

STARWARS OF THE BODY BESEIGED

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EVALUATION OF IMMUNITY: An Overview and Review

Harry R. Hill, M.D.

Objectives:

- To review the major portions of the immune system and relate the components to “Star Wars” of the body**
- To describe how patients with defects in different portions of host defense present clinically**
- To describe the laboratory tests utilized in defining defects in the immune system**

Immunologic Deficiencies in Physician Training

This editorial appeals for the repair of a major deficiency in the training of physicians (an immune deficiency), namely an appraisal of immunodeficiency as a cause of specific infections. A primarily “parasite oriented workup of an infection and especially recurrent infections will no longer suffice as the patient’s host response clearly determines the clinical presentation and outcome of specific infections.

Gene H. Stollerman, M.D. J. Chronic Diseases

Immunodeficiency in Training Physicians

- We teach about all of the parasites, viruses, fungi, & bacteria in great detail.
- We teach little, however, about how the body responds to each of these types of pathogens.
- The responses of the host determines the degree, severity, and type of symptomatology and the outcome of infection in all instances, i.e. HIV!!

CASE PRESENTATION

The patient was a 16 month old male infant who was brought in by his mother who complained that he was always sick. The patient had suffered from one to two upper respiratory infections per month since 4 months of age. There had also been two middle ear infections and a number of 'sore throats.' The patient had never been hospitalized and had grown normally. Because of the recurrent infections, the patient received numerous courses of antibiotics and was currently receiving 0.2cc of gammaglobulin per month.

CAUSES OF RECURRENT INFECTIONS

- The normal child may suffer 6-12 infections per year. Day Care Centers increase this.
- Structural and anatomic defects must be ruled out
- Immune deficiency is a possibility

Physical and Anatomic Defects

Foreign Bodies:	Pulmonary - peanuts, carrots, bacon, portacaths, vascular lines. catheters,
Breakdown Barriers:	Skin, mucous membrane, fractures, burns, eczema
Anatomic Problems:	Eustation tubes, ureters, sinuses, CF, dermal sinuses, basilar skull fx

The Normal Child with Too Many Infections

- 6 infections per year average in preschool, early school-aged child
- Normal to go as high as 12, however
- Daycare average is 9/year
- Infections generally last 2 weeks with prodrome, acute and convalescent phases
- 12 infections X 2 weeks = 24 weeks = 6 months/yr!!!!!! Oh No!!!!!!



MEN NEVER REALLY
LISTEN TO WHAT
WOMEN ARE SAYING,
DO THEY?

SCHULZ



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WE'VE GOT YOU OUTNUMBERED!



IT WAS TIME
TO GO HOME!



is reported, other in-
collected, such as
characteristics, day of
er a physician was
subsequent contacts,
ollowed up to its ter-
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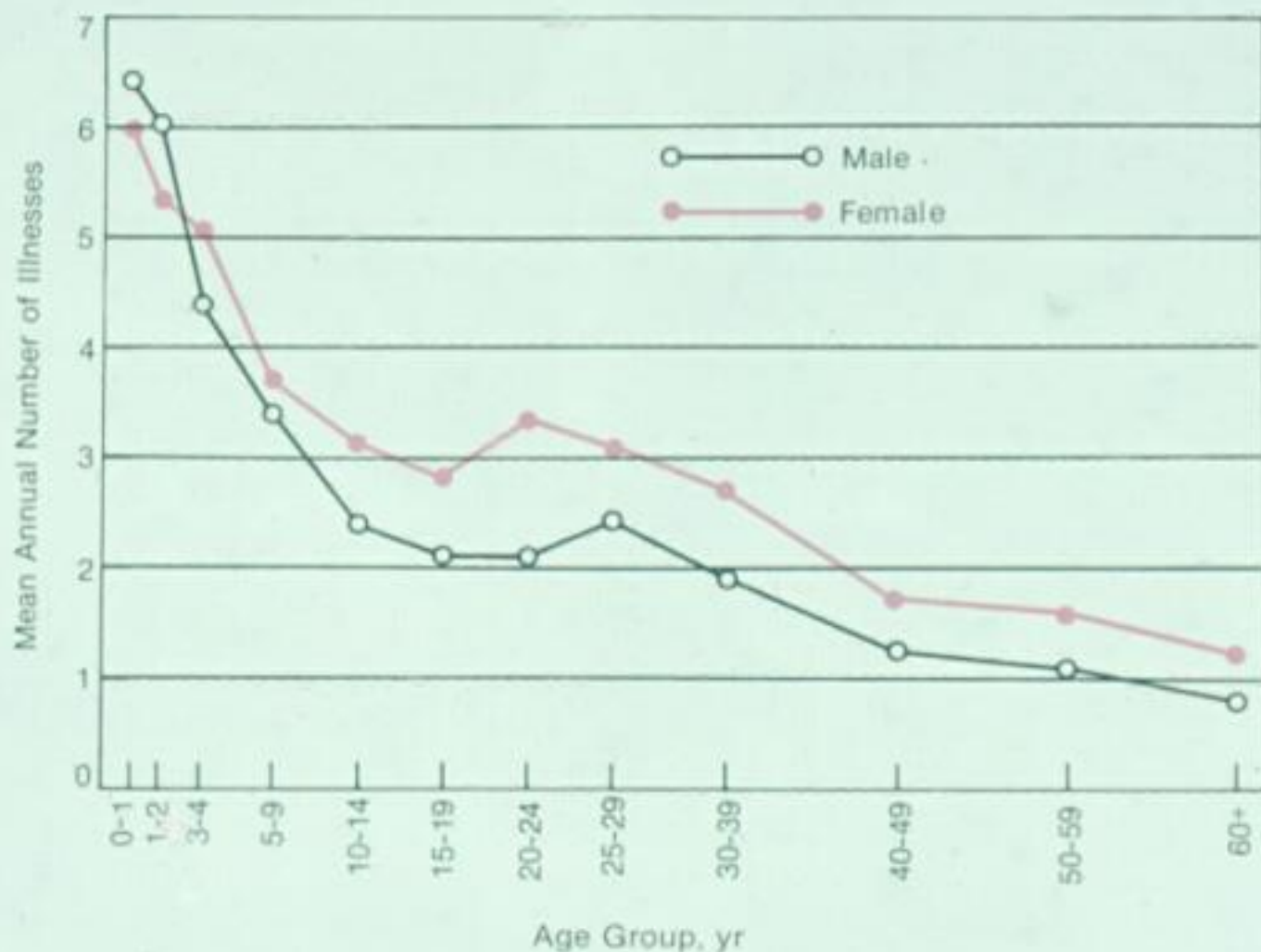


Fig 1.—Mean number of respiratory illnesses experienced per year.

A.S. Monto JAMA 227: 164, 1974

HA! I KNEW
YOU WEREN'T
ASLEEP!



“In teaching medical students, the primary requisite is to keep him (or her) awake.”

Chevalier Jackson
1865-1985

“Agreed!!!!”

Harry R. Hill, M.D.







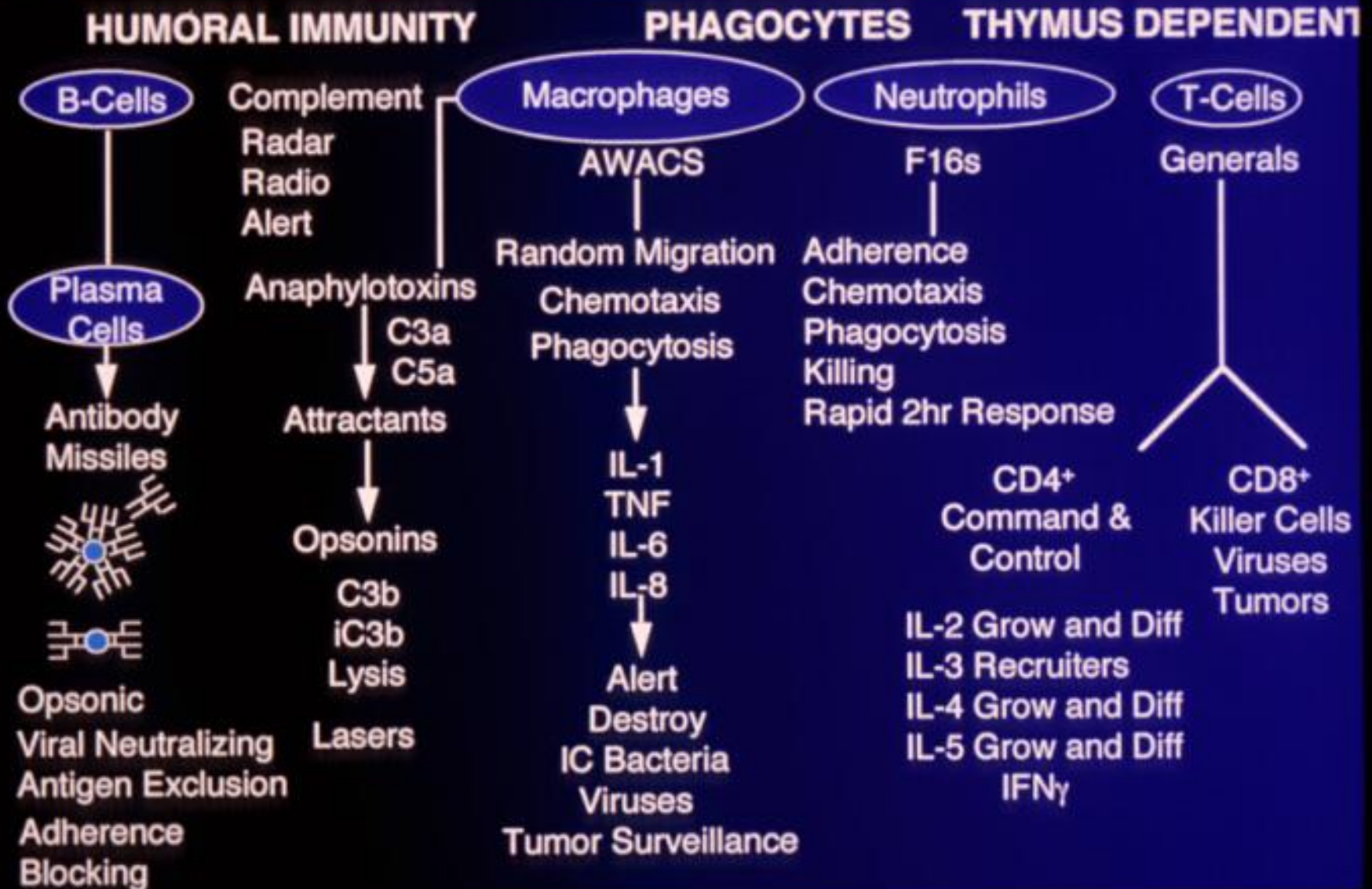




THE
RETURN OF
**STAR
WARS**

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# STAR WARS OF THE BODY BESIEGED



# STAR WARS MISSILE DEFENSE



INTERCONTINENTAL BALLISTIC  
MISSILE - IgM

M.W. - 950,000

80% Blood Stream

Antipolysaccharide Ab

Opsonin-Complement

Viral Neutralization



FIELD RANGE MISSILE  
IgG

M.W. - 150,000

Blood & Tissues

Opsonin-Complement

Viral Neutralization



POLARIS MISSILE  
IgA

M.W. - 400,000

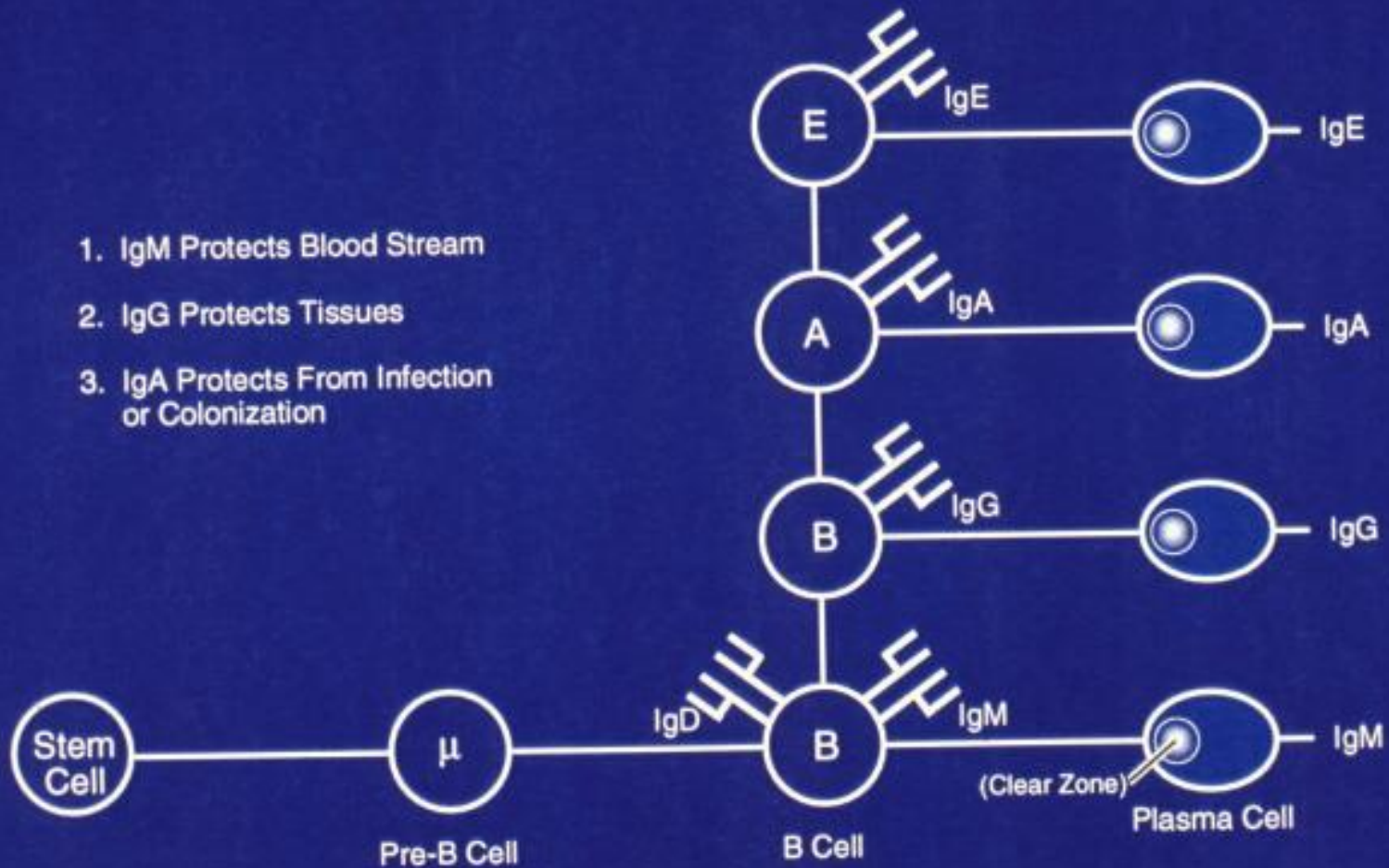
Immune Exclusion

Viral Neutralization

Prevents Bacterial  
Attachment



# DEVELOPMENTAL SEQUENCE OF ANTIBODY PRODUCTION



PICTURE THIS,  
IF YOU WILL...



# CASE HISTORY

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## 16 Year Old Male

6 mo – 8 yrs

Recurrent Otitis

8 yr – 16 yrs

Recurrent Sinusitis

12 -14 yrs

2-3 Episodes of  
Pneumonia





# LABORATORY DATA

## S.G.

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- IgG - 190 mg% (750-2000)
- IgA - 98 mg% (82-462)
- IgM - 32 mg% (63-250)
- Isohemagglutinins – Negative
- AOS - Negative; Schick - Positive
- Skin Tests - Positive
- T Cells - 40% (40-75)
- B Cells - 41% (10-25)

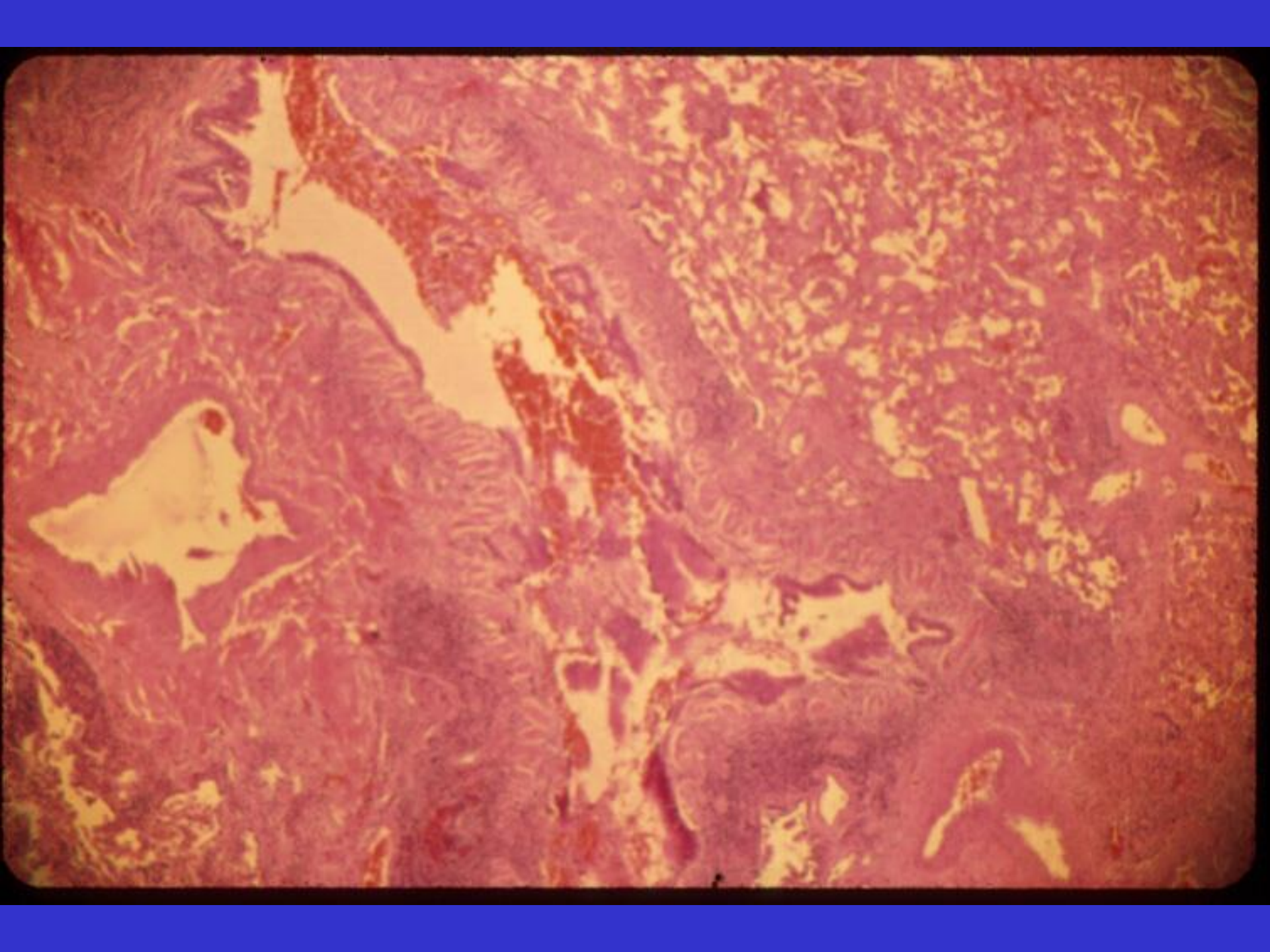




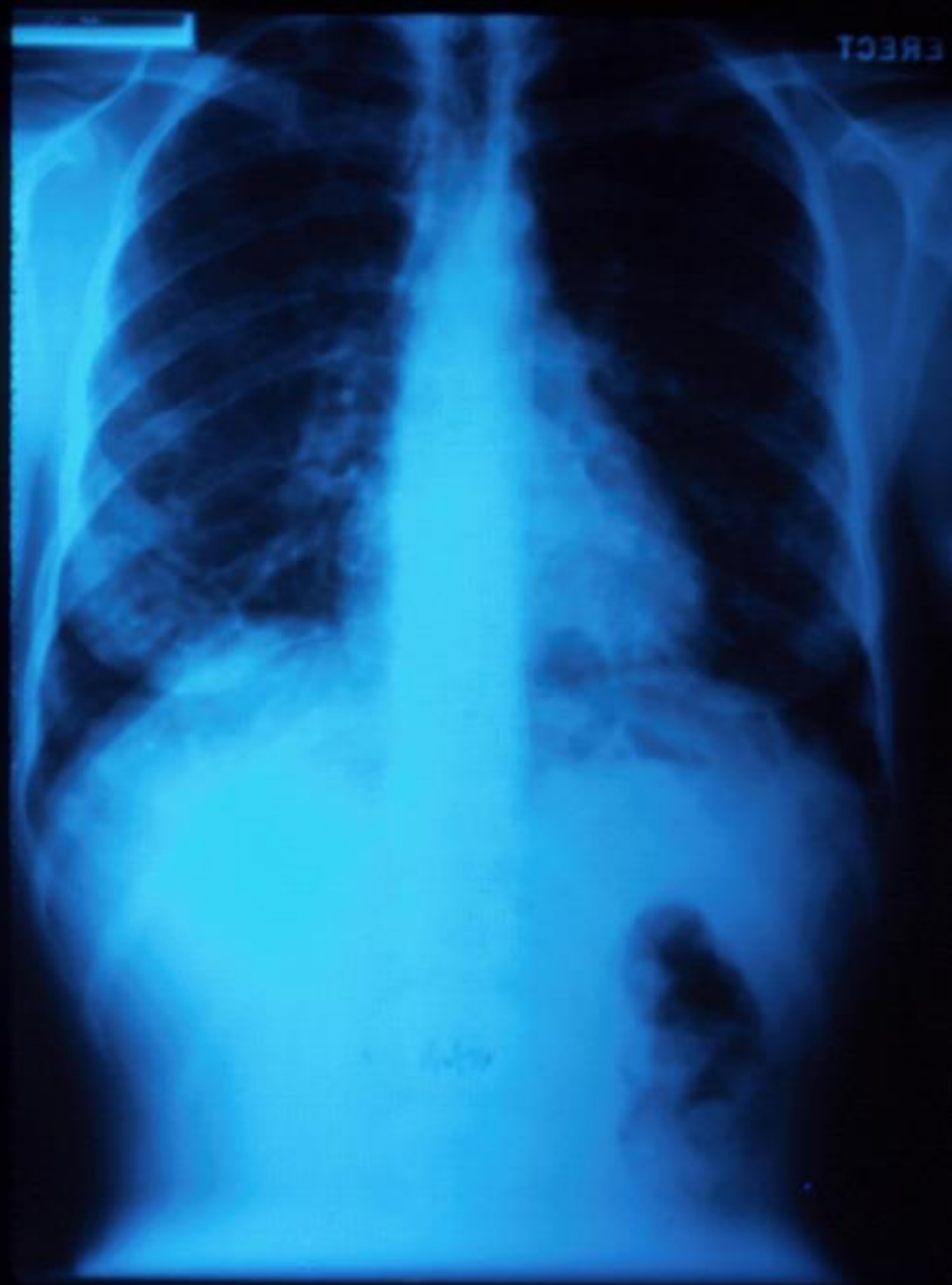
# Case History CVID

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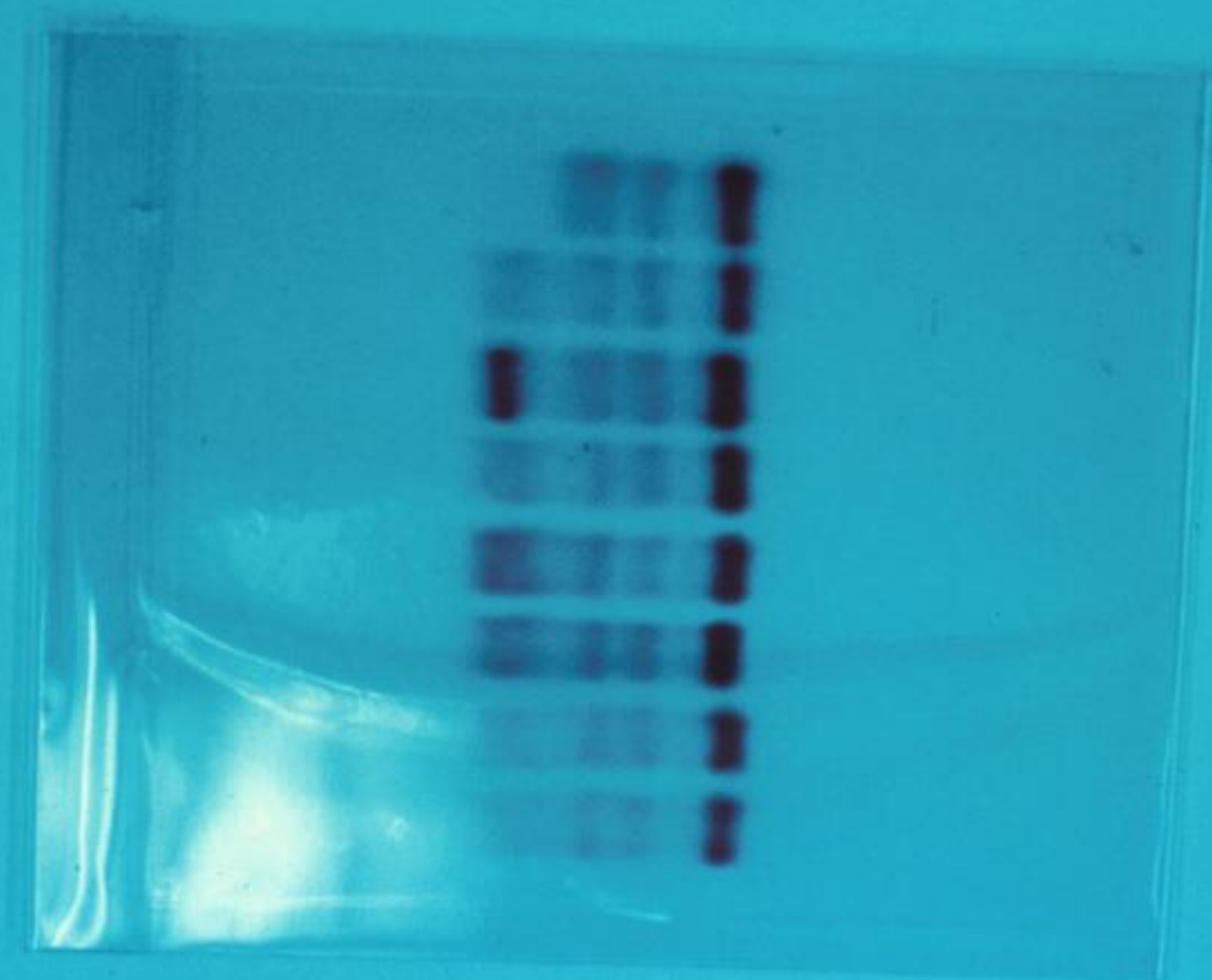
- 29 year old female who had 5 episodes of pneumonia over the past 2 years, hospitalized for 3 of these.
- Diffuse infiltrate on X-ray; ? Pigeon breeders hypersensitivity pneumonia??
- IgG 60 mg %; IgA < 6 mg%; IgM 25 mg %; (an IgG of  $\leq 250$  mg% called as a critical value!)
- B cells had normal surface immunoglobulin and were present in normal percentages



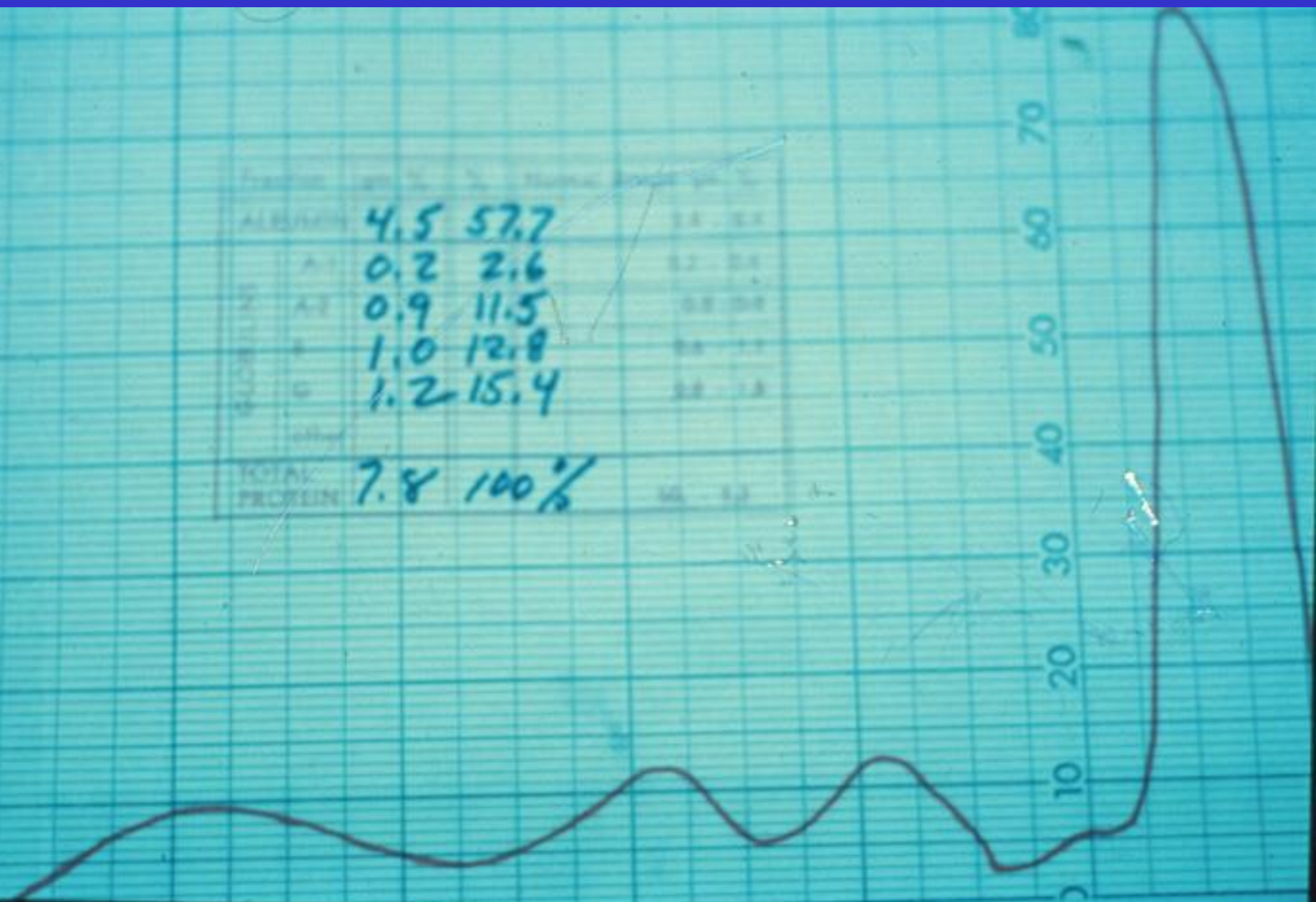








| Fraction      | wt. % | % of total protein | wt. % |
|---------------|-------|--------------------|-------|
| ALBUMIN       | 4.5   | 57.7               | 2.6   |
| A1            | 0.2   | 2.6                | 0.2   |
| A2            | 0.9   | 11.5               | 0.8   |
| B             | 1.0   | 12.8               | 0.8   |
| G             | 1.2   | 15.4               | 0.8   |
| TOTAL PROTEIN | 7.8   | 100%               | 6.2   |













70

60

50

40

30

20

10

0

| Fraction      | gm % | %    | Normal Range | gm %      |
|---------------|------|------|--------------|-----------|
| ALBUMIN       | 3.7  | 41.1 | 3.4 - 5.4    |           |
| GLOBULINS     | A1   | 0.2  | 2.2          | 0.2 - 0.4 |
|               | A2   | 0.9  | 10.0         | 0.5 - 0.9 |
|               | B    | 0.8  | 8.9          | 0.6 - 1.1 |
|               | G    | 3.4  | 37.8         | 0.8 - 1.5 |
| other         |      |      |              |           |
| TOTAL PROTEIN | 9.0  | 100% | 6.0 - 8.3    |           |





Destain

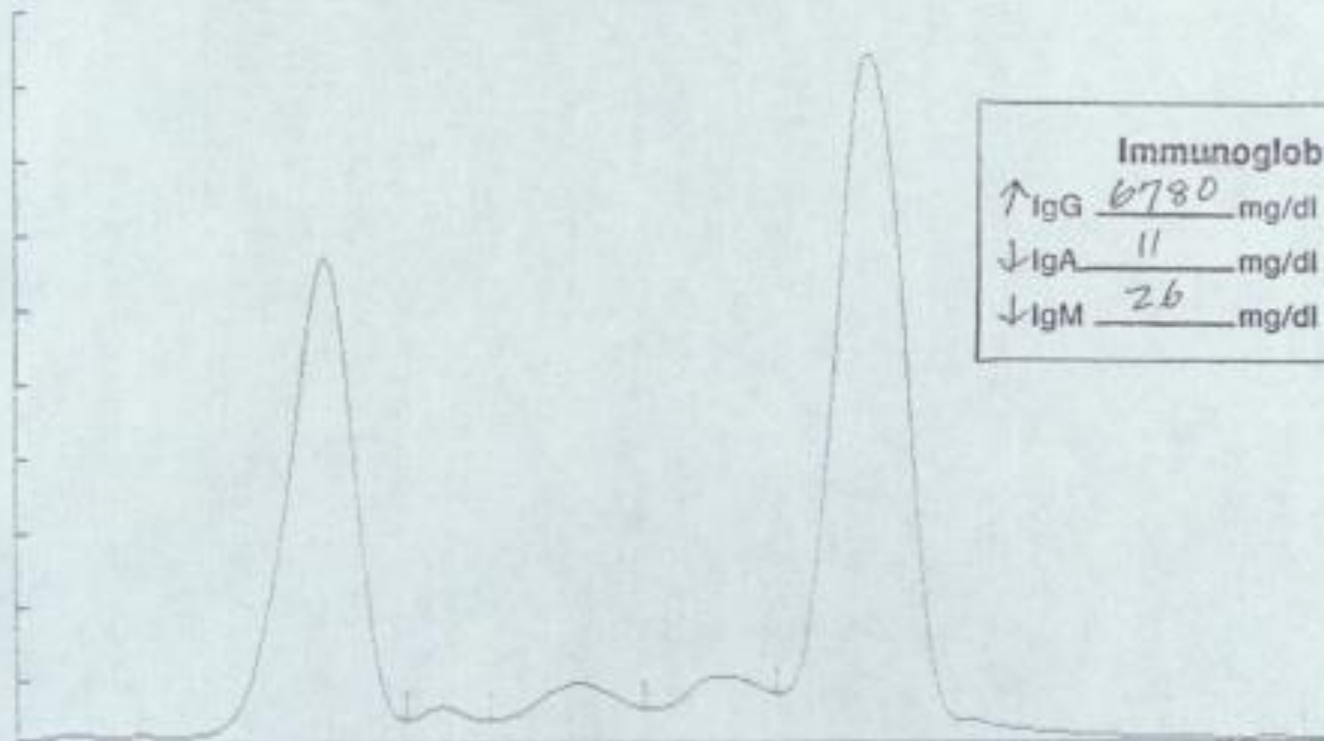
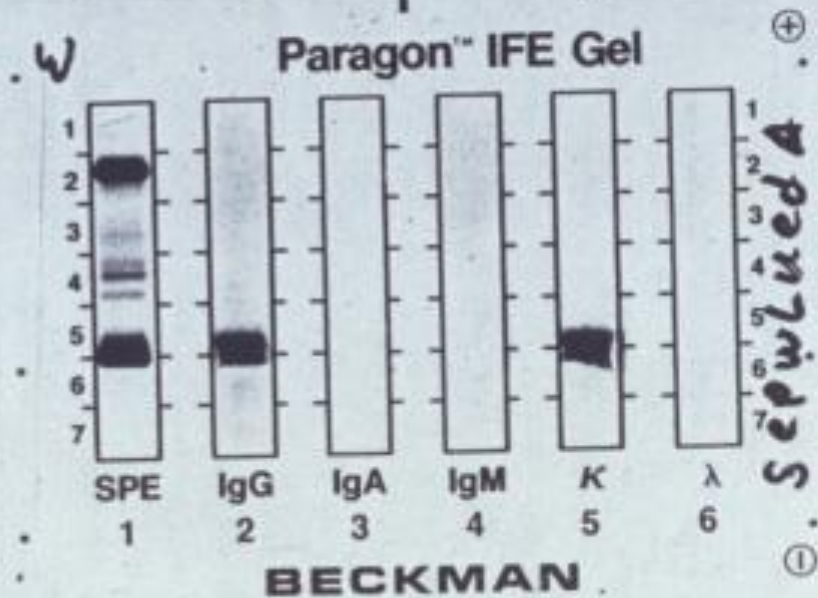
LOT # 0506-101  
EXP. 2009/10

Sebia Wash

Lot # 26037/11  
Exp. 2010/03

VWR  
Light Duty Floor Wipes  
VWR  
Light Duty Floor Wipes

SAVE for  
Antisera  
COVER



#### Immunoglobulin Levels

↑ IgG 6780 mg/dl (Normal 800-1800)

↓ IgA 11 mg/dl (Normal 90-450)

↓ IgM 26 mg/dl (Normal 60-260)



# IMMUNOGLOBULIN G SUBCLASSES\*

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| <u>Characteristic</u> | <u>IgG1</u> | <u>IgG2</u> | <u>IgG3</u> | <u>IgG4</u> |
|-----------------------|-------------|-------------|-------------|-------------|
| % in Serum            | 70          | 21          | 5           | 4           |
| Half-Life Days        | 23          | 23          | 11          | 23          |
| C1q Binding           | ++++        | ++          | ++++        | -           |
| Sensitize Cells       | -           | -           | -           | +           |
| Polysaccharide AB     | -           | +++         | -           | -           |
| Protein Ab (D,T)      | ++++        | -           | ++          | -           |
| Viral Protein AB      | ++          | -           | ++++        | -           |

\*Based on antigenic and structural differences of heavy chains.

*Luminex SD*

STATUS ● ● TEST

POWER ■

REGULATOR ●

100 L/min

100 L/min

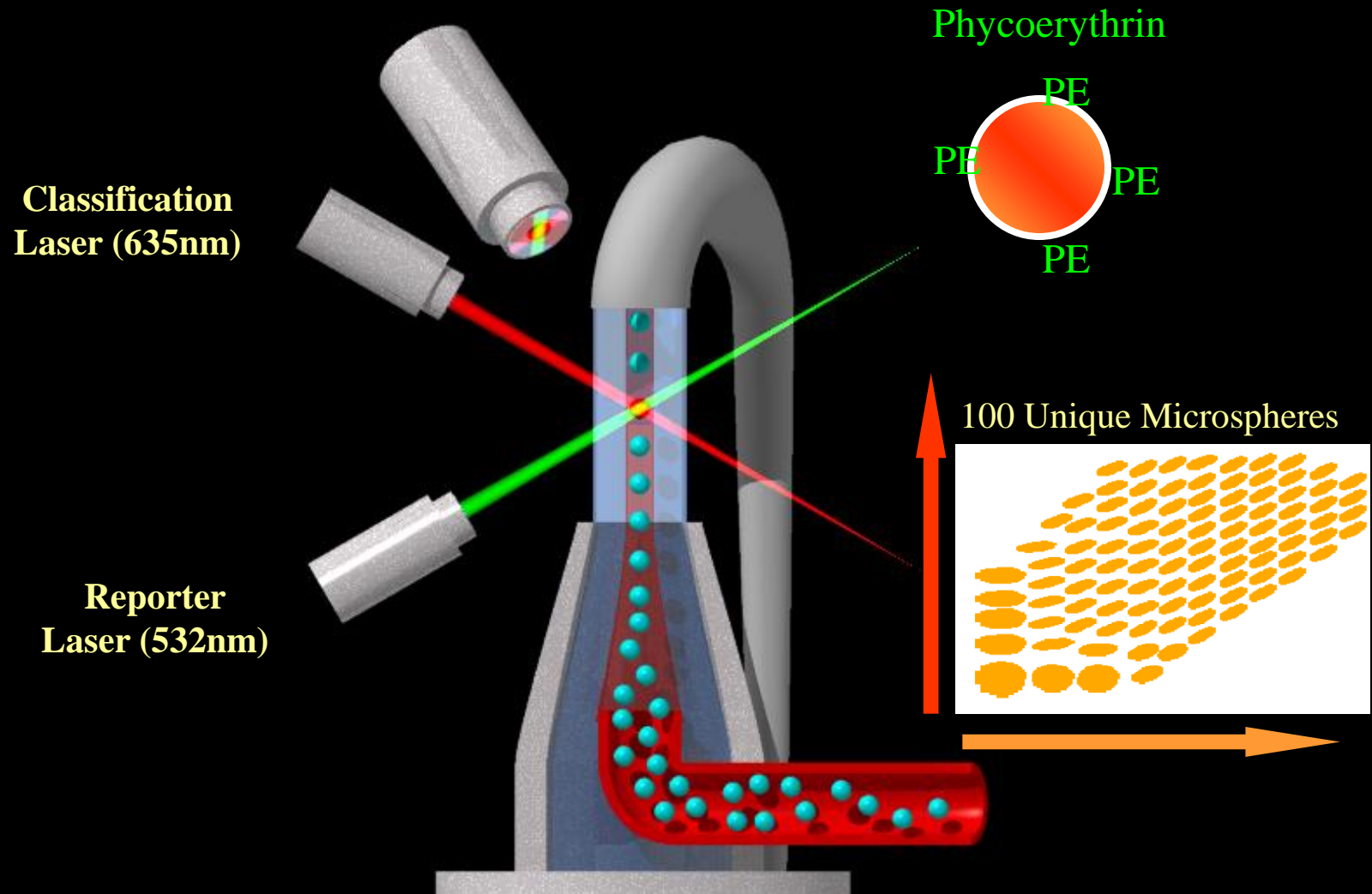
100 L/min

*Luminex<sup>100</sup>*

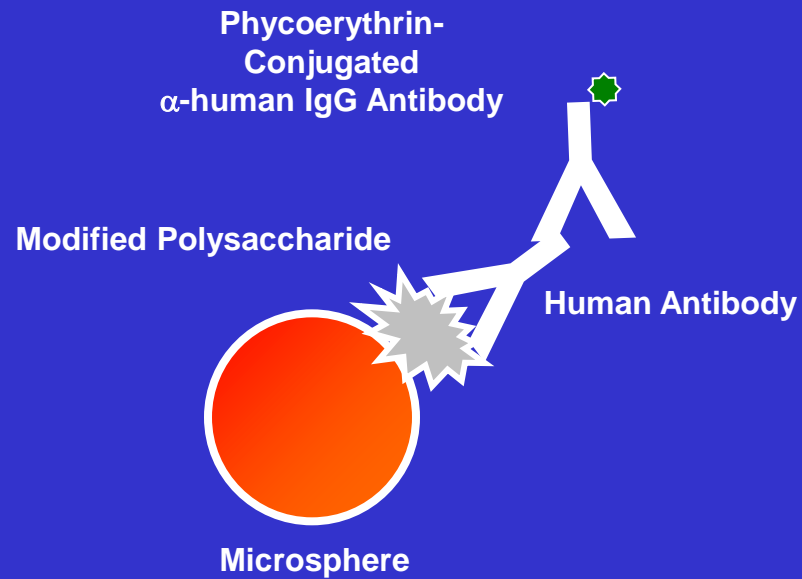
*Luminex*  
LUMINEX

*Luminex*

# Luminex 100 System

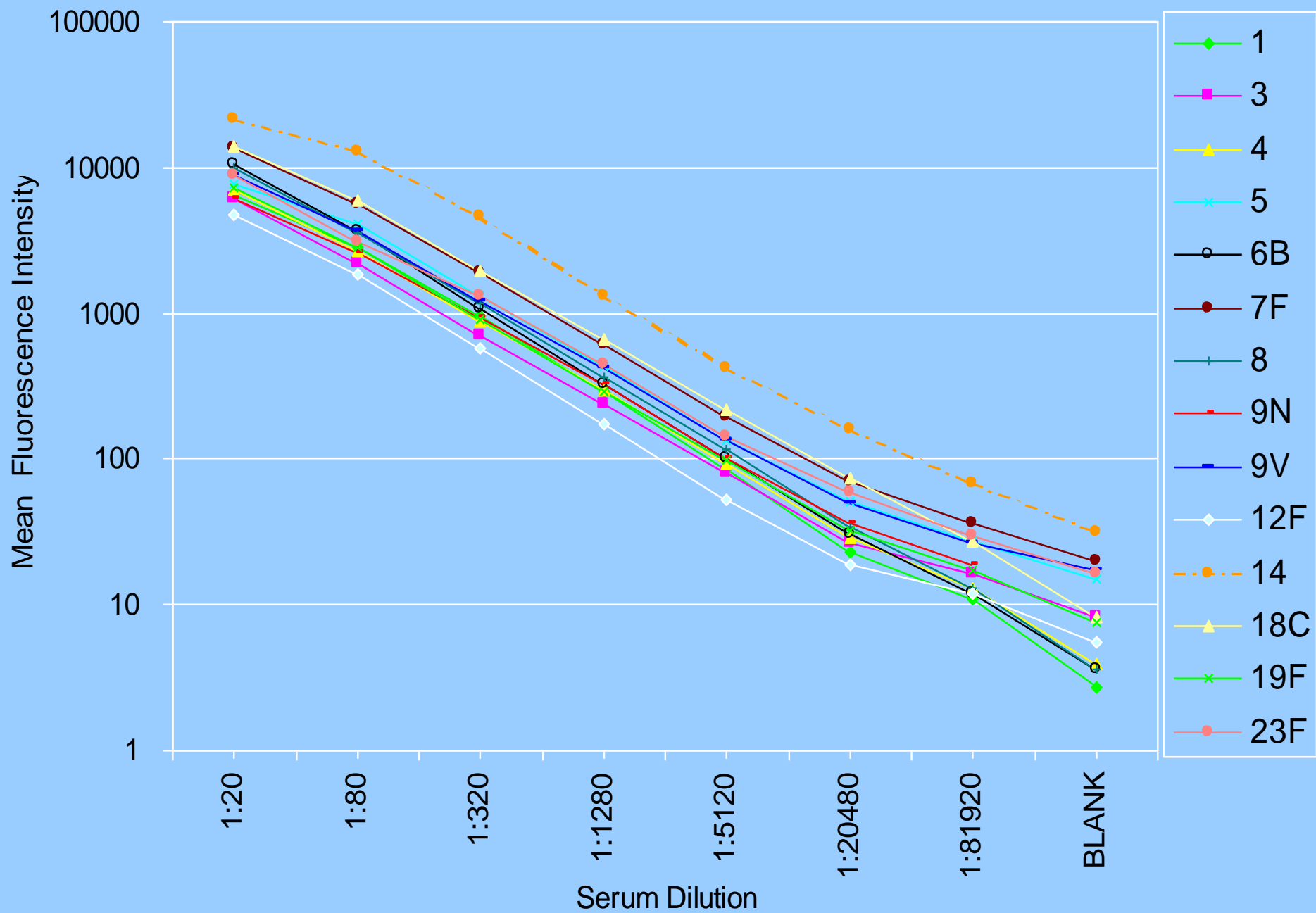


# Pneumococcal Serotype Assay

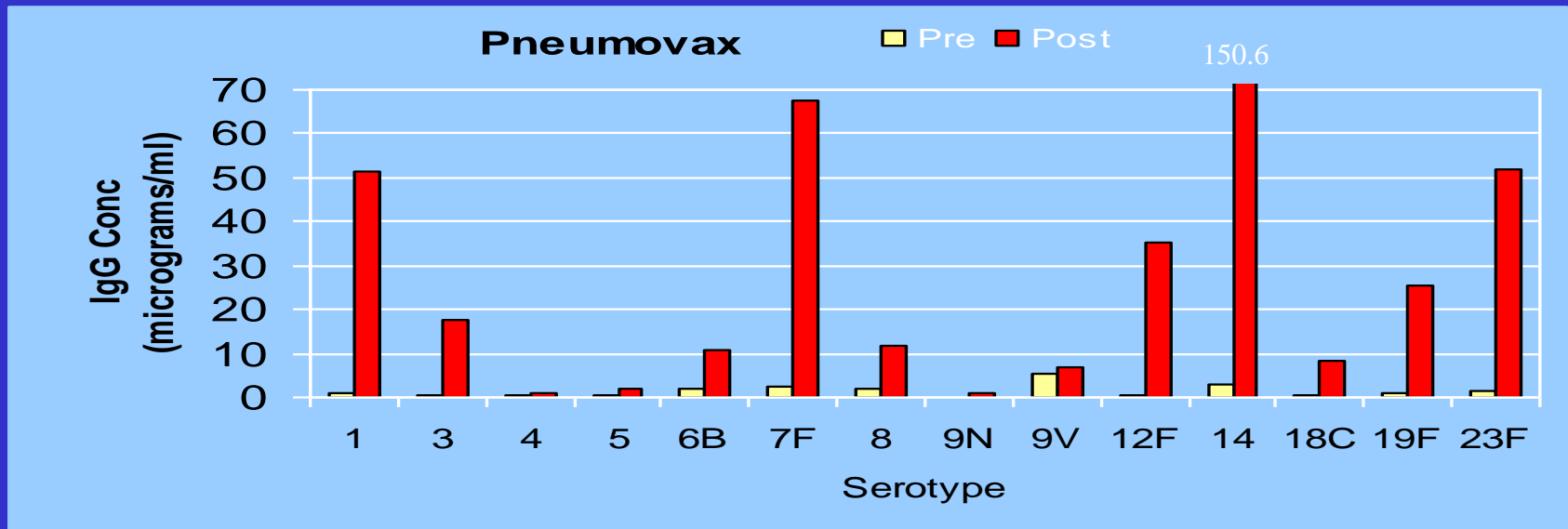
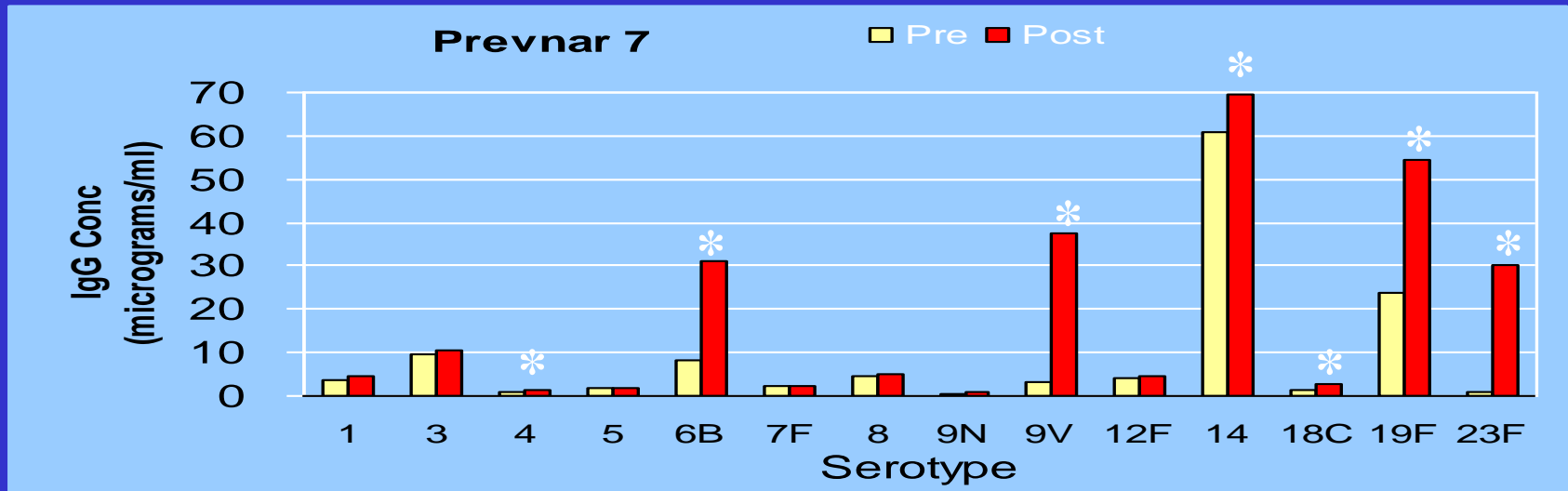




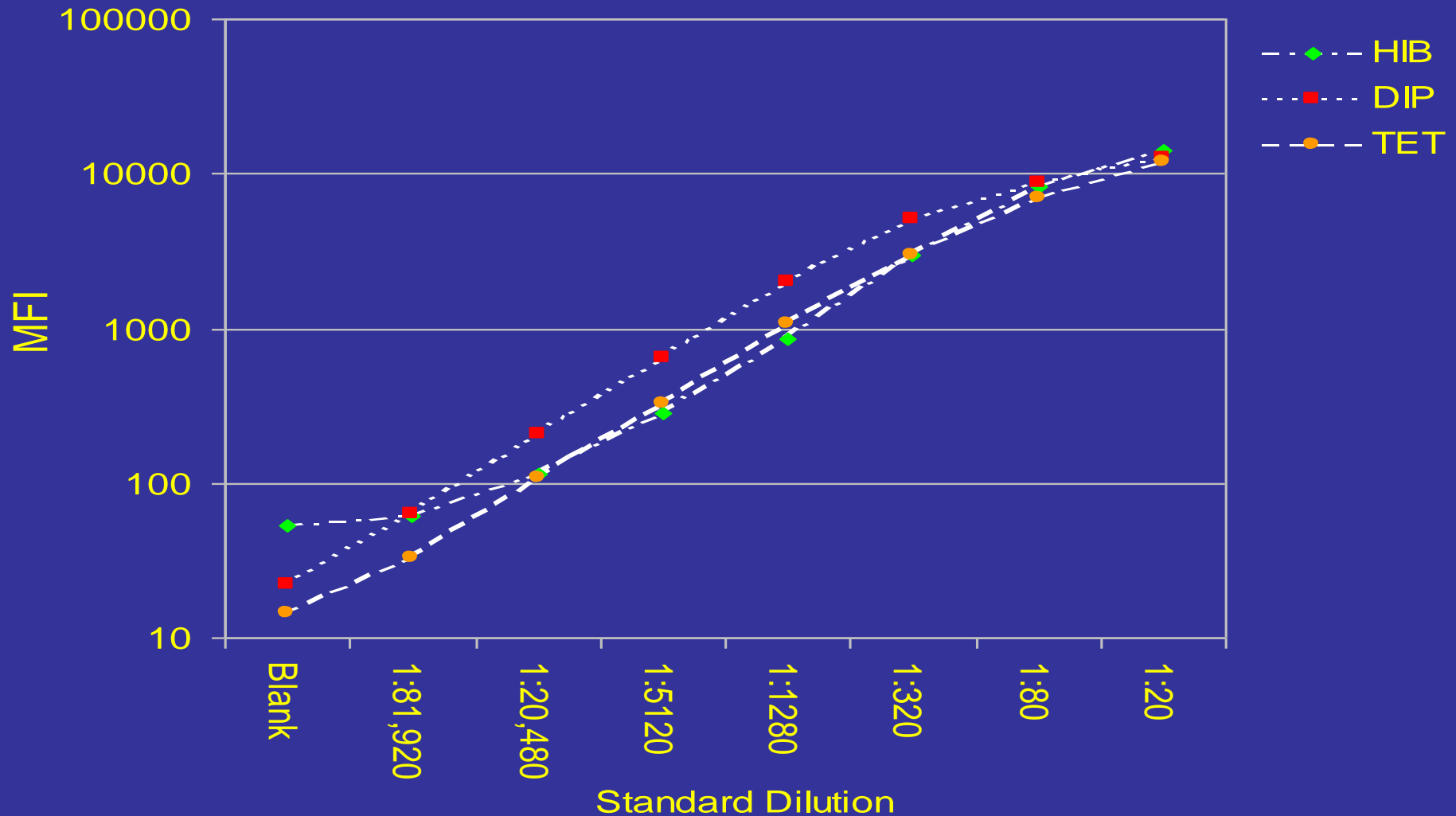
# Titration of Pneumococcal Reference Serum



# Pneumococcal Antibody Concentrations for Pre and Post Vaccination Sera determined by Luminex

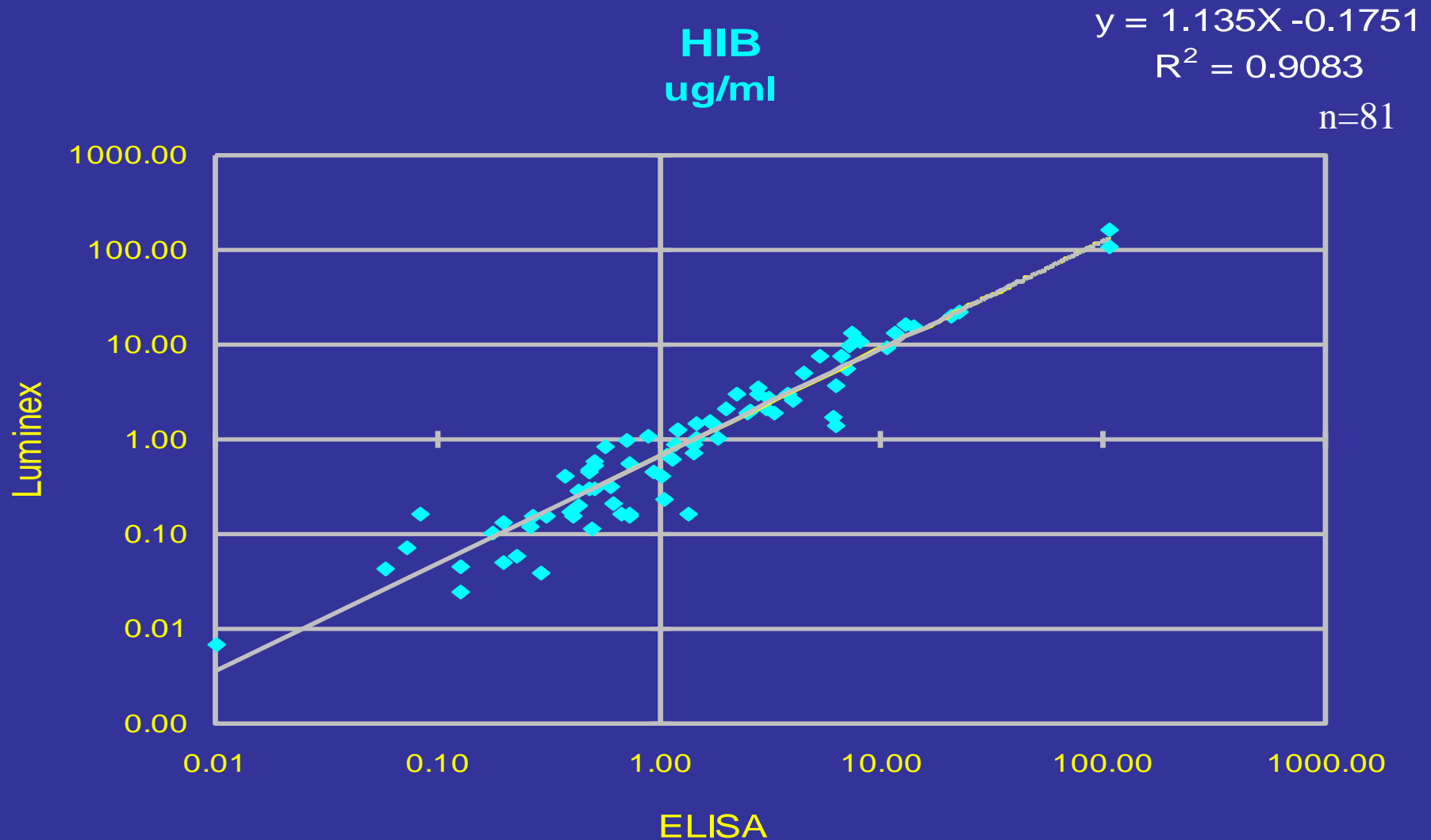


# Standard Curve for Multiplexed Luminex Assay for Tetanus, Diphtheria and *Haemophilus influenza* type b

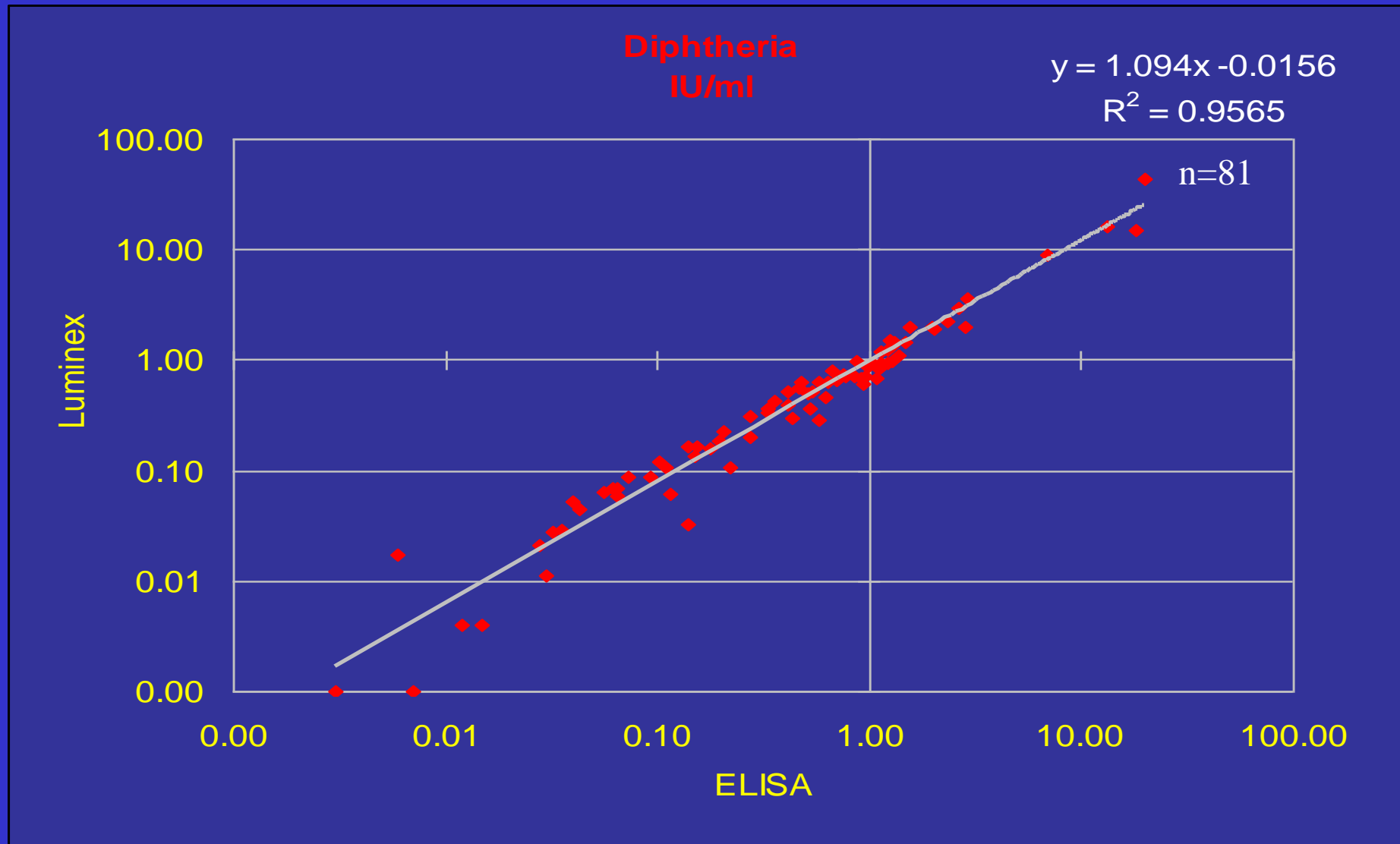




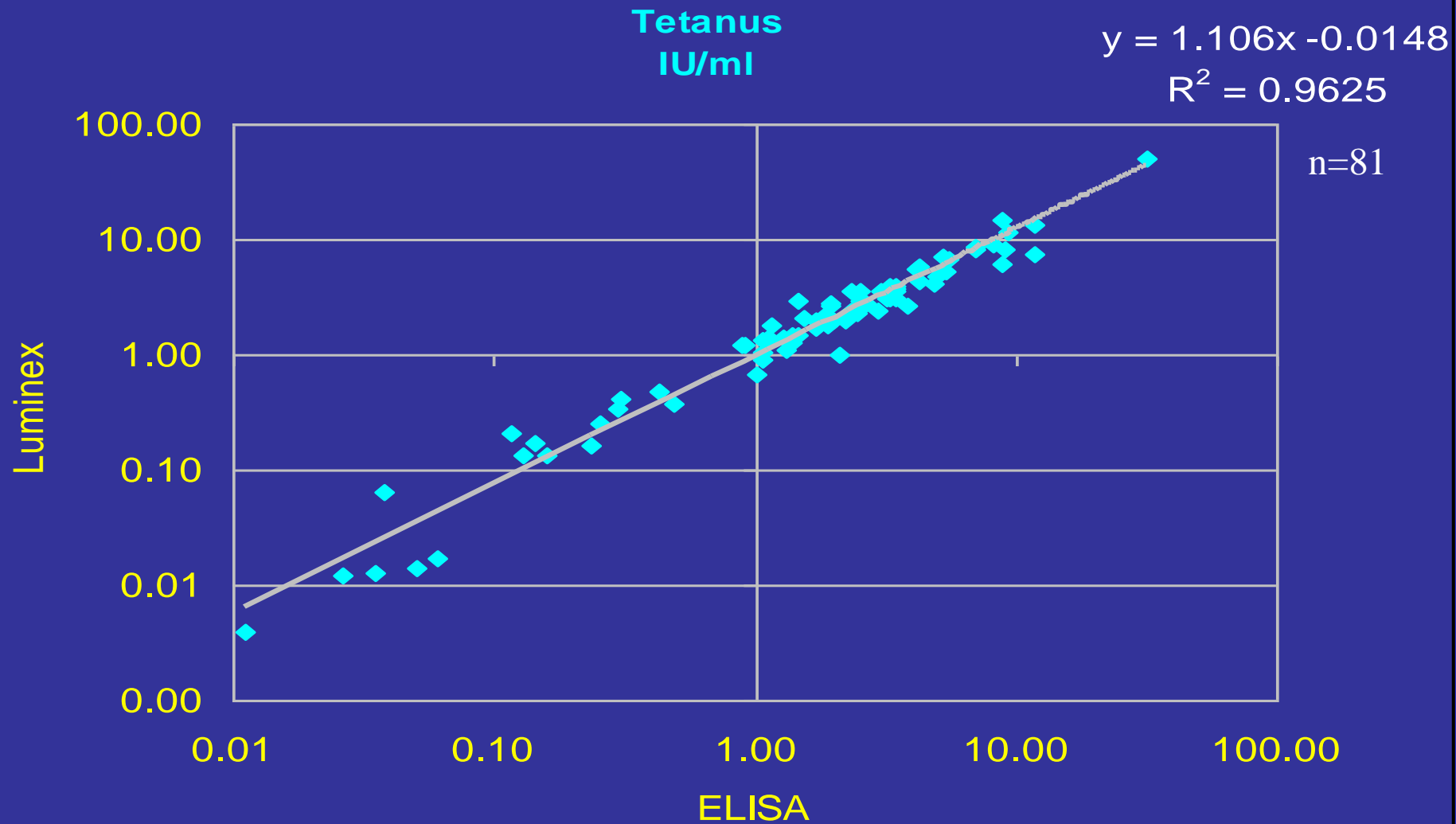
# Comparison of the Multiplexed Luminex Assay to an In-House ELISA for IgG Antibodies to *Haemophilus influenza* type b



# Comparison of the Multiplexed Luminex Assay to an In-House ELISA for IgG Antibodies to Diphtheria



# Comparison of the Multiplexed Luminex Assay to an In-House ELISA for IgG Antibodies to Tetanus





# Summary of IgG Concentrations for Pre and Post-Vaccine Samples for Dip, Tet and Hib determined by the Luminex Multiplexed Assay

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| Luminex<br>N=5  | Diphtheria<br>IU/ml | Tetanus<br>IU/ml | <i>H. influenza b</i><br>(ug/ml) |
|-----------------|---------------------|------------------|----------------------------------|
| Prevaccination  |                     |                  |                                  |
| Mean            | 0.45                | 0.15             | 0.33                             |
| Range           | 0.12-1.37           | 0.04-0.31        | 0.06-1.09                        |
| Postvaccination |                     |                  |                                  |
| Mean            | 12.32               | 28.16            | 58.32                            |
| Range           | 2.70-23.57          | 2.58-65.35       | 17.61-147.47                     |

# DIAGNOSIS OF ANTIBODY DEFICIENCY

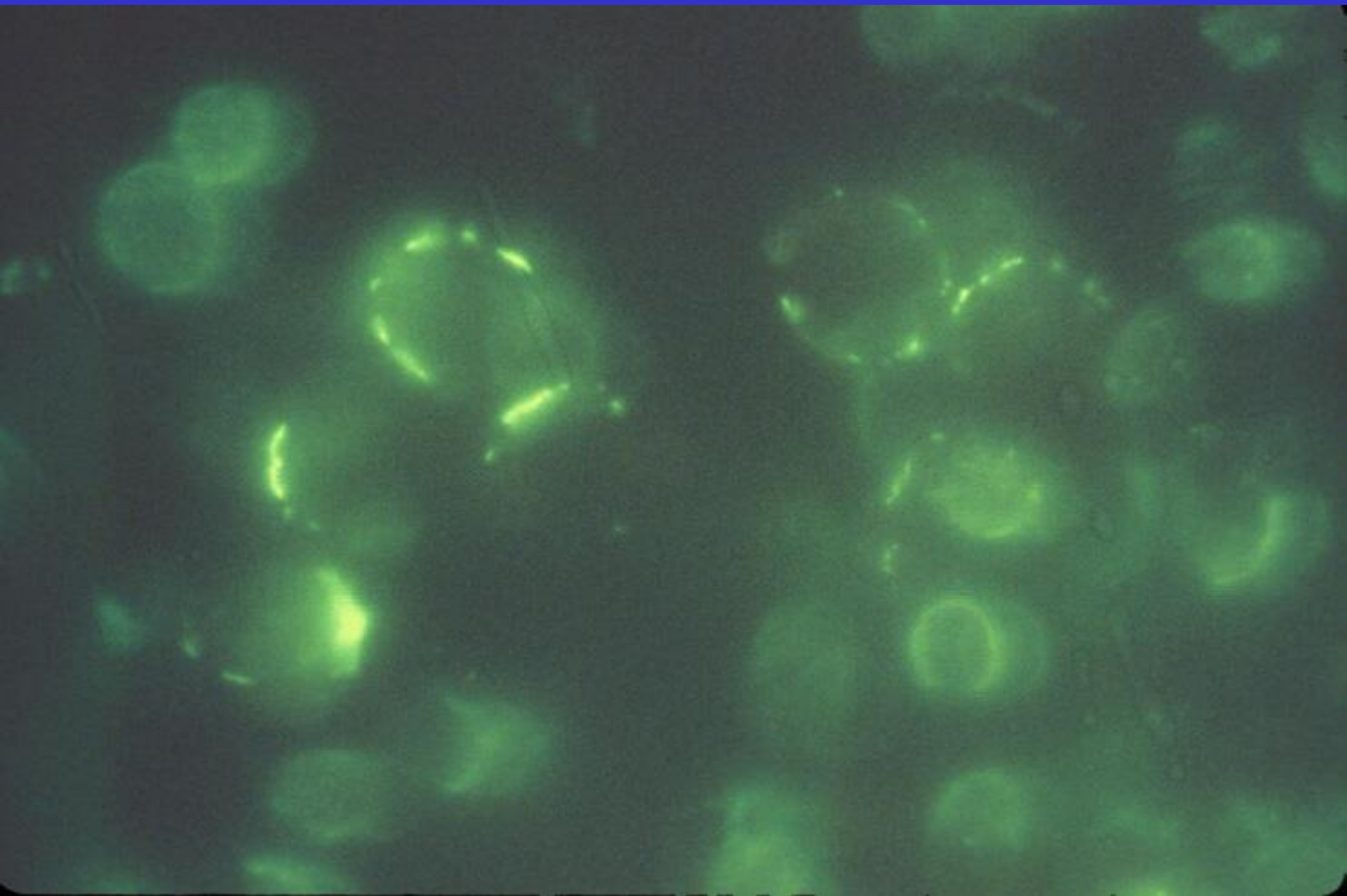
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- Quantitative immunoglobulins or IgG subclasses  
by Nephelometry; IgA subclasses  
Specific antibody production by multianalyte or ELISA
  - Diphtheria and tetanus titers, Hib - IgG1
  - Pneumococcal antibody titers, - IgG2
  - Influenza titers - IgG3
- B cell numbers with CD19 or 20 or surface IgM, IgD, IgG, IgA – B cell immunodeficiency profile, CD40, CD40L
- T-helper and suppressor, memory, naïve, NK & B cell numbers by flow cytometry – T cell Immunodeficiency Profile Extended









# **BRUTON'S AGAMMAGLOBULINEMIA - Cont'd**

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**Severe infections starting at 4-6 months of age when mother's immunoglobulin disappears**

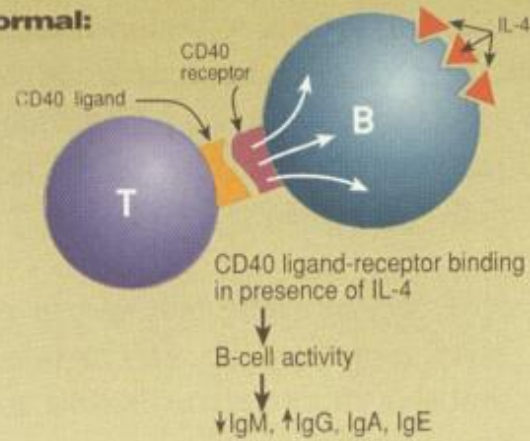
- Sinopulmonary infections**
- GI infections**
- Malignancies – lymphoreticular – 20%**
- Autoimmunity – 20%**



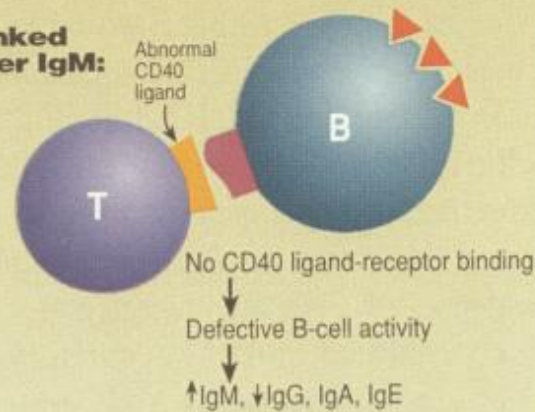


# DEFECT IN NON-X-LINKED HYPER-IGM SYNDROME

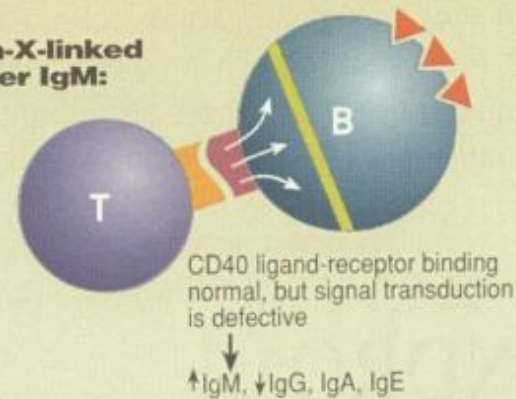
## Normal:



## X-linked hyper IgM:



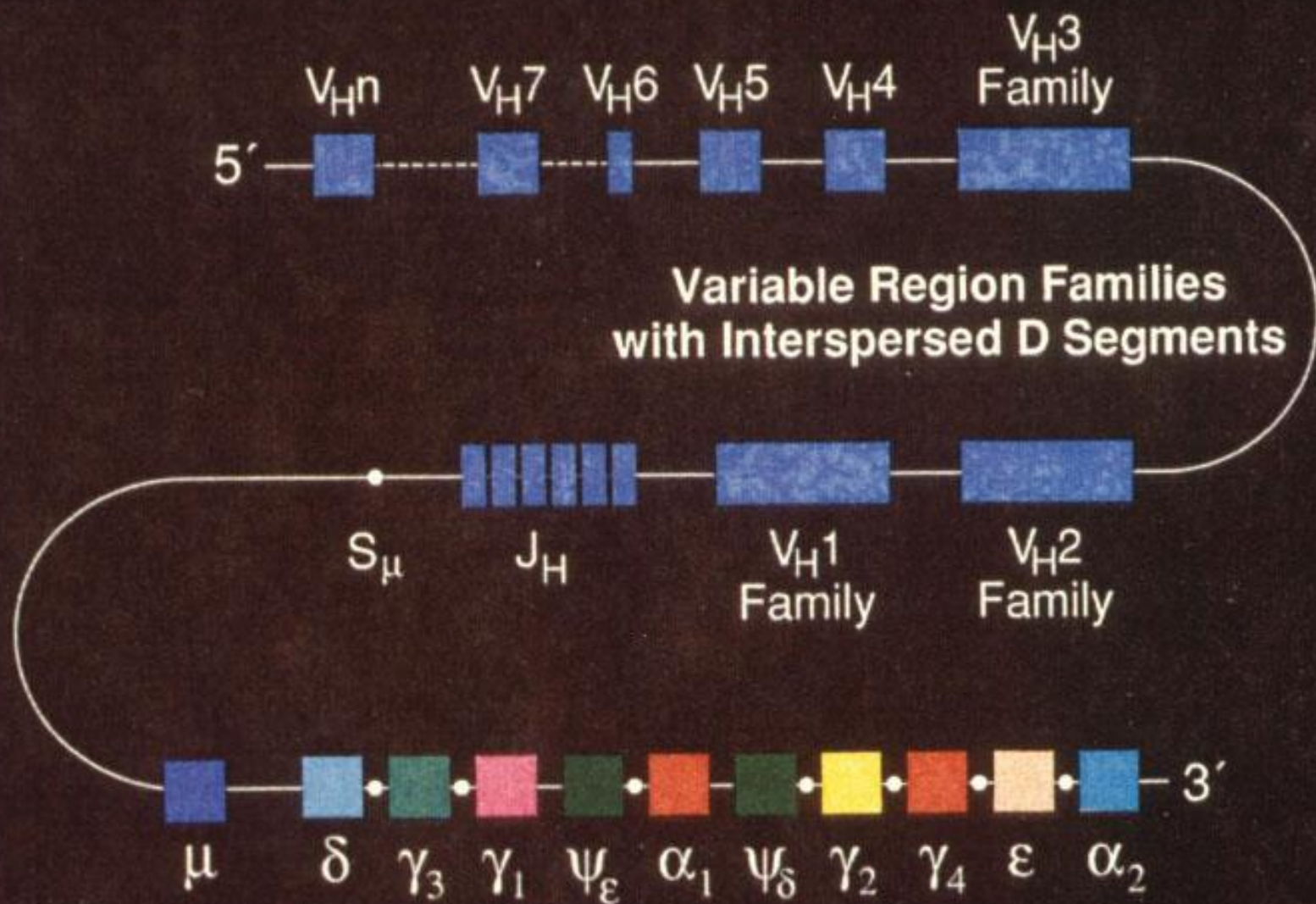
## Non-X-linked hyper IgM:











# **COMMON VARIABLE HYPOGAMMAGLOBULINEMIA**

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- **Starts several years after birth**
  - **Common**
  - **Variable immunodeficiency of B and T cells**
  - **One-quarter develop malignancies**
  - **Clinical manifestations:**
    - **Sinopulmonary infections 90-100%**
    - **Chronic diarrhea/giardia 50-60%**
    - **Sepsis, meningitis**
    - **Bronchiectasis**
    - **Autoimmune disease/arthritis**

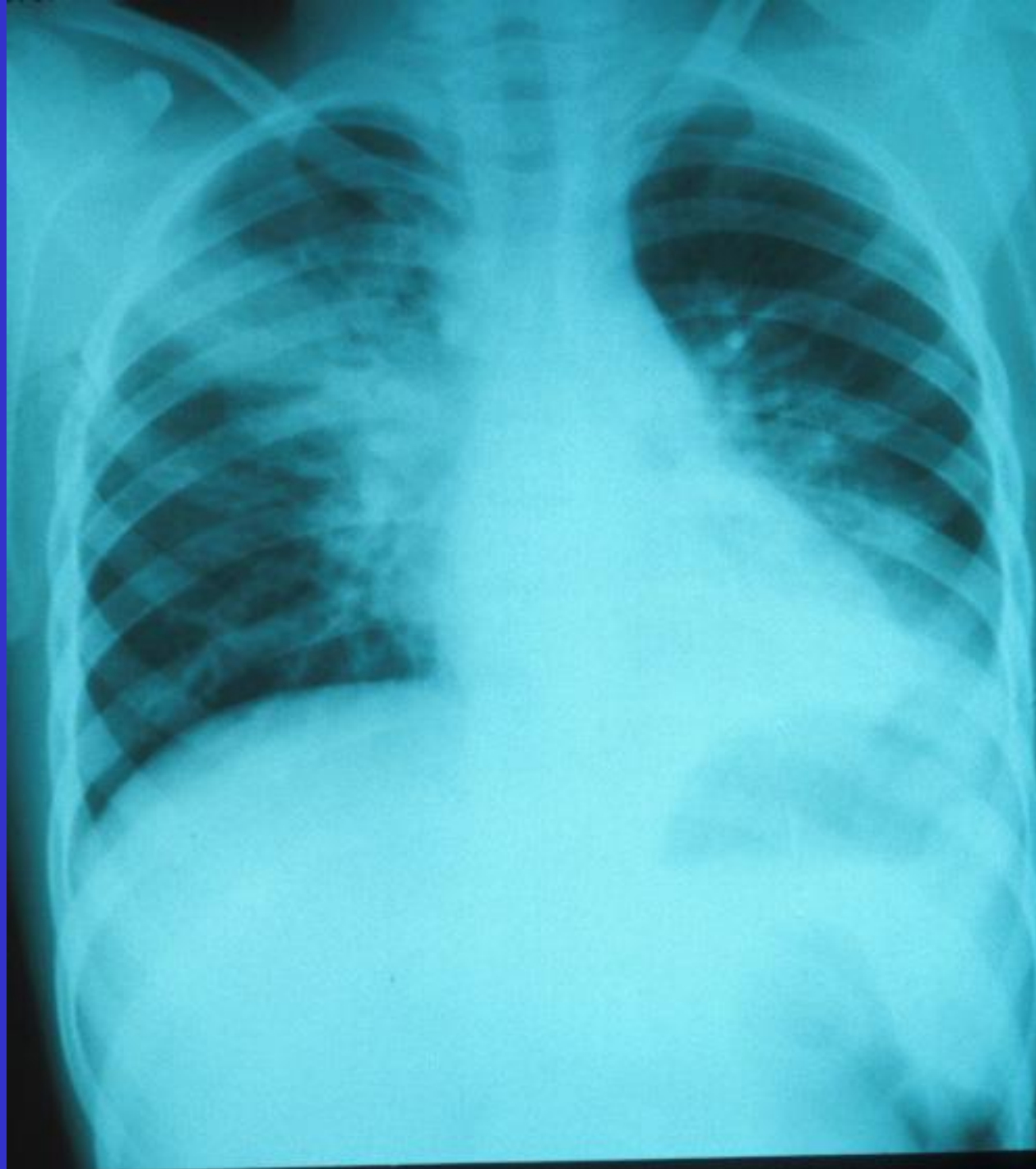


# **PATIENT R.P.**

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- **11 year old male with otitis media since birth**
- **Sinusitis, URIs**
- **Admitted – Temperature 103°**
- **LLL infiltrate**







# LABORATORY VALUES

## R.P.

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- IgG – 80      IgA – 16      IgM – 44
- Rubella Titer – negative
- Anti-A and B antibodies – 1:1
- B Lymphocytes – 23%
- T Lymphocytes – 48%
- Blood Culture – *H. influenzae b*

# COMMON VARIABLE IMMUNODEFICIENCY

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- Incidence: 1:50,000 – 1:200,000
  - Australia: 0.77/1000,000
- Onset: 3-90 years
  - Average: 2-3 decade – 25 years
- Diagnosis: 28 years

# CLINICAL FEATURES OF ACQUIRED HYPOGAMMAGLOBULINEMIA\*

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| <u>INFECTION</u> | <u>%</u> | <u>INFECTION</u> | <u>%</u> |
|------------------|----------|------------------|----------|
| Sinopulmonary    | 100      | Empyema          | 4        |
| Sinusitis        | 66       | Meningitis       | 4        |
| Otitis           | 32       | Bacteremia       | 5        |
| Pneumonia        | 86       | Giardiasis       | 34       |
| 1-10 episodes    | 68       | UTI              | 4        |
| 10 or more       | 18       |                  |          |
| Bronchiectasis   | 28       |                  |          |

***H. flu*, *S. pneumoniae*, *S. pyogenes*, *S. aureus***

# ASSOCIATED FINDINGS IN ACQUIRED HYPOGAMMAGLOBULINEMIA\*

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| <u>FINDING</u> | <u>%</u> |
|----------------|----------|
| Diarrhea       | 60       |
| Malabsorption  | 60       |
| Achlorhydria   | 53       |
| Giardia        | 64       |
| X-ray NLH      | 28       |
| Splenomegaly   | 28       |
| Conjunctivitis | 6        |

| <u>FINDING</u> | <u>%</u> |
|----------------|----------|
| Arthritis      | 8        |
| Allergy        | 40       |
| Malignancy     | 24       |
| Stomach CA     |          |
| Lymphoma       |          |
| Thymoma        |          |

\*Amer. J. Med. 61:221, 1976



# Mongenic Models of CVID

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- **Deficiency of Inducible Co-stimulator (ICOS)**  
T-cell costimulator molecules on activated cells  
– induces IL-4,5,6,17, GM-CSF, TNF $\alpha$ , IFN $\gamma$   
and superinduction of IL-10; AR in 4 families
- **Transmembrane activator and CAML  
interactor (TACI) +BAFF and APRIL induce  
IgA and antibody response to polysaccharides;  
13 of 162 CVID patients.**
- **CD 19 Deficiency - AR disorder with decrease  
in BCR stimulation, poor AB responses but no  
autoimmunity or lymphoproliferation.**

# **Other Genetic Causes CVID 2015**

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**CD 19, CD20, CD21, CD81,  
TACI, BAFF-R, ICOS,  
LRBA, PLCG2, PRKDC,  
NFKB2, PIK3CD, IKOS**

**Variable Phenotypes of  
RAG1, JAK3 with late  
onset cause picture  
similar to CVID but CID**

AH! HERE IT IS...



# **THERAPY OF HYPOGAMMAGLOBULINEMIA**

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- **Gammaglobulin or plasma**
- **Intermittent antibiotics**
- **Pulmonary therapy**
- **Atabrine or Metronidazole**
- **Close to follow-up – malignancies**



# **GAMMAGLOBULIN (IM)**

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## **Cohn Fractionation – 1946**

- **Cold Ethanol Extraction**
  - **25% Alcohol in the Cold**
- **Inactivates all Viruses**
- **16.5 Gram Percent**
- **95% IgG**
- **< 5% IgA**

# COMPLICATIONS OF IMIG

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- Pain at local site
- Aggregates into Vein
- Anaphylactic Reactions
  - Usually IgE or IgG4 to IgA
- Blocks Active Immunity



I HAVE NO  
DESIRE TO  
LEAVE MYSELF  
OPEN FOR A  
MALPRACTICE  
SUIT!

SCHULZ





SO CUT  
IT OUT !!



# Available IgG Products

| Brand Name       | Available Concentrations | Manufacturer     | Method of Administration | Osmolarity/<br>Osmolality    | PH        | IgA Content   |
|------------------|--------------------------|------------------|--------------------------|------------------------------|-----------|---------------|
| Gammagard S/D    | 5% / 10%                 | Baxter           | IVIg                     | 636 mOsm/kg /<br>1250 mOsm/L | 6.8 ± 0.4 | 1µg/mL<br>N/A |
| Gammagard Liquid | 10%                      | Baxter           | IVIg / SCIg              | 240-300 mOsm/kg              | 4.6 – 5.1 | 37 µg/mL      |
| HYQVIA           | 10%                      | Baxter           | SCIg                     | 240-300 mOsm/kg              | 4.6 – 5.1 | 37 µg/mL      |
| Gammaplex        | 5%                       | Bio Products Lab | IVIg                     | 460-500 mOsm/kg              | 4.6 – 5.1 | <4 mcg/mL     |
| Bivigam          | 10%                      | Biotest Pharm    | IVIg                     | 510 mOsm/kg                  | 4.0 – 4.6 | 200 µg/mL     |
| Carimmune NF     | 3% - 12%                 | CLS Behring      | IVIg                     | 192-1074 mOsm/kg             | 6.4 – 6.8 | 720 µg/mL     |
| Hizentra         | 20%<br>(200 mg/mL)       | CLS Behring      | SCIg                     | 380 mOsmol/kg                | 4.6 – 5.2 | 50 mcg/mL     |
| Privigen         | 10%                      | CLS Behring      | IVIg                     | isotonic<br>(320 mOsmol/kg)  | 4.8       | ≤25 mcg/mL    |
| Flebogamma DIF   | 5% / 10%                 | Grifols          | IVIg                     | 240-370 mOsm/kg              | 5.0 – 6.0 | <3 mcg/ml     |
| Gamunex-C        | 10%                      | Grifols          | IVIg / SCIg              | 258 mOsm/kg                  | 4.0 – 4.5 | 46 µg/mL      |
| Gammaked         | 10%                      | Kedrion          | IVIg / SCIg              | 258 mOsm/kg                  | 4.0 – 4.5 | 46 µg/mL      |
| Octagam          | 5%                       | Octapharma       | IVIg                     | 310-380 mOsm/kg              | 5.1 – 6.0 | <100 µg/mL    |

# INDICATIONS FOR IVIG THERAPY

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- Recurrent bacterial infections
- IgG < 200 mg – 500 mg%
- No antibody formation when immunized
- IgG subclass deficiency???

# **THERAPY FOR HYPOGAMMAGLOBULINEMIA**

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- **IVIG 300-400 mg/kg q 3-4 weeks or weekly, biweekly or monthly SQ IgG**
- **Treat acute infections promptly**
- **Occasional prophylactic antibiotics**
- **Pulmonary therapy**
- **Careful observations for malignancy**











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Continued...



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