

TOXOPLASMOSIS (1)

**Immuno Sorbent Agglutination Assay
(ISAgA ou ISAGA)**

Detection of IgM anti- *T.gondii*

Principal

- **Immunocapture** of IgM in the analyzed serum
- Revelation of the presence of **specific IgM** by adhesion of ***Toxoplasma*** (fixed, in suspension)
- $R^{\circ} +$: **agglutination** (cloudy background)
- $R^{\circ} -$: sedimentation (dot)

Human anti-IgM



Serum



Antigen



NEGATIVE =
Sedimentation



POSITIVE =
Agglutination

Material

- Wells sensitized by **human anti-IgM** mouse monoclonal antibodies
- Antigen : **suspension of *Toxoplasma* fixed by formaldehyde** (RH Sabin strain maintained in mouse)

Technique

- **1- Screening**

Has to be confirmed by:

- **2- Confirmation test**

1- Screening

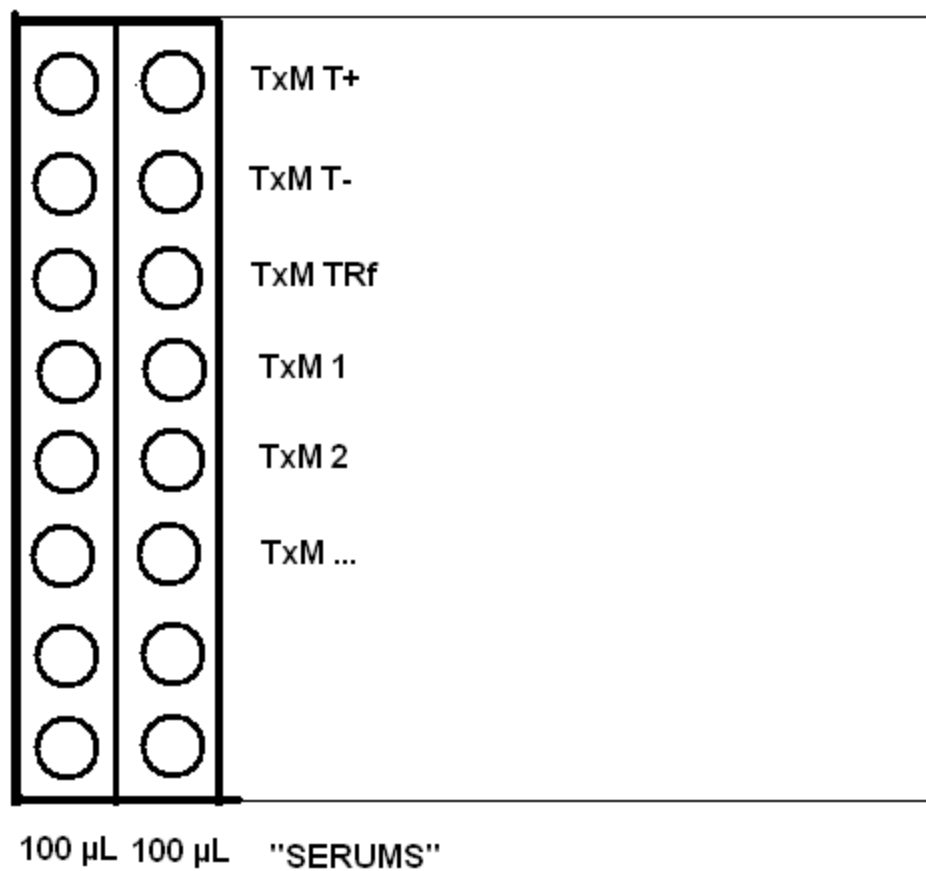
- **Identify** the frosted part of the stripe for a correct position in the frame
- **1- Incubation of sera**
 - **Dilute** serums in PBS
 - Serums of interest: 1/100
 - Control serum M+ : 1/10
 - Control serum M-: 1/100

– **Distribute :**

- **100 µL** of the dilution of **Control M+** serum in 2 juxtaposed wells
- **100 µL** of the dilution of **Control M-** serum in 2 juxtaposed wells
- **100 µL** of **PBS** (Control Reagent) in 2 juxtaposed wells
- **100 µL** of the dilution of serum of interest (Tx M 1, Tx M 2, ...) in 2 juxtaposed wells

– **Cover** the stripe with a self-sealing foil

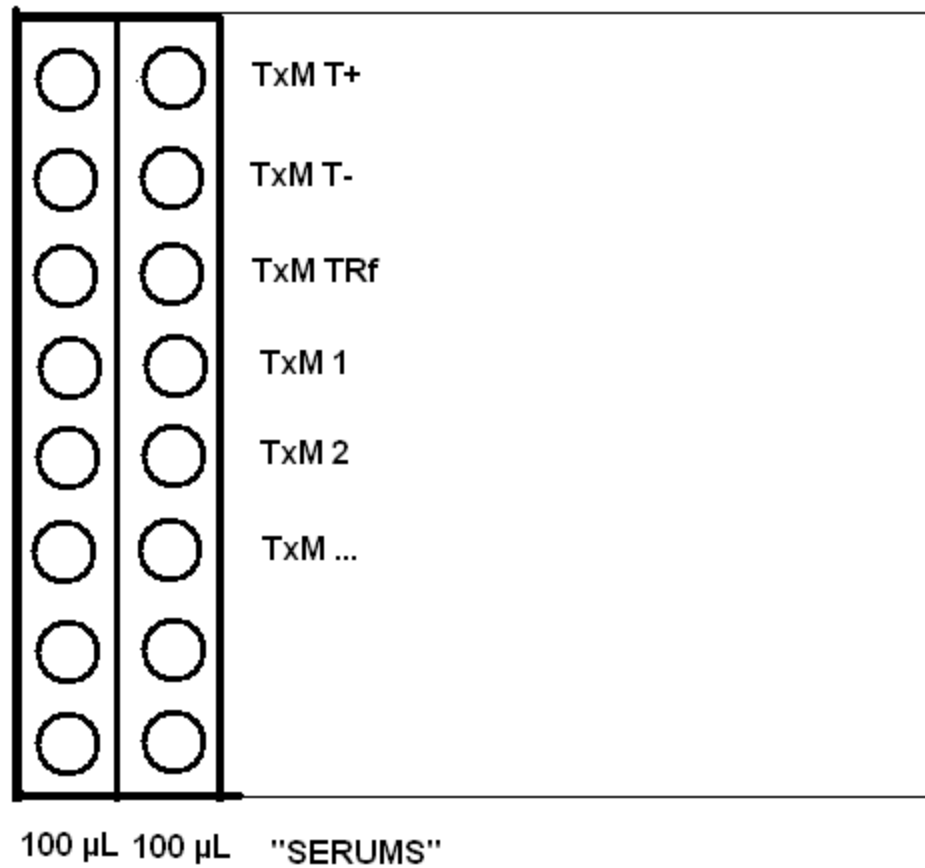
– **Incubate 2 hours at 37° C**



- **2- Washes with pissette**
 - **Empty the wells (turn upside down): careful !!!**
 - **Wash once with PBS-Tween (no overflow)**
 - **Empty immediately**
 - **Wash twice 5 min with PBS-Tween**
 - **Then wash twice with PBS**
 - **After each washing step, remove excess of liquid in each well on paper towell (don't let it dry)**

- **3- Revelation**

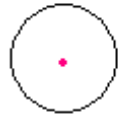
- **Distribute in all wells used the antigen diluted at 1/20 in BABS buffer**
 - **100 μ L** in the **first** wells (first column)
 - **150 μ L** in the **second** wells (second column)
- **Recover** the stripe with a self-sealing foil
- **Incubate** overnight at **37° C** (in a **humid atmosphere**)



100 μ L 150 μ L Suspension of fixed *Toxoplasma*

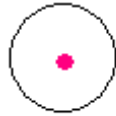
- **4- Reading**

- By eye
- Place the stripes at around 50 cm from a white support, properly illuminated
- **R° - : sedimentation of *Toxoplasma***
- **R° + : agglutination of *Toxoplasma* (cloudy background)**
- **In case of an excess of antigen / IgM: subsidiary sedimentation more or less important.**



0

Total sedimentation, comparable to the control « Antigen »



1+

Little dot of sedimentation



2+

Intermediate dot of sedimentation



3+

Large dot of sedimentation



4+

Cloudy background on the whole well, comparable to the serum Control M+

- **5- Results**

- **ISAgA index = sum of values** attributed for the 2 volumes of Antigen used
- Example

| Serum \ Ag | 100 µL | 150 µL | ISAgA Index |
|------------|--------|--------|-------------|
| | | | |
| Serum x | 3 + | 2 + | 5 |
| Serum y | 4 + | 4 + | 8 |

- **Analysis :**
 - **0 to 5 = negative R°**
 - **≥ to 6 = R° to be completed by a confirmation test**

2- Confirmation test

- Cf « screening »
- Ag : 150 et 200 μL /well
- ISAgA index

Sum of values for the 3 volumes of Ag used: 100 et 150 μL for the « screening » and 200 μL for the confirmation test (not done today)

- Results and analysis
 - Example

| Ag Serum | 100 µL | 150 µL | 200 µL | ISAgA index |
|-------------|--------|--------|--------|----------------|
| Serum x | 4 + | 2 + | 2 + | 8 |
| Serum y | 4 + | 4 + | 3 + | 11 |

– **Toxoplasmosis**

- Index 0 to 5 : negative reaction
- Index 6 to 8 : ambiguous reaction
- Index 9 to 12 : positive reaction