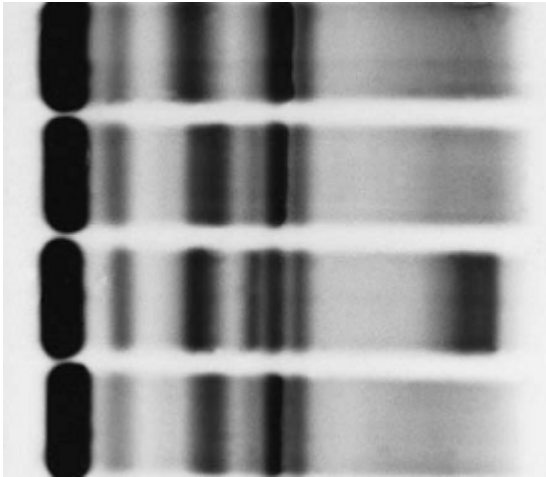
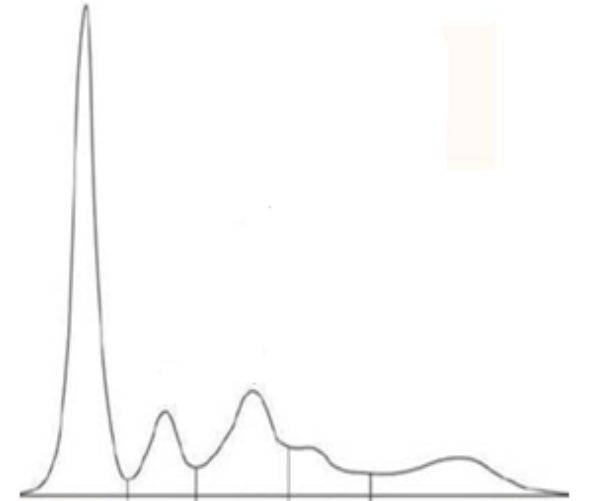


Klinik Laboratuvarda Güncel Elektroforez Uygulamaları



Doğan Yücel, PhD
S.B. Ankara EAH
Tıbbi Biyokimya
Bölümü



Tarihsel Gelişim



Arne Tiselius

(1902 – 1971)

1948 Nobel Kimya Ödülü

*Transactions of The
Faraday Society*

A NEW APPARATUS FOR ELECTROPHORETIC ANALYSIS OF COLLOIDAL MIXTURES.

BY ARNE TISELIUS.

Received 25th January, 1937.

In the course of earlier work¹ on the electrophoretic migration of proteins in various buffer solutions (using a photographic-microphotometric method to follow the migration), a detailed study was made of the different sources of error in electrophoretic measurements and the possibilities of their elimination. Special interest was devoted to the electrophoretic behaviour of mixtures of proteins. It was found that the individual components in such mixtures do not usually mutually influence migration to any great extent, so that a partial separation is brought about by the current. From this we can derive conclusions as to the electrochemical homogeneity of a given colloidal solution, similar

to those Svedberg derives from his ultracentrifuge molecular weight analysis. The importance of using physical methods for such a purpose is obvious: the usual methods of fractionation of biocolloids (which are very unstable and very sensitive towards even quite mild chemical agents), are often of doubtful value. The procedure was exemplified both on artificial and on natural mixtures, and was also used for the purification of proteins. Fig. 1 shows the apparatus used.

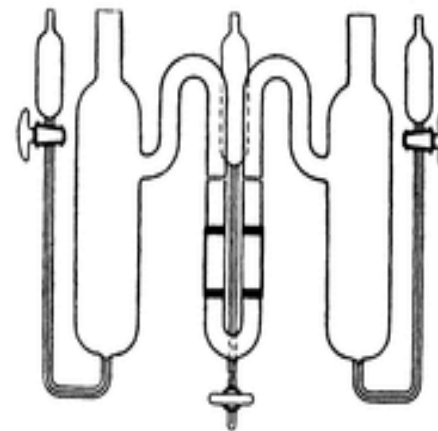
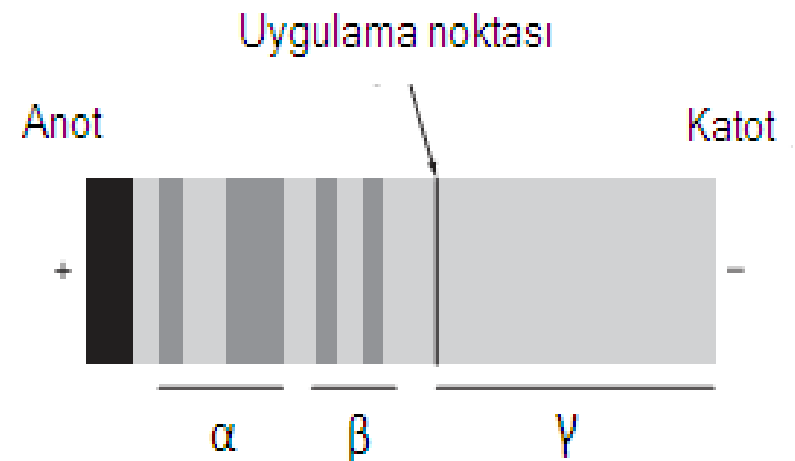


FIG. 1.—Electrophoresis apparatus for the moving boundary method (Tiselius, 1930).

The value of electrophoretic fractionation and purification of

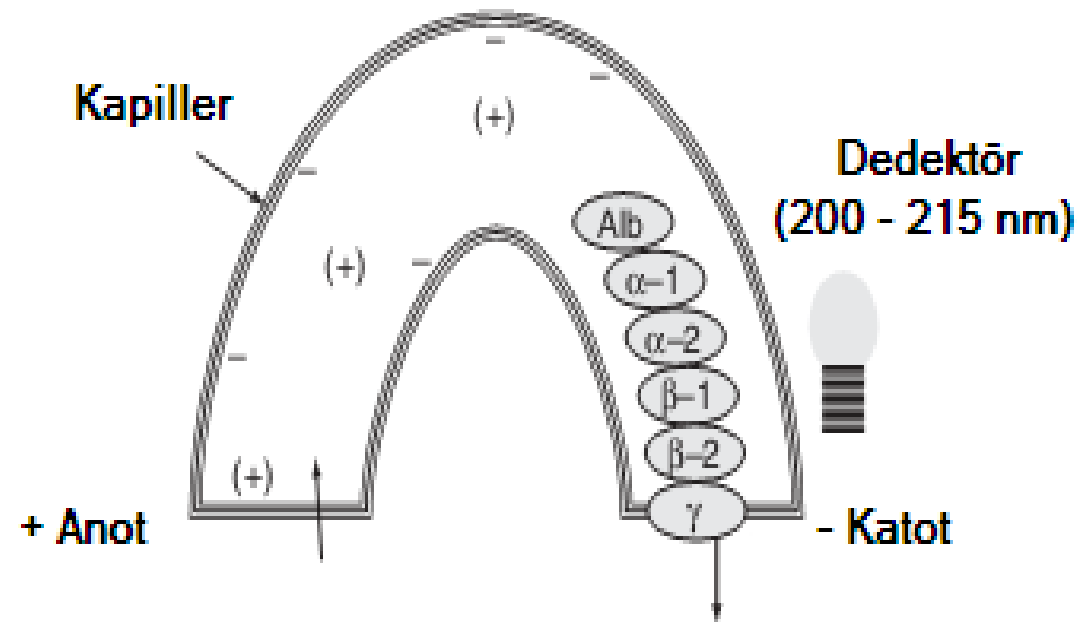
Tarihsel Gelişim: Klasik Elektroforez

- Sıvı ortamda “serbest akış” sistemiyle ayırım: **“Moving boundary electrophoresis”** ve **“free flow ”**
- Katı bir destek ortam üzerinde elektroforez: **“Zone electrophoresis”**
 - Kağıt, nişasta, selüloz asetat, agar, poliakrilamid, agaroz
 - **Kağıt:** 1950’ler
 - **Selüloz asetat:** 1960’lar
 - **Agaroz jel:** 1960’lar
 - Dansitometrik tarama: 1964

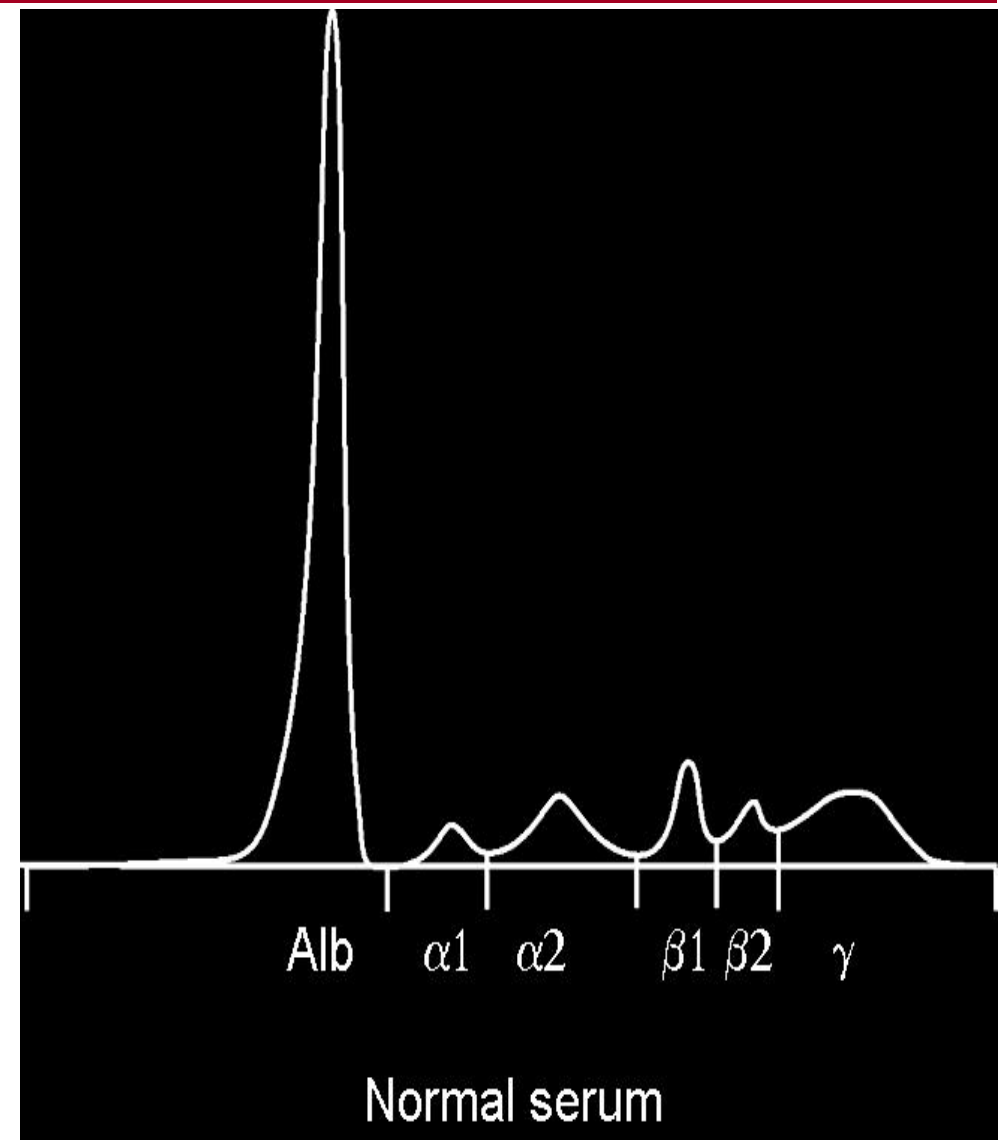
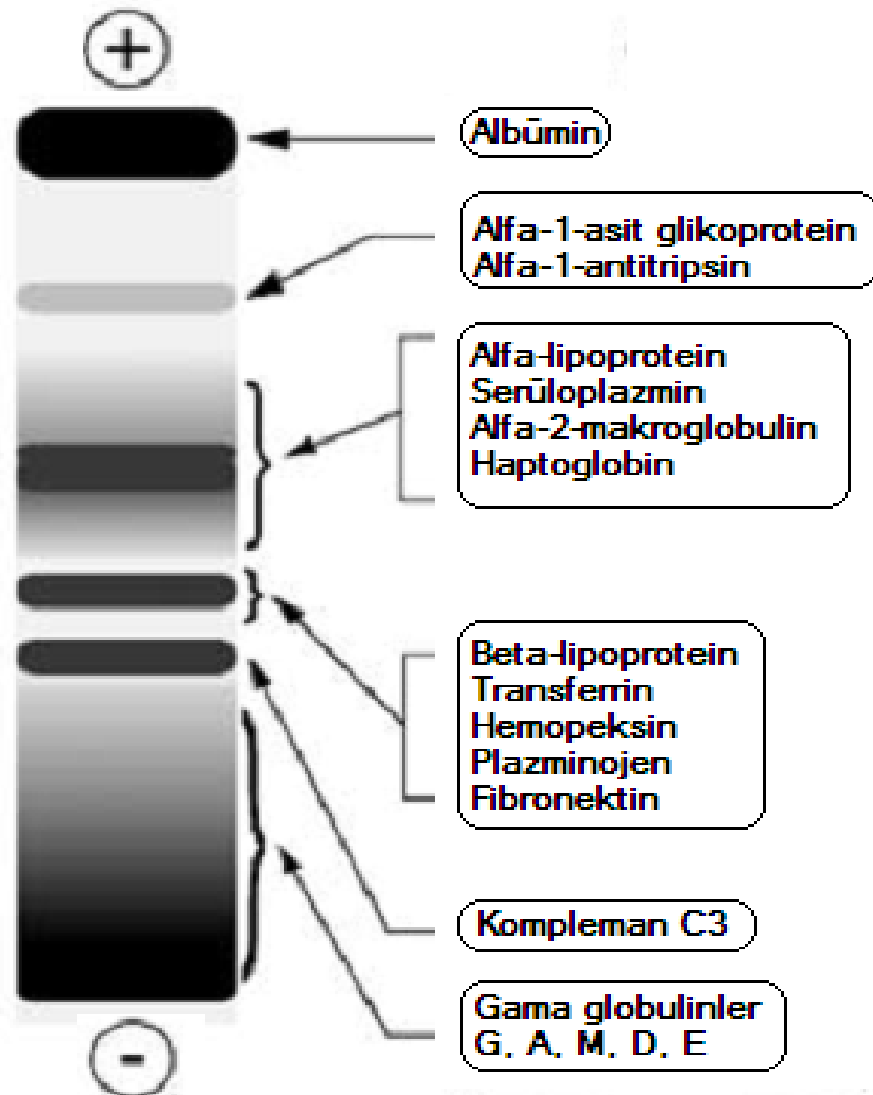


Tarihsel Gelişim: Kapiller ELEktroforez

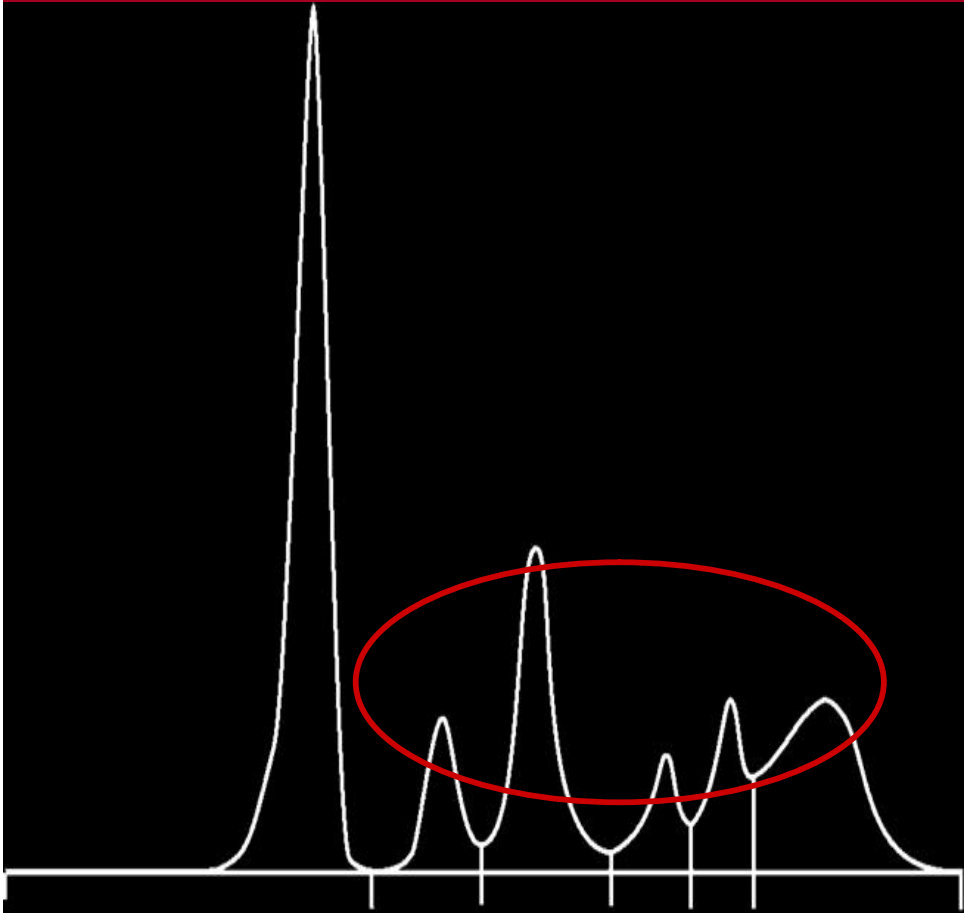
- 1960'larda geliştirilmesine rağmen rutin uygulamalar 1990'larda başladı
- Destek ortamı yoktur
- Boya yok
- Kapiller:
 - İç çapı 10 – 100 μm
 - Uzunluk: 20 – 200 cm
- Az örnek: 1 – 5 μL
- İyi rezolüsyon
- Yüksek voltaj: 10 000 V
- Isı oluşumu yok



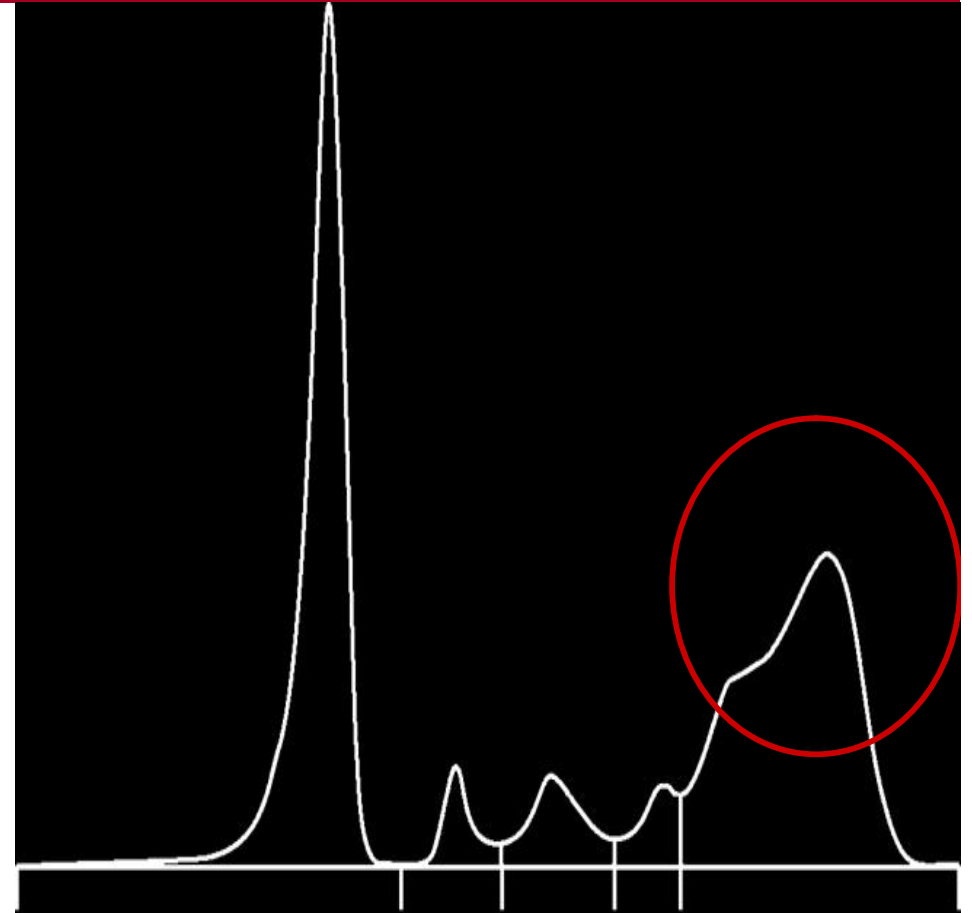
Serum Protein Elektroforezi



Örnekler

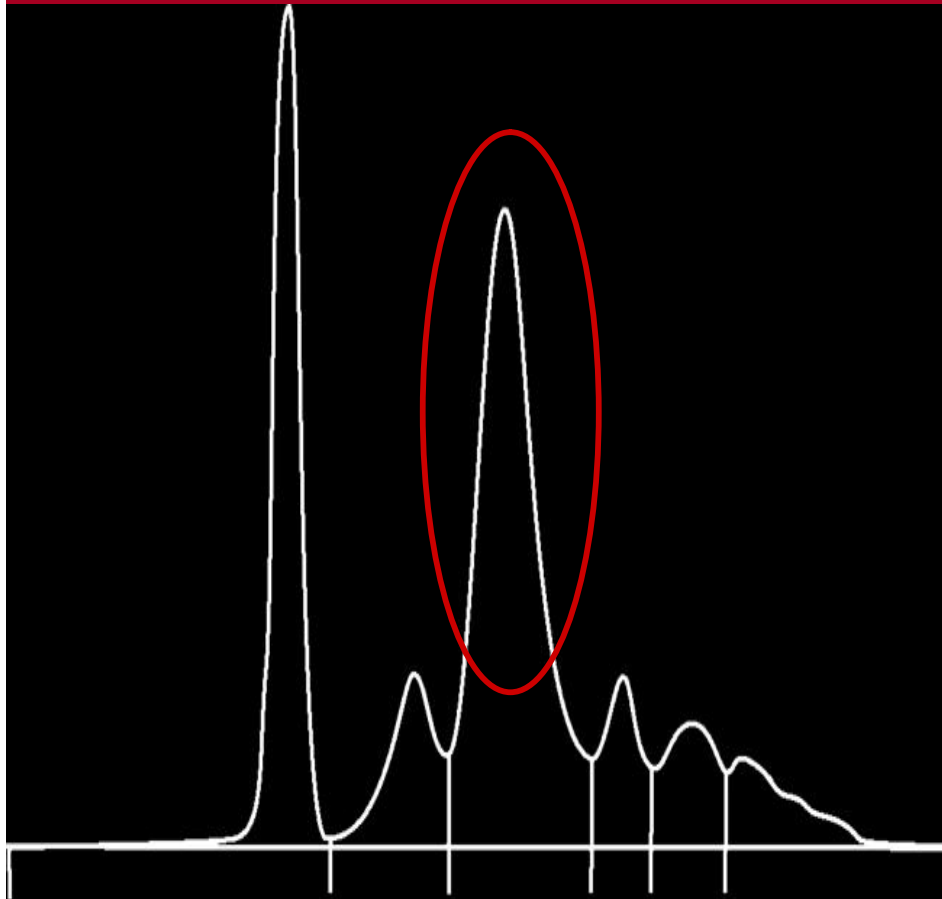


Kronik Enflamatuvar Hastalık

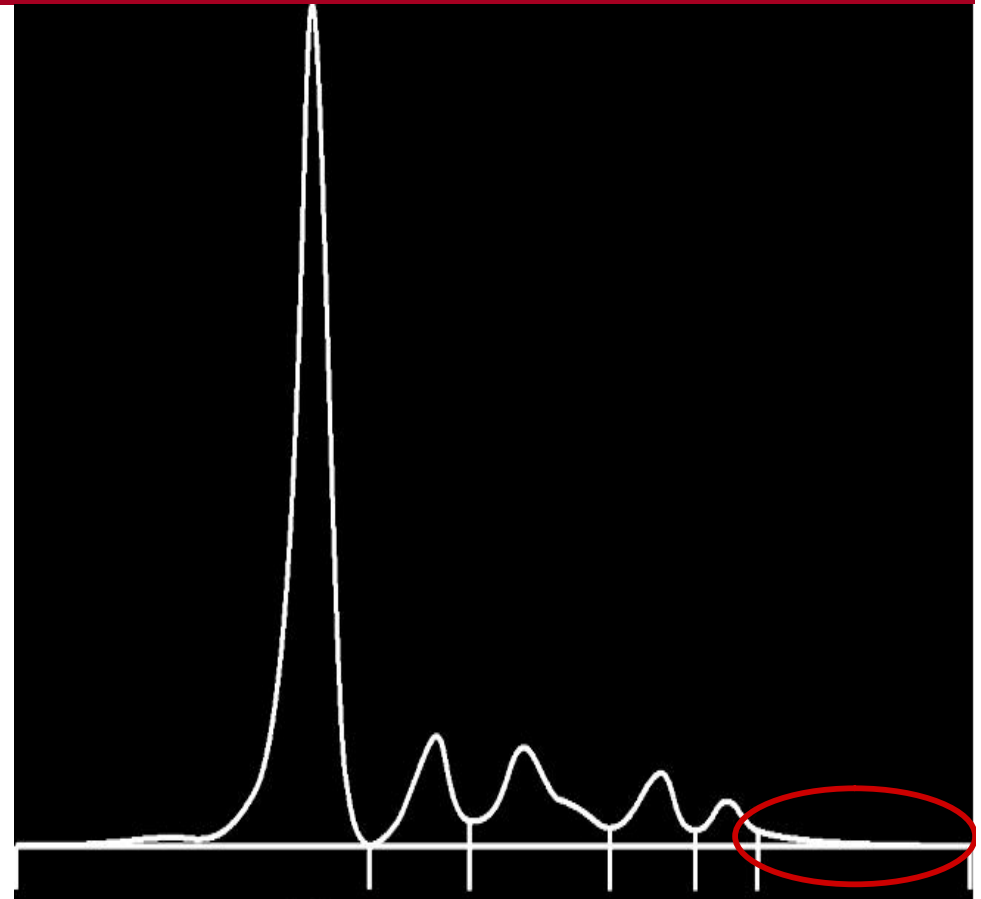


Beta -Gama Köprüsü

Örnekler

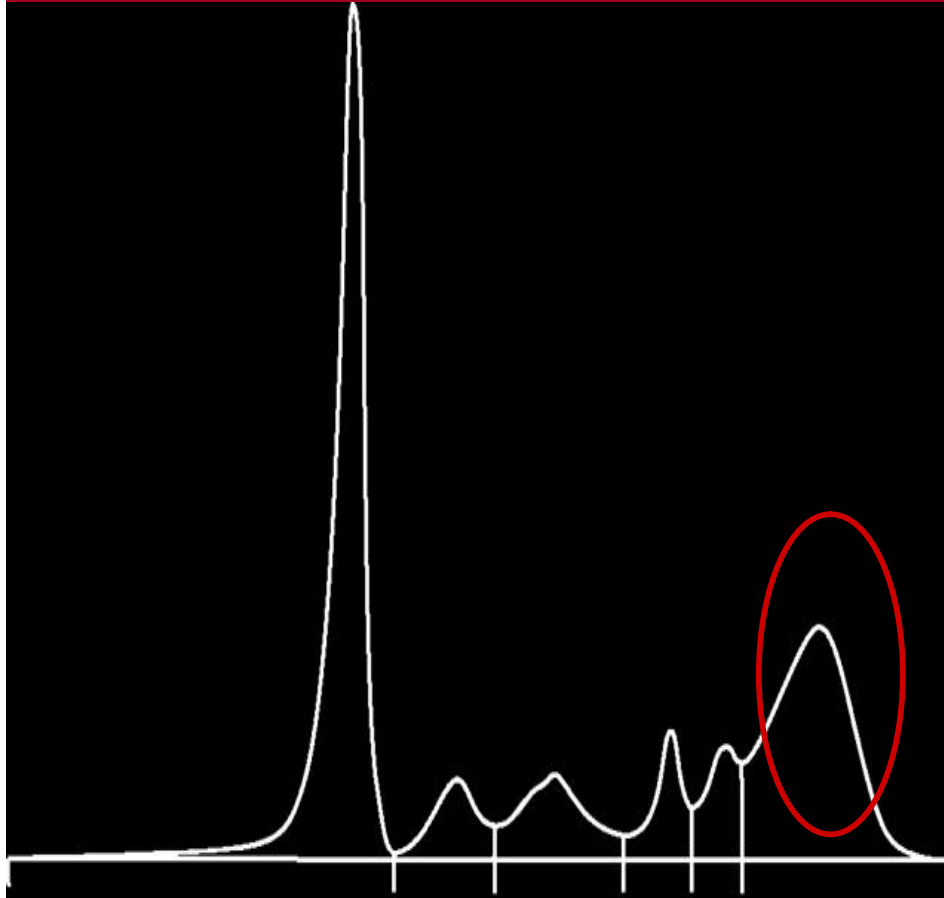


Nefrotik Sendrom

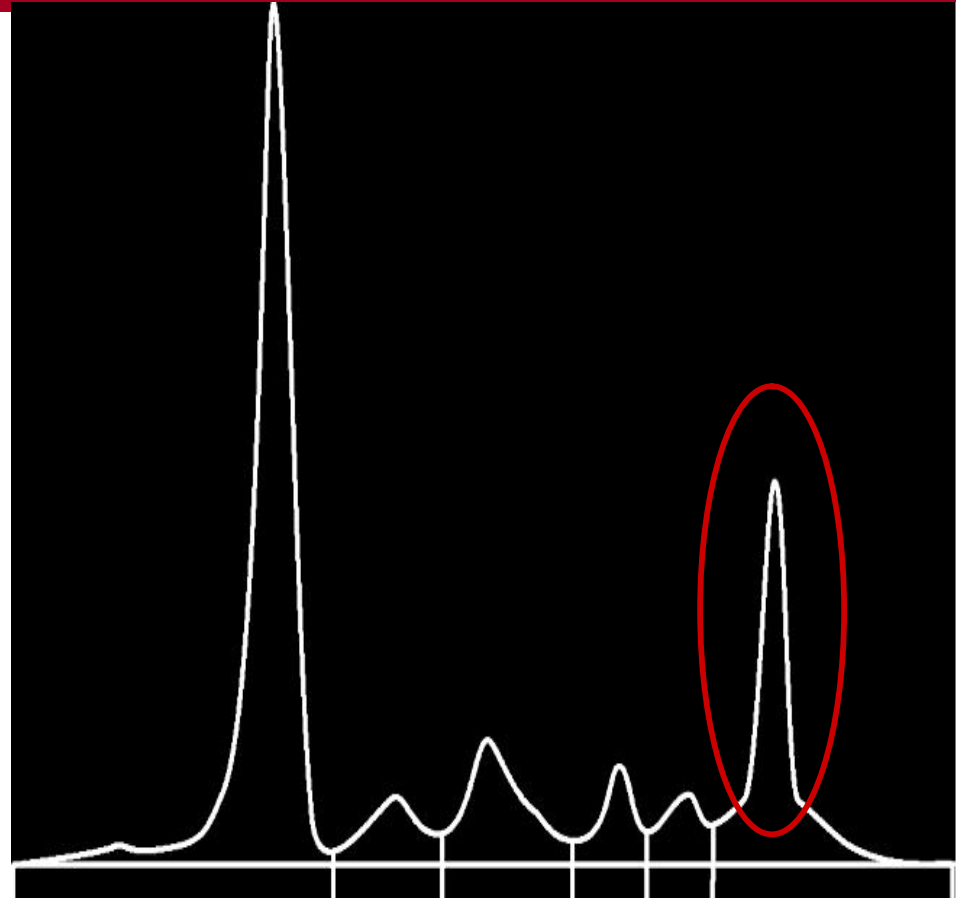


Hipogamaglobulinemi

Örnekler

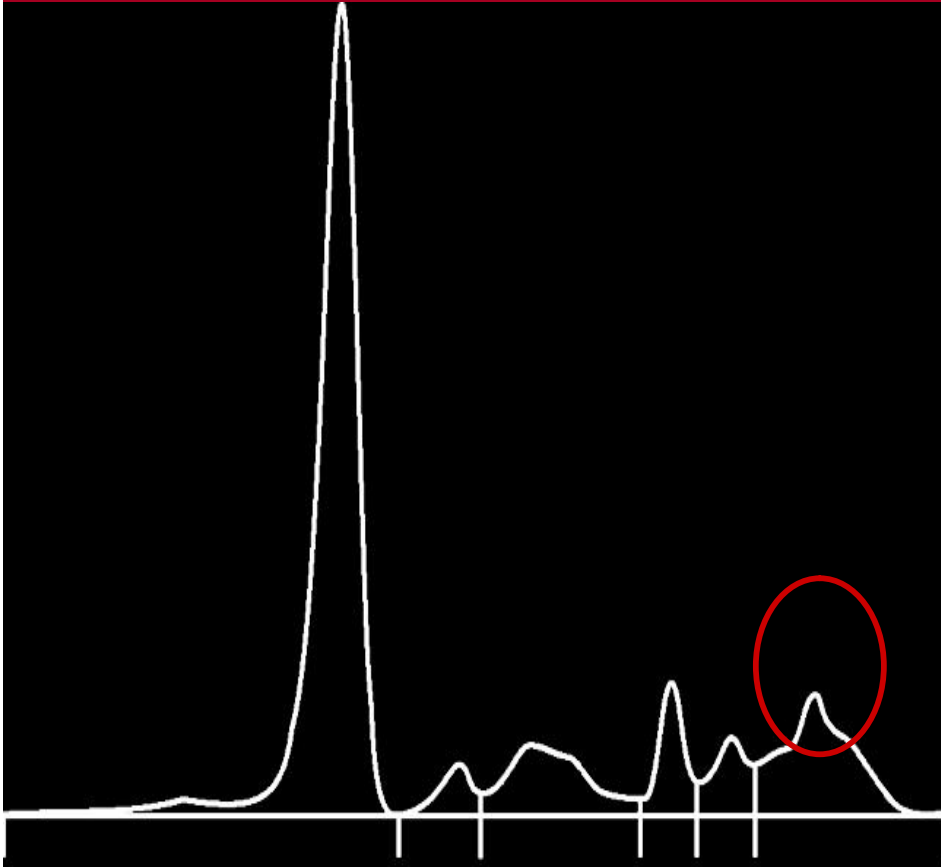


Poliklonal gamopati

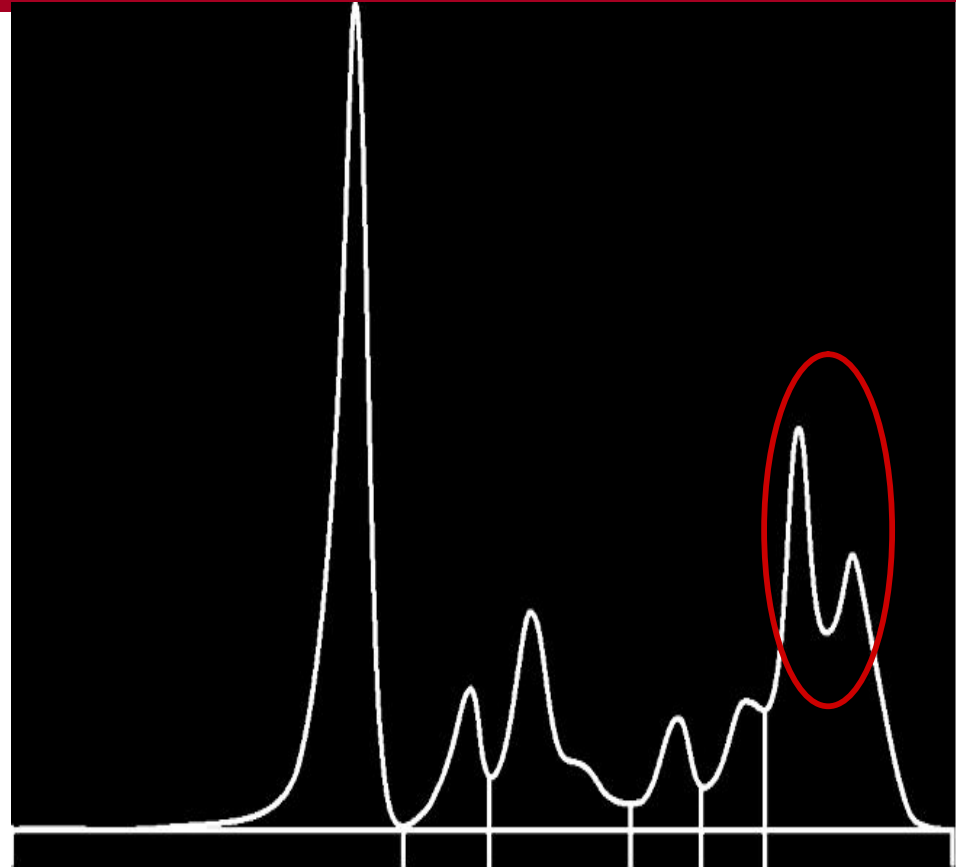


Monoklonal gamopati

Örnekler

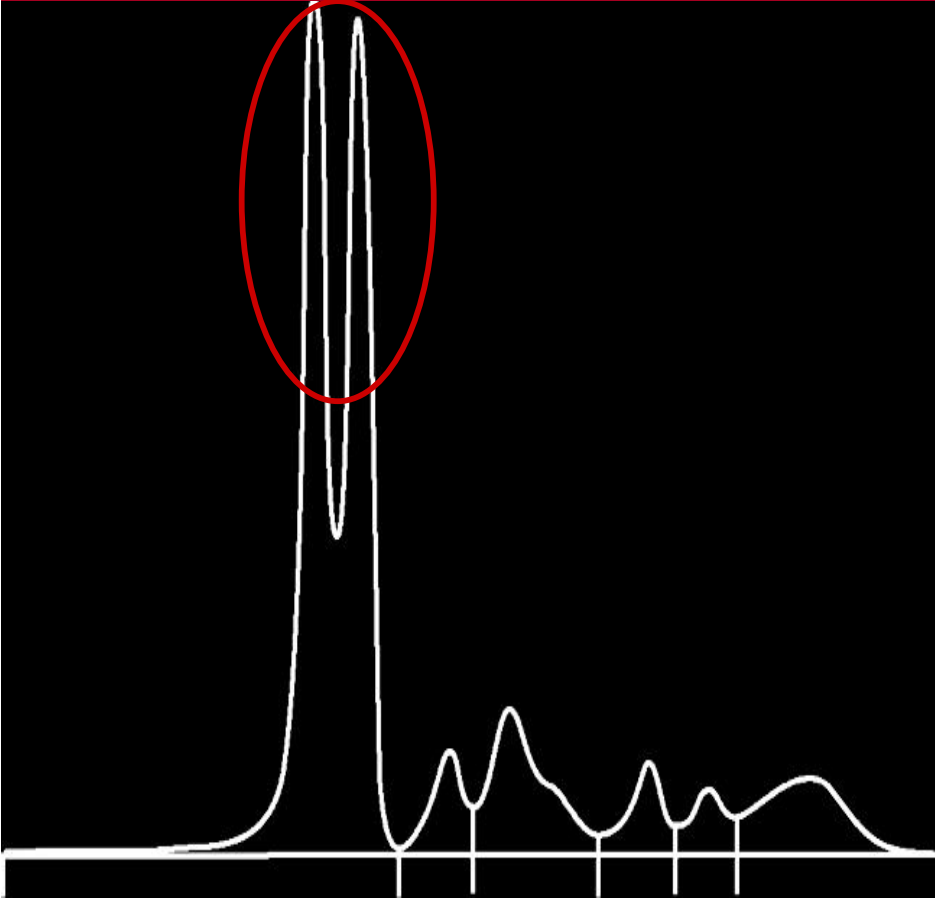


Monoklonal gamopati

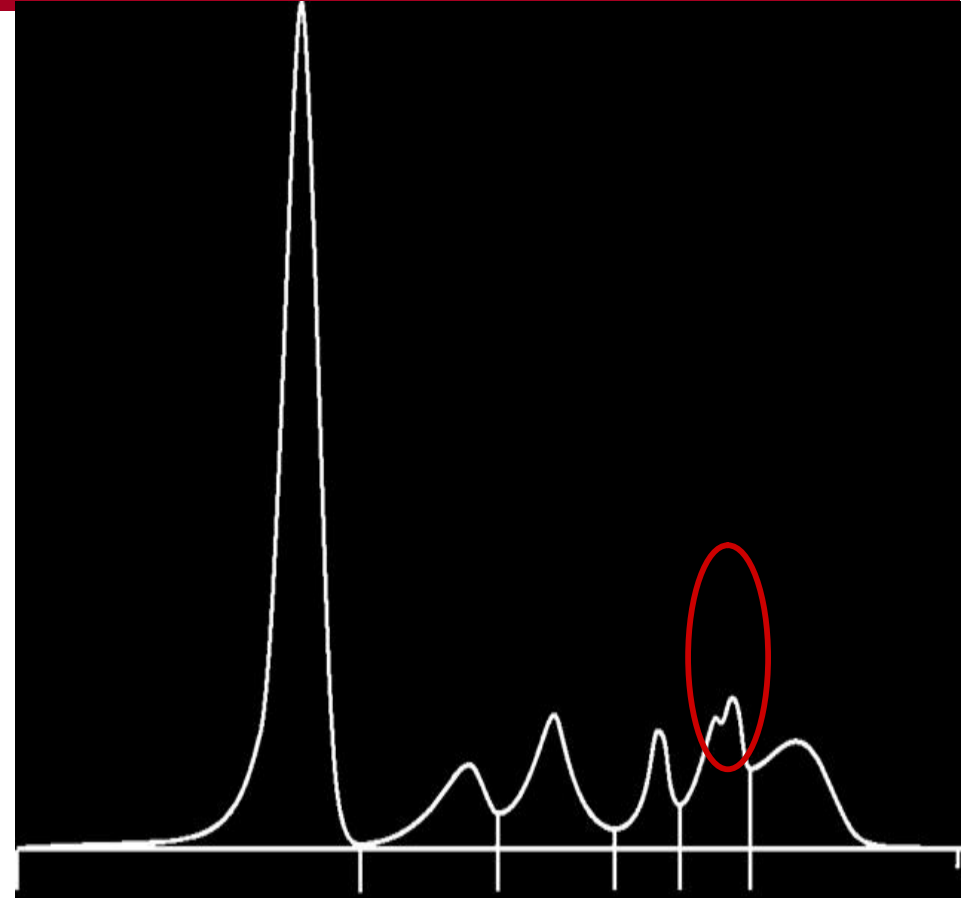


Biklonal gamopati

Örnekler

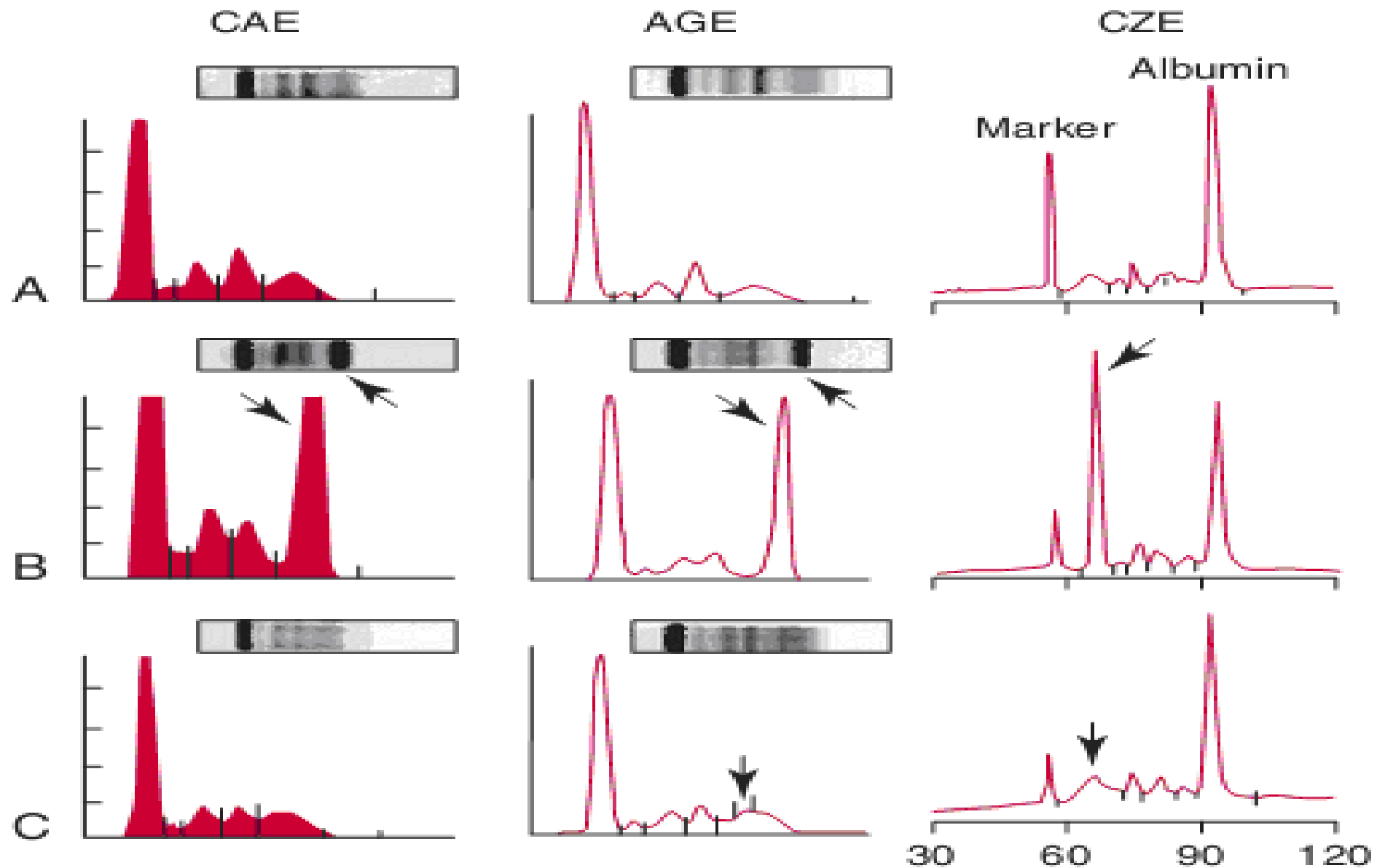


Bisalbüminemi

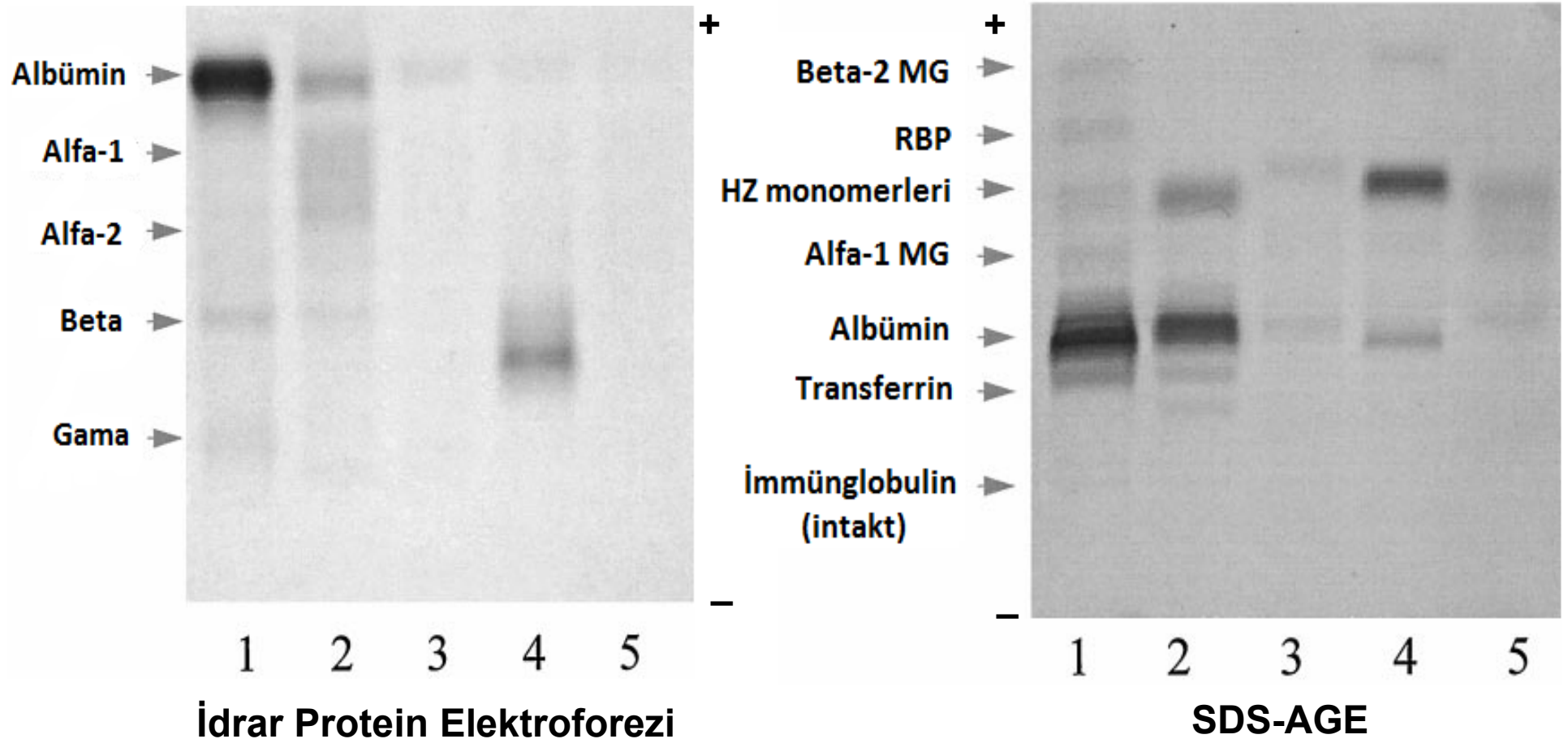


Fibrinojen varlığı (Plazma)

Karşılaştırma

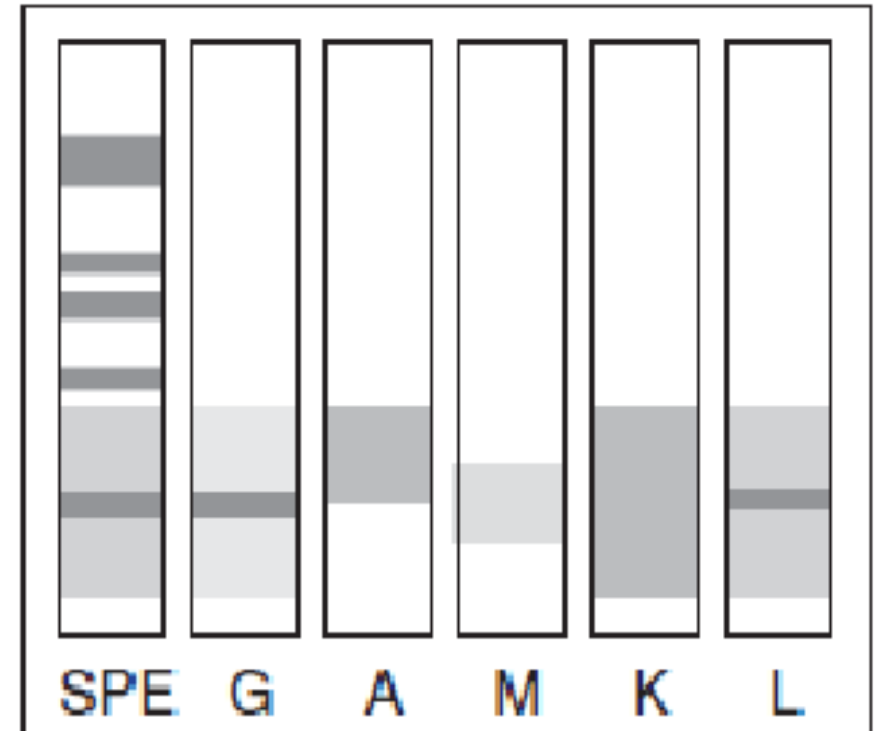
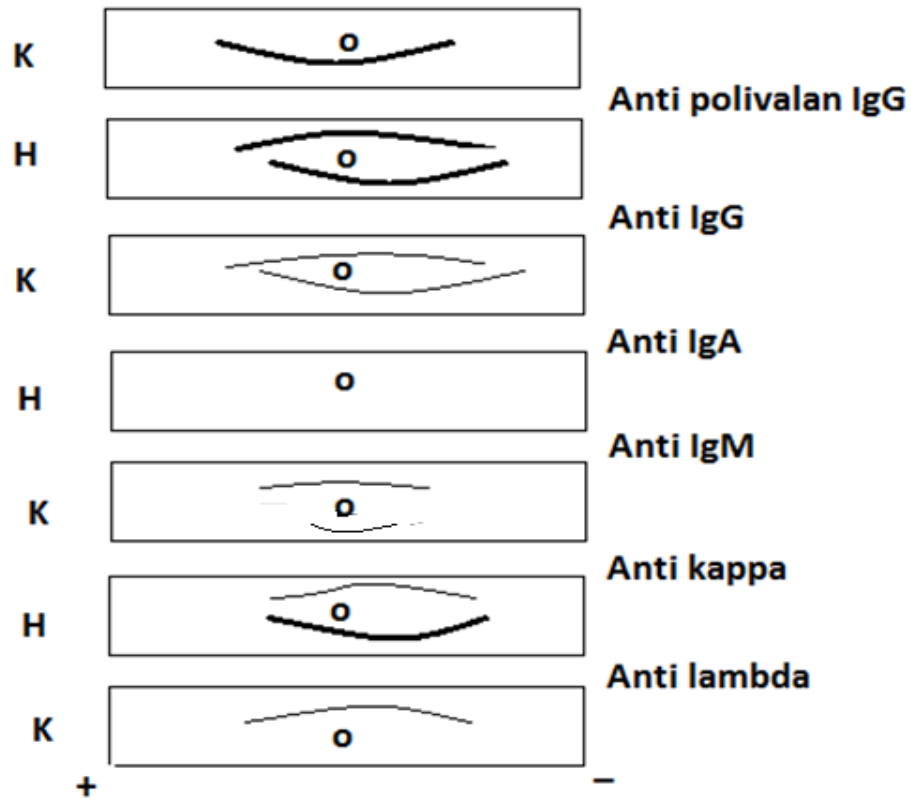


İdrar Protein Elektroforezi



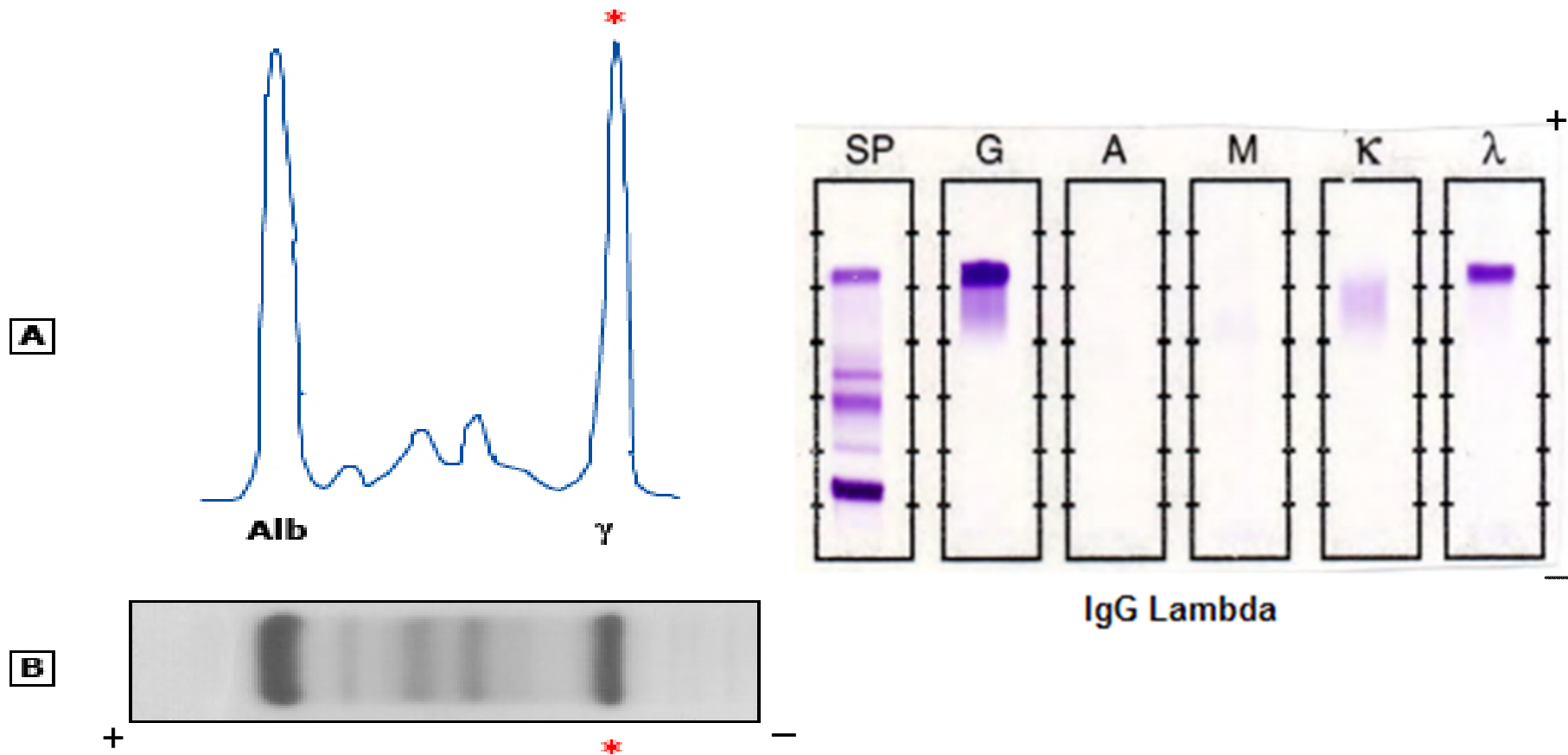
1: Mikst proteinüri, BJP (-); 2: Glomerüler proteinüri, BJP (+); 3: BJP %20; 4: BJP %60; 5: Tübüler Pr

İmmünelektroforez/İmmünfiksasyon

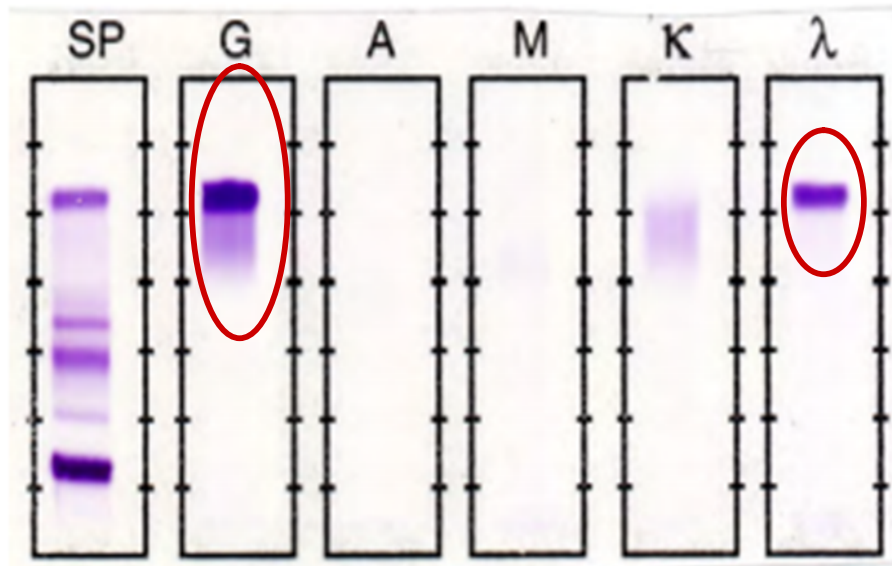


IgG lambda monoklonal gamopati

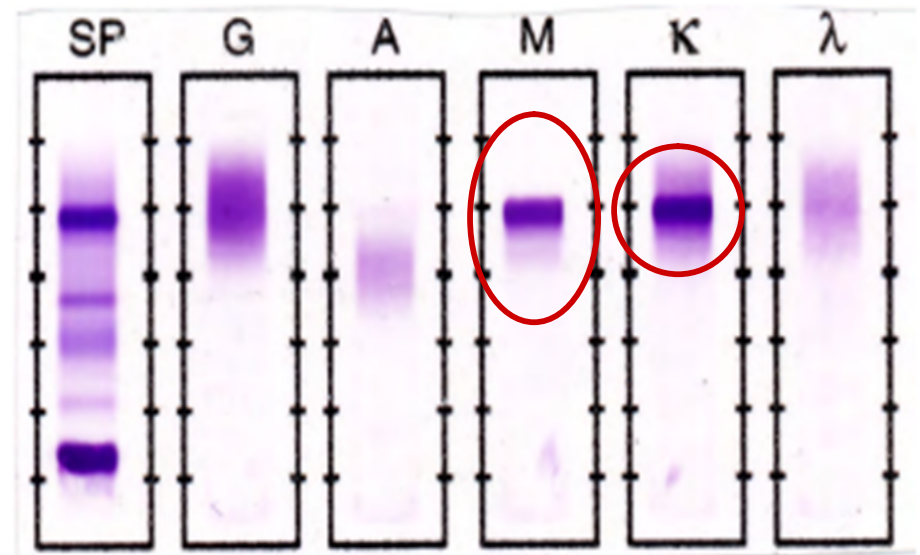
IFE: Değerlendirme



IFE: Örnekler

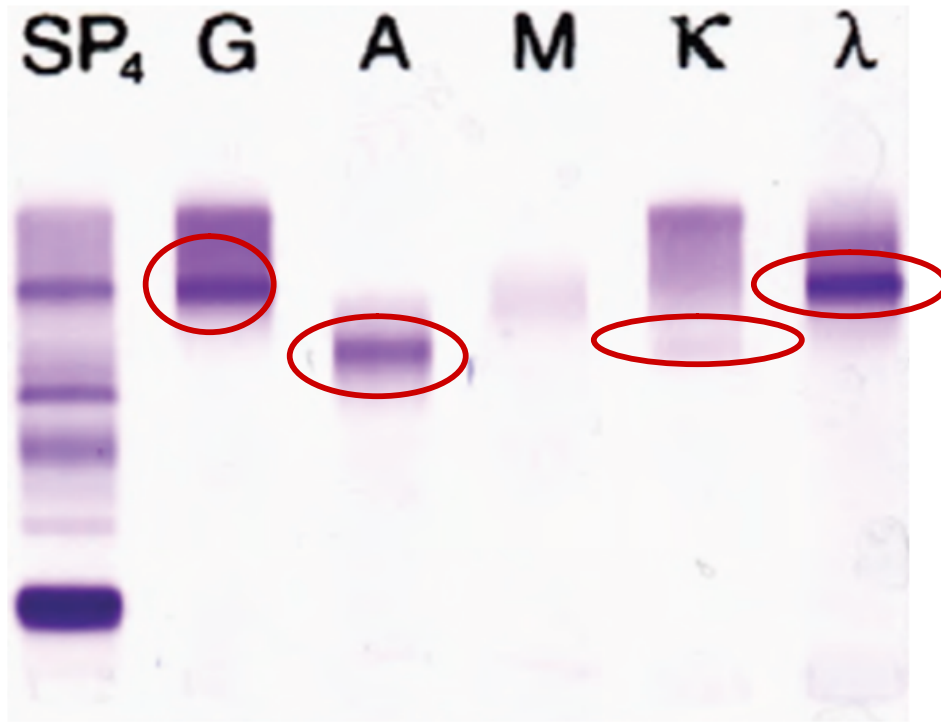


IgG Lambda

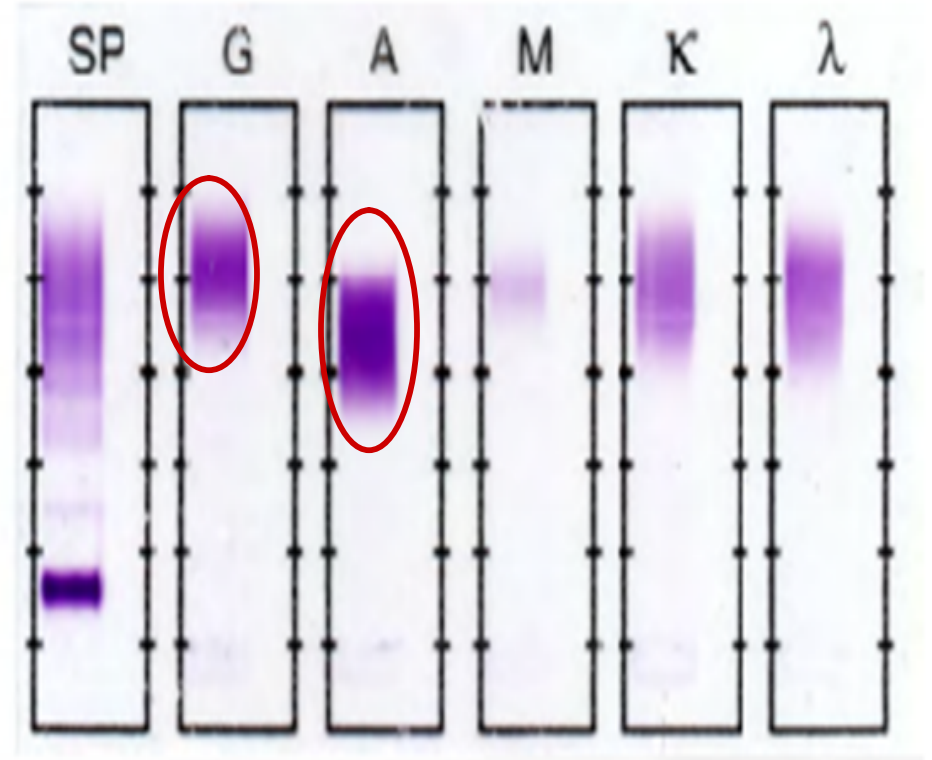


IgM Kappa

İFE: Örnekler

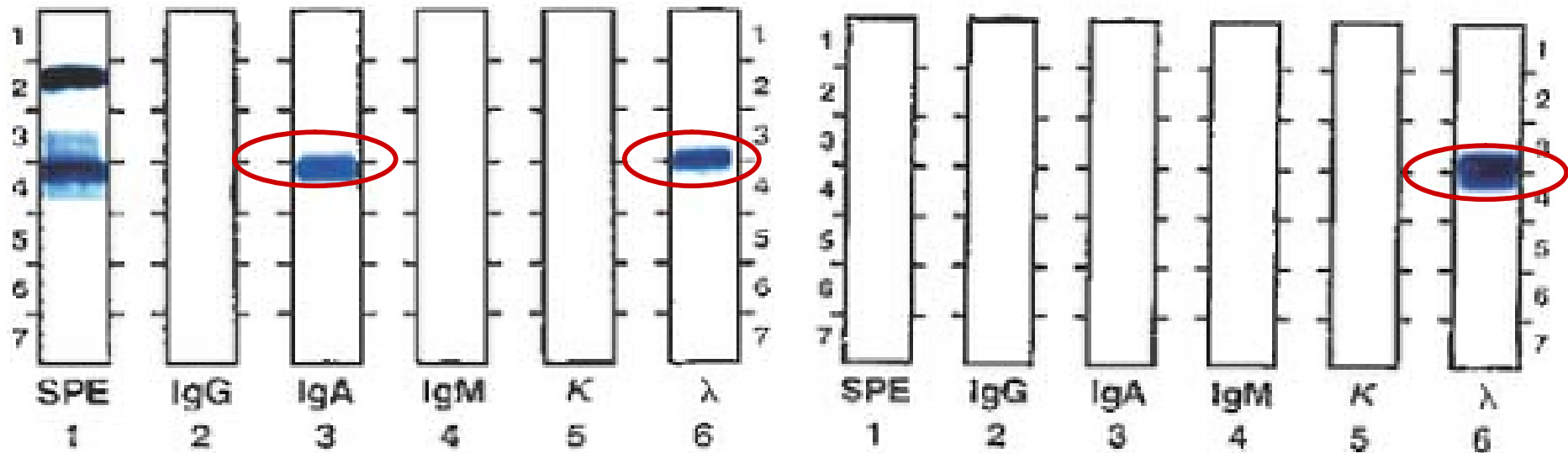


IgG Lambda, Ig A Kappa Biklonal



Poliklonal artış

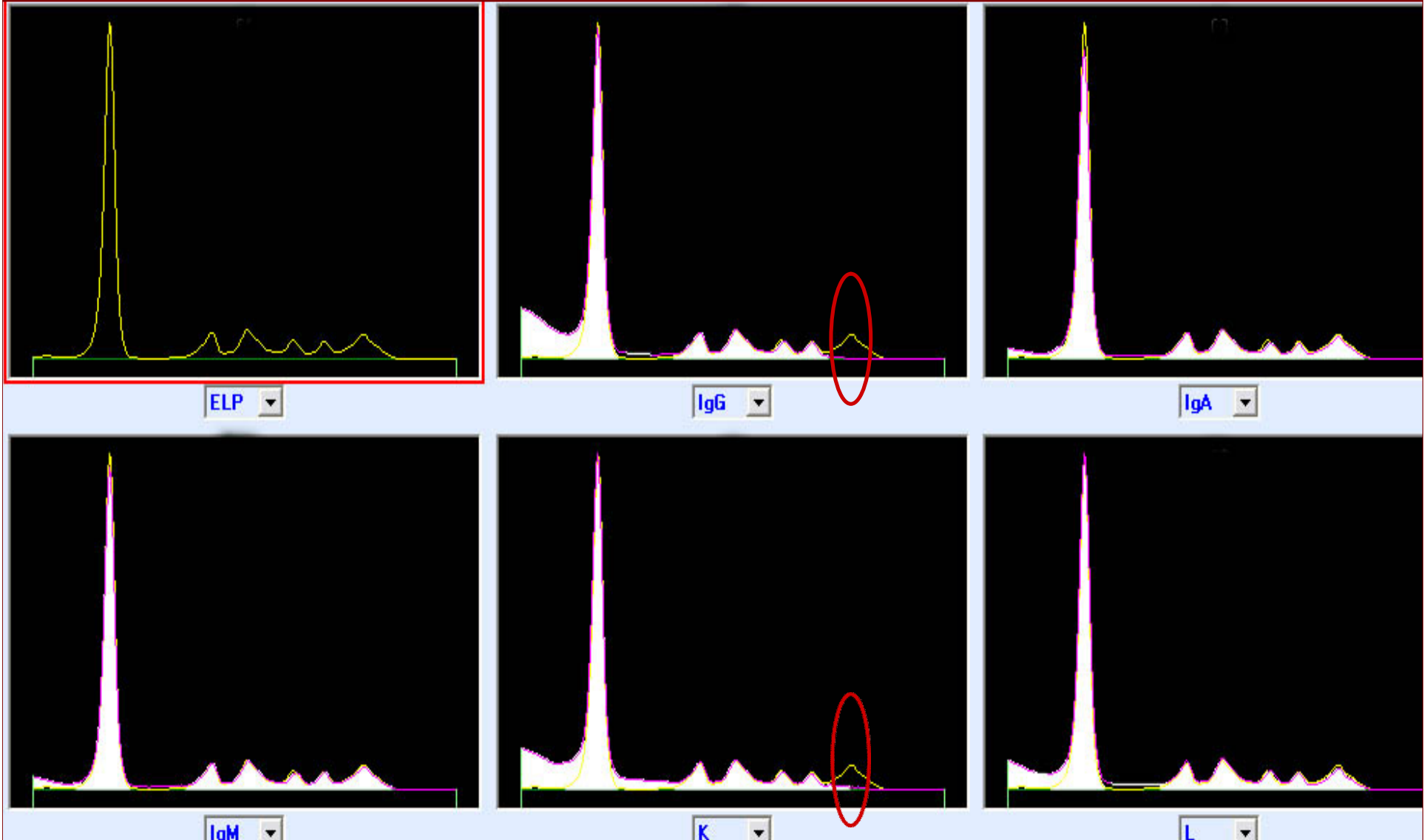
iFE: İdrar



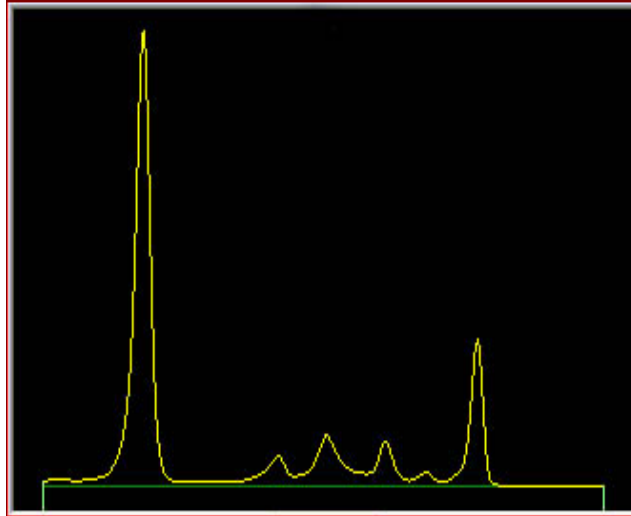
Serum

İdrar

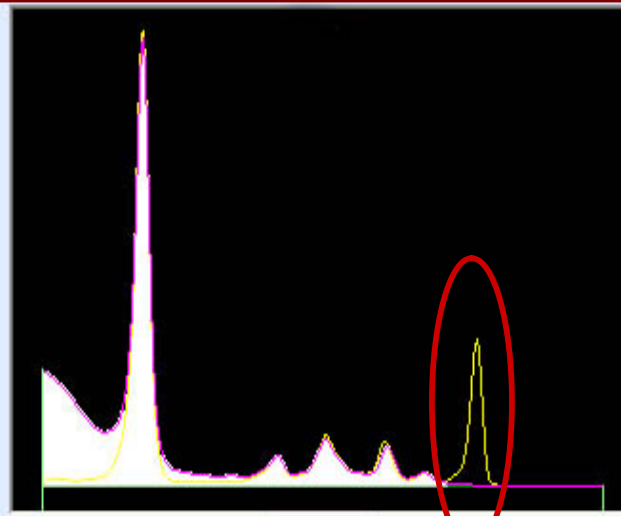
“İmmün Çıkarım”/ IgG Kappa



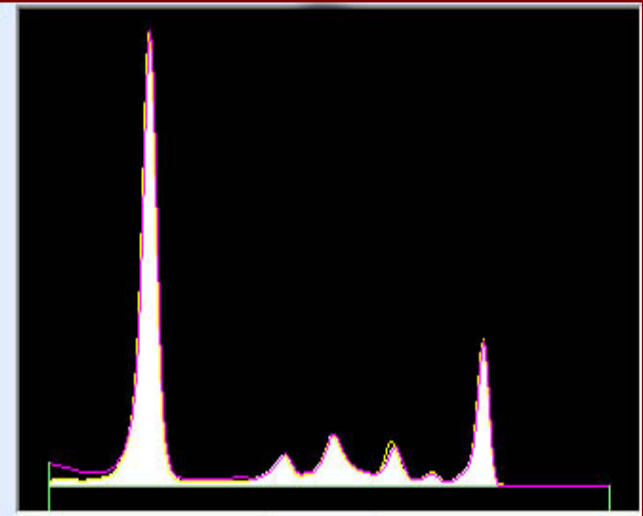
IgG Lambda



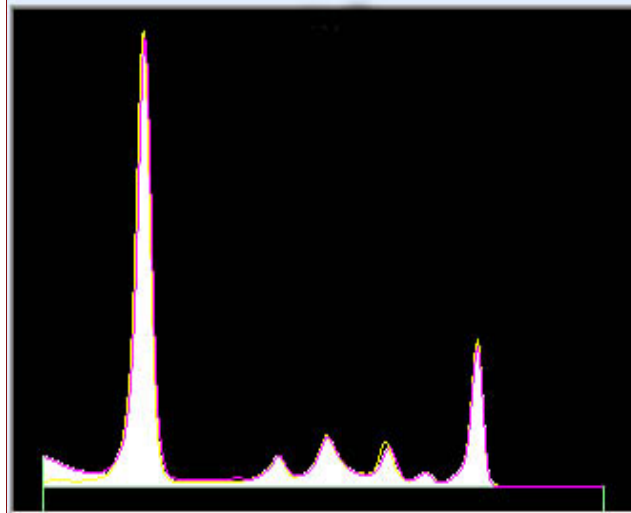
ELP



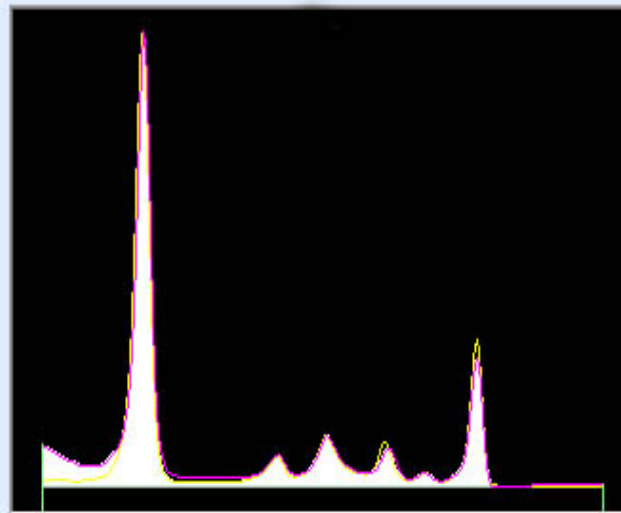
IgG



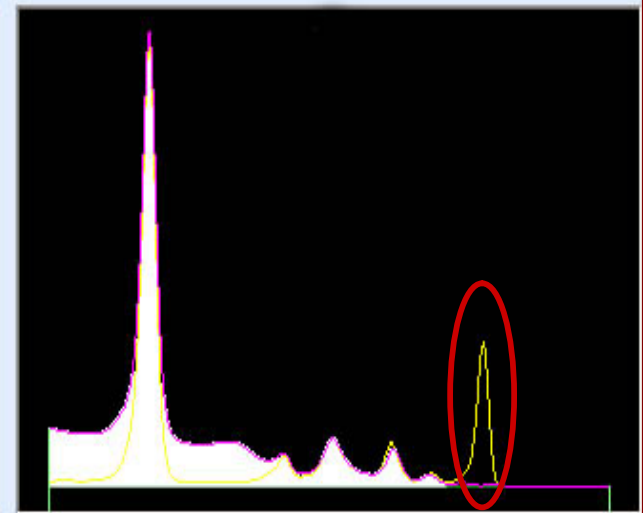
IgA



IgM

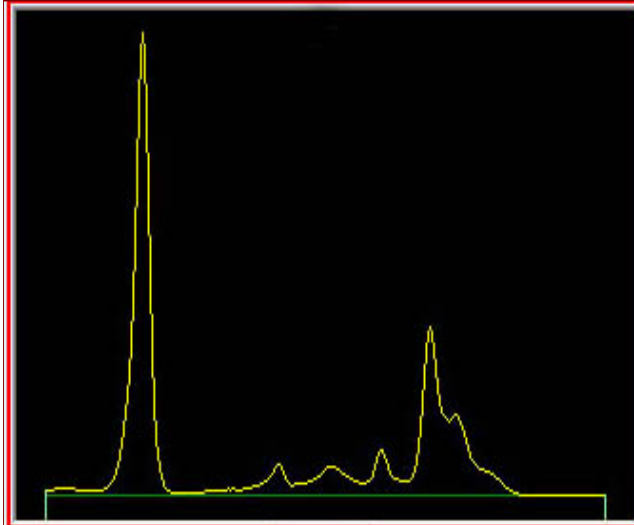


K

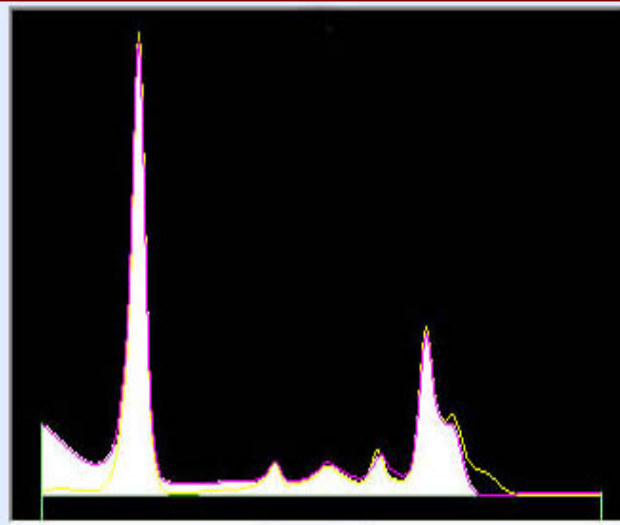


L

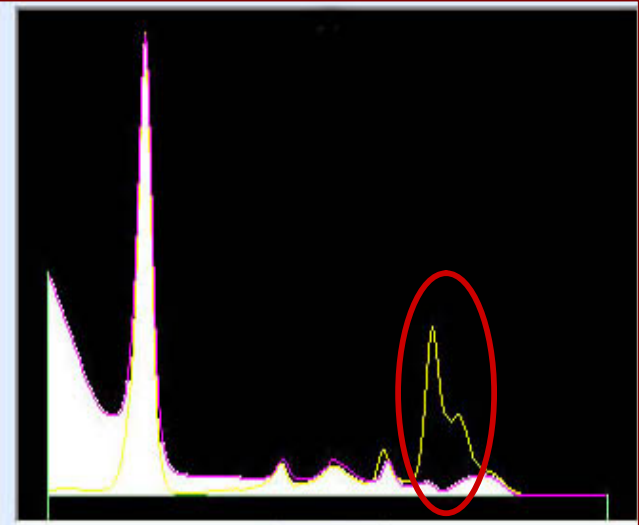
IgA Kappa



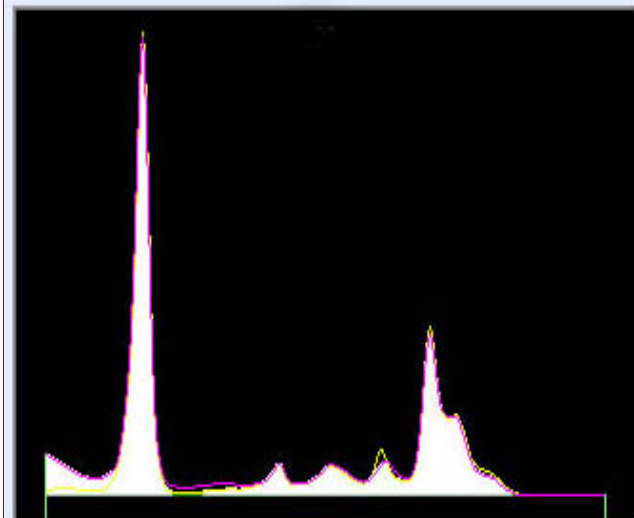
ELP



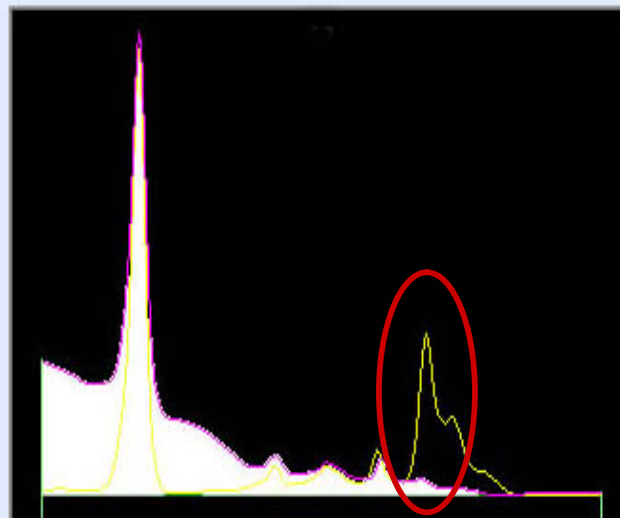
IgG



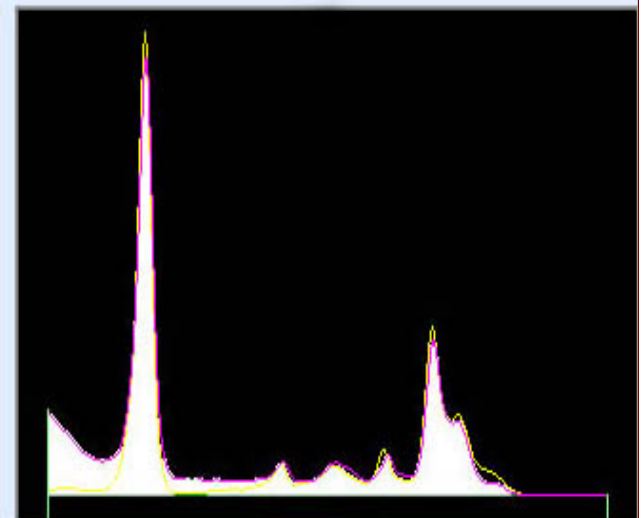
IgA



IgM

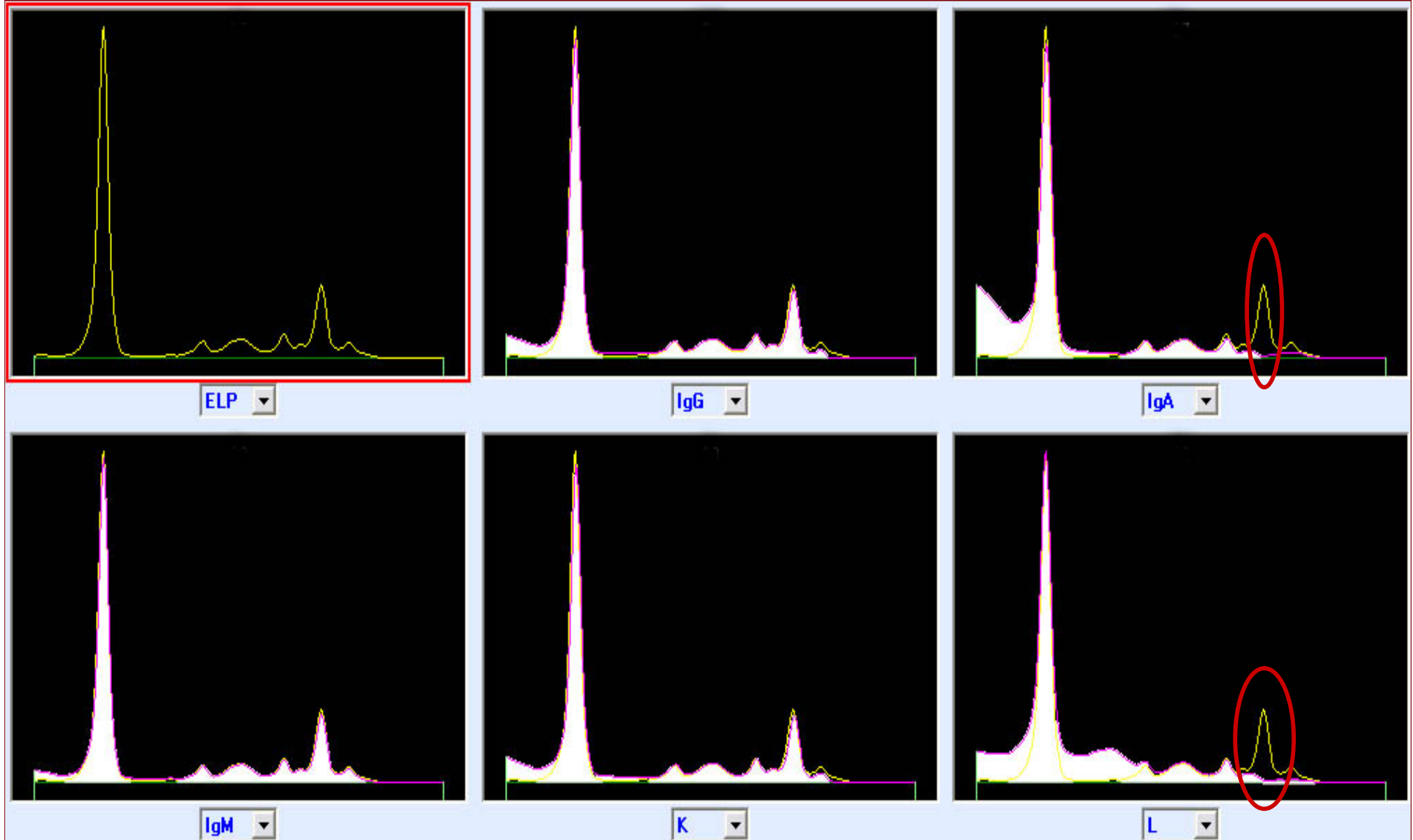


K

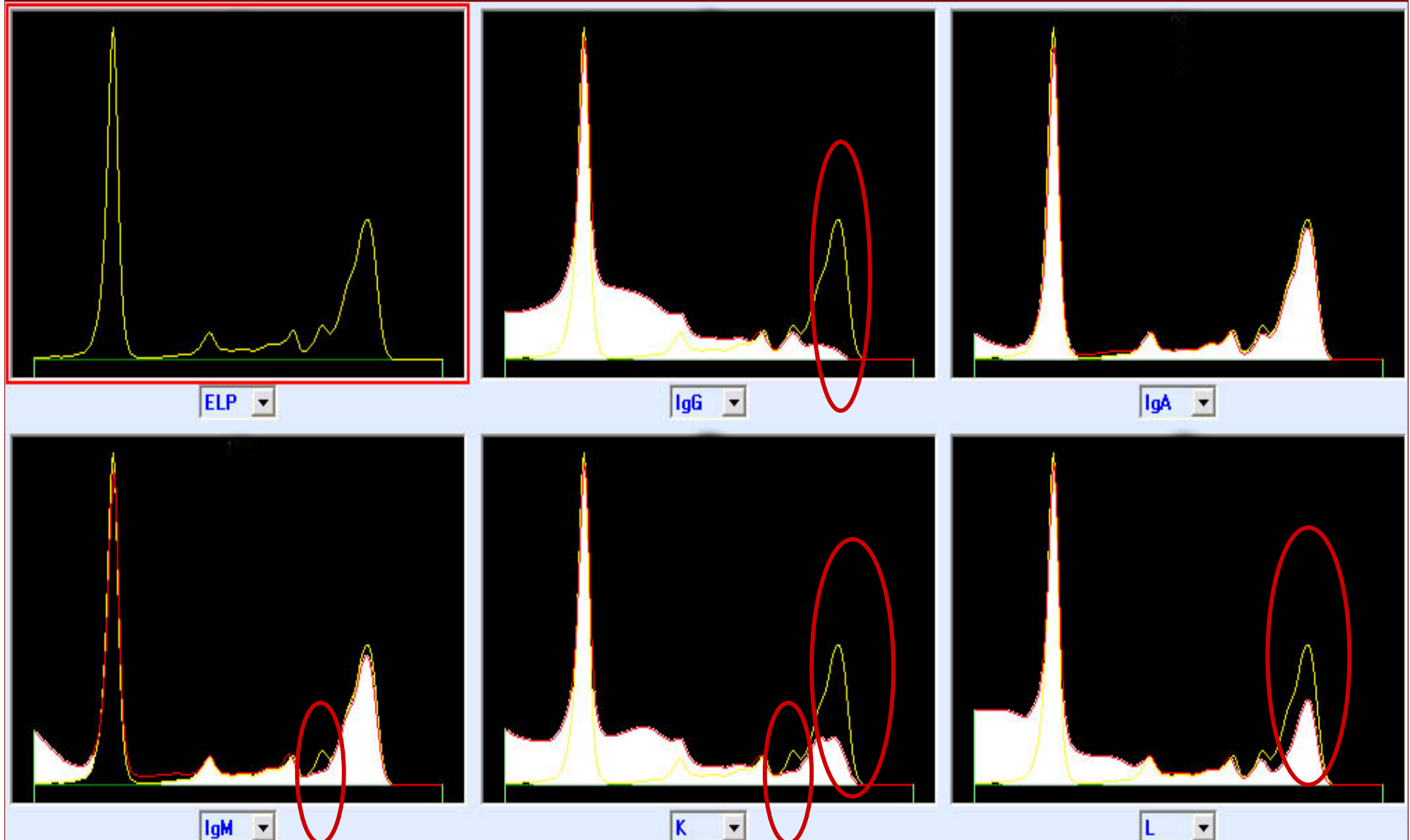


L

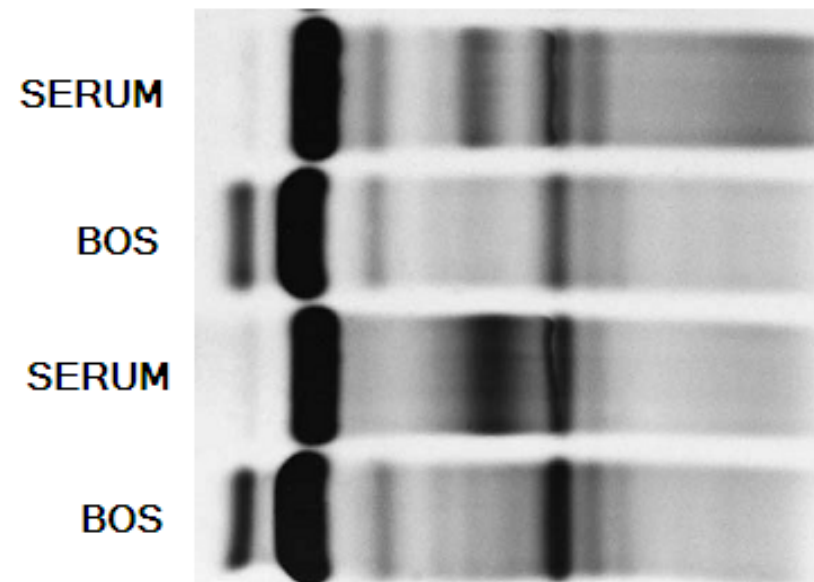
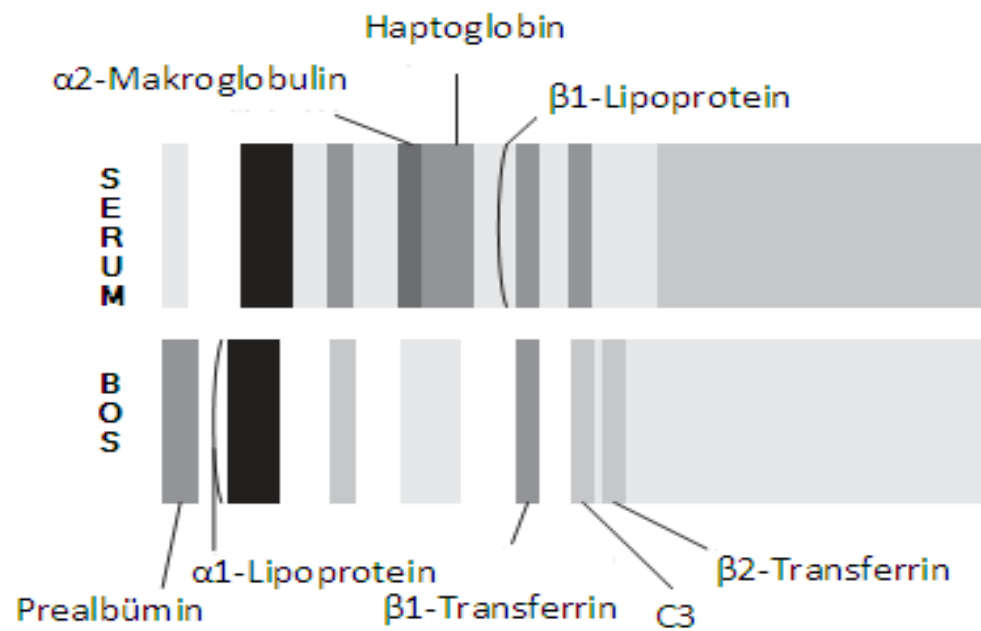
IgA Lambda



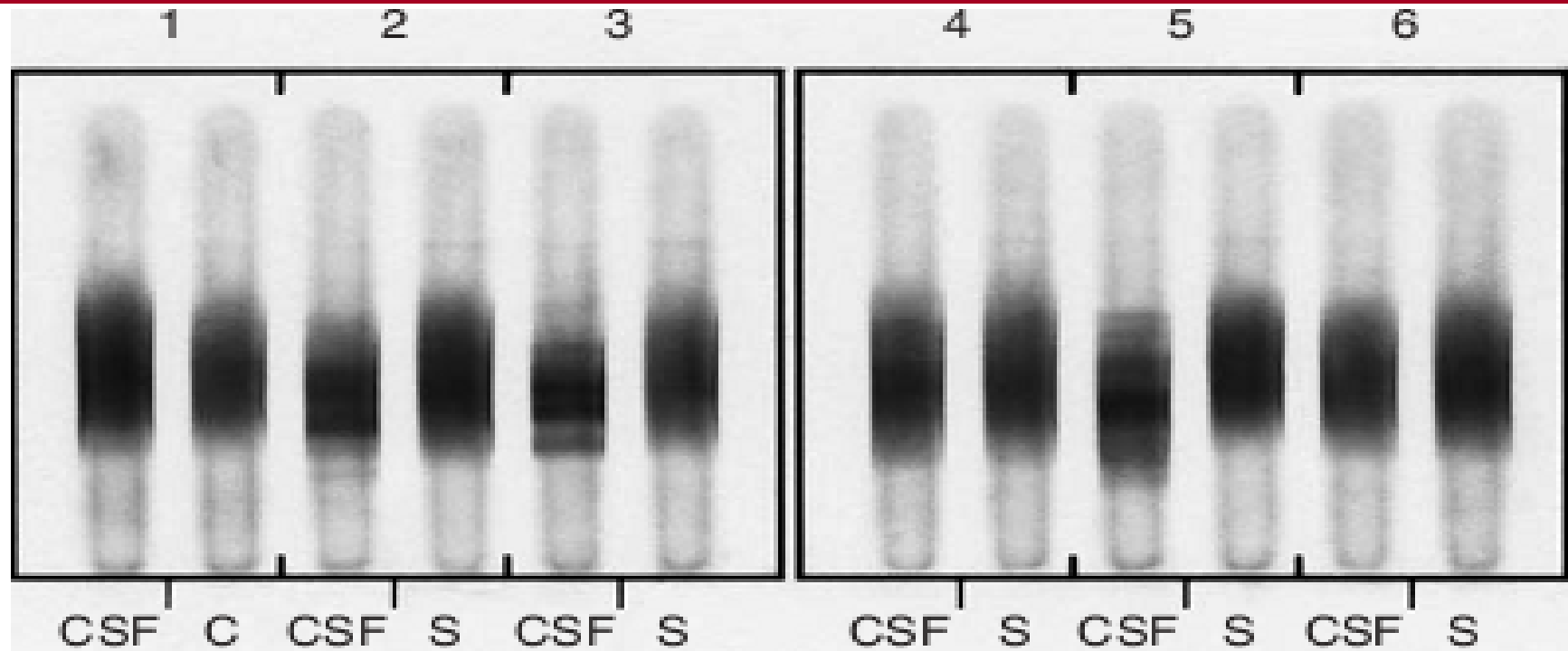
IgG Kappa + IgG Lambda + IgM Kappa



BOS

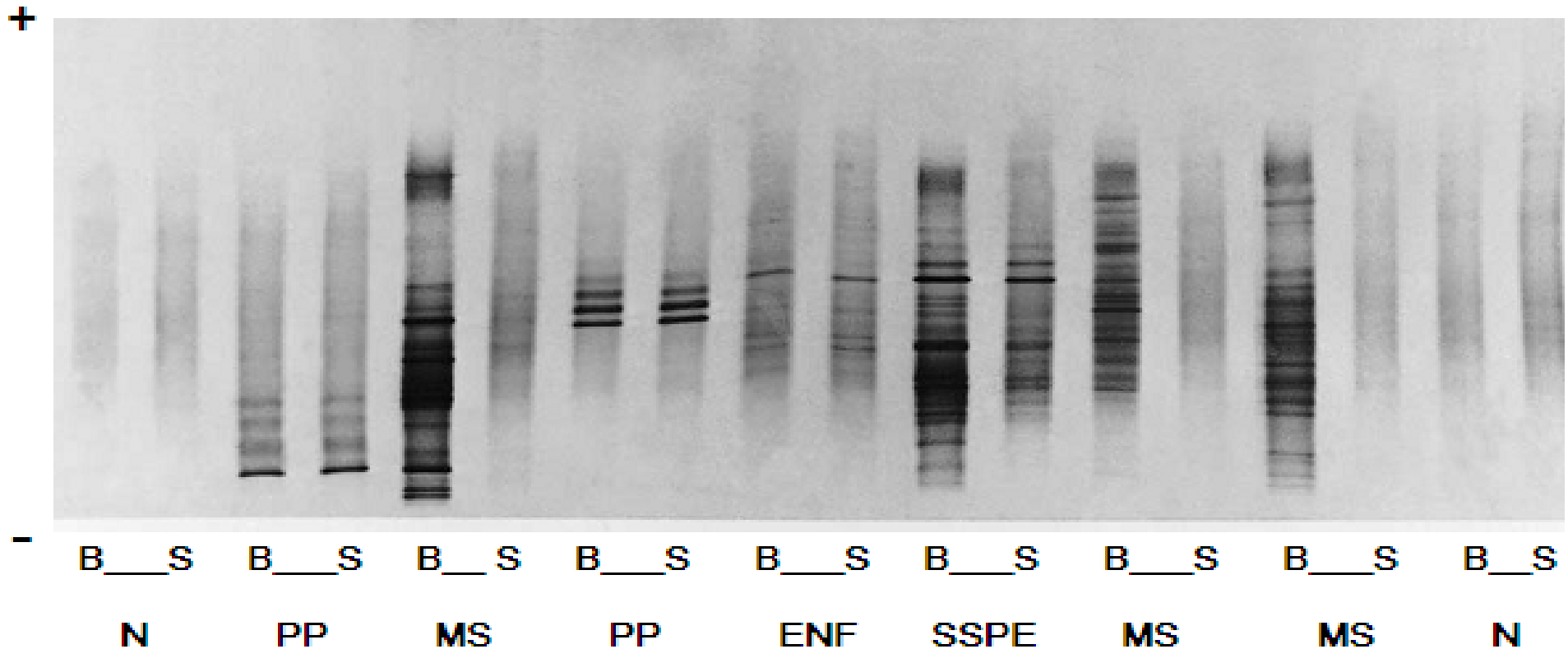


BOS İFE: Oligoklonal Bant

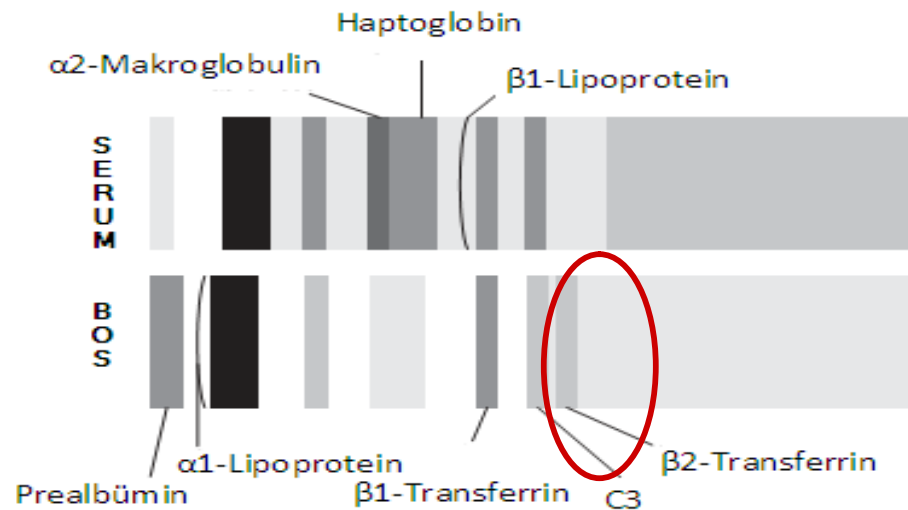


2, 3 ve 5: Oligoklonal Bant pozitif
4 ve 6: Negatif

İzoelektirik Odaklama: Oligoklonal Bant



β_2 -Transferrin

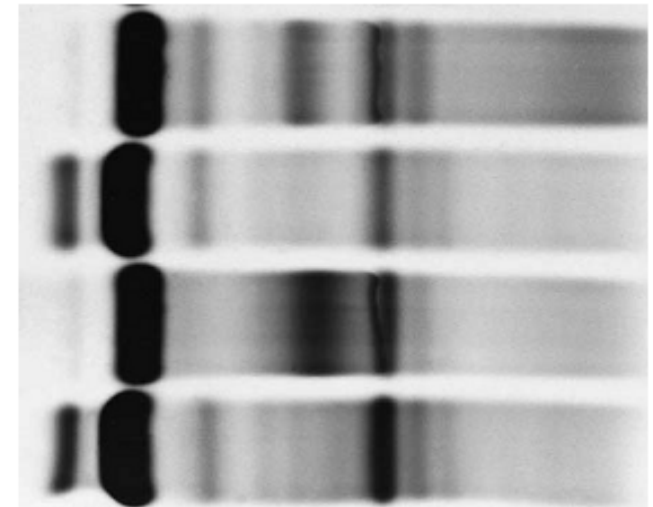


SERUM

BOS

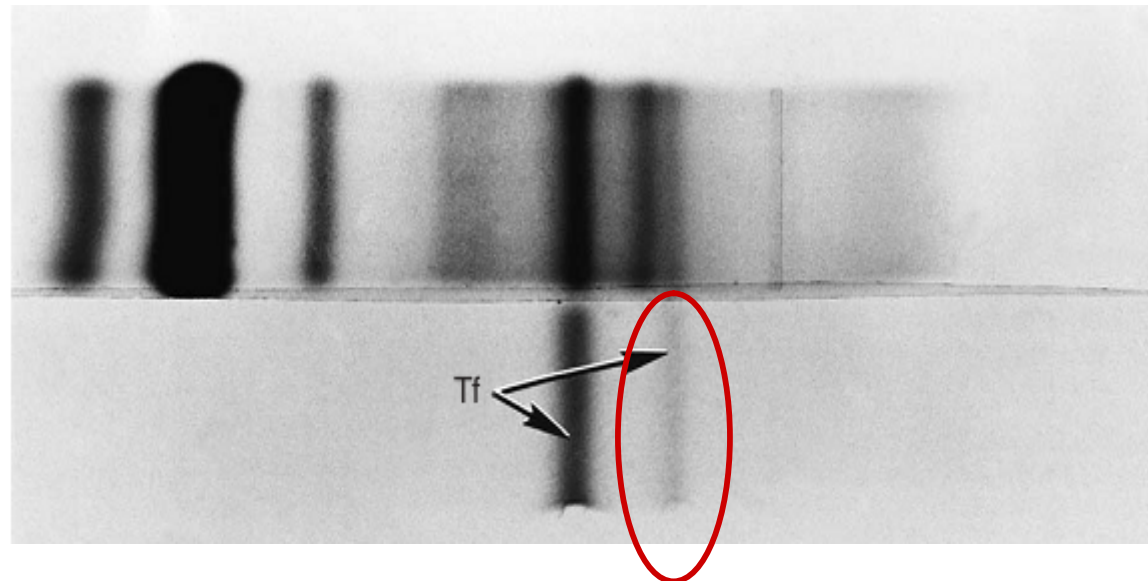
SERUM

BOS

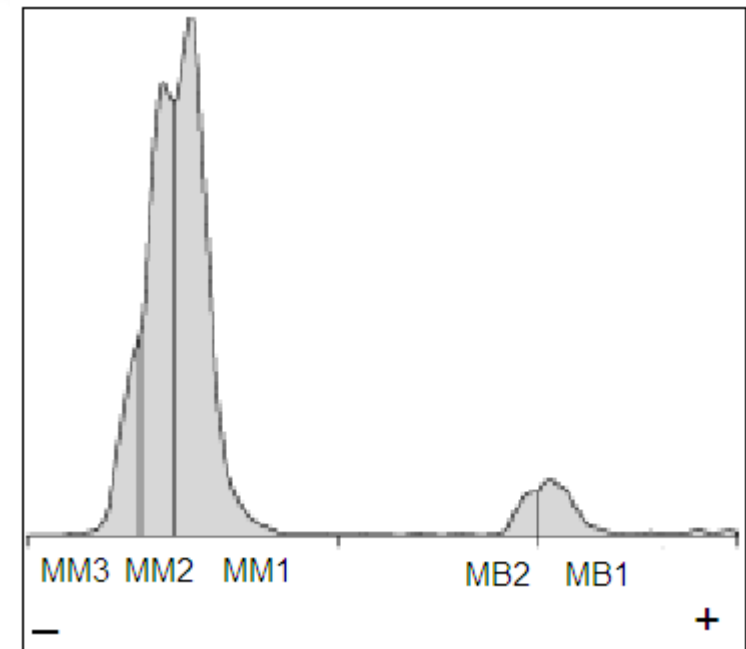
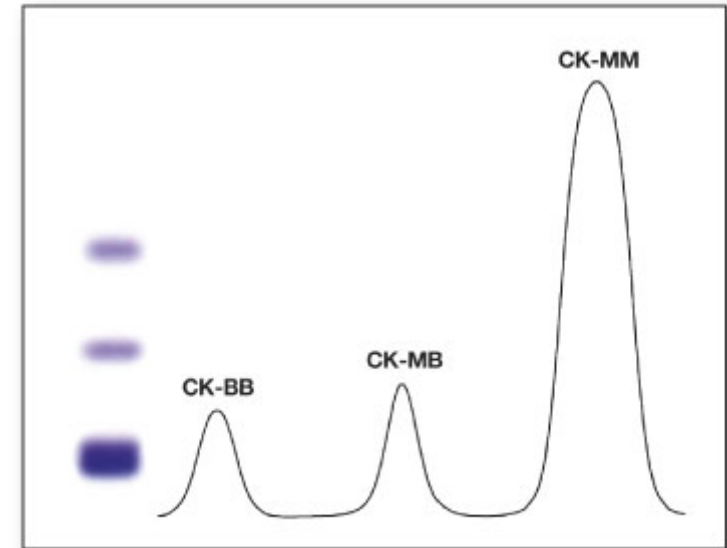
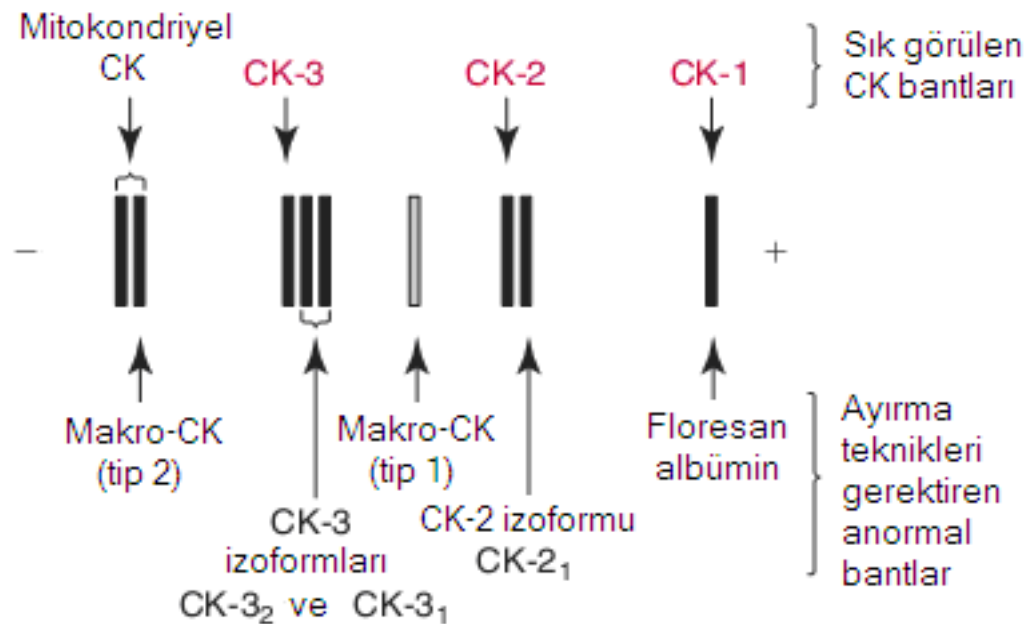


KONSANTRE BOS

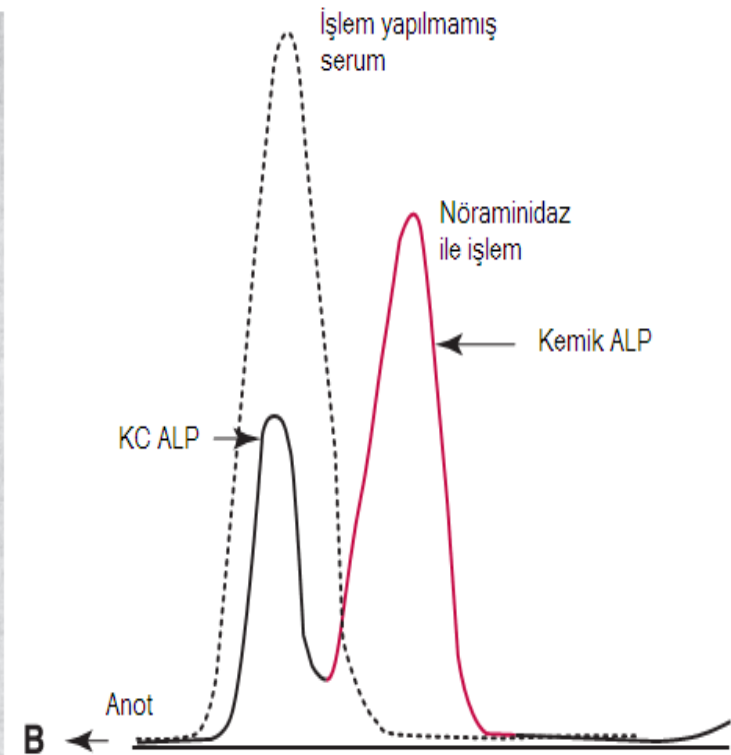
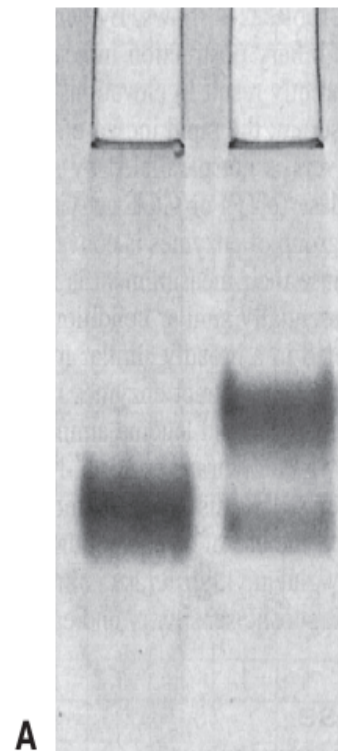
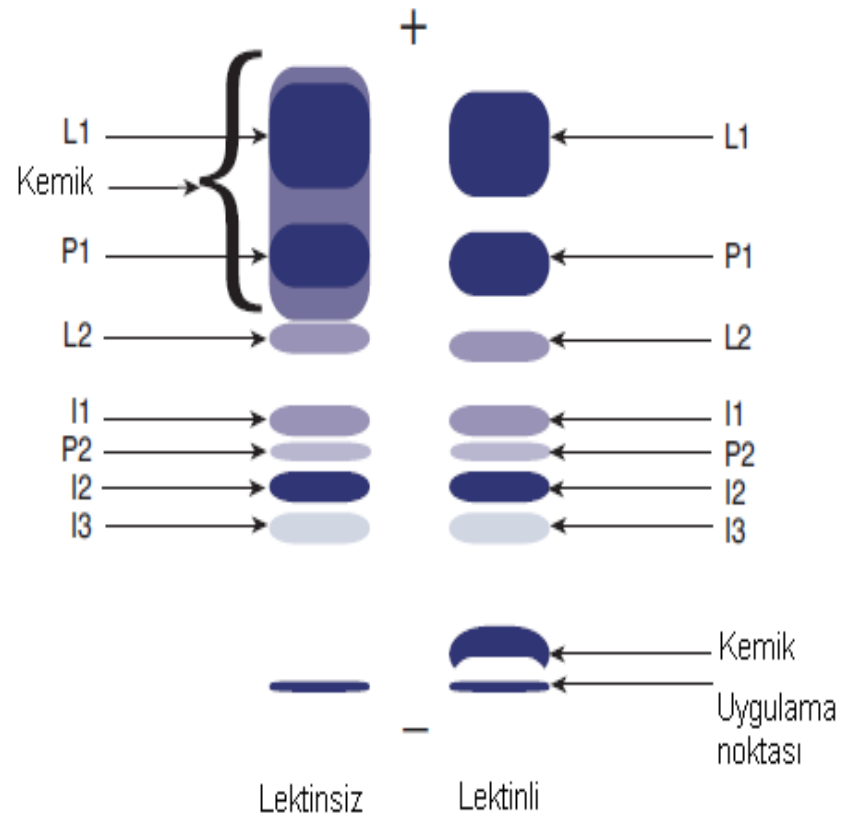
İFE



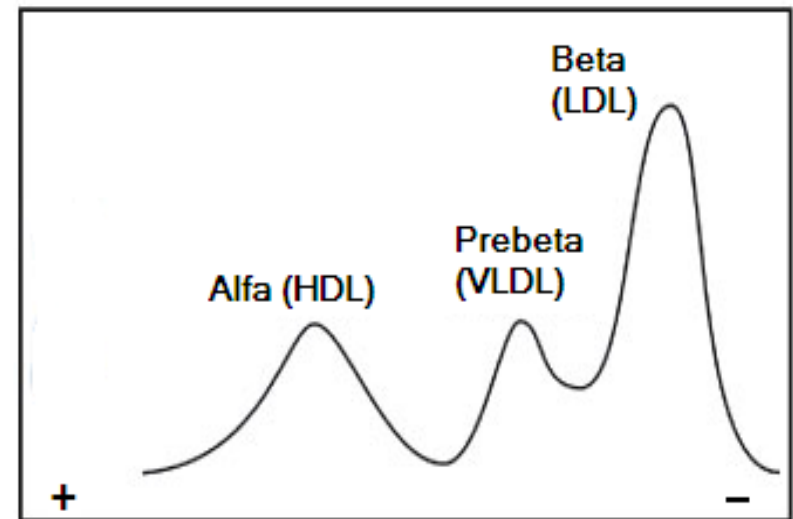
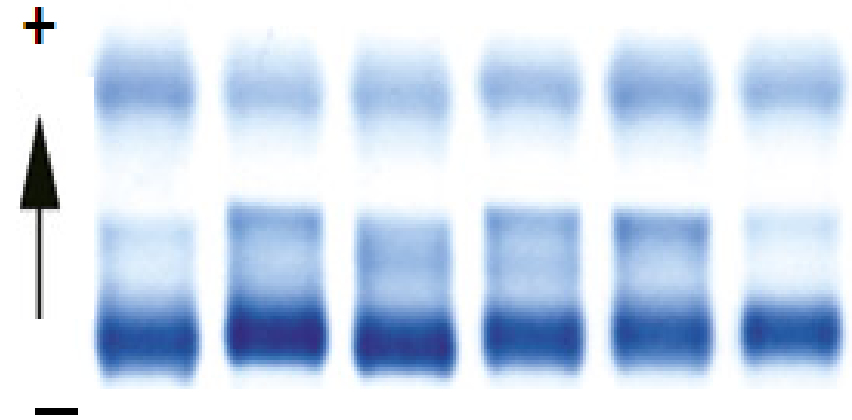
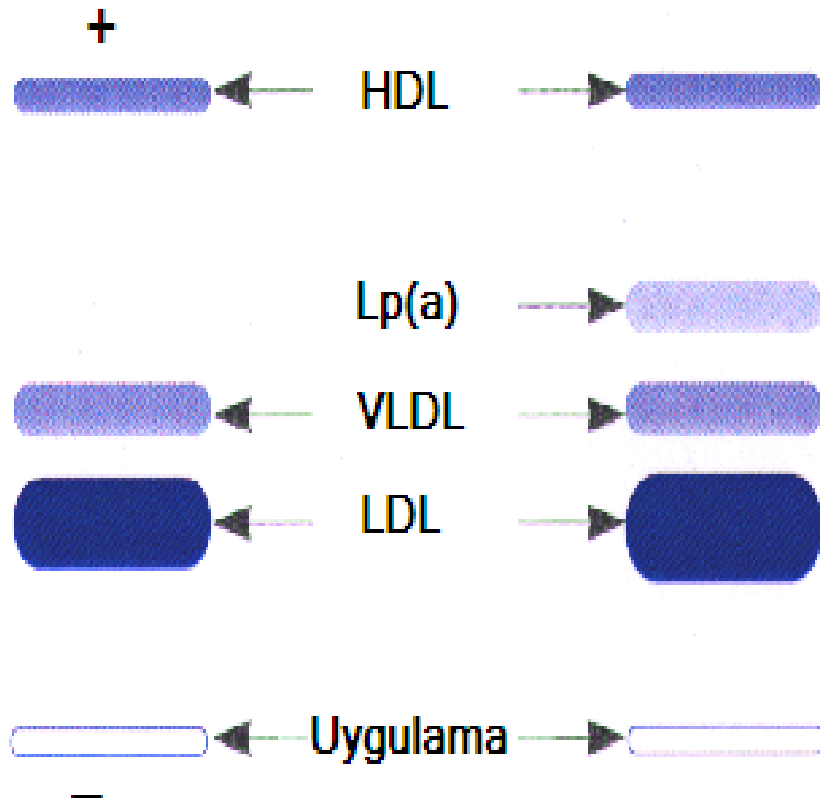
İzoenzim Elektroforezi: CK



İzoenzim Elektroforezi: ALP

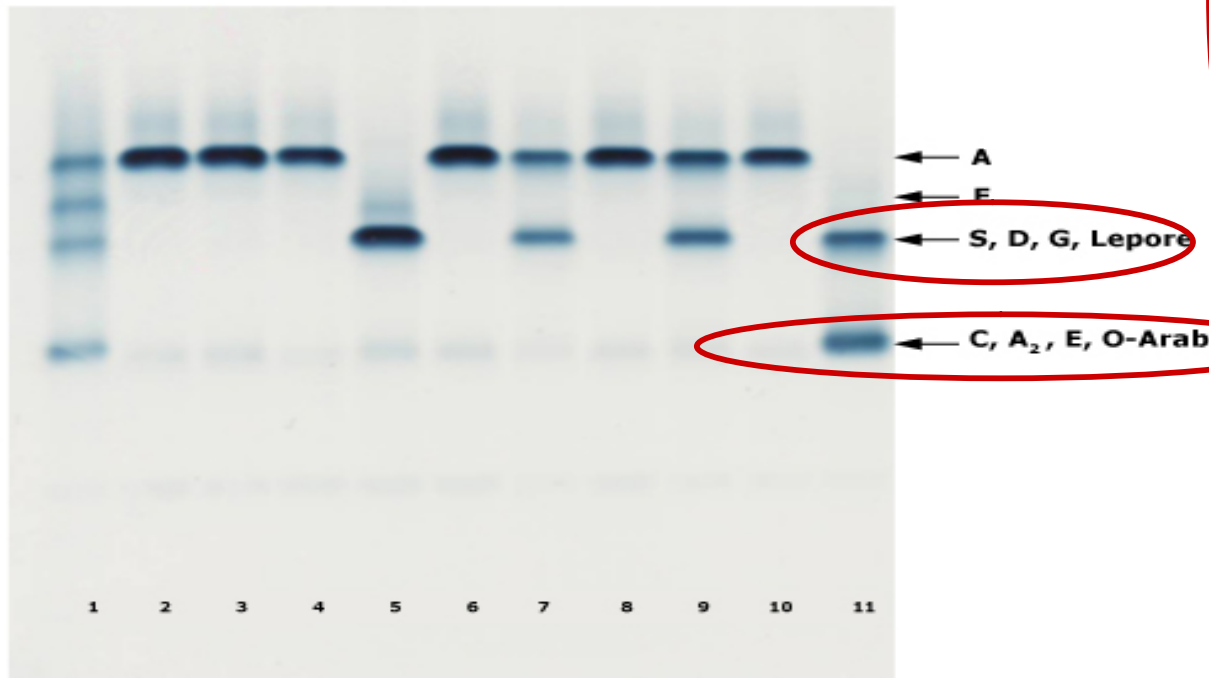
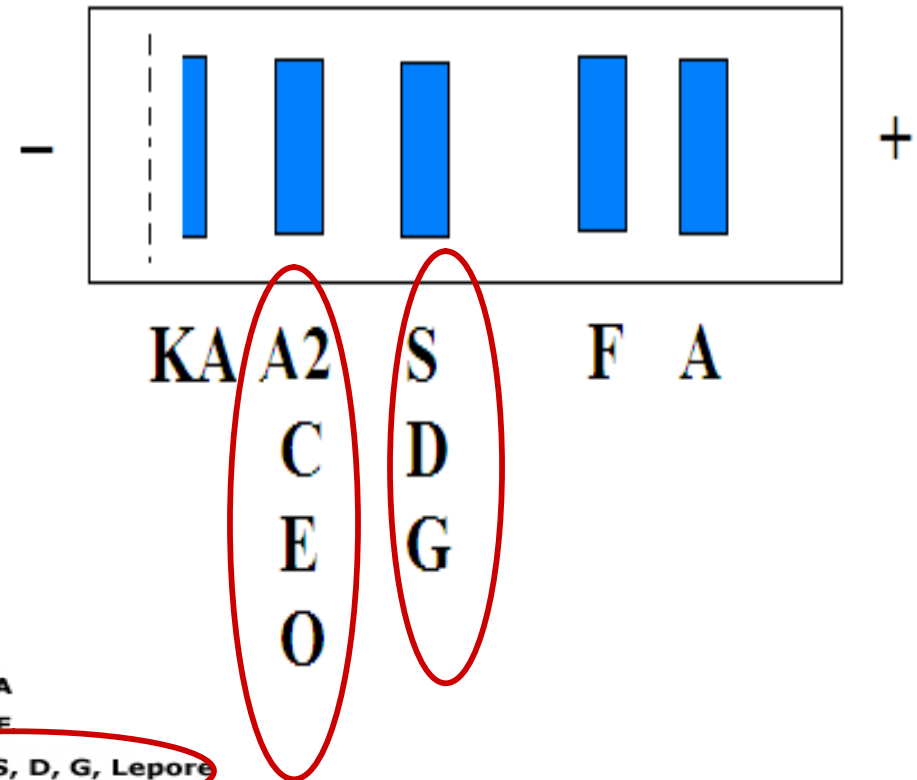


Lipoprotein Elektroforezi



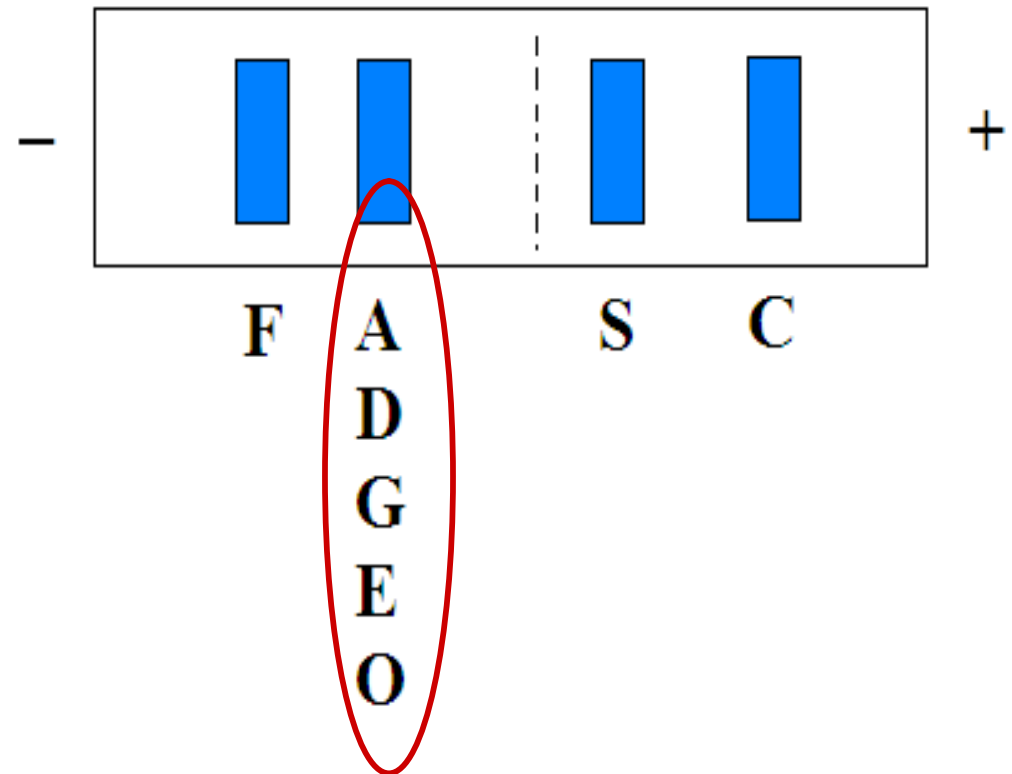
Hb Elektroforezi: Alkali

- Selüloz asetat
 - pH: 8.6
 - Ponceau S
- Agaroz jel
 - pH 9.2
 - Amido black, G-250



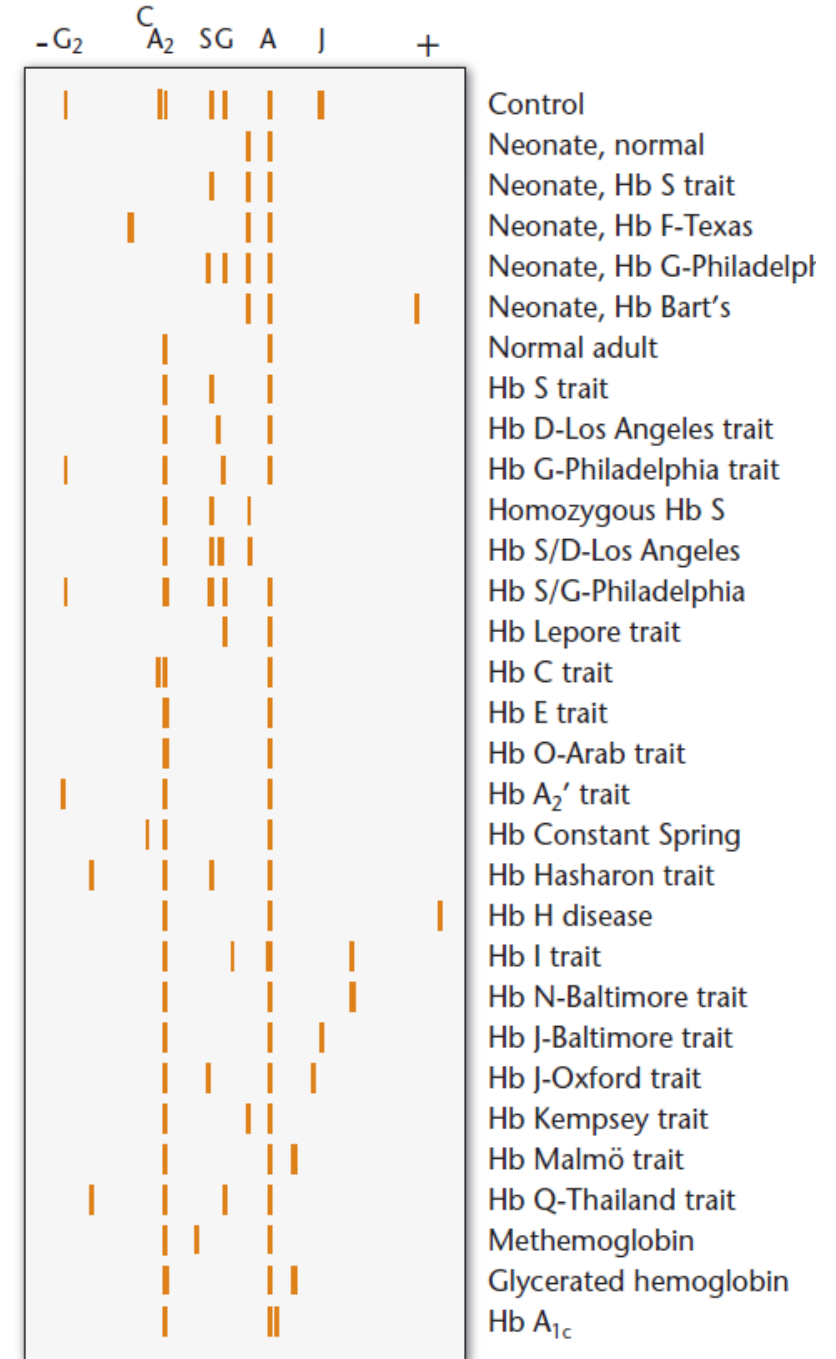
Hb Elektroforezi: Asit

- Sitrat agar elektroforezi
- pH: 6.0 - 6.2
- Boya: "Acid blue" veya "Acid violet"
- Agar: Agaroz + Agaropektin
- Alkali elektroforezin tamamlayıcısıdır

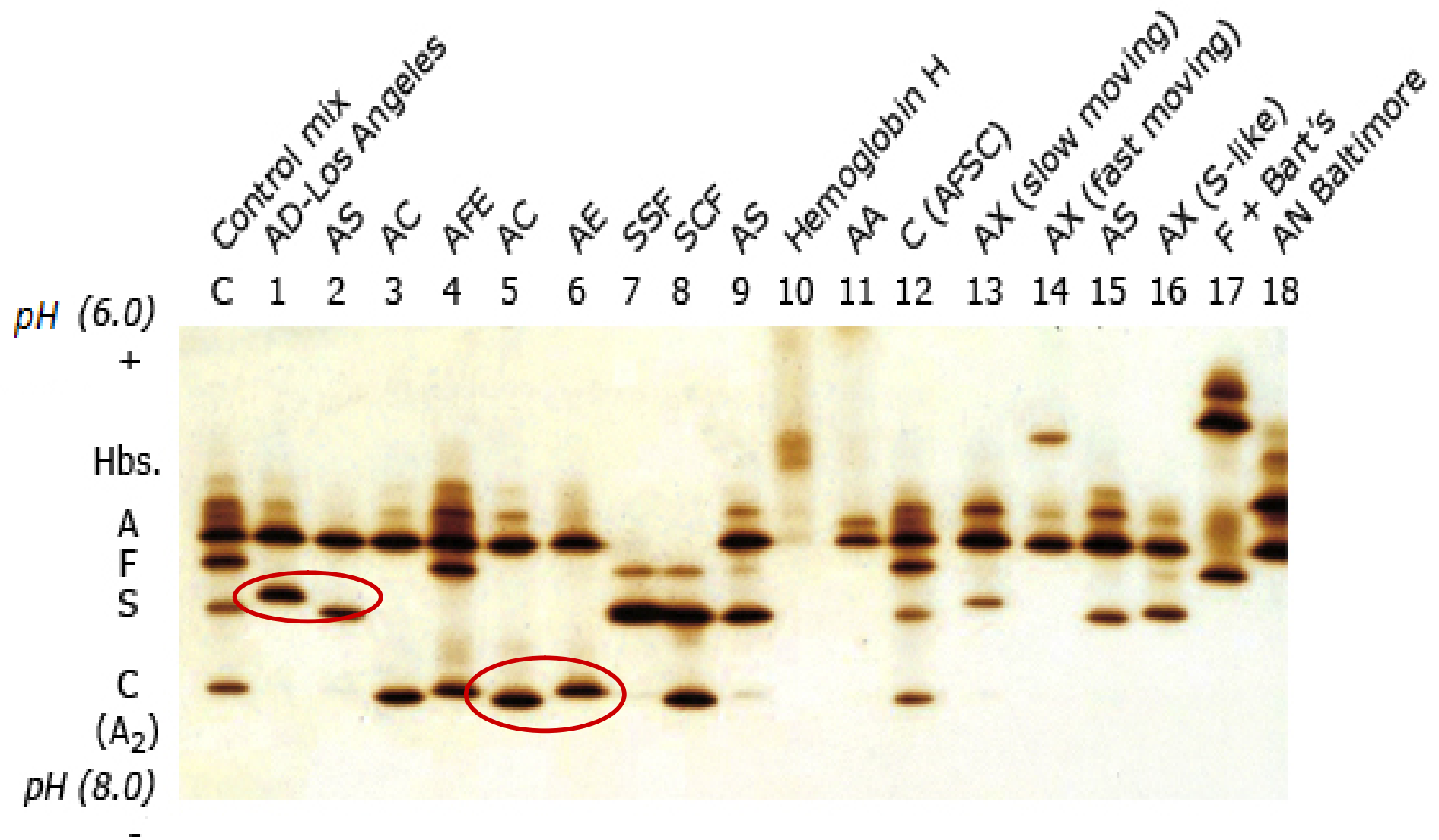


İzoelektrik Odaklama

- Ayrım ve bantların keskinliği daha iyidir
- Pahalı ve zaman alıcı
- 6.0-8.0 arasında pH gradyanı
- Destek ortamı genellikle agaroz
- Hb A₂ ve Hb F miktarı için uygundur
- Topuk kanından çalışmak mümkün
- Yorumu zor

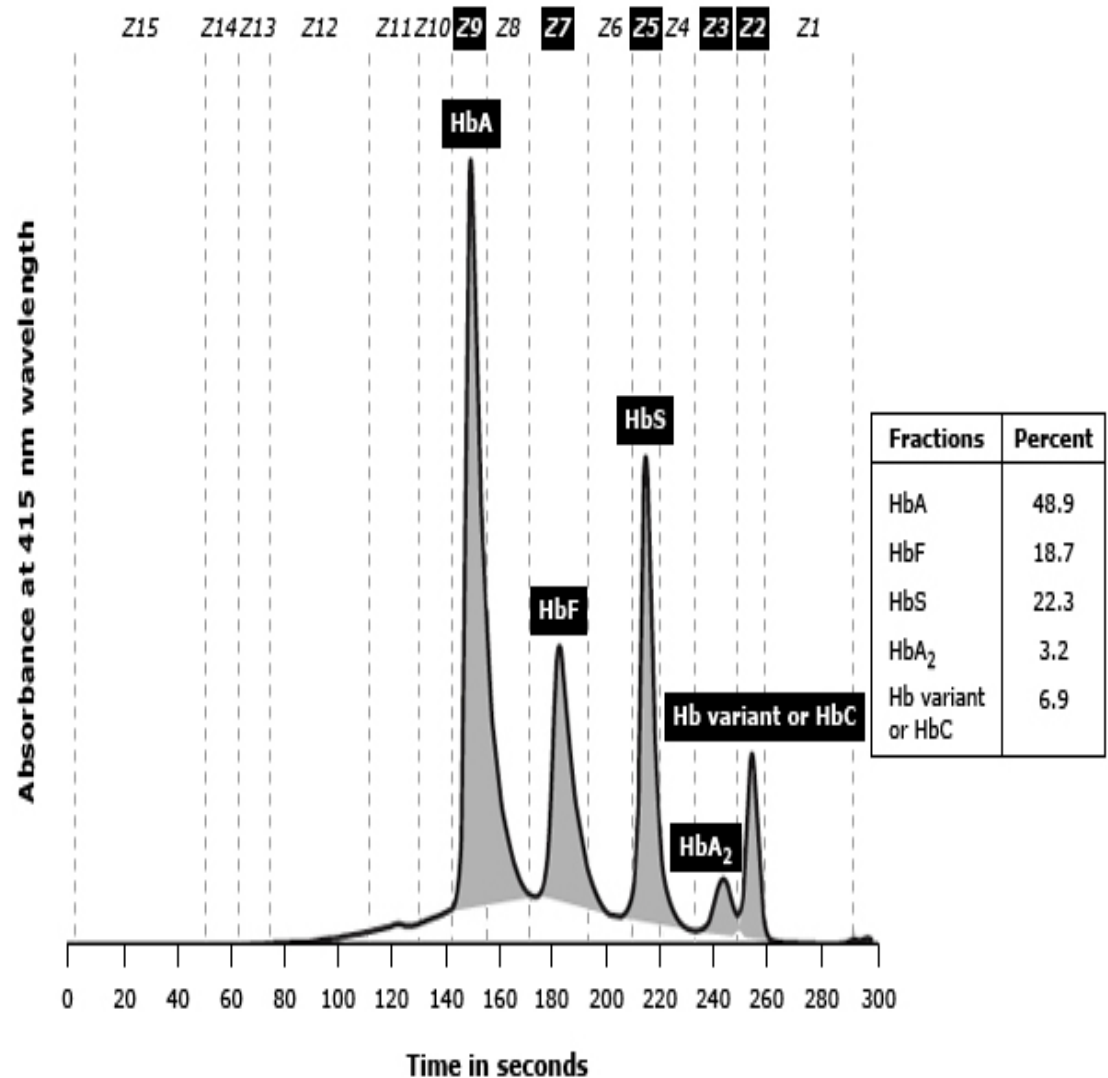


İzoelektrik Odaklama

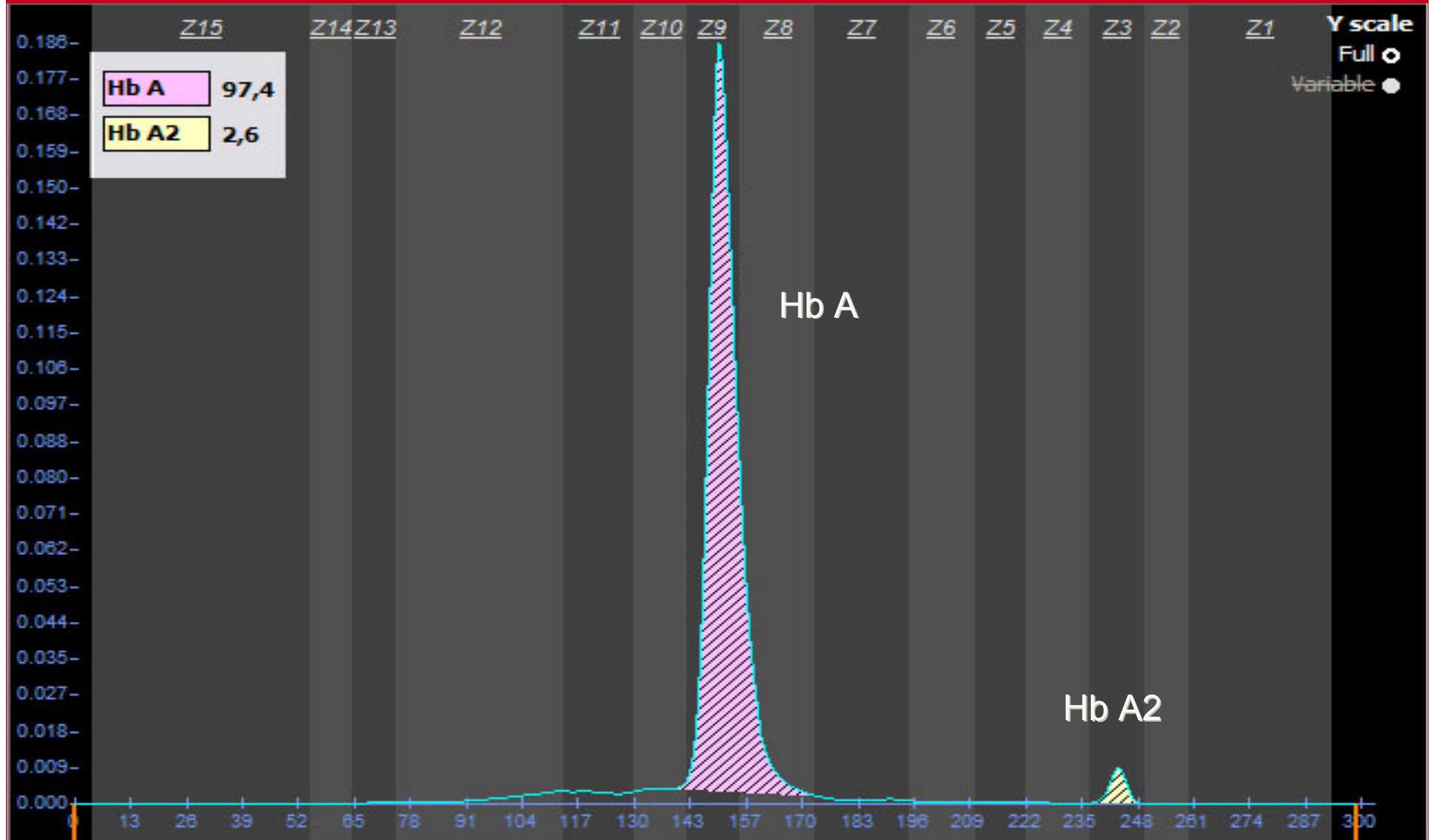


Hb Elektroforezi: Kapiller

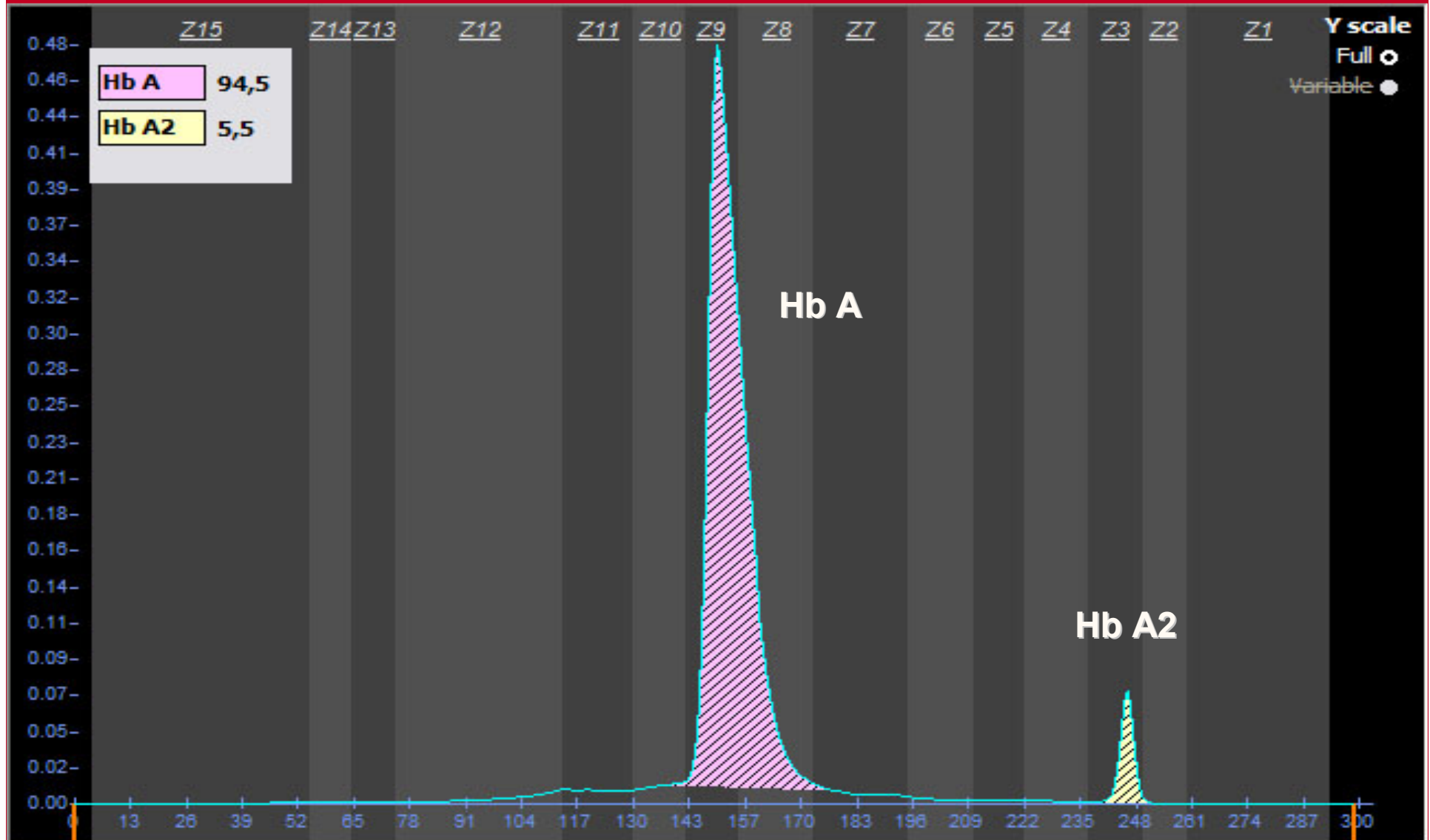
- Alkali pH (9.4)
- Çok yüksek akım: 10 000 V
- Elektroendozmoz ve yük
- 415 nm'de 300 okuma
- 7 - 8 dakikada sonuç
- Alıkonma zamanı
- Hb A ve Hb A2 piklerinin yerleşimine göre diğer Hb'lerin tanımlaması yapılır
- Hb A2 ve Hb F miktarı verilebilir
- Hb A2 ile E, Hb H ve Hb Lepore ayrılabilir



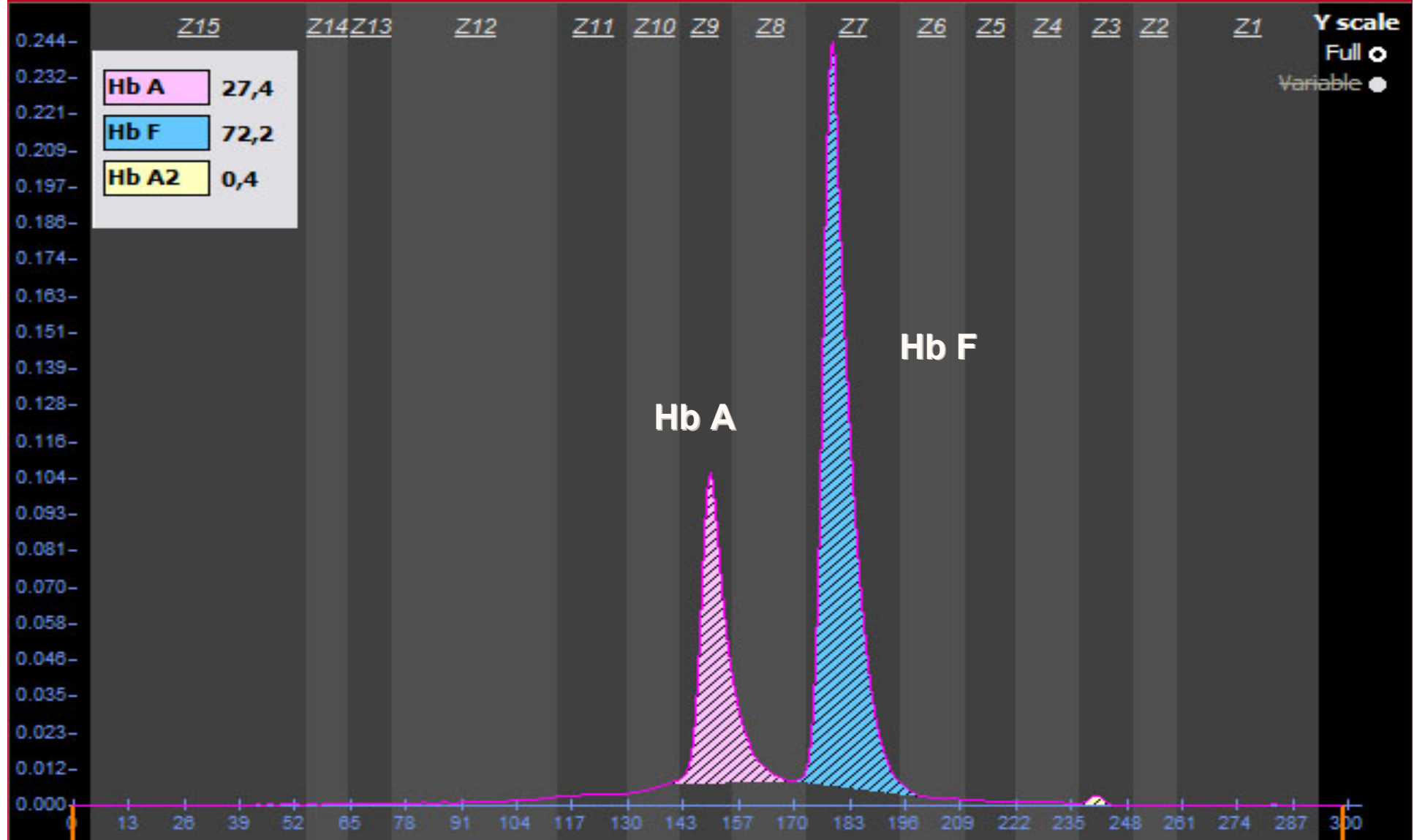
Örnek: Sağlıklı



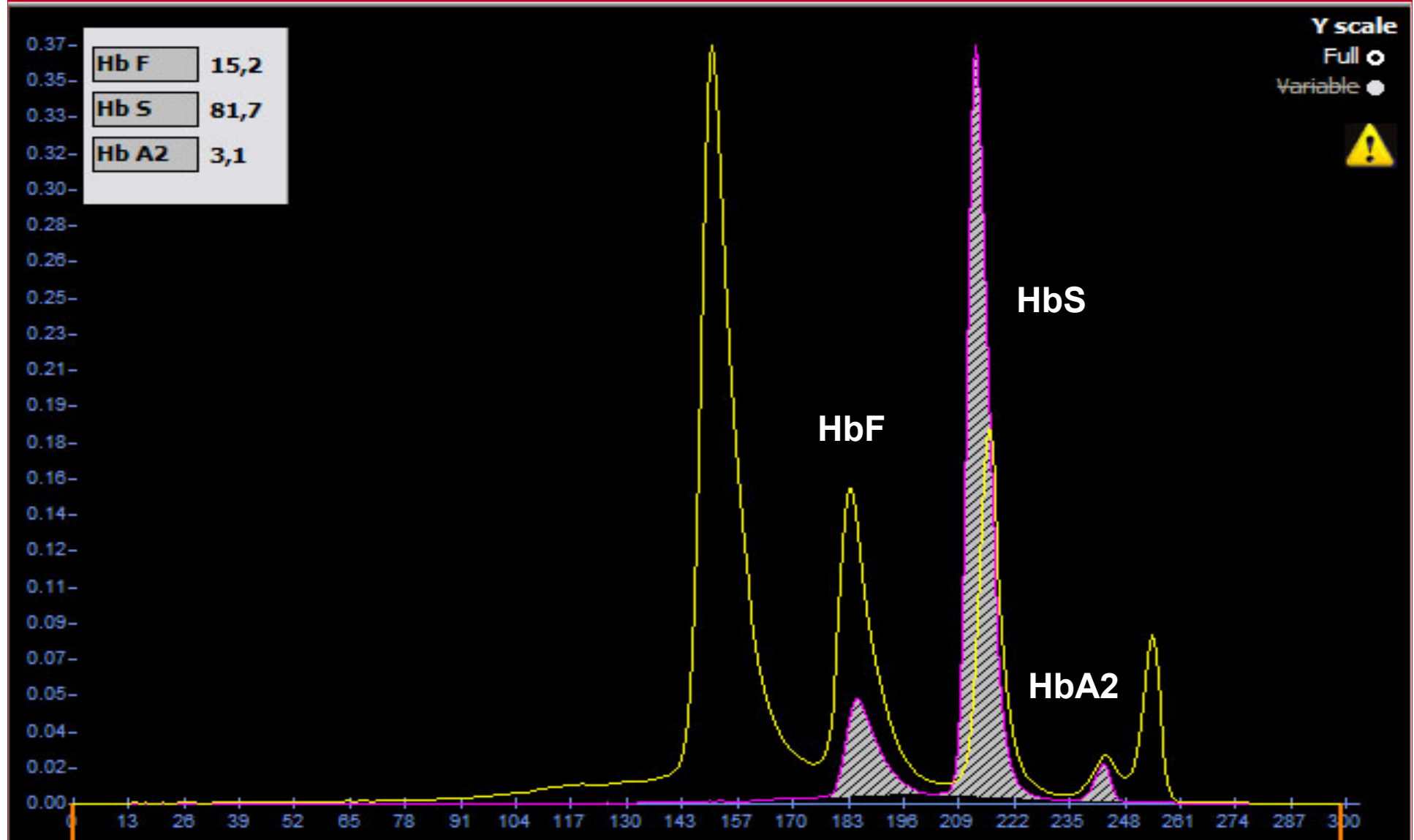
Örnek: Beta Talasemi “Trait”



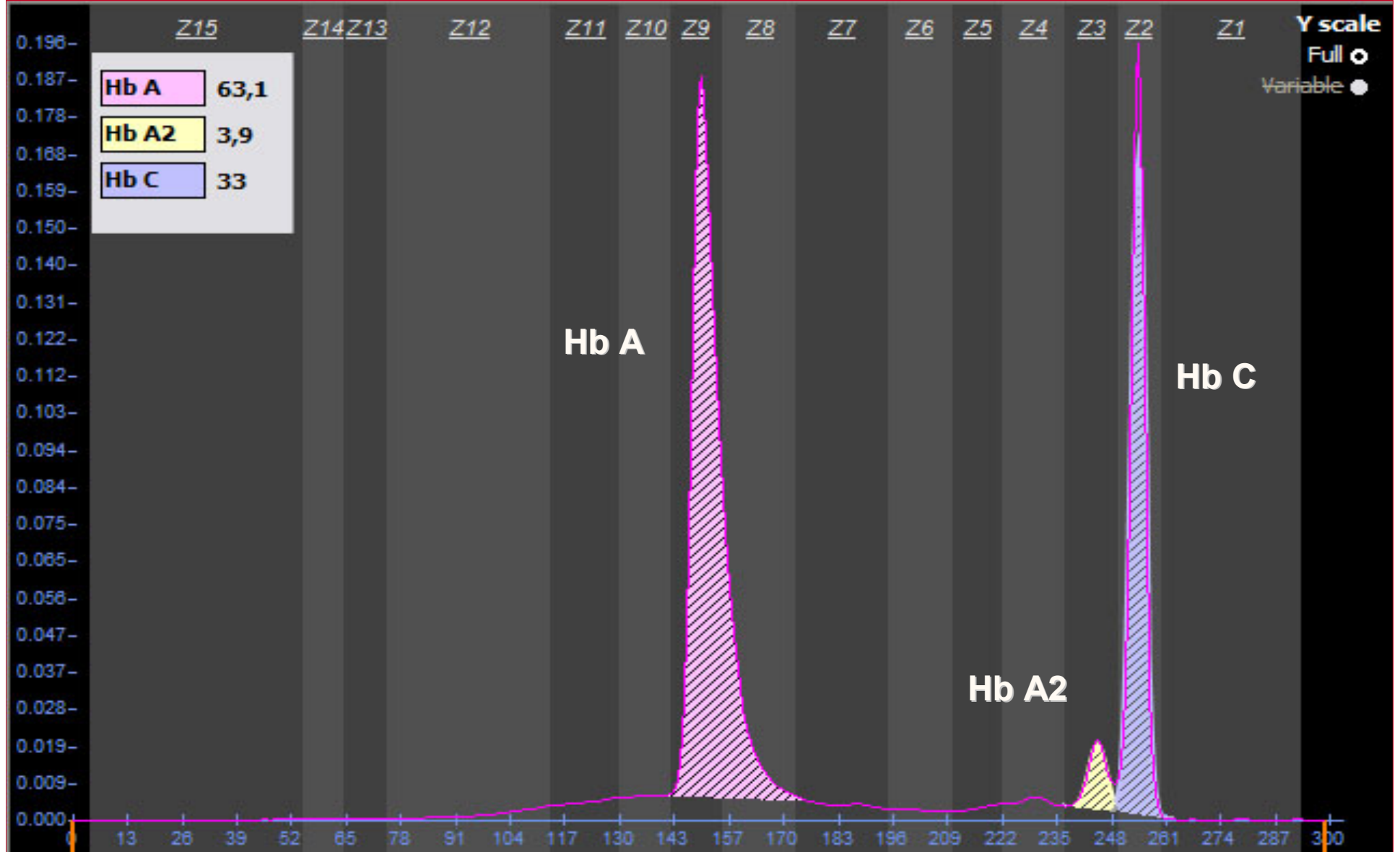
Örnek: Yenidoğan



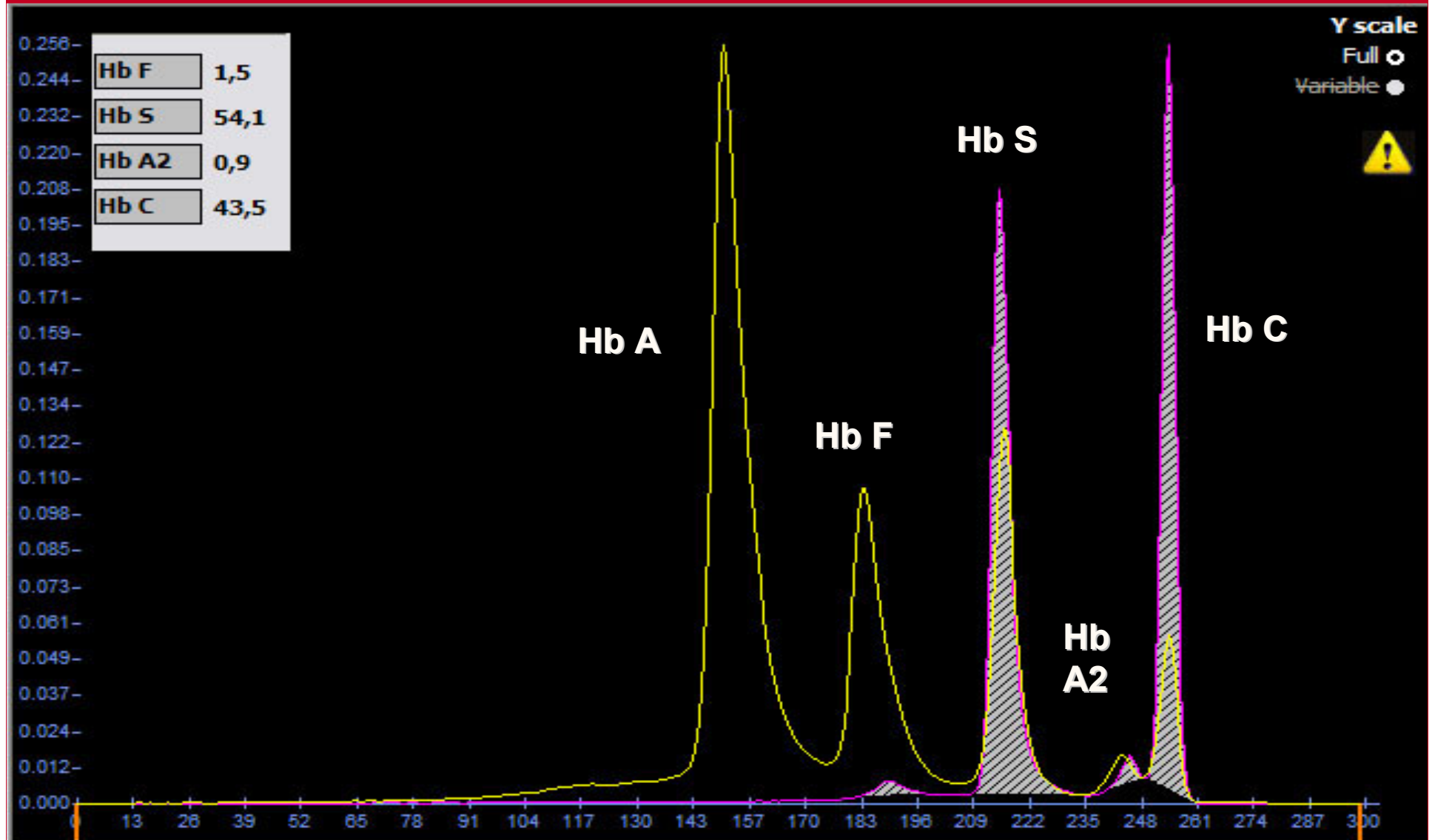
Örnek: Homozigot Hb S + AFSC Kont.



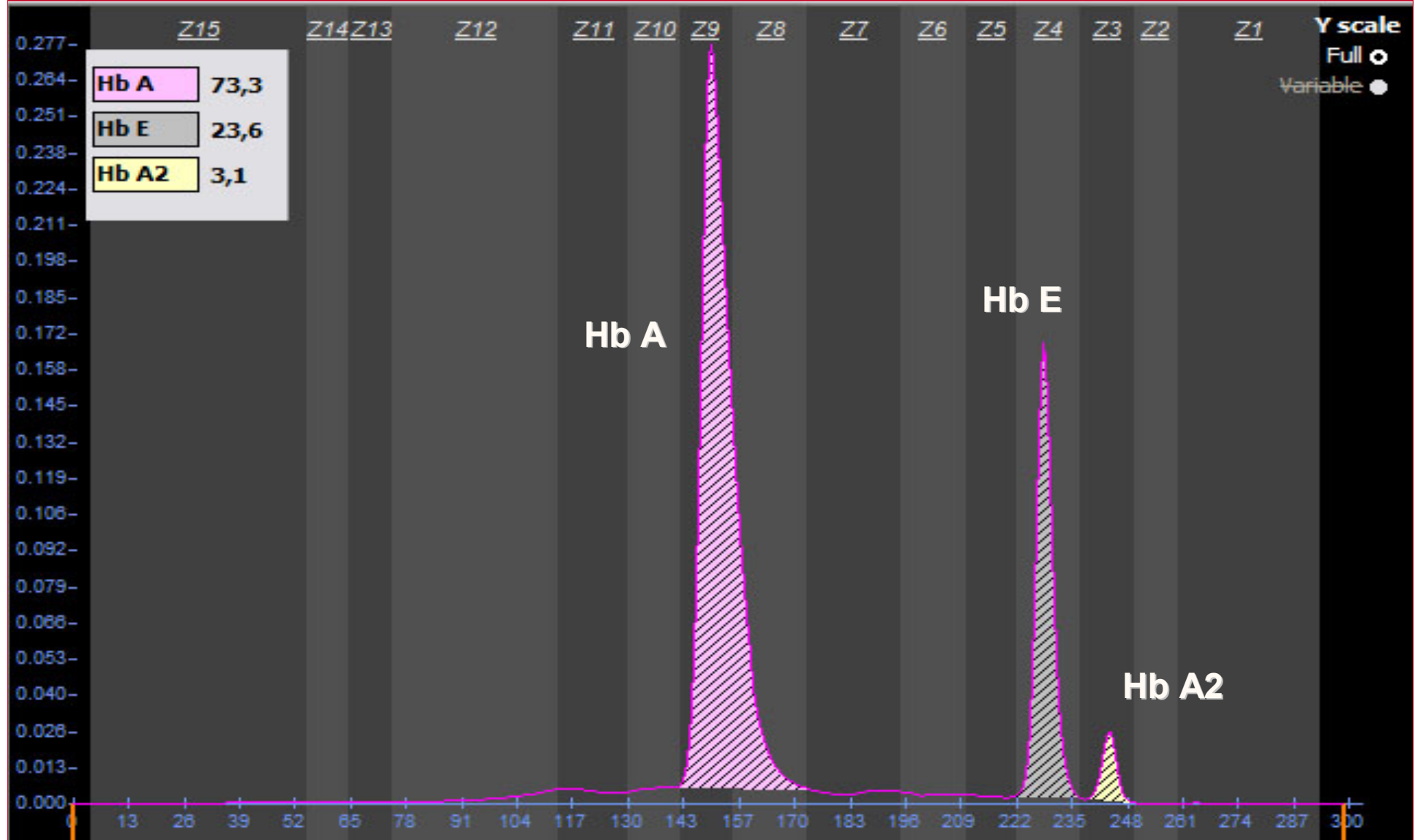
Örnek: Hb AC



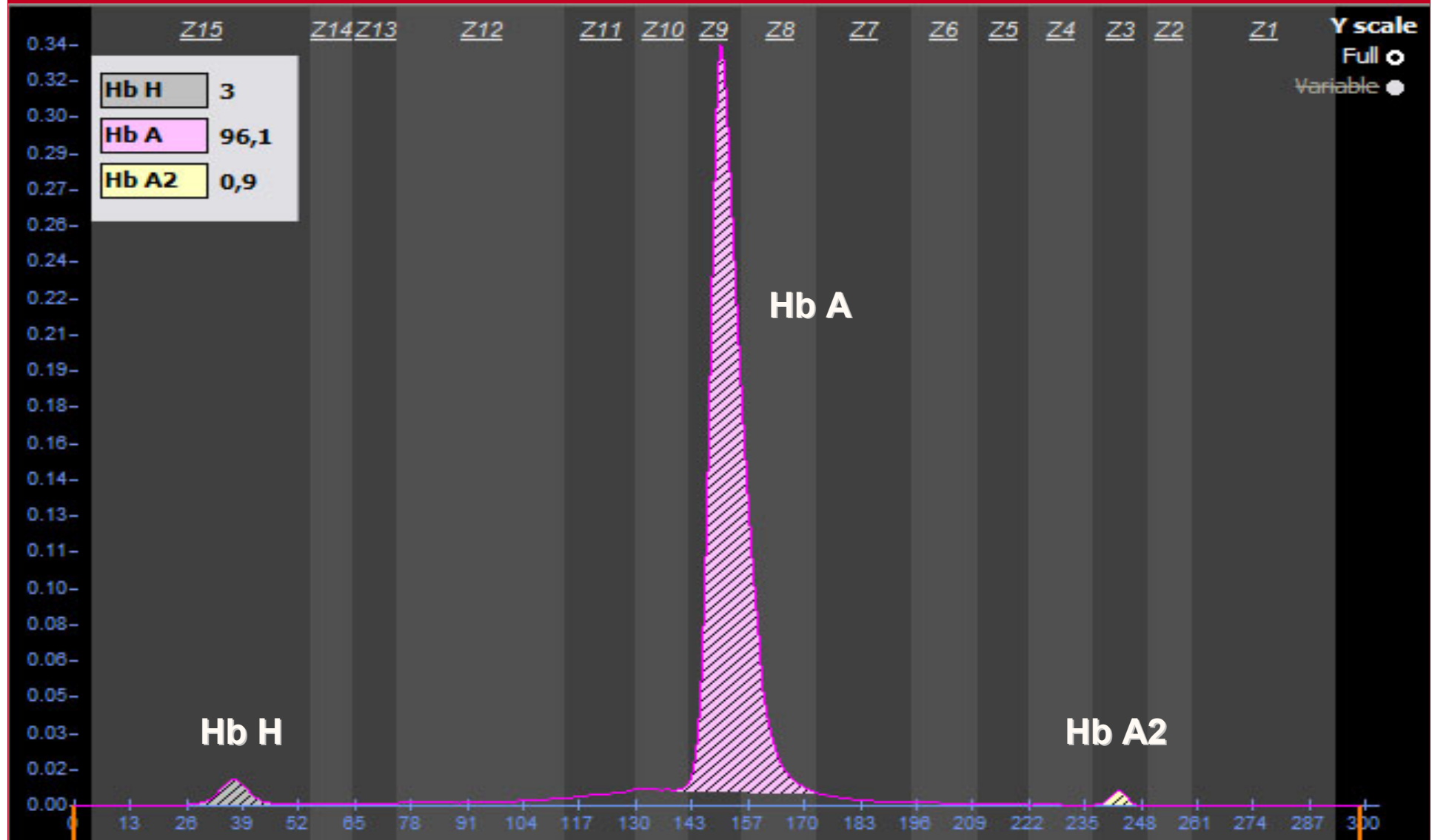
Örnek: Hb SC



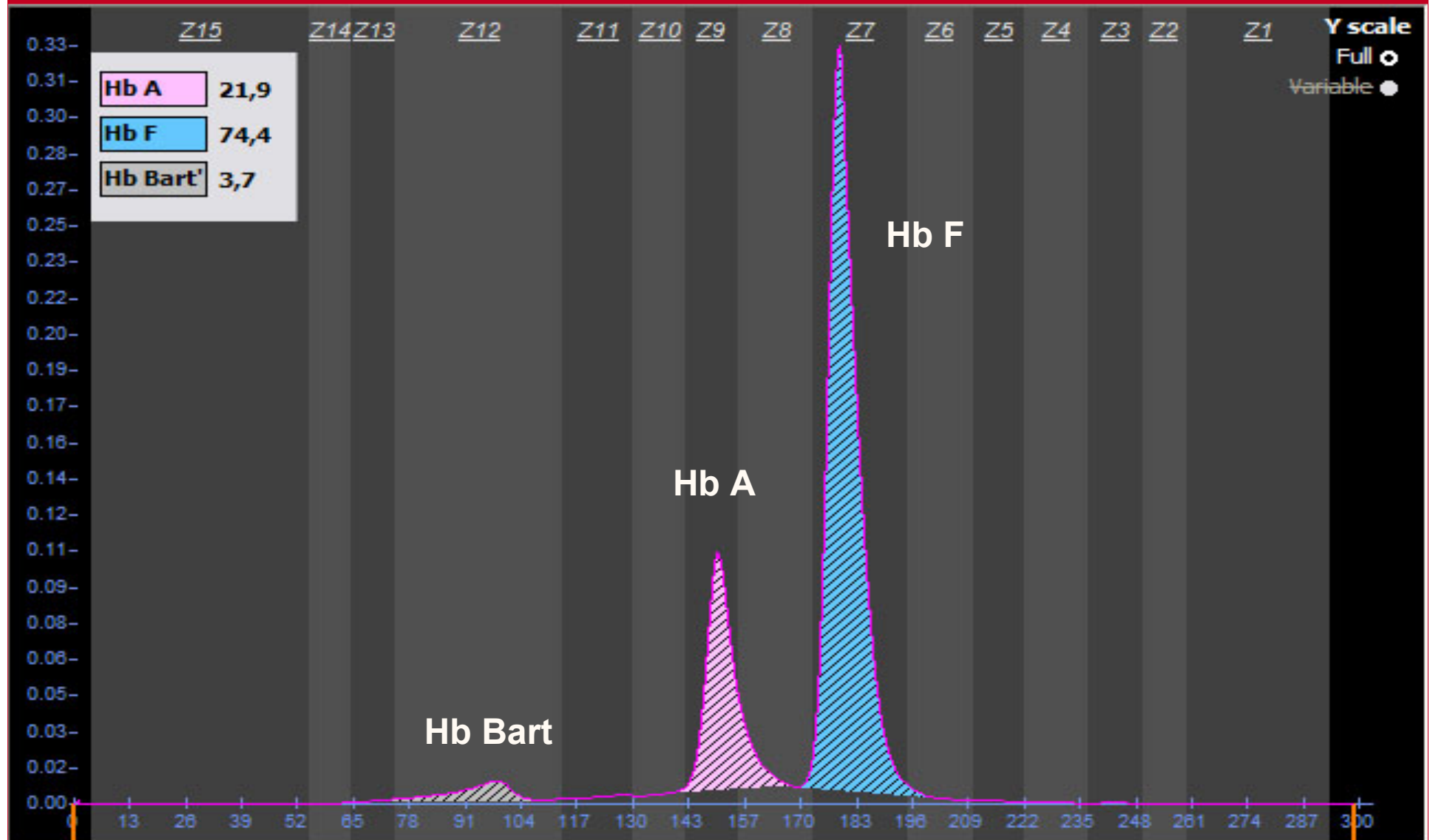
Örnek: Hb AE



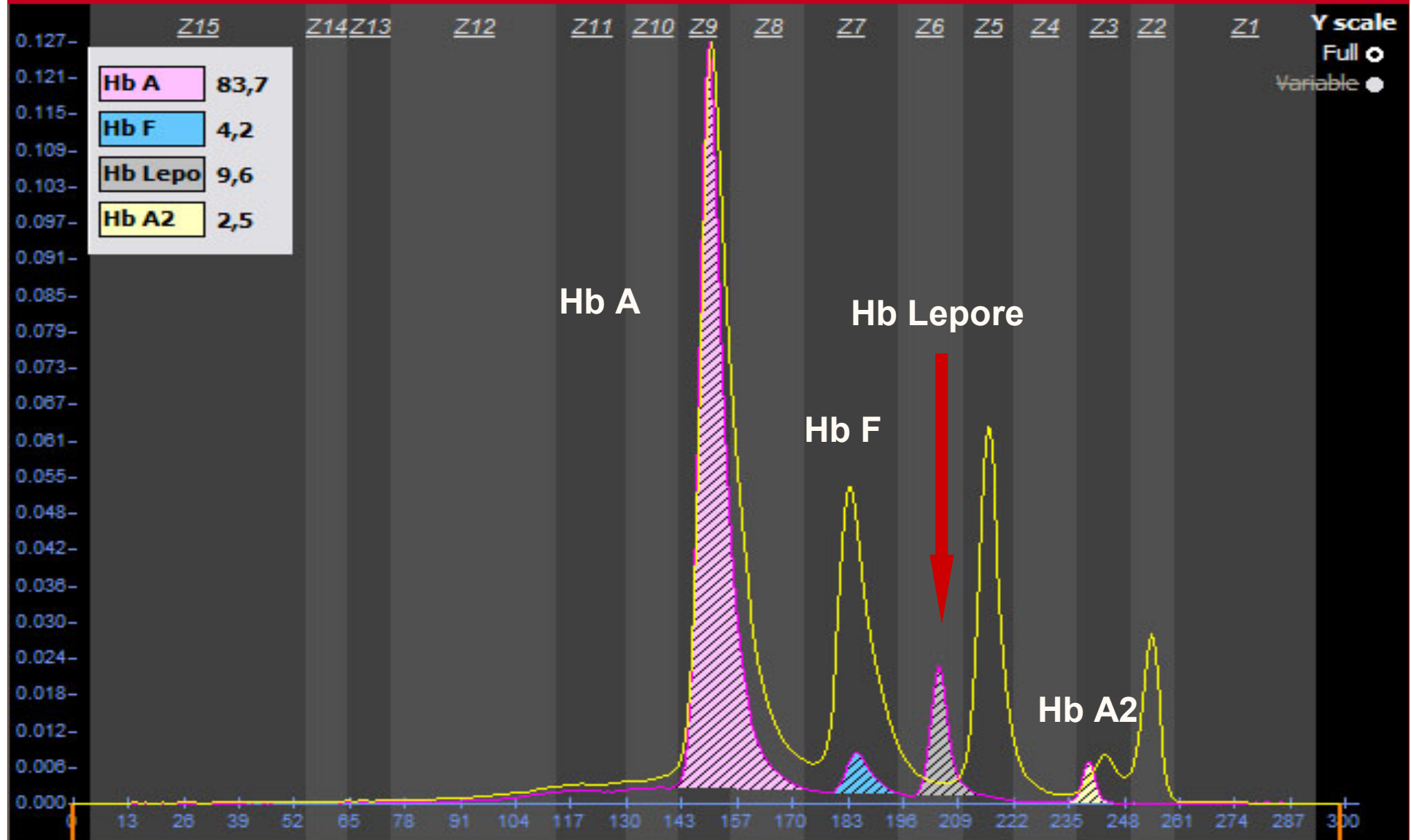
Örnek: HbH



Örnek: Hb Bart

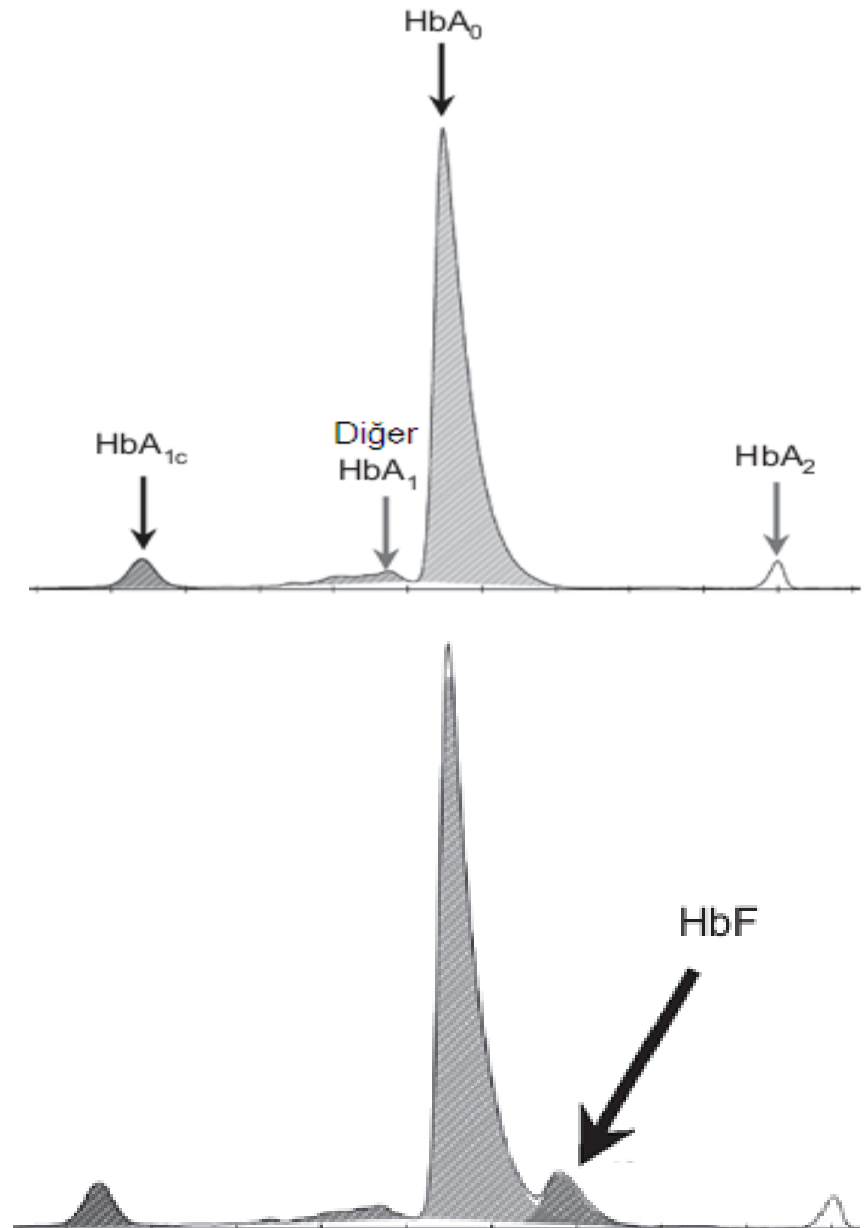


Örnek: Hb Lepore

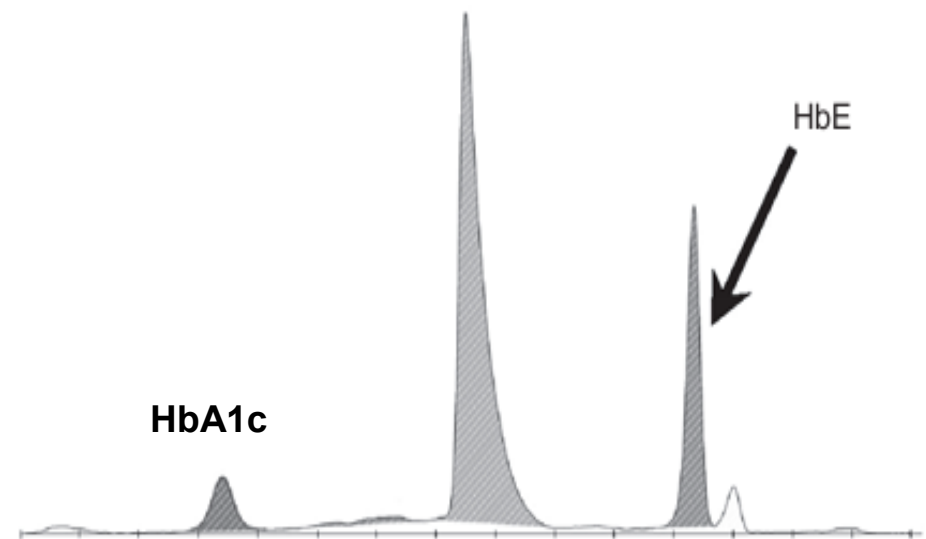
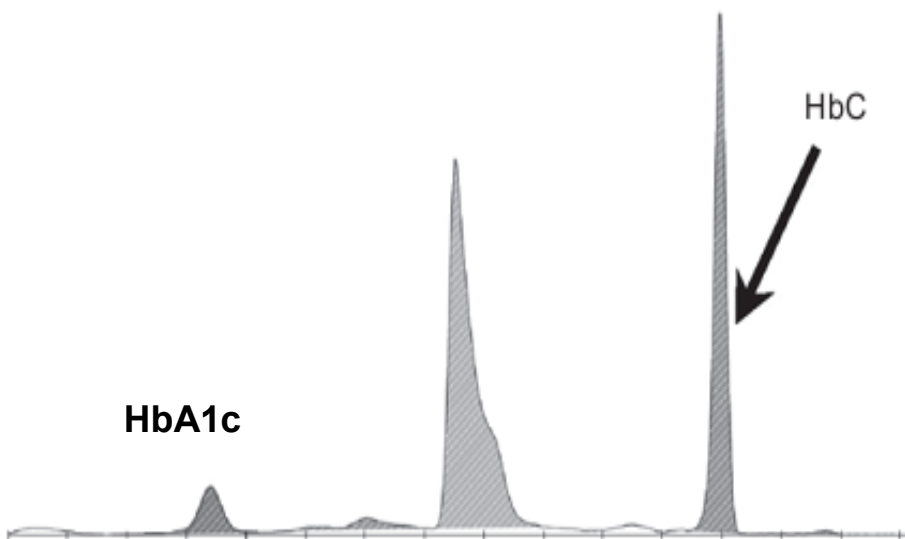
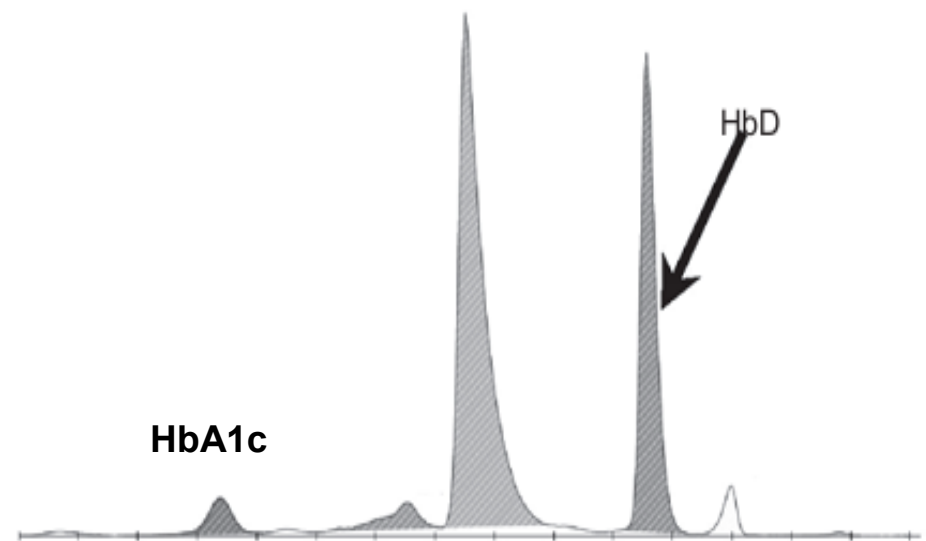
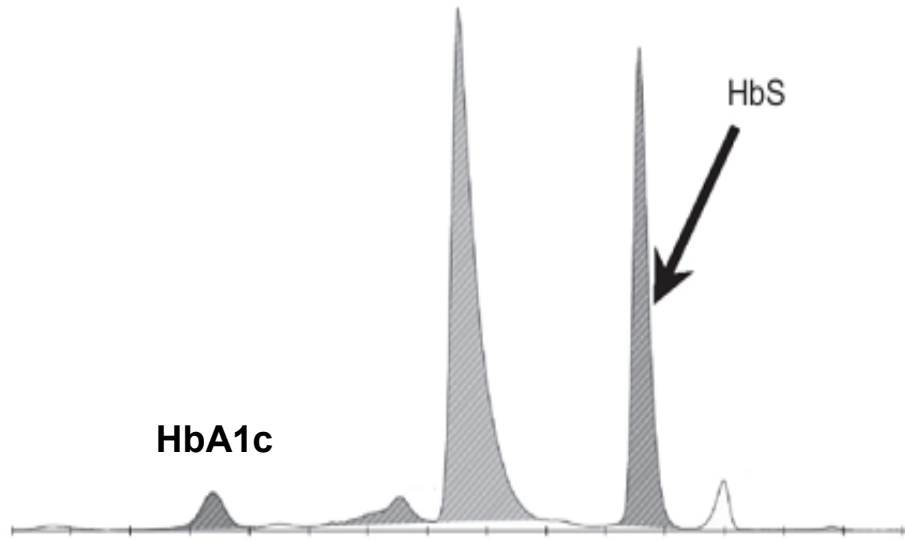


HbA1c: Kapiller

- Kesinlik
 - Çalışma içi:
%1.62
 - Çalışmalar arası:
%1.45
- Doğrusallık:
%3.9 - %16.9
- Karbamil Hb,
HbF, S, C, D, E
etkilemiyor



HbA1c: Kapiller



Sonu ve Teekkr

