Electrophoresis testing

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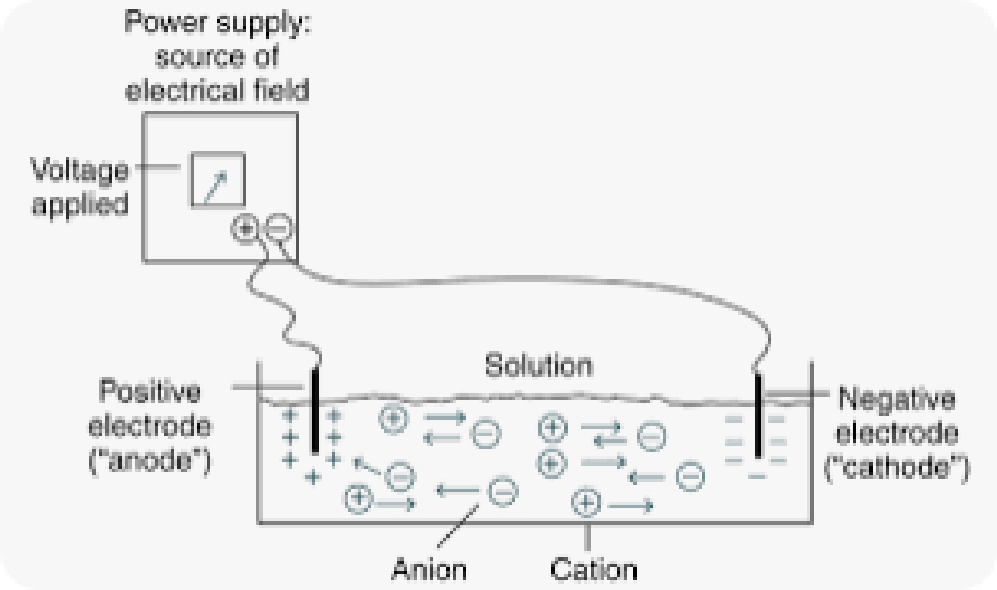
Principles of electrophoresis

- Electrophoresis testing uses an electrical field to separate molecules based on their charge

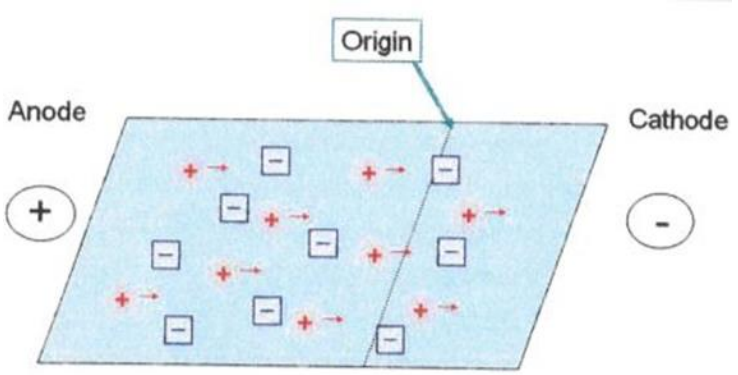


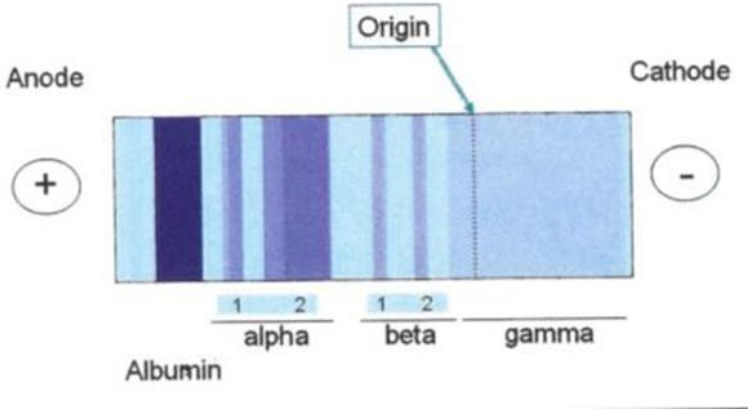
Oncohema Key

Electrophoresis Techniques | Oncohema Key

Factors which effect electrophoretic mobility

- 3 major components of electrophoresis techniques
  - Support media (paper, cellulose acetate, agarose) – usually net negative charge that doesn't move
  - Buffer/solvent - usually net positive charge that flows toward cathode (electro-endosmosis)
  - Patient sample – most major serum proteins net negative charge at pH used in assays (8.6-10.0)





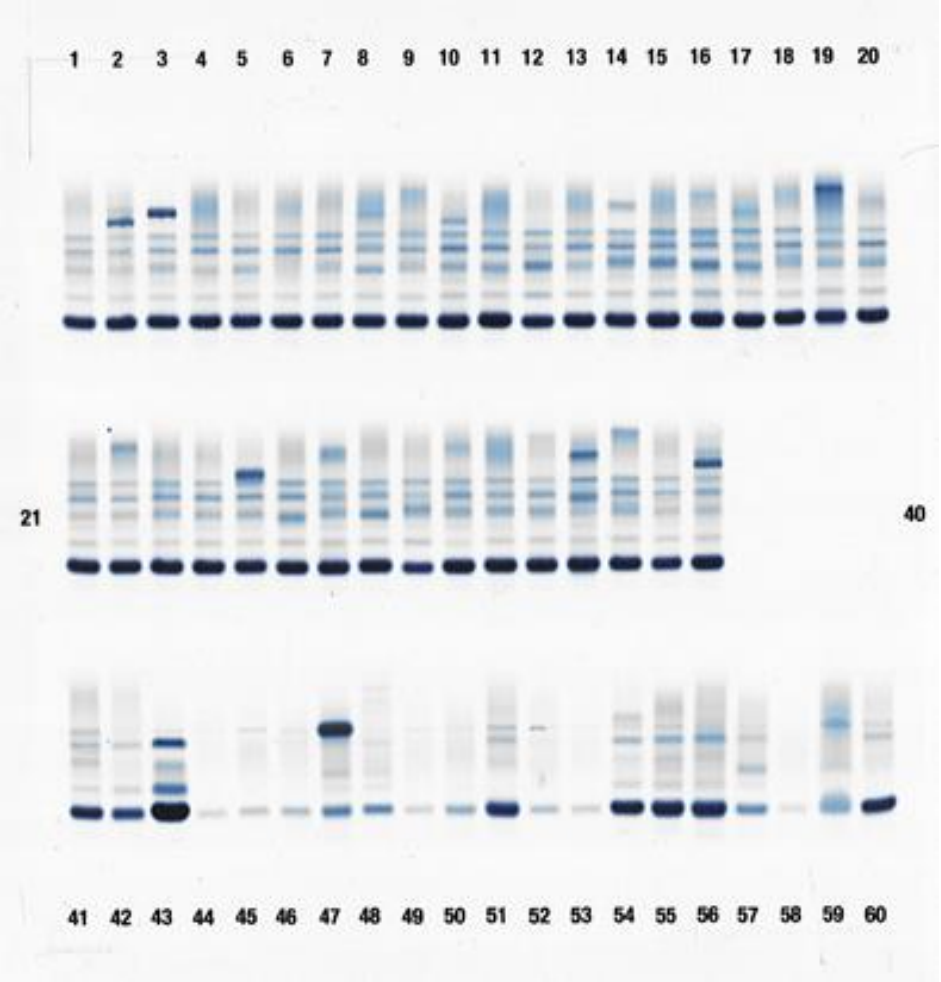
- Migration on gel depends on strength of negative charge for a given protein vs the endoosmotic "current" they're swimming against

Clinical applications of electrophoresis

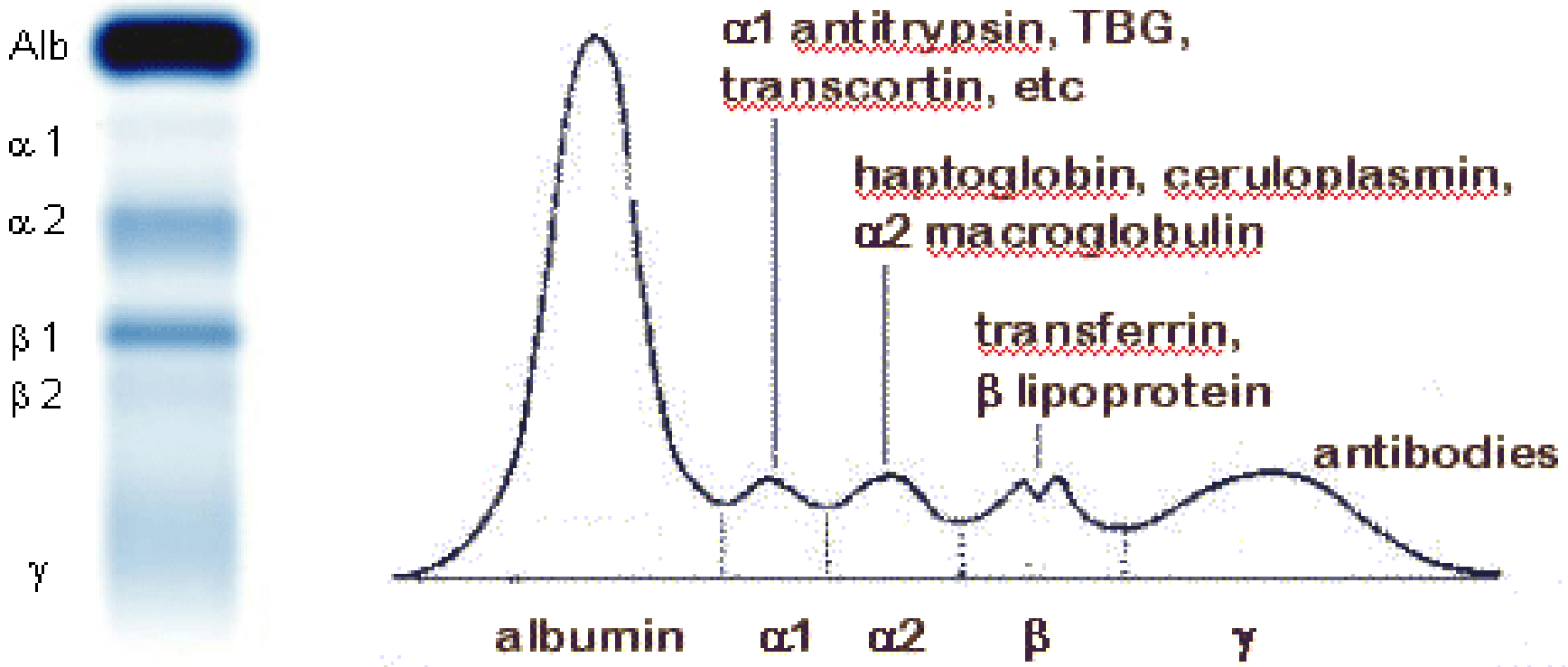
- 3 main clinical scenarios where ELP testing is used
  - Identification of monoclonal immunoglobulins (multiple myeloma, MGUS, amyloid)
  - Identification of oligoclonal bands in CSF (multiple sclerosis, CNS inflammatory disease)
  - Identifying the source of an abnormal serum enzyme level (ALP, CK, LDH, A1AT)
- 2 major techniques used for this testing
  - Protein electrophoresis: separate by charge, visualize with non-specific stain
  - Immunofixation: separate by charge, visualize with antibody-specific fixation and staining
- Testing is usually done on serum or urine, although other types may be used in specific situations

Protein electrophoresis

- Serum/urine applied to agarose gel and current applied.
- Electrophoresed sample stained with non-specific (acid blue) protein stain
- Banding pattern is examined to look for abnormalities relative to normal distribution

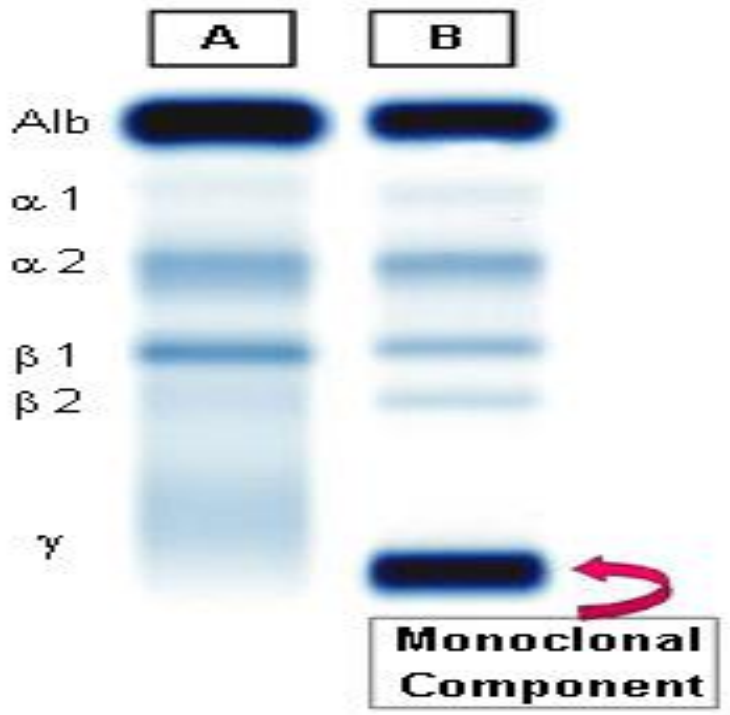


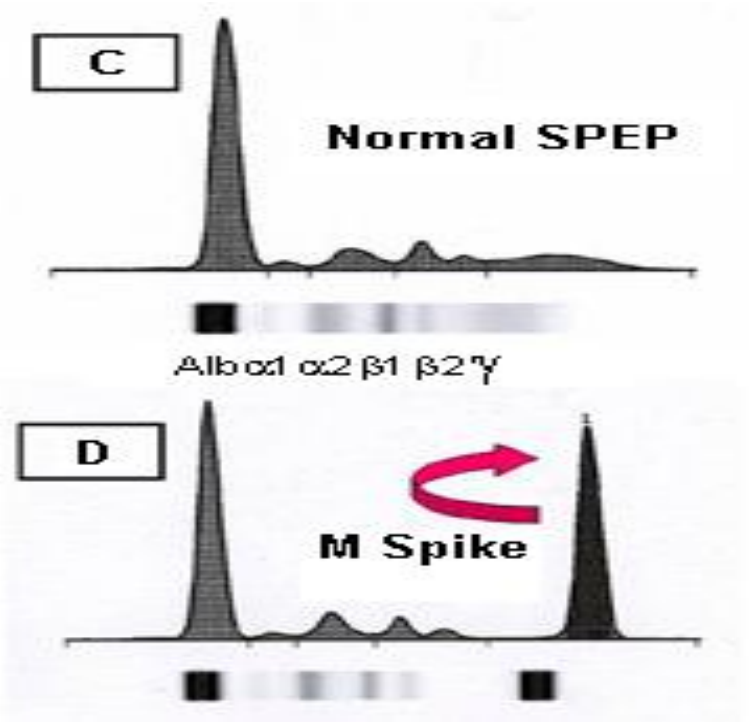
Normal serum protein migration pattern



- The locations and intensity of bands are fairly consistent between individuals, allowing identification of abnormal bands

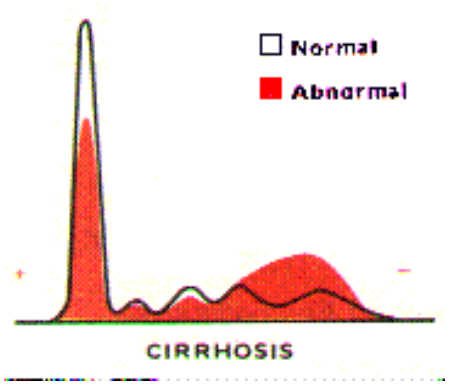
ELP patterns in multiple myeloma

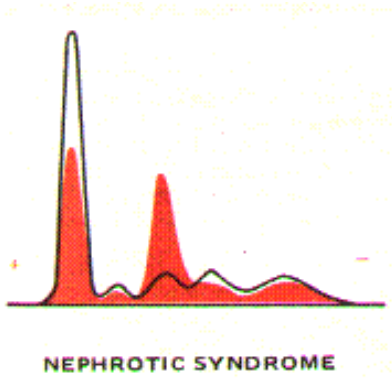


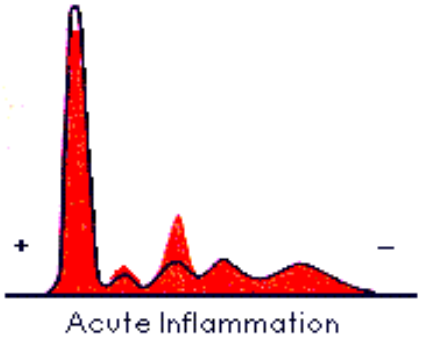


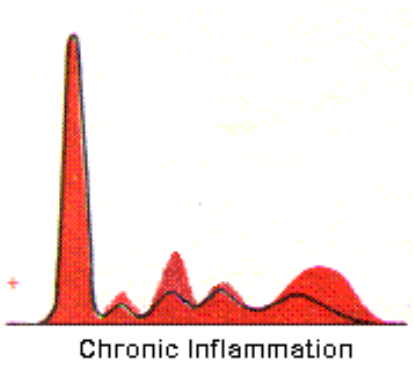
Advantages	Limitations
High-throughput, inexpensive screen	Abnormal PE bands can be due to other causes than monoclonal gammopathy
Provides semi-quant estimate of amount of M protein present	M proteins can "hide" behind normal serum proteins
Can detect other abnormalities besides monoclonal gammopathy	Technique is less sensitive than IFE for low-level M proteins

ELP patterns in other common diseases







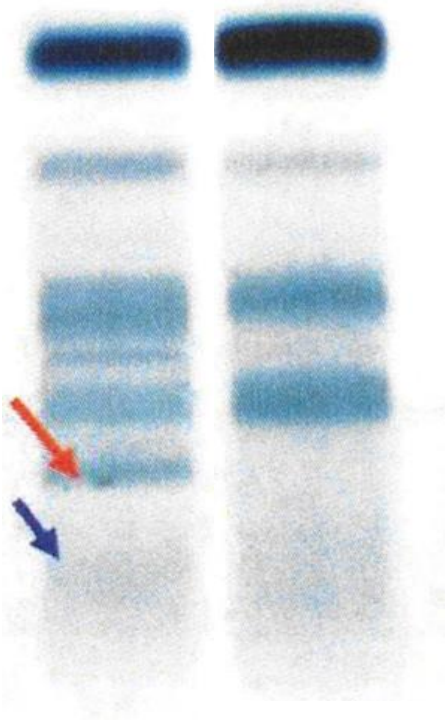


Low albumin/α2 due to decreased liver synthetic function **Beta-gamma bridging due to increased IgA**	**Low albumin due to renal losses** **α2 increase from liver compensatory increases in synthesis**	**α2 increase due to hepatic synthesis of acute phase proteins**	**α2 increase due to hepatic synthesis of acute phase proteins** **Polyclonal increase of immunoglobulins**

- **However, without an IFE one cannot definitively rule out a monoclonal Ig as the cause of a band**

Common serum EP artifacts

Plasma

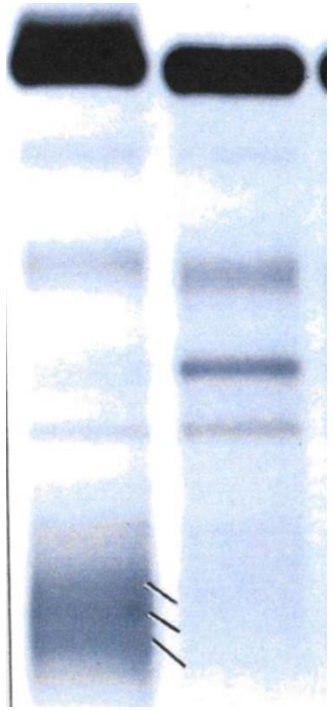


Sharp band in γ from fibrinogen

Hemolysis

Shoulder on α2 region from hemoglobin-haptoglobin complex

Hepatitis

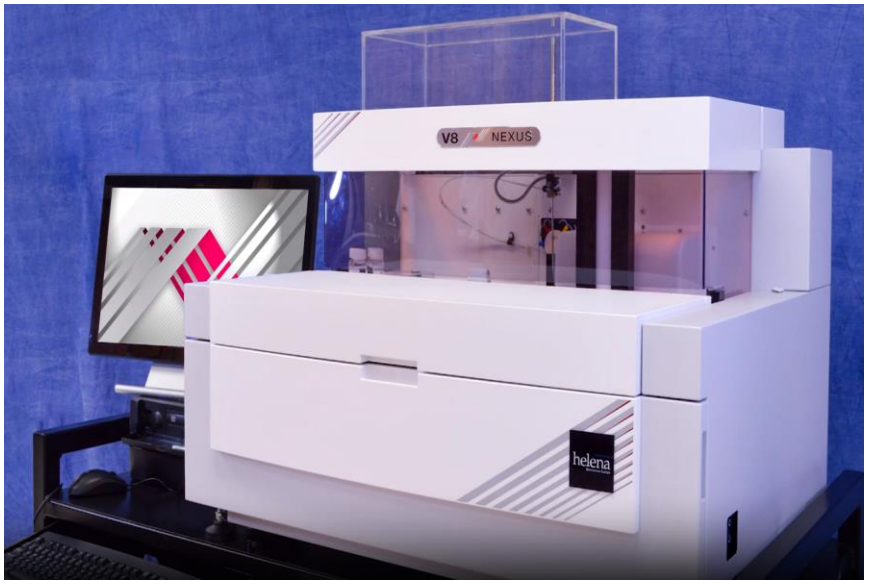


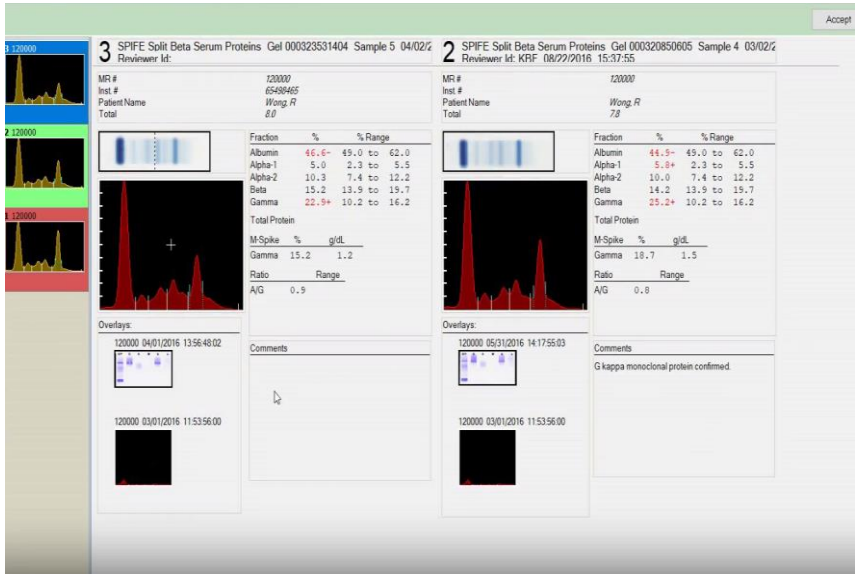
Multiple faint γ bands on polyclonal background from chronic inflammation

V8 Nexus Capillary Electrophoresis

Fully automated, faster, more connected and flexible

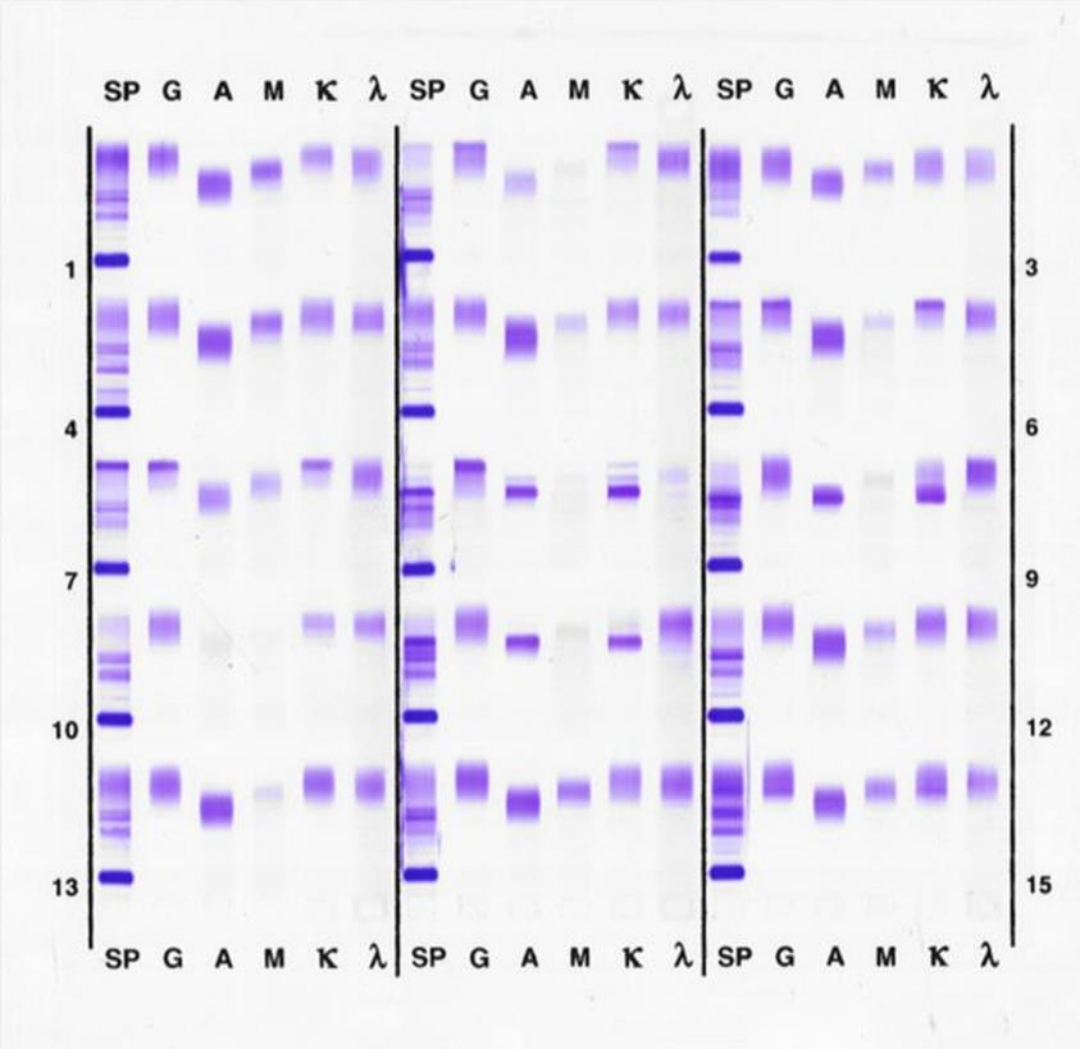
Patient's results can be integrated with gel results in tracking of treatment



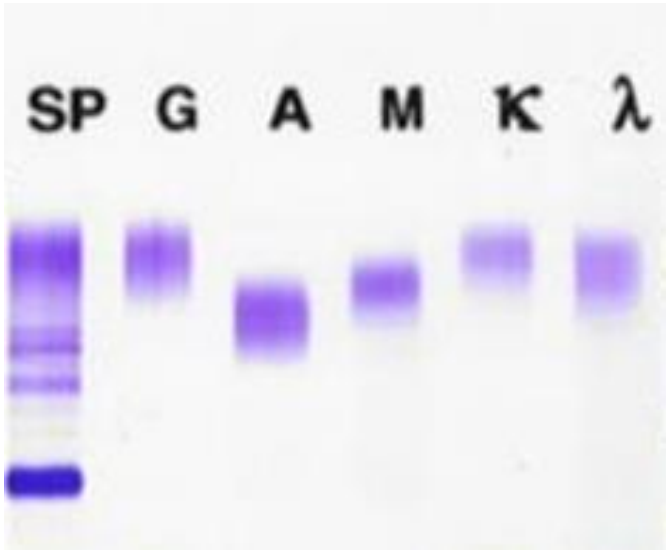


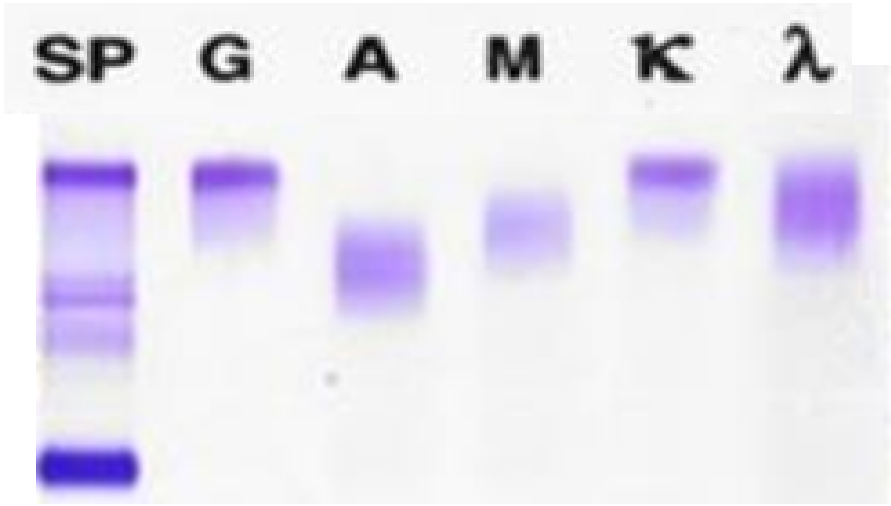
Immunofixation electrophoresis

- Serum/urine applied to agarose gel and current applied.
- Antisera applied to gel which precipitates the apppropriate isotype (G, A, M, K, or L)
- Unreacted proteins washed away and precipitated complexes stained with non-specific (acid violet) stain



Normal and abnormal IFE patterns

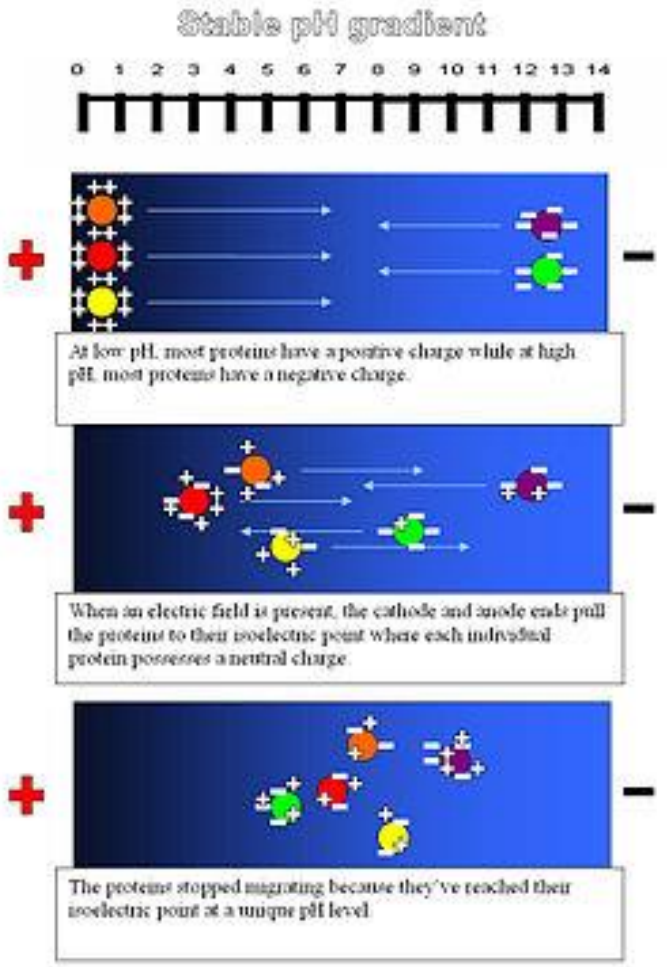




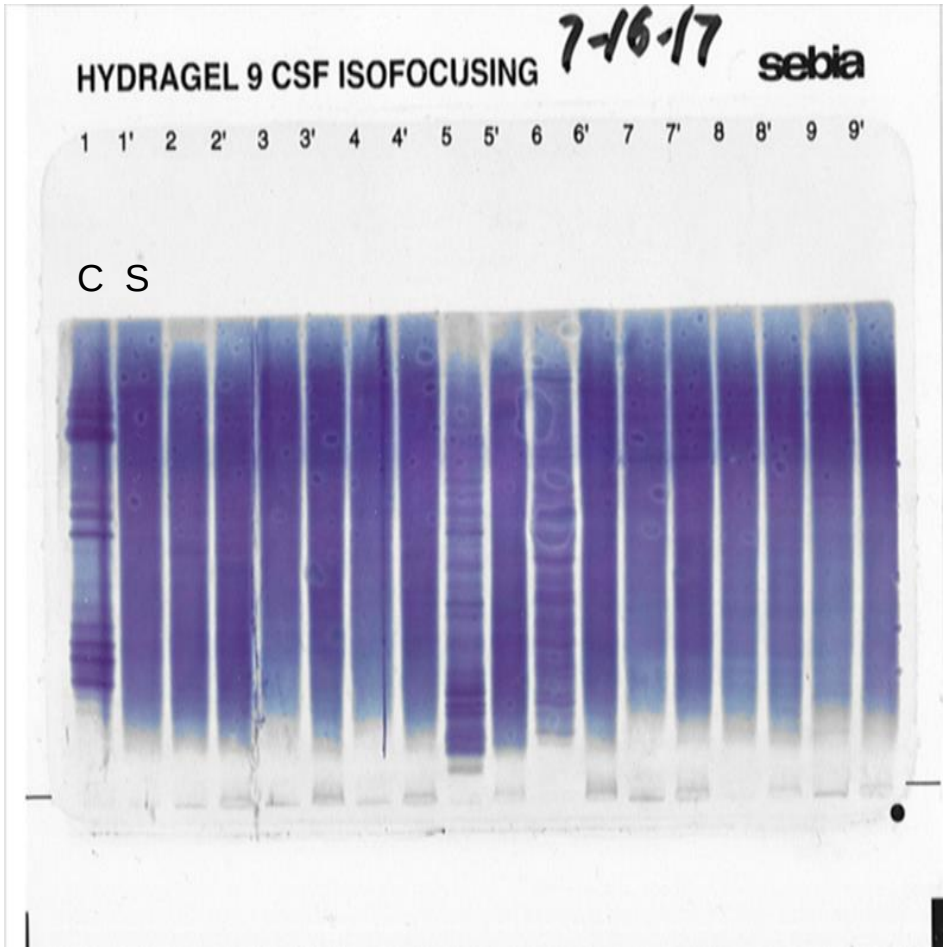
Advantages		Limitations
Confirms that abnormal band is a monoclonal immunoglobulin		Does not quantitate amount of M protein present
Detection not affected by normal serum protein bands		Can detect low-level bands on unclear signficance
Provides information on isotype		Lower throughput

Isoelectric focusing

- Paired serum and CSF are applied to agarose gel and current applied.
- Samples migrate through a pH gradient where protein charges change
- Proteins stop migrating when they reach zero charge (isoelectric point)
- Provides improved separation of proteins with similar charges at biological pH

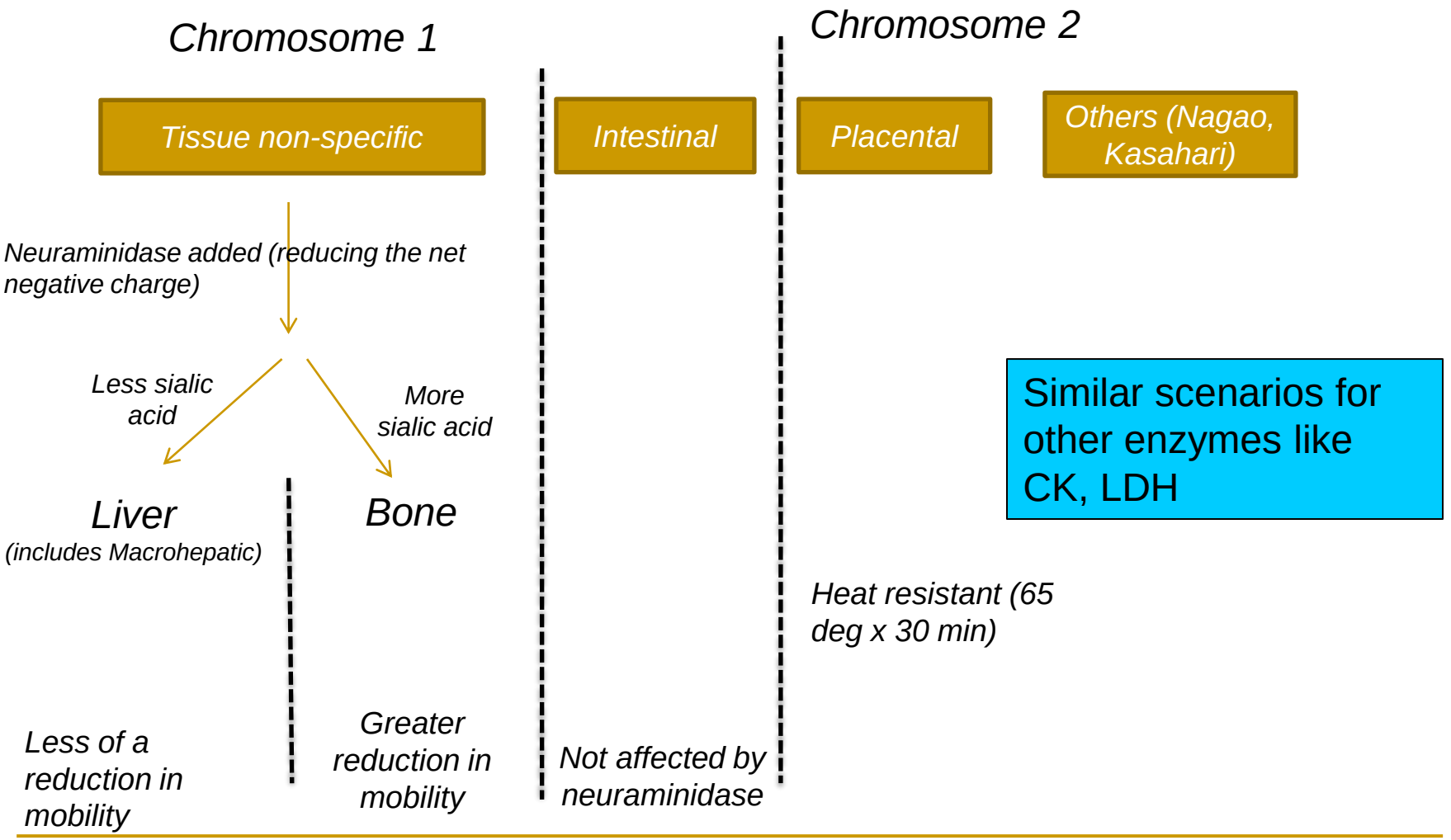


IEF in multiple sclerosis



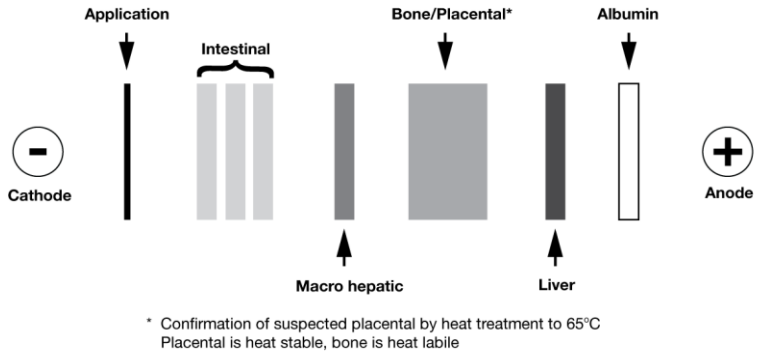
- Normal appearnace is no bands in CSF or serum
- Bands in CSF > serum : intrathecal synthesis of IgG
  - Can be due to causes other than MS
- Bands in CSF = serum : usually systemic inflammatory process with crossover into CSF
  - Crossover can be *in vivo* or due to contamination during spinal tap

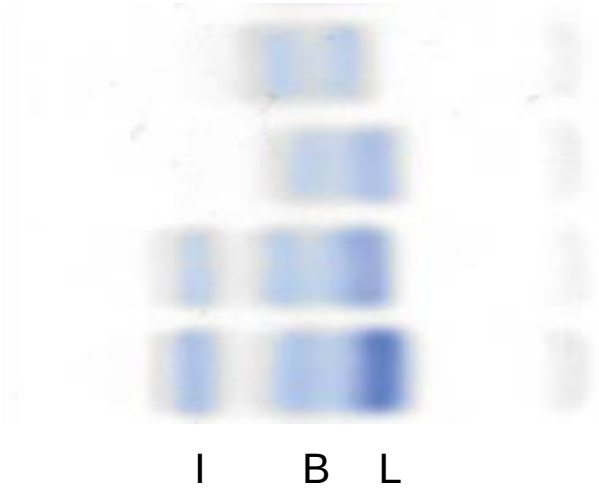
Isoenzyme testing (alkaline phosphatase)



Alkaline phosphatase isoenzyme testing

- Serum is treated with neuraminidase (cleaves sialic acid to improve bone/liver separation)
- Samples electrophoresed on agarose gel in constant pH buffer
- Gels treated with NBT (nitro blue tetrazolium) which is cleaved to blue stain by alkaline phosphatase
- Fractions in bone, liver, and other are quantitated as % of whole





Conclusions

- Electrophoresis techniques take advantage of charge differences to characterize proteins
- Immunofixation can be used to increase the sensitivity and specificity of testing for monoclonal immunoglobulins
- Although most testing can be done with routine techniques, specialized adaptations such as isoelectric focusing and isoenzyme analysis can be used for specific clinical conditions.