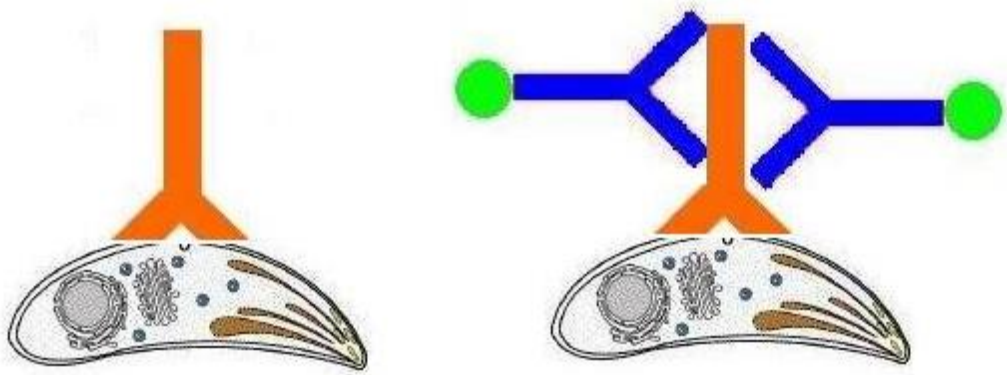
TOXOPLASMOSIS (2)

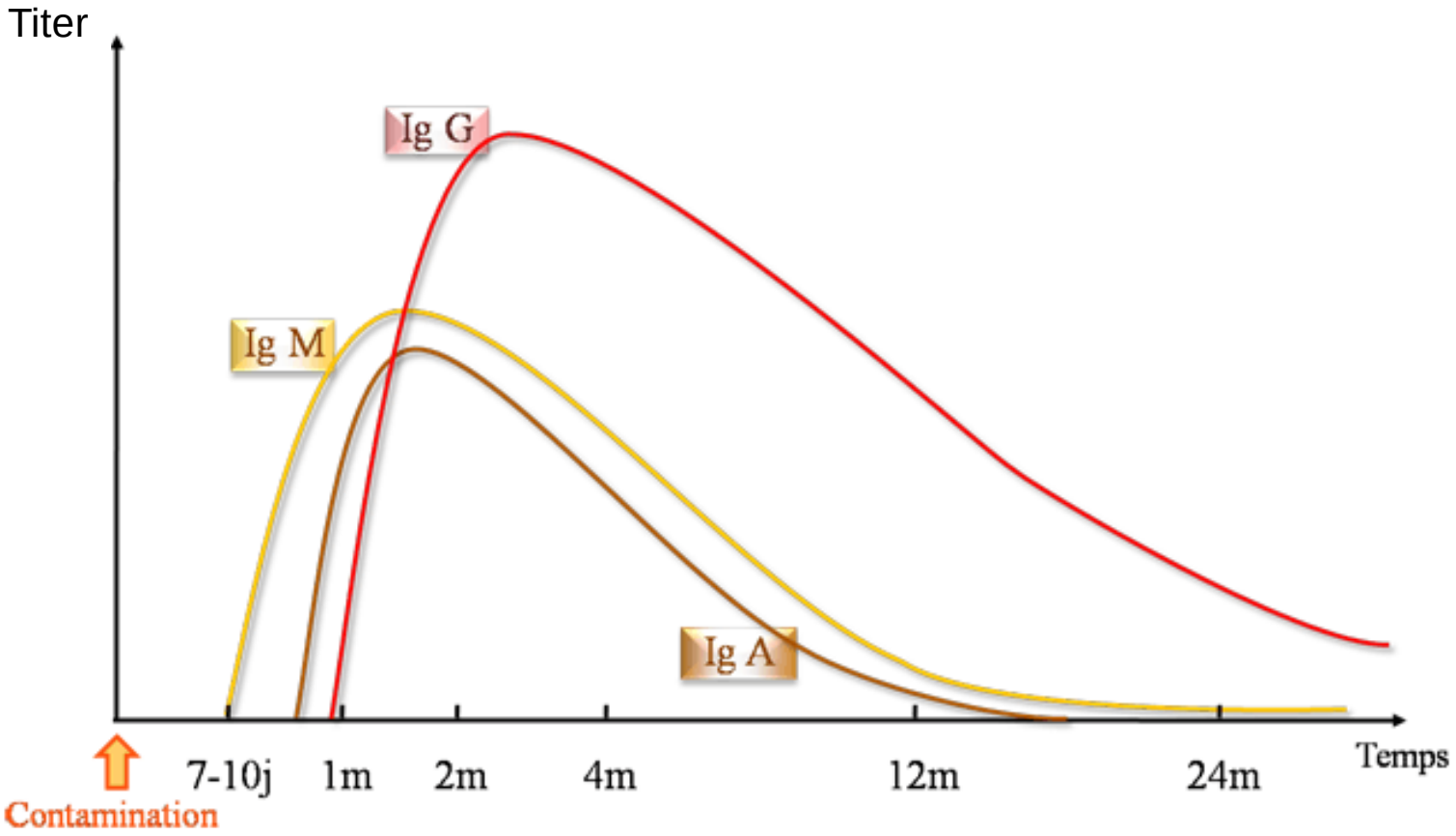
Indirect Immunofluorescence (IFI)
Titration of anti-*T.gondii* IgG



ANTI-*Toxoplasma* ➔ Antibody titration

Toxoplasma = Ag

Evolution kinetics of anti-*Toxoplasma* antibodies

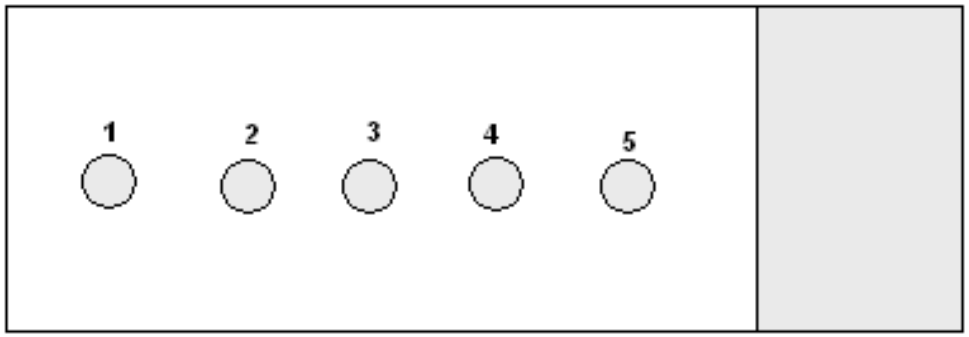


Seroconversion = négative serology → positive

The appearance of IgG allows to affirm the serconversion

Slides used for IFI

- Antigen bound to the slide : *Toxoplasma gondii*
- Slide of 5 wells: 1 for 2 groups



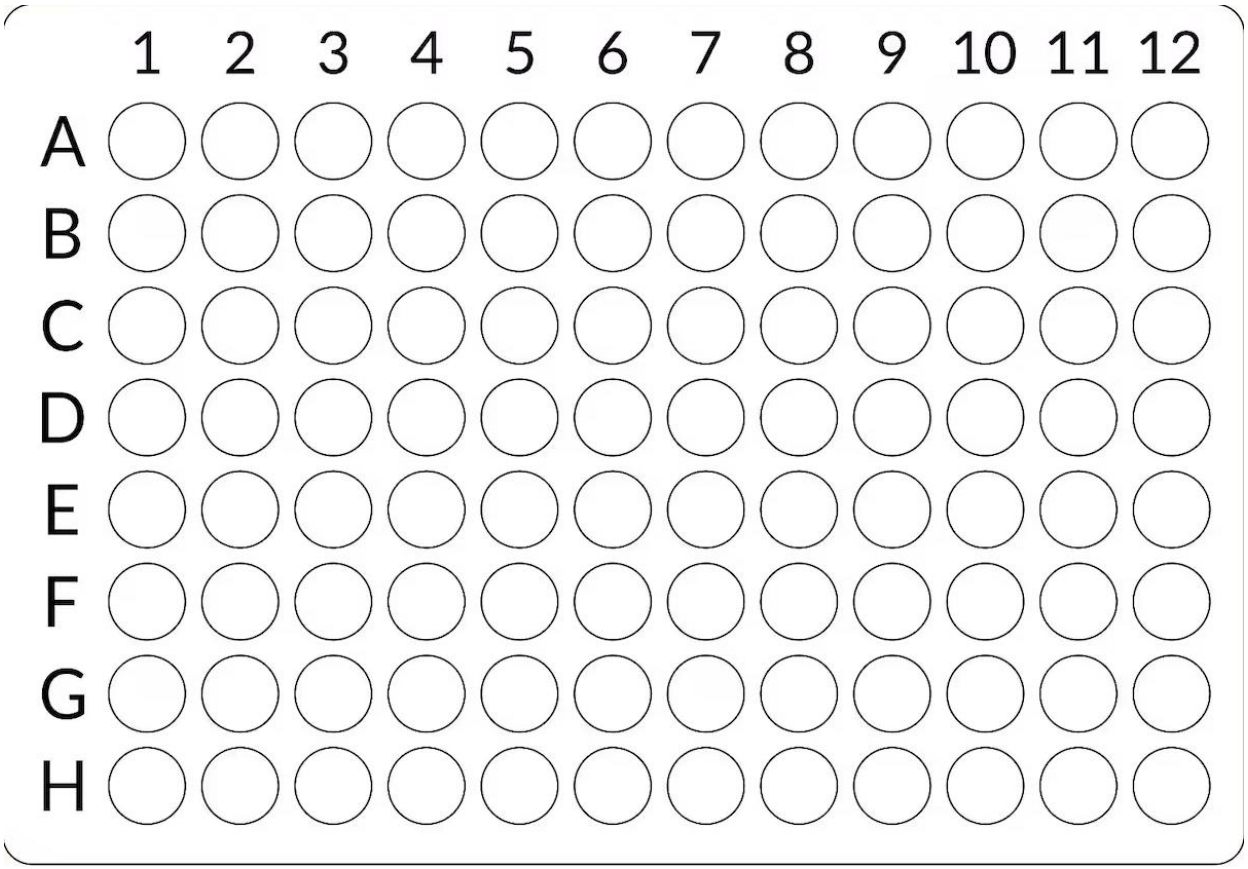
Dilution of serums

- The serums Tx1 to Tx6 are given diluted at 1/4 and have to be diluted at 1/64 in PBS:
- Dilutions are performed in PBS, in a microtitration plate:
  - $^1/_2$ ⇒ **1/8** 50µL+ 50
  - ½ ⇒ **1/16** 50 + 50
  - ½ ⇒ **1/32** **…**
  - ½ ⇒ **1/64**

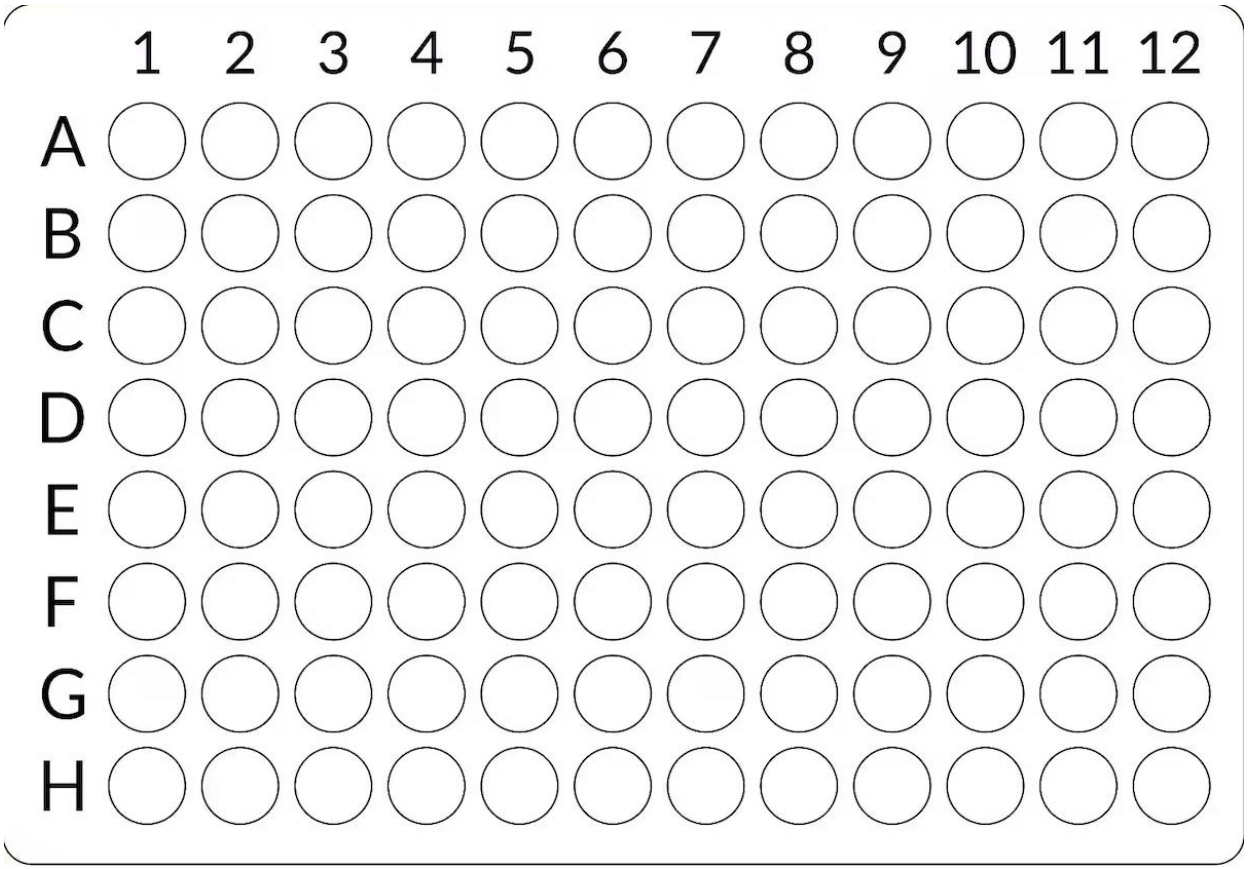
- Control serums TxT+, TxT-, TRf are ready to use → ask for the deposition of TxT+ on the slide

PBS

50 µl | 50 µl | 50 µl | 50 µl



Dilution	1/8											
Serum (1/4)	50 µl											
PBS	50 µl	50 µl	50 µl	50 µl								



Dilution		1/8	1/16									
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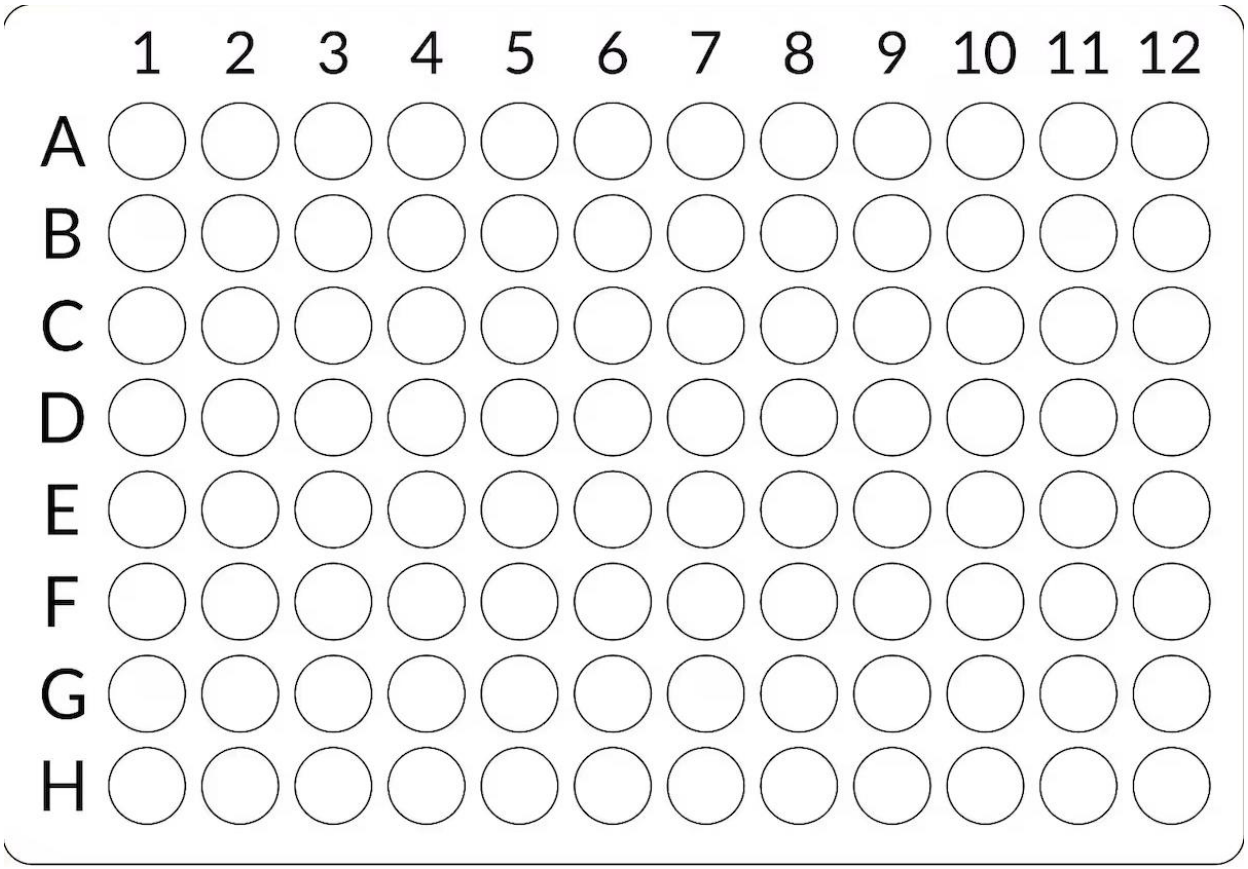

Dilution	1/8	1/16	1/32	
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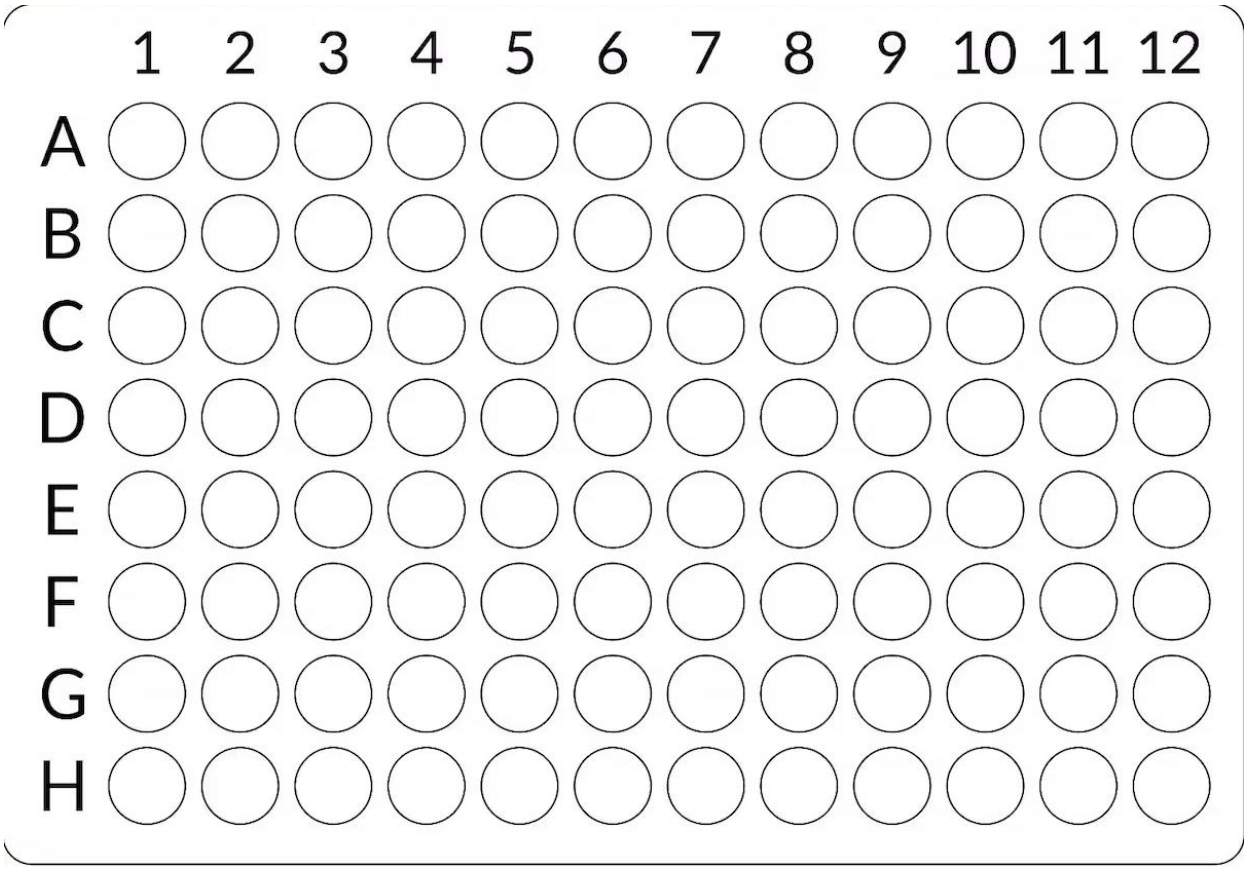

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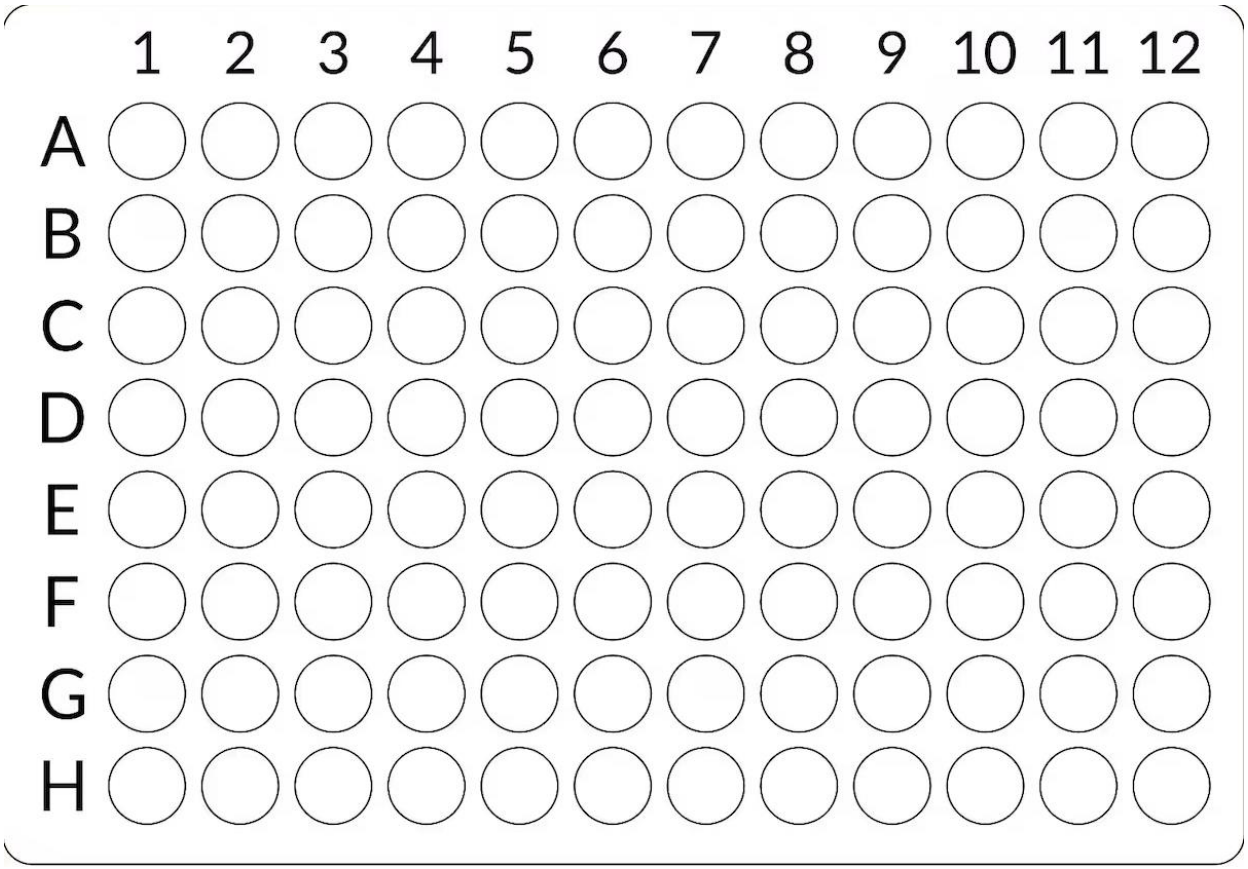
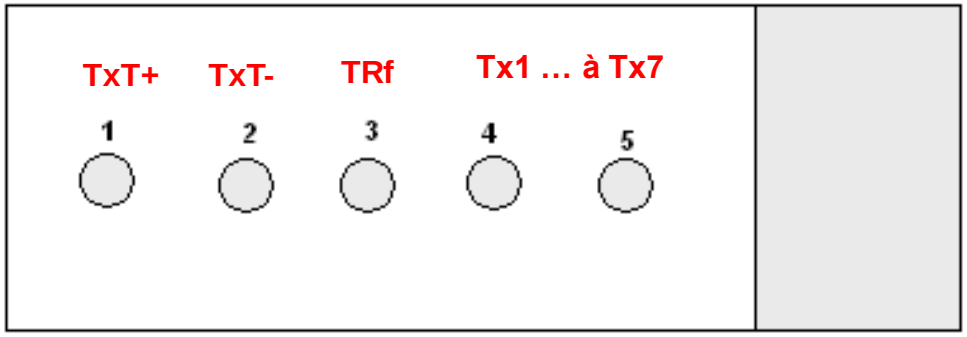


Serum loading

- Put the slide in a humid chamber

- **20µL of diluted serums** in the wells as illustrated:



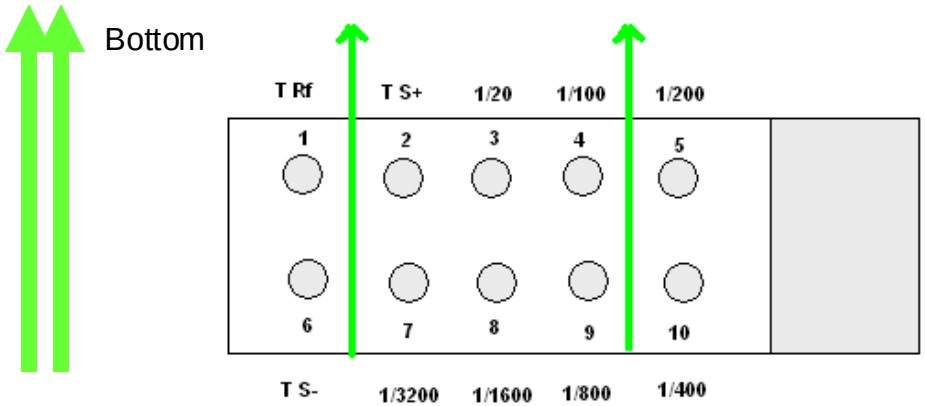
TxT+ = positive control
TxT- = negative control
TRf = Control reagent

Incubation

- In a humid chamber
- Incubator **37˚ C**
- **30 min**

Washes

- Rinse gently with **PBS** (pissette)



- 1st wash in **PBS : 5 min**
- 2nd wash in **PBS : 5 min**
- 3rd wash in **PBS : 5 min**

Slides drying

- Very gently !!!

- Between the wells as illustrated →

  Coton bud

  Paper towel

Secondary antibody loading

- Human anti-IgG conjugated with fluorescein.
- Put the slide in a humid chamber
- Anti-IgG is ready to use: ask for the deposition on the slide

Incubation

- 37° C for 30 min in a humid chamber

Washes

- 3 washes 5 min in PBS (as the first time)
- **At the last washing step**: add 3 to 5 drops of Evans Blue in PBS bath

Drying

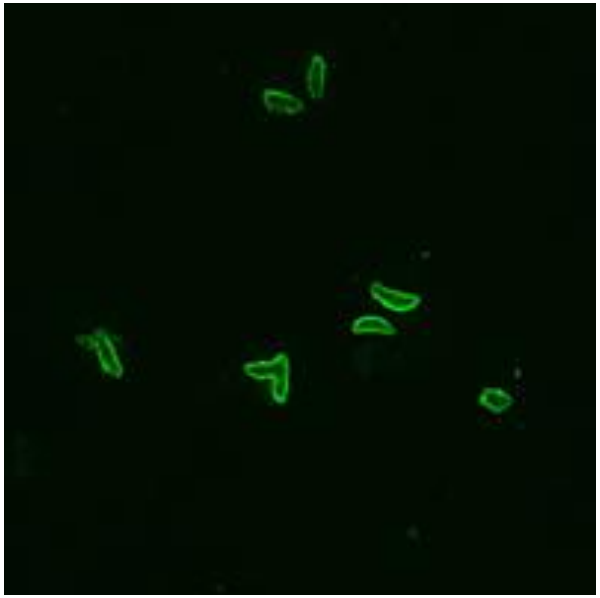
- Always gently!!!
- More gently than the first time

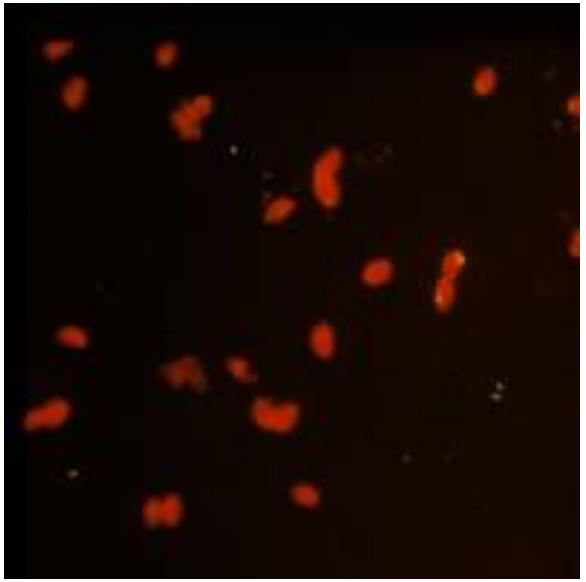
Mounting

- In buffered glycerol
- 5 drops deposited in each well
- Recover with large coverslip 24 x 60 mm
- Remove the excess of mounting medium if necessary
- Possibility to keep the slides mounted several days at +4° C

Reading

- **Epifluorescence** microscope
- Objective **x 40**
- **The interpretation is not possible if the controls are not correct**
- What can we see?





Analysis of results

- **Membrane fluorescence: positive**
- If fluorescence only in the cytoplasm, or no fluorescence: **negative**
- **Positivity threshold of the technique: 1/64**
- **Conclude** and indicate the **patient follow-up**