Serum Protein Analizinde Kapiller Elektroforez ve Kullanım Alanları

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ACIBADEM ÜNİVERSİTESİ

Neden Serum protein elektroforezi yapılıyor? Kime yapılmalı?

- Kronik Karaciğer hastalığı
- Sistemik lupus eritematozus
- Romatoid artrit
- Romatolojik hastalıklar
- Inflammatuvar cevap
- Protein kaybettiren hastalıklar
- Nefrotik sendrom
- Gebelik
- Genetik hastalıklar
- Malnutrisyon

Durumundaki hastalara yapılmamalı!!!!

Gerçek Endikasyonlar

Klinik olarak;

- Multipl Myelom, Waldenström makroglobulinemisi, primer amiloidoz, Kİ maligniteleri
- Açıklanamayan kemik ağrıları veya kırıkları
- Reküran enfeksiyonlar
- Açıklanamayan periferik nöropati (DMII veya kemoterapi dışında)
- Prognoz ve tedavi takibi

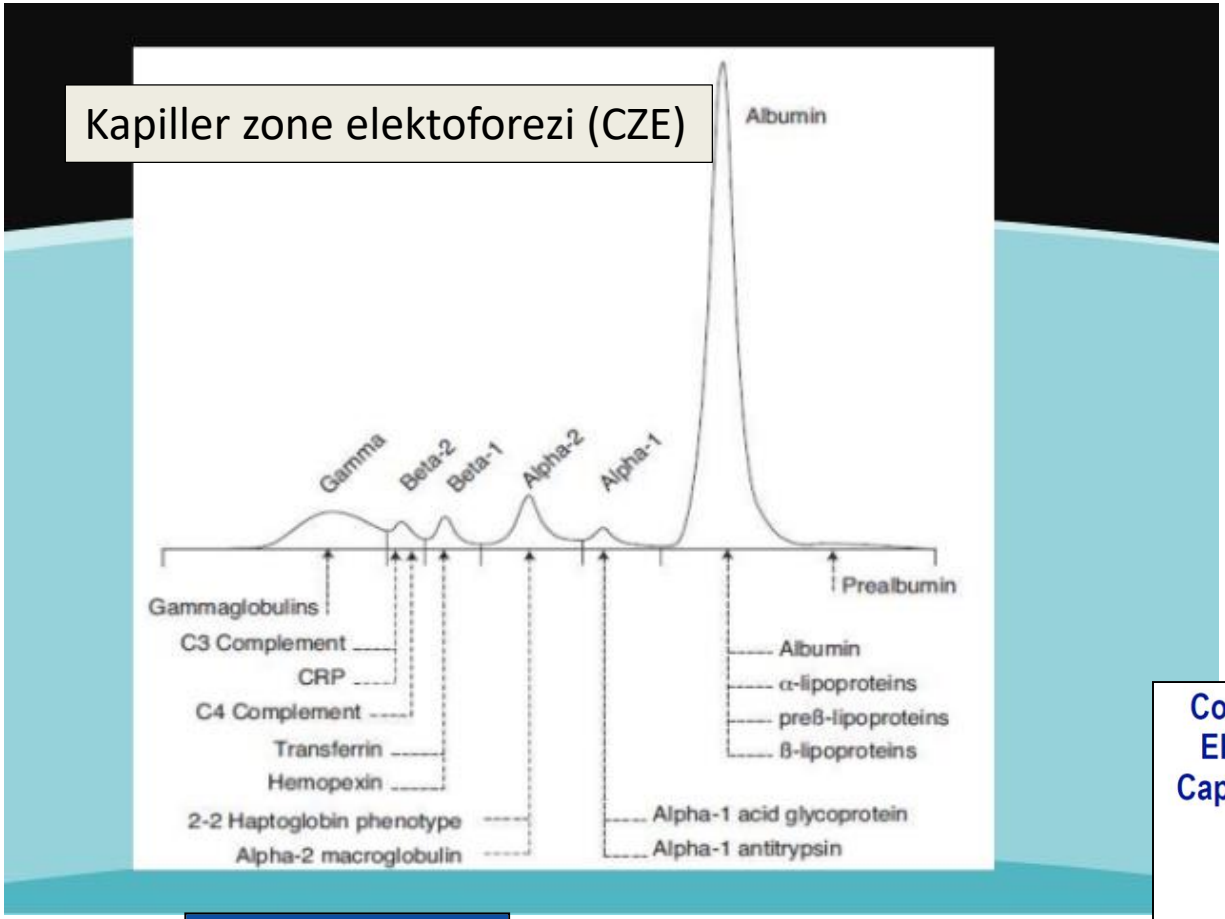
Radyolojik olarak;

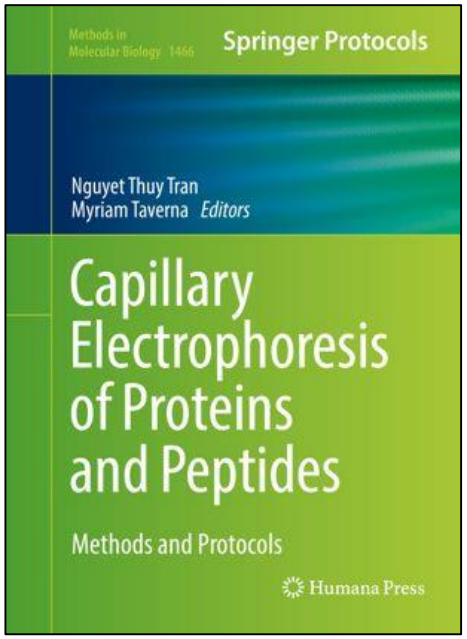
- Litik kemik lezyonları
- Açıklanamayan osteopeni

Laboratuvarda;

•TP>10 g/dL, Ca>10,5mg/dL
•Çok yüksek lenfosit sayımı
•Açıklanamayan anemi
•Açıklanamayan ESR>50mm/Saat
•Açıklanamayan renal fonksiyon bozukluğu
•Periferik yaymada RBC rulo formasyonu
•İdrarda albumin normalken total protein miktarının artması
•İdrar serbest hafif zincirlerinin artmış bulunması (Bence-jones proteinürisi)

Kapiller zone elektoforezi (CZE)





Comparison of Agarose Gel Serum Protein Electrophoresis and Immunofixation with Capillary Electrophoresis and Immunotyping

A Compendium of Selected Cases

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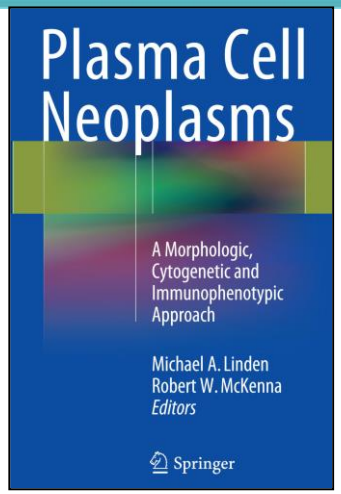


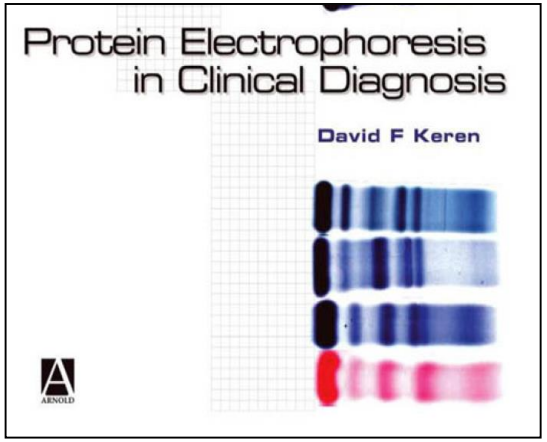


TABLE 52–3 Some Functions of Plasma Proteins

Function	Plasma Proteins
Antiproteases	Antichymotrypsin α_1-Antitrypsin (α_1-antiproteinase) α_2-Macroglobulin Antithrombin
Blood clotting	Various coagulation factors, fibrinogen
Enzymes	Function in blood, for example, coagulation factors, cholinesterase Leakage from cells or tissues, eg, aminotransferases
Hormones	Erythropoietin[a]
Immune defense	Immunoglobulins, complement proteins, and β_2-macroglobulin
Involvement in inflammatory responses	Acute phase response proteins (eg, C-reactive protein, α_1-acid glycoprotein [orosomucoid])
Oncofetal	α_1-Fetoprotein (AFP)
Transport or binding proteins	Albumin (various ligands, including bilirubin, free fatty acids, ions [Ca^{2+}], metals [eg, Cu^{2+}, Zn^{2+}], metheme, steroids, other hormones, and a variety of drugs) Corticosteroid-binding globulin (transcortin) (binds cortisol) Haptoglobin (binds extracorpuscular hemoglobin) Lipoproteins (chylomicrons, VLDL, LDL, HDL) Hemopexin (binds heme) Retinol-binding protein (binds retinol) Sex-hormone-binding globulin (binds testosterone, estradiol) Thyroid-binding globulin (binds T_4, T_3) Transferrin (transport iron) Transthyretin (formerly prealbumin; binds T_4 and forms a complex, with retinol-binding protein)

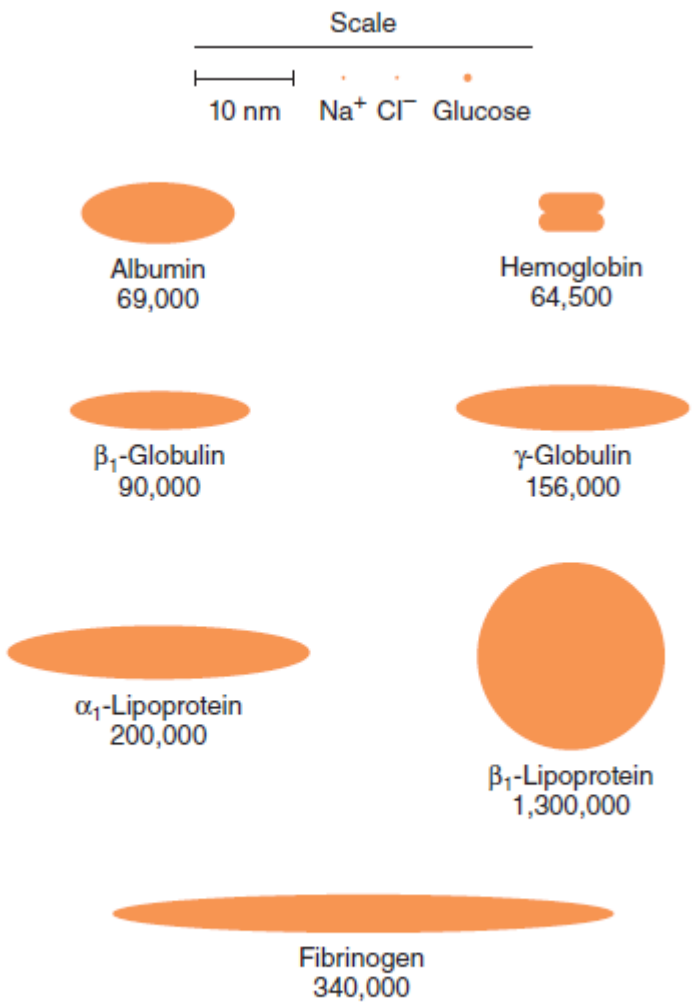
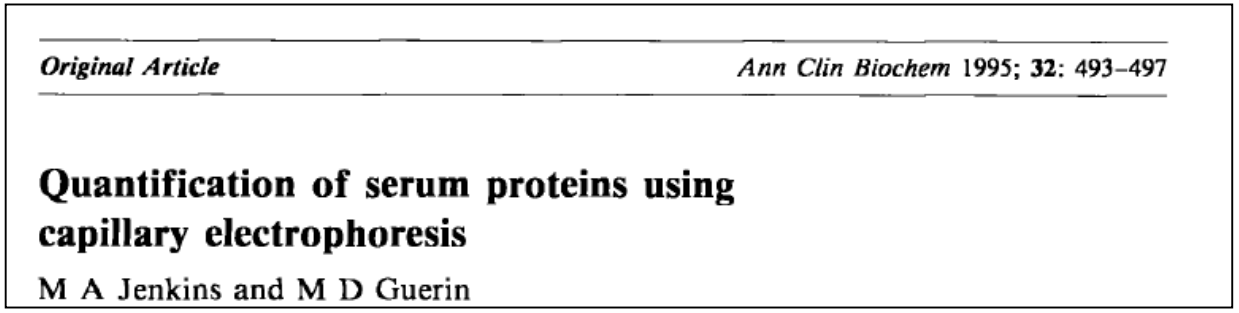


FIGURE 52–2 Relative dimensions and approximate molecular masses of protein molecules in the blood.

«So What's the Big Deal with Those Little Tubes?»*

*Şu küçük tüplerle ilgili büyük olay nedir?

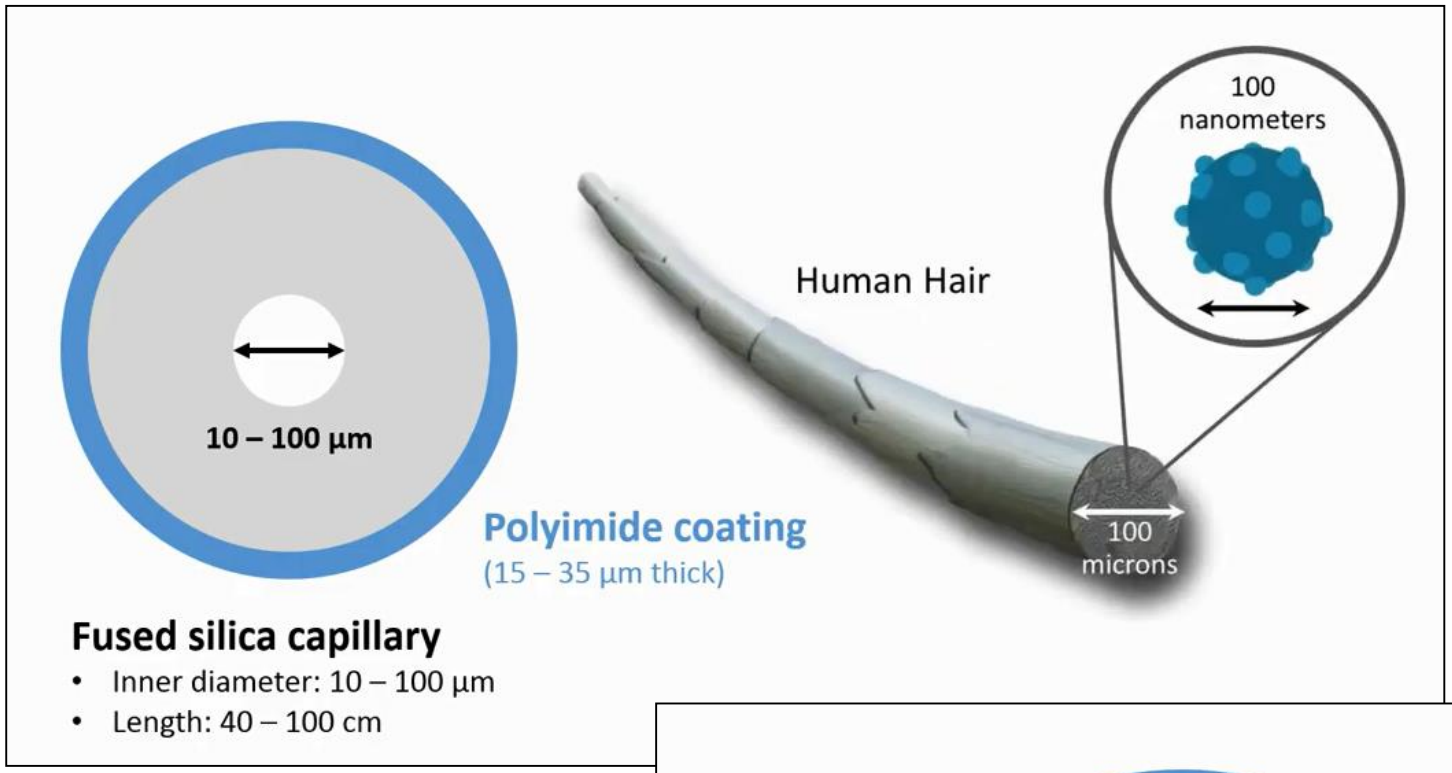
Capillary Electrophoresis



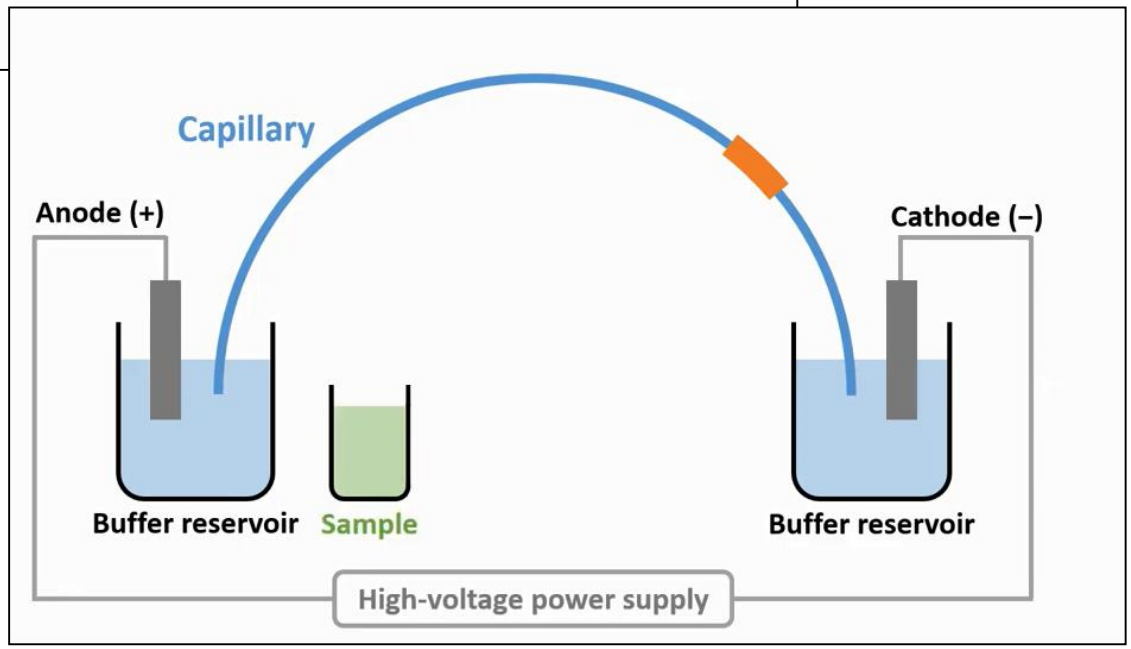
Original Article *Ann Clin Biochem* 1995; **32**: 493–497

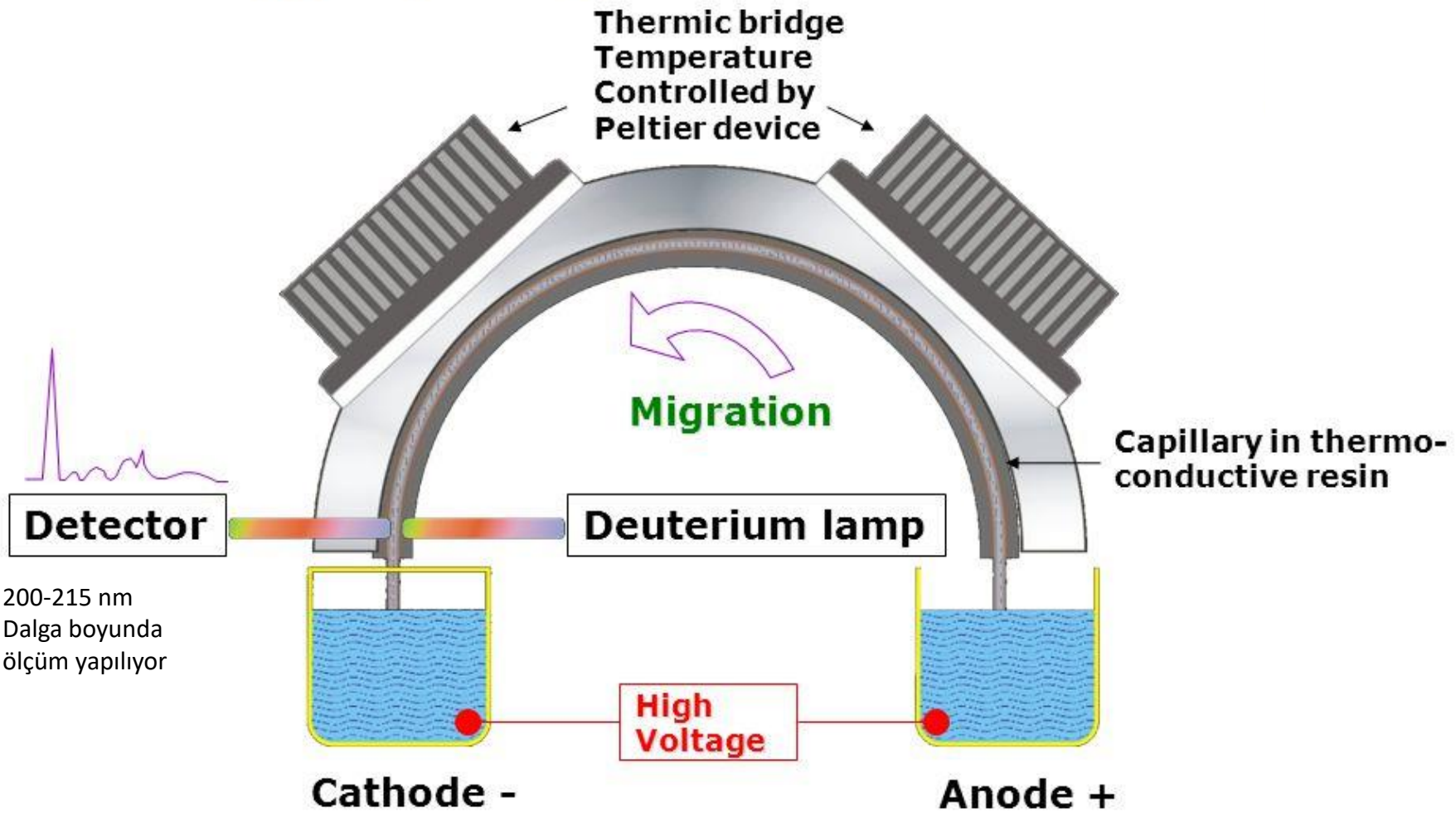
Quantification of serum proteins using capillary electrophoresis

M A Jenkins and M D Guerin

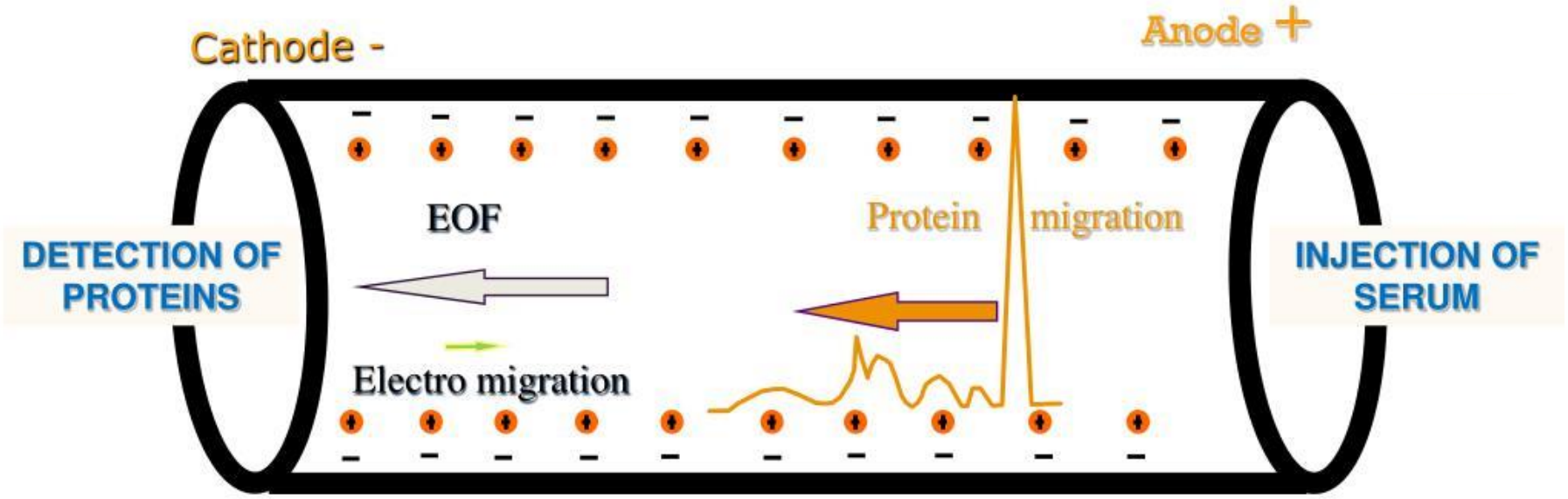


CE de 20-100um çapında, 20-100cm uzunluğunda kapiller cam tüpler/kolonlar kullanılmaktadır.



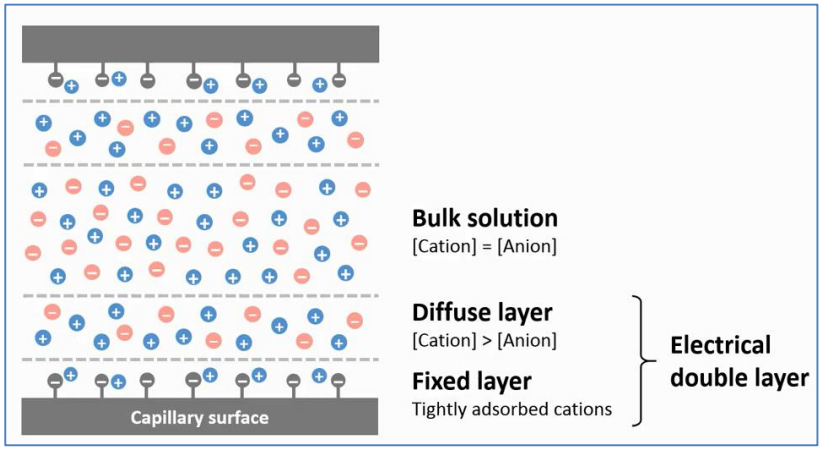

Thermic bridge
Temperature
Controlled by
Peltier device
Migration
Capillary in thermo-
conductive resin
Detector
Deuterium lamp
200-215 nm
Dalga boyunda
ölçüm yapılıyor
High
Voltage
Cathode -
Anode +

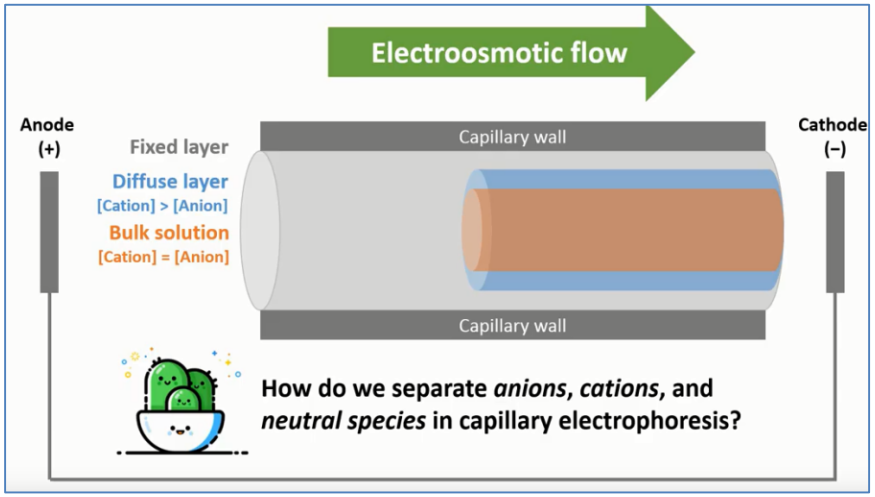
Capillary Electrophoresis

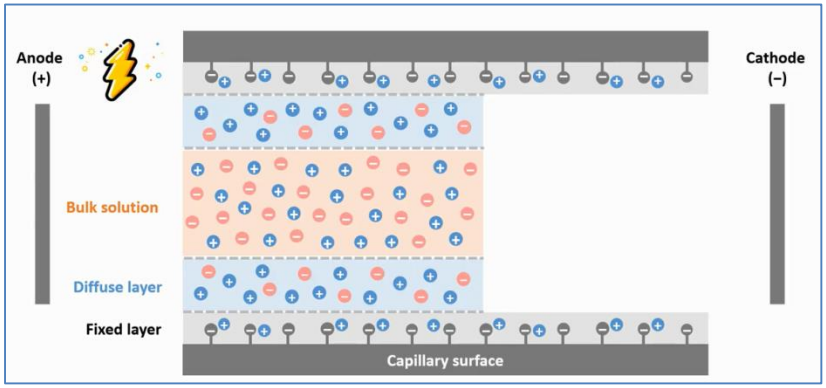


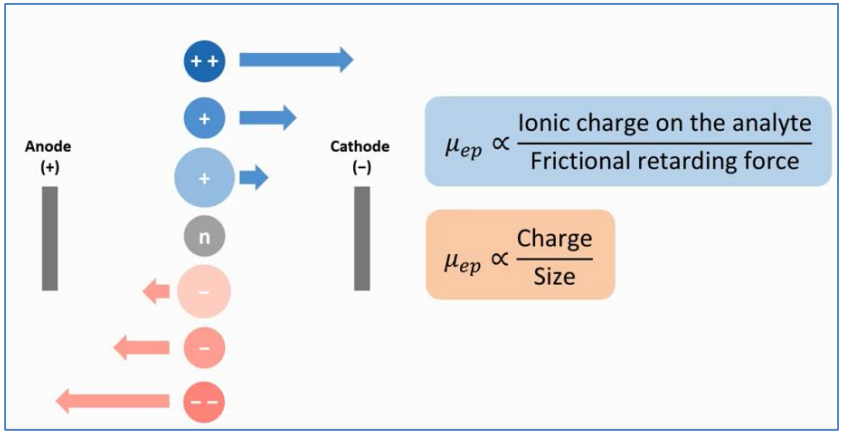
- Positive charges of the buffer solution
- Negative charges of capillarywall

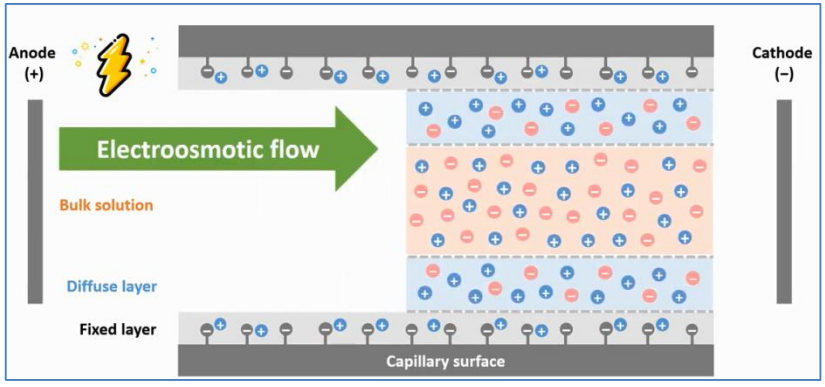
The Electro-Osmotic Flow (EOF) is a stronger force than the Electrical Field.
As a result, all proteins are carriedtowards the cathodic end of the capillary.

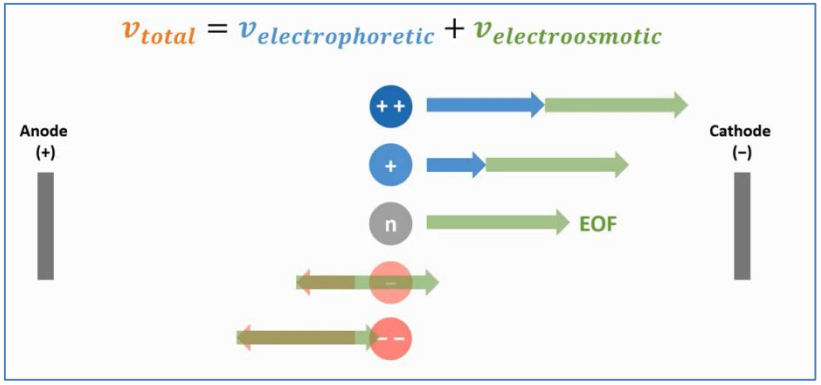

Bulk solution
[Cation] = [Anion]
Diffuse layer
[Cation] > [Anion]
Fixed layer
Tightly adsorbed cations
Electrical double layer
Capillary surface


Electroosmotic flow
Anode (+)
Cathode (−)
Fixed layer
Diffuse layer
[Cation] > [Anion]
Bulk solution
[Cation] = [Anion]
Capillary wall
Capillary wall
How do we separate *anions*, *cations*, and *neutral species* in capillary electrophoresis?

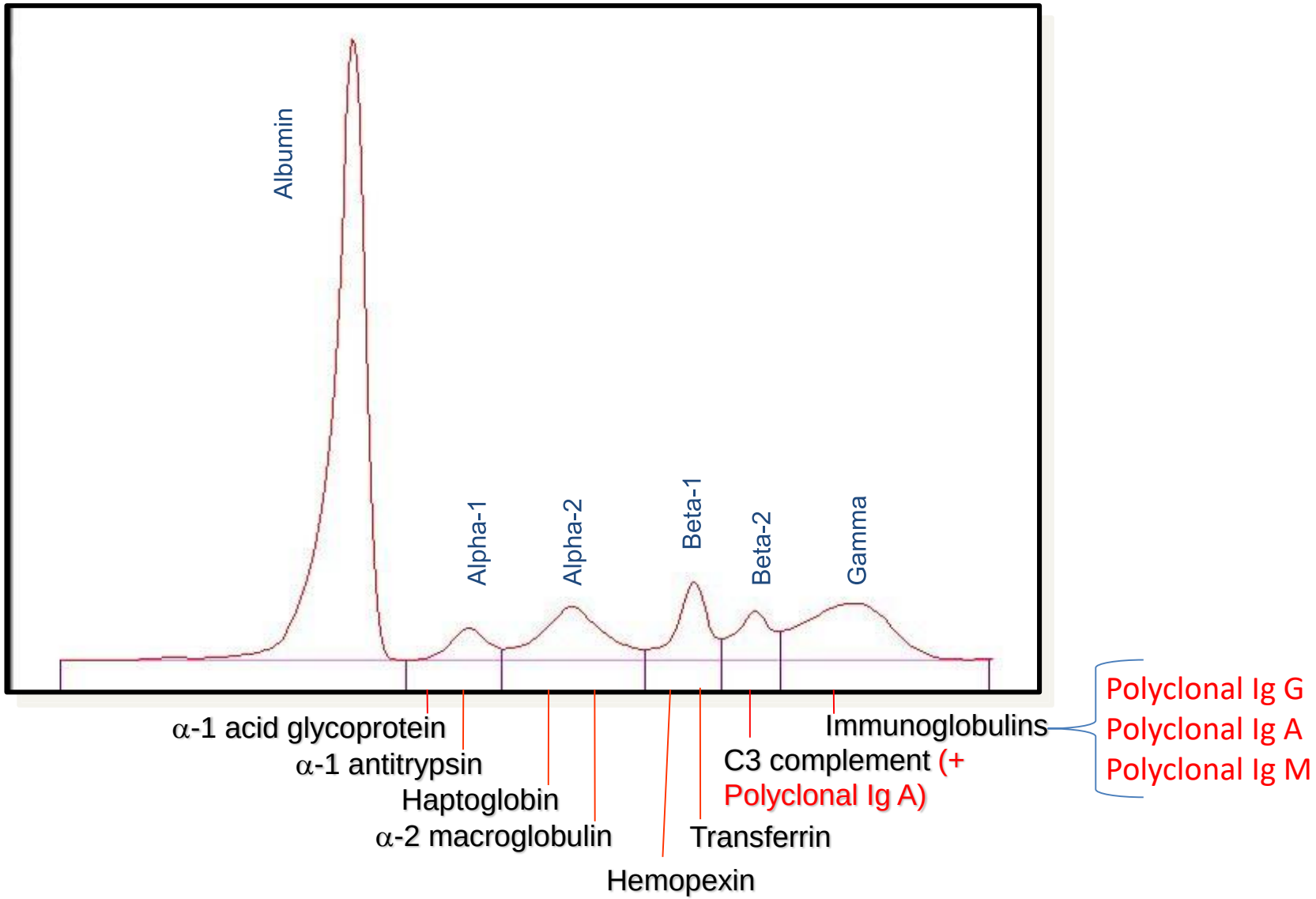

Anode (+)
Cathode (−)
Bulk solution
Diffuse layer
Fixed layer
Capillary surface


Anode (+)
Cathode (−)
++
+
+
n
−
−
−−
$\mu_{ep} \propto \frac{\text{Ionic charge on the analyte}}{\text{Frictional retarding force}}$
$\mu_{ep} \propto \frac{\text{Charge}}{\text{Size}}$


Anode (+)
Cathode (−)
Electroosmotic flow
Bulk solution
Diffuse layer
Fixed layer
Capillary surface


$v_{total} = v_{electrophoretic} + v_{electroosmotic}$
Anode (+)
Cathode (−)
++
+
n
EOF
−
−−

Kapiler elektroforezde fraksiyonlar



Kapiler Elektroforez/ Agaroz jel ile SPE ve IFE

Kapiller Elektroforez

- Yeni Yöntem (Ortam Su bazlı)
- Hızlı sonuç
- Rezolüsyonu iyi
- Örnek miktarı az (1-5uL)
- Boyama yok
- Dansitometre kullanımına gerek yok
- Yorumlaması nispeten zor
- Monoklonal proteinlerde deteksiyon limiti daha yüksek (25 mg/dL)

Agaroz jel

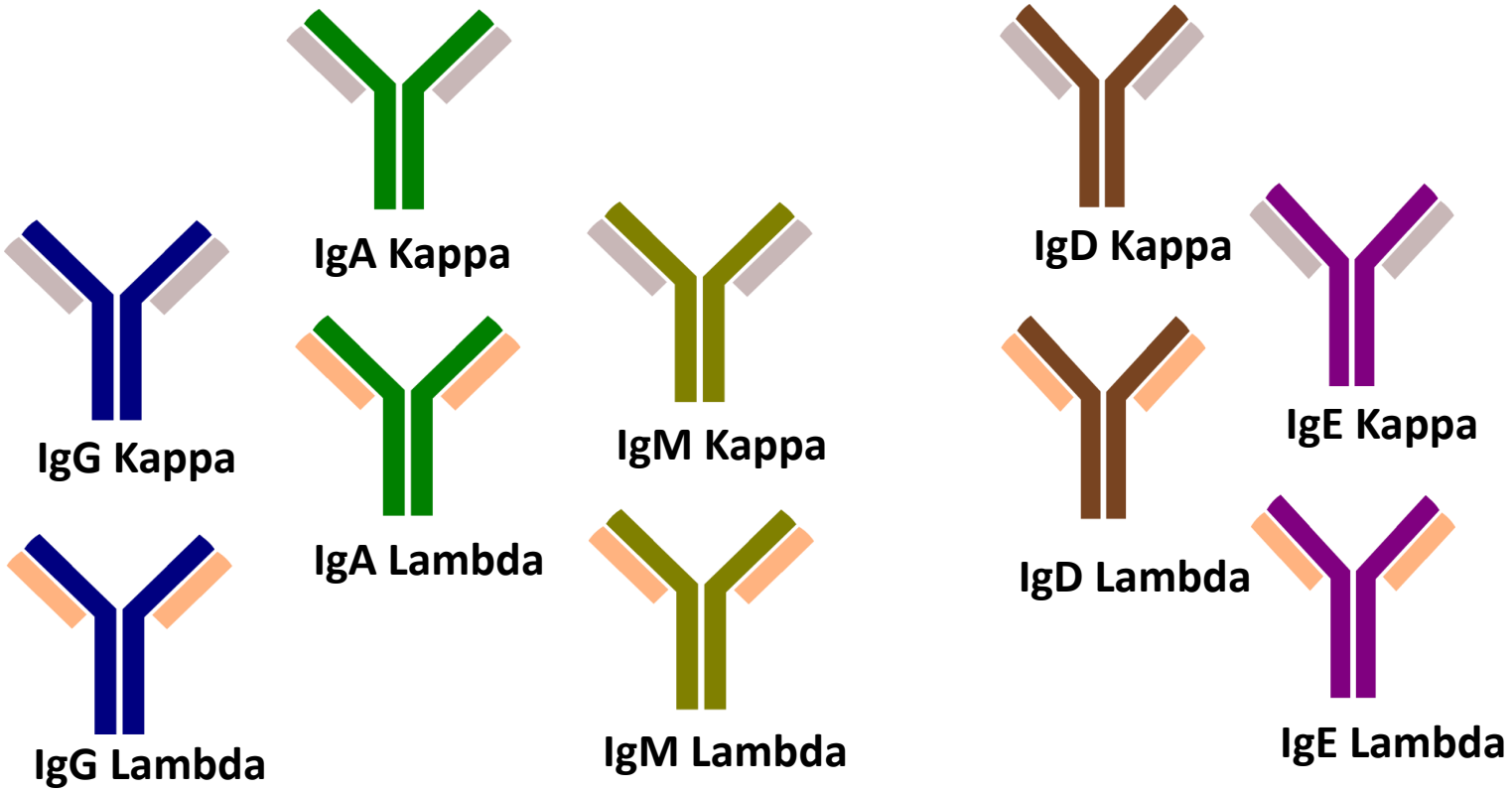
- Klasik yöntem (Ortam Jel)
- Yavaş sonuç
- Hala gold standart
- Örnek miktarı (20-35uL)
- Boyama yapılıyor
- Dansitometre ile kantitasyon
- Yorumlamasına daha alışkınız
- Monoklonal proteinlerde deteksiyon limiti daha aşağıda (12 mg/dL)

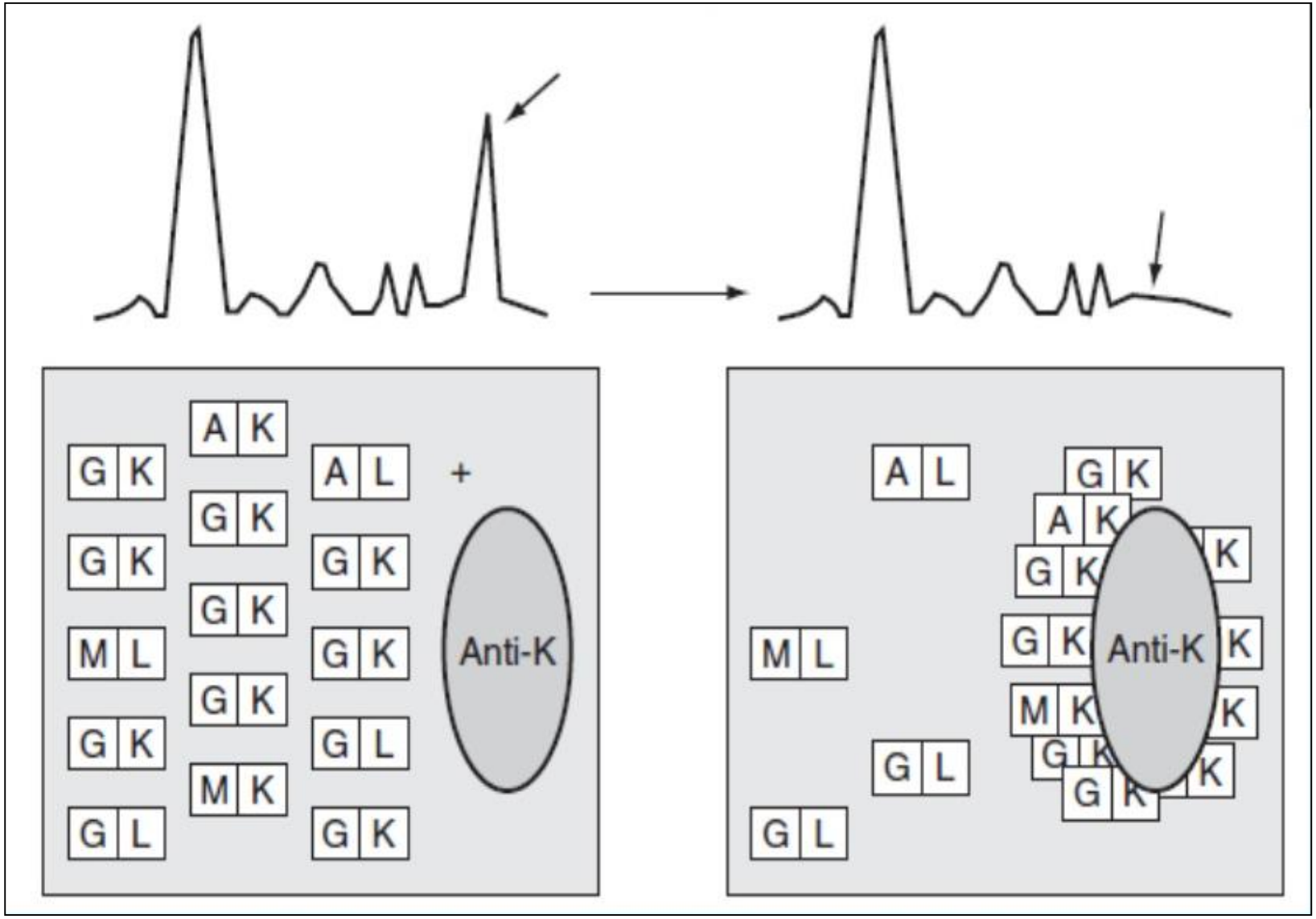
Nerede kullanıyoruz kapiller elektroforez sistemlerini?

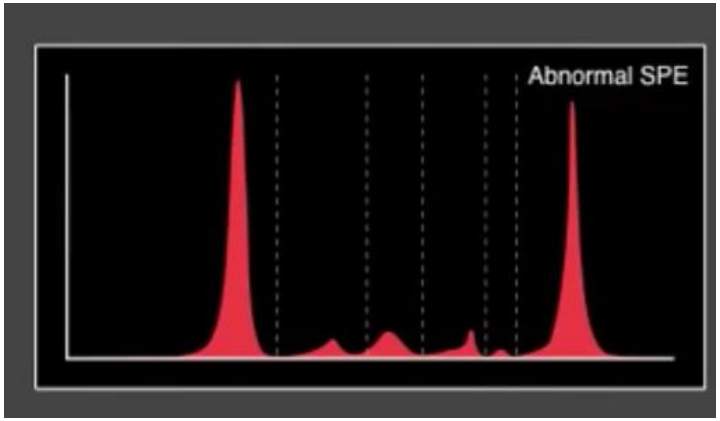
- SPE
- Serum monoklonal protein tiplendirme
- İdrar protein elektroforezi
- İdrarda hafif zincir araştırılması (Bence-jones)
- İdrar monoklonal protein tiplendirme
- Kriyoglobulin tiplendirme
- CDT (Carbohydrate deficient transferrin)
- …
- ……..

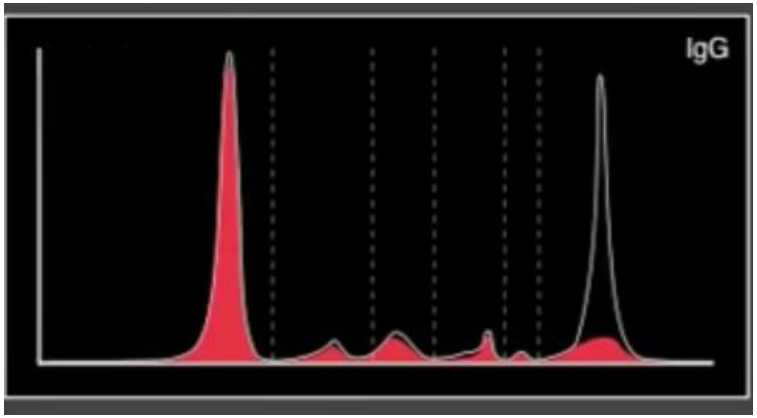
Monoklonal proteinlerin saptanması ve miktarının değerlendirilmesinde Kapiller «Immunodisplacement» Yöntemi

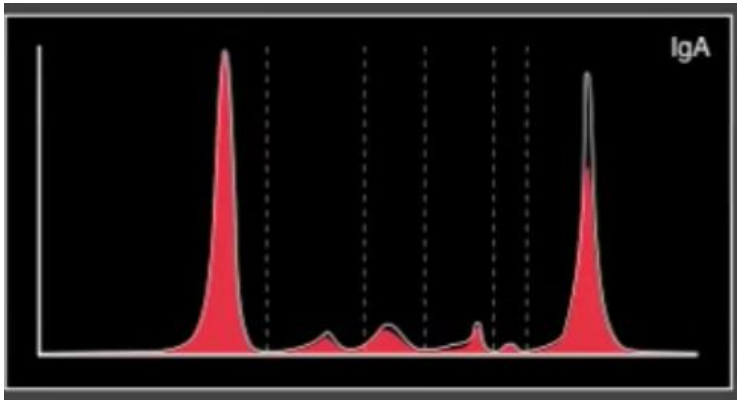
10 farklı immunoglobulin kombinasyonu

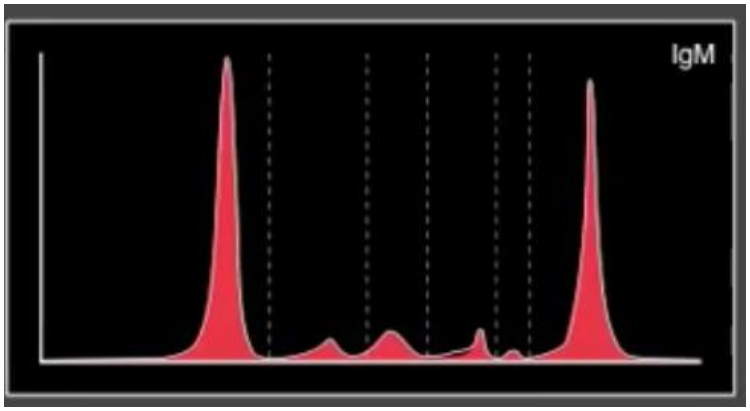


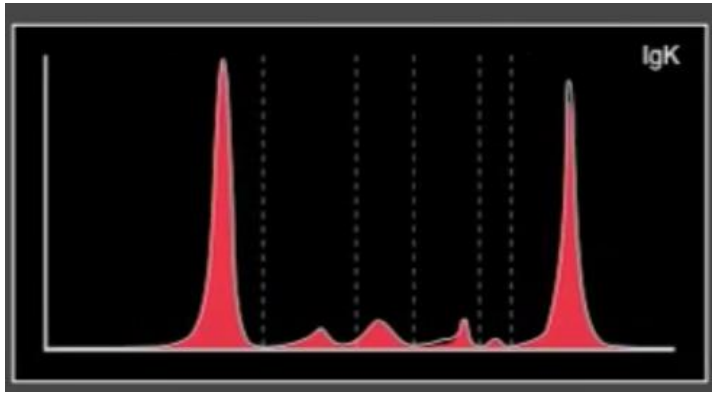

A K
G K
A L
+
G K
G K
G K
G K
M L
G K
G K
G K
G K
G L
M K
G K
Anti-K
A L
G K
A K
G K
K
G K
K
M L
Anti-K
M K
K
G L
G K
K
G K
G L

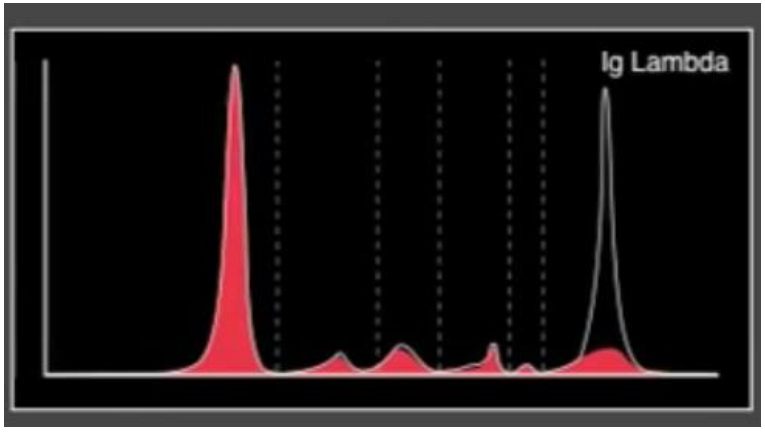

Abnormal SPE

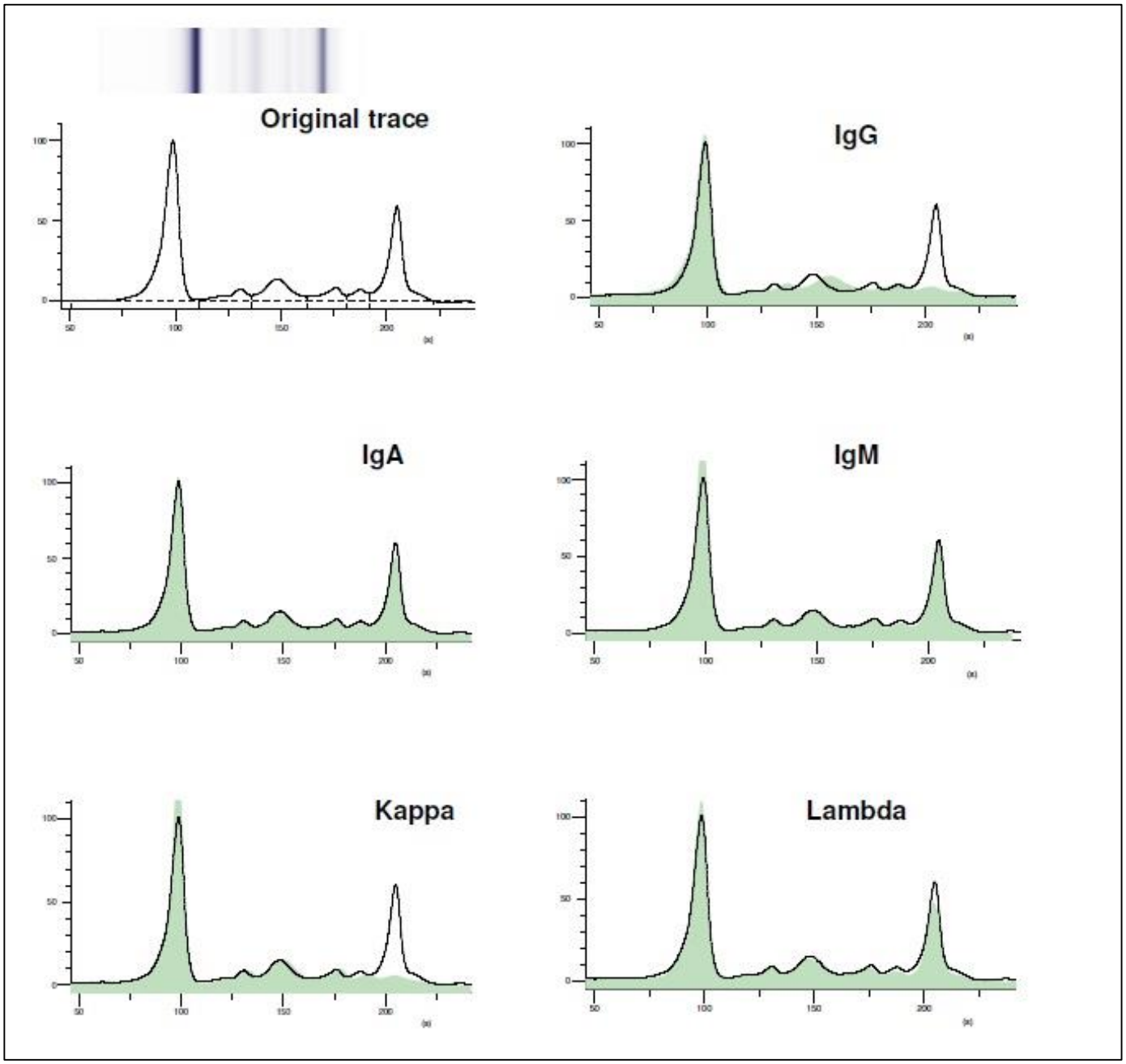

IgG


IgA

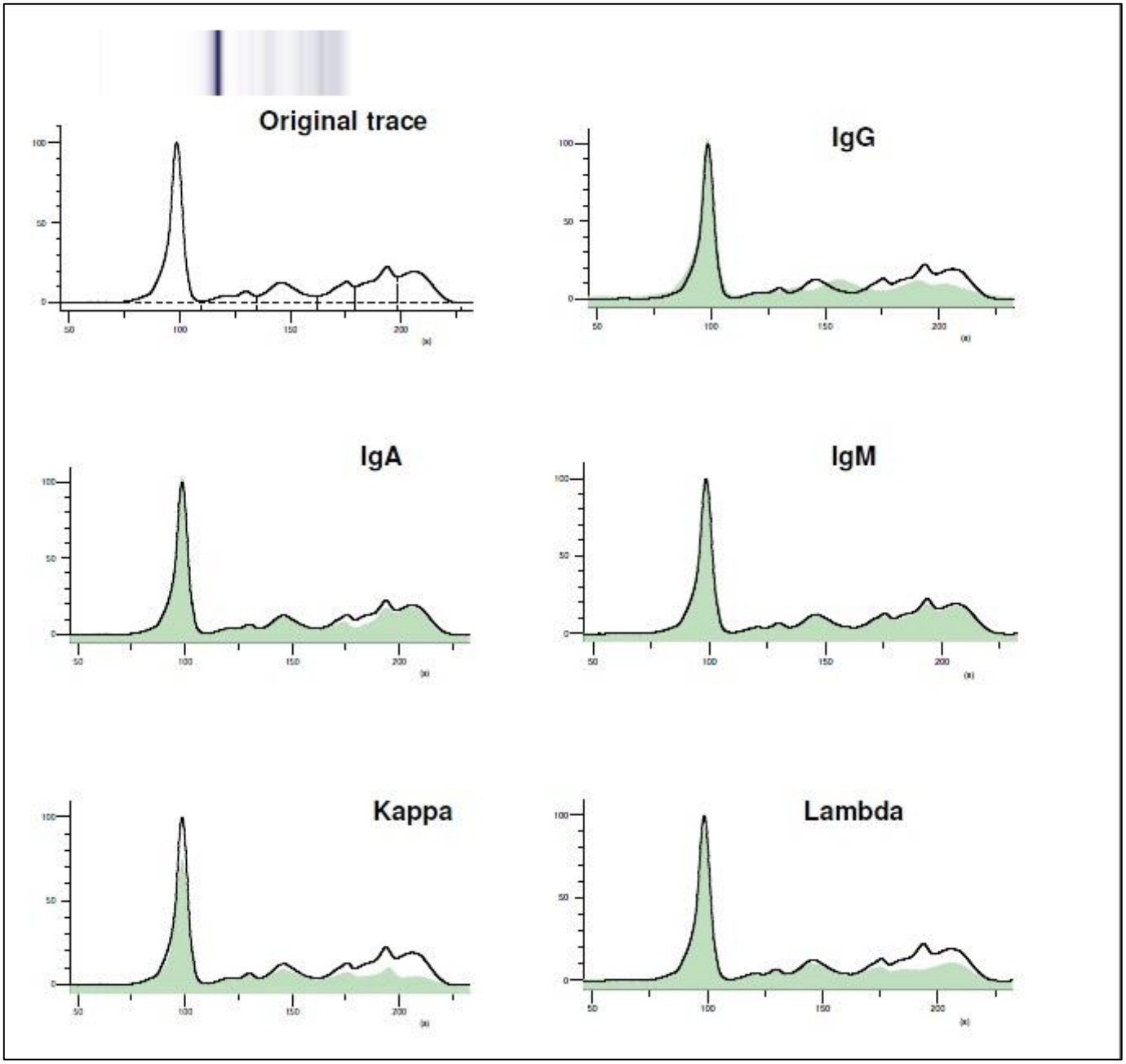

IgM


IgK


Ig Lambda


Original trace
IgG
IgA
IgM
Kappa
Lambda

?


Original trace
IgG
IgA
IgM
Kappa
Lambda

?

79 years old male with known prostate cancer was admitted with severe anemia (hemoglobin 6.1), renal failure, and hypertension.

Total Protein:	6.8 g/dL (6.4-8.3 g/dL)	Immunoglobulins:	
Agarose gel electrophoresis:			
Albumin:	3.8 g/dL (3.8-5.8 g/dL)	IgG:	1160 mg/dL (751-1560 mg/dL)
Alpha-1 Globulin:	0.2 g/dL (0.1-0.2 g/dL)	IgA:	158 mg/dL (82-453 mg/dL)
Alpha-2 Globulin:	1.0 g/dL (0.4-0.9 g/dL)	IgM:	16 mg/dL (46-304 mg/dL)
Beta Globulin:	0.9 g/dL (0.5-1.1 g/dL)		
Gamma Globulin:	1.1 g/dL (0.5-1.3g/dL)		

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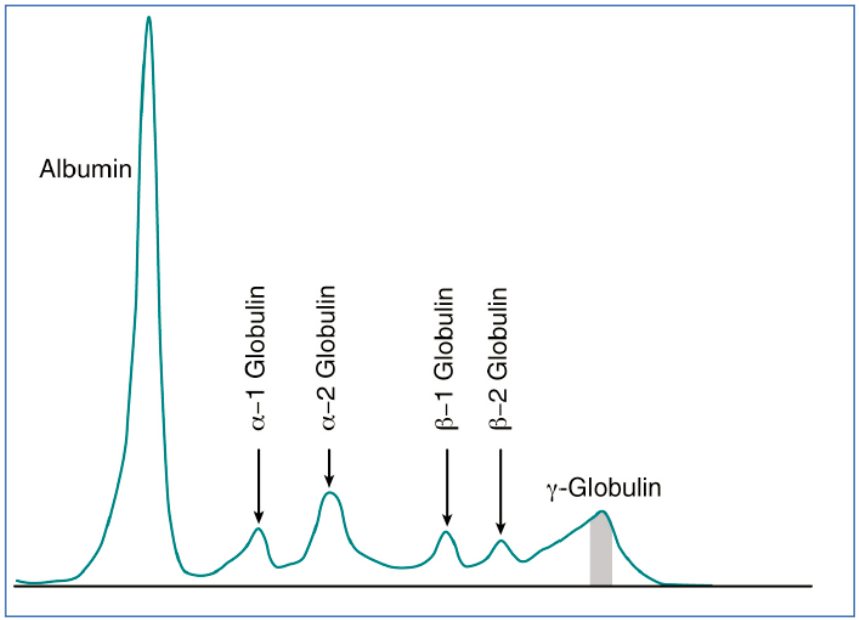
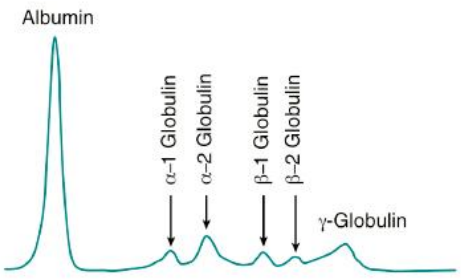
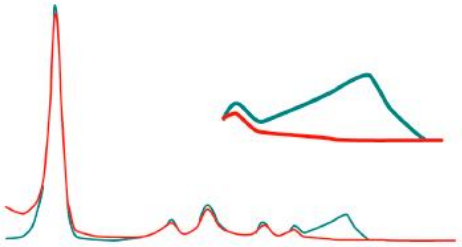
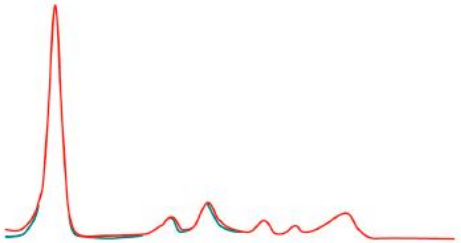




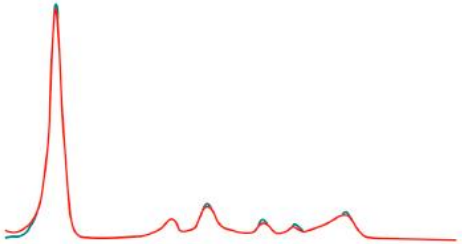
Serum Protein Capillary Electrophoresis



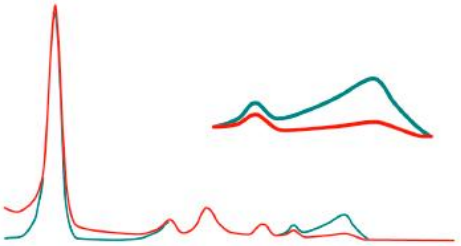
IgG



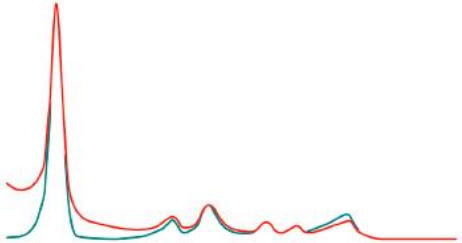
IgA



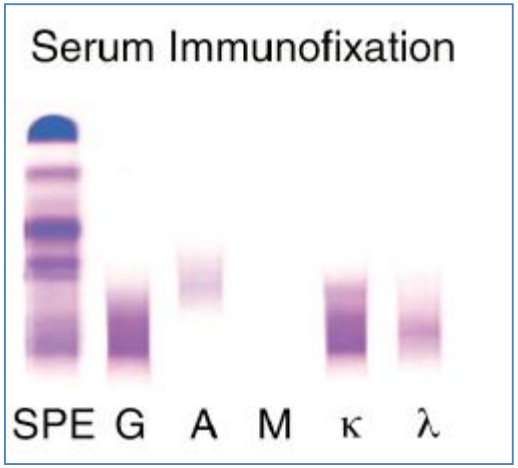
IgM



Kappa



Lambda



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DOI: 10.12998/wjcc.v4.i3.81

World J Clin Cases 2016 March 16; 4(3): 81-87
ISSN 2307-8960 (online)

CASE REPORT

Hepatitis C virus positive patient diagnosed after detection of atypical cryoglobulin

Belkiz Ongen, Fehime Benli Aksungar, Bahattin Cicek, Isin Akyar, Abdurrahman Coskun, Mustafa Serteser, Ibrahim Unsal

Figure 1 Agglutinations on the complete blood count tube. It was anticoagulated with K2-EDTA, analyzed at room temperature and red blood cell, Hemoglobin and Hematocrit results were discordant with each other.

- **Initial Laboratory Findings**
  - **Discordance in RBC, Hb and Htc values !!!!!!**
  - Clinical Chemistry
    - **Urea: ↑**
    - **Crea: N**
    - **Bilirubins: ↑↑↑**
    - **ALT, AST, GGT: ↑↑↑↑**
    - **Total Protein, Albumin: ↓**
    - **PT and INR: N**

Detection of cryoglobulin

- Pre-warm test tube at 37 °C
- Tube transfer at 37 °C to the lab
- Incubation at 37 °C until serum separates
- Keep separated serum at 4 °C for 7 days
- Observe each day for any precipitate

- Incubation of cryoprecipitate at 37 °C for 30 min dissolves

Figure 2 Cryoglobulin sediment at 4 ℃.

Kriyoglobulin tiplendirmesi

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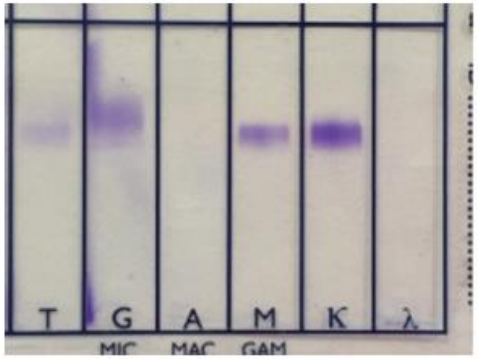


Figure 3 Cryoglobulin immunofixation electrophoresis with SAS-1 agarose gel (Helena, United Kingdom). A polyclonal IgG and monoclonal IgM kappa are detected. T lane shows total protein electrophoresis of cryoglobulin and an absent albumin band shows washing and isolating of the cryoprecipitate is successfully performed.

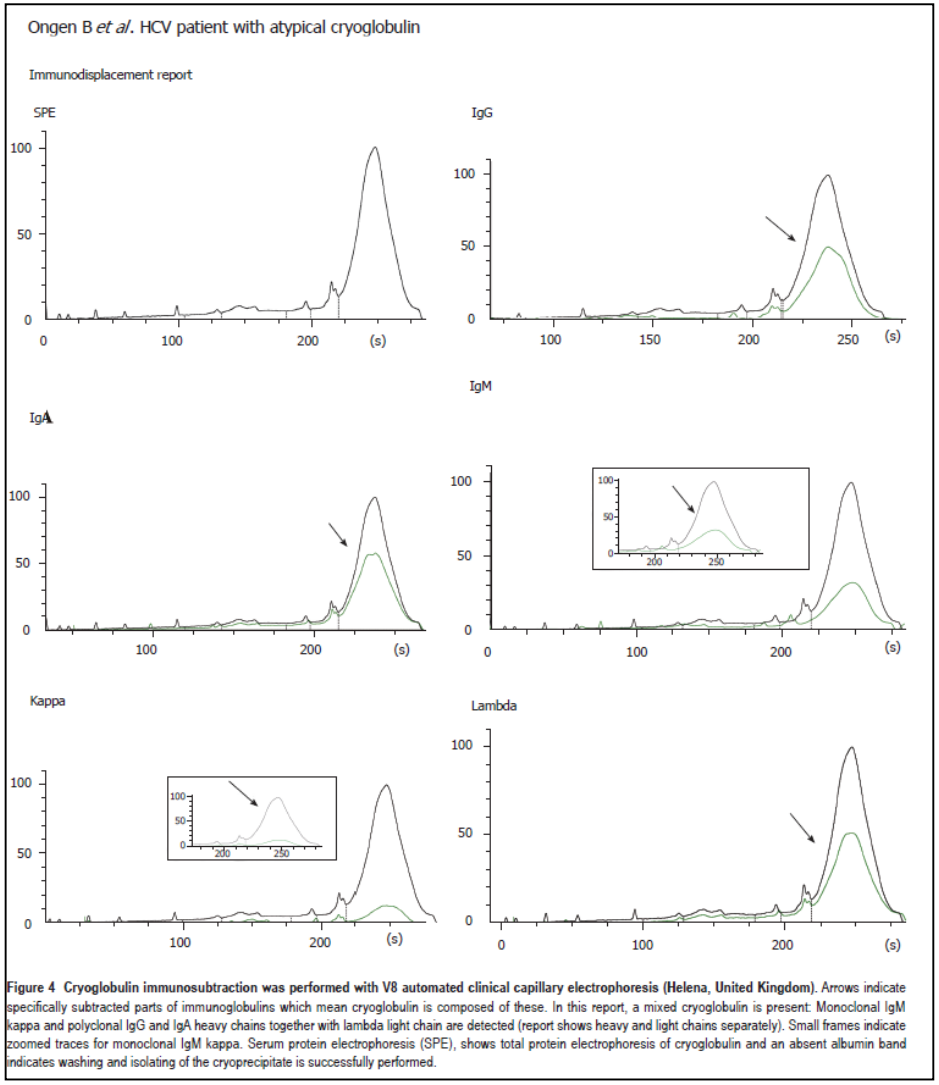


Figure 4 Cryoglobulin immunosubtraction was performed with V8 automated clinical capillary electrophoresis (Helena, United Kingdom). Arrows indicate specifically subtracted parts of immunoglobulins which mean cryoglobulin is composed of these. In this report, a mixed cryoglobulin is present: Monoclonal IgM kappa and polyclonal IgG and IgA heavy chains together with lambda light chain are detected (report shows heavy and light chains separately). Small frames indicate zoomed traces for monoclonal IgM kappa. Serum protein electrophoresis (SPE), shows total protein electrophoresis of cryoglobulin and an absent albumin band indicates washing and isolating of the cryoprecipitate is successfully performed.

Table 2 Classification of cryoglobulins and laboratory findings[4,18]

Cryoglobulin type	Content	Related diseases	Laboratory findings
Type I	Monoclonal immunoglobulins (IgG, IgM or IgA) or Bence Jones protein/ monoclonal free light chains	Multiple Myeloma Waldenstrom Macroglobulinemia Lymphoproliferative disease related monoclonal gammopathy Light chain disease	Precipitation within 24 h Hyperviscosity
Type II (mixed)	Monoclonal immunoglobulins (IgG, IgM or IgA) and polyclonal immunoglobulins (usually IgG)	HCV Essential cryoglobulinemia Sjogren's syndrome Rheumatoid arthritis Chronic lymphocytic leukemia	Precipitation within 7 d HCV positivity Decreased C3 Decreased C4 Decreased CH50
Type III (mixed)	Polyclonal immunoglobulins	Essential cryoglobulinemia Sjogren's syndrome Systemic lupus erythematosus Viral infections (HBV, CMV, EBV, HIV) Endocarditis Biliary cirrhosis	Increased autoantibodies such as ANA, ENA, AMA

Ongen B *et al*. HCV patient with atypical cryoglobulin

Case report

DE GRUYTER Turkish Journal of Biochemistry – Türk Biyokimya Dergisi; 2016; 41 (5): 367–372

Clinical Biochemistry
Case Report – 48752

Belkız Öngen*, Fehime Benli Aksungar, Arzu Tiftikçi, Abdurrahman Coşkun, Mustafa Serteser, İbrahim Ünsal

A rare association: celiac disease and multiple myeloma in an asymptomatic young patient

Asemptomatik genç bir hastada çölyak hastalığı ve multipl myelom'un nadir birlikteliği

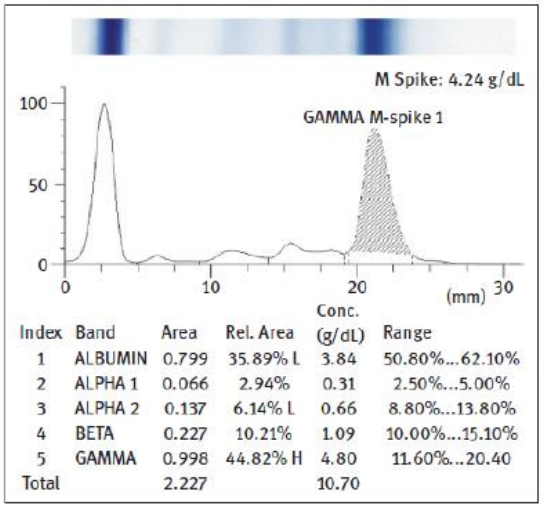
Test name (Unit)	Result	Reference range
Hemoglobin (g/dL)	9.1	11.5–15.5
Hemotocrit (%)	29.2	35.5–48
MCV (fL)	74.9	80–95
Iron (ug/dL)	21	50–150
Total Iron Binding Capacitiy (ug/dL)	347	250–450
Ferritin (ng/mL)	6	10–291
Vitamin B12 (pg/mL)	326	200–835
Folic Acid (ng/mL)	3.6	>5.38
Urea (mg/dL)	10	13–43
Creatinine (mg/dL)	0.68	0.6–1.0
ALT (U/L)	56	14–59
Total Protein (g/dL)	11.4	6.4–8.2
Albumin (g/dL)	2.94	3.4–5
Calcium (mg/dL- Corrected for albumin)	9.82	8.5–10.5
Beta-2 microglobulin (mg/L)	3.26	1.09–2.05
IgA (mg/dL)	5894	40–350
IgG (mg/dL)	201	690–1600
IgM (mg/dL)	<8	50–300
Free Kappa, serum (mg/L)	0.69	3.3–19.40
Free Lambda, serum (mg/L)	81.66	5.71–26.3
Gliadin Antibodies IgA (U/mL)	17.9	<12
Gliadin Antibodies IgG (U/mL)	26.5	<12
Transglutaminase Antibodies IgA (U/mL)	>300	<12
Transglutaminase Antibodies IgG (U/mL)	51.1	<12
Endomysium Antibodies IgA (Titer)	1:32	<1:10
Endomysium Antibodies IgG (Titer)	1:320	<1:10

- 42 y/o Female
- Dysphagia (3 d before because of hot food ingestion)
- Physical examination, personal history and family history: No remarkable features
- Gastroesophageal reflux?

- Upper GIS endoscopy
  - Esophageal mucosa normal,
  - Small hyperemic areas on antral mucosa,
  - Scalloping of folds, fissures, mosaic appearence in duodenal mucosa

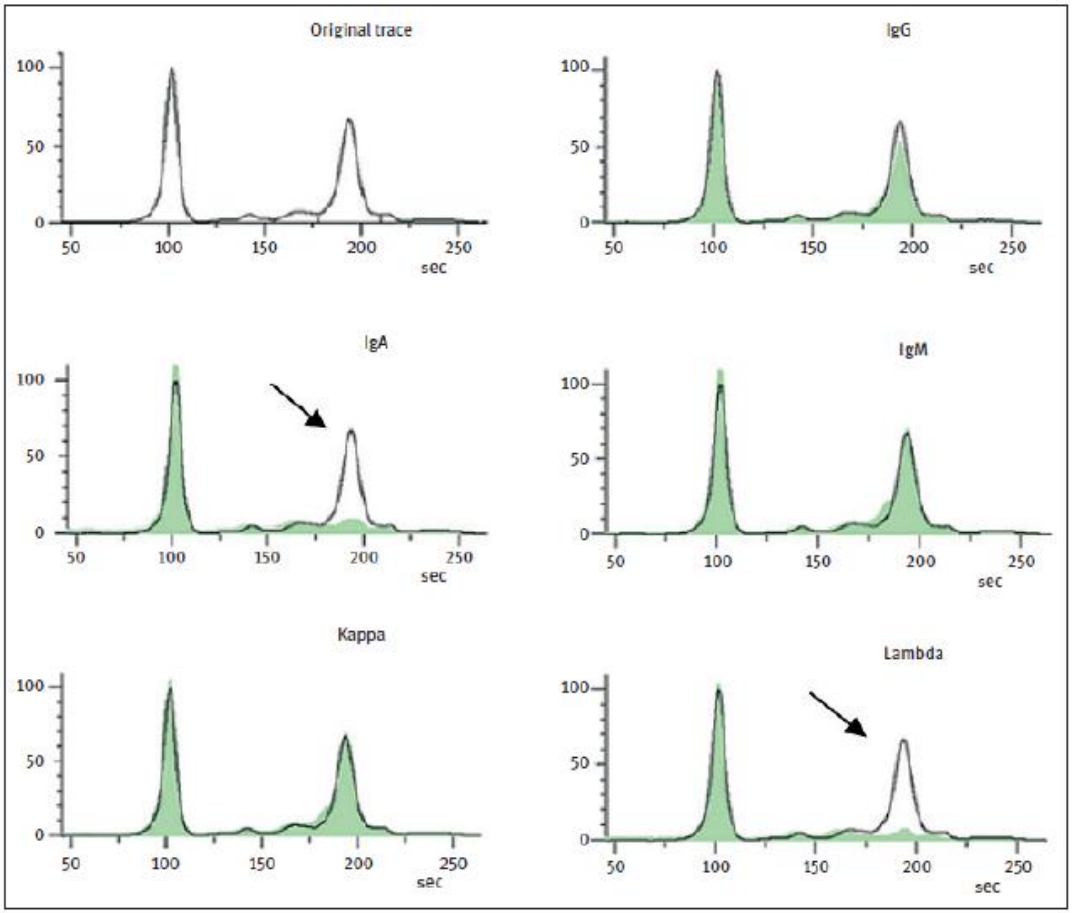
Biopsy (diff dx: eosinophilic esophagitis, duodenal atrophy)
- Celiac Diseae (Marsh type 3c)

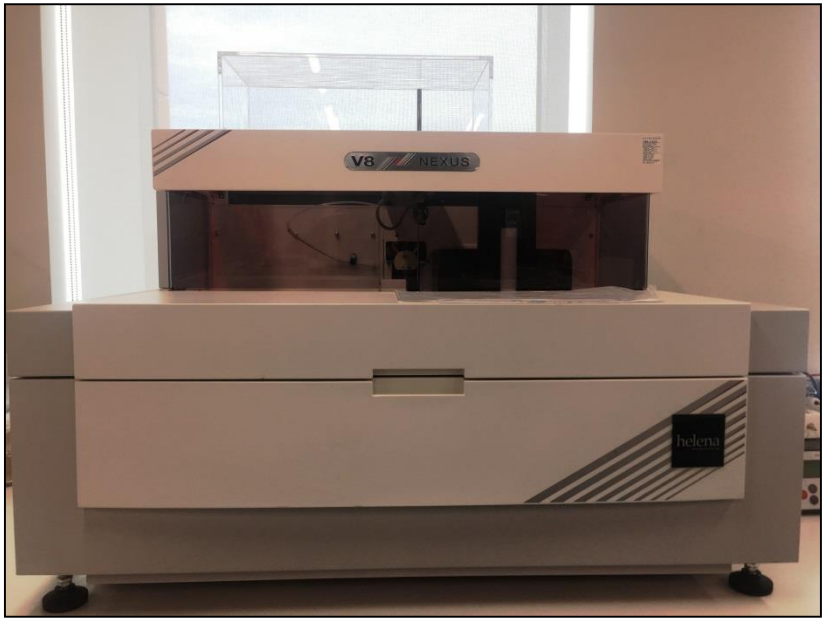
After high serum total protein

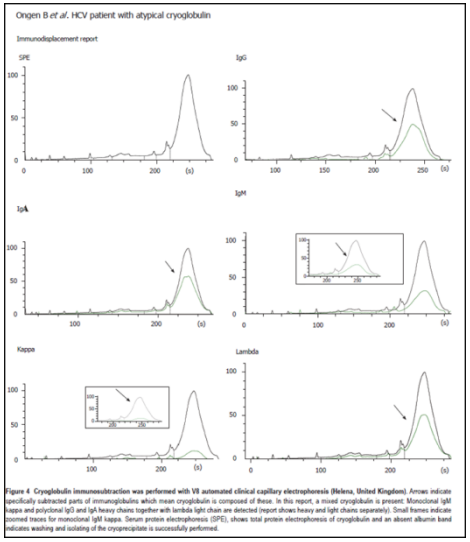


Index	Band	Area	Rel. Area	Conc. (g/dL)	Range
1	ALBUMIN	0.799	35.89% L	3.84	50.80%...62.10%
2	ALPHA 1	0.066	2.94%	0.31	2.50%...5.00%
3	ALPHA 2	0.137	6.14% L	0.66	8.80%...13.80%
4	BETA	0.227	10.21%	1.09	10.00%...15.10%
5	GAMMA	0.998	44.82% H	4.80	11.60%...20.40
Total		2.227		10.70	

Figure 2: Protein electrophoresis by agarose gel showed a remarkable M protein in the gamma region.



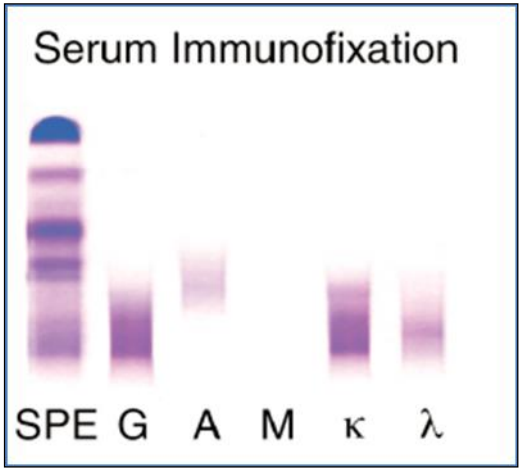



Ongen B *et al*. HCV patient with atypical cryoglobulin

Immunodisplacement report

SPE IgG IgA IgM Kappa Lambda

Figure 4 Cryoglobulin immunosubtraction was performed with V8 automated clinical capillary electrophoresis (Helena, United Kingdom). Arrows indicate specifically subtracted parts of immunoglobulins which mean cryoglobulin is composed of these. In this report, a mixed cryoglobulin is present: Monoclonal IgM kappa and polyclonal IgG and IgA heavy chains together with lambda light chain are detected (report shows heavy and light chains separately). Small frames indicate zoomed traces for monoclonal IgM kappa. Serum protein electrophoresis (SPE). shows total protein electrophoresis of cryoglobulin and an absent albumin band indicates washing and isolating of the cryoprecipitate is successfully performed.



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