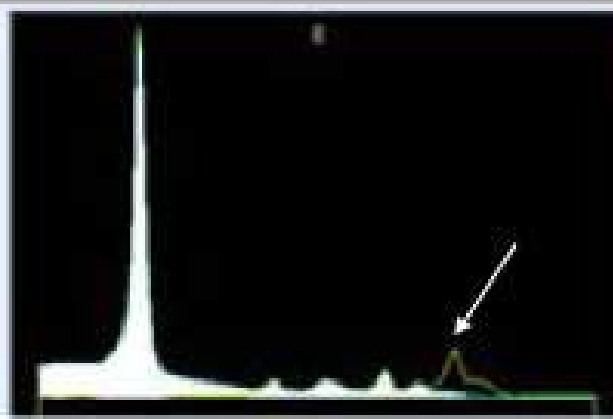
Protein(e) 6



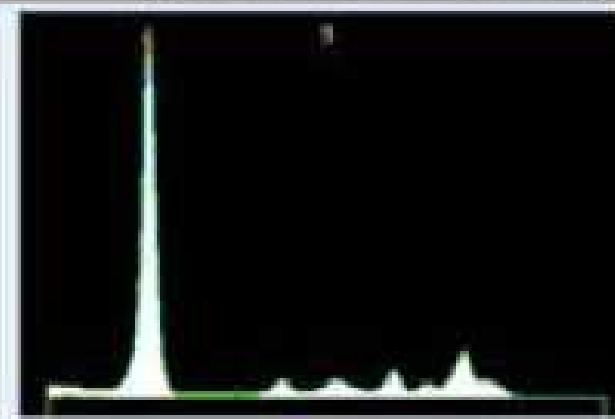
IgG/L



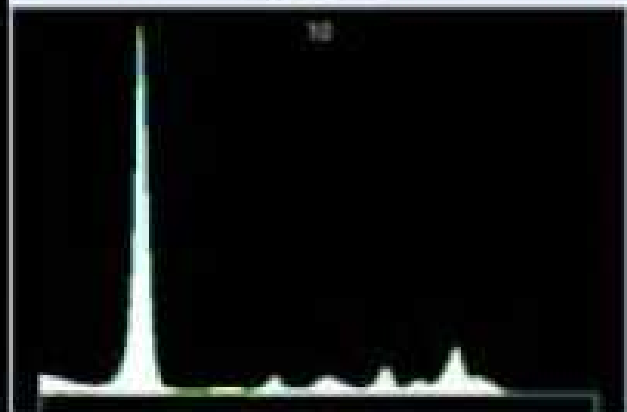
ELP



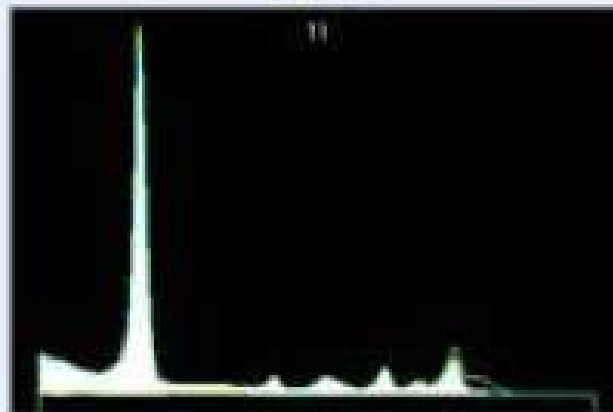
IgG



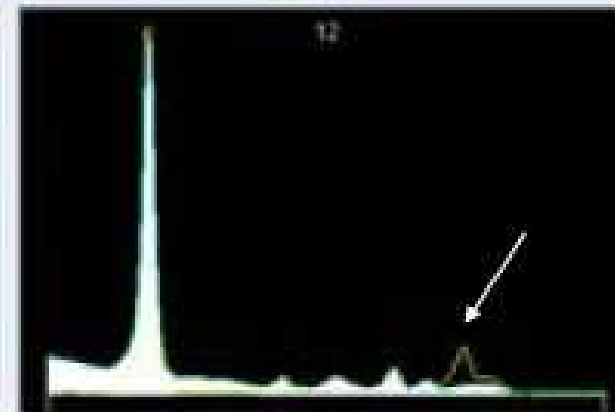
IgA



IgM



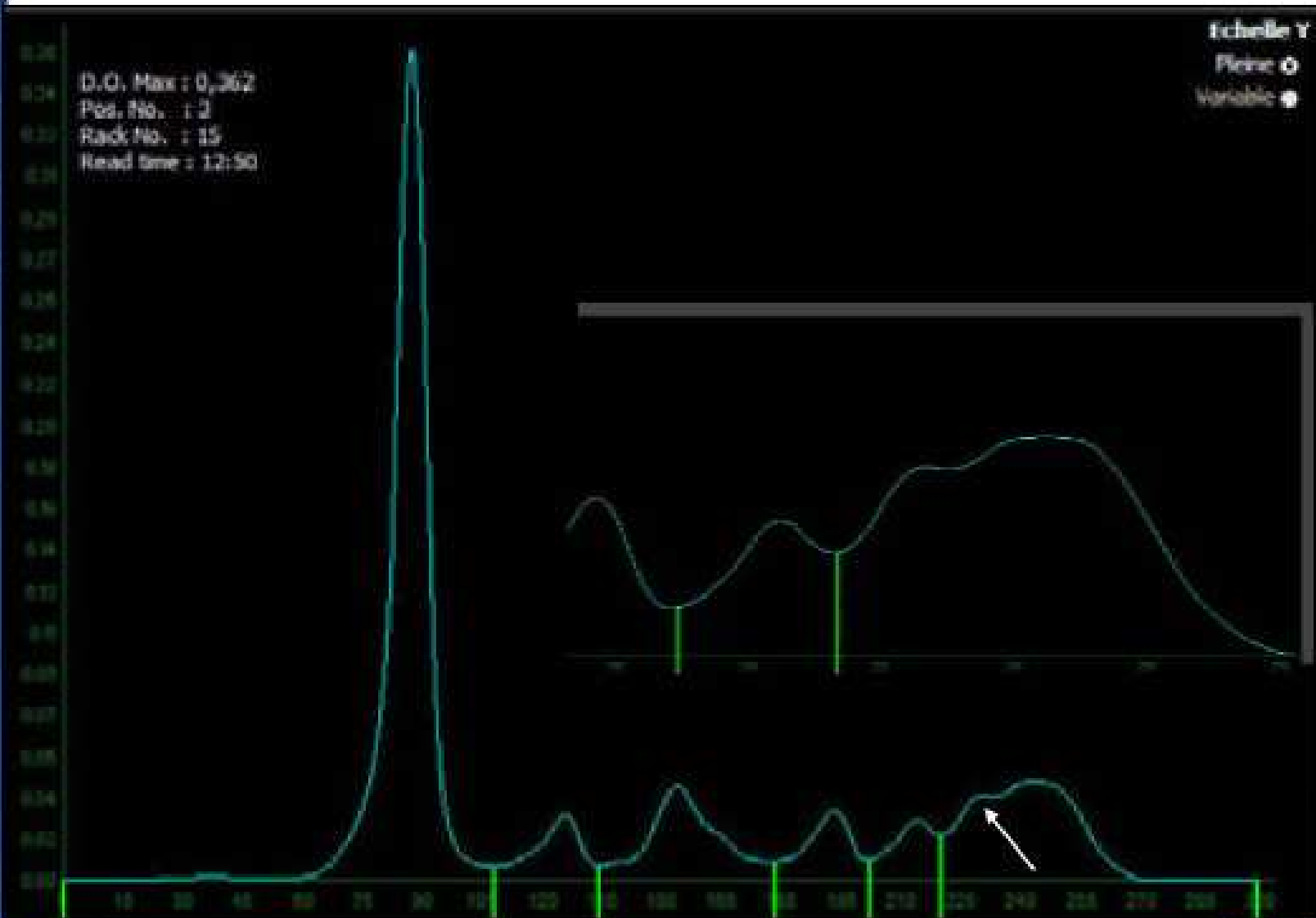
K

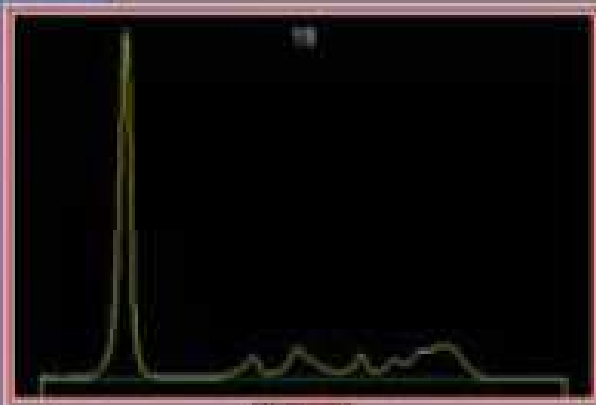


L

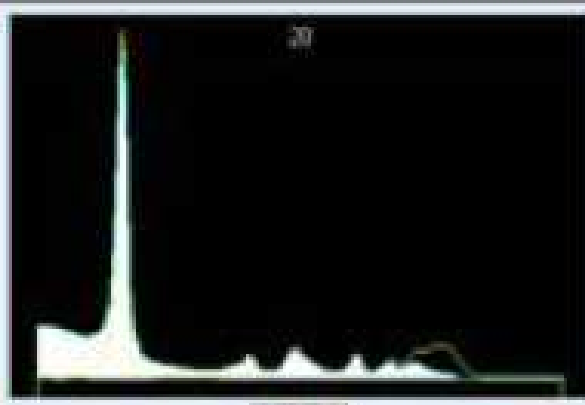
Y

Protein(e) 6

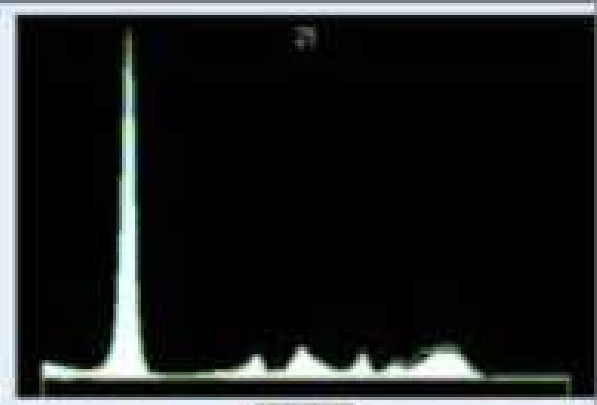




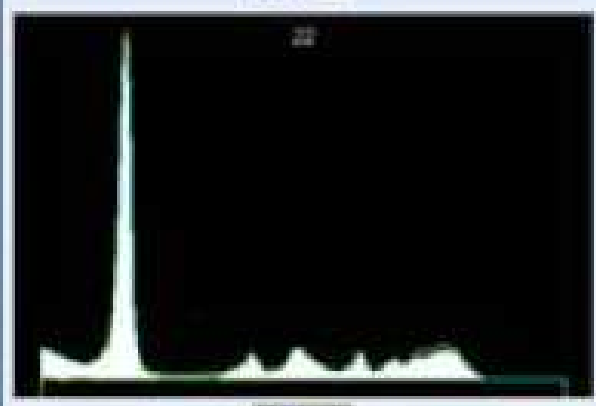
ELP



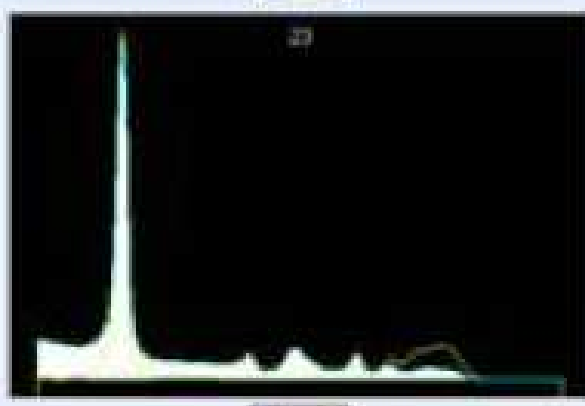
ELP



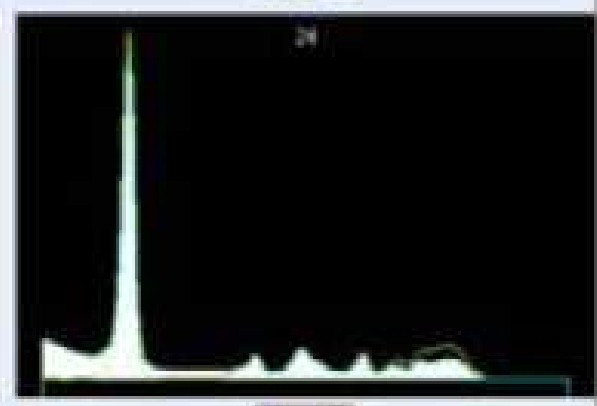
ELP



ELP

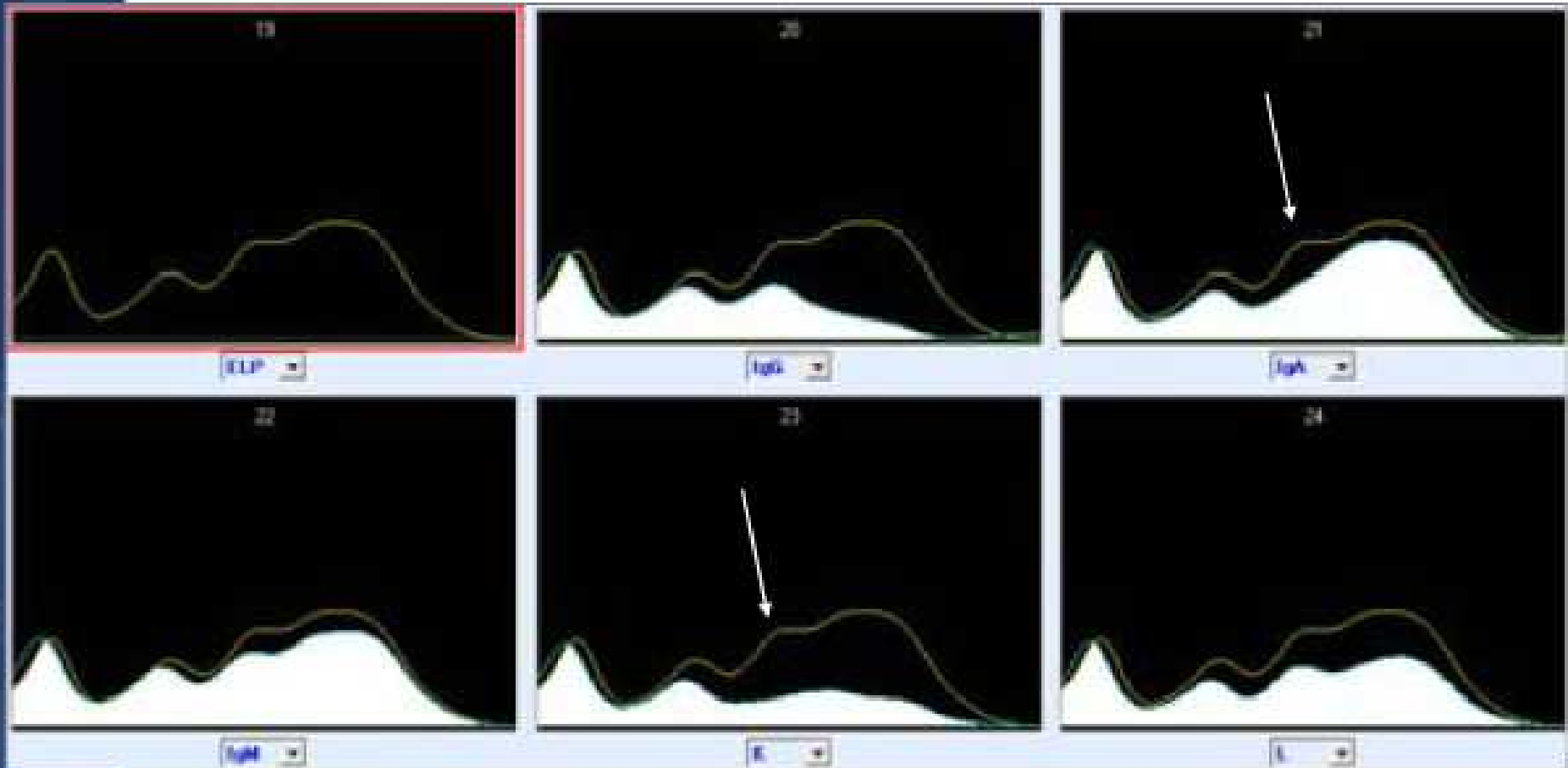


ELP

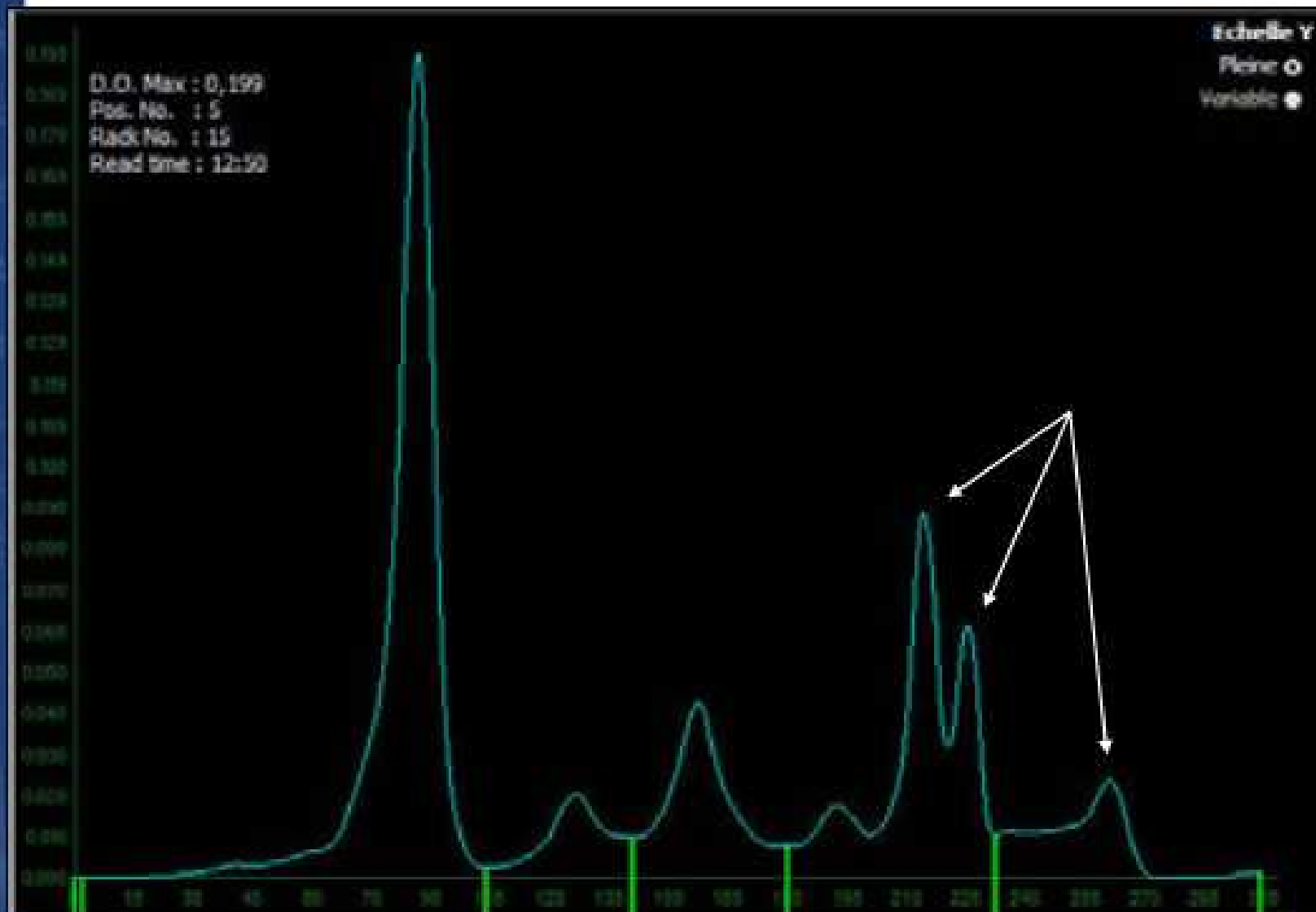


ELP

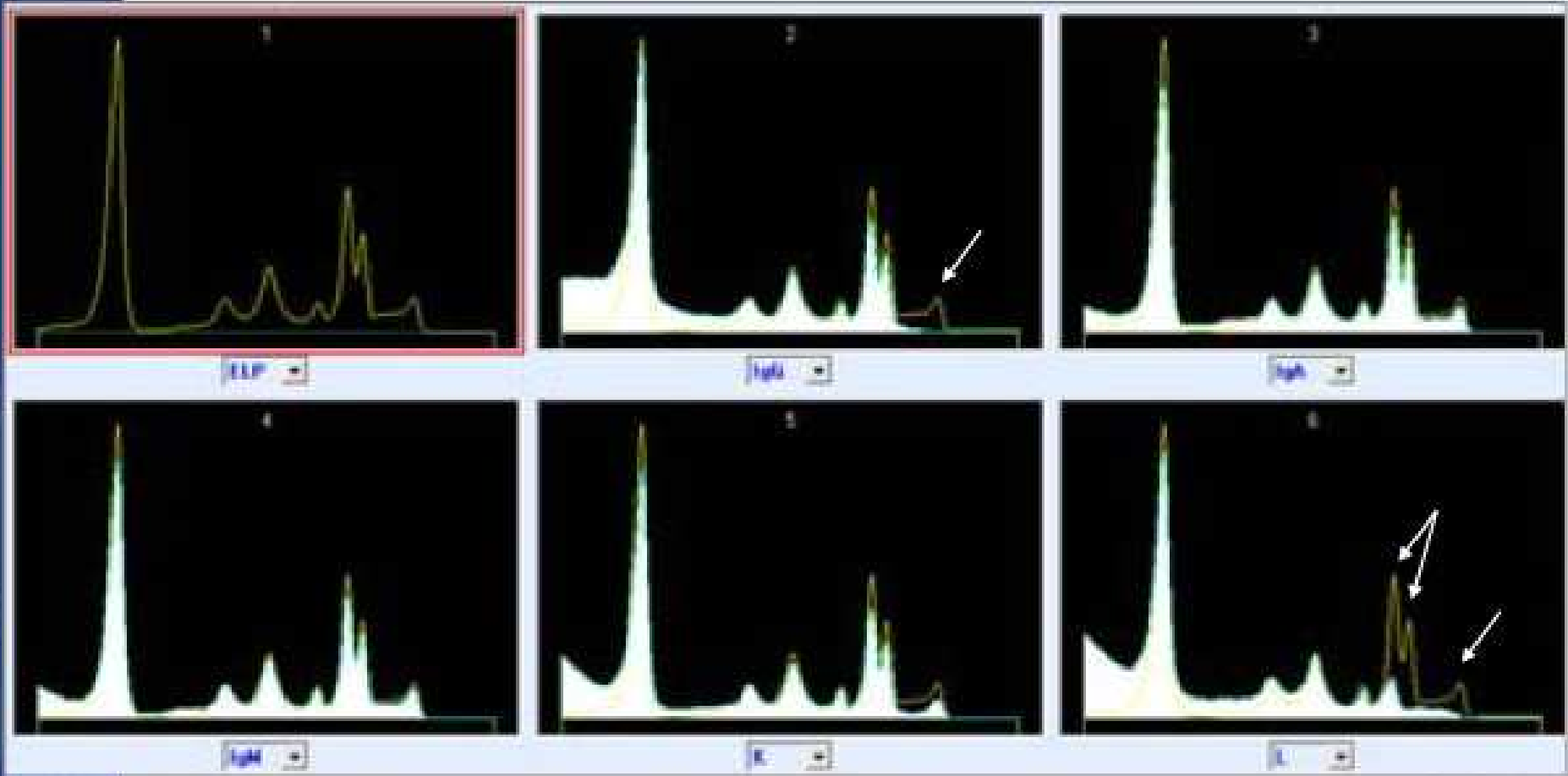
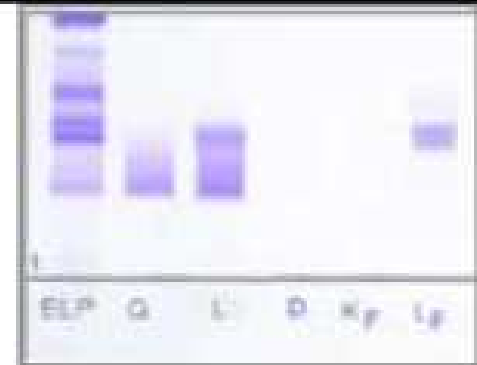
IgA/K



Protein(e) 6



IgG/L + free L



IT: FACTS TO REMEMBER

- Principle: capillary electrophoresis in free solution
- Abnormal fractions in serum protein electrophoregrams are suspect of being monoclonal proteins
 - CAPILLARYS IT utilizes specific antibodies to ID these abnormal fractions
- Detection limit for monoclonal component:
25 – 50 mg/dL
 - depends on the location of the monoclonal component
- Throughput: 10 samples/hour
- Sample volume required: 240 μ l
 - Dead volume required: 340 μ l
- Full automation
 - No variation due to operator's technique issues

THIS IS NOT THE END OF THE STORY....

- Current tests available on CAPILLARYS:
 - Protein(E) 6
 - Protein(E) HR
 - IT
 - CDT
 - Hemoglobin(E)
 - Urine Protein(e)

More tests are coming soon!

THANKS FOR YOUR ATTENTION!