CHU – Liège (Belgium)
Medical Microbiology

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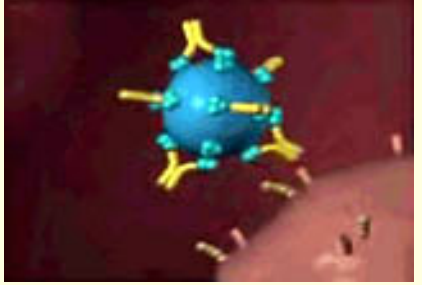
SEROLOGICAL DIAGNOSIS IN 2006 AND FUTURE PROSPECTS

- Introduction
- From specimen collection to result
  - Pre- analytic
  - Analytic
  - Post- analytic: - CMV
    - Influenza Virus
    - Lyme disease
- Future prospects
- Conclusion



Infectious Serology : definition

= detection, in the serum or CSF,
of **antibodies** produced during the humoral immune response,
before, **during** or **after** the infection

B Lymphocytes and antibodies

BL differentiation:

- in **PLASMOCYTE** : IgA and IgM production, afterwards IgG.

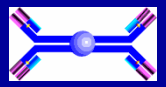
B Lymphocytes and antibodies

- in **MEMORY BL** :

  when re-exposure to the same antigen

→ Ab synthesis : faster,
IgG > IgM
higher level

Different types of antibodies

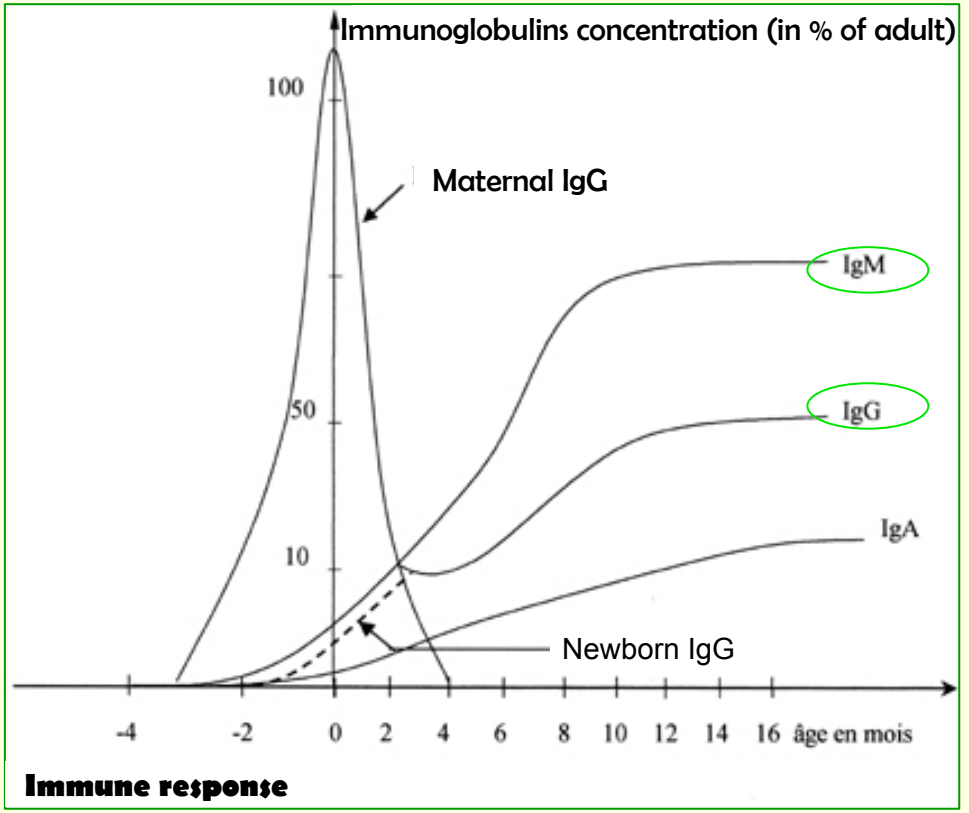
		early detection time	% pool
IgA ⇒ local *and* general immunity		**4-10 days**	**15 %**
IgM + sites ⇒ **+ cross-reactions**		**4-10 days**	**10 %**
IgG Stimulate phagocytosis, activate C' **immunity** ***Only Ig to go through placenta!***		**2-3 weeks**	**75 %**
IgD, IgE		**few days**	**<1 %**

In fœtus and newborn

- ***Fœtus:***
  - **IgM / A** synthesis > 21st week
  - **IgG** passively acquired maternal Ab
- ***Newborn:***
  - **IgM / A** and **IgG** production

⇒ **IgM / A** presence evocates recent infection

⇒ **IgG** follow-up is non sense!



From specimen collection to result

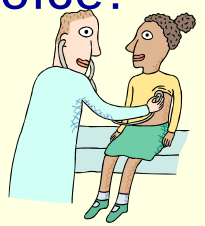
1 – **PRE- ANALYTIC**	→	prescription, specimen collection
2 - **ANALYTIC**	→	antibodies detection
3 - **POST- ANALYTIC**	→	interpretation of results

PRE-ANALYTIC: Prescription

CLINICIAN

- **choice analysis** should be based upon **clinical** and **anamnestical** informations

  BUT usually, clinician <u>subjectivity</u> influences this choice!

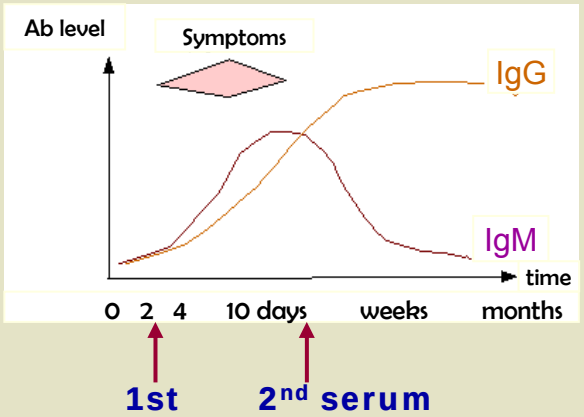


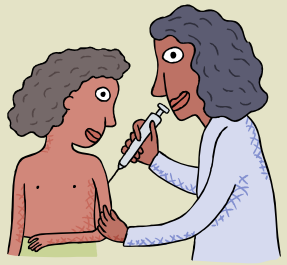
- **request form:**

  - name,...
  - <u>clinical informations</u> help the biologist:
    - ✓ to adjust prescription
    - ✓ to choose a rapid diagnosis test
    - ✓ to interpret results

1- « Is it a recent infection ? »

IgM and/or **IgA** ***AND*** **IgG** on **2** serums (**10-15 days** apart)



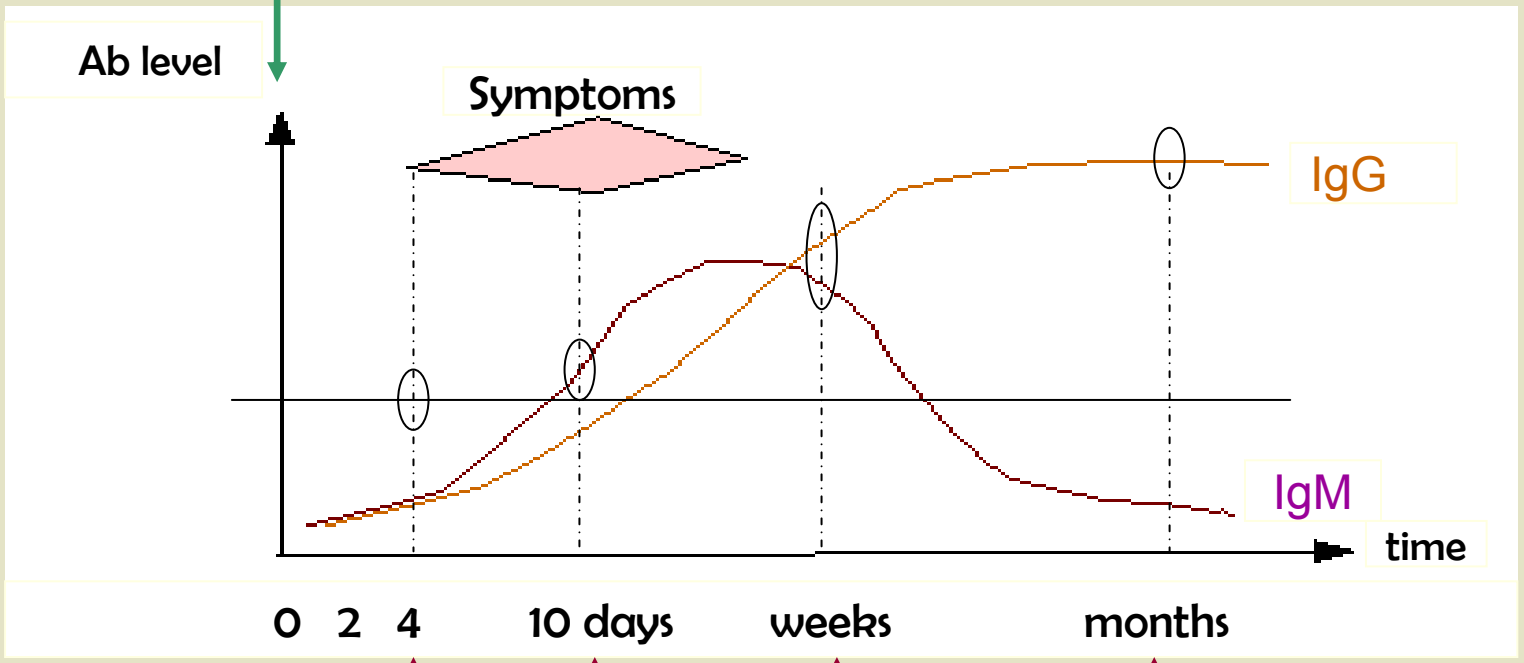


2- Assessment of specific immunity :

IgG on 1 serum sample (blood, organ donors, women before pregnancy,...)

Absence of IgG = no specific immunity
(post-vaccination or post-infectious)

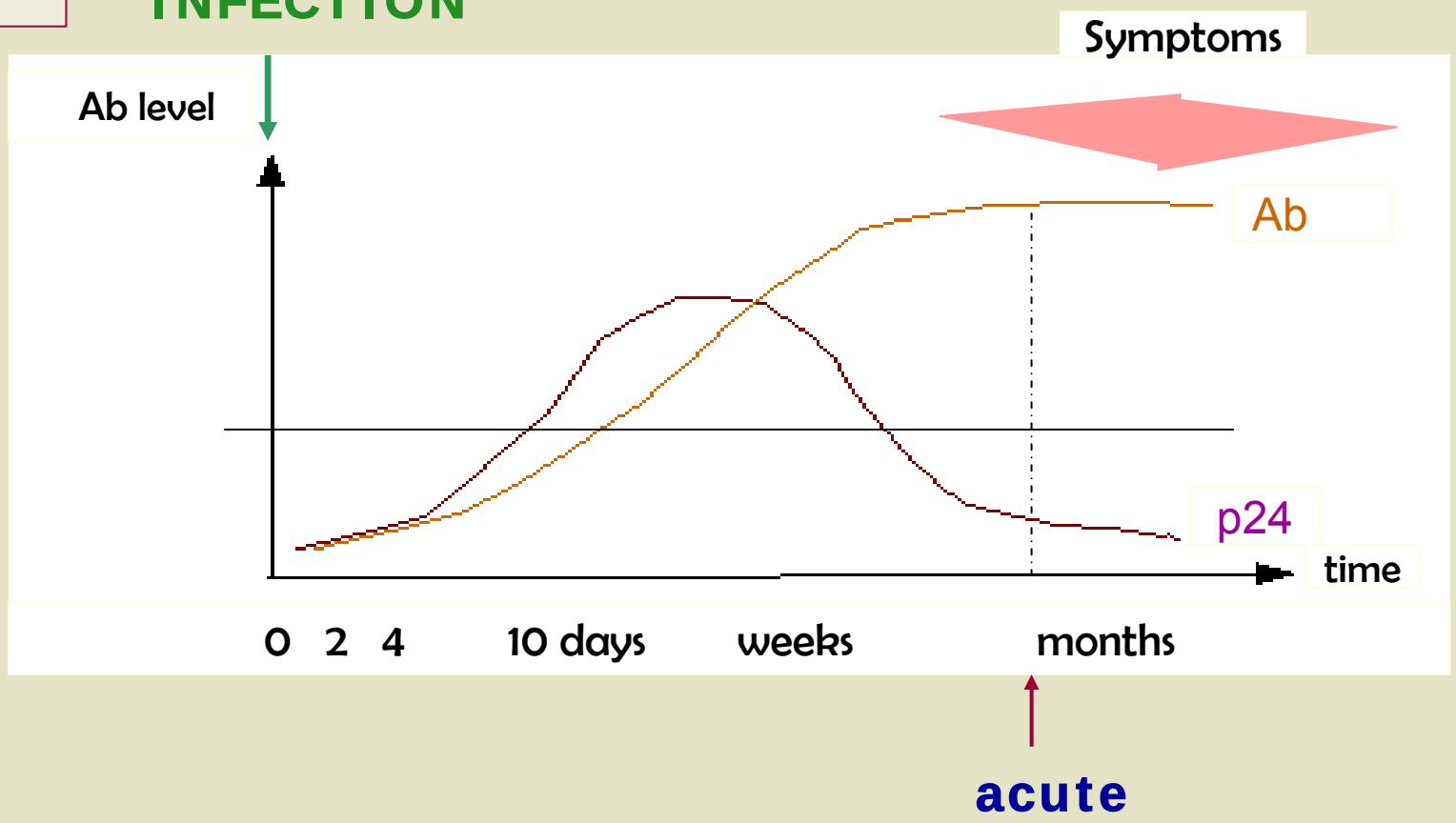
usually **INFECTION**



	acute	recent		immunity
IgM	neg	POS	POS	neg
IgG	neg	neg	POS	POS

HIV

INFECTION



Indications of serological analysis

- ✓ Etiologic diagnosis of infectious disease
- ✓ Infectious monitoring in immunodeficient patients
- ✓ Follow-up of chronic infections (Hepatitis,…)
- ✓ Screening and follow-up of pregnant women
- ✓ Therapeutic follow-up (Syphilis)
- ✓ Risk assessment in Healthcare workers injuries (HIV, HCV)
- ✓ Evaluation of immunisation efficiency
- ✓ Epidemiologic survey

PRE-ANALYTIC: specimen

CLINICIAN

<u>Specimen collection:</u>

- ***serum***: dry test tube (+/- gel), WITHOUT anti-coagulant
- ***CSF***: <u>dry</u> test tube

- Quality ⚠ haemolysis!
- Volume
- Labels, leak proof bag

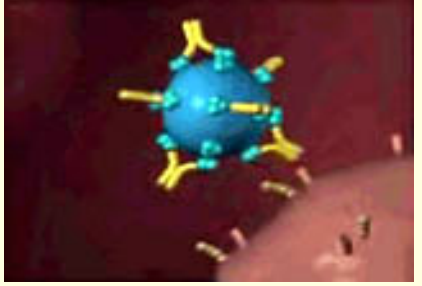
TRANSPORT

- Time to transfer to the lab (<48h)

LABORATORY

- Centrifugation within 48h after collection
- Storage of specimens:

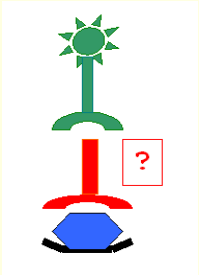
• long conservation → – 20°C
• rapid analysis (< 1 week) → + 4°C



ANALYTIC

Methods:

1- Screening



- Immuno– assay (enzymology, fluorescence)
- Agglutination (i.e. RPR)
- Inhibition of hemagglutination (i.e. Influenza)
- Complement Fixation (i.e. CMV, Poliovirus)

2- Confirmation methods

- PCR (i.e. HIV, HCV)
- Western- blot (i.e. Borrelia)

sensitivity and specificity ≠ :

+ sensitive	→	screening
+ specific	→	confirmation

Methods

Interferences in dosage:

- ***Cross reactions*** (IgM >> IgG)

  - Rhumatoïd Factor
  - Other Ab
  - Heterophile Ab
  - Reagent is never « pure »

  → **false– positives**

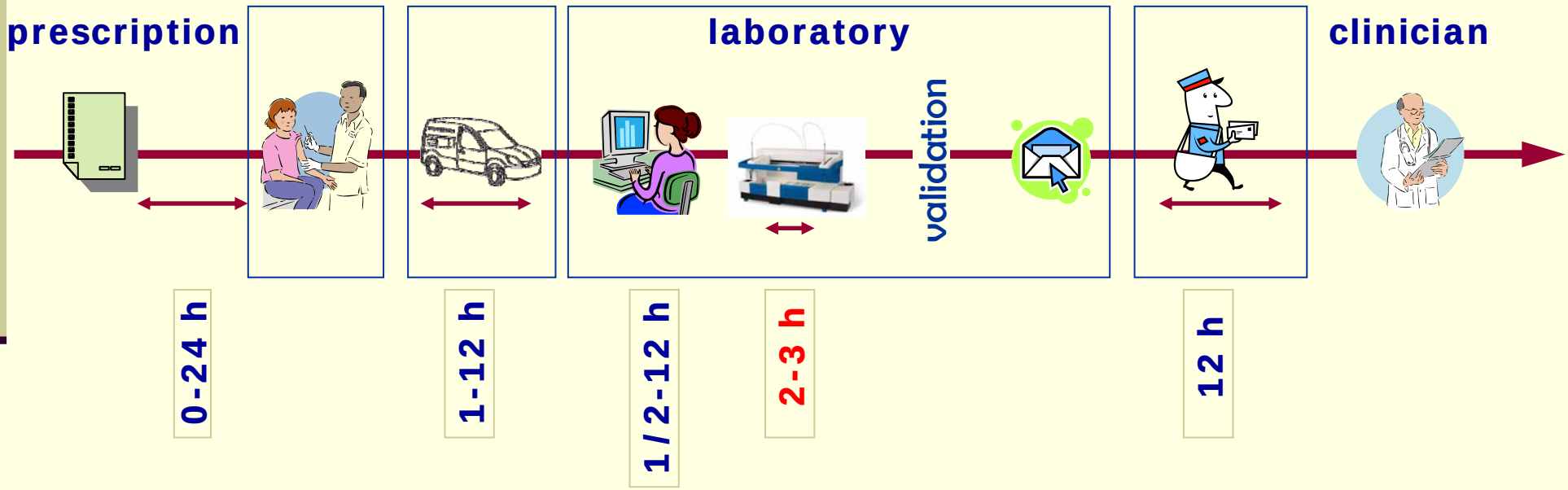
- ***Saturation:*** high quantity of Ig *(i.e. IHA, CF)*

  ⇒ saturation → **false– negatives**

- ***Pregnancy:*** Frequent polyclonal activations *and* reactivations

Expected turn-around time: CHU example

5h → 5 days

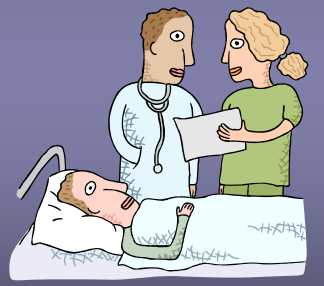


POST-ANALYTIC

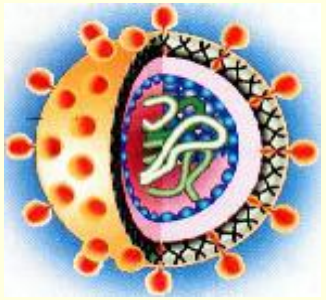
= interpretation of the results

Illustrations:

- *CMV*
- *Influenza virus*
- *Lyme disease*



CMV INFECTIONS



Pathogenicity

Primo-infection → persistence at latent state (heart, kidney)

↓

reactivation

↓

secondary infection(*)

(*) Usually asymptomatic in immunocompetent patients

Clinical manifestations

I. Primo-infection in immunocompetent patient

→ asymptomatic *OR* mononucleosis like syndrome

II. Primo-infection in immunocompromised patient

→ symptomatic

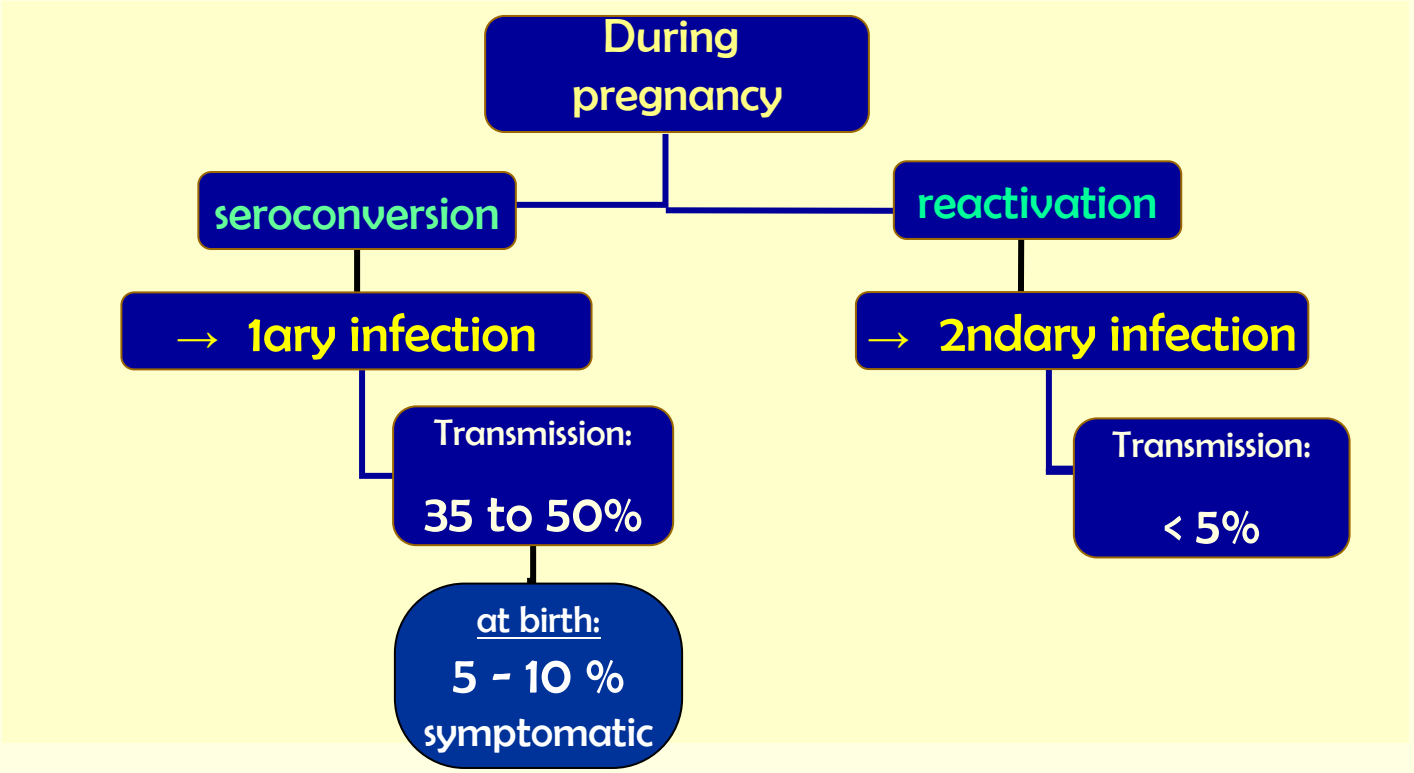
Cellular immunodepression => frequent **reactivations**

Clinical manifestations

III. Materno –fœtal transmission

CMV = **1st european cause of congenital infection**.

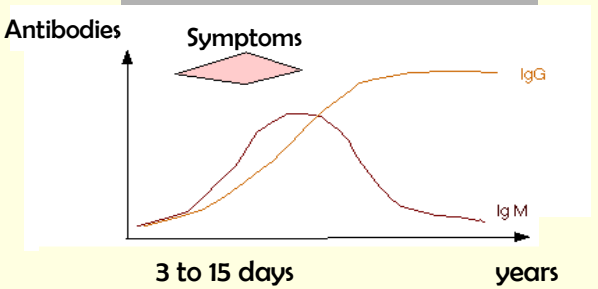
CONSEQUENCES: related to maternal immunologic status



CMV serology: interpretation

Case 1

♀ 17 years, pregnant, tired



INFECTION

	2/7/05	13/7/05	27/07/05	
IgM	Neg	**Pos**	**Pos**	
IgG	<0,4	**1,9**	**6,2**	UI/ml (Pos >0,6)

→ **Primo-infection**

Case 2

♀ 22 years, pregnancy follow-up, asymptomatic

	previously 5/1/03	*pregnancy* 29/12/04	10/2/05	23/2/05	
IgM	Neg	Neg	**Pos**	**Pos**	
IgG	<0,4	<0,4	<0.4	**5,1**	UI/ml (Pos>0,6)

INFECTION

→ **Primo-infection**

Case 3

♀ 24 years, early pregnancy

	previously		*pregnancy*		
	2002	↔	9/8/05	21/8/05	
IgM		?	**Pos**	**Pos**	
IgG	<0.6		6,8	15,3	UI/ml (Pos >0,6)

1- *Recent infection?*

2- *Primo-infection* OR *reactivation?*

AVIDITY : low (12%)

CONCLUSION:

consistent with a **recent primo-infection**

(< 3 months)

IgG Avidity

Principle: **recent** infection ⇒ **weak** link Ag-Ab
old infection ⇒ **stable** link Ag-Ab

→ Dosage of **IgG** with and without **urea**:

if link is weak ⇒ urea will break the link

Indication: to determine the time of infection when
presence of IgM* and *IgG in **pregnant woman**
whom previous serological status is unknown

Interpretation:

HIGH avidity → consistent with an **OLD** primo-infection (**> 3 months**)
LOW avidity → consistent with a **RECENT** primo-infection (**< 3 months**)

Case 4 ♀ 25 years, pregnant (13 weeks), **none previous serology**

	5/06/04	19/06/04	
IgM	**Pos**	**Pos**	
IgG	**13,2**	**14,1**	UI/ml (Pos >0,6)

1- *Recent infection?*

non significant ↑ IgG → **persistent IgM** after an acute episode (WHEN?)
or **non specific IgM**

2- *1ry or 2ndary infection?* impossible to know without prior results!

⇨ **AVIDITY : high** (74%)

CONCLUSION:
results consistent with an **old primo-infection**

Case 5

♀ 31 ans, pregnant (19 weeks)

	previously	*pregnancy*			
	25/11/03	2/10/05	18/10/05		
IgM	Neg	**Pos**	**Pos**		
IgG	**3,9**	**9.5**	**19.5**	UI/ml	(Pos >0,6)

1- *Recent infection?*

2- *1ry or 2ndary infection?*

⇒ **viral reactivation**

Avidity not useful !

Case 6

immunocompromised patient, prior results: presence of IgG

	22/5/05	8/06/05	
IgM	**Neg**	**Neg**	
IgG	**7**	**20**	UI/ml (Pos >0,6)

→ significative ↑ of IgG

⇒ consistent with a **reactivation**

Immunodeficiency

presence of IgM: non-constant or delayed

→ ✓PCR
✓Antigen

IMPORTANT!

1- To compare 2 levels of IgG:

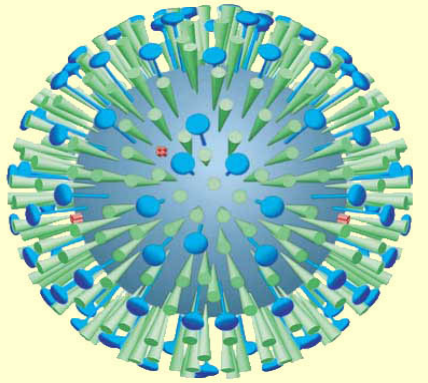
- advised to test **PAIRED SERUMS** in parallel
- at least performed in the **SAME LABORATORY,**

2- To determine the time of an infection:

- impossible from results obtained from ONE SERUM ONLY!
- need for ≥ 2 serums (2-3 weeks apart)

3- To respect **TIME** between the 2 serums.

4- To never interpret definitively a doubtful serology before obtaining a **CONTROL** 2 to 4 weeks after the first specimen!

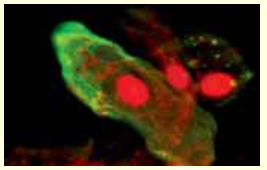


INFLUENZA VIRUS

Laboratory diagnosis: acute phase

acute phase

- **Antigenic detection** of *Influenza* virus in a nasopharyngeal specimen
  - *Immunoflorescence*



Epithelial cells infected by Infuenza A.

  - *Rapid immuno- assays*

(Immunocards, strips,…)

- **PCR, isolation of the virus:** essential to study antigenic variability

Laboratory diagnosis: retrospective

retrospective

❑ Detection of antibodies:

1st serum: in the acute phase
2nd serum: 10 to 15 days later

Methods

- **ELISA:**

and/or | **IgA Pos** (or IgM)
IgG Pos (with apparition *or* significant ↑ between 2 serums)

- **CF, Inhibition of hemagglutination :**

high titer *OR* **x4** between 2 serums in CF

! *Cross reactions between IgA (or IgM) Influenza A et B*

⇒ **consistent with an acute infection**

Inhibition of hemagglutination reaction (IHA):

→ detection of **specific Ab** against the hemagglutinin of Influenza virus (virulence factor)

(HA: agglutinates red cells)

⇒ allows **serotyping**

Influenza outbreak in a Belgian nursing home (march '05)

Flu symptomatology in 30 vaccinated patients

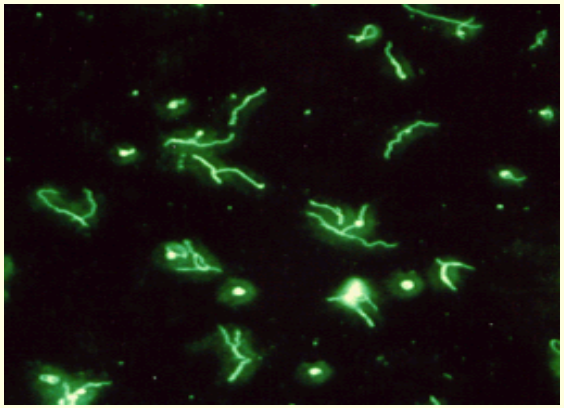
1st sample collection: performed 15 d. after the beginning of the outbreak

2nd sample collection: performed 2-3 weeks later

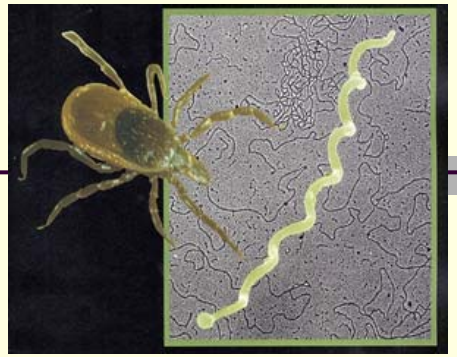
	Influenza A		*Influenza B*		IHA:		
	IgA	IgG	IgA	IgG	NY	NC	JI
Mrs F 31/3	Neg	>200	**Pos**	115	**480**	30	30
Mrs F 18/4	VS	1227	**Pos**		**1280**	40	40
Mrs T ?/3	Neg	74	Neg	85	**20**	20	20
Mrs T 31/3	Neg	346	Neg	129	**160**	10	5
Mrs P ?/3 N		493	Neg	74			
Mrs P 16/4 N		555			**1280**	20	20

serotype: NY= New York NC= New Caledonia JI= Jiangsu

LYME DISEASE



Reminder



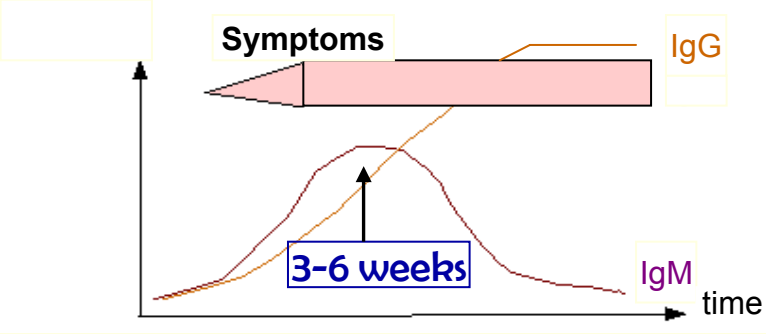
Spirochete: *Borrelia burgdorferi*

Vector: tick

Most important species:

	Region
B afzelii	Europe and parts of Asia
B burgdorferi sensu stricto	North America and Europe
B garinii	Parts of Asia and Europe

Serological diagnosis



The usual laboratory diagnosis is based on the SEROLOGICAL ANALYSIS.

Case 1

♀ 54 years, migrant erythem, tick bite

Antibiotics ↓

	10/8/05	5/9/05	22/9/05	20/10/05		
IgM	**Pos**	**Pos**	Neg	Neg		
IgG	181.8	158,5	159	73	UI/ml	(Pos > 15)

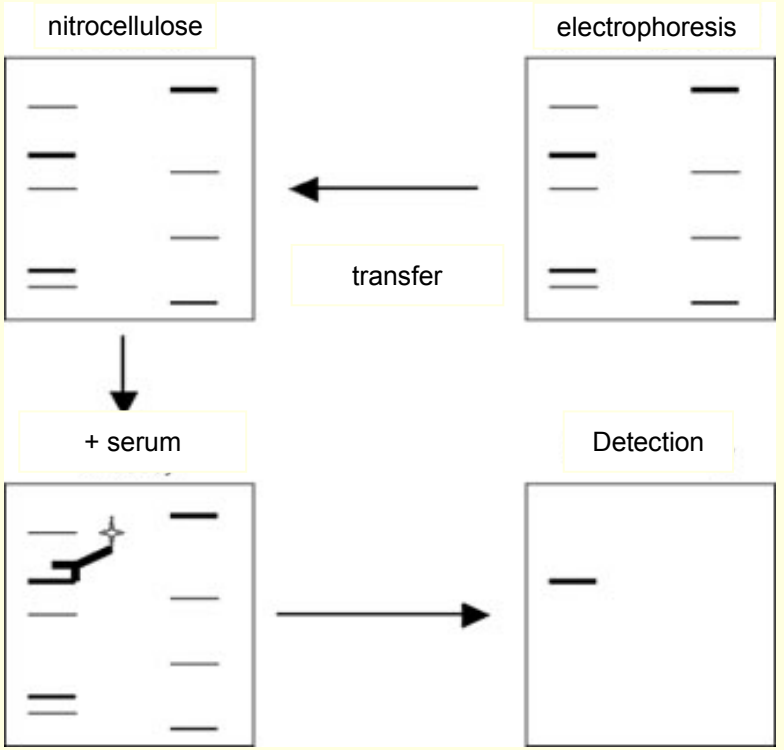
Skin biopsy :
PCR **Pos**

➡ **Recent infection**

⇒ ~~Western-blot~~

Confirmation test: Western-blot

- Principle: **detection of Ab** against different specific Ag of the micro-organism, after having separated them by electrophoresis.



- **High specificity** (> ELISA)

- Recommended use:

  confirmation of a serological diagnosis

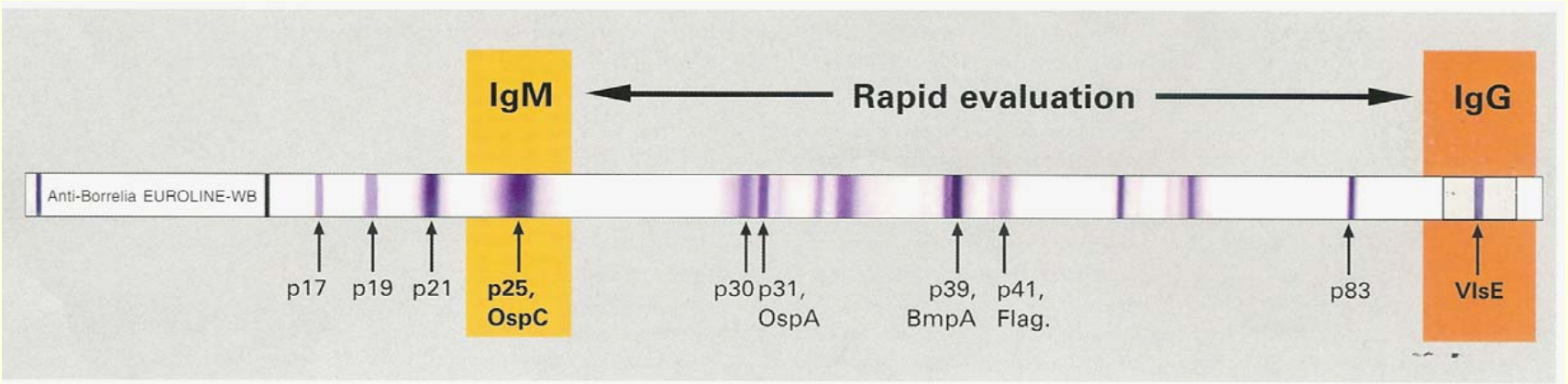
  (*Borrelia*, HCV, HIV)

Borrelia burgdorferi

Surface proteins:

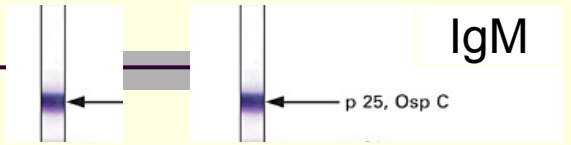
Osp A, B, **C** (early infection), D; **VlsE**

(VlsE: *Variable major protein-like sequence expresse)*



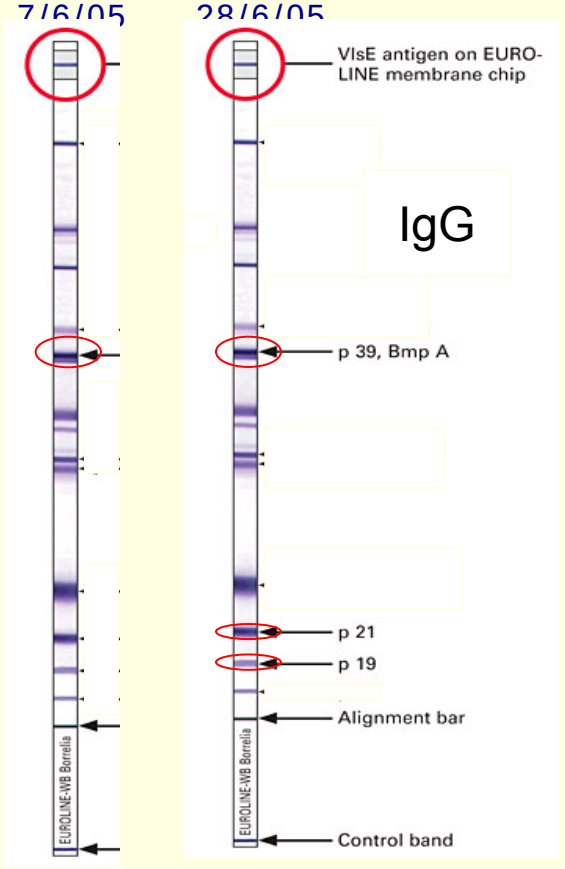
Case 2

♀ 48 years, arthralgies left knee , atypical erythem left leg



	7/6/05	28/6/05
IgM	**Pos**	**Pos**
IgG	**25**	**85** UI/ml (Pos > 15)

W.B. IgM	**Pos** (OspC)	**Pos** (OspC)
W.B. IgG	**Pos** (2 bands)	**Pos** (4 bands)



➡ **Recent infection (phase 2)**

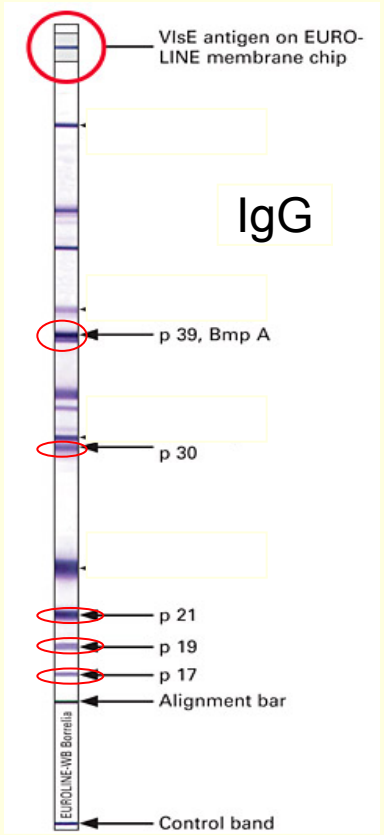
Euroimmun®

Case 3

♂ 12 years (Ardennes), atypical erythem (right leg), facial paralysis

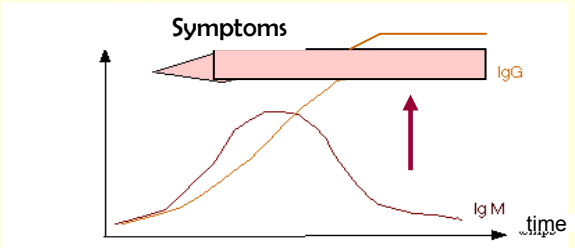
serum	21/8/06	31/8/06	
IgM	Neg	Neg	
IgG	**165**	**164**	UI/ml (Pos > 15)
W.B. IgM	Neg		
W.B. IgG	**Pos** (VlsE, p39, p30, p21, p19, p17)		
CSF			
IgG	**69** UA/ml	(Pos > 5.5)	
	(Index CSF/serum: 4.5)		
PCR	Neg		

⇨ Skin Biopsy: PCR: **Positive**



Euroimmun®

➡ **Recent infection (phase 2)**



Case 4

♂ 23 years, tick bite, asymptomatic

	9/8/05	2/9/05
IgM	**Pos**	**Pos**
IgG	< 10	<10 UI/ml
W.B IgM	Neg	Neg
W.B. IgG	Neg	Neg

→ ***Treated infection***
or ***Non specific IgM***

➡ **non specific IgM**

Future prospects

Evolution of serological dosage techniques

- **methods**:

  CF, IHA, IF: *total Ab* → ELISA: *IgA,M,G*

  ⇒ interpretation

- **↑ sensitivity, specificity**

  of new methods

- **ELISA detection systems**

  colorimetry to chimiluminescence

- **automation:**

  - result
  - chain
  - open versus closed system

Integrated laboratories

Currently:

TECHNICAL integrated laboratories

Future:

serological results integrated with MEDICAL FILE

Conclusion

- The optimal diagnosis of an infection is **direct diagnosis**, based on identification of the pathogen *or* its components

BUT it is not always possible

⇒ infectious serology is the main alternative

- **Serological results:**
  - lack of specificity
  - retrospective diagnosis
  - difficult to interpret

- Results must be **integrated** in clinical context

⇒ collaboration between clinician and biologist is mandatory

Acknowledgments

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