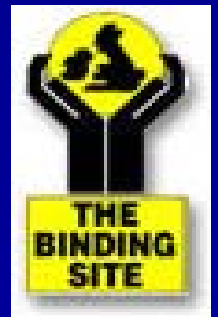




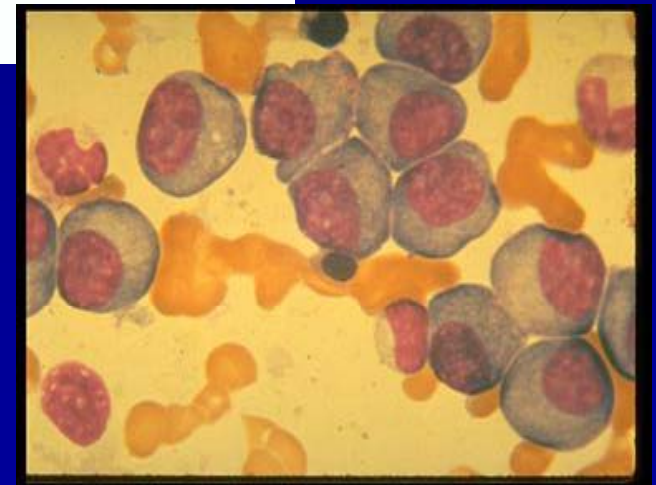
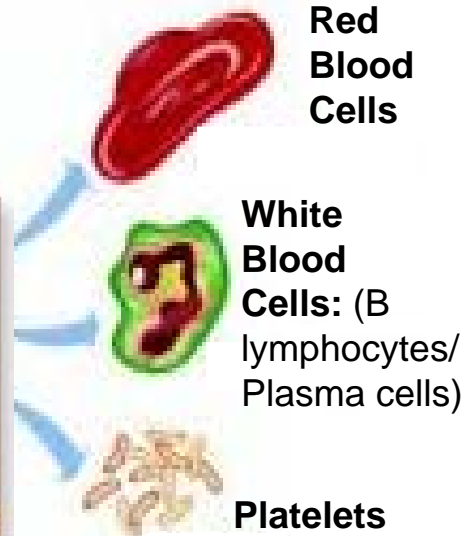
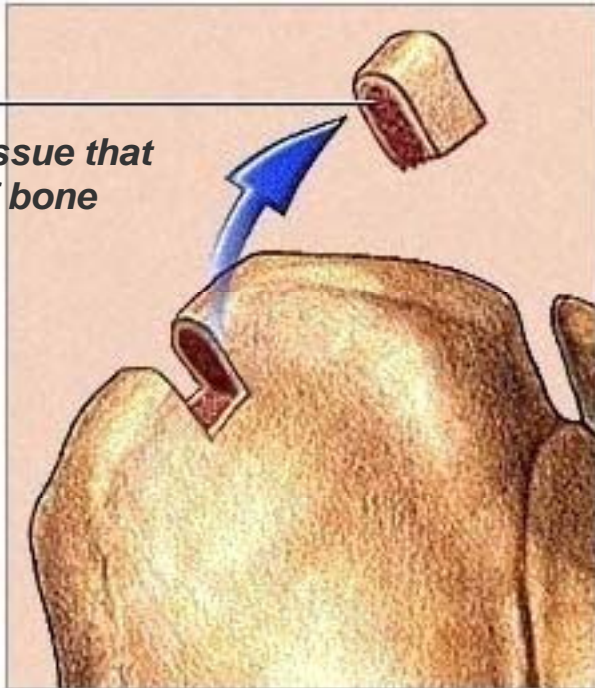
Understanding the Serum Free Light Chain Assays

Anne L Sherwood, PhD
Director of Scientific Affairs
The Binding Site, Inc.

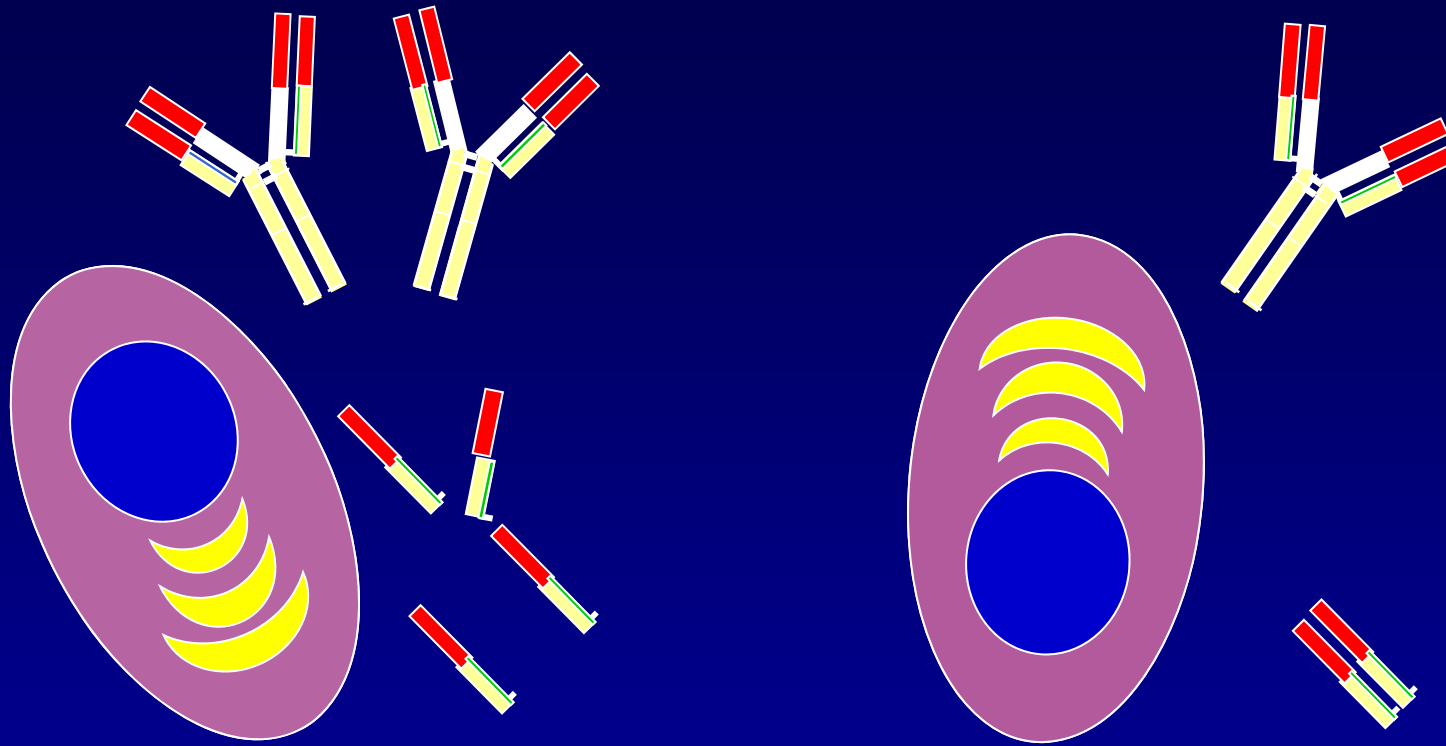


AL Amyloidosis: abnormality of proteins from Plasma Cells in the Bone Marrow

Bone marrow
Soft blood-forming tissue that fills the cavities of bone



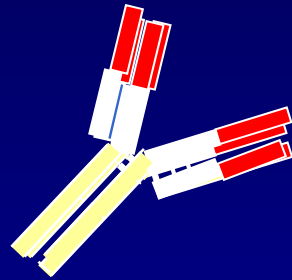
Plasma Cells produce Antibodies and Free Light Chains



Kappa

Lambda

Antibodies Are Made Up Of Heavy Chains And Light Chains

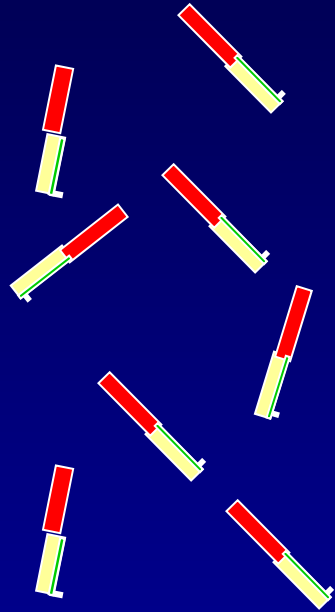


Immunoglobulin
(Antibody)

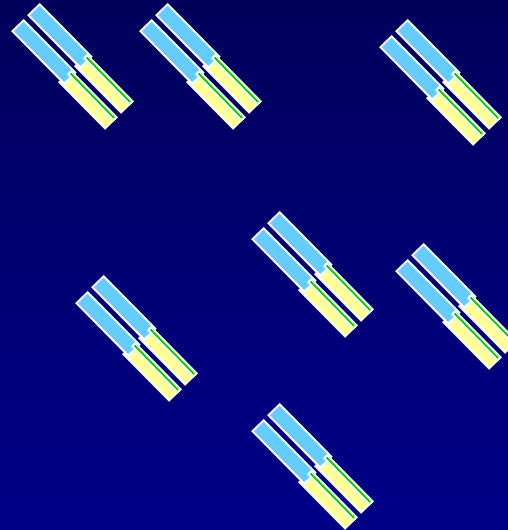
Light Chains

Heavy Chains

There Are Two Types Of Light Chains – Kappa And Lambda



Kappa Free
Light Chains



Lambda Free
Light Chains

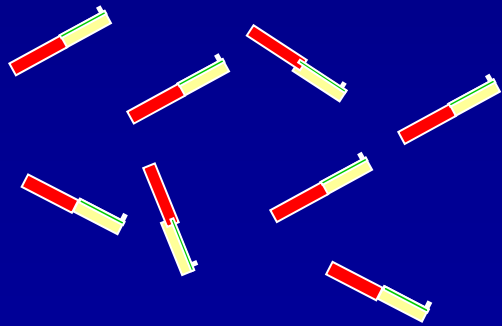
MULTIPLE-MYELOMA PROTEINS

III. The Antigenic Relationship of Bence Jones Proteins to Normal Gamma-Globulin and Multiple-Myeloma Serum Proteins

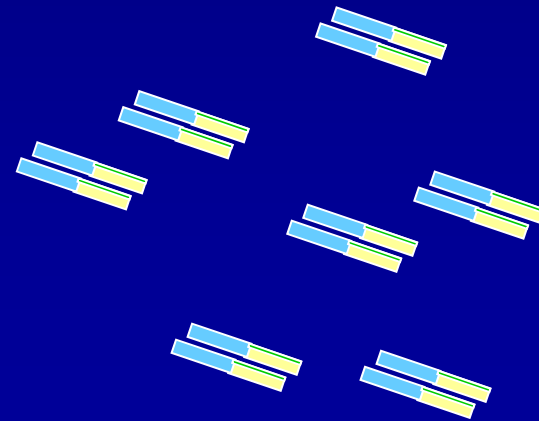
LEONHARD KORNGOLD, PH.D., AND ROSE LIPARI, B.A.

Cancer. 1956;9:262-272.

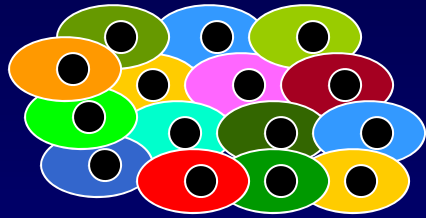
Korngold "Kappa"



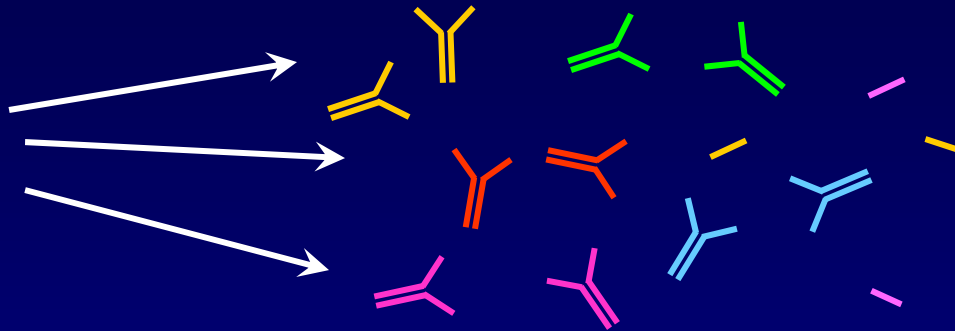
Lipari "Lambda"



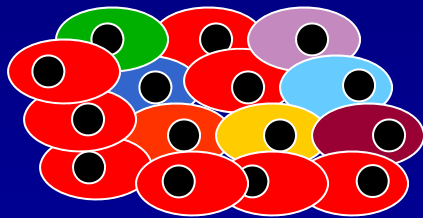
Normal vs AL Amyloidosis



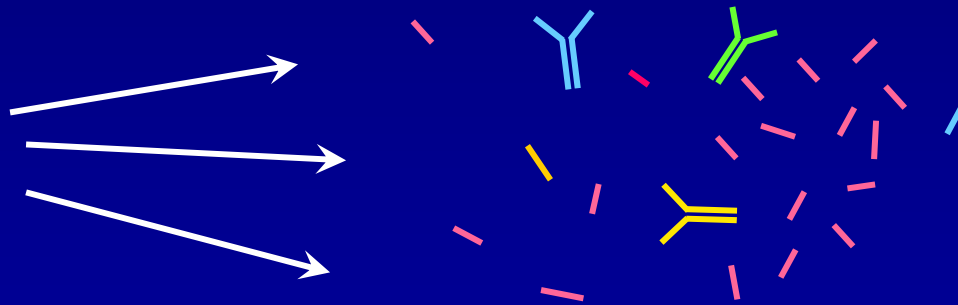
Normal



Lots of different types of whole antibodies



AL Amyloidosis



Too much of a Toxic, precipitating light chain produced

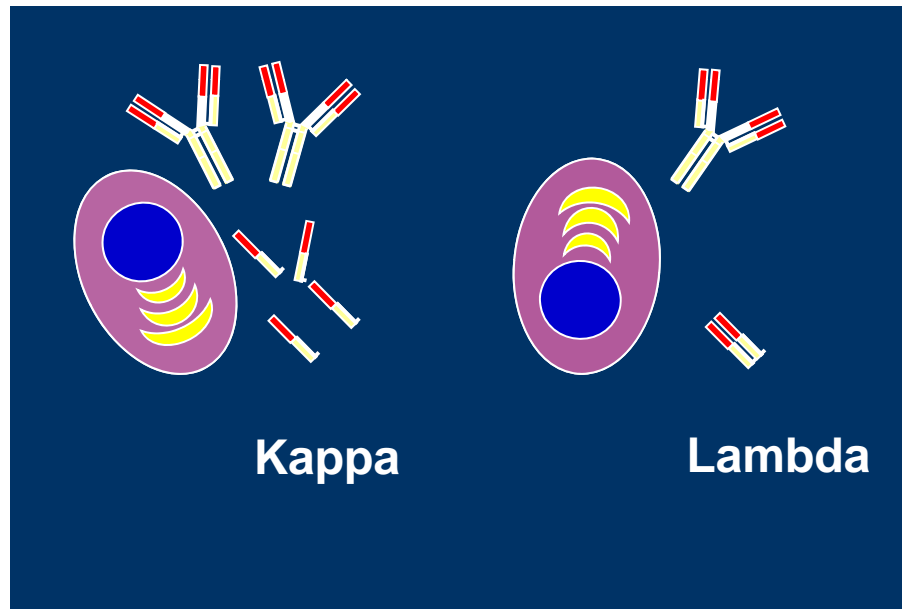
What is AL Amyloidosis?

“AL” = Antibody Light chain

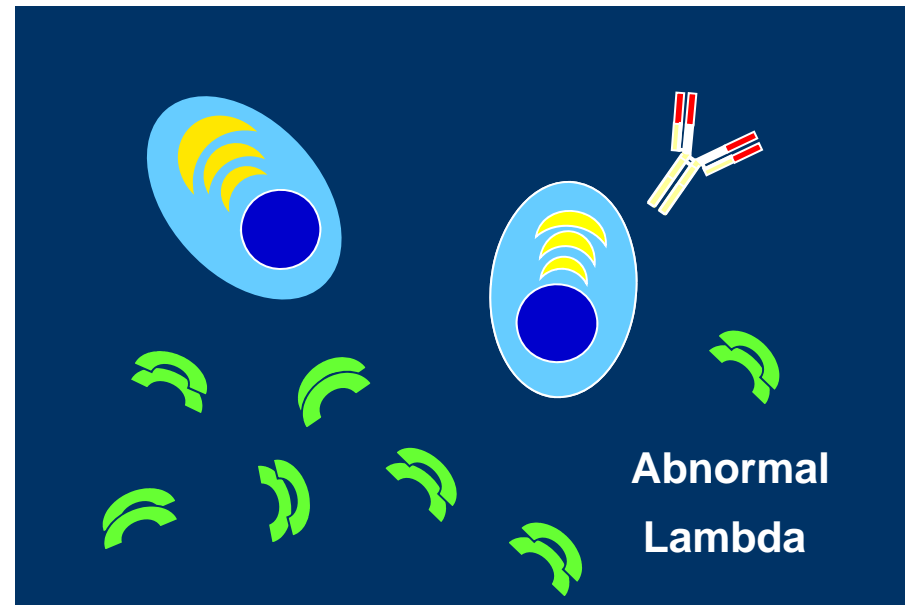
(a disease of protein misfolding)

“Amyl”oid = Starch-like (Greek)

Primary [AL] Amyloidosis is a plasma cell disorder

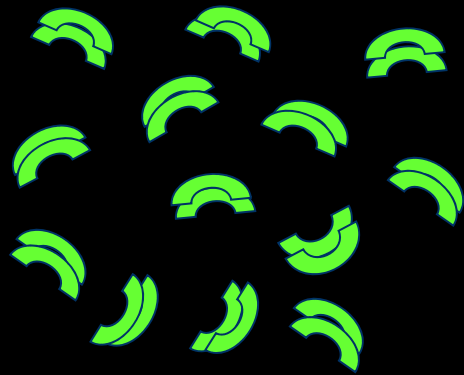


Normal Healthy Plasma cells



Mutated (diseased) Plasma cells

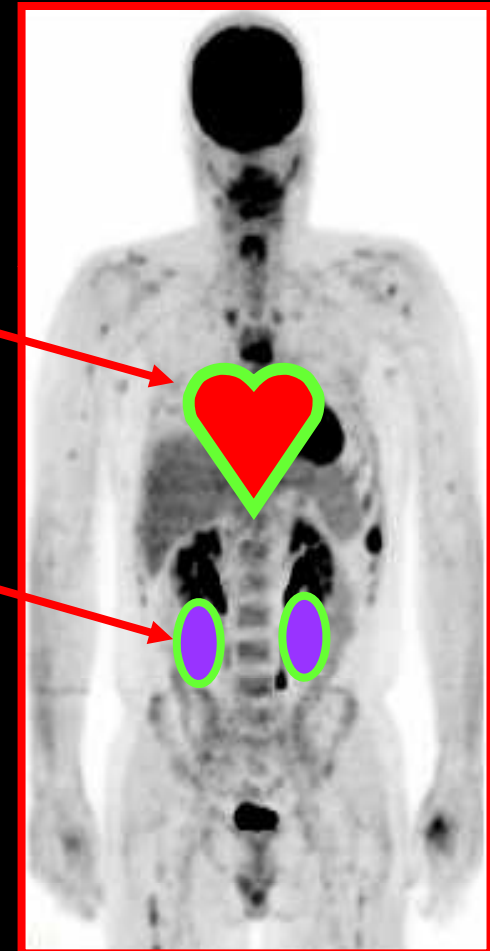
These abnormal light chains have mutations which make them “sticky”



They bind together to form an amyloid protein complex



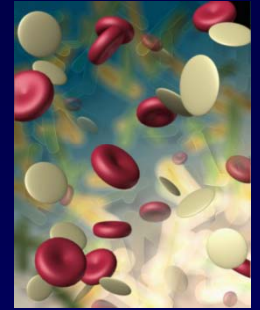
Amyloid proteins accumulate in target organs such as the **HEART** and **KIDNEYS**



AL Amyloidosis

- In AL amyloidosis, proteins which deposit can damage critical organs (e.g., heart, kidneys)
- Important to use testing to monitor disease and guide therapy

Free Light Chain Review



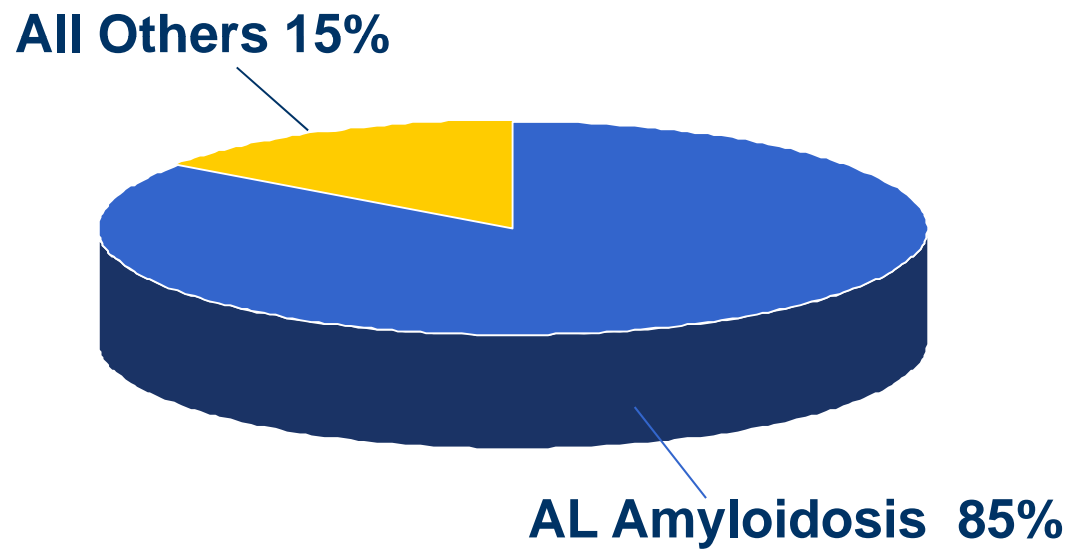
- Free light chains are normally found in the blood.
- Free light chains circulate in the blood at abnormally high levels in many patients with AL amyloidosis.
- Levels of FLCs are associated with the number of malignant plasma cells in a patient with ALA.
- In ALA, abnormal free light chains stick together to form amyloid protein which can damage important organs like the kidneys and heart.

Types of Systemic Amyloidosis

- Primary (AL) Amyloidosis
 - Kappa or Lambda Immunoglobulin light chain associated
- Secondary (AA) Amyloidosis
 - Amyloidosis secondary to another disorder (such as RA, Alzheimer's disease, MS, Type II diabetes)
 - Serum Amyloid A associated
- Hereditary Amyloidosis
 - Associated with certain genotypes
 - TTR – mutant transthyretin associated

More than 25 different amyloid proteins have been identified!

Relative Frequency



Amyloidosis Incidence

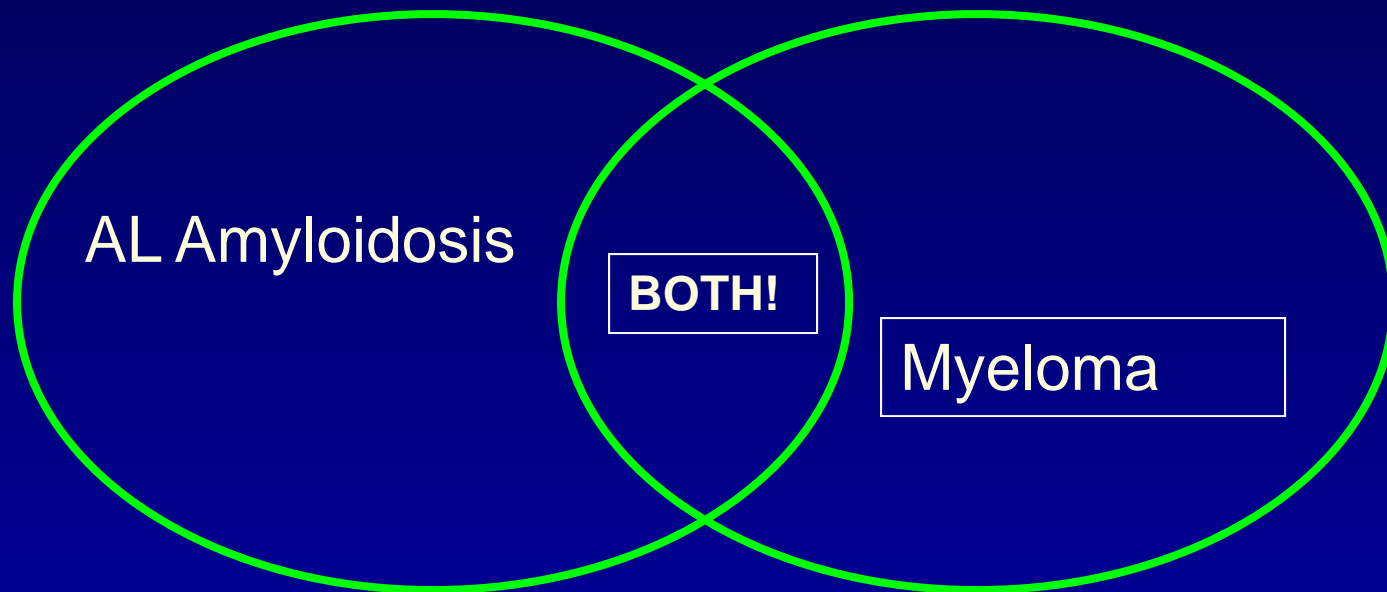
- AL Amyloidosis
 - Incidence in the population 1/5th of Multiple Myeloma (Annual Incidence 9 per million)

Palumbo, A, Rajkumar SV. *Leukemia* 23:449 2009

Kyle , RA, Rajkumar SV. *N. Engl. J of Med* 351:1860 2004

Bradwell , Serum Free Light Chain Analysis, 5th ed, 2008, p 125

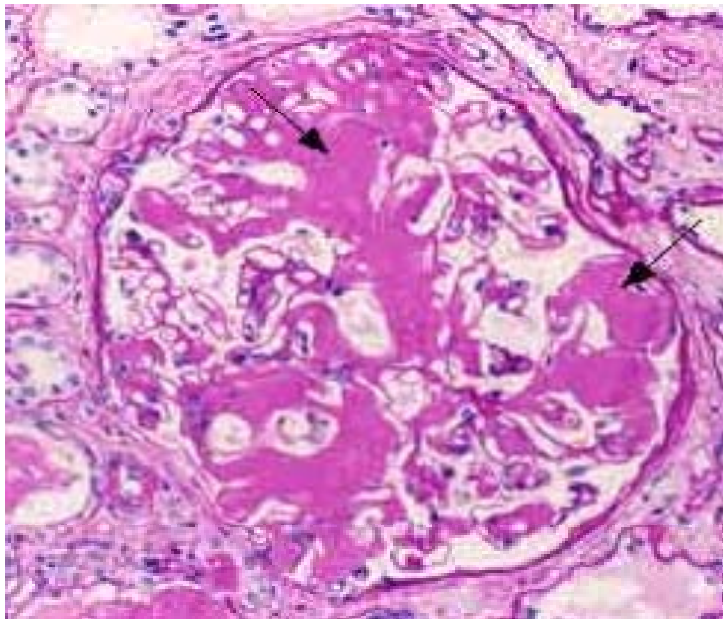
Overlapping Diseases



Amyloid

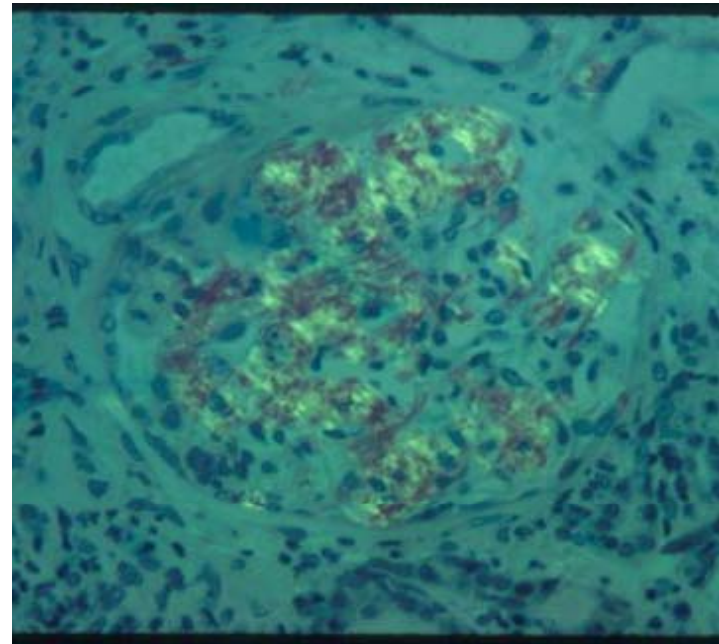
The definitive diagnostic test for amyloidosis is a tissue biopsy showing “apple green birefringence” when stained with congo red dye and viewed under a microscope using polarized light

“Pink” deposits when stained with hematoxylin and eosin

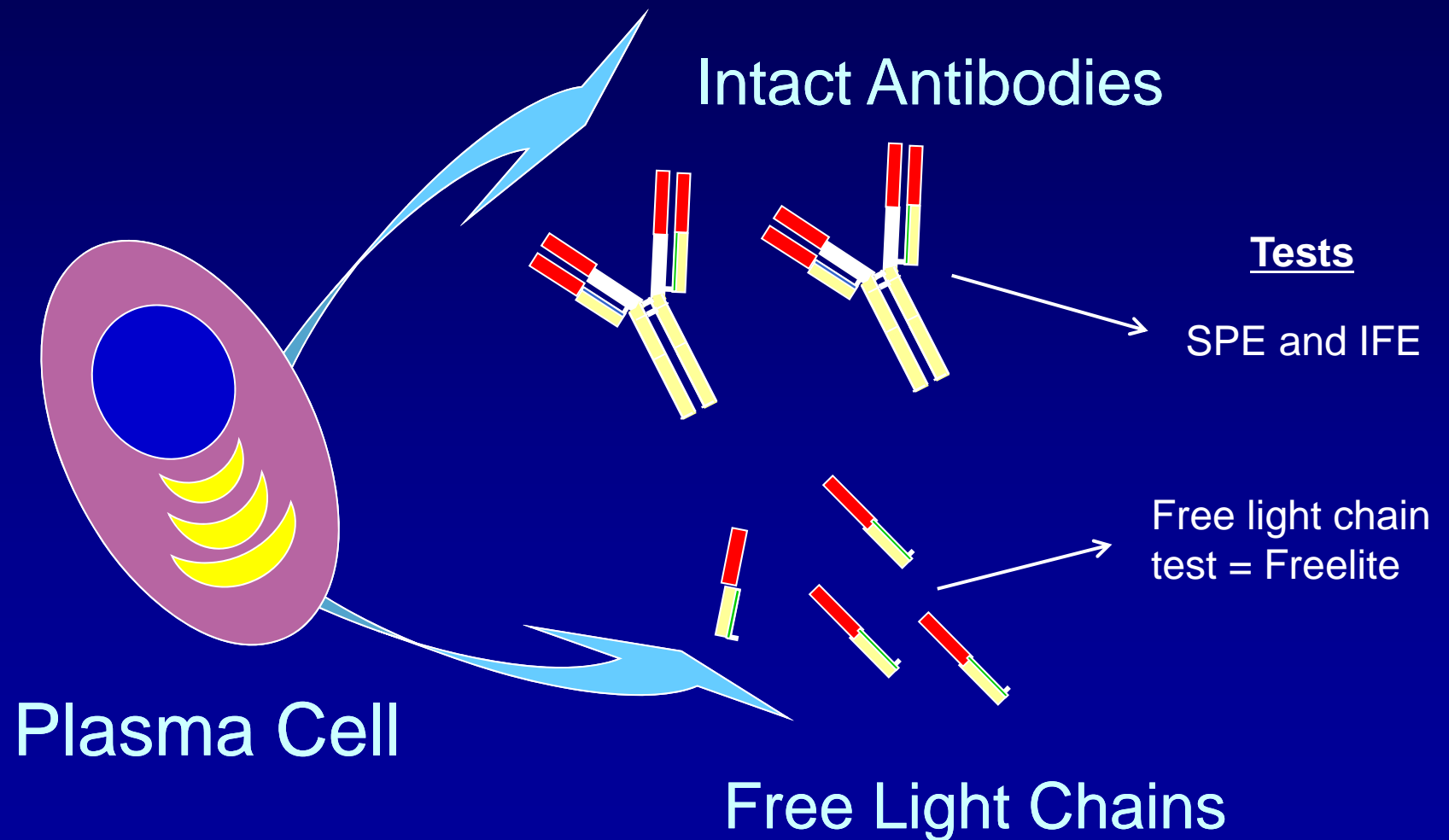


Glomerular amyloidosis

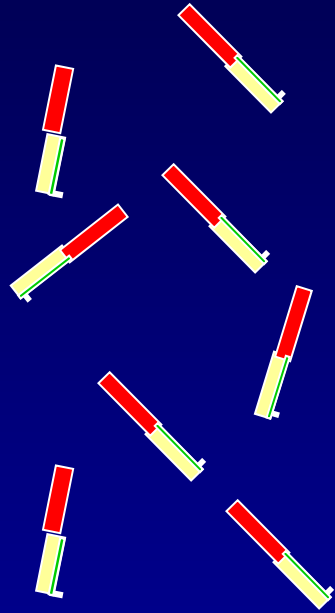
The pink deposits appear “apple green” with congo red staining and polarized light



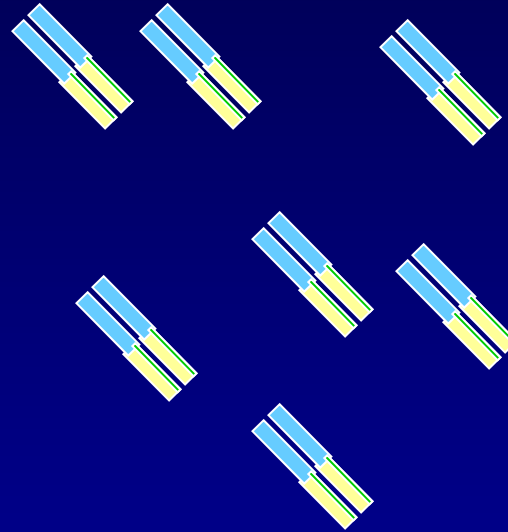
Plasma Cells Produce Intact Antibodies AND “Free Light Chains”



The FreeLite Test Measures both types of Free Light Chains



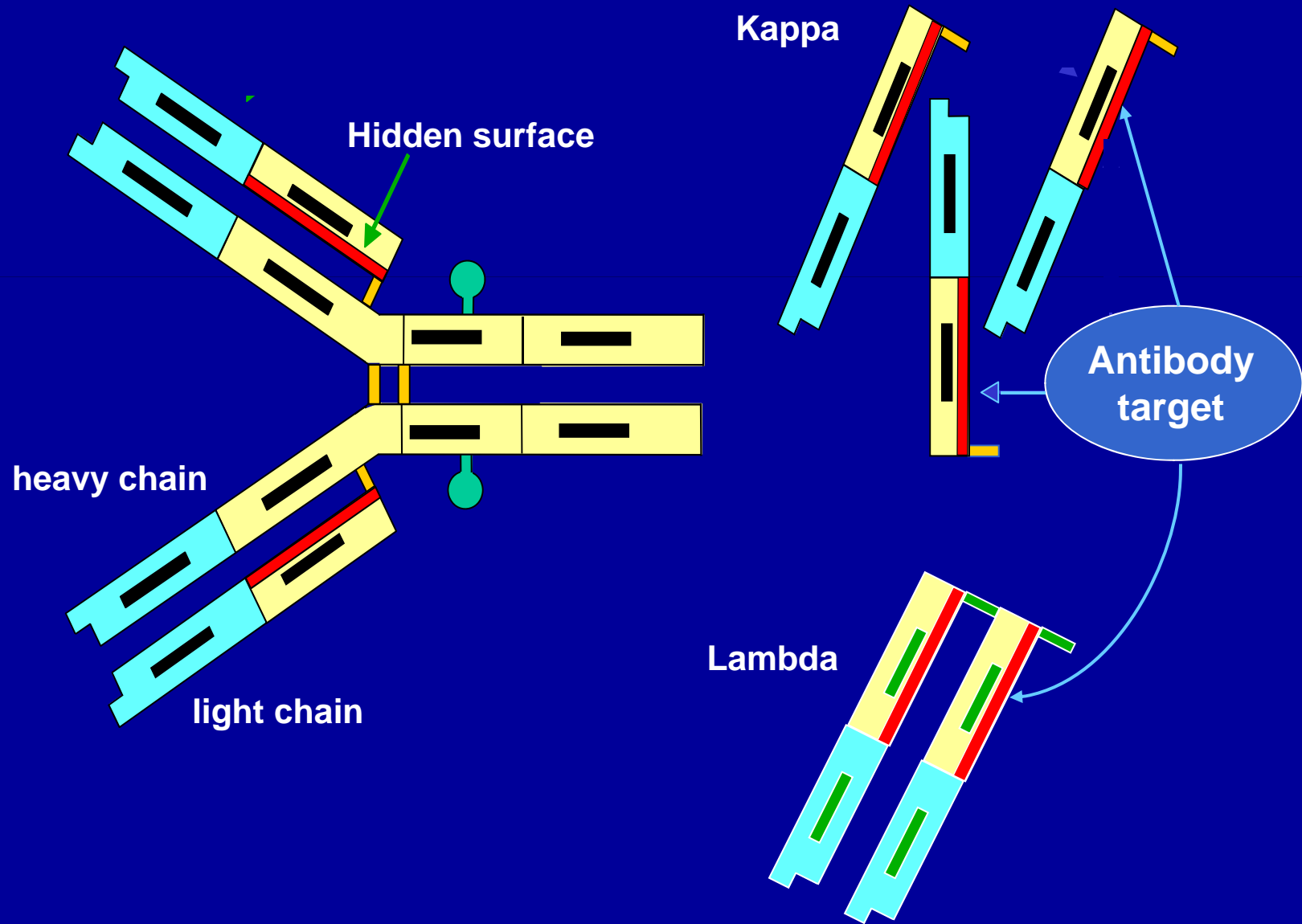
Kappa Free
Light Chains



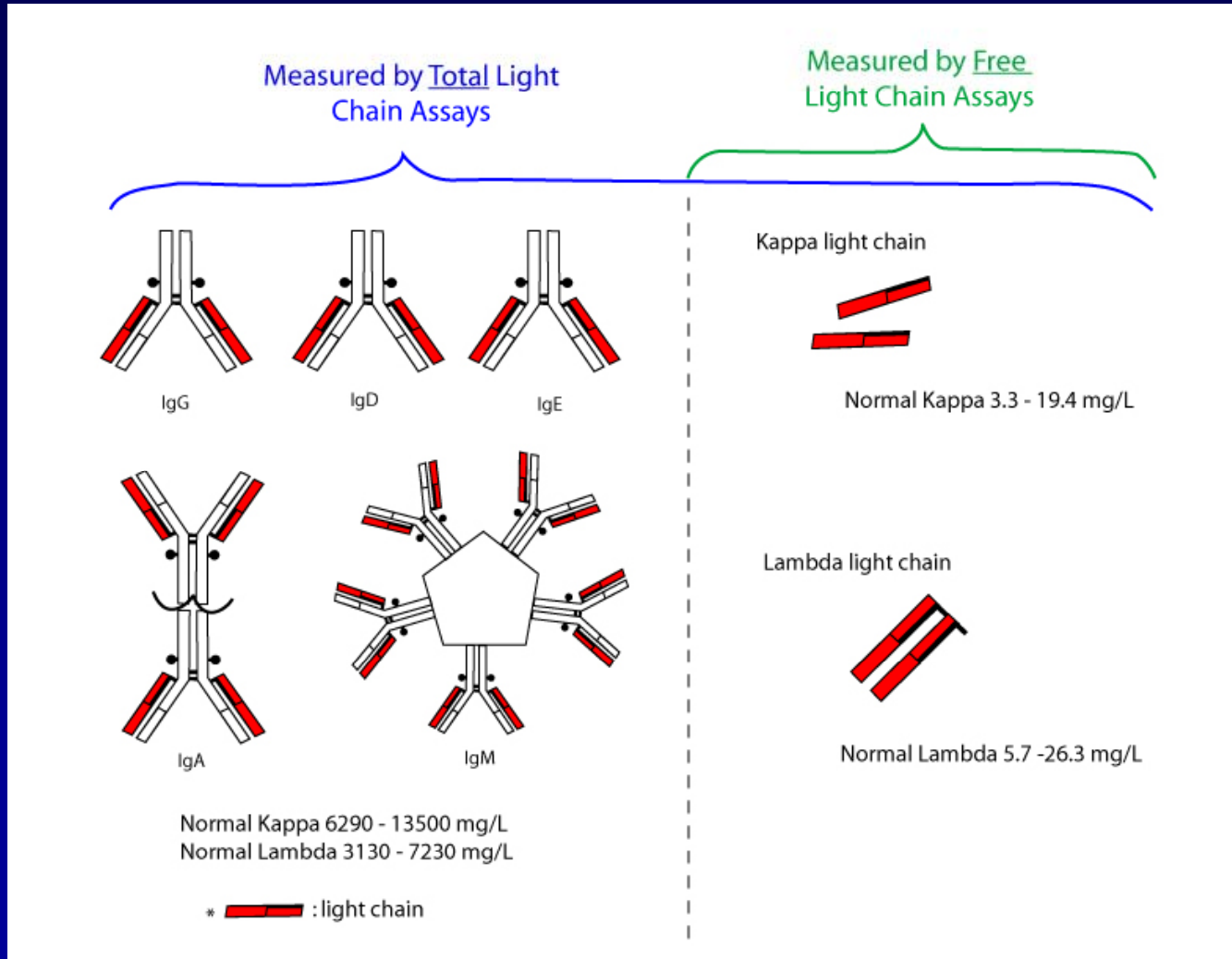
Lambda Free
Light Chains

FREELITE™:

Polyclonal Sheep Abs Only Bind to Free Light Chains



Total Light Chain Assay



Normal Ranges for Serum Free Light Chains

Units (mg/L)

Units (mg/dL)

Kappa: 3.3–19.4 mg/L

Kappa: 0.33–1.94 mg/dL

Lambda: 5.7–26.3 mg/L

Lambda: 0.57–2.63 mg/dL

κ/λ ratio: **0.26–1.65**

κ/λ ratio: **0.26–1.65**

Examples

May

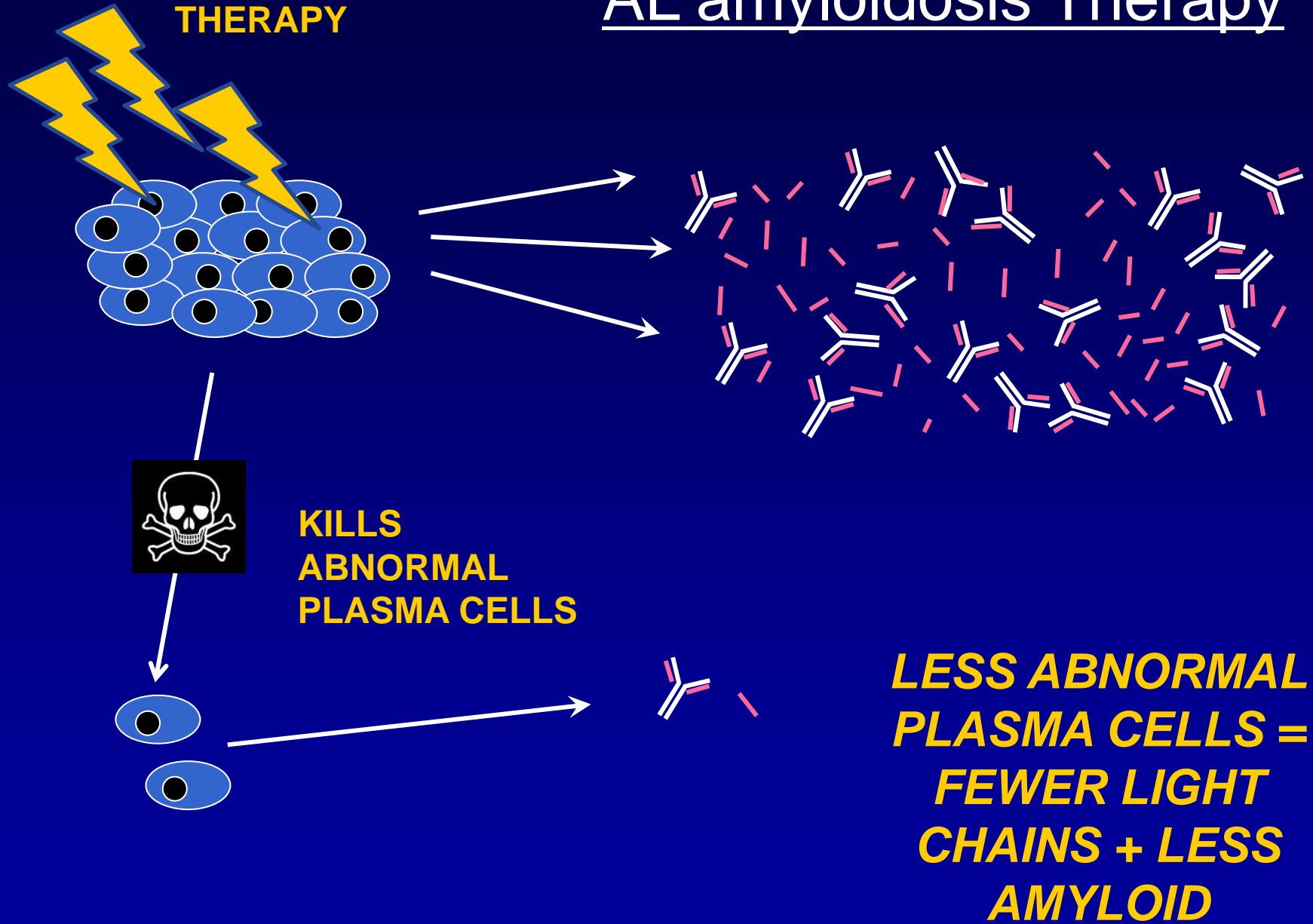
Description	Flag	Result	Normal Range
KAPPA LAMBDA FREE LIGHT CHAIN			
KAPPA FREE LIGHT CHAIN	H	38.60 MG/DL	0.33 - 1.94
LAMBDA FREE LIGHT CHAIN	L	0.31 MG/DL	0.57 - 2.63
KAPPA LAMBDA RATIO	H	124.19 %	0.26 - 1.65

= 385 mg/L

July

Free K+L Lt Chains, Qn, S				
Free Kappa Lt Chains, S	303.00	High	mg/L	3.30 - 19.40
Results verified by repeat testing				
Free Lambda Lt Chains, S	0.56	Low	mg/L	5.71 - 26.30
Results verified by repeat testing				
Kappa/Lambda Ratio, S	541.08	High		0.26 - 1.65

AL amyloidosis Therapy

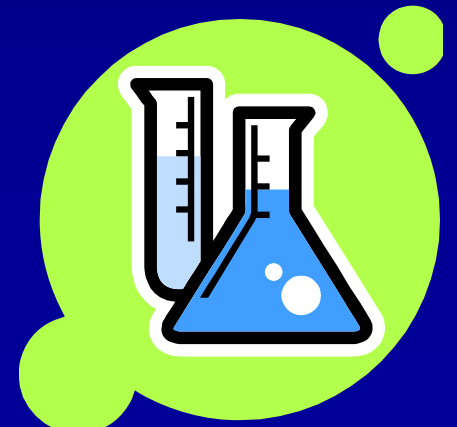




Lab Reports

Laboratory Variability

- Absolute values in the serum free light chain assay can vary from lab to lab
 - Different analytical instruments
 - Different kits for different instruments
 - Normal lab to lab variation



Laboratory Variability

If you change the laboratory where your serum free light chains are measured:

- If possible, obtain some of the last sample and run it along with the new sample or
- Establish a new baseline for your serum free light chain levels.

The change in your absolute sFLC level should not effect the clinical interpretation.

Additional Resources

- www.wikilite.com (web version of our “red book”)
- Google “Binding Site”
- Email us info@thebindingsite.com
- Call The Binding site 800-633-4484
 - Experts are on hand to assist you
- Understanding Serum Free Light Chain Assays (IMF booklet)



Q & A