Screening and Immunotyping Monoclonal Antibody using the V8 Nexus

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Biosciences Europe

Ankara – July 2019

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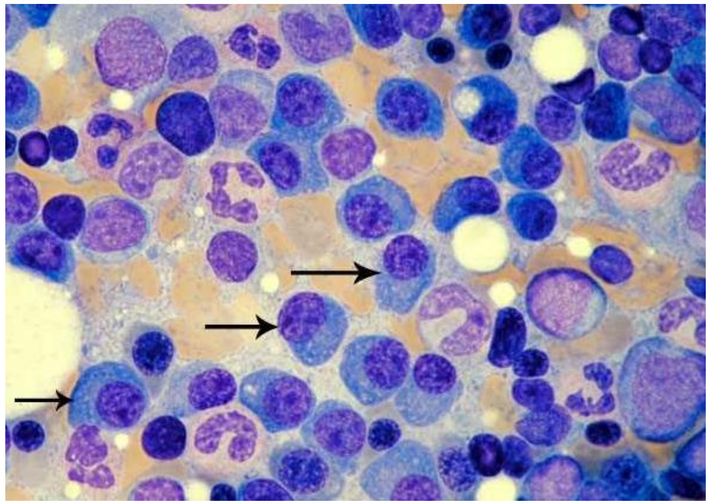
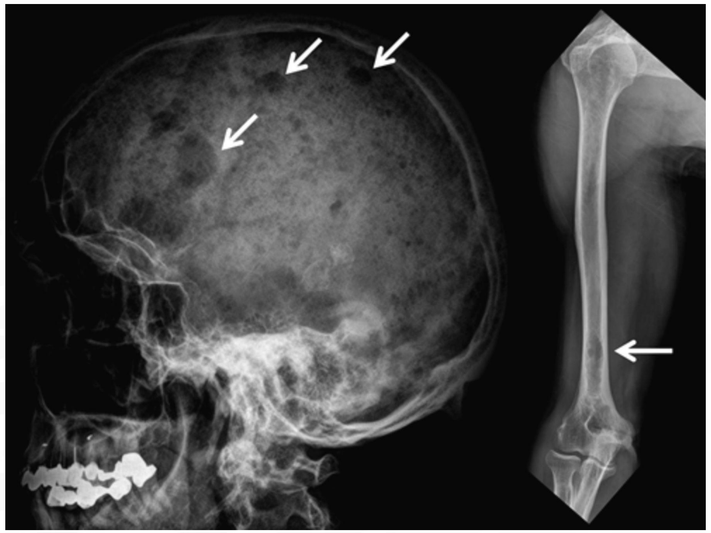
Multiple Myeloma

- Cancerous plasma cell tumour mass
- Clonal expansion of Plasma & B Cells
- Monoclonal antibody production
- Serum monoclonal antibody level

  indirectly proportional to tumour mass

- Serum Protein Electrophoresis

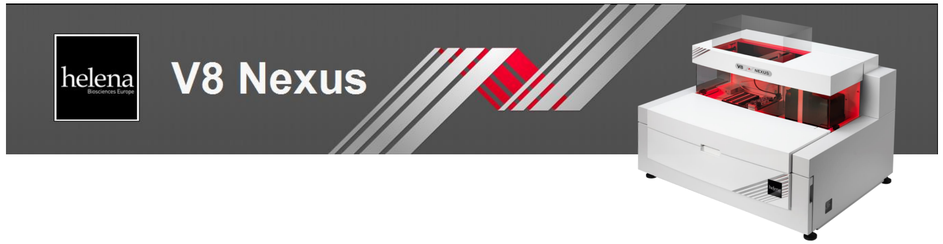




Serum Protein Screening using Helena

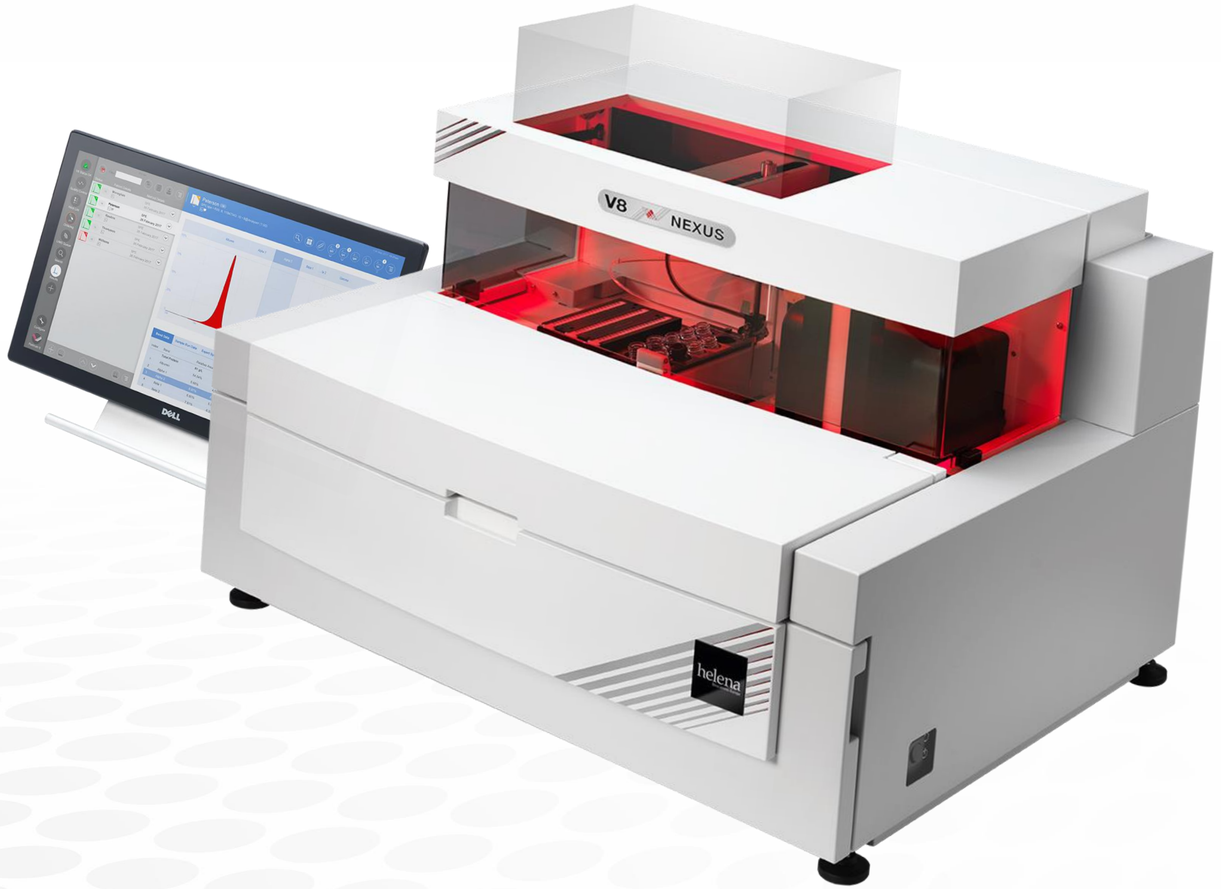
Helena platform can address entire serum protein screening requirements:

- High throughput CZE screening
- Sensitive immunotyping
- Powerful integration
- Comprehensive training

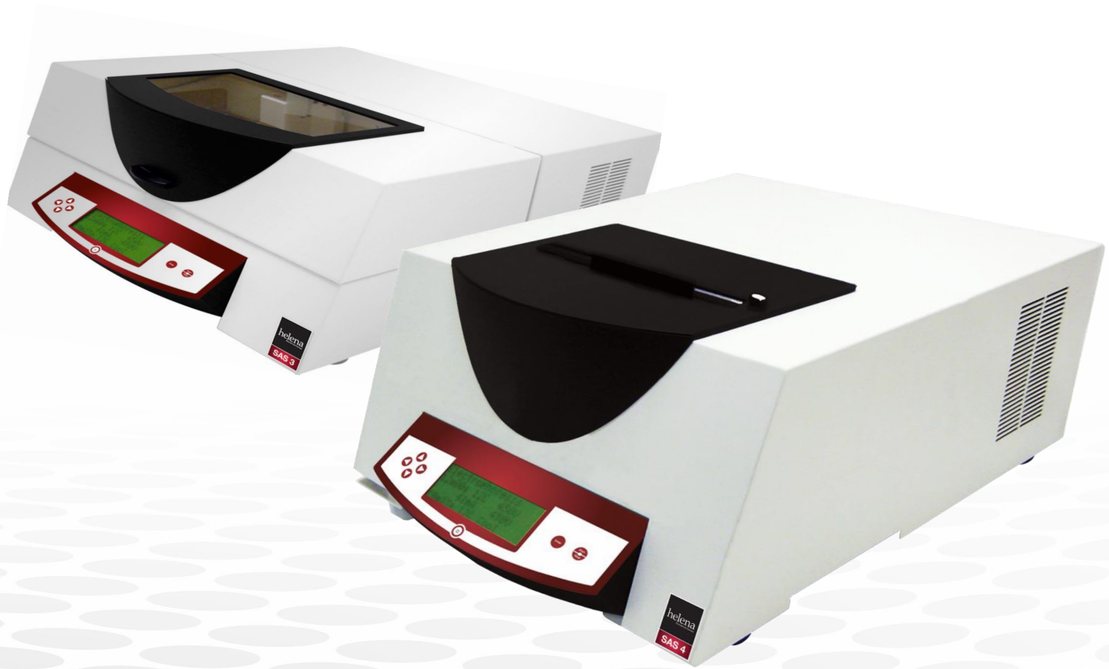


V8 Nexus

- Capillary electrophoresis
- 8 Channel
- Full walk away automation
- Multi test analyser
- Automated maintenance
- Gel integration
- Track and network integration

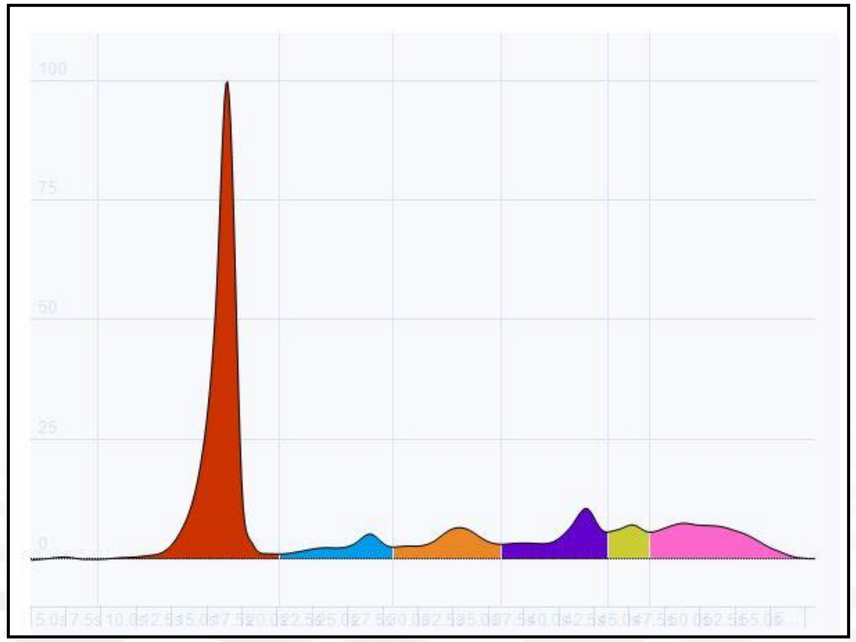


SAS Gel Electrophoresis



- Semi automated
- SAS-3 Electrophoresis
- SAS-4 Stainer
- Automated sample prep using V8 Nexus
- Common database for Gel and CZE results

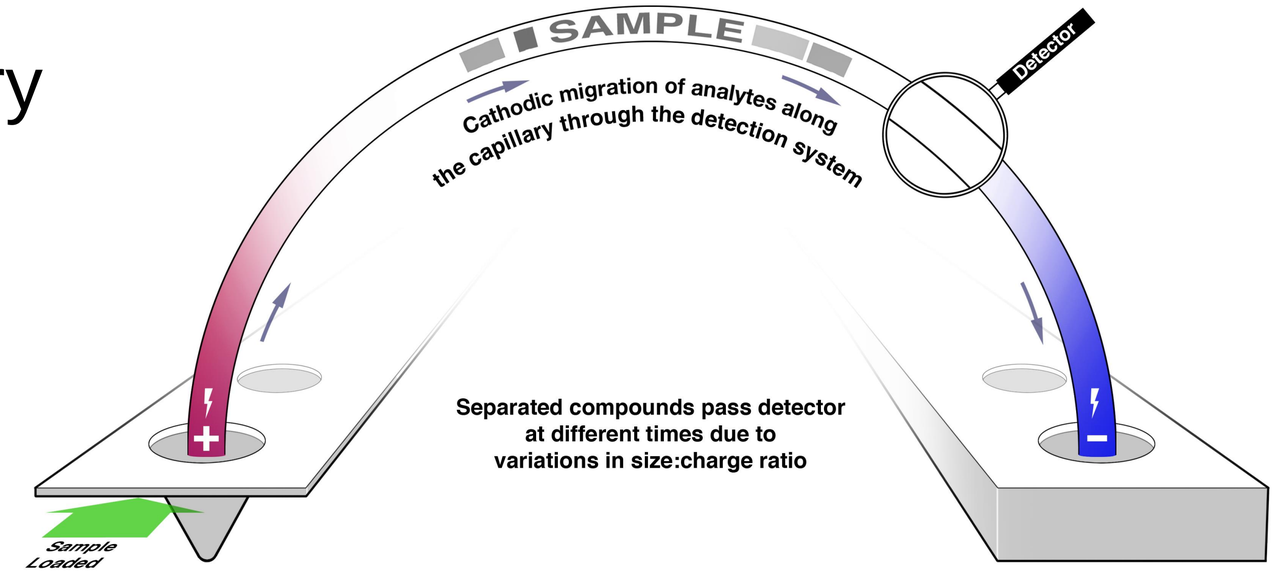
Serum Protein Screening



- CZE separation on the V8
- Split beta separation
- High throughput
- Accurate monoclonal measurement
- Easily compare to historic results

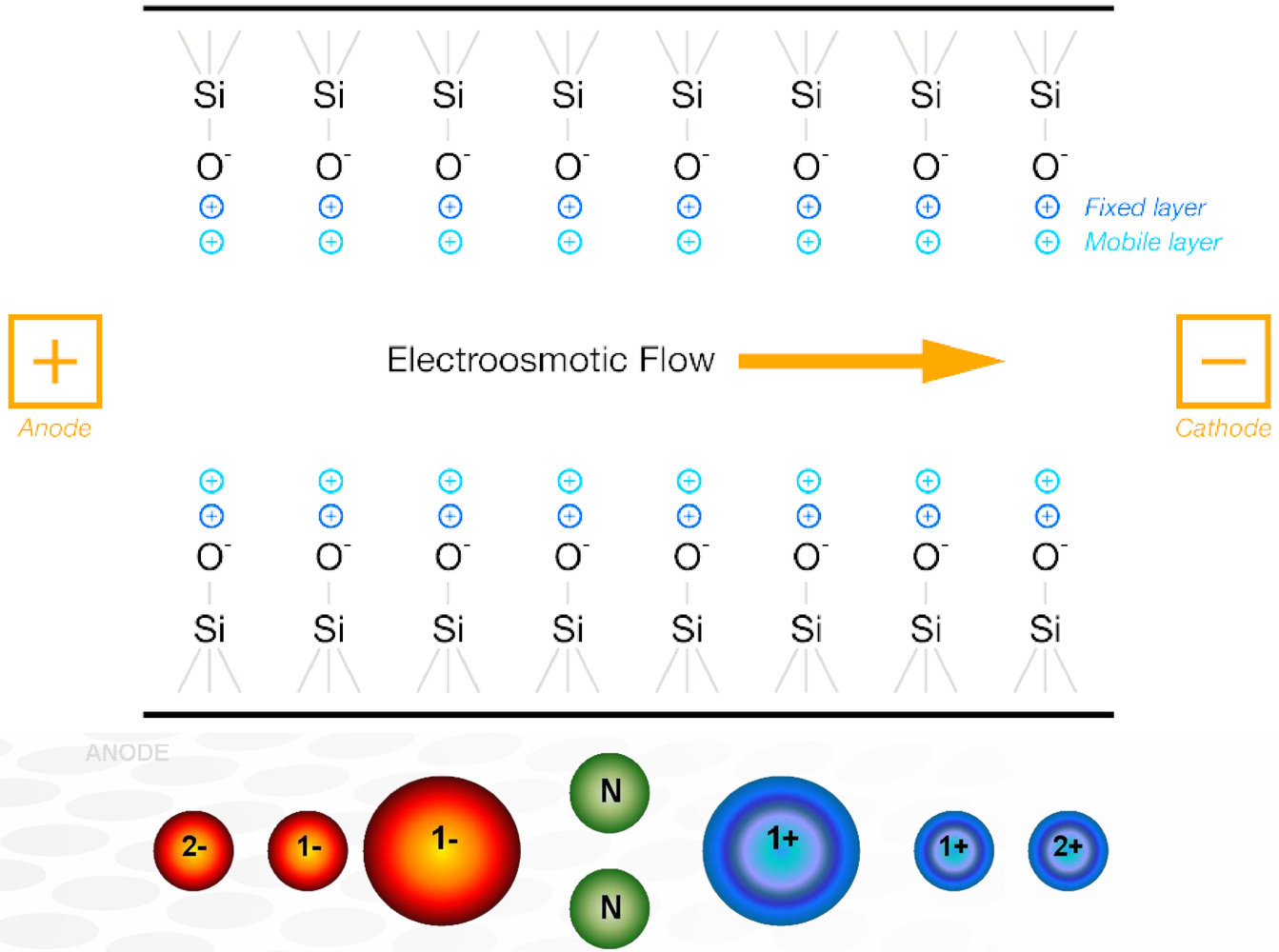
Capillary Electrophoresis

- Sample cups loaded at anode
- Vacuum applied to load capillary
- Current applied to facilitate separation
- Absorbance measured as protein passes the detector



Electro-Osmotic Flow (EOF)

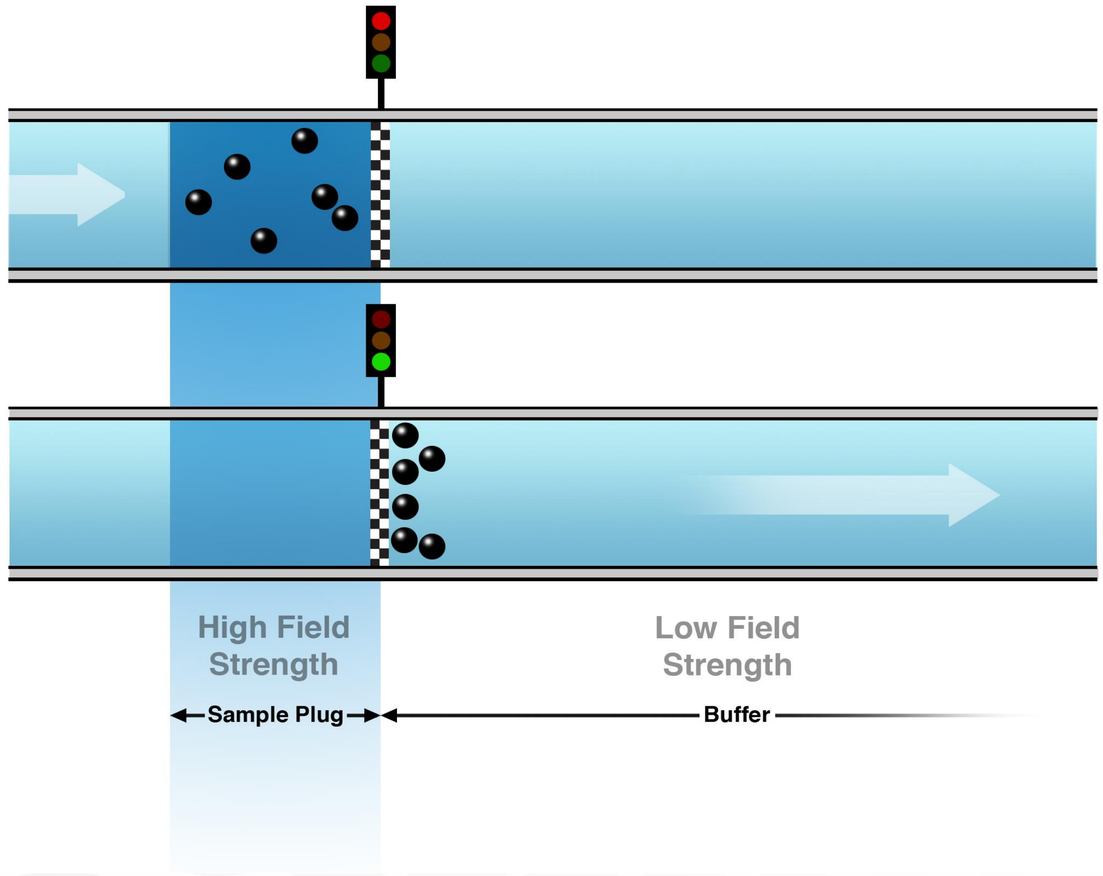
- Silica capillaries have negative charge
- Run buffer coats capillary neutralising charge
- Remaining buffer flows through capillary



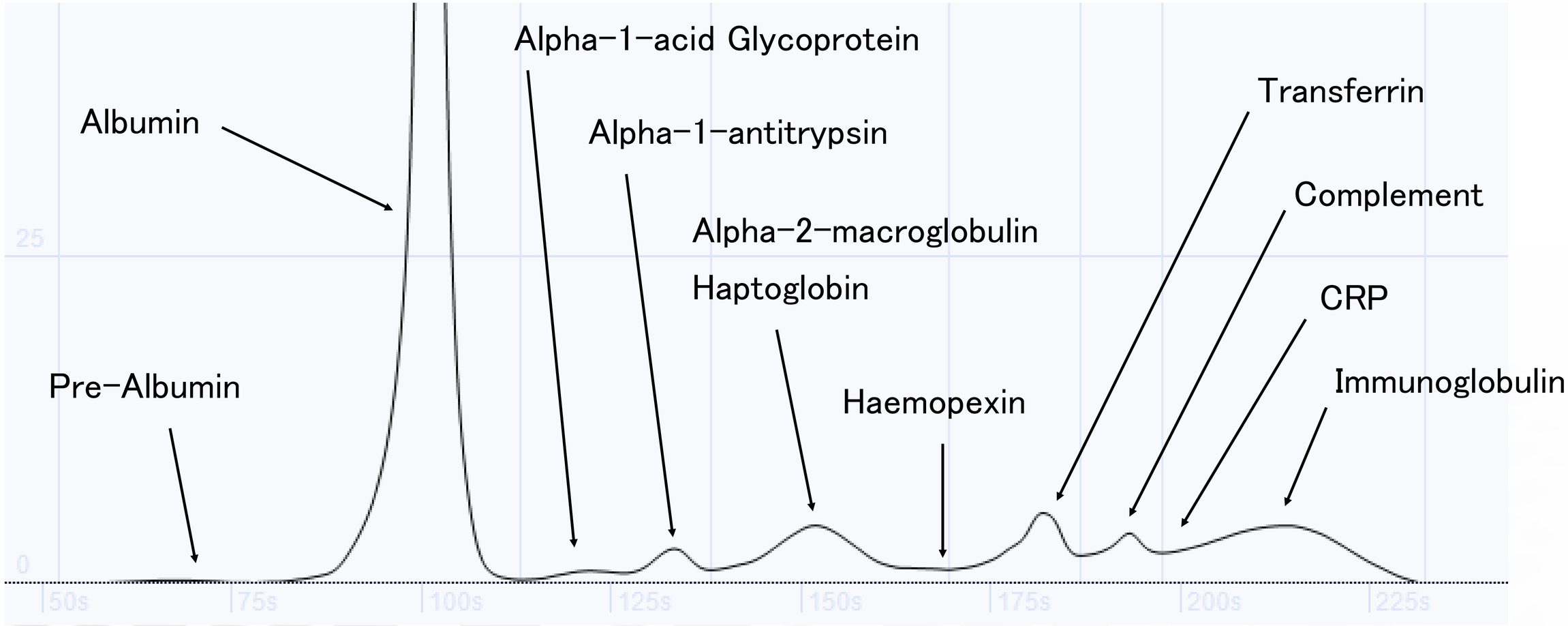
Sample Stacking

- Differences in protein mobility between diluent and buffer stack protein prior to separation

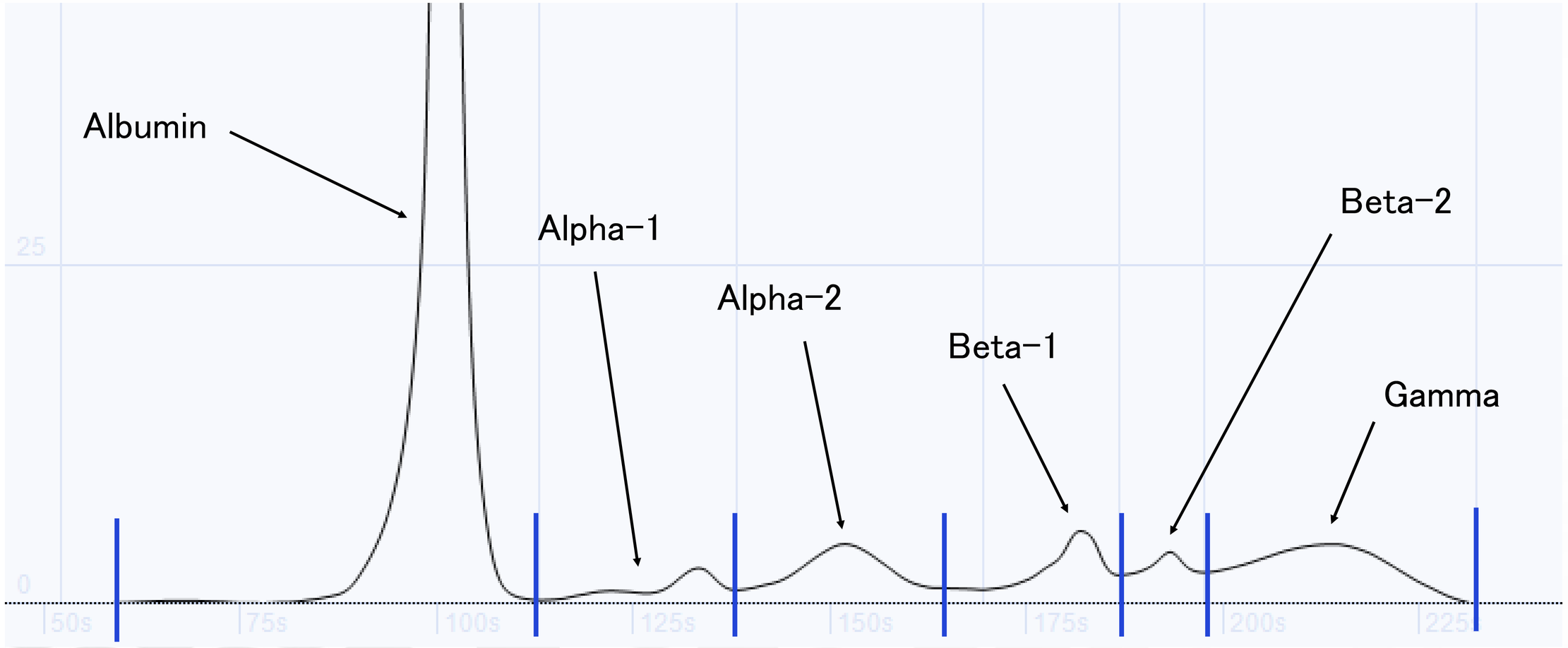
- Diluent – fast mobility
- Run buffer – slow mobility



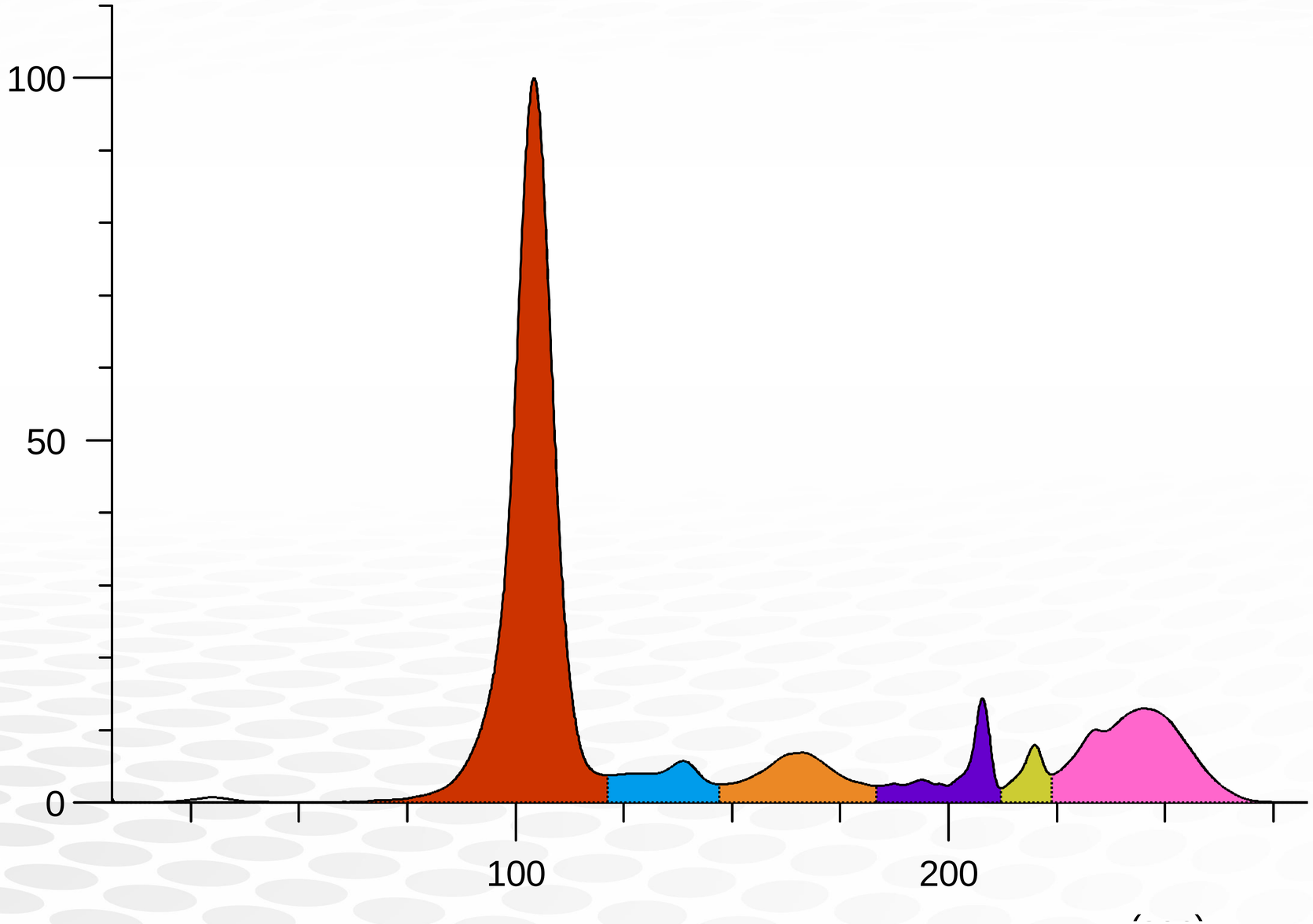
Serum Protein Electropherogram



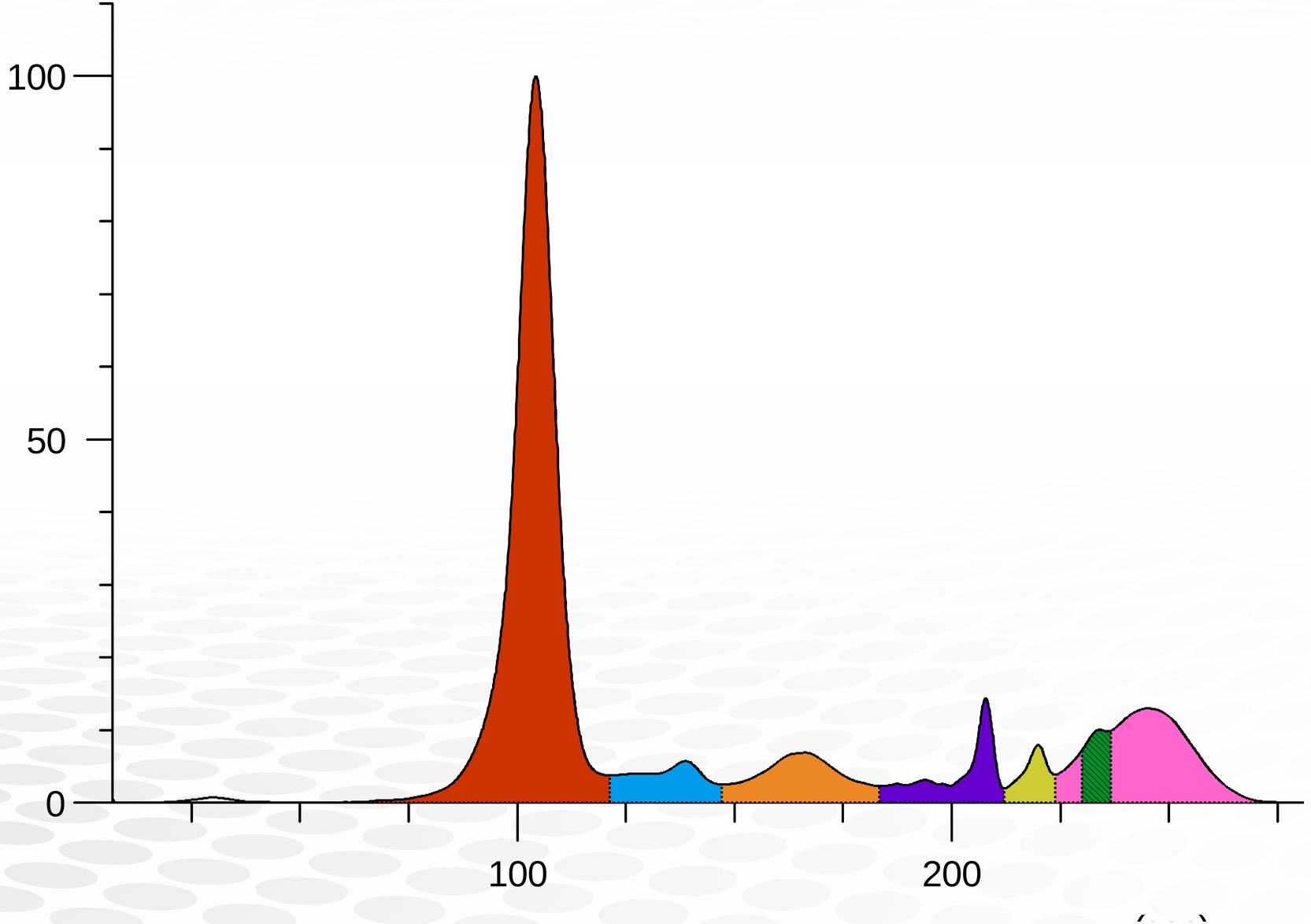
Six Band Serum Protein Trace



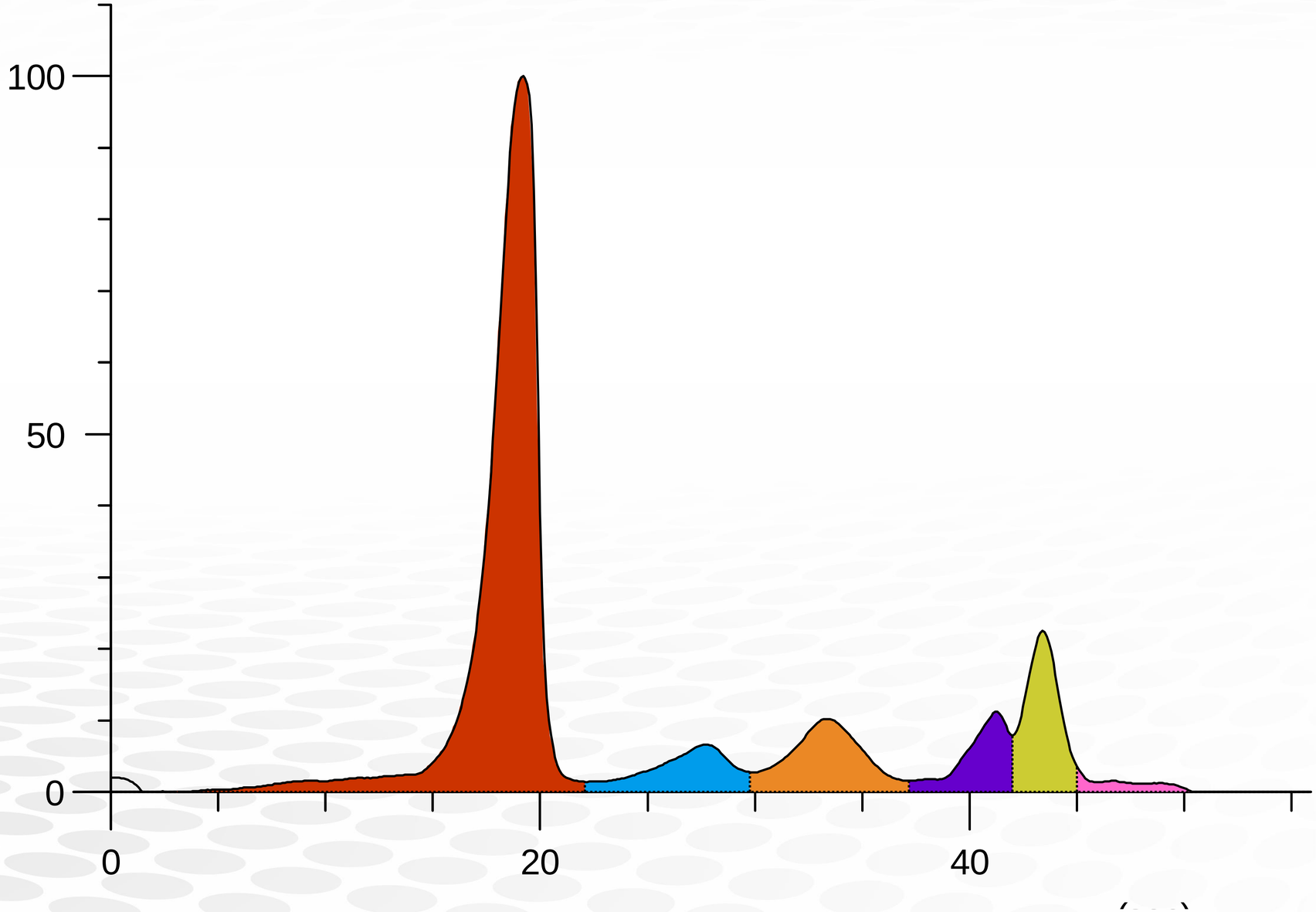
Additional Peak



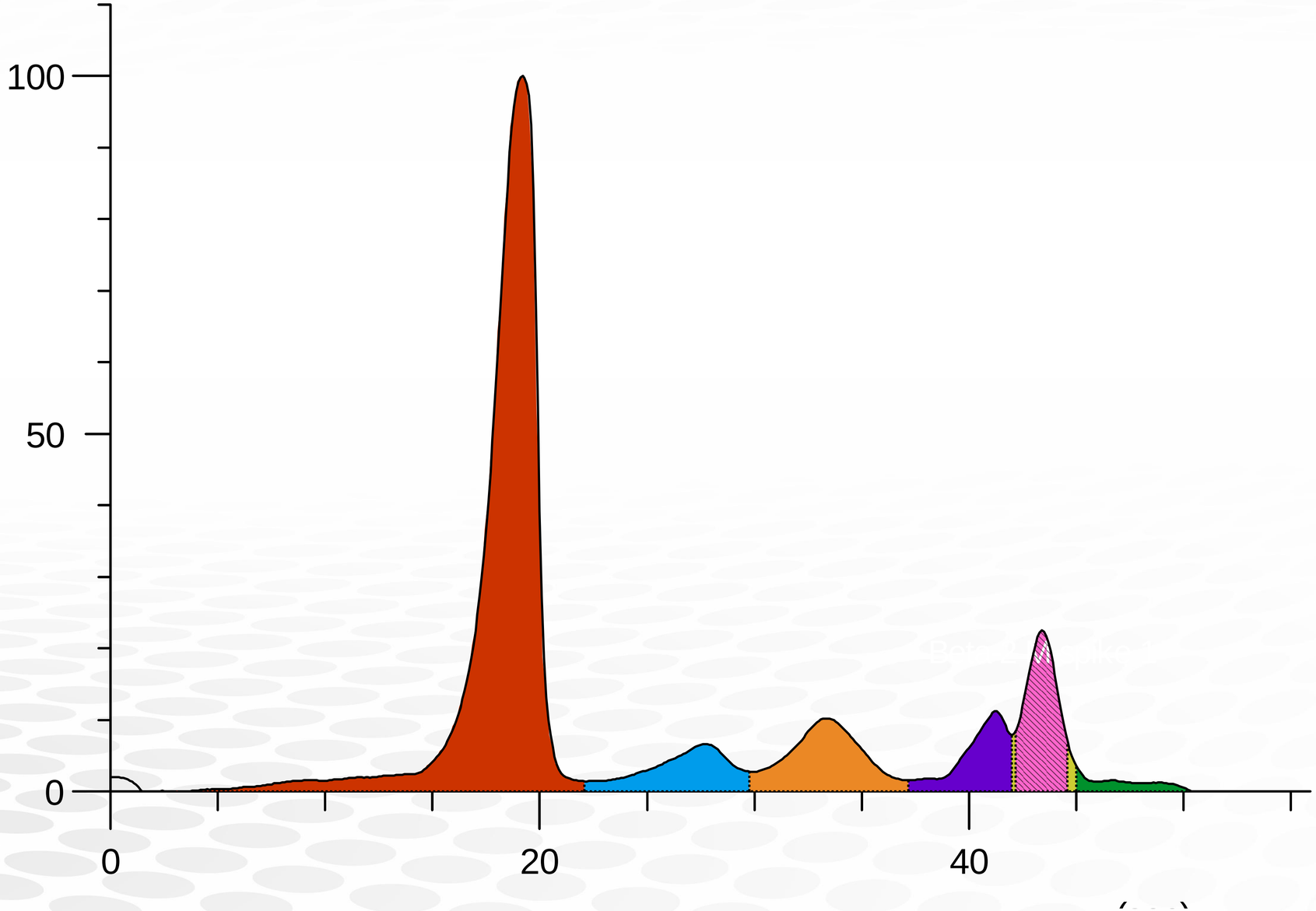
Additional Peak



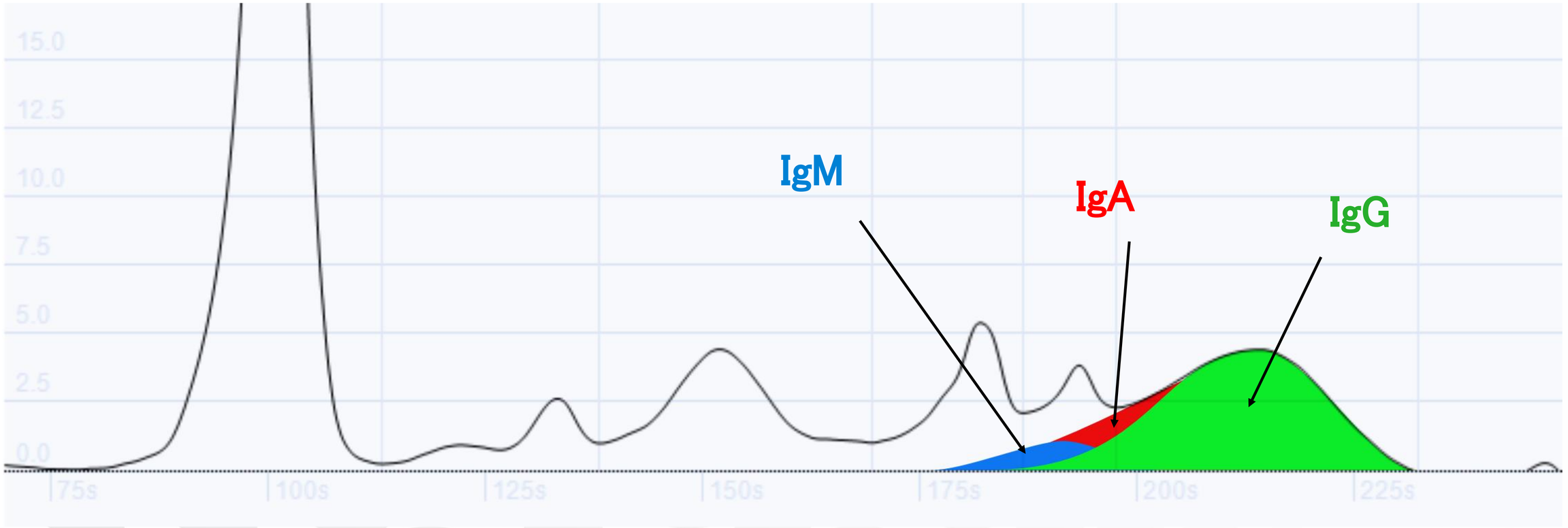
Relative Peak Percentages



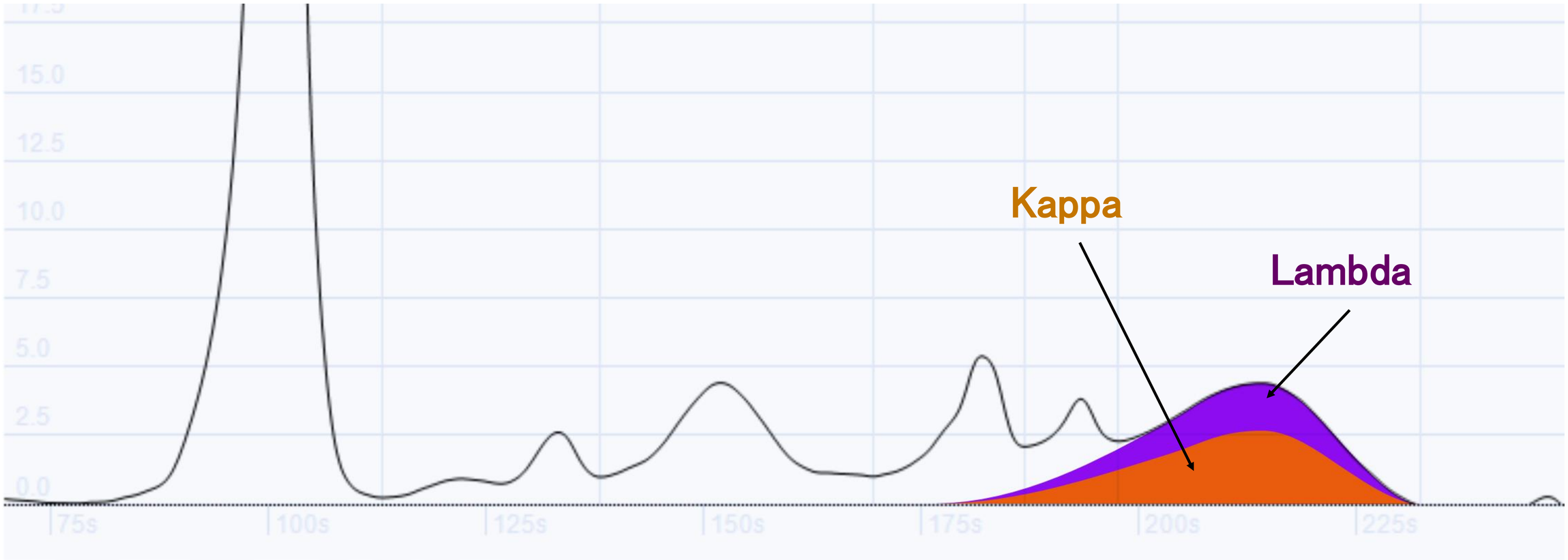
Relative Peak Percentages



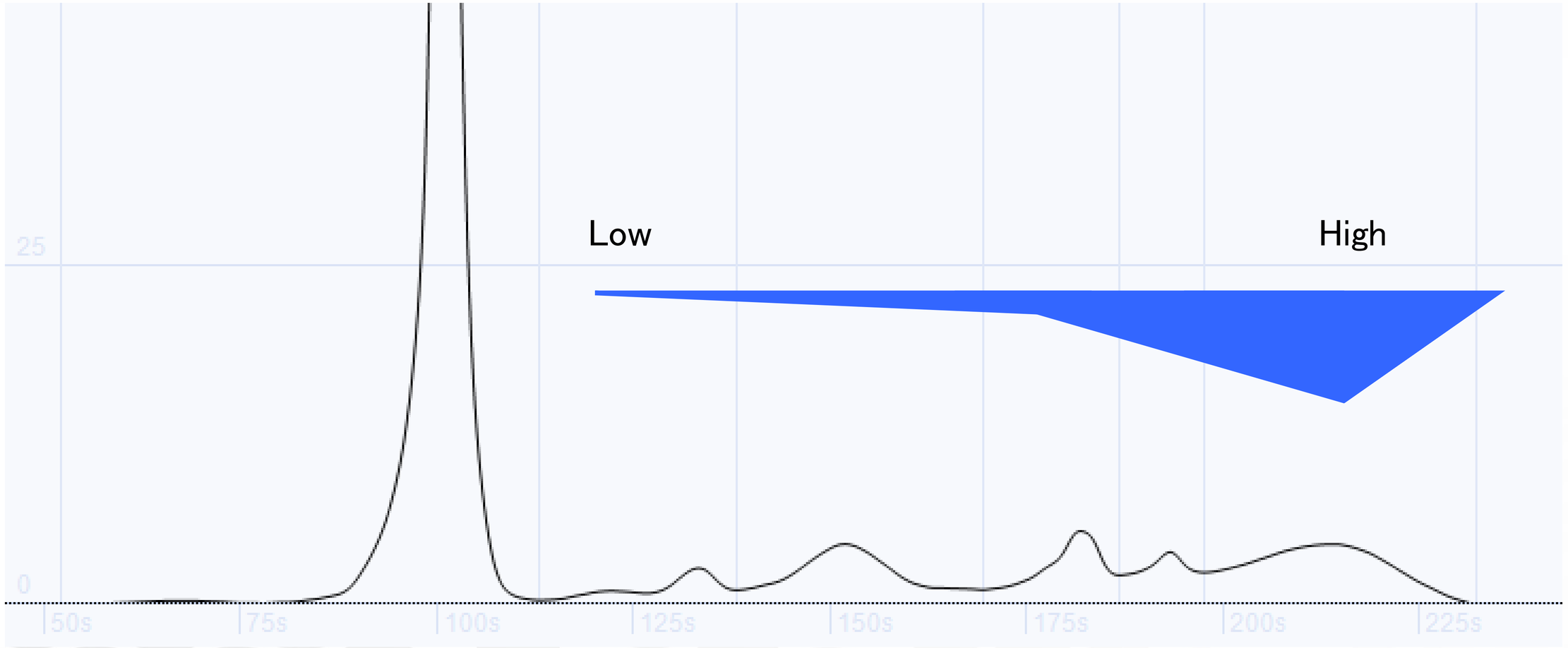
Immunoglobulin Migration – Heavy Chains



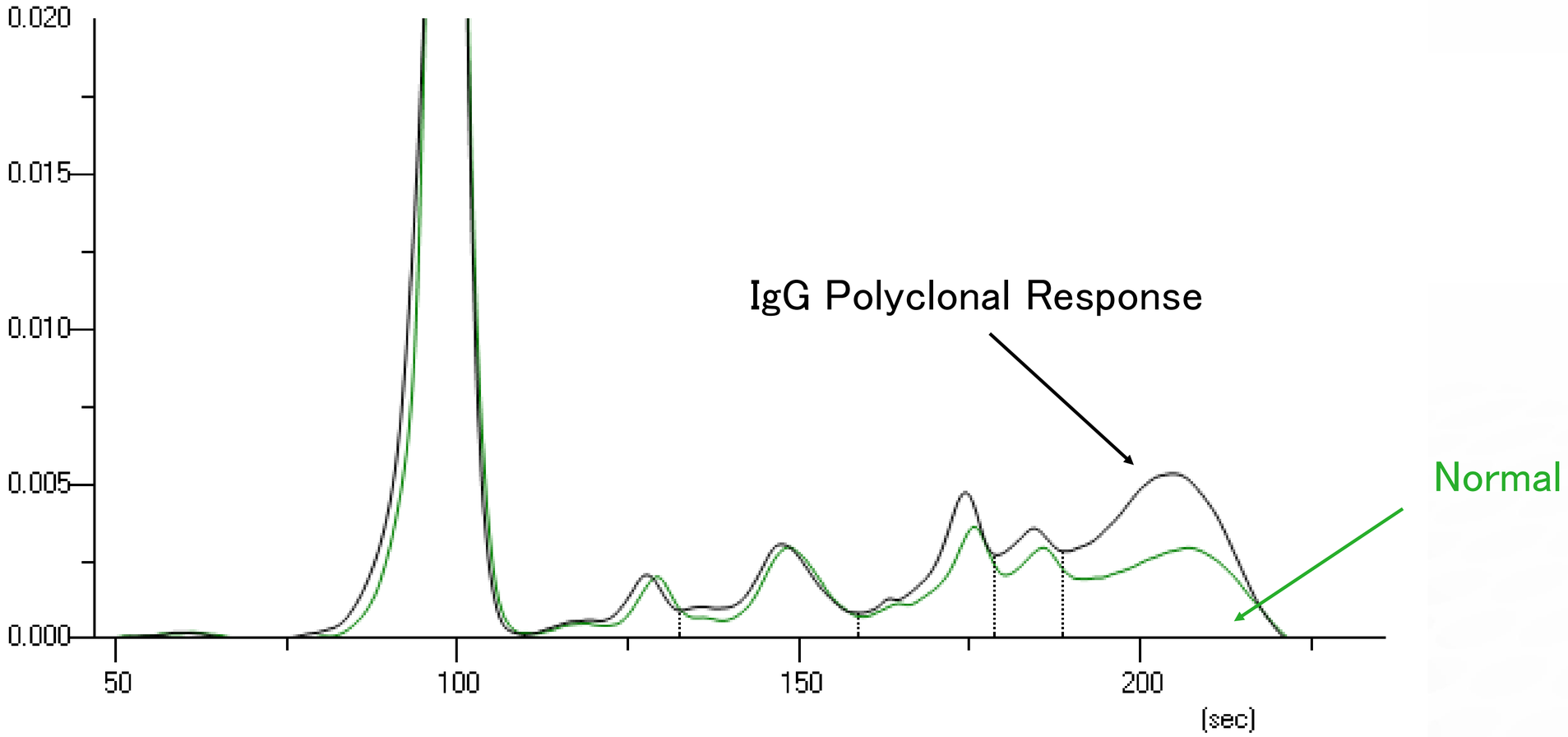
Immunoglobulin Migration – Light Chains



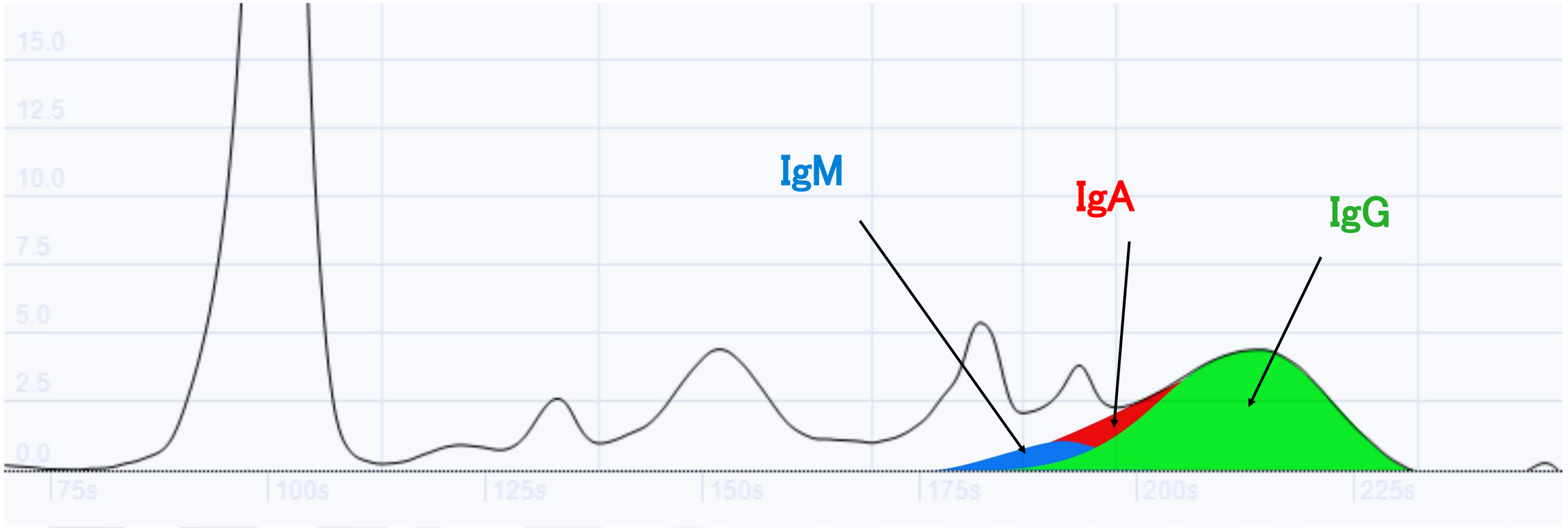
Monoclonal Migration – Relative Incidence



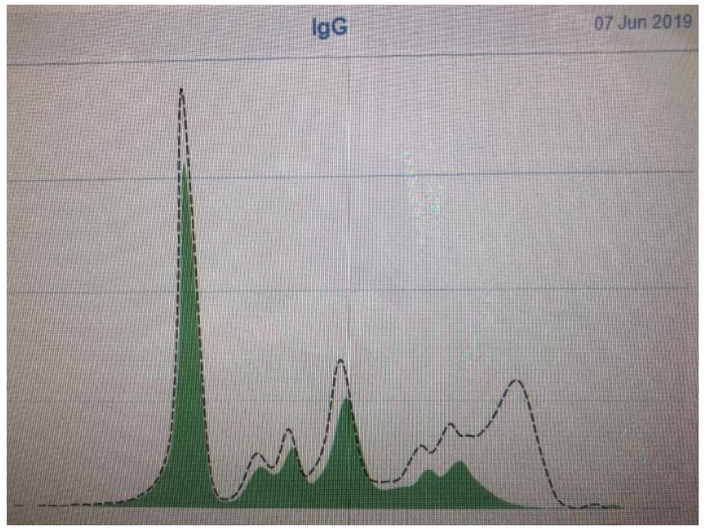
Polyclonal Response

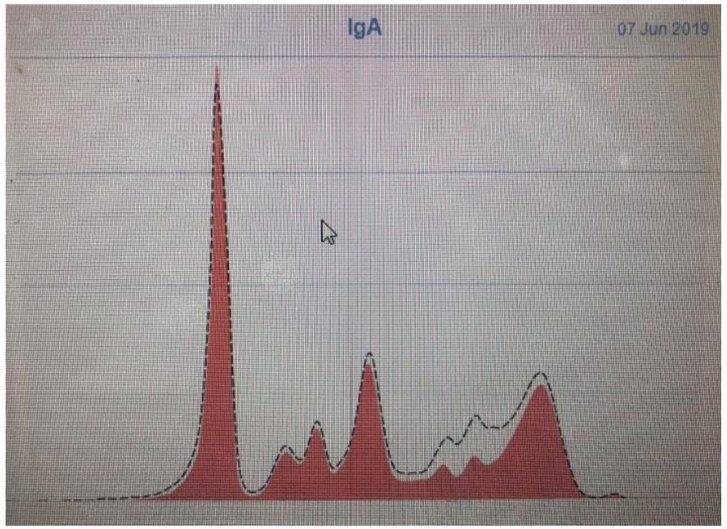


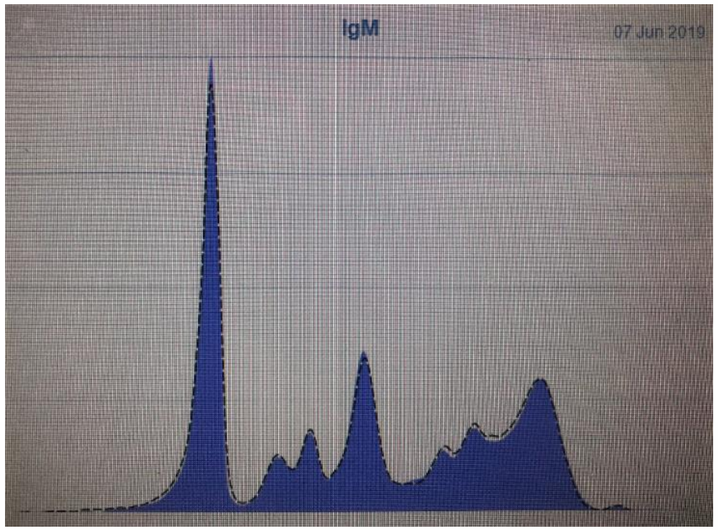
Polyclonal Response – Clone Specific

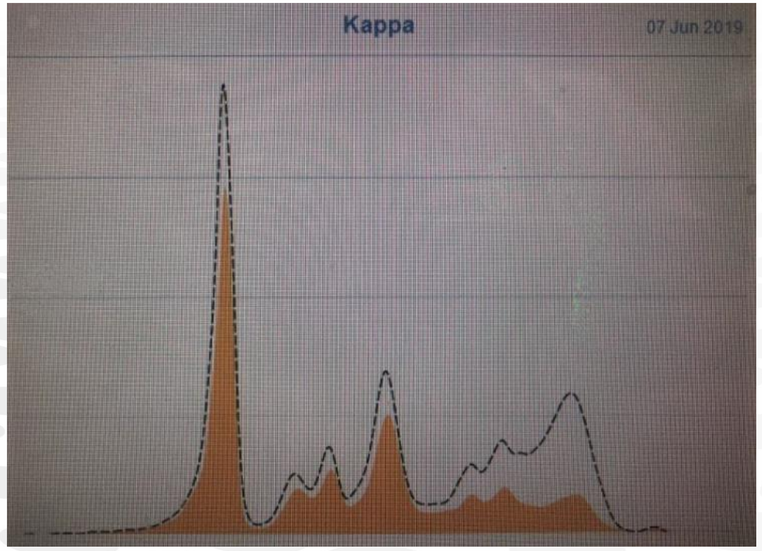


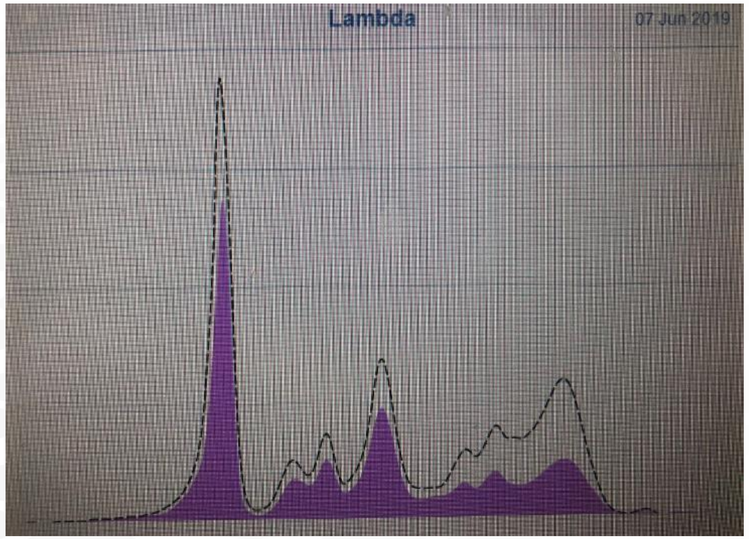
Polyclonal Response – IgG and IgA Response





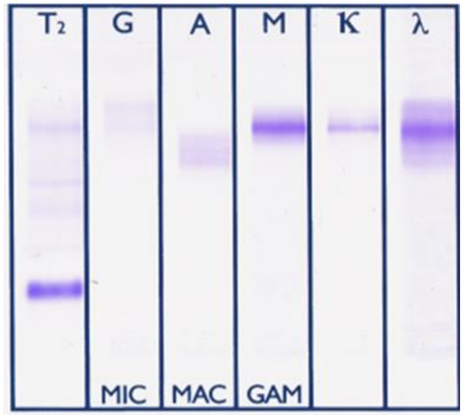




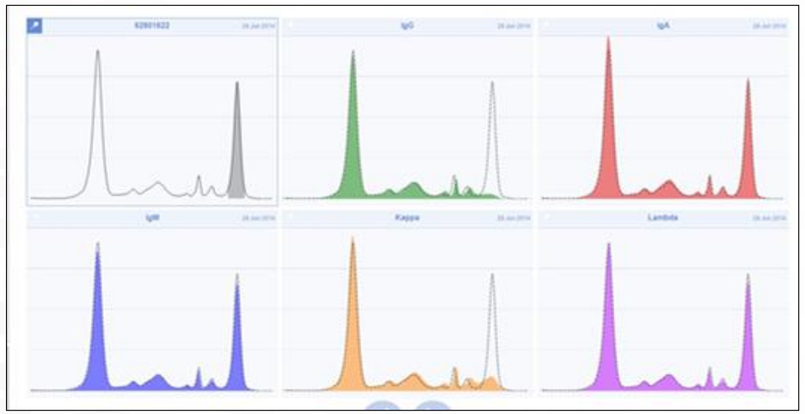


Immunotyping Monoclonal Antibody

- Confirm the presence of monoclonal
- Immunotype monoclonal

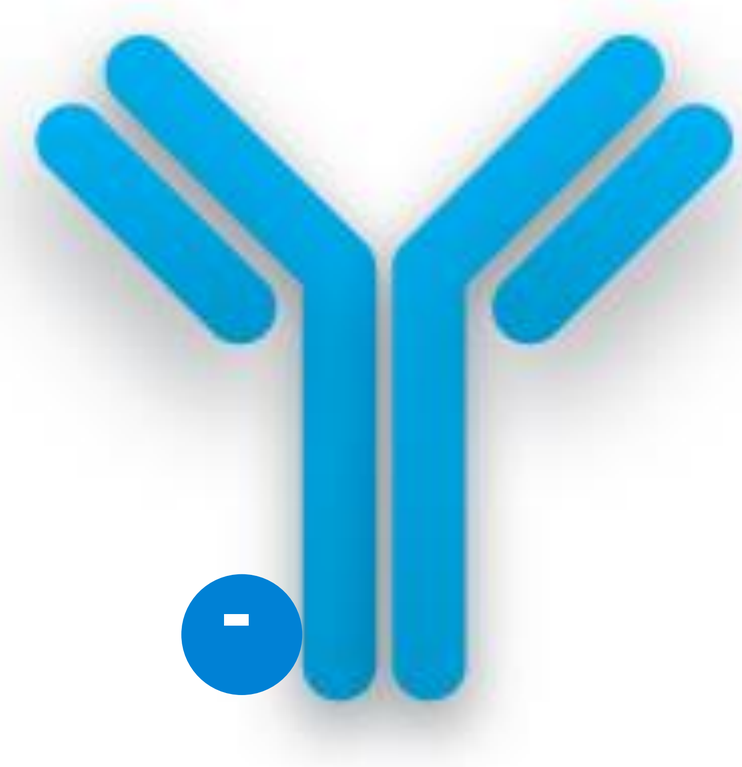


- Two methodologies
- CZE immunodisplacement
- Gel immunofixation
- Both have pros and cons



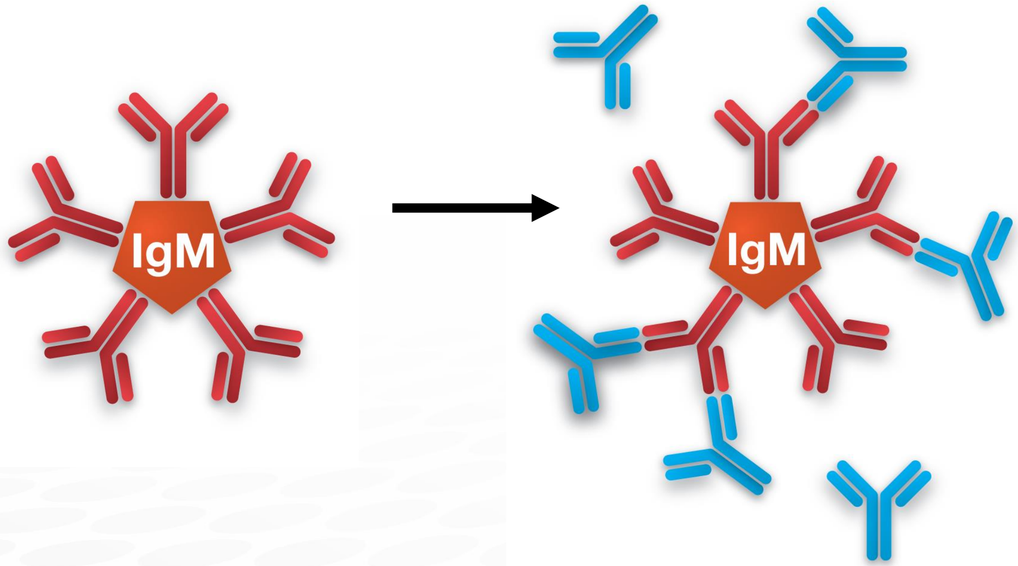
Immunodisplacement Antisera

- One antisera for each major chain
- IgG, IgA, IgM, Kappa & Lambda
- Chemically treated to add strong negative charge to antibody
- Reduces electrophoretic mobility



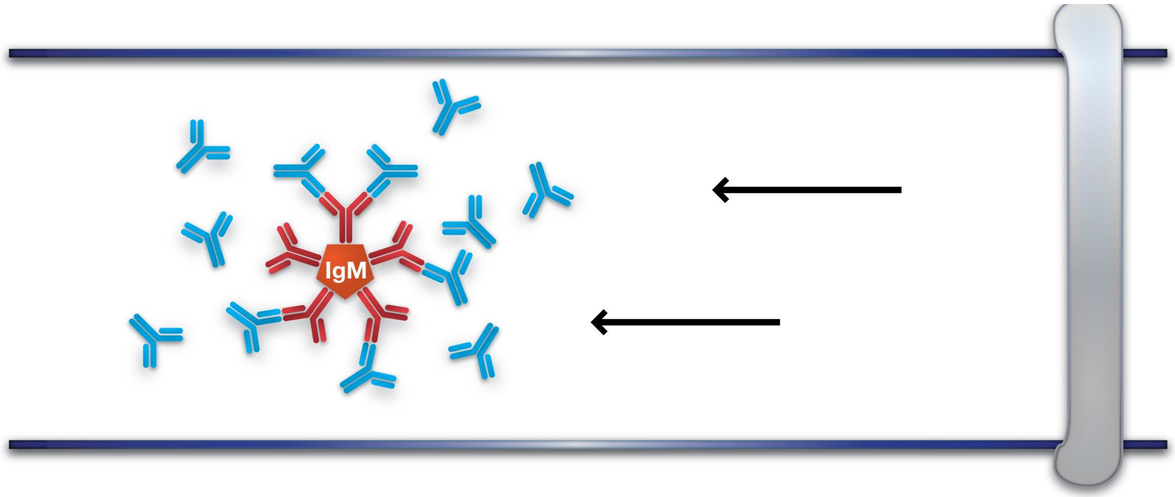
Immunodisplacement

- Antisera added to sample
- Antisera will bind all immunoglobulin of a specific immunotype
  - Monoclonal
  - Polyclonal

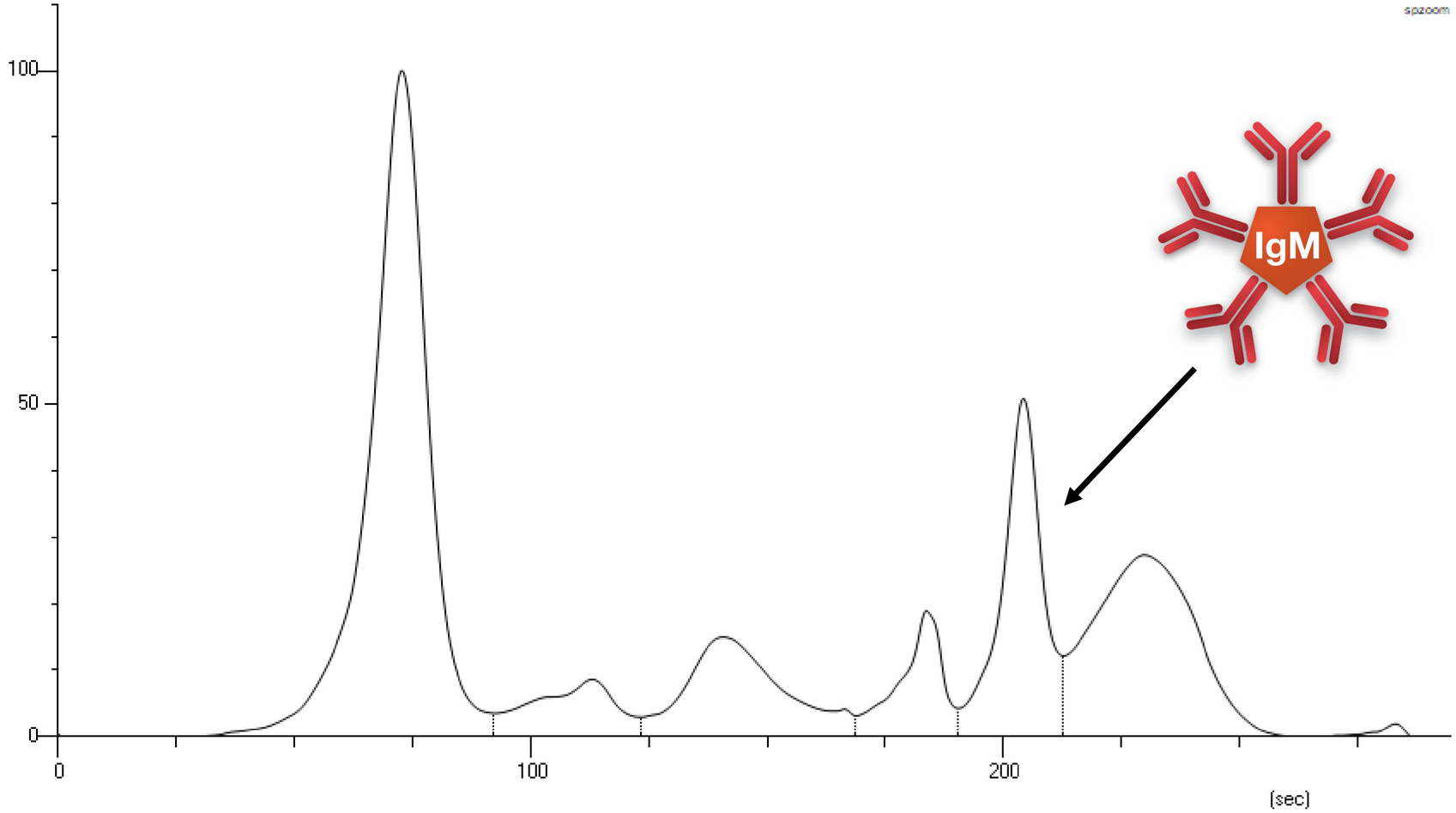


Reduced Mobility

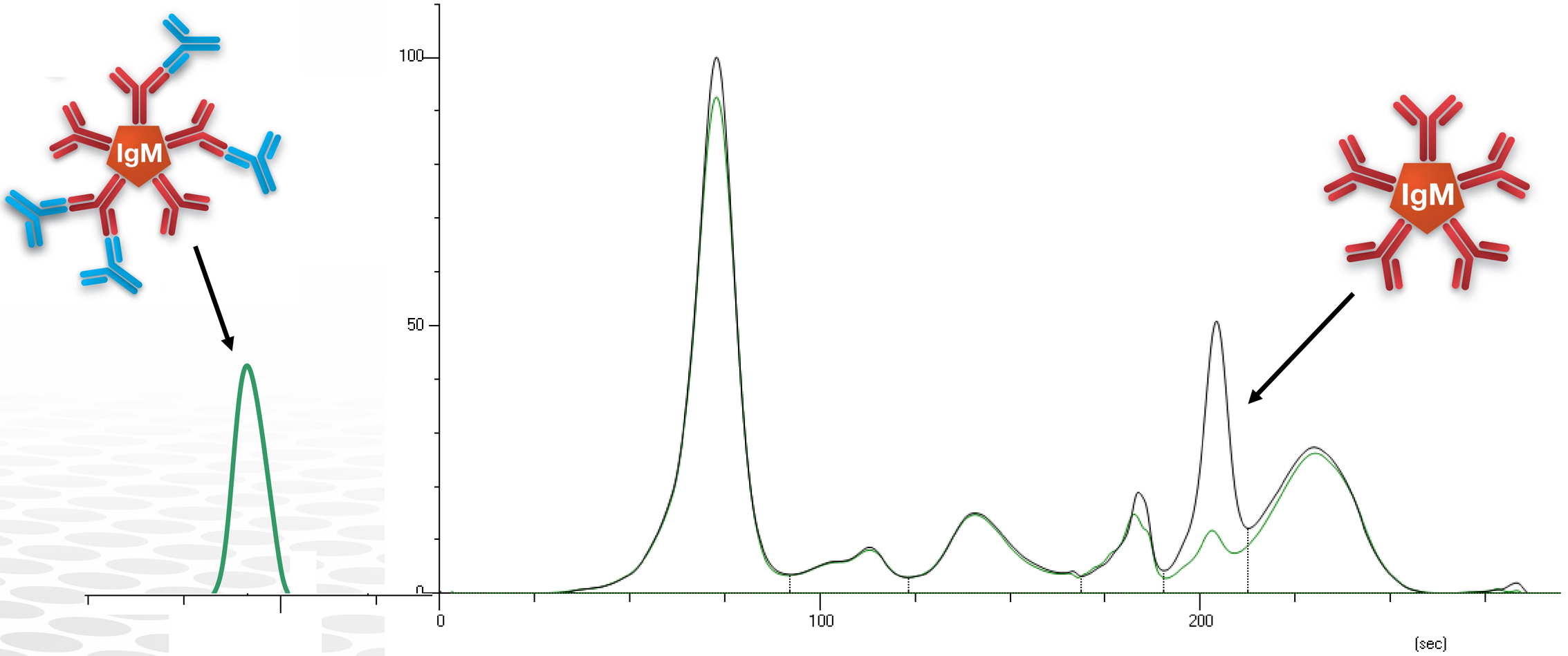
- Antisera bound immunoglobulin migrates significantly slower than unbound immunoglobulin
- Immunoglobulin removed from trace



Immunodisplacement – IgM Monoclonal

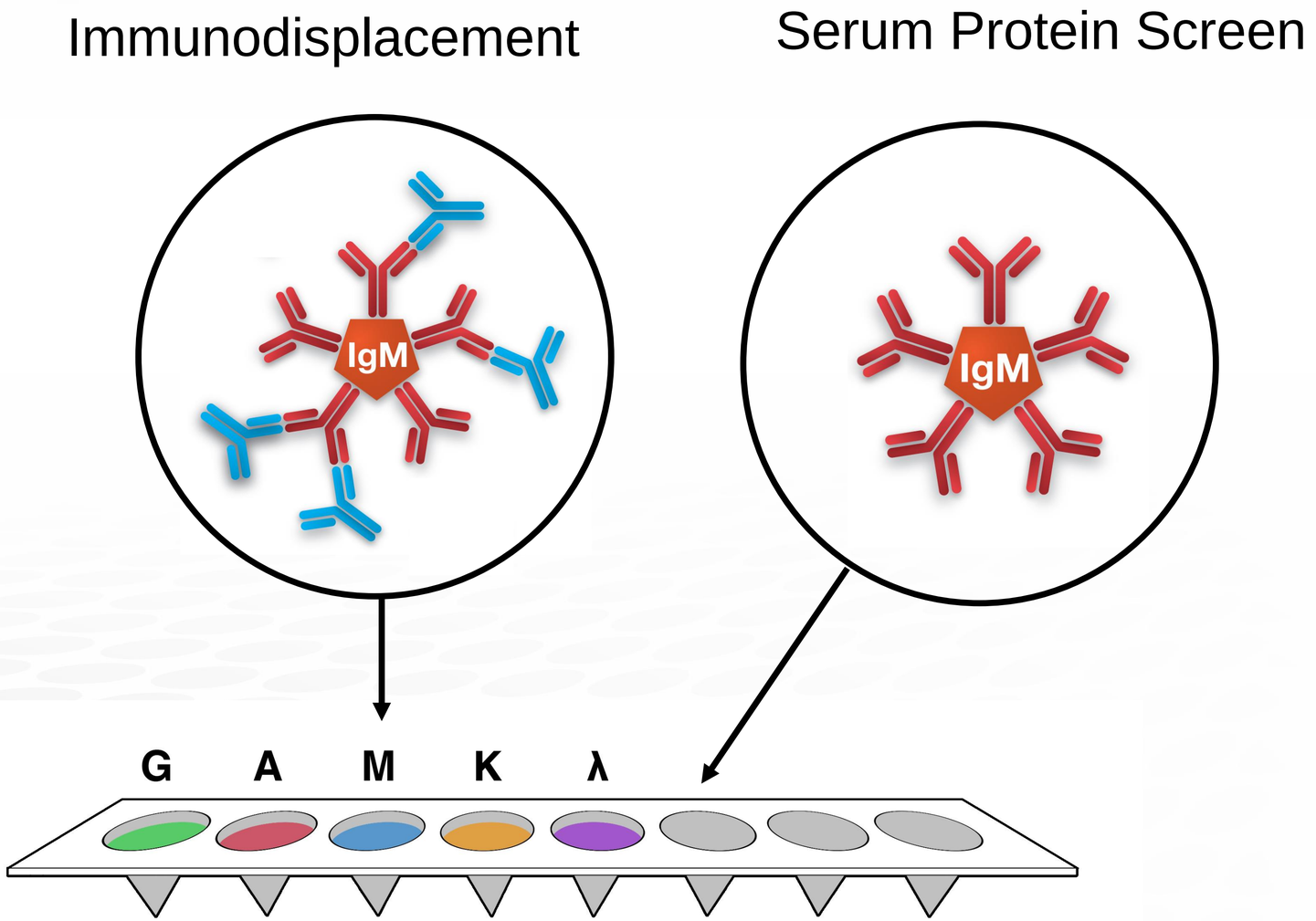


Immunodisplacement – IgM Monoclonal

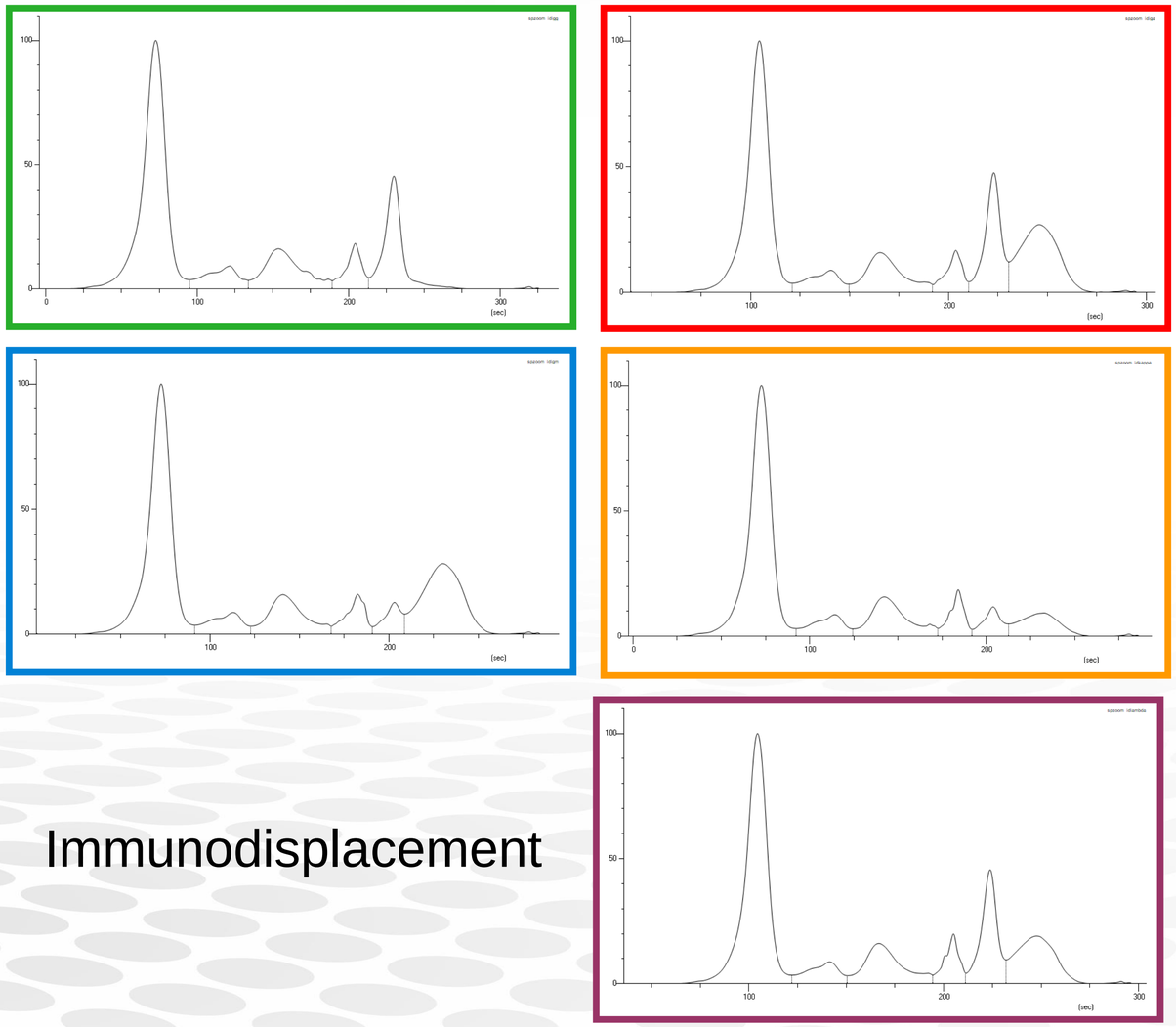


Immunodisplacement

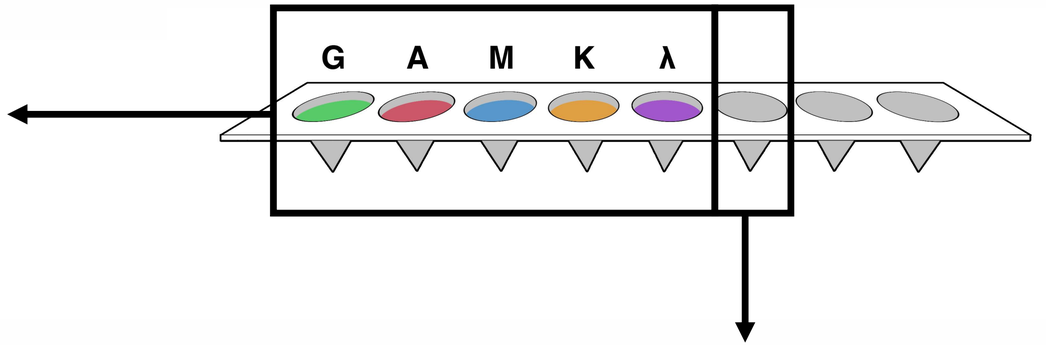
- Five antisera required
  - IgG, IgA, IgM
  - Kappa & Lambda
- Five capillaries
- Linked to original screening trace

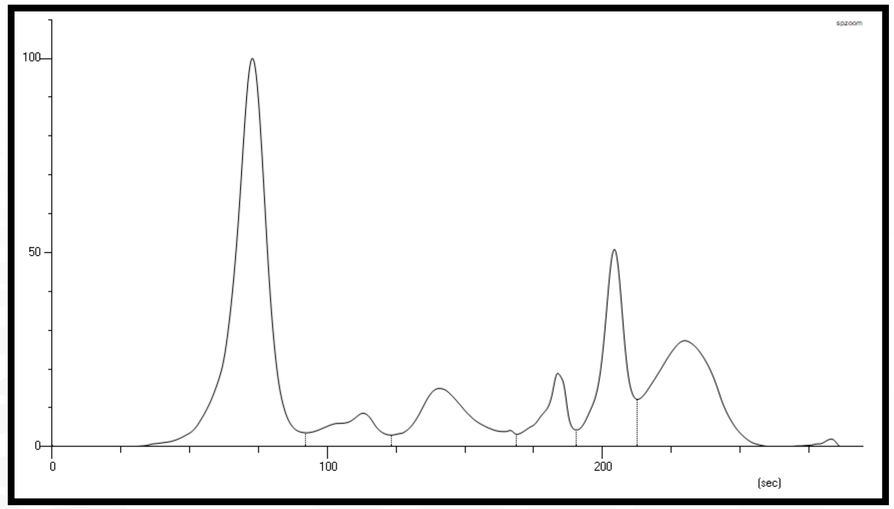


Immunodisplacement



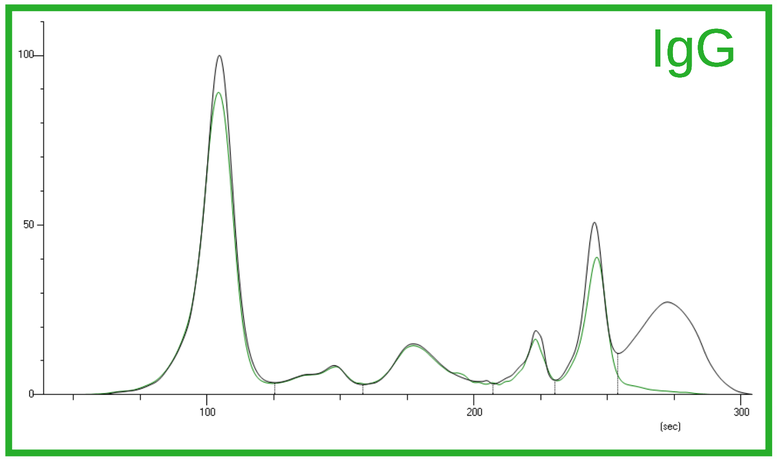
Immunodisplacement

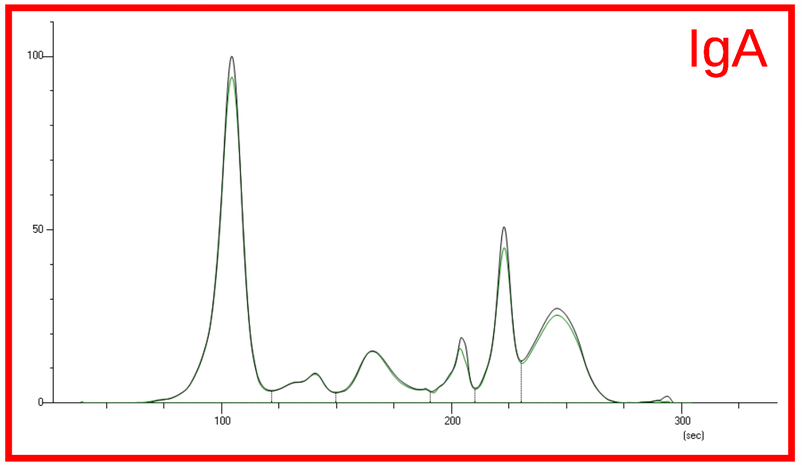


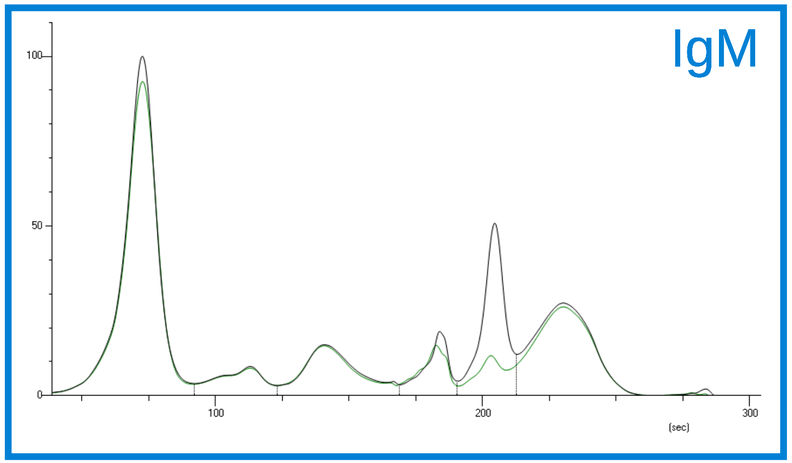


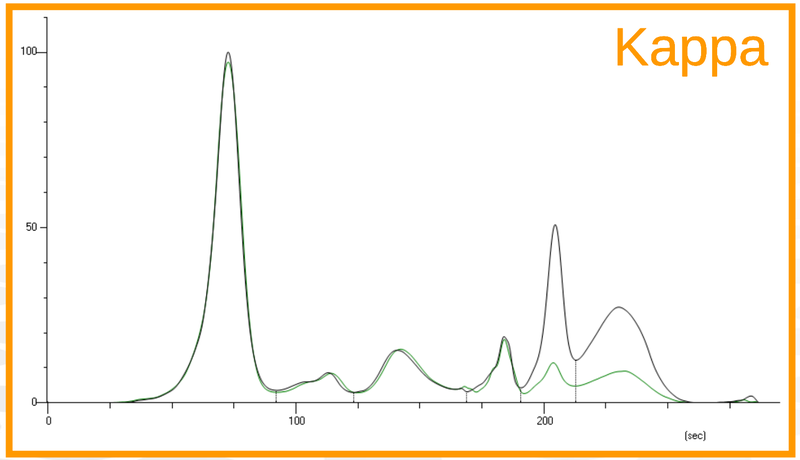
Serum Protein Screen

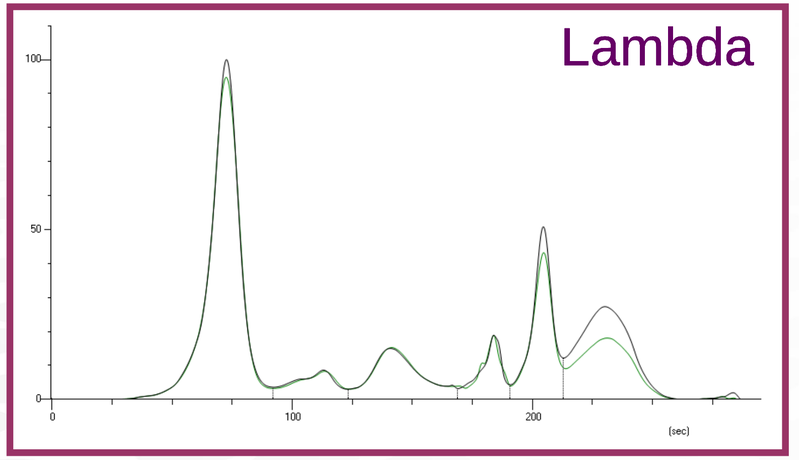
Immunodisplacement Overlay



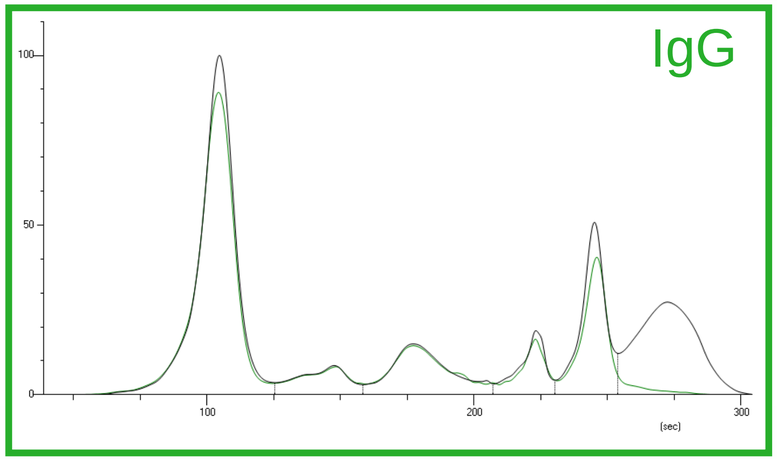


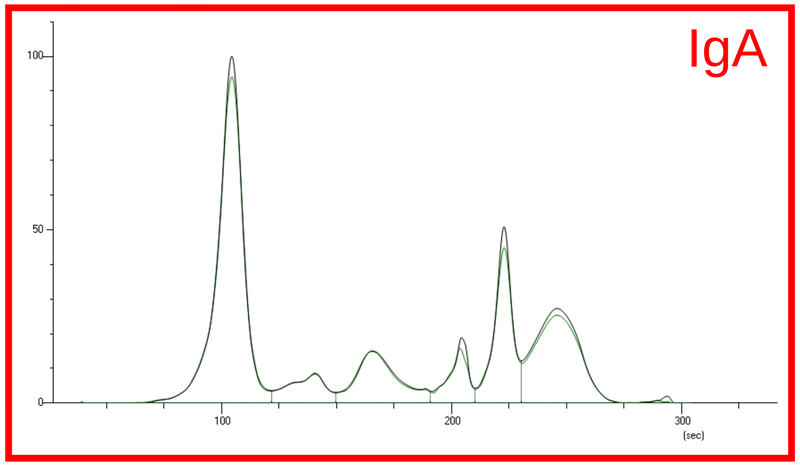


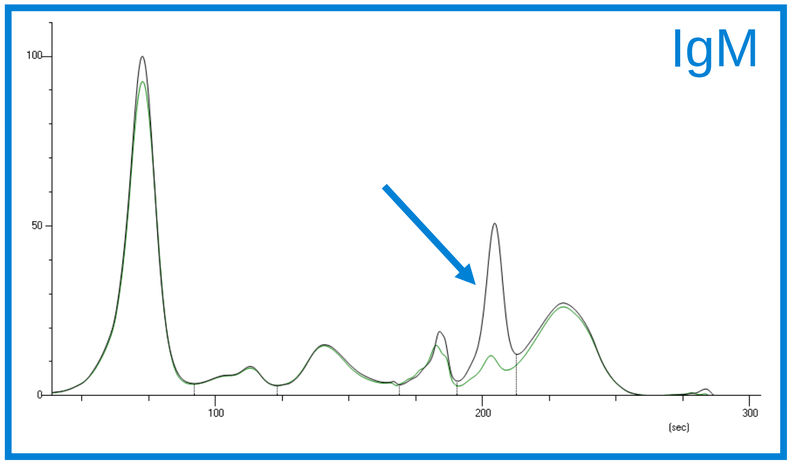


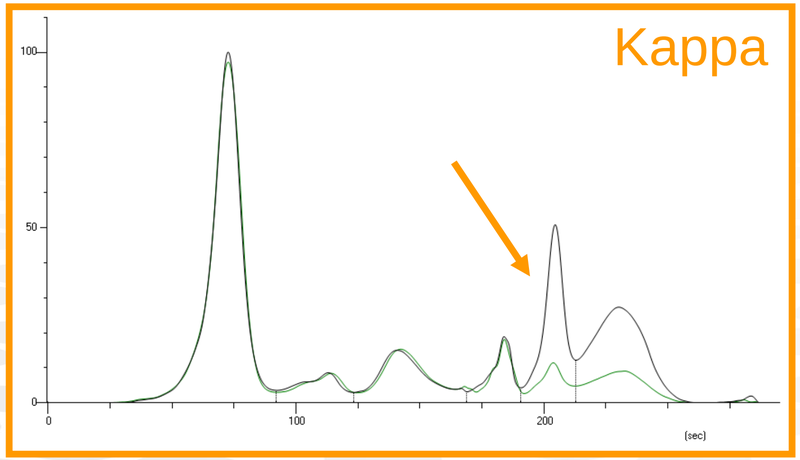


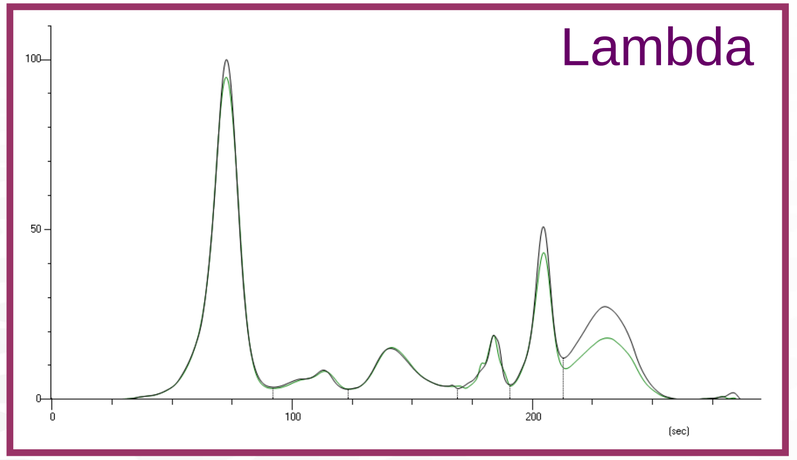
IgM Kappa Monoclonal



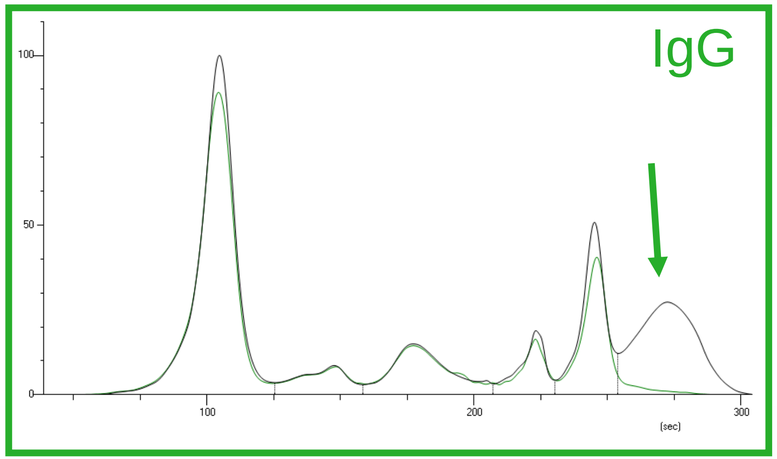


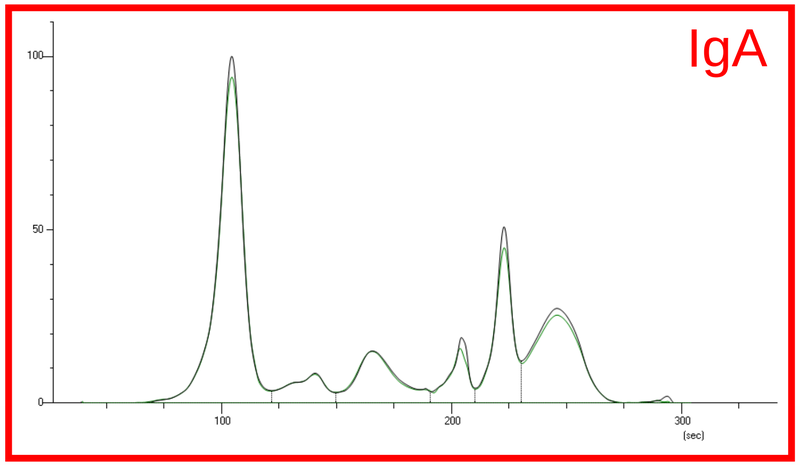


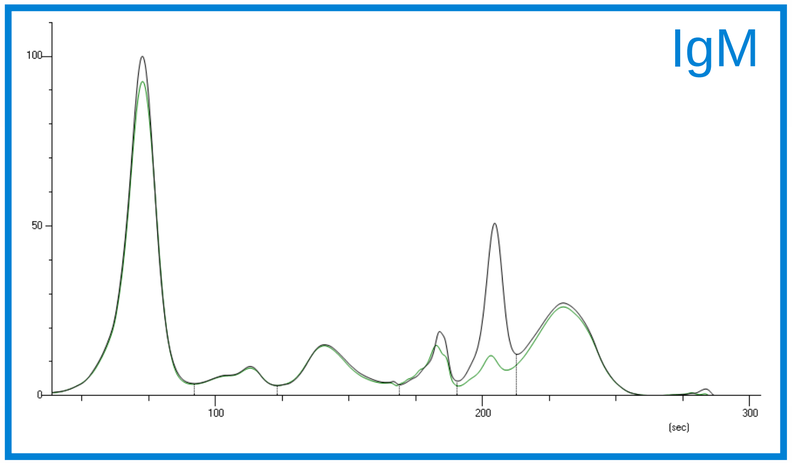


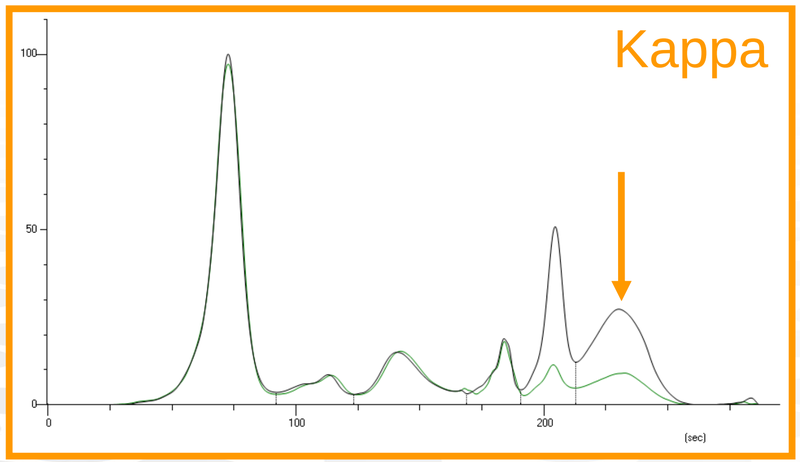


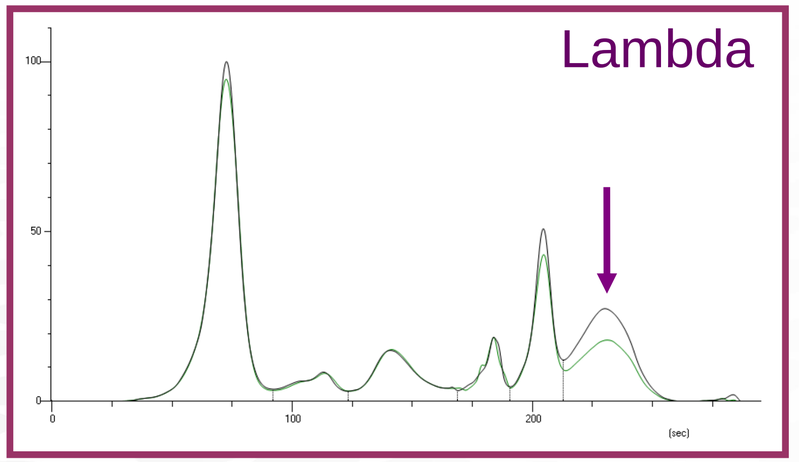
Polyclonal Removal





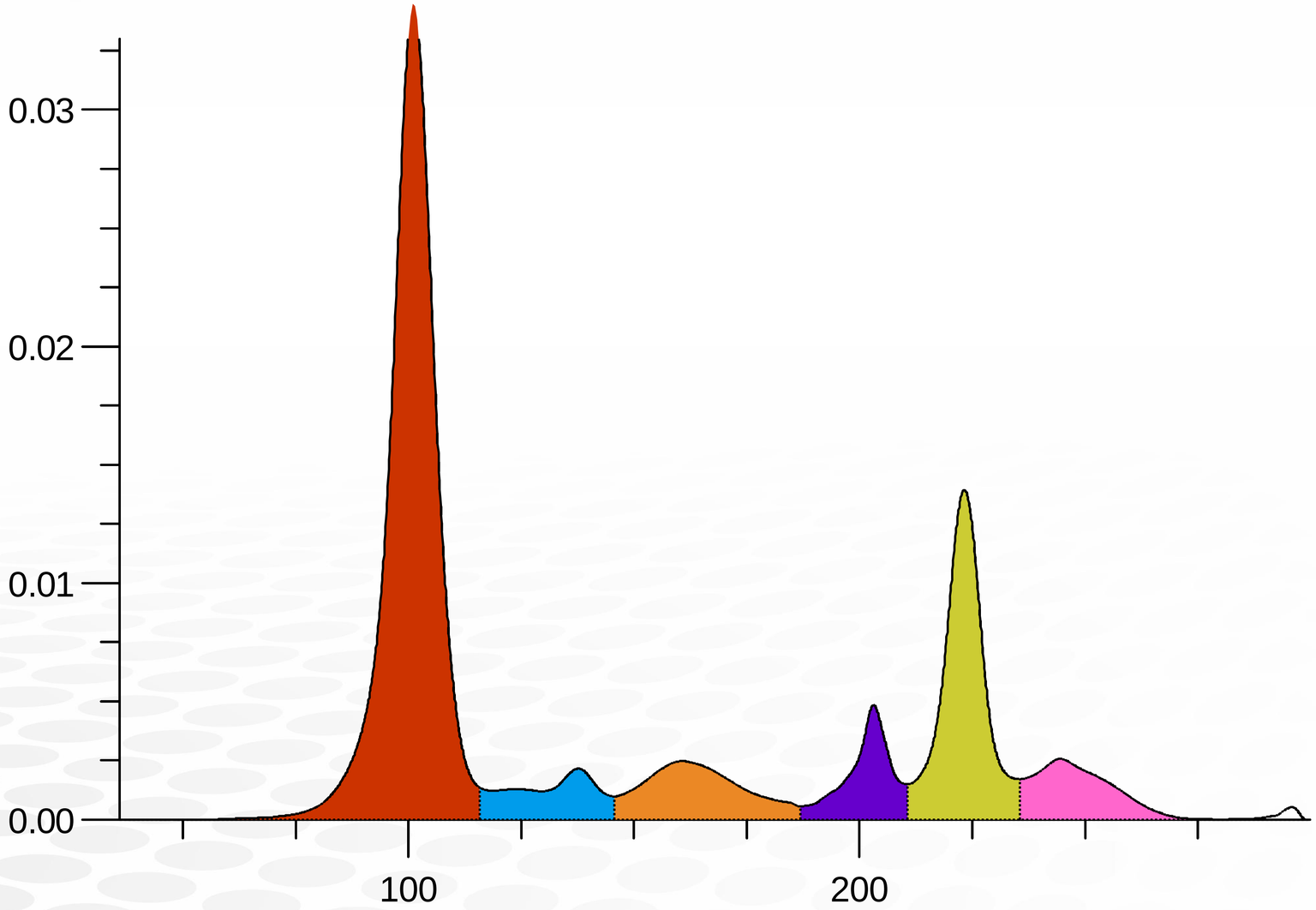






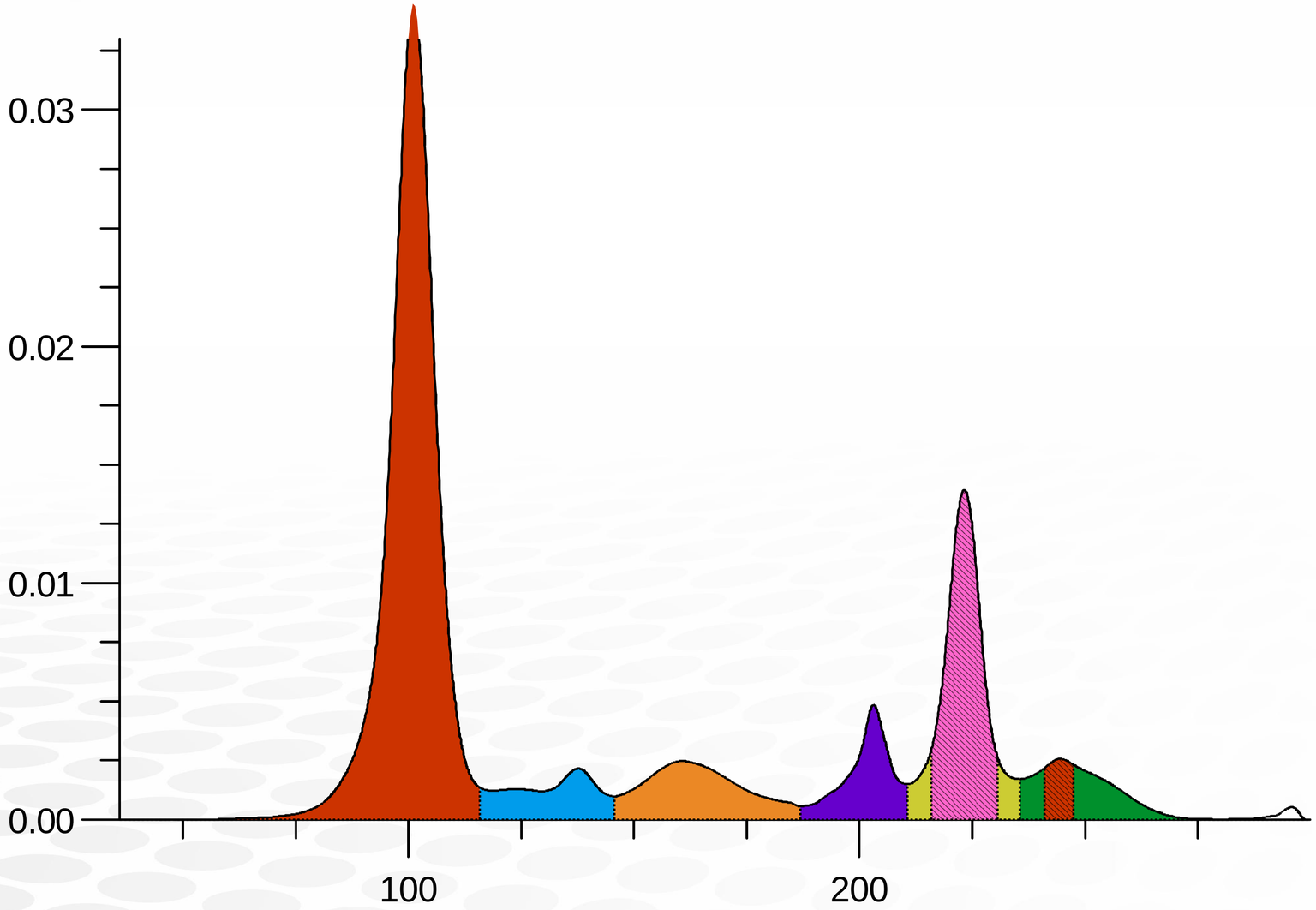
Example Case Study

- Serum protein screening trace



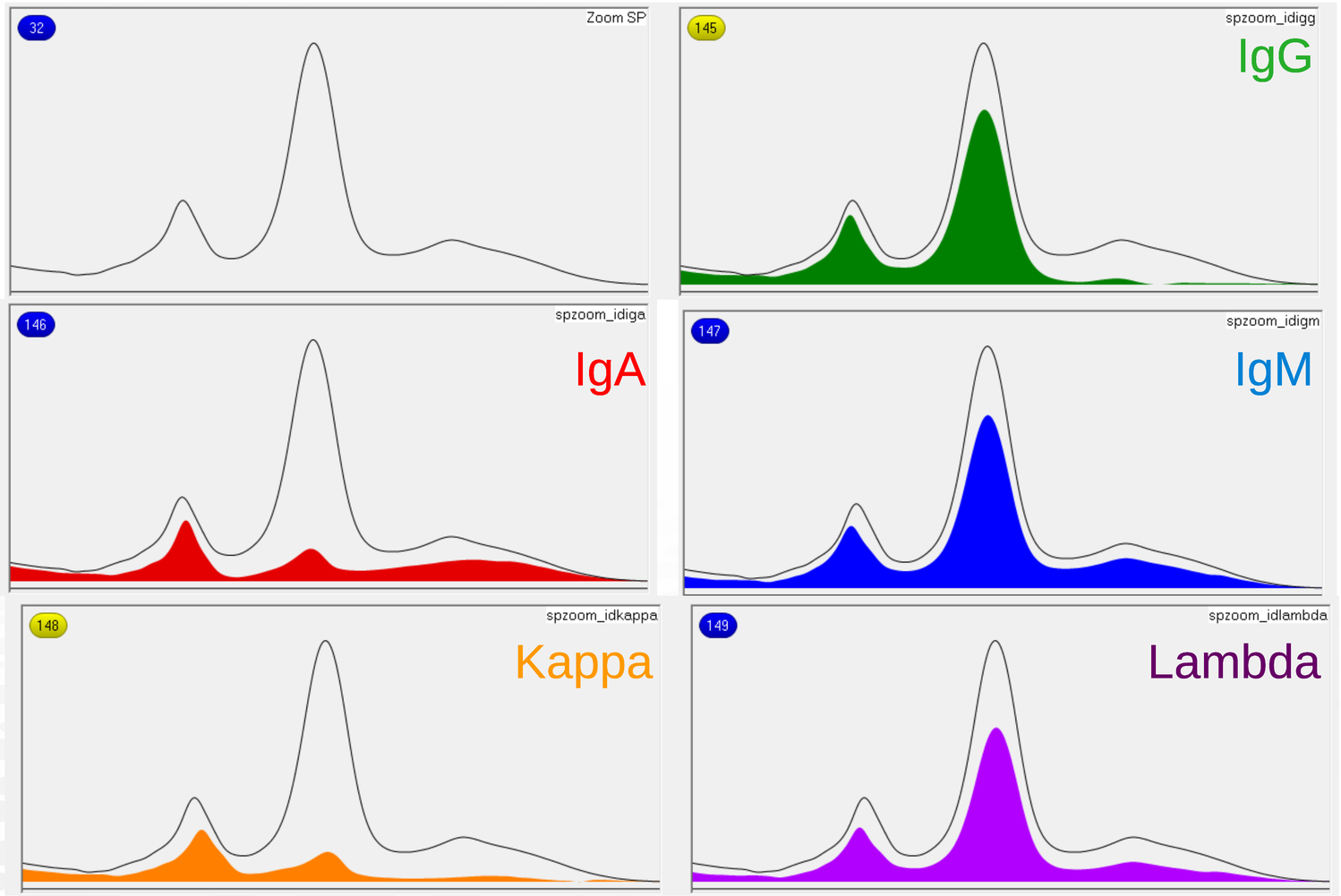
Example Case Study

- Two monoclonal peaks
- Beta-2 monoclonal
- Gamma Monoclonal



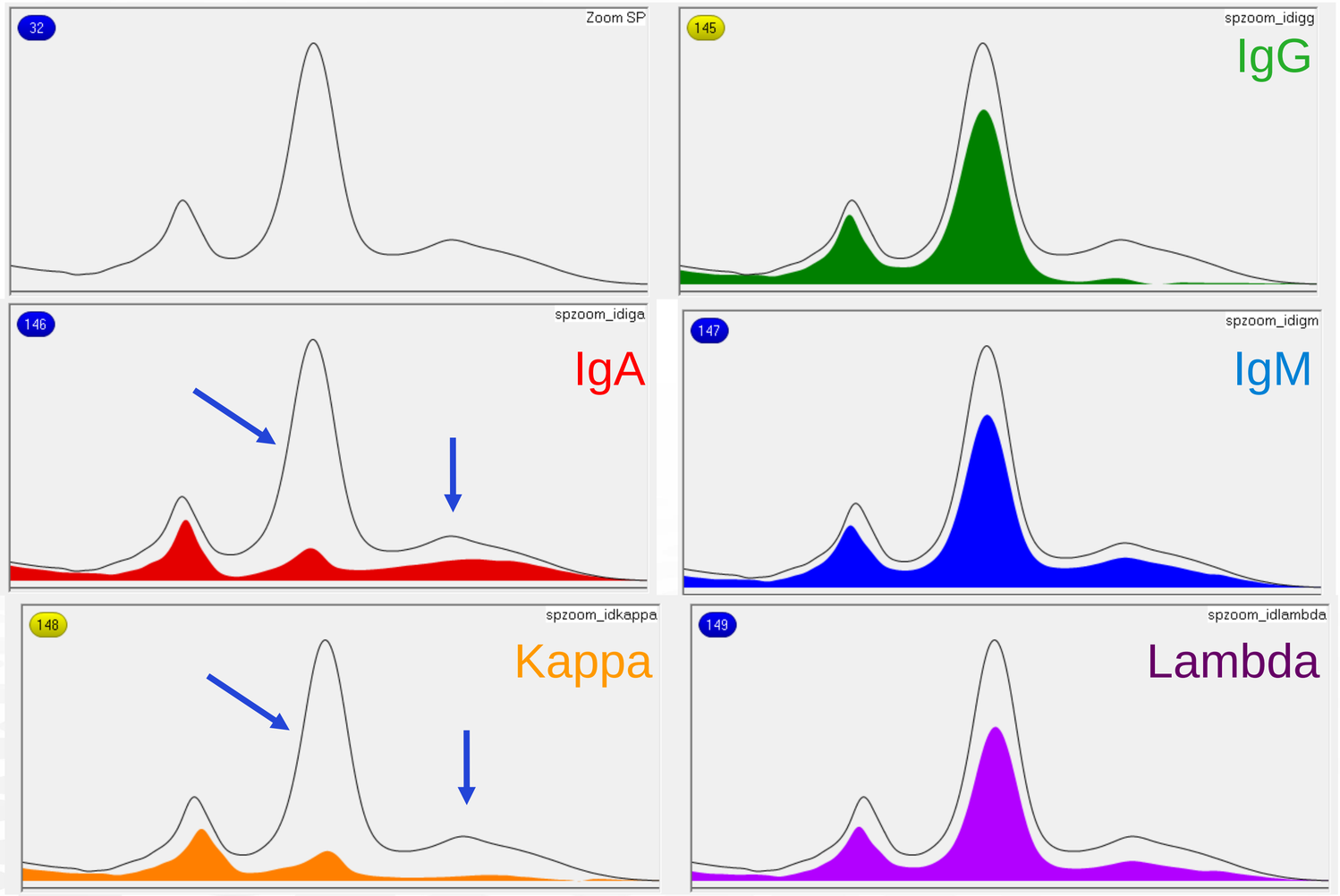
Example Case Study - Immunodisplacement

- Two monoclonal peaks
- Beta-2 monoclonal
- Gamma Monoclonal



Example Case Study - Immunodisplacement

- Two monoclonal peaks
- IgA Kappa x2

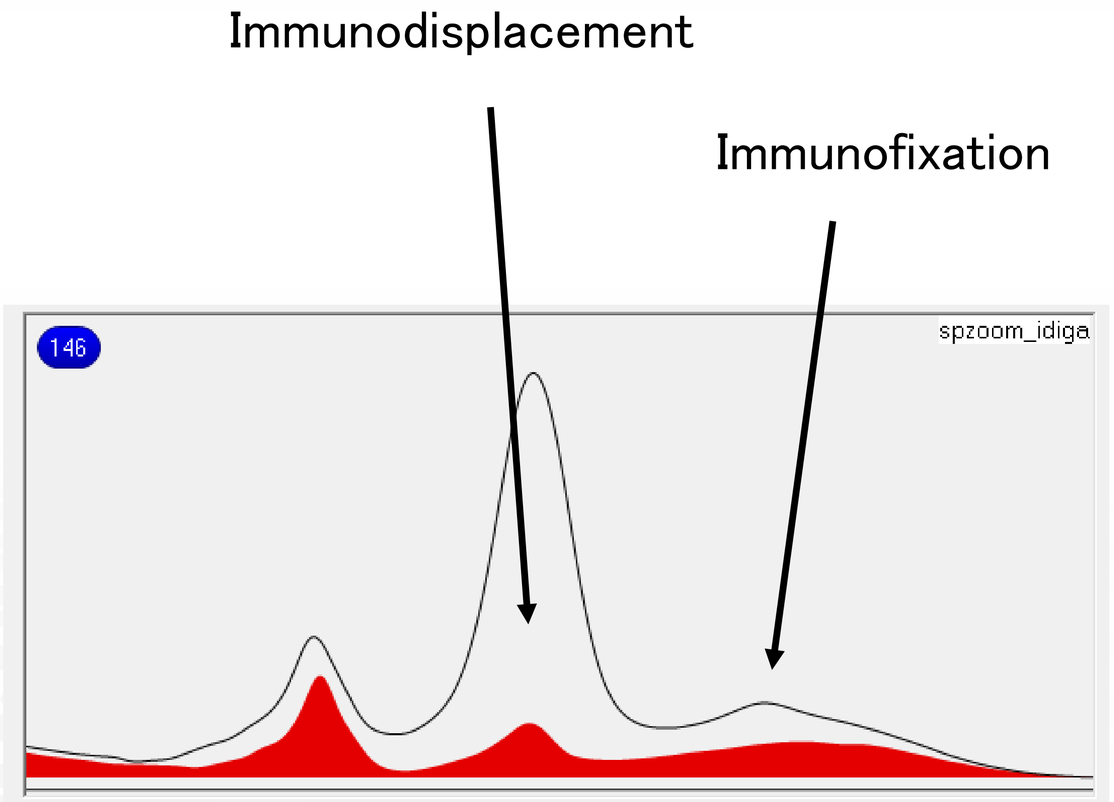


Immunotyping

How to choose between methods

- Immunodisplacement
  - Medium & large peaks
  - Suspected co migrating peaks
  - Fast / automated

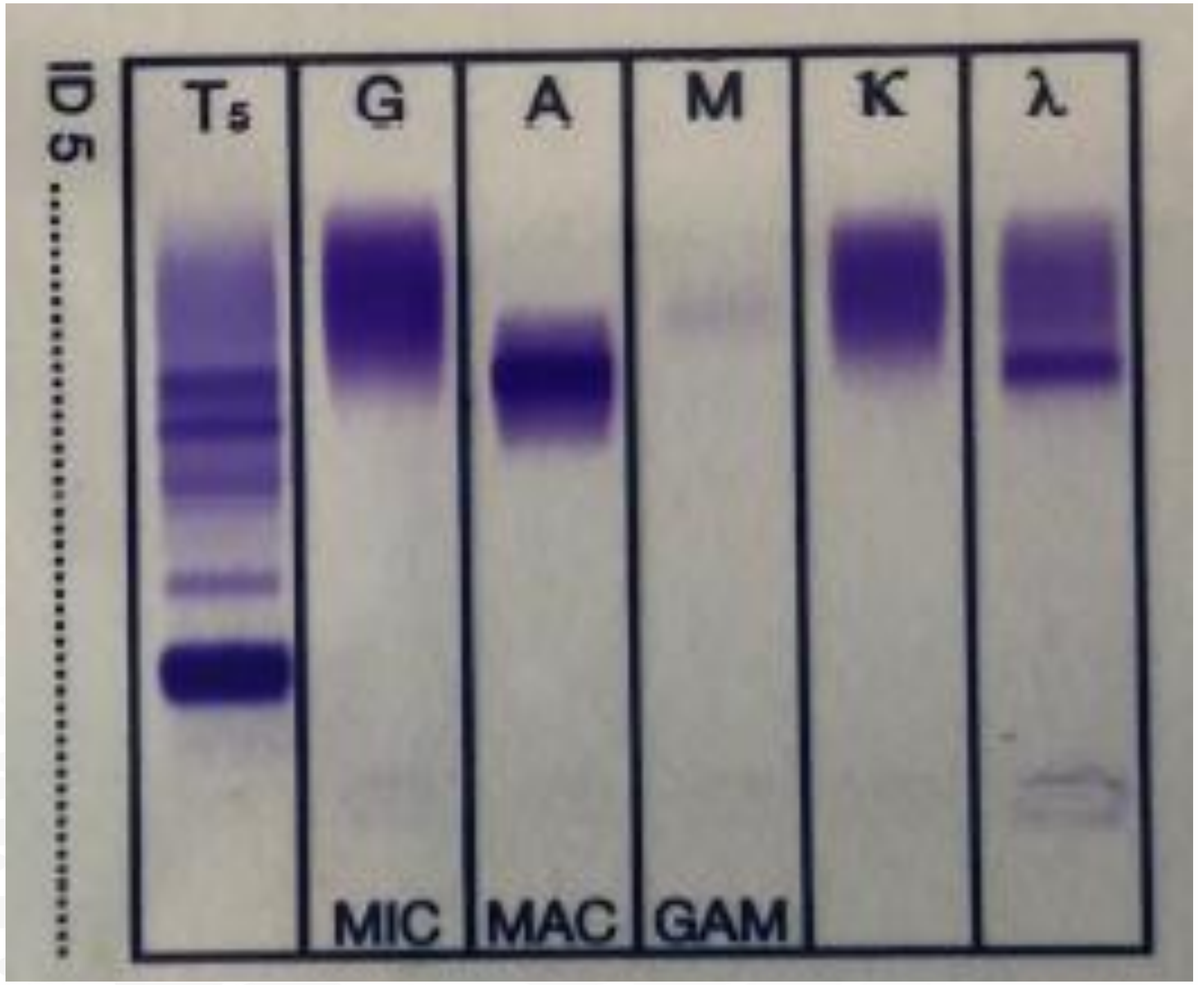
- Immunofixation
  - Small peaks
  - Manual
  - Batches



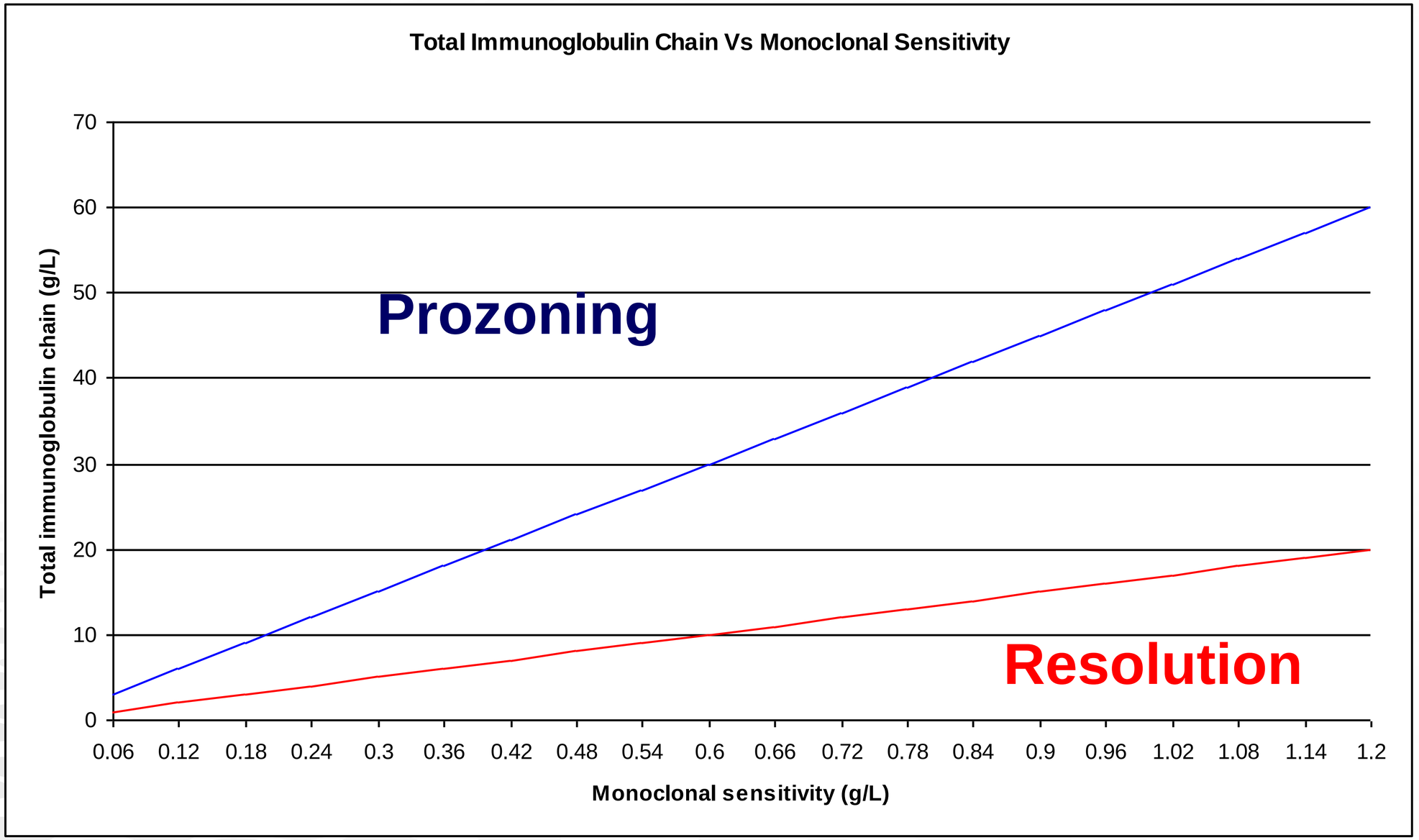
Immunofixation

- Customise for purpose
- Screening versus / typing

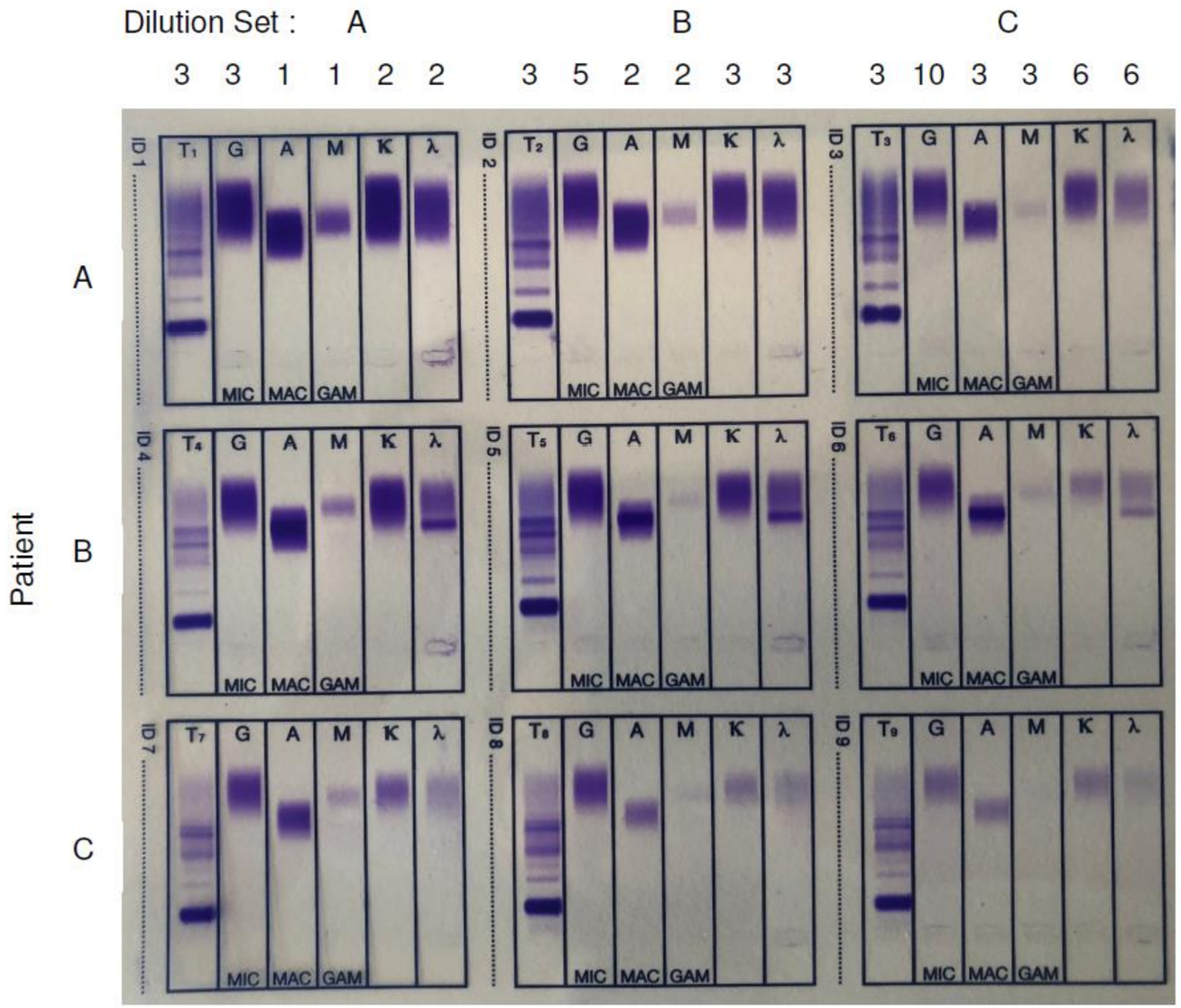
- Suggested dilution schemes
  - Screening
    - 52233
  - Immunotyping
    - Monoclonal specific



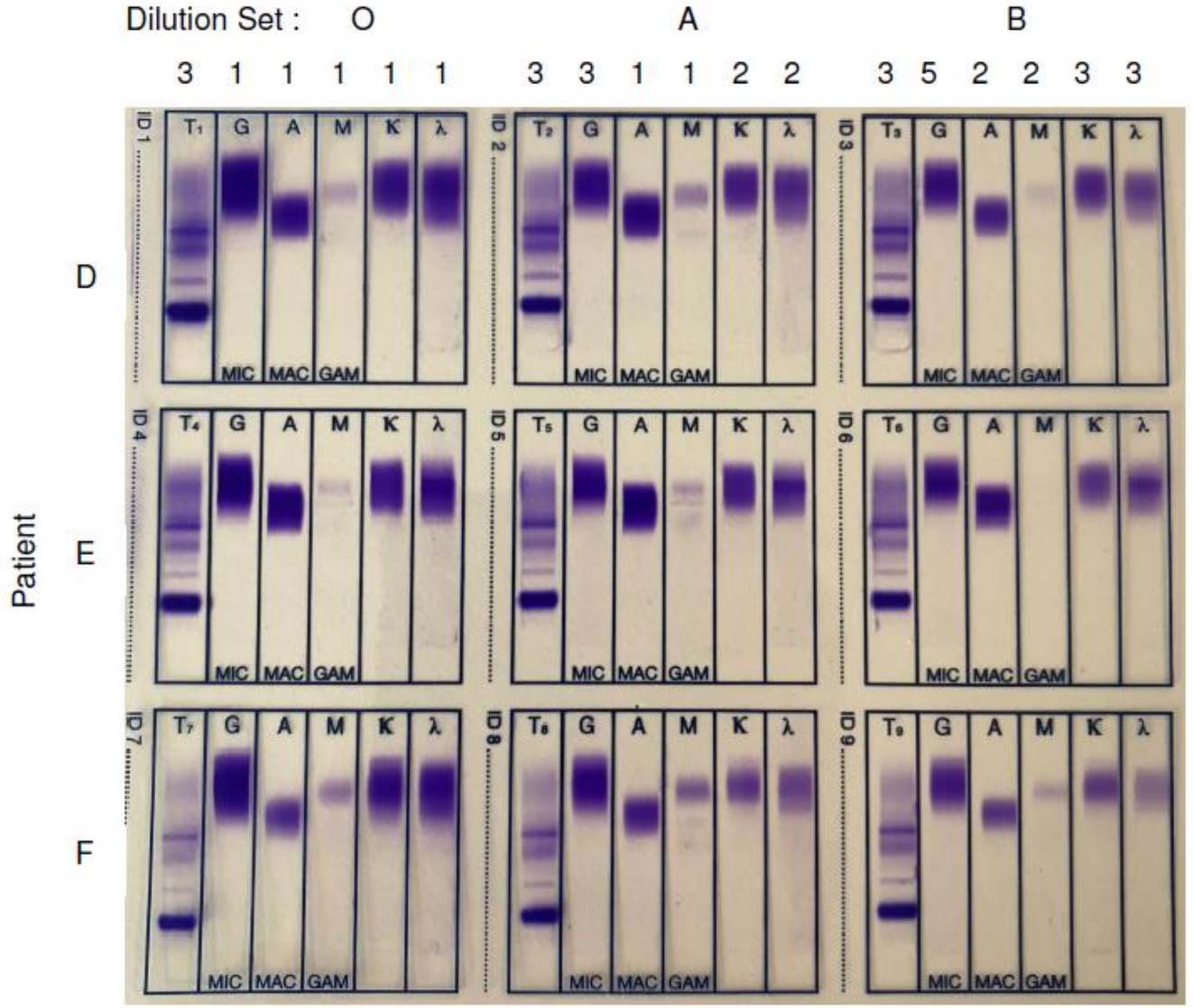
IFE Dilution Scheme



IFE Dilutions



IFE Dilutions



Patient D – Total IgG 5.9g/L. Unknown patient

Patient E – Total IgG 5.7g/L – Previous IgG Lambda (large clot in sample)

Patient F – Total IgG 5.7g/L – Unknown patient

Any Questions

Ankara – July 2019

Tony Aitchison – Helena Biosciences