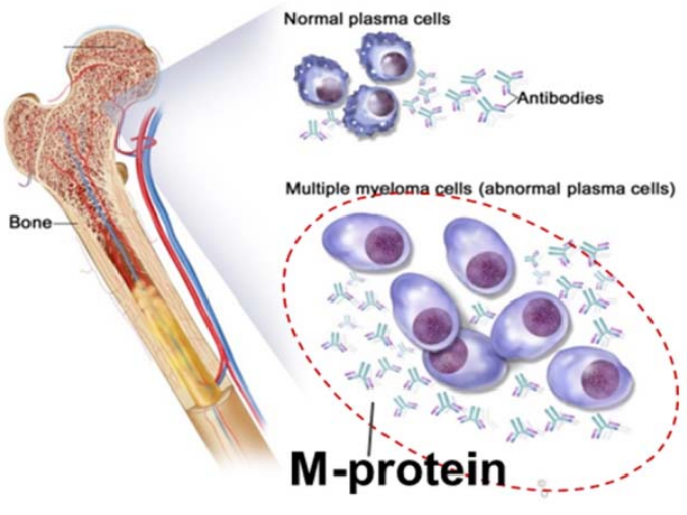
The Disease: Multiple Myeloma - Detection and Identification



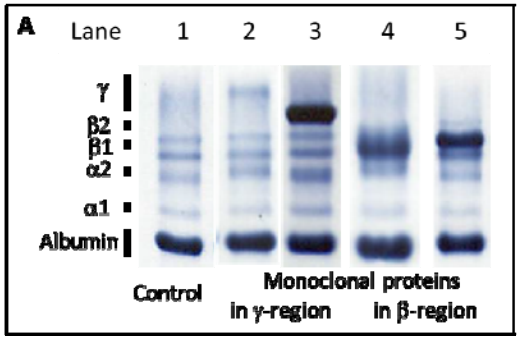
Multiple myeloma (MM)

- Blood cancer (2nd most common)
- Prevalence: 95K in U.S. (230K globally)
- New cases: 30K/year in U.S.
- MM produces **M-proteins**
  = Patient specific **immun0globulins**
- Immunoglobulins are detected by **PEP (A)** and identified by **IFE (C)**
- Tx: Chemotherapy, transplantation
- MM is not curable but recent therapies increase survival

A - M-proteins: Detected by protein electrophoresis (PEP) = blue gels
M-proteins migrate in beta and gamma region

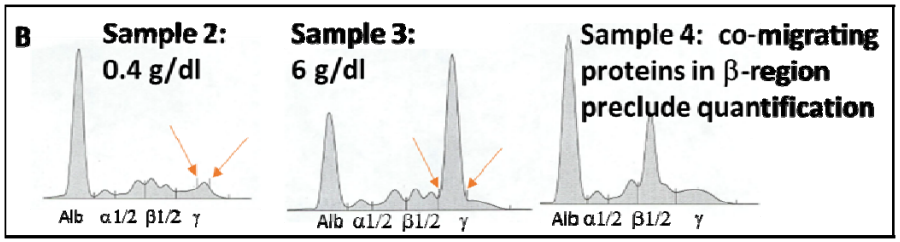
B - Densitometry is used to quantify the amount of M-protein

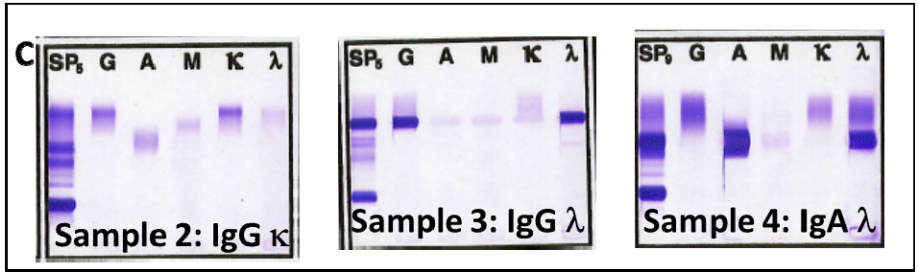
C- Immunofixation (IFE= purple gels) identifies the type of protein *
IFE is ~ 10 x more sensitive than PEP
PEP and IFE: State of the art to follow up MM treatment



*Immunoglobulins- combination of a 'heavy chain' with a 'light chain', or light chains alone

6 heavy chains : G, D, M, A, D, E
2 light chains : Kappa or Lambda

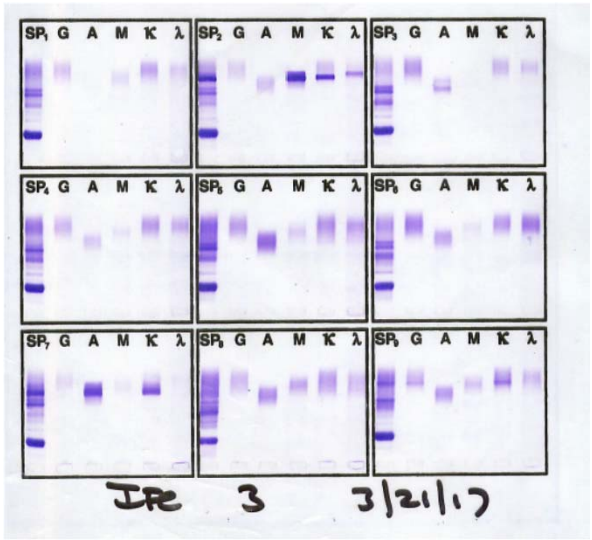




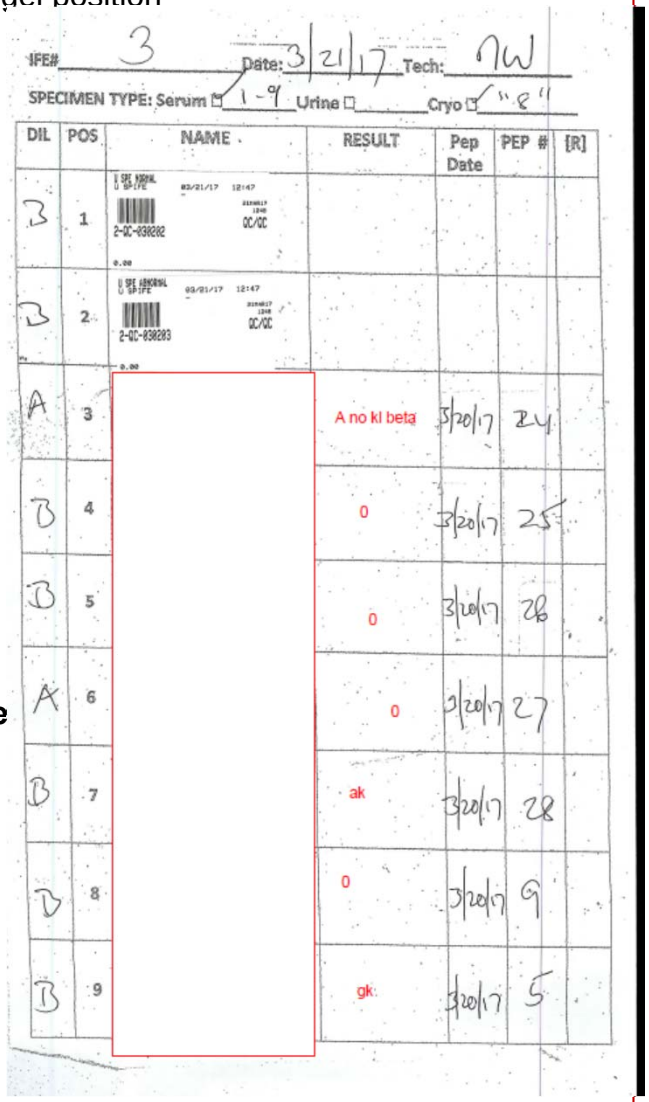
Workflow - Gel Interpretation

Step 1: Start with blue PEP gels. Each lane is one patient. Sample 1: negative control. Sample 2 : positive control
1- I mark the positive and 'suspicious' lanes
2- I transcribe the positive cases into an excel file that has the patient's name and gel position

3/21/17	3/21/17	
Gel # 336038		
NAME	SPEP	IFE
Patient lane 3		0
Patient lane 4	4	gk
Patient lane 5		
Patient lane 6		0
Patient lane 7	7	gk
Patient lane 8		gk?
Patient lane 9		gl beta
Patient lane 10		0
Patient lane 11		0
Patient lane 12	12	gk
Patient lane 13		0
Patient lane 14	14	gk
Patient lane 15		0/ gl (A)
Patient lane 16		gk
Patient lane 17	17	gk



Step 2: Purple IFE gels: 9 patients per she
3- I identify if monoclonal immunoglobulin is present or not, type, and migration pattern (beta, gamma), multiple immunoglobulins possible, too
4- I transcribe into a scanned in pdf that has Patient name and technical data that can be relevant to interpretation
4- I transcribe the result into the same excel sheet. Very manual process.



How is the final diagnosis made ?

1) Techs independently transcribe their result into the 'system'
2) I review their assessment and compare with my assessment (from the excel sheet and additional gel and patient history review in specific cases)
Techs and I agree in ~ 85 % of cases. Those are reviewed by me again. My results prevail in almost all cases.

What are the goals for a pattern recognition project?

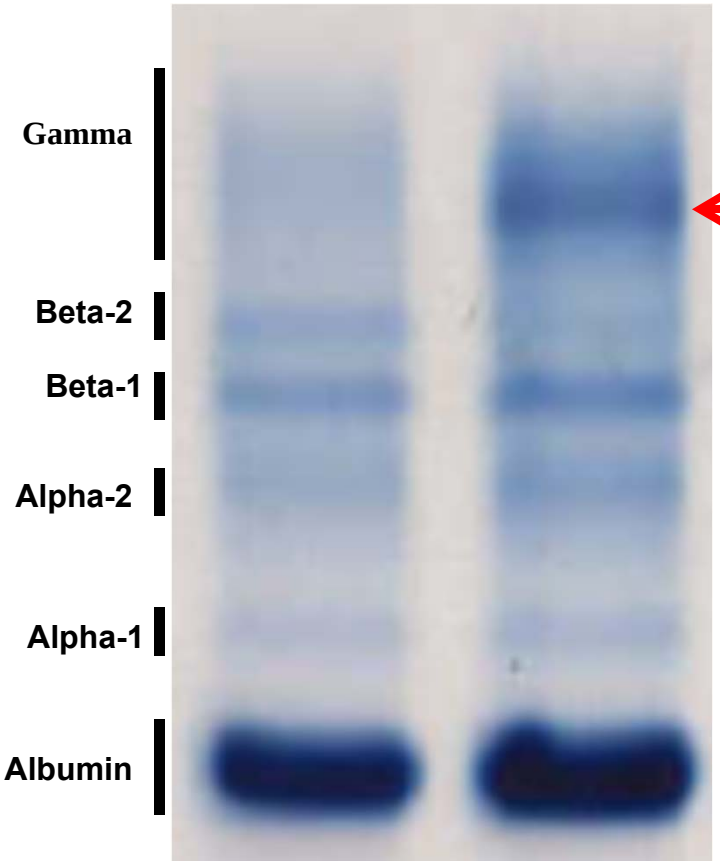
Ideal outcome would be an independent assessment of the gels run in parallel with tech and MD results
Hope is that the machine gets 'better' over time

Final product would be to confidently assess 85 % (or more !) cases

Quantitation of abnormal clone is not really necessary as it is done by the vendor machine

Pattern recognition could be used for other types of gels that are very systematic, i.e. hemoglobinopathies

On SPEP we look for abnormal bands



Other proteins also migrate on SPEP

Gamma — IgG, IgM, IgA, IgD, IgE
Kappa, Lambda
CRP

Beta-2 — C3, C4, Fibrinogen, IgM, IgA

Beta-1 — Transferrin, Ig A
Apo B (VLDL, LDL)

Alpha-2 — α2 -macroglobulin
Haptoglobin
Ceruloplasmin

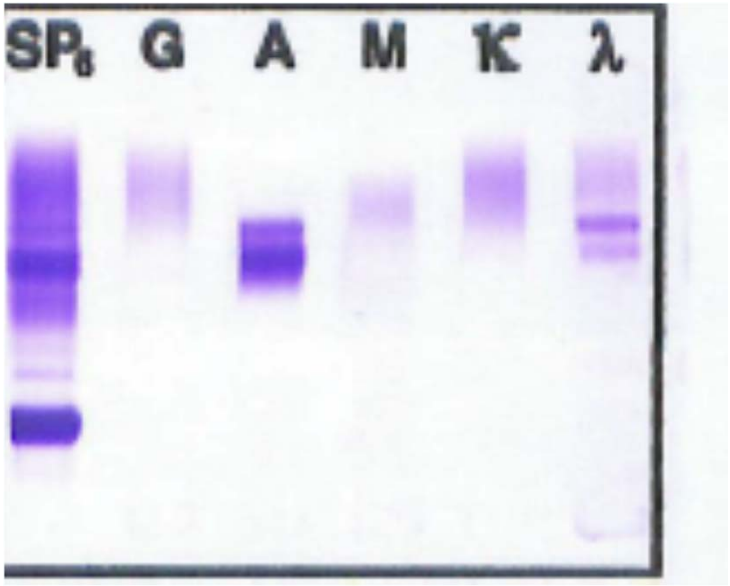
Alpha-1 — α1-lipoproteins Apo A (HDL)
α1-antitrypsin
α1-fetoprotein

Albumin

Healthy

Problem for quantifying IgA or IgM !

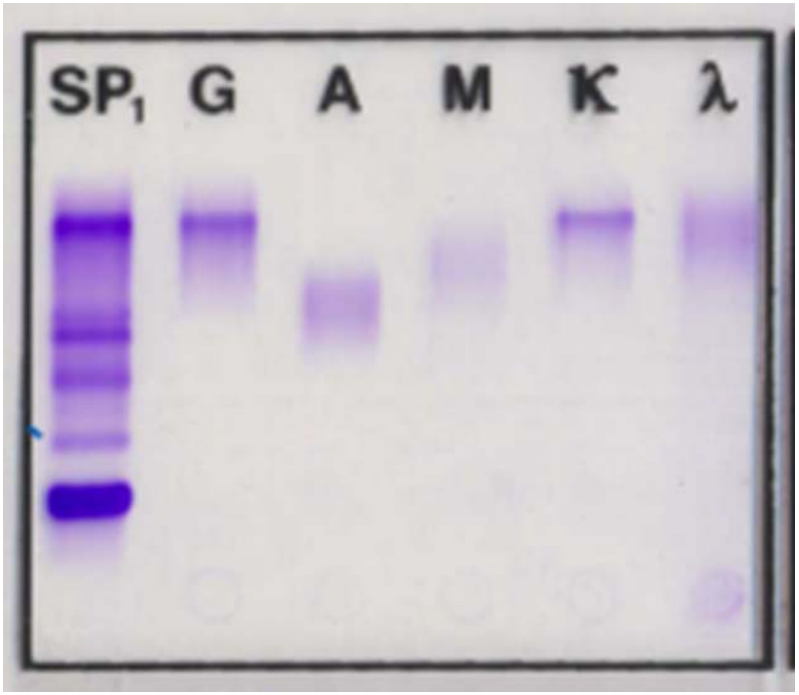
Abnormal bands are identified by Immunofixation (IFE)



Example for an IgA Lambda in beta

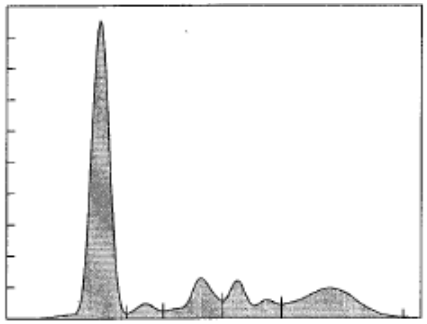
Abnormal bands are identified by Immunofixation (IFE)

How is this done?



For each patient 6 lanes are loaded with serum and then ‘probed’ with antibodies that are specific for the immunglobulins

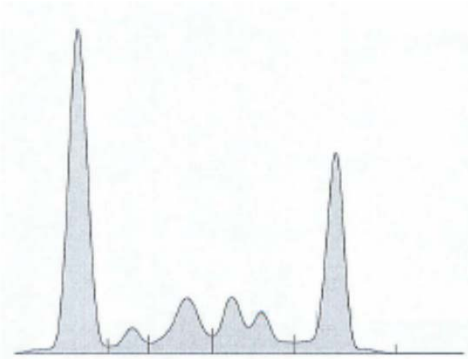
Abnormal proteins are quantified by densitometry



Fraction	g/dL	g/dL Range	
Albumin	4.0	3.6	4.8
Alpha 1	0.2	0.2	0.4
Alpha 2	0.8	0.8	1.1
Beta	0.8	0.6	1.0
Gamma	1.2	0.8	1.8
Total	7.0	6.7	8.6

Ratio	
A/G	1.3

Healthy



Fraction	g/dL	g/dL Range	
Albumin	3.4-	3.6	4.8
Alpha 1	0.3	0.2	0.4
Alpha 2	0.9	0.6	0.9
Beta	1.1+	0.6	1.0
Gamma	2.0+	0.8	1.8
Total	7.7	6.7	8.6

Restricted Band	g/dL
Gamma	1.8

Ratio	
A/G	0.8

Abnormal band