Plasma proteins, methods in protein chemistry

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The protein paradox

Daily protein turnover

75g intake

300g – total body protein synthesis

- **75g – muscles**
- **70g – digestive fluids**
- **20g – blood plasma**
- **20g – leukocytes**
- **8g – hemoglobin**
- **2g – skin**

200g – recycling **10g loss**

Composition of blood plasma - the protein paradox

By origin: cell constituent

product (e.g. export from liver)

Molecular anatomy: monoclonal – albumin

polyclonal – all globulins

Fate of plasma proteins

Biological half-life: production - elimination

rapid: coagulation factors, hormones, acute phase proteins, biological response modifiers

slow: albumin, immunoglobulins

Total plasma protein determinations

Salting out, precipitation - gravimetry
UV absorbance - 280nm, 220nm
Fluorescence - aromatic amino acids

Photometry - Biuret reaction (all proteins)
- bromochresol green (albumin)
Reference range total protein: 60-80g/l
albumin: 35-45g/l

Interpretation of total protein measurements

Hyperproteinemia: dehydration
multiple myelome

Hypoproteinemia: increased plasma volume
decreased synthesis
protein loss

Hypoalbuminemia: decreased synthesis
albumin loss

Not nutritional parameters!

Analysis of individual proteins
Protein separation techniques

Column chromatography: gel filtration, ion exchange, affinity, HPLC (peptides)

Electrophoresis: free (capillary)
paper, starch, agar,
agarose, cellulose acetate,
polyacrylamide

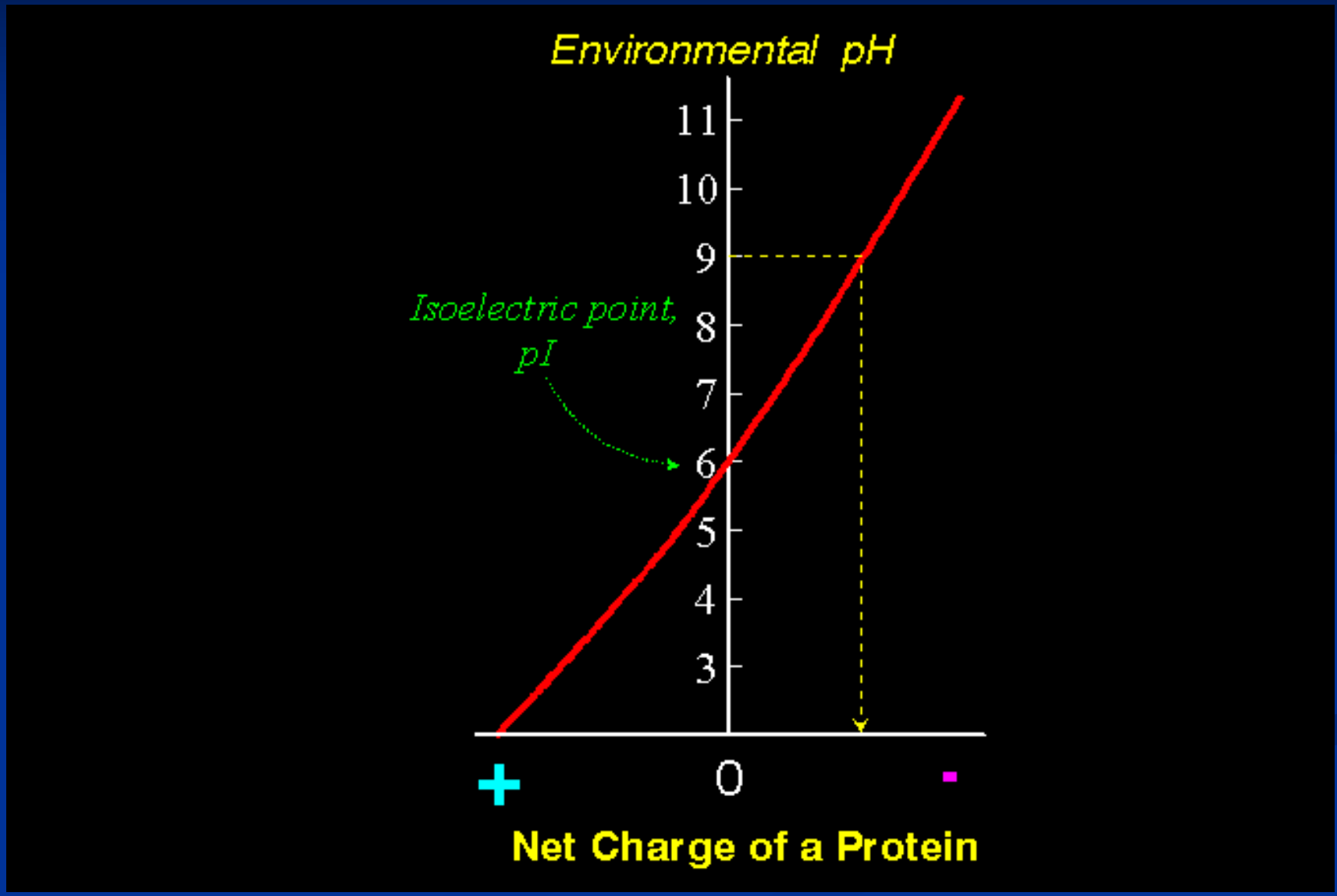
Principles of electrophoresis

Amphoteric amino acids:

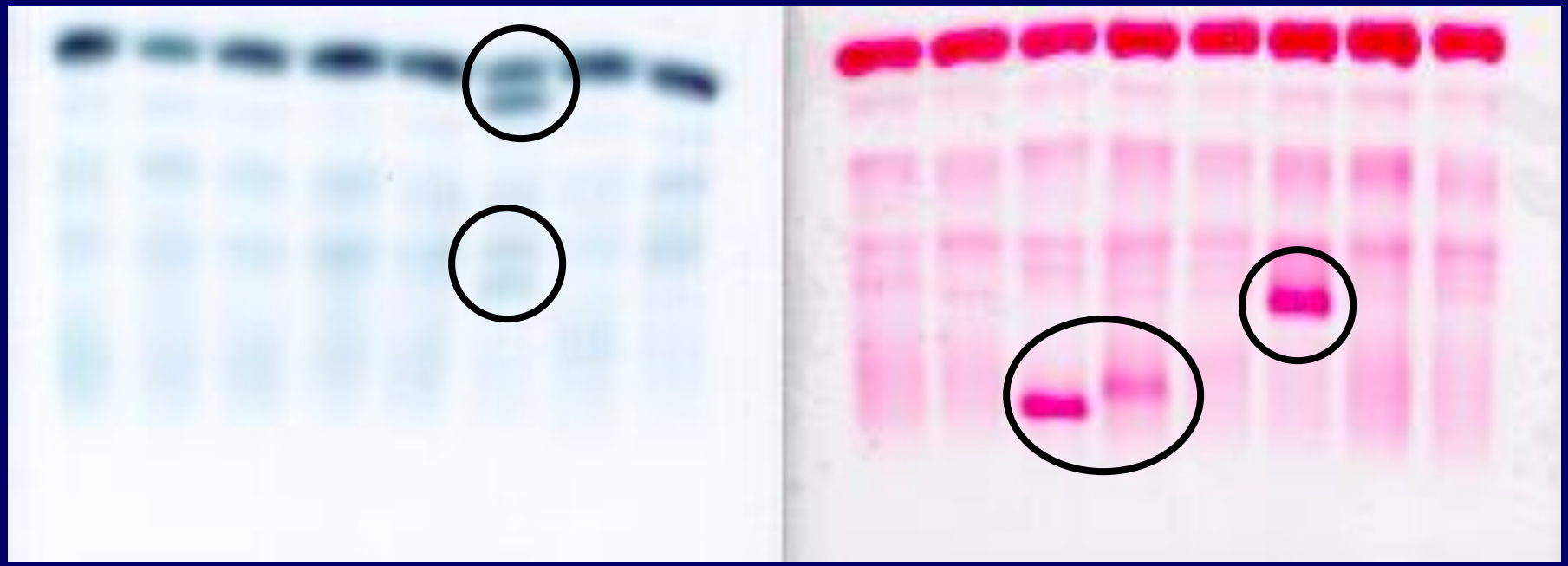
monoamino monocarbonic
monoamino dicarbonic
diamino monocarbonic

Posttranslational modifications!
Isoelectric point, dissociation (albumin)
Running medium, fixation, staining
by charge – by molecular mass

Charge of a protein



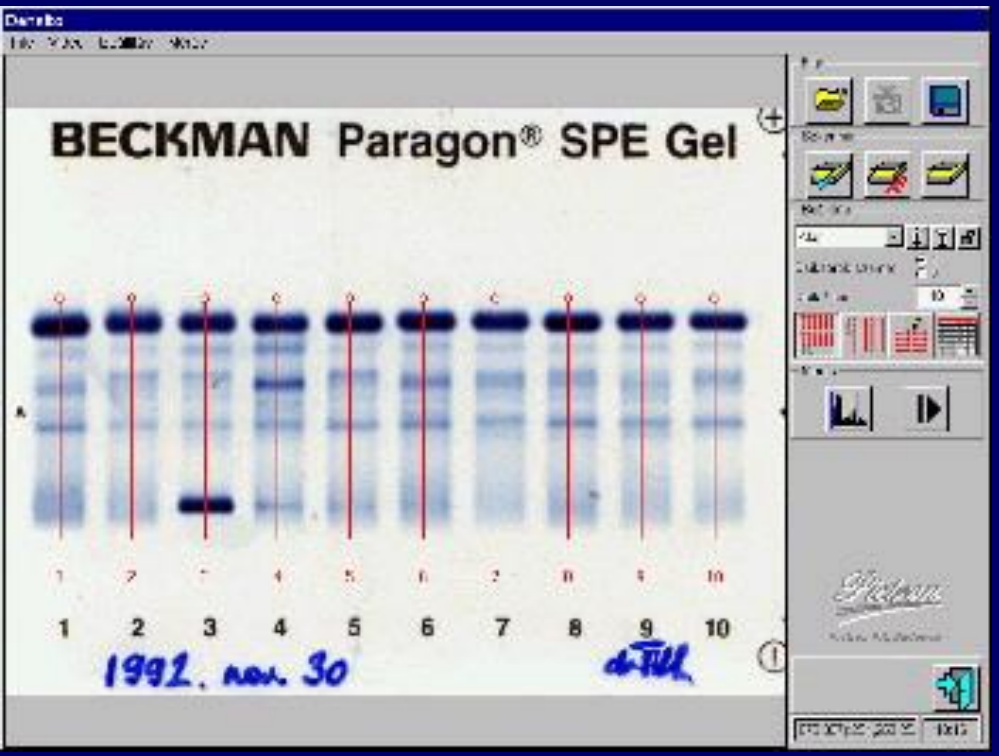
Protein electrophoresis

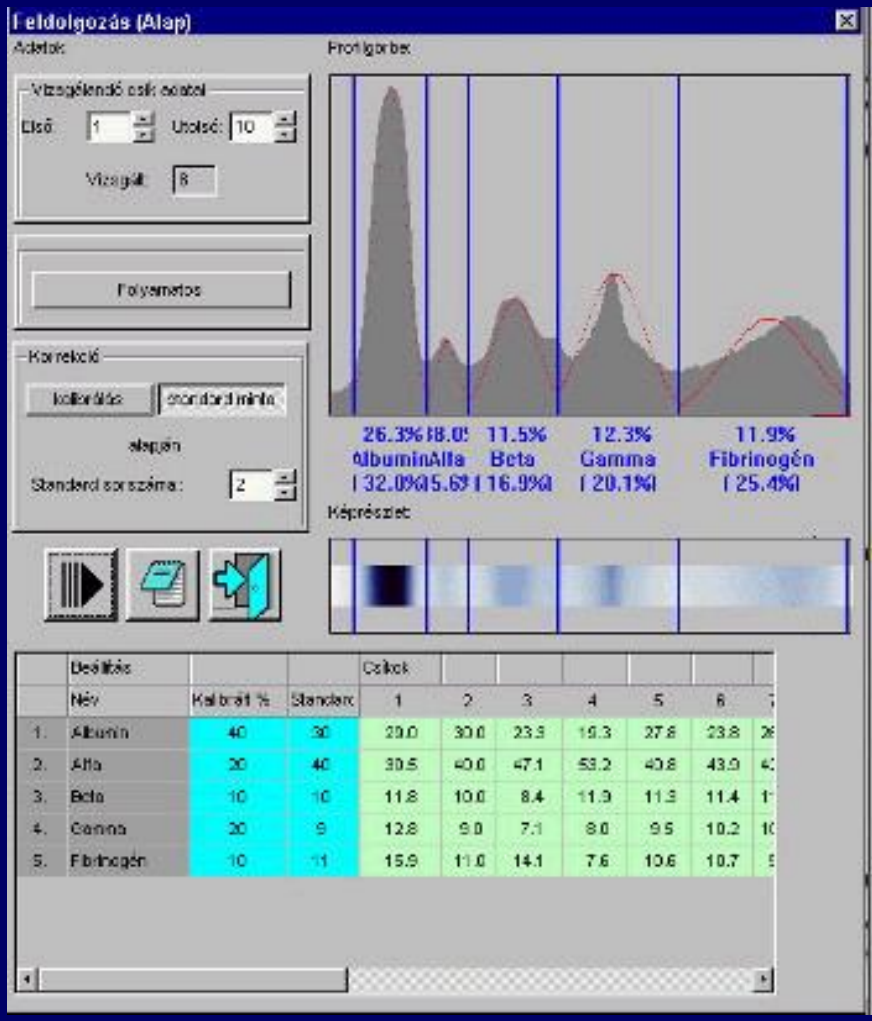


Nitrocellulose membrane

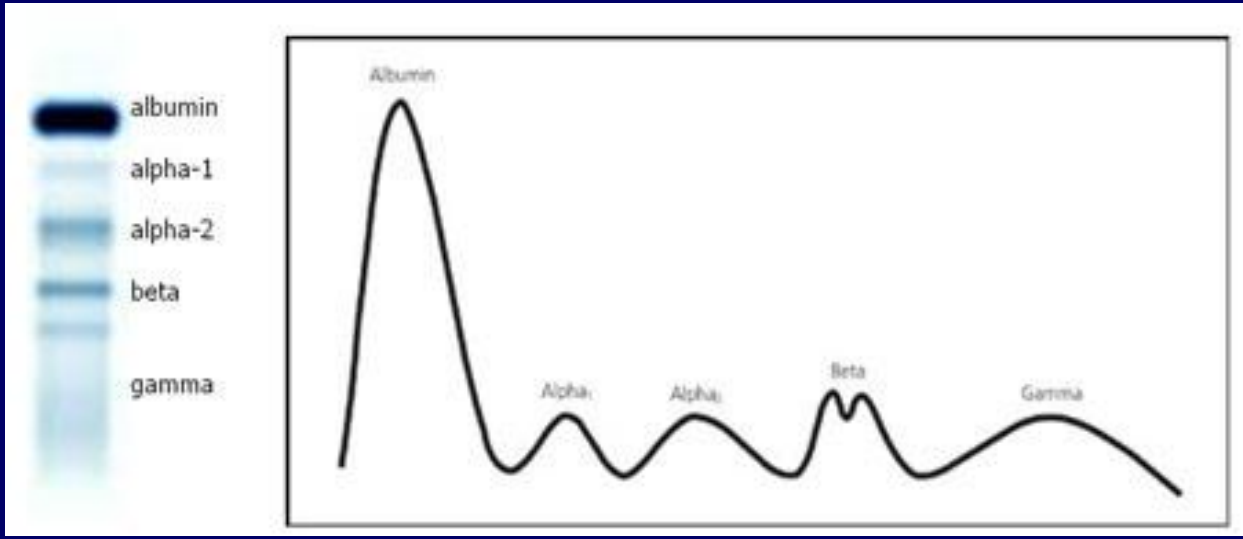
Amido black **Ponceau S**

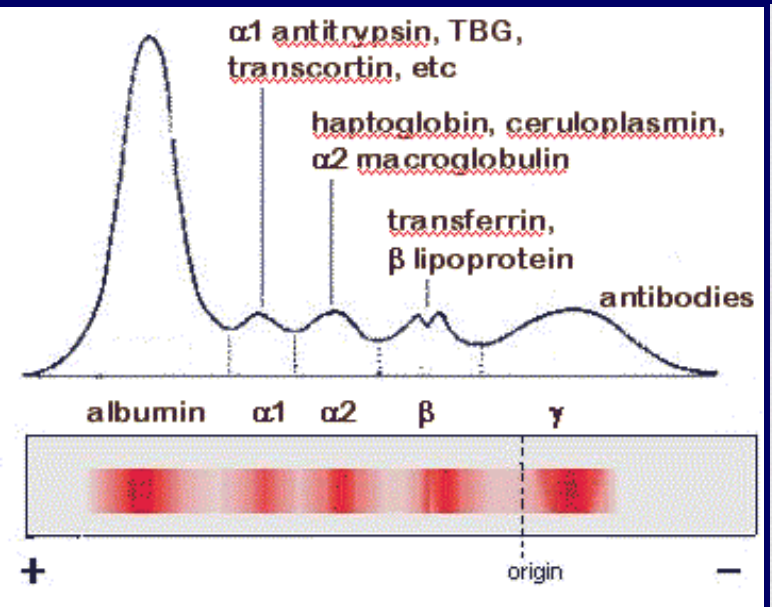
Protein electrophoresis
Densitometry

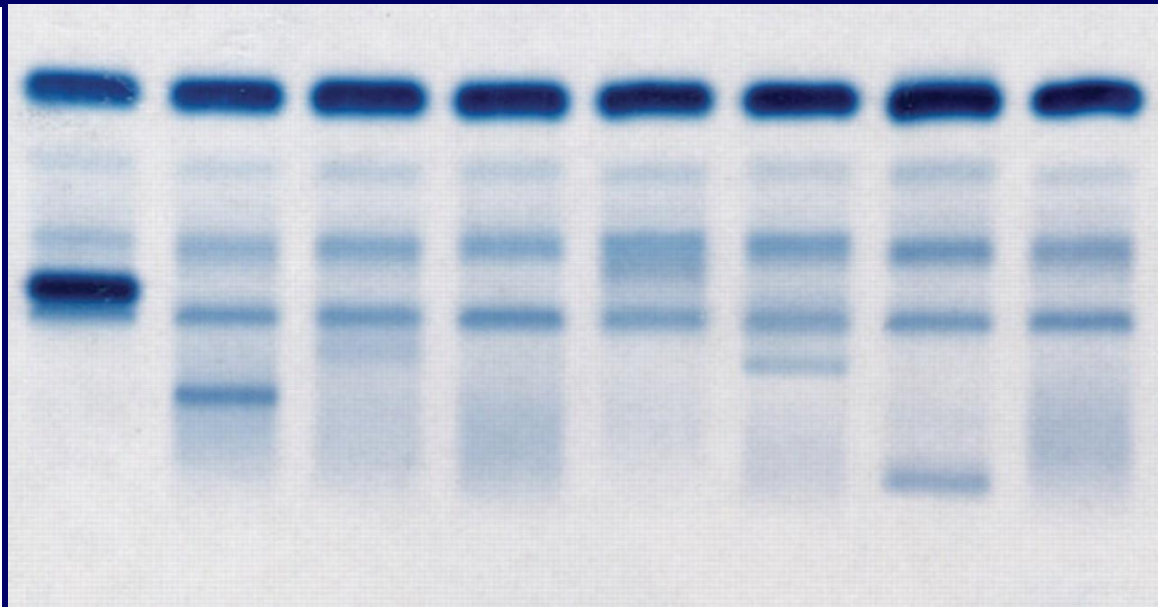




Protein electrophoresis







Immune fixation

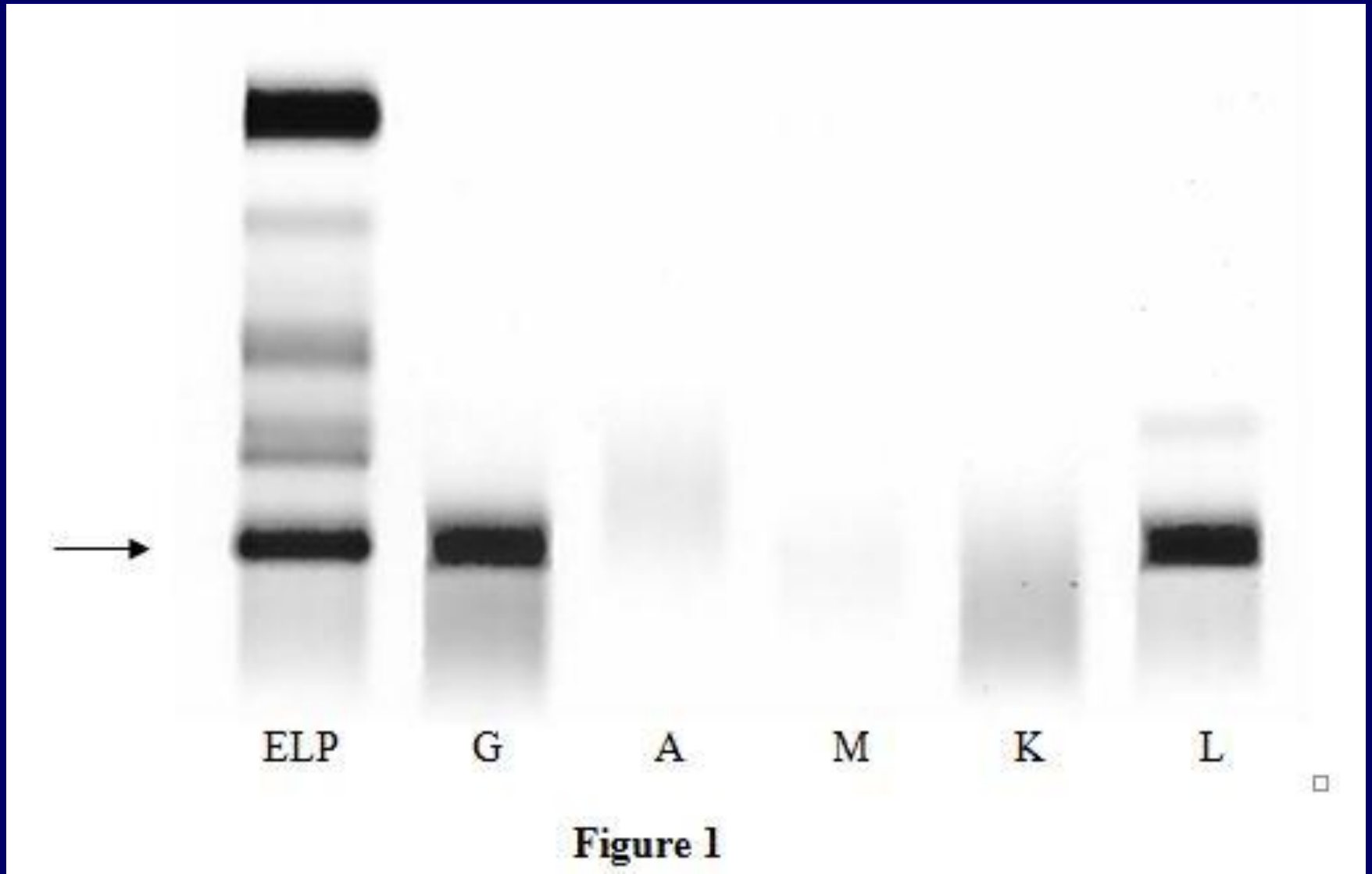
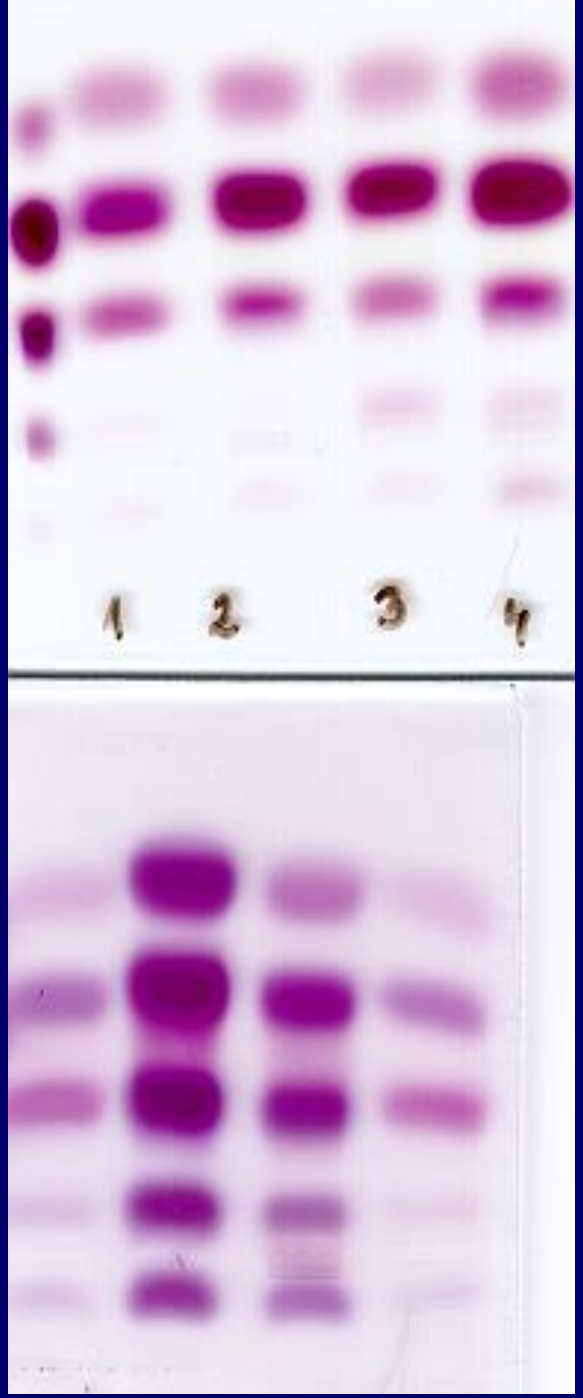


Figure 1

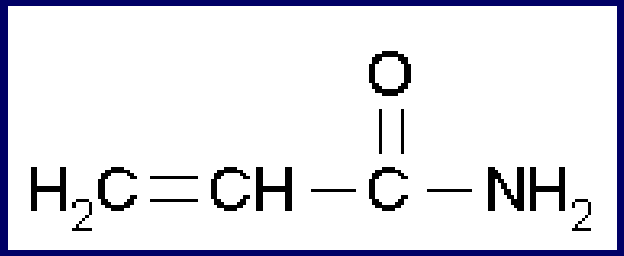


LDH isoenzyme test Agarose gel

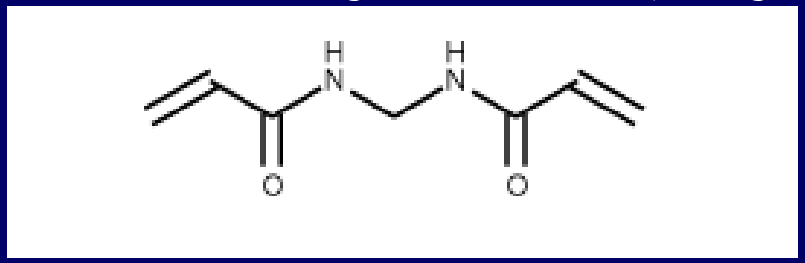
LDH 1
2
3
4
5

Protein electrophoresis
Polyacrylamide gel

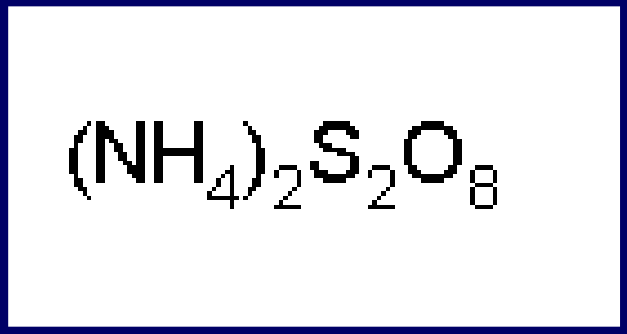
Acrylamide



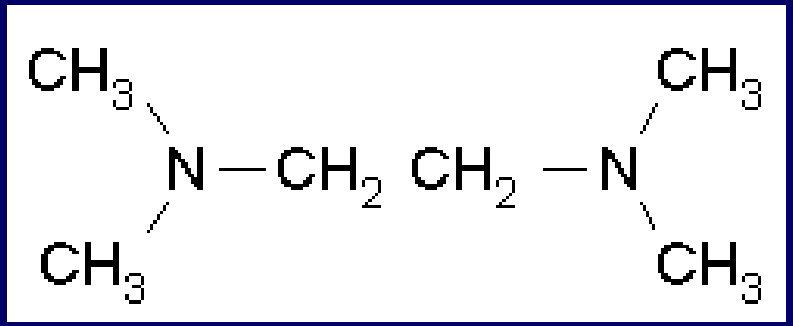
Bis, [N,N'-methylene-bis(acrylamide)]



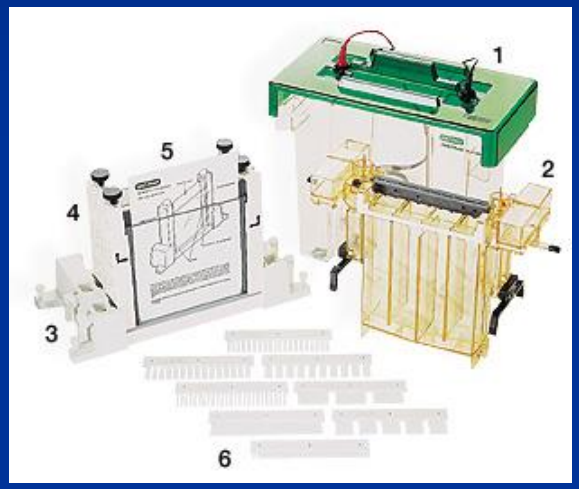
Ammonium persulfate



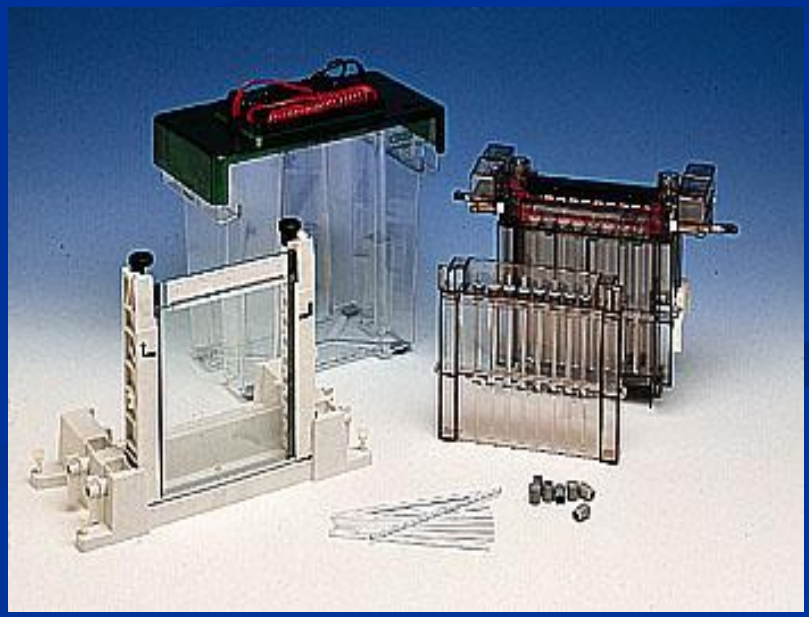
TEMED
(tetramethylethylenediamine)

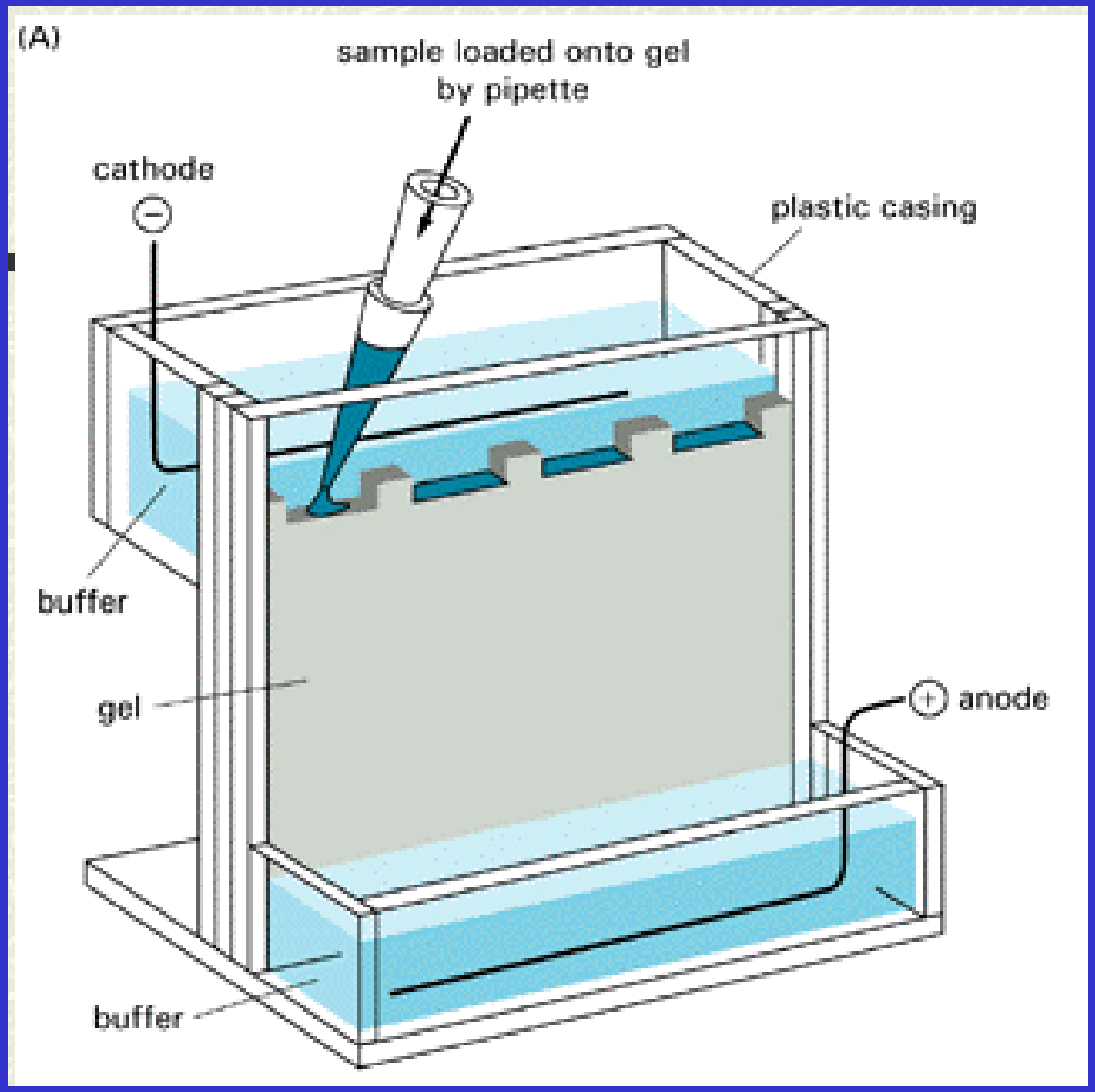


Choice of electrophoretic system



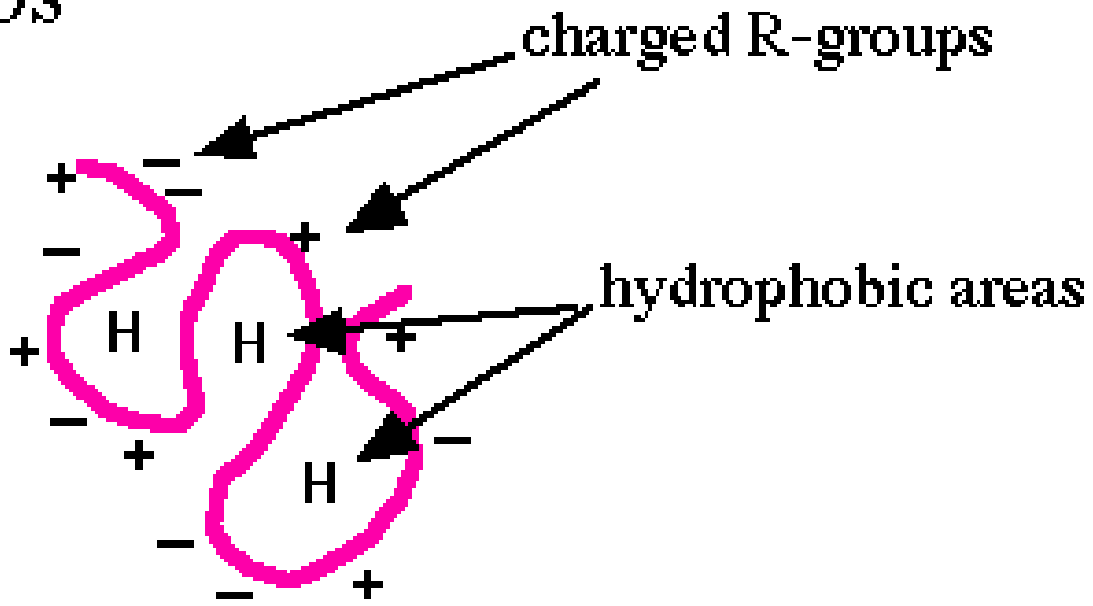
Bio-Rad



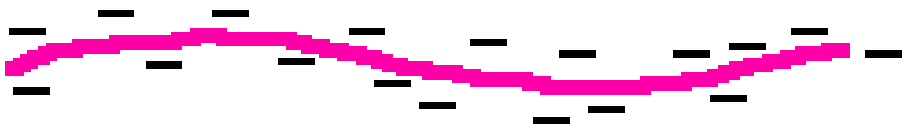

(A)
sample loaded onto gel
by pipette
cathode
plastic casing
buffer
gel
anode
buffer

Denaturing

BEFORE SDS

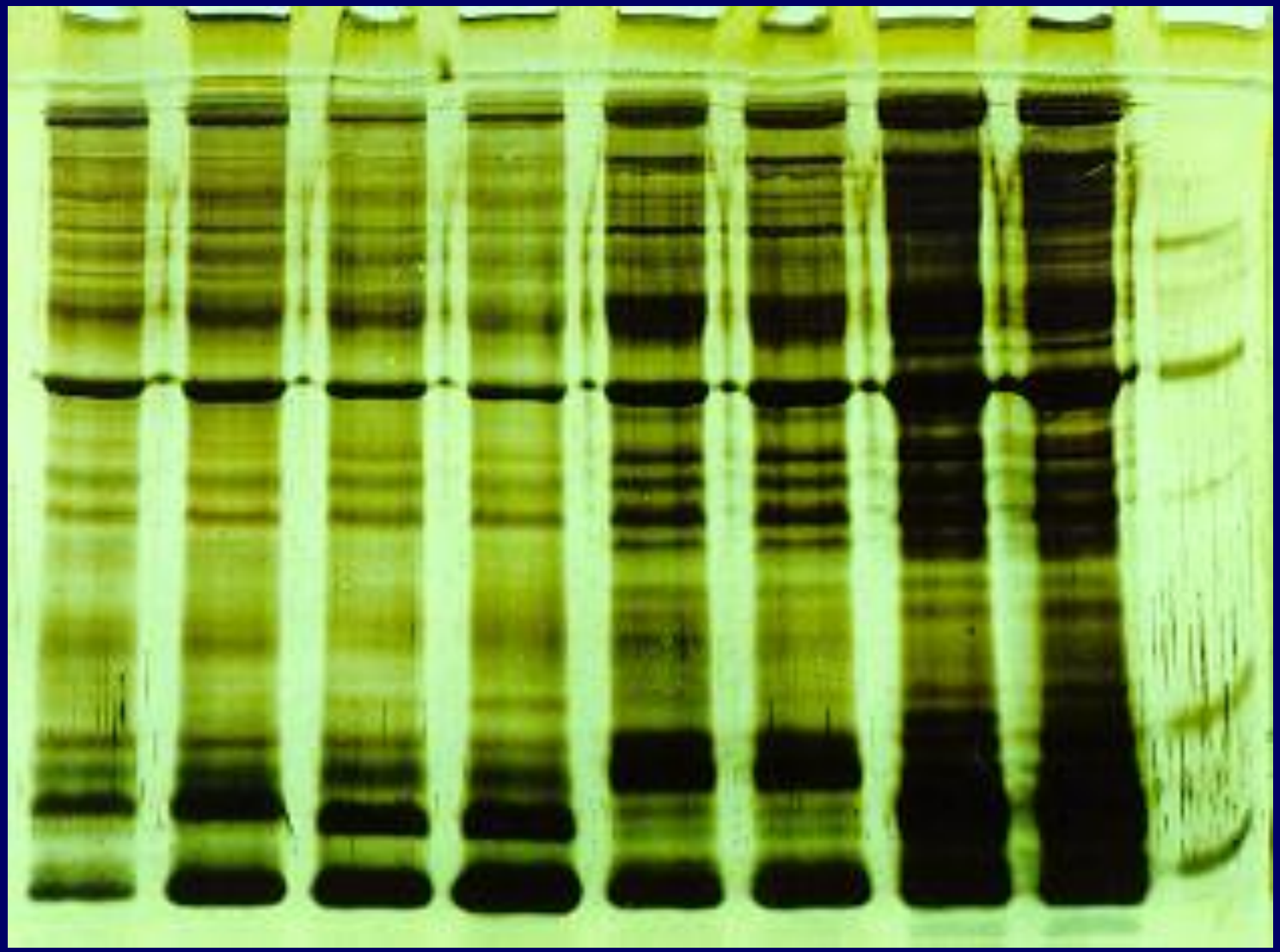


AFTER SDS

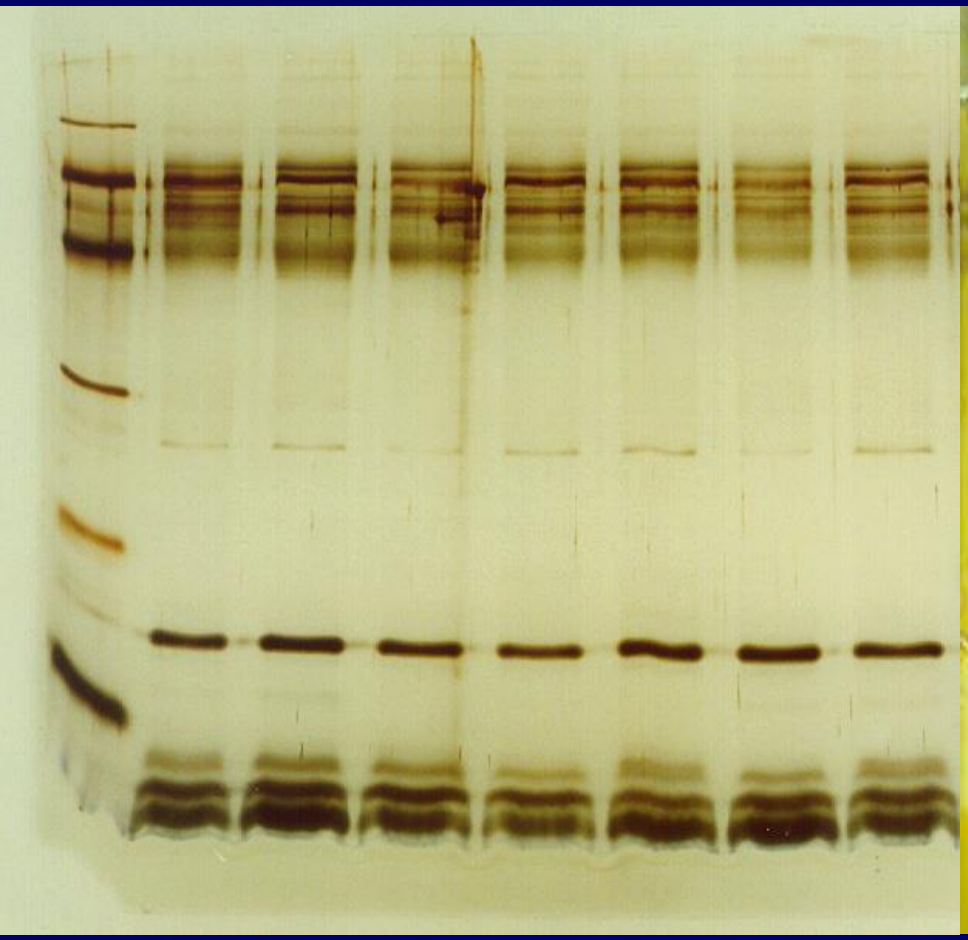


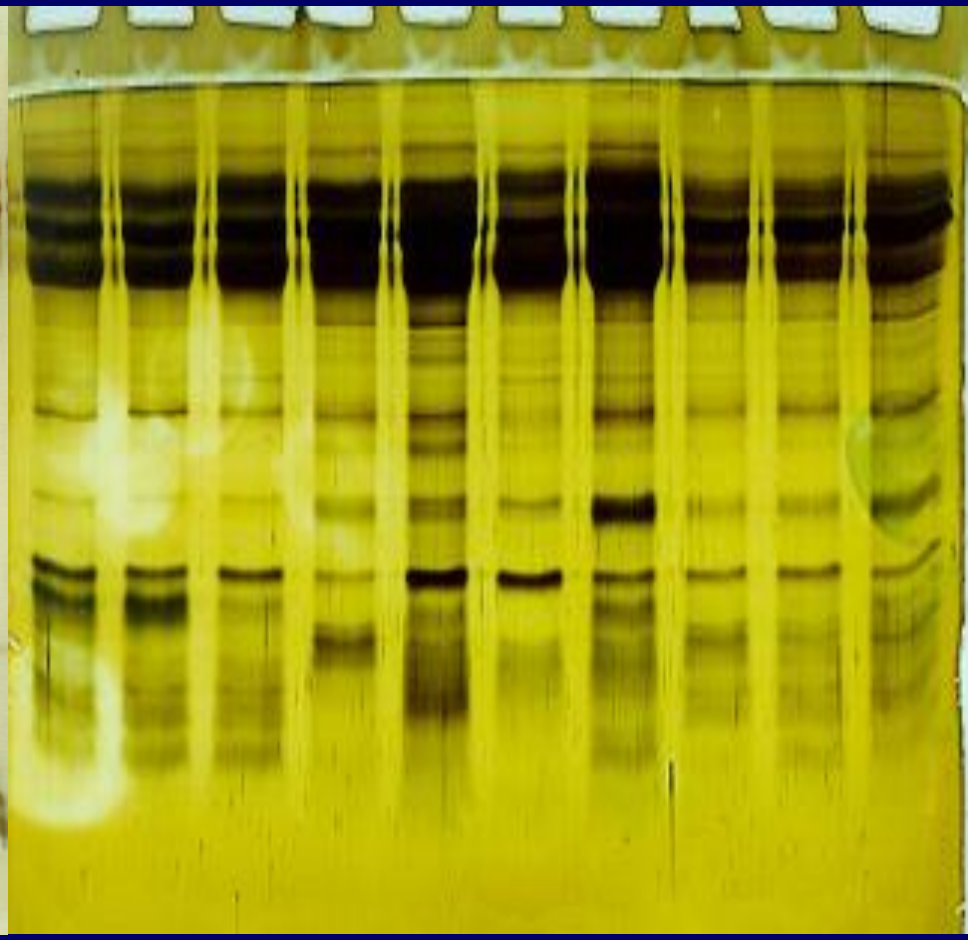
http://www.davidson.edu/academic/biology/courses/Molbio/SDSPAGE/SDSPAGE.html

SDS-PAGE of human platelets

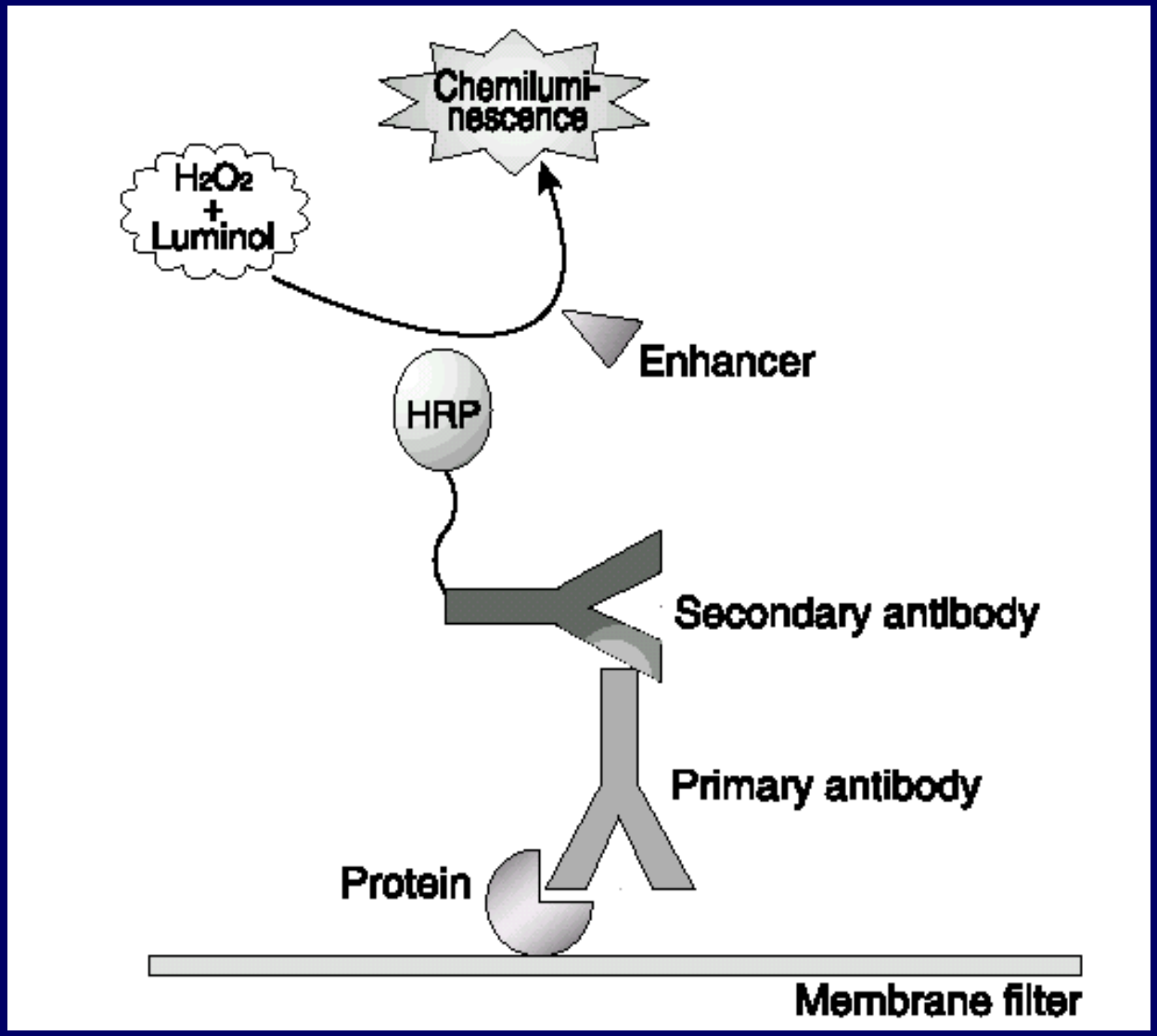


Protein pattern of healthy individuals and cancer patients



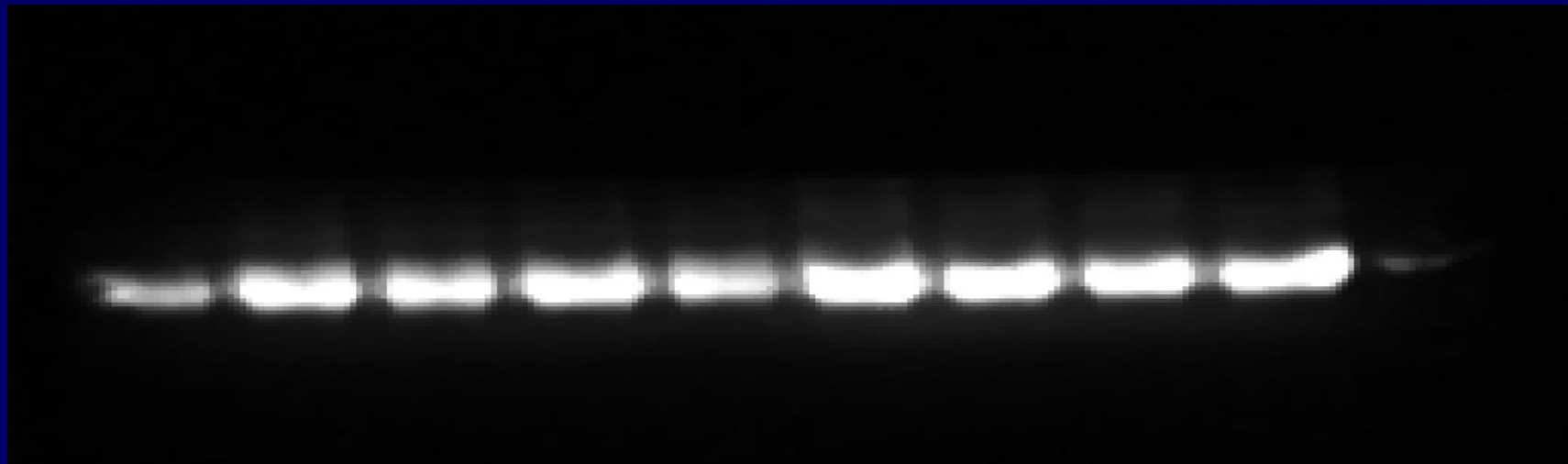


Principles of immunoblotting



Chemiluminescent detection of dot blots

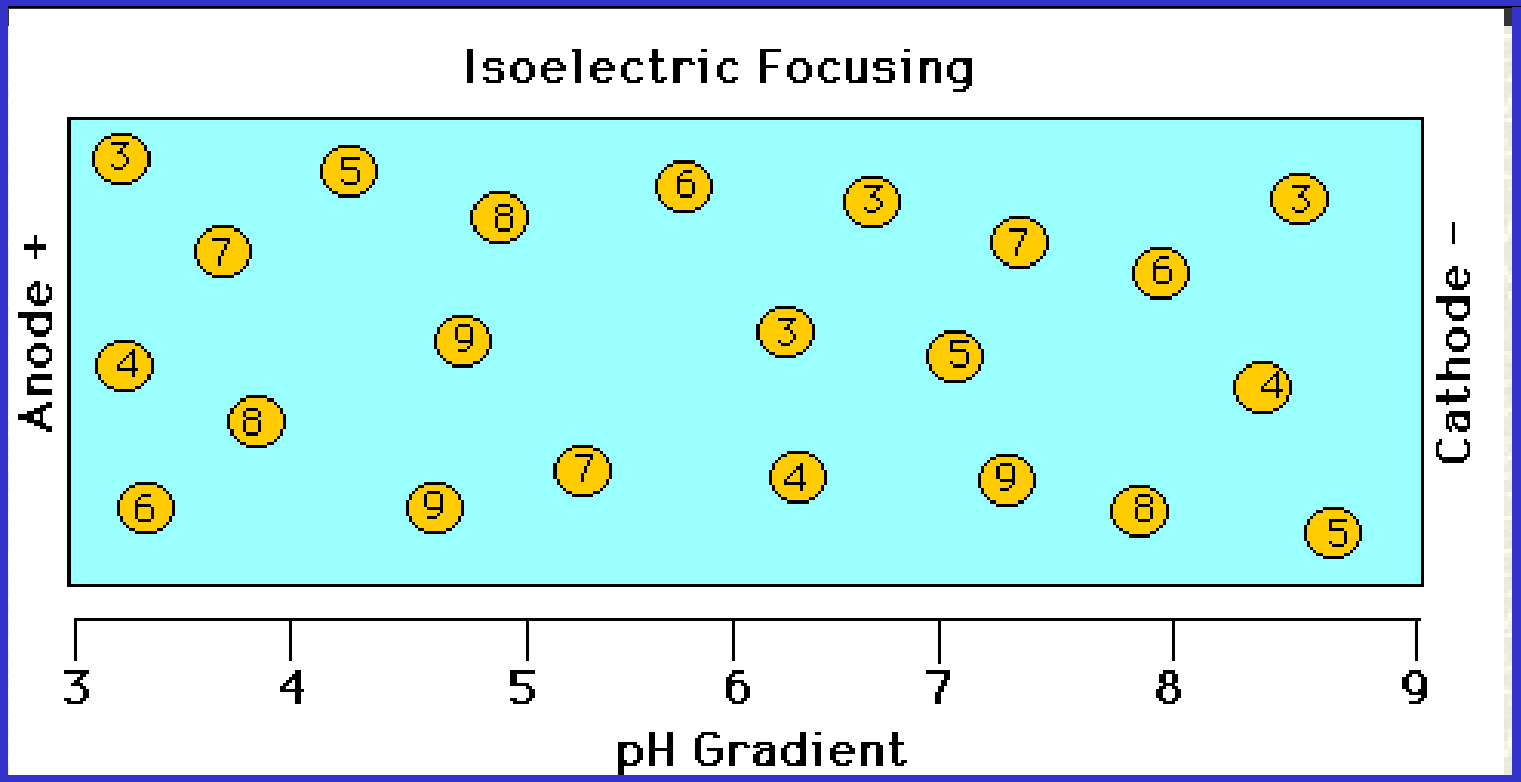
Chemiluminescent detection of orosomucoid



Anti-human (rabbit) orosomucoid glycoprotein

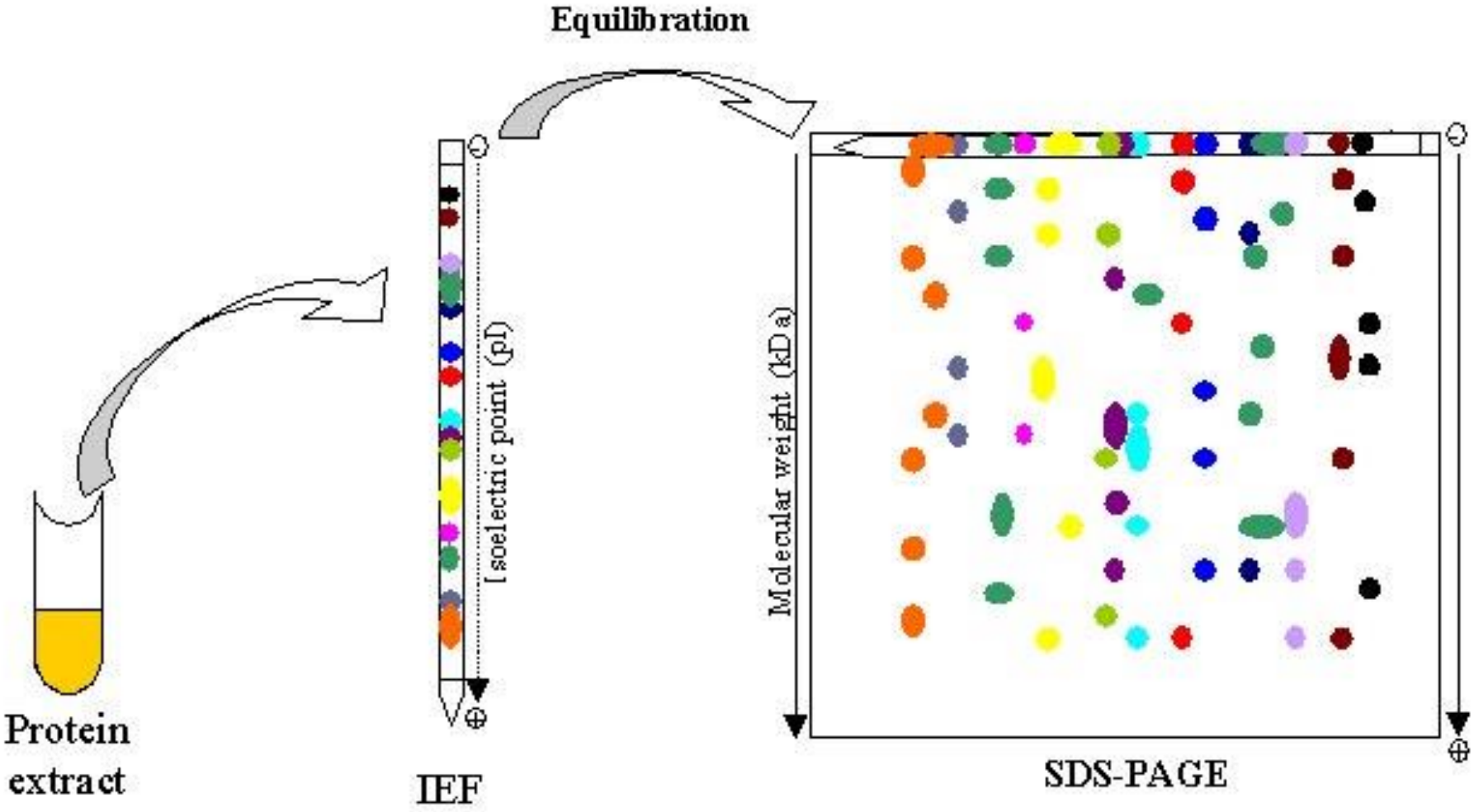
HRP labeled anti-rabbit IgG

Isoelectric focusing

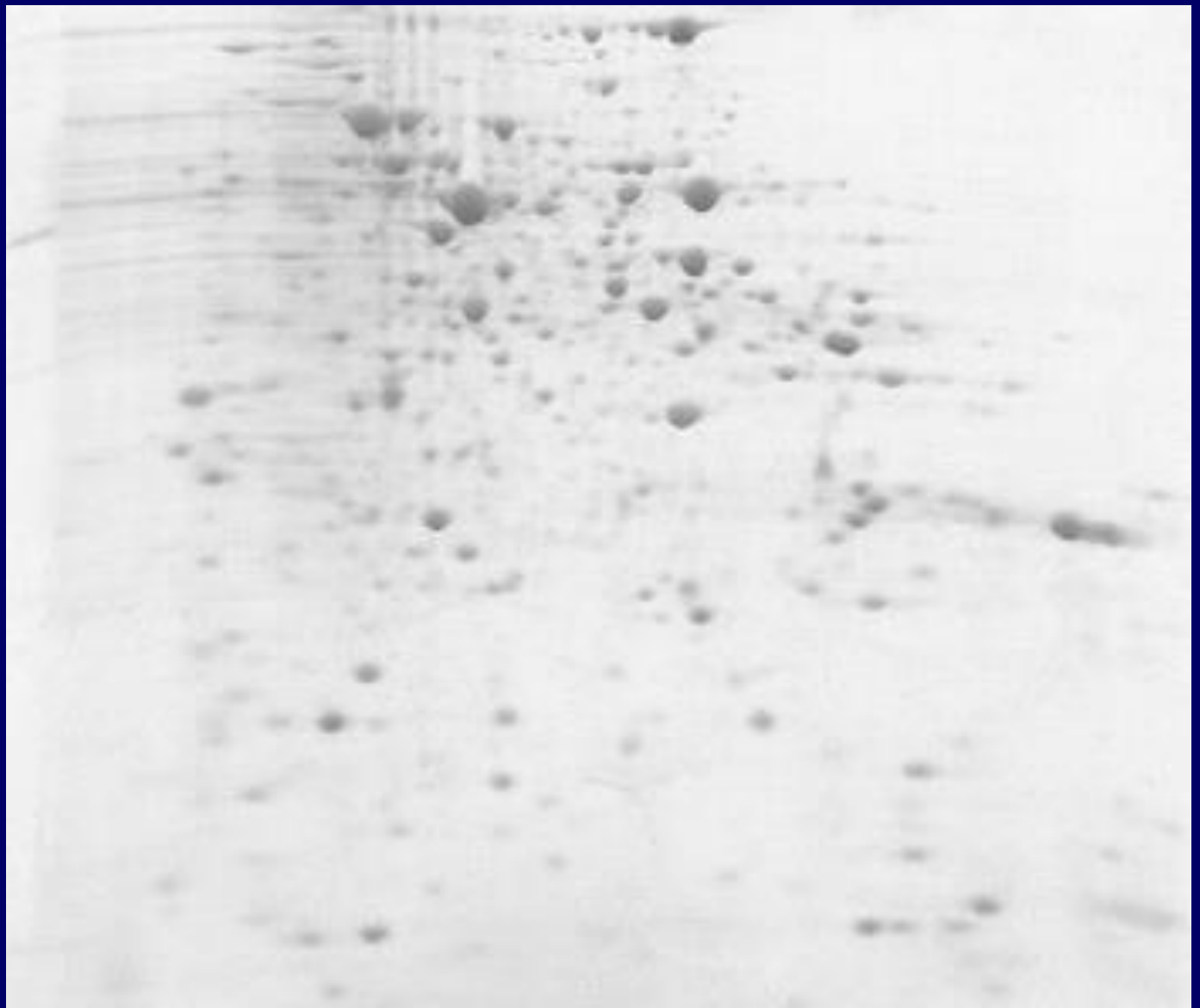


http://www.aber.ac.uk/parasitology/Proteome/Tut_2D.html#Section%203

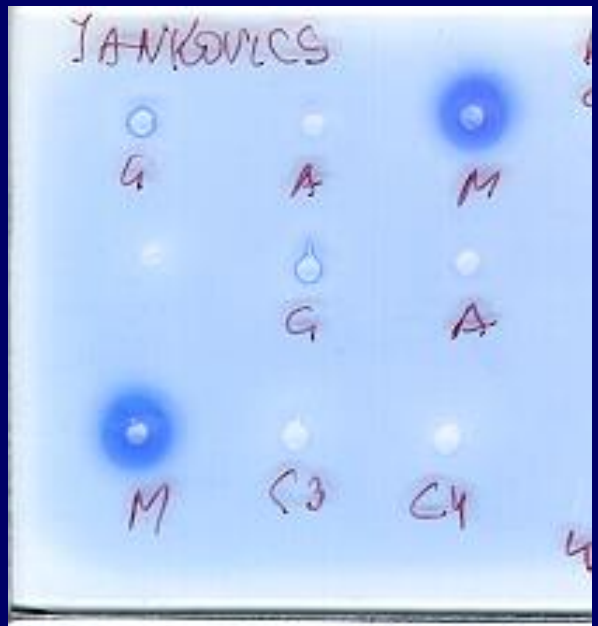
2D electrophoresis



2D electrophoresis



Quantitative measurement of individual proteins



Immune diffusion

Rocket immune elpho

Antigen-antibody reaction detection with quantitative analysis

Automated quantitative measurement of individual proteins

- Immune turbidimetry
- Nephelometry
- Immuno assays