

Immunoglobulins

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INTRODUCTION

- Prophylactic and therapeutic use of immune milks has been shown to be successful in preventing and treating enteropathogenic *Escherichia coli* infections, rotavirus gastroenteritis in infants, Cryptococcosis and diarrhoea in AIDS and other immunodeficient patients, caries formation, and others.
- In all these instances colostrum containing immunoglobulin has been obtained from animals immunised with specific pathogens.
- Kummer (1992) showed that colostrum from non-immunised cows may prevent gastrointestinal disease in infants.

IMMUNE SYSTEM

- Immune system - a natural defence mechanism that helps ward-off or combat disease.
- Immunoglobulins have an integral role in this defence system in that they form antibodies.
- Most prevalent immunoglobulin in all species of animals is IgG.
- In human milk and colostrum IgA is predominate immunoglobulin
- Specific antibodies exist in bovine colostrum and milk which are effective against enteropathogenic and enterogenic organisms.

IMMUNE RESPONSE

- Defence mechanism by which the body fights infection.
- Innate immune system vs adaptive immune system
- Innate immune system
 - ∪ first line of defence
 - ∪ non-specific but usually effective
 - ∪ resistance not improved by repeated exposure
- Adaptive Immune system
 - ∪ second line of defence
 - ∪ specific response - antibodies
 - ∪ involves memory
 - ∪ resistance improves with repeated exposure

Immune Response

- Most infectious agents enter the body proper via the epithelial surfaces of either the upper respiratory, digestive or genito-urinary tract.
- Variety of physical and chemical mechanisms involved in protection.
- Specialised set of cells called Leukocytes or white blood cell help combat infection and disease.
- Leokocytes fall into two broad categories of cell types: Phagocytes and Lymphocytes

Immune Response

- Phagocytes

- Innate Immune system
- Non-specific response
- Neutrophils, basophils , eosinophils, monocytes and macrophages

- Lymphocytes

- Adaptive Immune System
- Specific Response
- Involves memory
- Two types: B cells and T cells

Immune Response

- B cells

- Differentiated in bone marrow
- Mainly found in lymph nodes and spleen
- antibodies (immunoglobulins)

- T cells

- Differentiated in the thymus
- Regulate activity of B cells : T helper cells and T suppressor cells
- Directly attack infectious agents: T killer cells

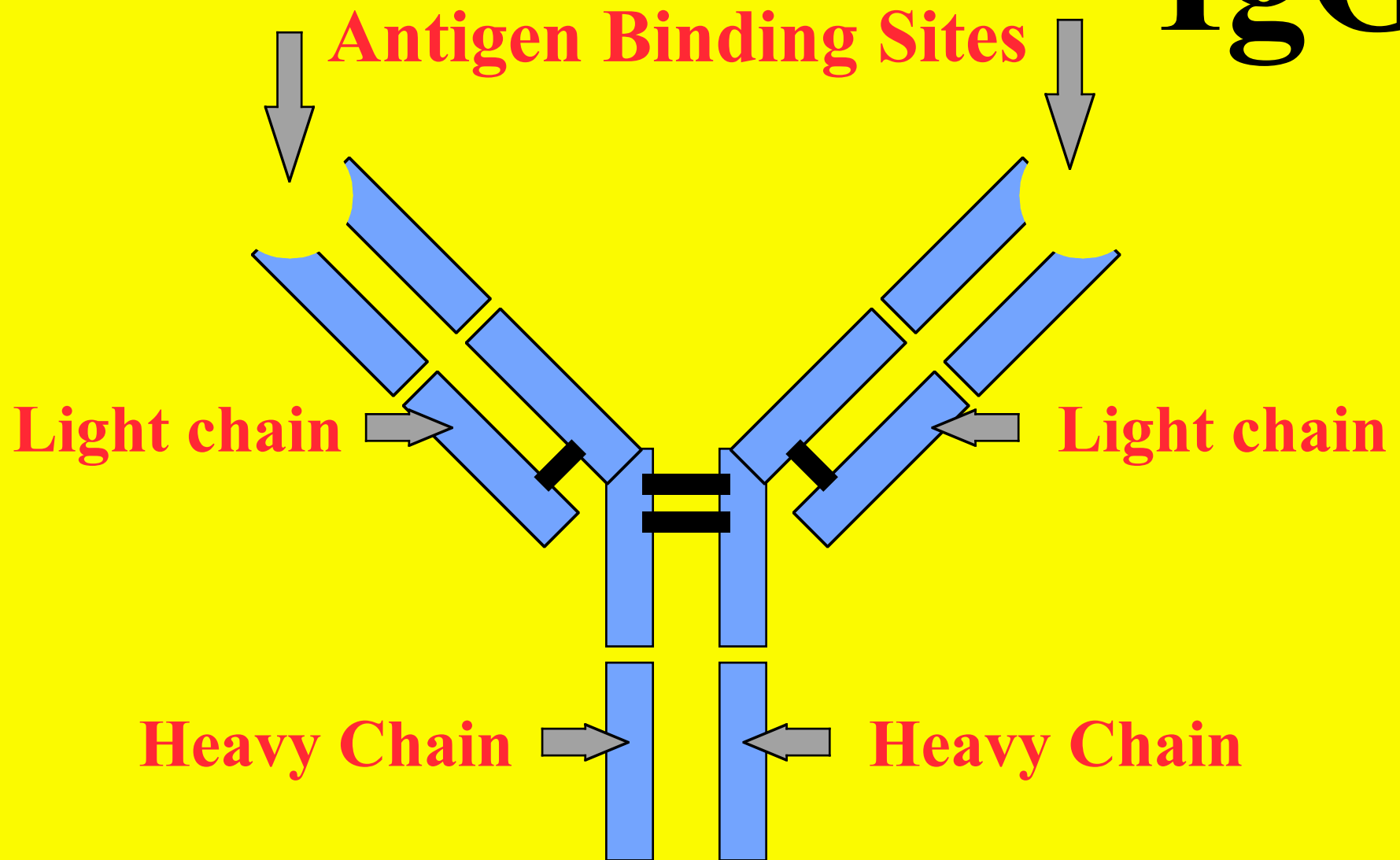
What Are Antibodies?

- Specialized group of bioactive proteins called immunoglobulins (Ig's)
- Present in serum and other tissue fluids , including milk of mammals and egg yolk of egg laying species
- Produced in response to hosts exposure to immunogenic foreign substance (antigen); such as infectious microbes.
- Adaptive immune response - impart memory - prepares the body to effectively repel any later invasion by same organism

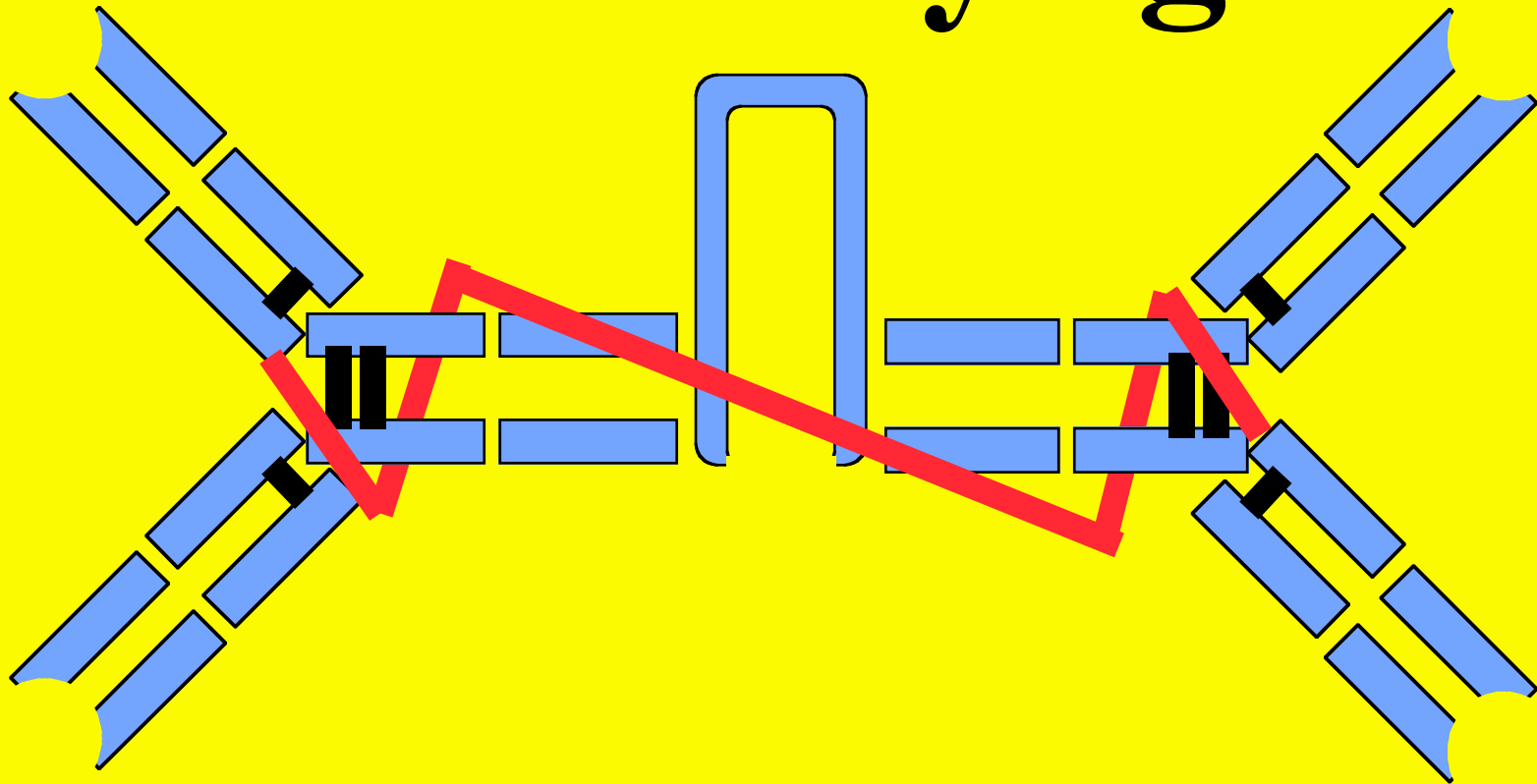
Antibodies Cont.

- Produced by activated B cell Lymphocytes (plasma cells)
- Each plasma cell secretes only one class of immunoglobulin or antibody
- Antibody produced by a single plasma cell is of the same specificity - directed towards same antigen
- Five classes of immunoglobulin: IgG, IgA, IgM, IgD and IgE
- Most prevalent class of immunoglobulin in all species IgG
- In Human milk and colostrum IgA is predominate immunoglobulin
- Function is to bind to invading organisms and activate specific actions to rid body of disease causing agent
- Function in cell killing, inflammation and prevention of microbial and viral attachment

IgG

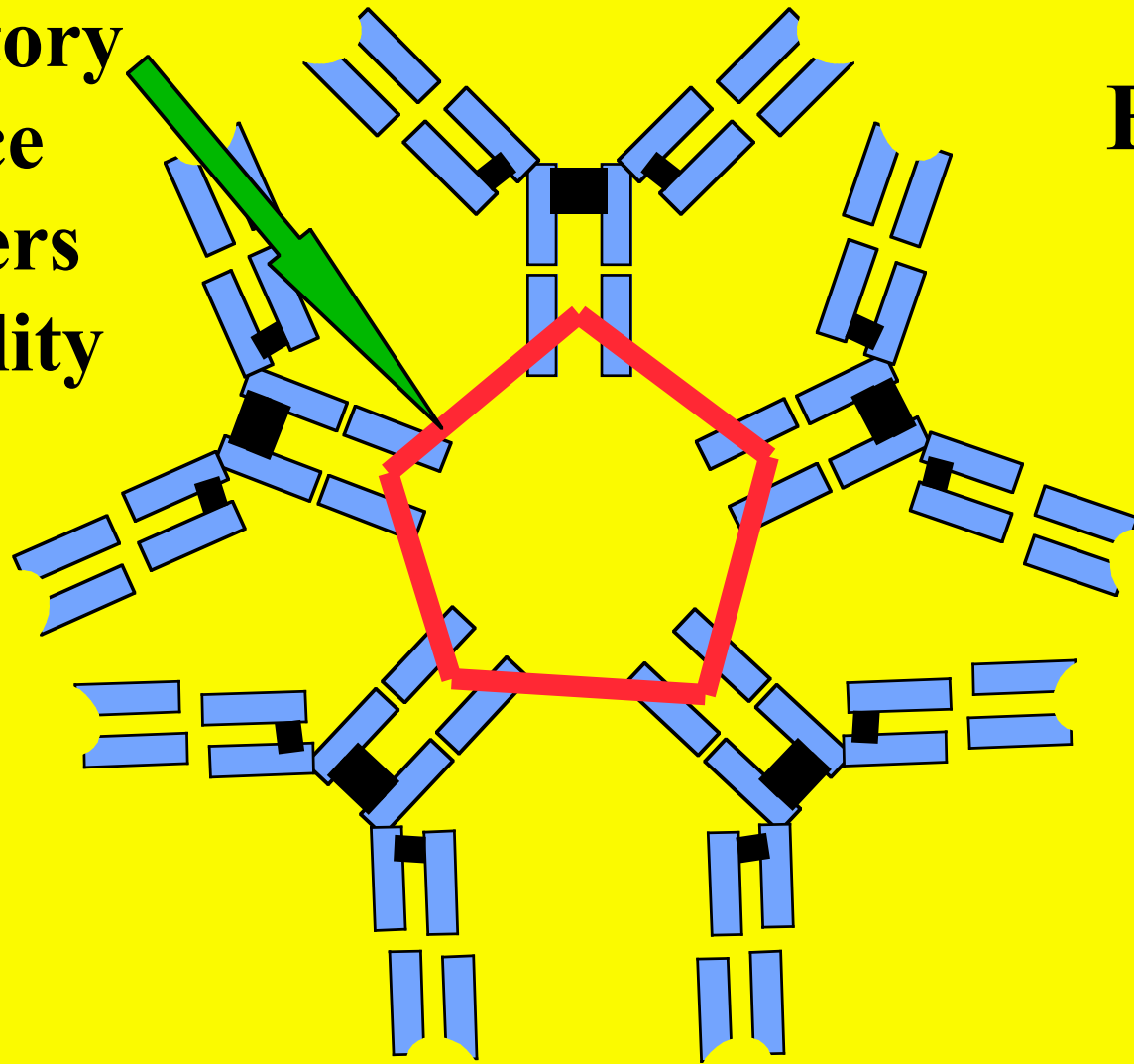


Secretory IgA



These modifications allow secretory IgA to be secreted into mucus, intestinal juices, and tears, where it protects those areas from infections.

**Secretory
Piece
confers
Stability**



**10
Binding
Sites**

SECRETORY IgM

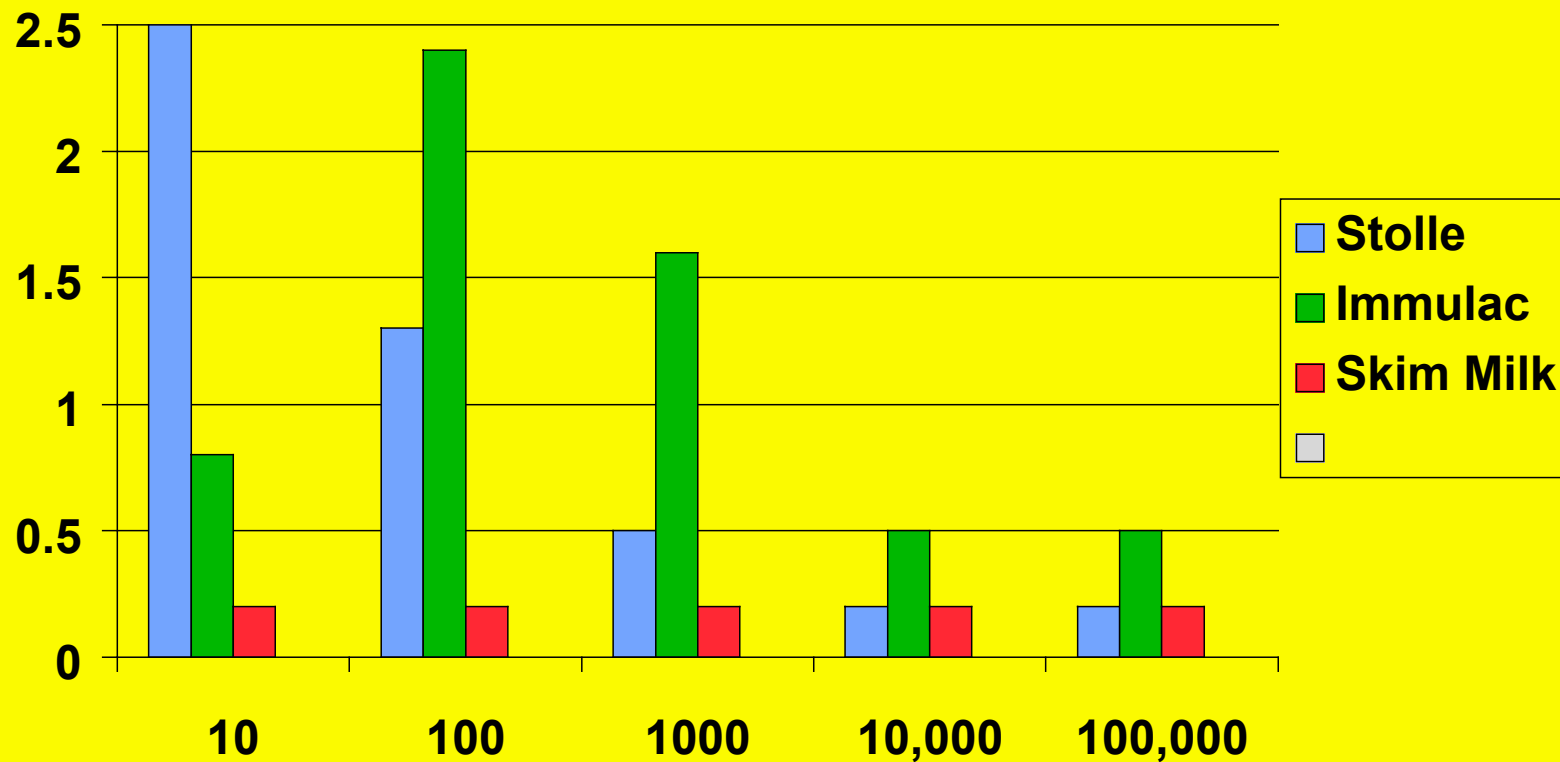
The Differences in Clostrum

IgG	sIgA	sIgM
♣45 mg/ml	♣5.5 mg/ml	♣6.9 mg/ml
♣~80%	♣~10-15%	♣~10-15%
♣150,000 Daltons	♣380,000 Daltons	♣980,000 Daltons
♣2 ABS	♣4 ABS	♣10 ABS
♣Specific	♣Specific & Non-specific	♣Specific & Non-specific
♣Blood, Tissue Fluids, Milk & Colostrum	♣Mucous Fluids, Tissue Fluids, Milk & Colostrum	♣Mucous Fluids, Tissue Fluids, Milk & Colostrum
♣ 90 ABP	♣ 22 ABP	♣69 ABP

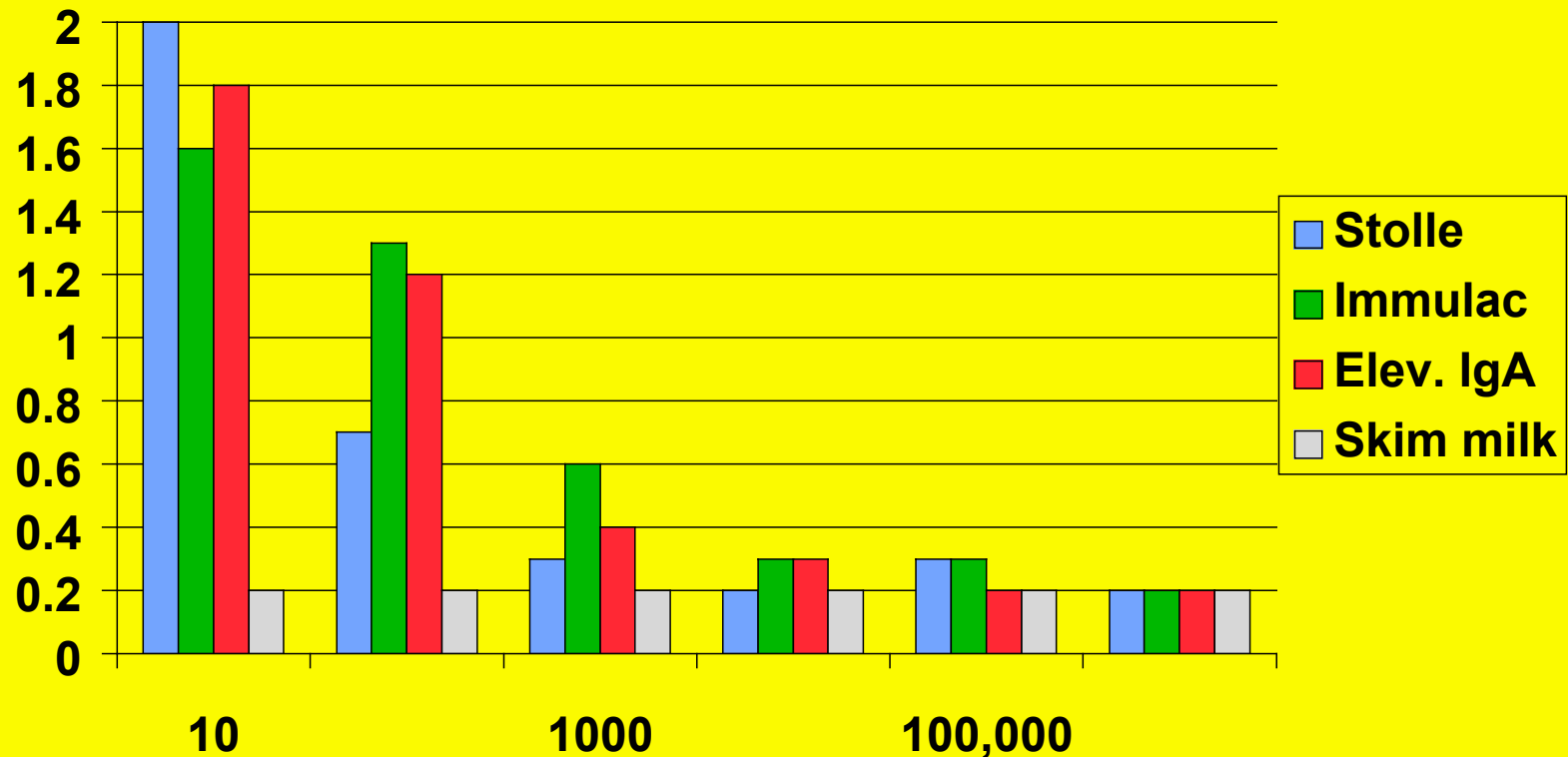
Antibody Titre

- The titre or potency of antibody solution is measured by limiting diluting
- The more potent or concentrated the antibody solution the greater it can be diluted

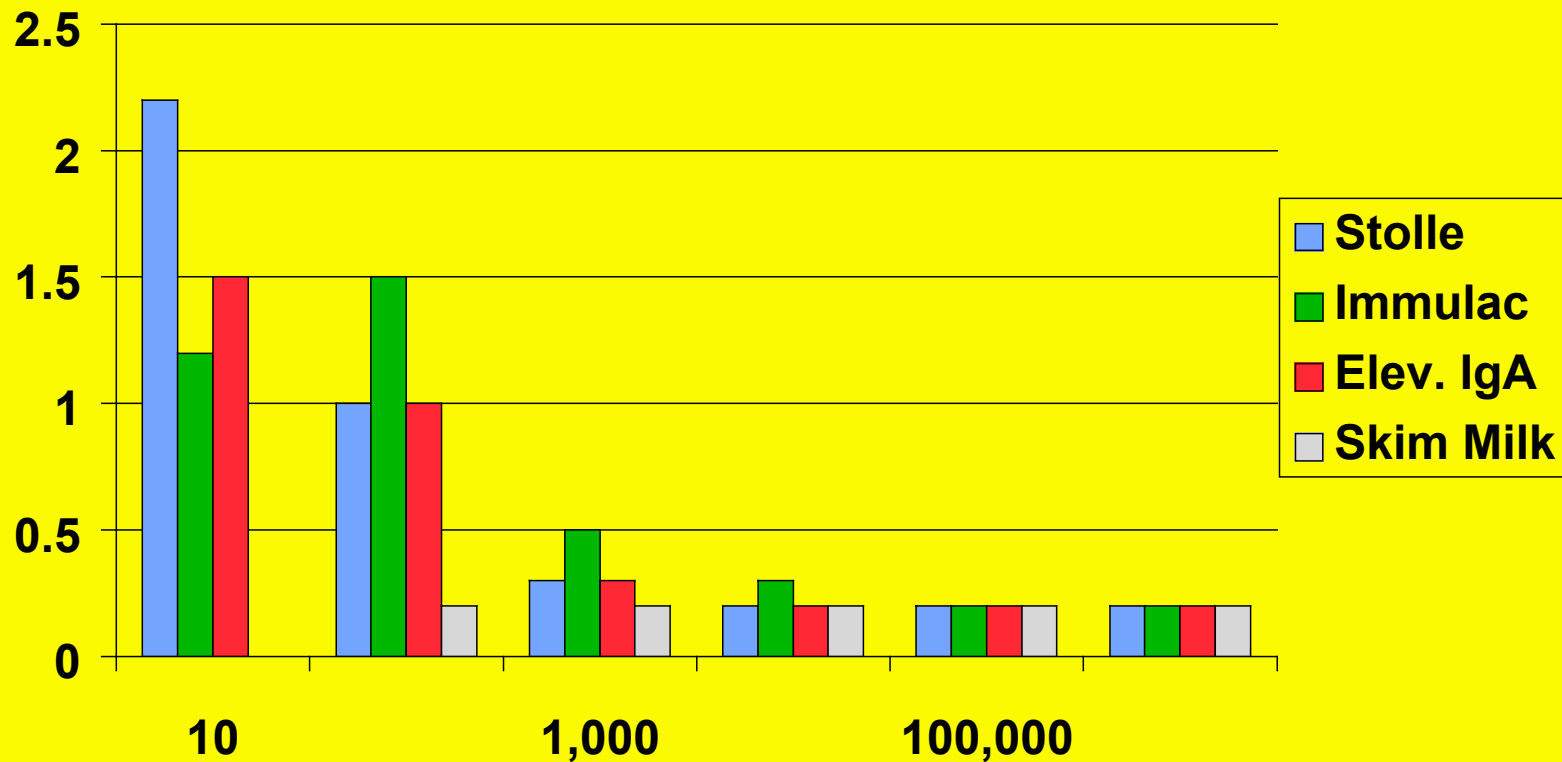
Specific IgG Antibody Titre to *Candida albicans*



Specific IgA Antibody Titre to Candida albicans



Specific IgM Antibody to *Candida albicans*



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Antigen Binding Potential (ABP)

*“A quantifiable measure of
affinity, avidity, and antibody action”*

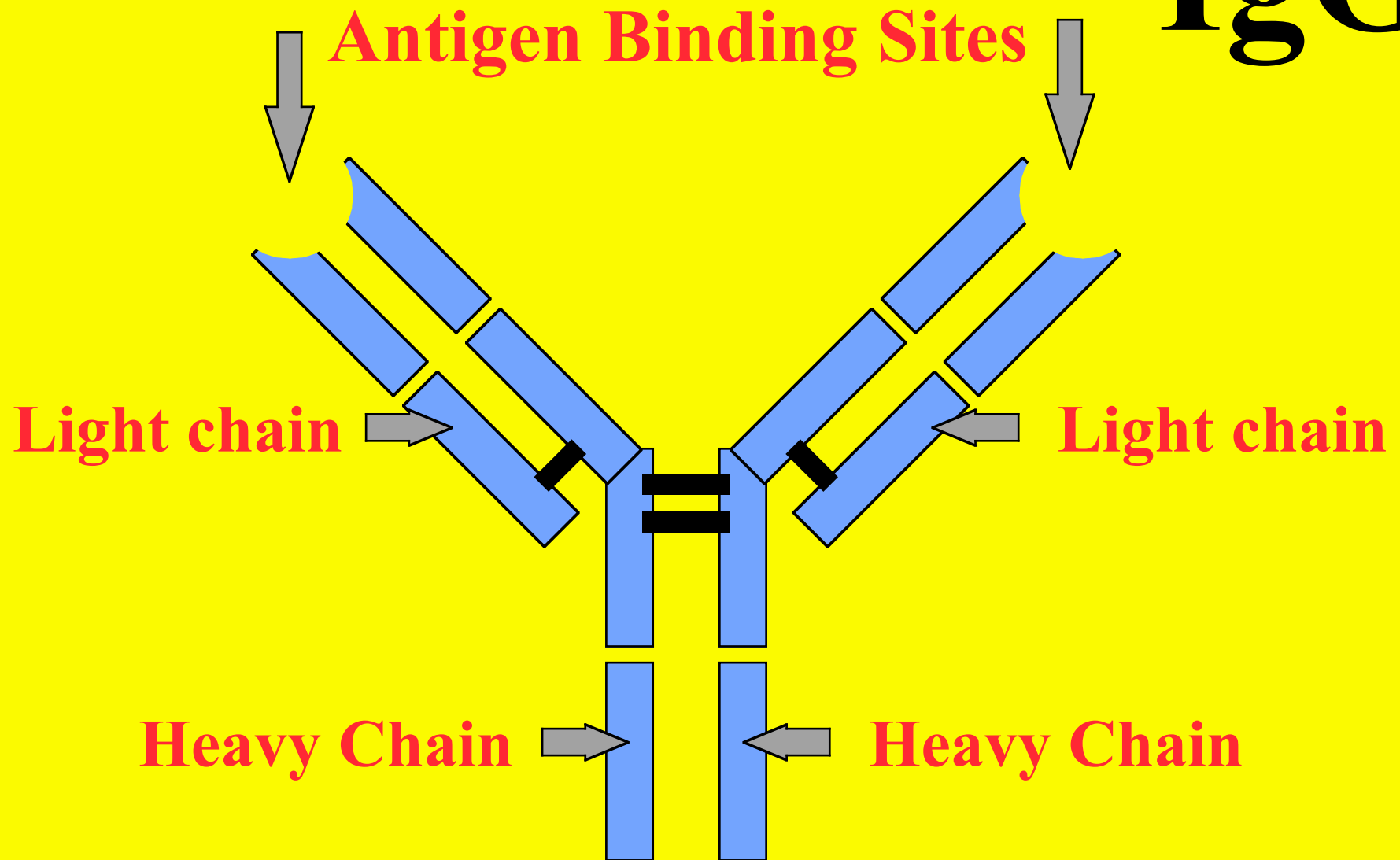
ABP of IMMUNOGLOBULINS

IgG	IgA	IgM
♣45 mg/ml	♣5.5 mg/ml	♣6.9 mg/ml
♣~80%	♣~10-15%	♣~10-15%
♣2 ABS	♣4 ABS	♣10 ABS
♣90 ABP	♣22 ABP	♣69 ABP
♣90 Pathogens/ml	♣22 Pathogens/ml	♣69 athogens/ml
♣50% Activity	♣12% Activity	♣38% Activity

Immunoassay vs Affinity assay

- Antibody antigen reaction vs Attracton
- Qualitative assay
- Quantitative assay
- Types of immunoassays
- The choice

IgG



Radial Immuno-Diffusion

- Simple, inexpensive
- Monospecific antibody in gel
- Sample (antigen) added to well
- Sample migrates out
- At equivalence migration stops and precipitin ring forms
- Ring area proportional to concentration
- Sample ring sizes compared to standards for quantitation
- An accepted immunoassay system
- Specific
- Can be used quantify many proteins and substances

Protein G HPLC

- Recombinant Protein G binds Fc portion of IgG
- Protein G assay developed by NZ Dairy Research Institute
- Standard test method for Asian/South Pacific Rim
- NZ, Australia
- Capture incorporating HPLC
- Amount of IgG is measured spectrophotometrically
- Precise objective
- Nice trick
- Only good for IgG

Protein G vs RID

- Protein G
- 7 minutes
- automated
- precision of HPLC
- Nice trick
- Expensive
- Only good for IgG
- RID
- 4 days
- manual
- Relative
- Technical expertise
- Immunoassay
- Inexpensive
- All Biochemicals

IgG ISSUES

- Solubility
- Test Method
- Standardisation

SOLUBILITY

- processing
- solvent
- concentration
- dissolution

Processing can affect results

- Solubility Index of UF'ed colostrum:
 - Sample 1 (7.5)
 - Sample 2 (5.5)
 - Sample 3 (8.0)
- Solubility Index of Skim colostrum:
 - Sample 4 (0.1)
 - Sample 5 (0.1)
 - Sample 6 (0.2)

Hypothesis

- *Sample preparation affects results obtained.*

Dissolution solvent may affect result.

DI Water 7.0 mg/ml

PBS 16.3 mg/ml

Dissolution time is not long enough to solubilize product for test

Time (min)	Stirred	Not Stirred
10	60 mg/ml	
70	154	78 mg/ml
130	241	120
250	310	288

Dissolution temperature can affect results

Time(min)	0C	24C	40C
10	331mg/ml	347 mg/ml	405 mg/ml
70	369	455	526
130	338	494	474
190	414	465	458
360	473	470	

Sample concentration can affect result

Sample	recon conc	DRI-HPLC
1	2	26.5
	10	22.0
2	2	17.8
	10	13.3
3	2	26.7
	10	19.9
4	2	19.1
	10	11.7
5	2	15.5
	10	10.7

Complete Dissolution at 40C for Two Hours

Conc mg/ml	Quant mg/ml
2	20.1
5	20.6
10	20.6
25	20.6

RID and Protein G Test Methods Give Different Results

Sample	DRI-RID	DRI-HPLC
1	30.2 mg/ml	23.2 mg/ml
2	9.6	16.6
3	13.0	28.6
4	9.2	24.4
5	10.0	17.5
6	7.8	12.0

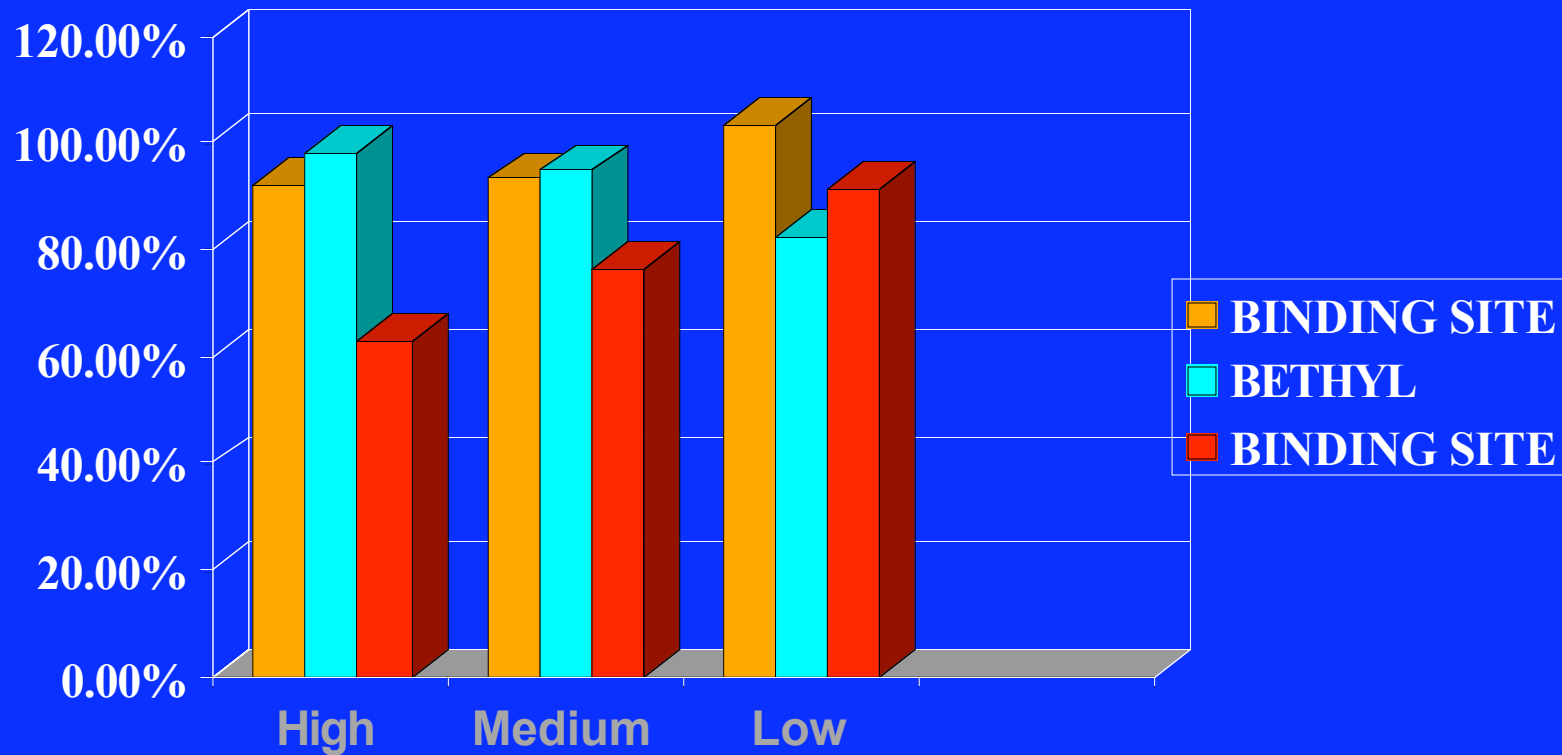
TEST METHODS

- *WHY ARE THE RESULTS DIFFERENT?*
- *WHAT MAKES THE RESULTS DIFFERENT?*

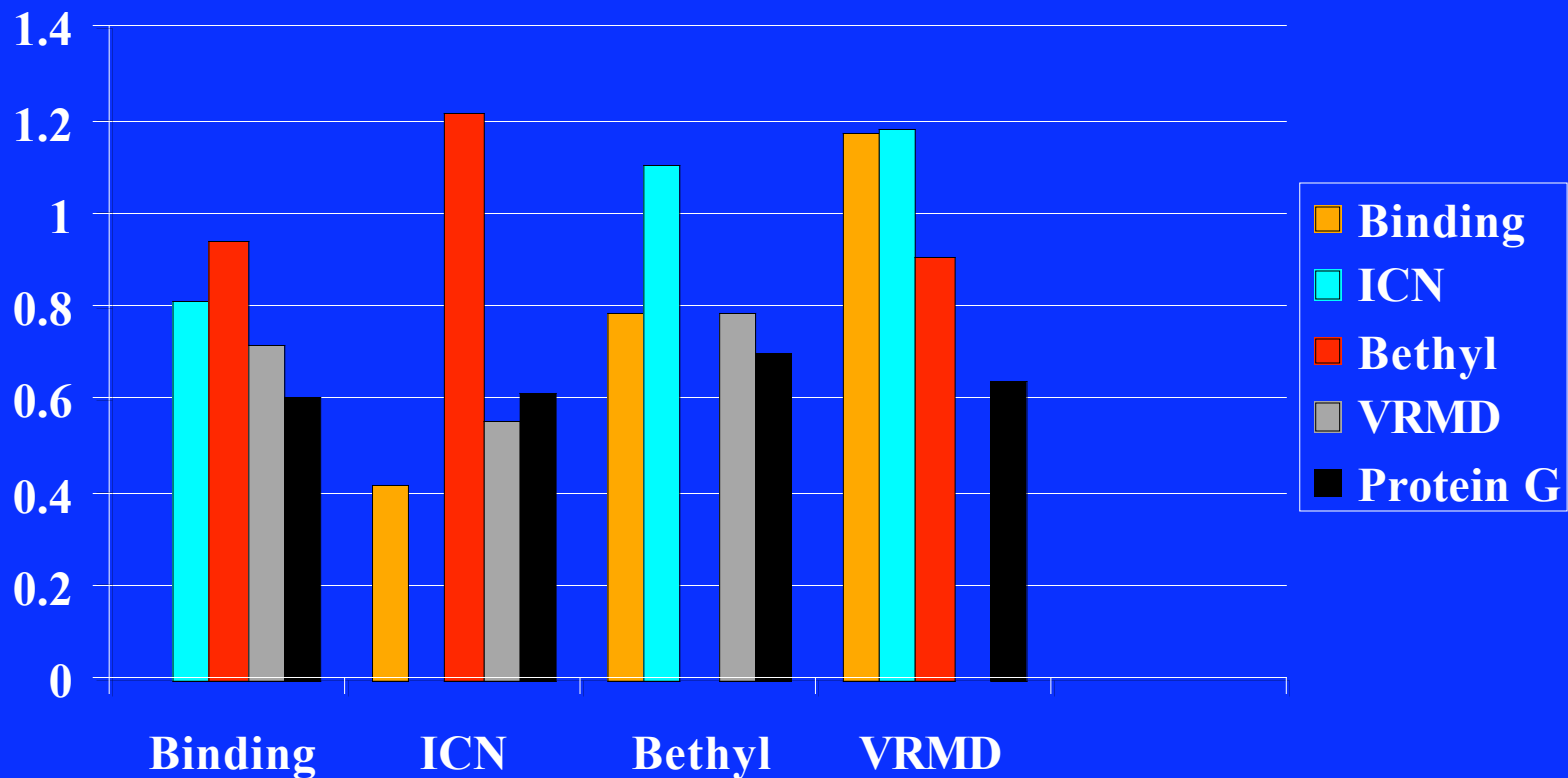
TEST METHODS

- Comparison study incorporating four commercially available RID test kits vs HPLC Protein G quantification.
- BETHYL
- VMRD
- BINDING SITE
- ICN

COMPARISON STUDY RID STANDARDS BY PROTEIN G



Comparison Study - Protein G and RID Standards By RID



*The Imperial “Foot Measure” is
Based on the Length of Henry
VIII’s Foot*

QUESTIONS