Traps in serology

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Espiroqueta 25 de abril 2012
Aims

- Presentation of serology
- Tests and their qualification
- Definition of application range
- Explain limits of utility
- Describe some traps and pitfalls
Immunity
Pathogen

innate
acquired

Immunity
Pathogen
Specific immunity

T

CD 4  -  CD 8

B

IgM  -  IgA  -  IgG
IgM

First reaction
Rapid production
IgG

2 recognition sites
Production delayed
Avidity increasing
Persistence (memory cells)
Protective (immunity)
IgA

In serum and secretion
Primo and reactivation
Selon Ch. A. Alford, 1971

Ontogenesis

Immunoglobulinspiegel (% d. Erw. Norm)

mütterliche Ig G

Ig M

Ig G

Ig A

Ig G

Ig A

Ig M

Erw. Werte

Ig G

Ig A

Ig M

Fetalzeit

Wochen

38

Monate

1 2 3 4 5 6 7 8 9 10

Kindesalter

Jahre

1.700

Selon Ch. A. Alford, 1971
Humoral responses

- IgM
- IgG
- IgA

- Few days
- 2 months
- 6 months
- Years
Pathogen

Multiplication
Dissemination

Gravité variable

Symptoms

contact

no
weak
early
late
chronic
end
Microorganism contact → Humoral response

ANALYSIS
Antigen choice

Test results
- reactive
- reactive
- reactive
- Non reactive
# Antibody panel

<table>
<thead>
<tr>
<th>Test</th>
<th>Ig Detected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agglutinations</td>
<td>Ig totaux</td>
</tr>
<tr>
<td>Fixation du complément</td>
<td>Ig totaux</td>
</tr>
<tr>
<td>Séroneutralisation</td>
<td>Ig totaux</td>
</tr>
<tr>
<td>Immunofluorescence</td>
<td>IgM, IgA, IgG</td>
</tr>
<tr>
<td>EIA - ELISA</td>
<td>IgM, IgA, IgG</td>
</tr>
<tr>
<td>Immunoblots IB</td>
<td>IgM, (IgA), IgG</td>
</tr>
<tr>
<td>Immunochromatographie ICT</td>
<td>Ig, IgM, (IgA), IgG</td>
</tr>
</tbody>
</table>
## Antigenic presentation

<table>
<thead>
<tr>
<th>Method</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agglutinations</td>
<td>cellules - molécules</td>
</tr>
<tr>
<td>Fixation du complément</td>
<td>extraits de cellules</td>
</tr>
<tr>
<td>Seroneutralisation</td>
<td>culture</td>
</tr>
<tr>
<td>Immunofluorescence</td>
<td>cellules</td>
</tr>
<tr>
<td>EIA</td>
<td>extraits - molécules</td>
</tr>
<tr>
<td>Immunoblots</td>
<td>extraits – molécules</td>
</tr>
<tr>
<td>ICT</td>
<td>molécules</td>
</tr>
</tbody>
</table>
Koch’s Postulate #2

The microorganism must be isolated from a diseased organism and grown in pure culture
**Diagnosis - pathogen related**

- Direct
  - Pneumocoque
  - *S. aureus*
  - *Campylobacter spp*
  - *E. coli*
  - *C. trachomatis*
  - *Giardia lamblia*
  - *Plasmodium falciparum*

- Indirect
  - *Borrelia burgdorferi*
  - HIV
  - TBE
  - Chikungunya
  - Viral hepatitis
  - Leptospirosis
Diagnosis – infection site

Direct

Nervous
ORL
Respiratory
Gastro-intestinal
Urinary
Sys. genital
Osteo

Indirect

Fever
Hepatitis
Diagnosis by stage of infection

Direct

active

severe

reactivated

chronic

convalescent

immunity

Indirect

Time
Dengue Virus

- **Dengue primaire**
- **Dengue secondaire**

**Ingémination**

**Début des signes cliniques**

**Virémie**

**Ag NS1**

**Présence d'anticorps spécifiques**

**Sérologie (Elisa, IC, blot,...)**

**Seuil de détection des IgG avec la technique Elisa capture**

**IgG**

**IgM**

J-7, J-2, J-0, J+5, J+7, J+10, M 3-M 6
What does the physician expect from serology?

1. Simplicity
   - One tube - one serum
   - Many requests
   - One answer = positive or negative

2. Rapidity
   - ICT 15-30 minutes
   - EIA 1-2 heures
   - An answer at least within the same day
What can we effectively obtain from infectious serology?
All of that, but ....

Serology requires **2 sera in a variable laps of time**

*Seroconversion* is a proof of recent contact with pathogen

*Adequate knowledge* of microbiological diagnosis
Indications et Pièges

1. Primary infection ➔ IgM
2. CSF Infection ➔ Intrathecal production
3. Chronical Infection ➔
4. Treatment monitoring ➔ Antibody decline ?
1. Reactivation ➔ IgA
1. Activity ➔ direct
1. Immunity, past infection ➔ IgG
2. Qualification of blood product ➔ indirect and direct
3. Prevalence ➔ role in test qualification
4. Prozone effect ➔ Ig quantity
1. Primo - infection

- Role of IgM
- Delay of IgA et IgG
- Dynamic of antibodies
- Seroconversion

- EBV – TBE – Measle
- Borreliosis – Syphilis
- Toxoplasmosis
Question

With fever, skin eruption in epidemic contexte. Does this result confirm measles infection?

IgM: positive
IgG : négative

YES
Measle: Confirmation

Indirect

✓ Seroconversion IgG in 2-3 weeks
✓ Increase of 4 fold IgG titer

Direct by PCR within 0- 15 days

✓ Vaccinated patients (suspecting vaccine problem)
✓ Atypical présentation (weak pre-test probability)
Question

Does this result confirm the etiology of a cutaneous lesion?

HSV, IgM: positive
HSV 1, IgG: positive
HSV 2, IgG: negative

NO
IgM are markers of primary infection but might be also unspecific so it does not confirm an HSV infection.
Cutaneous lesions: Confirmation

Indirect
- Seroconversion IgG HSV2

Direct
- Moleculaire or antigenic detection of herpès simplex
  - Site d’infection
- Possible infection with varicella zona virus (VZV)
- Multiplex detection needed
Question

Patient presenting with a small ulcerated lesion.

Do these results confirm syphilis?

VDRL / RPR : Titer 4 positif
TPPA / TPHA : Titer 80

Highly presumptive
Confirmation can be made by immunoblots, at early stage IgM are positive and IgG negative
Question

Patient presenting fever headache after a tick bite.

Does this result exclude a TBEV infection?

IgM: negative
IgG: negative

NO

Serology should be repeated within 7 days.
Fever is the first unspecific clinical sign
Neurological phase start 1-33 days later and at this stage serology is positive.
Diagnosis: VIS, PCR → serological tests → viremia → neurological symptoms → IgM antibodies in CSF

Graph showing:
- Infection timeline
- 4-14 d. incubation period
- 2-5 d. phase 1
- ~1 week interval
- ~3 weeks phase 2
- Fever
- IgM ab
- IgG ab
- Weeks post infection
2. CSF infection

- Intrathecal antibody production
- Role of the serum

TBE
Borreliosis – syphilis
Herpès simplex
Neuroborreliose has to be diagnosed directly by PCR on CSF as for enterovirus or HSV?

NO
It shows low sensitivity (5-10%)
Intrathecal production of specific IgM or IgG is complementary and useful even if sensitivity stays <80% up to 8 weeks after symptoms.
Intrathecal antibody index

\[
\frac{\text{Borrelia-specific Ig in CSF}}{\text{Borrelia-specific Ig in serum}} = Al
\]

Positive: Ratio > 2.0 (EUCALB)
Neuroborreliosis kit (Dako, Oxoid)

✓ Using enriched flagella as antigen
✓ Capture test IgG and IgM
✓ CSF and Serum
✓ Positive if DO Ratio IgG or IgM > 0.3
✓ Follow up needs > factor 5 variation
3. Infection chronique

- Follow up of specific markers
- IgM - IgG – IgA
- Antigens of different nature

Hepatitis B
Coxiella burnetti
Coxiella burnettii

IgM phase I et II

IgG phase I

IgG phase II

10 jours  1 mois  3 mois  10 ans
### Chronic coxiellosis

<table>
<thead>
<tr>
<th>C. burnettii</th>
<th>limites</th>
<th>19.07.07</th>
<th>30.07.07</th>
<th>03.10.07</th>
<th>03.07.08</th>
<th>29.09.09</th>
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</thead>
<tbody>
<tr>
<td>IgM ph. II</td>
<td>20</td>
<td>640</td>
<td>5120</td>
<td>2560</td>
<td>40</td>
<td>40</td>
</tr>
<tr>
<td>IgA ph. II</td>
<td>20</td>
<td>&lt; 20</td>
<td>&lt; 20</td>
<td>&lt; 20</td>
<td>&lt; 20</td>
<td>&lt; 20</td>
</tr>
<tr>
<td>IgG ph. II</td>
<td>20</td>
<td>20</td>
<td>5120</td>
<td>1280</td>
<td>1280</td>
<td>320</td>
</tr>
<tr>
<td>IgM ph. I</td>
<td>20</td>
<td>&lt; 20</td>
<td>160</td>
<td>40</td>
<td>40</td>
<td>20</td>
</tr>
<tr>
<td>IgA ph. I</td>
<td>20</td>
<td>&lt; 20</td>
<td>&lt; 20</td>
<td>&lt; 20</td>
<td>&lt; 20</td>
<td>&lt; 20</td>
</tr>
<tr>
<td>IgG ph. I</td>
<td>20</td>
<td>&lt; 20</td>
<td>&lt; 20</td>
<td>160</td>
<td>2560</td>
<td>640</td>
</tr>
</tbody>
</table>
4. Treatment monitoring

- Antibody titer
- Antigen dependant
- Slow kinetics
- Sera to be tested together

Syphilis ➔ RPR / VDRL
Borreliosis ➔ VlsE ?

Direct assays.
HIV, HBV, HCV, HDV, CMV, ... viraemia
## Treatment monitoring: primary syphilis

<table>
<thead>
<tr>
<th></th>
<th>Unit</th>
<th>30/7/09</th>
<th>19/11/09</th>
<th>25/5/10</th>
<th>24/8/10</th>
<th>11/3/11</th>
<th>9/12/11</th>
<th>21/3/12</th>
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</thead>
<tbody>
<tr>
<td><strong>time</strong></td>
<td>mois</td>
<td>0</td>
<td>3.5</td>
<td>10</td>
<td>13</td>
<td>19.5</td>
<td>28</td>
<td>32</td>
</tr>
<tr>
<td><strong>RPR</strong></td>
<td>titre</td>
<td>512</td>
<td>256</td>
<td>64</td>
<td>32</td>
<td>16</td>
<td>8</td>
<td>4</td>
</tr>
<tr>
<td><strong>TPPA</strong></td>
<td>titre</td>
<td>40’960</td>
<td>10’240</td>
<td>10’240</td>
<td>2’560</td>
<td>1280</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>RPR</strong></td>
<td>Dil 2</td>
<td>-1</td>
<td>-2</td>
<td>-3</td>
<td>-4</td>
<td>-5</td>
<td>-6</td>
<td></td>
</tr>
<tr>
<td><strong>TPPA</strong></td>
<td>Dil 2</td>
<td>-2</td>
<td></td>
<td>-2</td>
<td>-3</td>
<td></td>
<td>-4</td>
<td></td>
</tr>
</tbody>
</table>
5. Reactivated infection

- Role of IgA
- Increase of IgG titers
- Absence of IgM

Zona (VZV)
*Chlamydia pneumoniae*

⇒ more specific with direct assay = PCR
7. Immunity – past infection

- Role of IgG
- Persistence of IgG
- Usefullness of IgG quantification for vaccine control
- Serological scar

Bilan de grossesse: Toxoplasmose – CMV
Vaccinations: HBV
Return from vacation: Chikungunya
Epidemiological study: legionellosis
## 8. Transfusion blood qualification

### Indirect
- **Infectious markers**
  - HIV
  - Anti-HBc
  - HCV anticorps
  - Syphilis TPPA

### Directe
- **Antigen HBs**
- **Nucleic amplification test (NAT) reducing the serological windows**
  - HIV, HBV HCV
9. Prevalence

- Very crucial point for test evaluation
- Influences predictive values (NPV, PPV)

All pathogens
All diagnosis
Valeur Prédicitive Positive et Prévalence de l'infection
sensitivity 100% specificity 99.8%
Valeur Prédicitive Positive et Prévalence de l'infection
sensibilité 100% spécificité 99.8%

67%
Faux Pos.
**Lyme borreliosis**

<table>
<thead>
<tr>
<th>Screening</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>EM</td>
<td>45 %</td>
<td>95 %</td>
<td>69.2 %</td>
<td>87.4 %</td>
</tr>
<tr>
<td>20 % prevalence</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EM</td>
<td>87.6 %</td>
<td></td>
<td>47.4 %</td>
<td>86.4 %</td>
</tr>
<tr>
<td>20 % prevalence</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EM</td>
<td>16 %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 % prévalence</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EM</td>
<td>3.5 %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 % prévalence</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Late stage</td>
<td>99 %</td>
<td>87.6 %</td>
<td>66.4 %</td>
<td>99.7 %</td>
</tr>
<tr>
<td>20 % prévalence</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Late stage</td>
<td>29.6 %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 % prévalence</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Late stage</td>
<td>7.4 %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 % prévalence</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Seroprevalence – serological scar
Asymptomatic or disease?

- Seropositivity does not mean disease
- Seropositivity does not implicate treatment
- And seroconversion?
- Activity if direct evidence of pathogen’s presence
- But watch carriers

Presence of parasites, bacteria or virus
HBV: AgHBe
HIV: Ag p24
Urinary antigens for legionella
Herpès simplex 1 ou 2, CMV, VZV on clinical samples
Seroconversion

45 - 43 kD

t0 : at tick bite

t2 : 2 months later

Only one person showed an erythema migrans
10. Prozone

- Very high quantity of Ig
- Weak quantity of antigen
- Sterical conformation inhibiting complexes
- = False Negative

Precipitations, Agglutinations
Also direct antigenic assay!
Even possible in genomic assay!
Primary syphilis

<table>
<thead>
<tr>
<th>Assay</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>First serum at consultaion</td>
<td>RPR undiluted serum negative</td>
</tr>
<tr>
<td>Contact with physician</td>
<td>RPR dilution to 1/32 positive</td>
</tr>
<tr>
<td>Complementary tests</td>
<td>TPPA 10240</td>
</tr>
<tr>
<td>IgM blot</td>
<td>positive</td>
</tr>
<tr>
<td>IgG blot</td>
<td>positive</td>
</tr>
</tbody>
</table>

Physician had a **strong suspicion** of primary syphilis and was surprised by lab results.
Conclusions

- Introduction of a test goes through a complete technical qualification.
- Its correct manipulation includes an adequate training.
- Interpretation requires a precise and critical vision between the technical, clinical and diagnostic aspects.
- Proficiency includes identification of potential sources of errors.
Conclusions

- Choice of a method depend on the patient clinic, the physician diagnosis, the knowledge of the pathogen and test quality and validity.

- The dialogue between the physician and the microbiologist is the clue to obtain accurate and optimum results in a appropriate delay for a reasonable cost.
Merci
pour votre attention