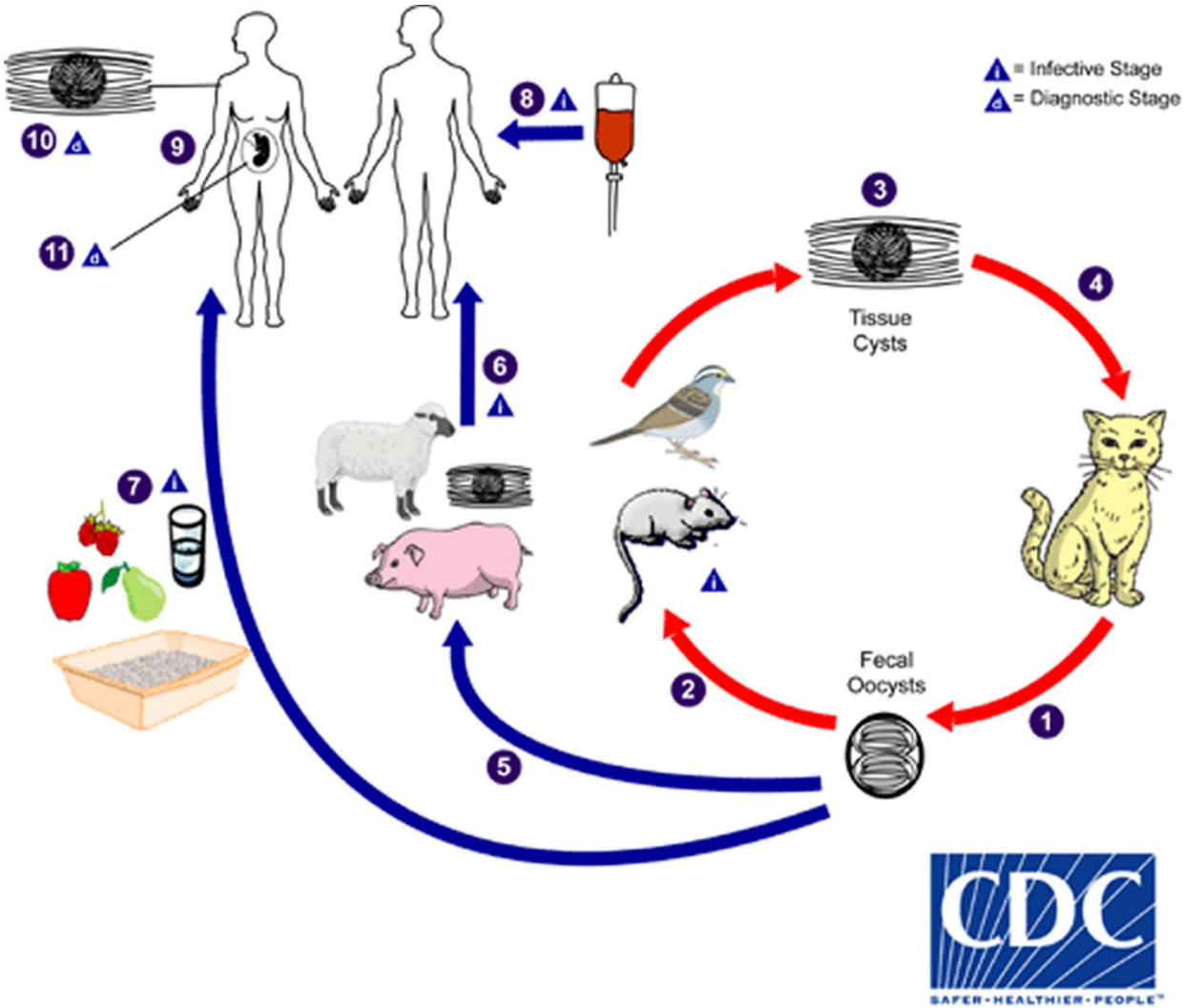
# DIAGNOSIS OF TOXOPLASMOSIS

# GENERALITIES

# *Toxoplasma gondii* life cyle

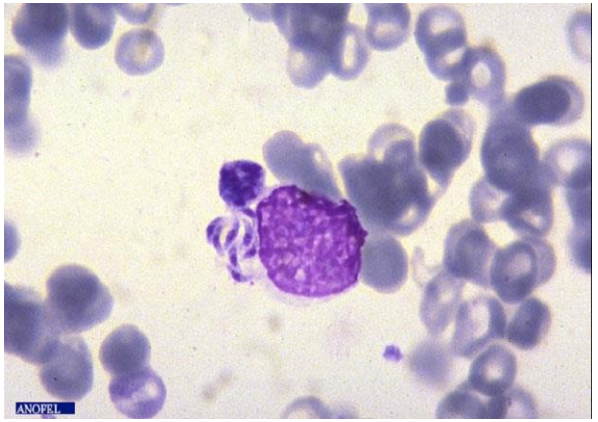


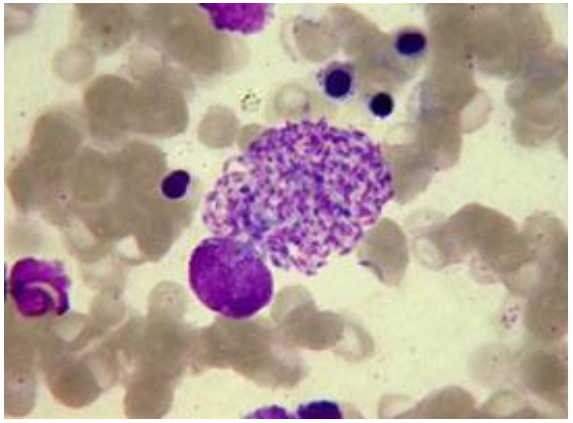
# 1- ORIENTATION ARGUMENTS (HEMATOLOGY)

- **Discrete mononucleosis syndrome**
- **Moderate hypereosinophily** (generally < 0.1 % in proportion)

# 2- PARASITOLOGICAL DIAGNOSIS

- **Evidence** of the **parasite** (or its constituents)
- On CSF, **bronchoalveolar lavage**, **amniotic liquid**, **placenta**, **biopsies** …
- **A- Microscope analysis**
  - Mainly **immunosupressed**
  - Giemsa staining or equivalent



- **B- Mouse inoculation**
  - Mainly **cerebral toxoplasmosis** (**placenta, CSF**)
  - Or **immunosuppressed** (**diverse** biological products)
  - **Long** technique (4 to 6 weeks) but 90% **reliable**

- **C- *In vitro* cell culture**
  - Same indications
  - But faster results (4 to 5 days)
  - Cells used
    - Human embryonic fibroblast (MRC 5)
    - Monocyte line (THP 1)
  - Staining (Giemsa ou fluorescence)
  - **Less used than PCR but sometimes useful**: hemorrhagic samples (Hemoglobin inhibits Taq pol)

- **D- PCR**
  - **Parasite DNA** investigation
  - Different target-sequences can be amplified: preferentially **B 1, P 30 genes**…
  - **Limit**: **hemorragic sample** ($\Rightarrow$ cell culture)

# 3- IMMUNOLOGICAL DIAGNOSIS

- **A- Legislation**

  - **Mandatory toxoplasmosis serology**

    - Declaration of **pregnancy**
    - Sampling of organs, tissues and cells (**donor** and **recipient**)
    - Checkup for **HIV infection**
    - **Diverse follow up :** pregnant woman without any previous contact with *Toxoplasma*, immunosuppression

– **J.O. avril 1995 puis TNB version N° 37 (janvier 2012) (France)**

- **All exams have to** precise
  - The technique
  - The positivity threshold of the reagent (Name)
  - The reagent lot number

- The **biologist should conclude**
  - For the presence or absence of anti-*T. gondii* antibodies
  - **Probable** date of infection in case of positivity
  - Interpretation

- The biologist **proposes the modalities of an eventual follow up**
- **Conservation** of serums: at least **1 year at –30° C**

# Toxoplasmosis serology

## Mandatory

- **Association of 2 different isotypes**
  - Titration of IgG in UI/mL
  - IgM investigation
- **Write an argumented conclusion**
  - Interpretation of the serologic profile.
  - Modalities of follow up if necessary
- **Precise on the report**:
  - Techniques
  - Reagents
  - Positivity threshold value

**Conserve serums**
**Frozen for 1 year**

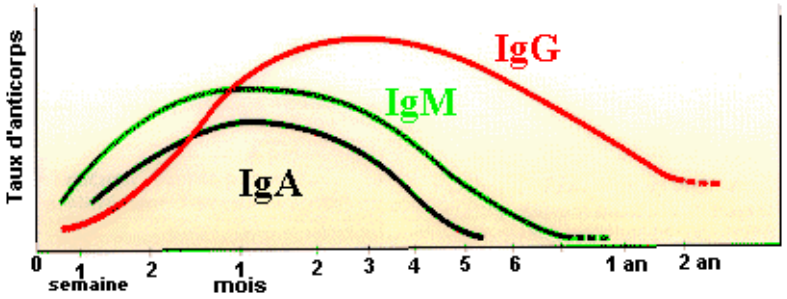
# The report should mention :

- The techniques used
- The lot number, the laboratory name
- Positivity thresholds (significative) for the quantitative techniques
- The antigens used
- The titer in UI/mL of IgG anti-Tx
- Indicate the presence or absence of IgM anti-Tx
- Analyze the results and conclude
- Indicate the modalities of follow up

- **3 cases mentioned**
  - Case of immunosuppressed
  - Case of pregnancy
  - Case of newborn

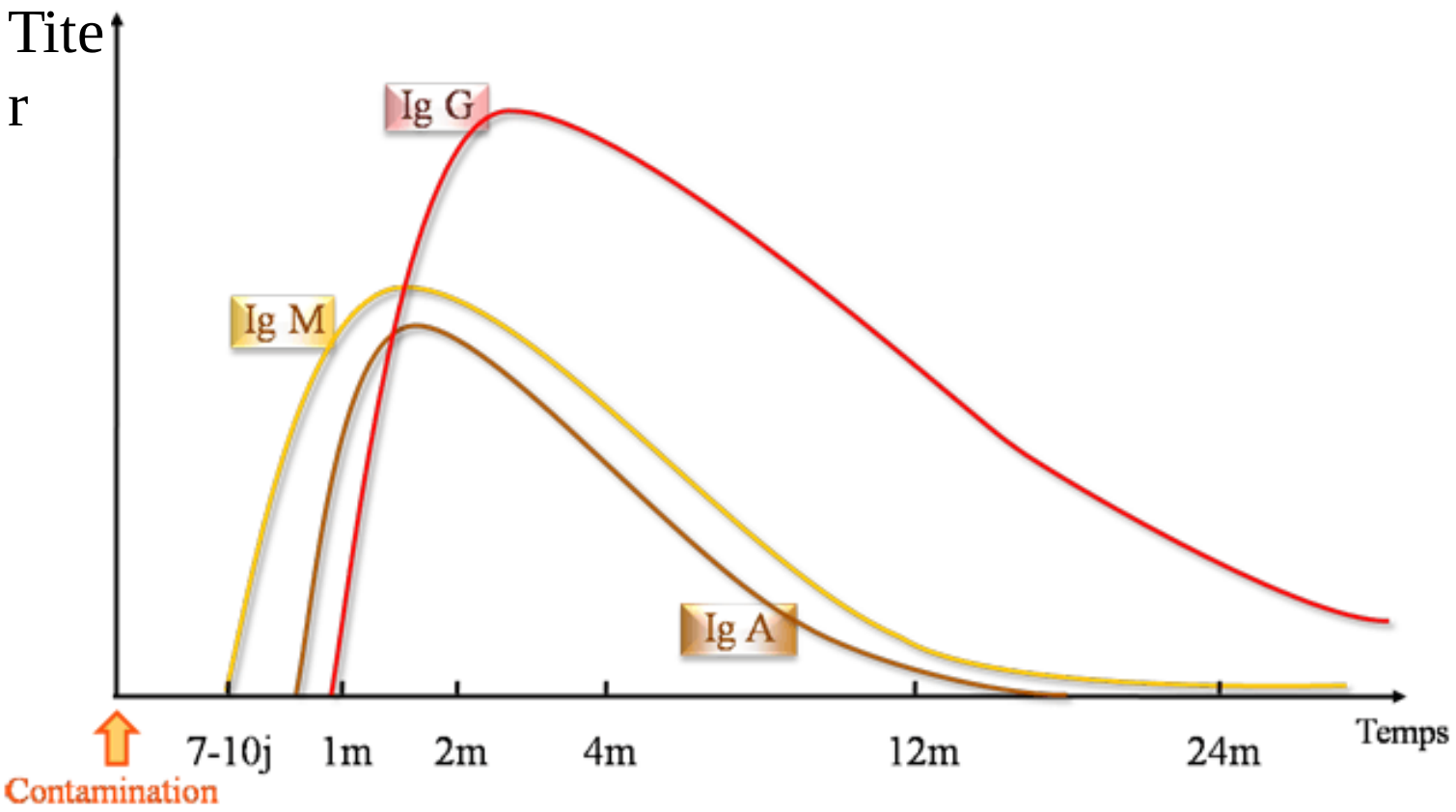
- **Identification and titration**
  - **At least 2 different Ig isotypes** (including IgG)
  - By at least 2 different techniques (selected by the biologist)
  - Follow up: *idem* with an analysis of the previous serums in parallel in the same series

# – Antibodies kinetics




- Anti-*Toxoplasma* Ig M
  - First to appear (8 to 10 days)
  - Recent or evolutive disease
  - Time? → has to be determined
- Anti-*Toxoplasma* IgA
  - Appear ≈ in the mean time as IgM
  - Disappear before IgM ⇒ **very recent disease**
  - Not much used
- Anti-*Toxoplasma* Ig G
  - **Appear later** (1 to 3 weeks)
  - Reach a threshold
  - **Persist for life**

# Evolution kinetics of anti-*Toxoplasma* antibodies




Seroconversion = negative serology → positive

The appearance of IgG allows to affirm the serconversion

# Toxoplasmosis serology: where are the problems?

- IgG+, IgM- : positive serology, immunized patient (IgG+).

- IgG-, IgM- : negative serology, non-immunized patient.

  In pregnancy monthly follow-up, with a last control after delivery.

  If immune defisciency semestrial follow-up

- IgG-, IgM- ⇒ IgG-, IgM+ ⇒ IgG+, IgM+

  -, then possible seroconversion, confirmed seroconversion (IgG+)

- IgG ambiguous, IgM- : immunized or not?

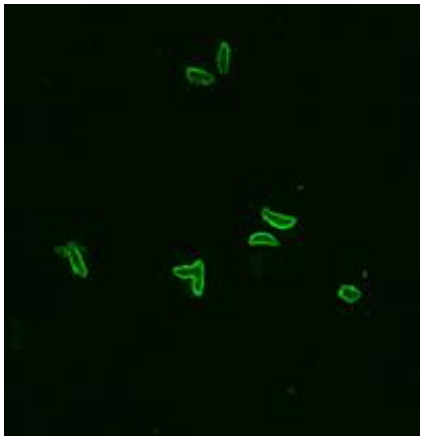
- IgG+, IgM+ : residual IgM or recent toxoplasmosis ?

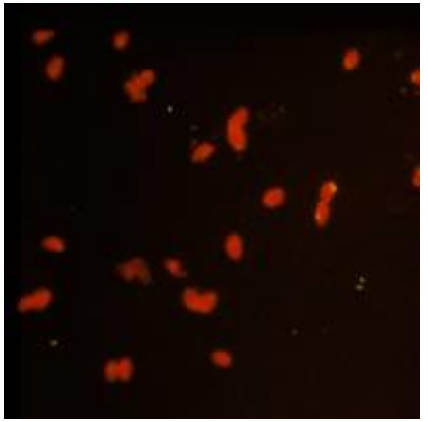
# C- Techniques

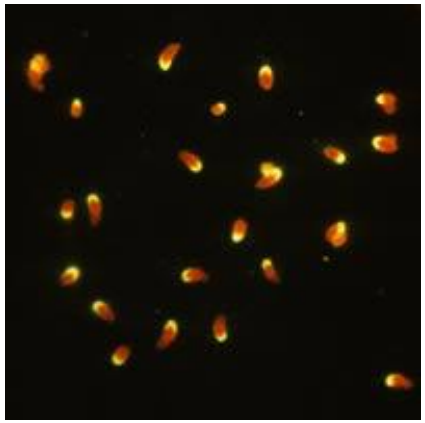
- Standard serum
  - IgG titer in UI Ab antitoxo/ml; ex 300 UI/ml

- Always T + T- and Treagent

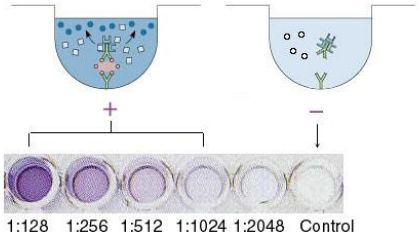
- IFI
  - Fixed antigen bound to a support (commercialised)
  - IgG
    - Threshold : 6 to 10 UI/mL
    - Human anti-IgG labeled with a fluorochrome
  - IgM (REMINGTON test)
    - Threshold : 1/40- 1/50
    - Human Anti-IgM labeled with a fluorochrome
  - Careful of false
    - Positive reactions
    - Negative reactions





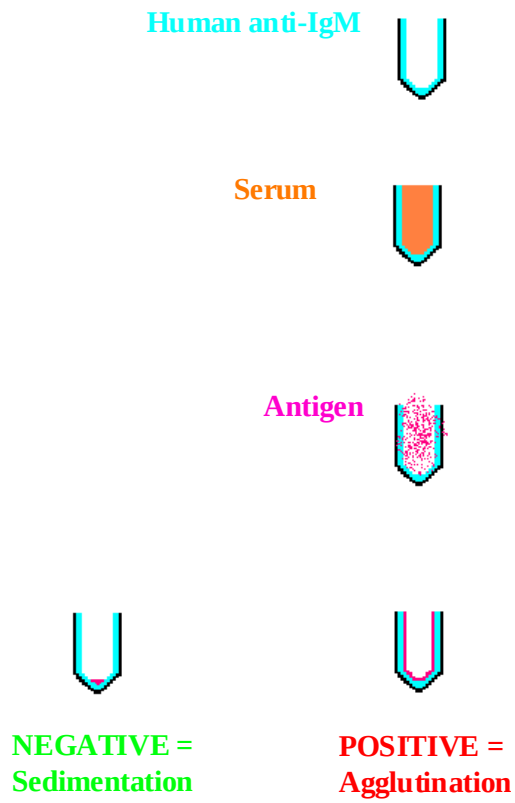


- **E.L.I.S.A. (much used, non subjective reading)** (Enzyme-Linked ImmunoSorbent Assay)
  - **Soluble antigens** (enriched in membrane fractions)
  - **IgG**
  - **Positivity threshold: 8 to 10 UI/mL**
  - **Some ELISA kits usable for IgM** (mini Vidas®, Biomerieux)
  - Avidity of IgG, increases with time (urea 6M)
  - Avidity index to date the infection



- **Techniques of immunobinding**
  - **Bound** or **soluble antigens**
  - **IgM**, **IgA**
  - **Principal :**
    - **Human anti-IgM bound to a solid support**
    - The IgM of the serum are then captured
  - **Methodologies** : multiple
    - **ISAgA** (3 steps)
      - **Antigen bound and digested by trypsine** (80% memb., 20% cyto.)
      - Results expressed as an **index** or **score**
      - **Positivity treshold** : score 8+
    - ELISA-double sandwich (5 steps)
    - « Reverse-ELISA » (4 steps)
  - **Very specific**, very (too much?) **sensitive**

# ISAgA (Desmonts)



# Toxoplasmosis and immunosupression

# TOXOPLASMOSIS:

- **Opportunistic parasitosis** → remains contemporary
- **Frequently starts** with the beginning of HIV disease **(CD4≤ 100/µl)**
- Can also be observed in cases of **immunosupression due to other causes than HIV:**
  - **Grafts**
  - **Neoplasia and their treatment**
  - **Other viruses**

# Toxoplasmosis in immunosuppressed:

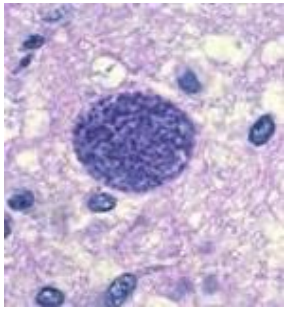
- Serious disease
- Fatal without or with late treatment (except for isolated ocular localization)
- Classically
  - **Localized Tx**
  - **Disseminated Tx**
  - **But** generally not that clear-cut…

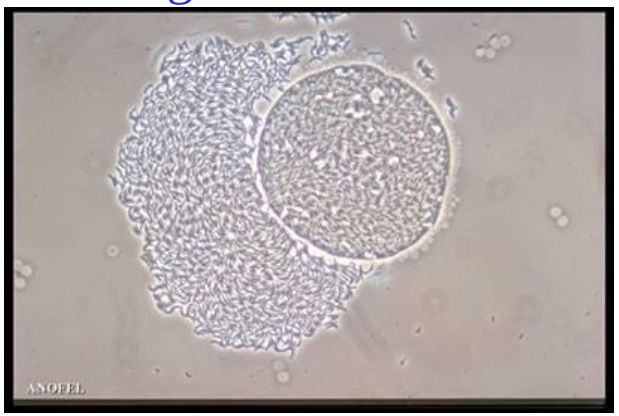
# Toxoplasmosis in immunosuppressed

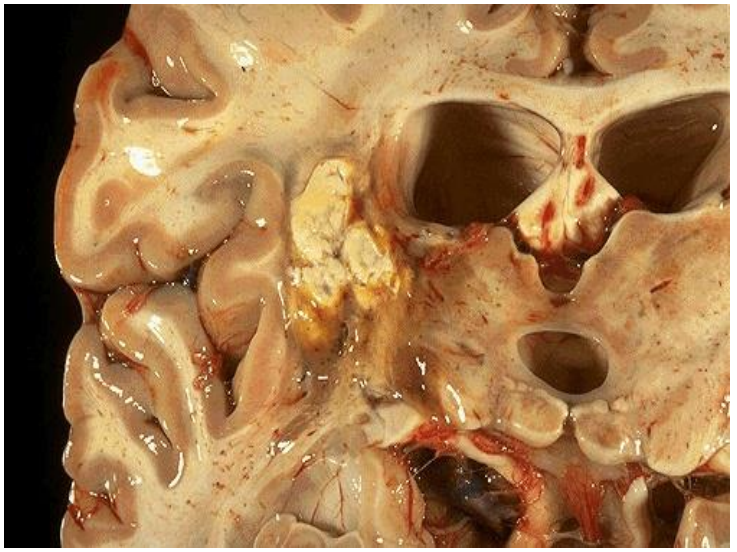
- Toxoplasmosis in immunosuppressed is fatal without treatment, except for isolated ocular localization
  - Reactivation of a former toxoplasmosis
  - Primary infection in immunosuppressed
- Severity of the disease more linked to immunological state of the patient than the parasite genotype (Ajzenberg, J. Infect. Dis. 2009)
  - HIV immunosuppressed : more cerebral localizations
  - Non HIV immunosuppressed : more pulmonary localizations

# Toxoplasmosis in immunosuppressed

- Reactivation of a former toxoplasmosis
  - HIV infection & CD4 < 100 $mm^3$
  - Bone marrow graft +++







Rupture of a cyst  Tachyzoites multiplication & tissue necrosis

- Primary infection in immunosuppressed
  - Solid Organ Transplantation (SOT), mainly heart (donor toxo + / recipient toxo-)
  - Primary infection in immunosuppressed without prophylaxis

# Biological diagnosis of toxoplasmosis in immunosuppressed

- Positive serology : possible diagnosis of a toxoplasmosis.

- Negative serology :

  diagnosis of a toxoplasmosis excluded, except for the beginning of a primary infection.

  Possible delay for the seroconversion : associate serology and parasite detection

- Diagnosis of certainty:

  → evidence of the parasite

# Can be due to:

- **Reactivation of a former toxoplasmosis (secondary)**
  - Important defisciency of cellular immunity
  - Often **HIV+ with CD4 ≤ 100/µL**
  - **Serology ± contributive, preferable to perform a direct diagnosis**

# Can be due to:

- **A primary infection:**
  - Often **secondary** to the transmission of a graft (solid organ: **heart** +++)
  - Sometimes with alimentary origin
  - **Contributive serology (but sometimes synthesis of antibodies is delayed, so it should be associated with a direct detection of the parasite)**

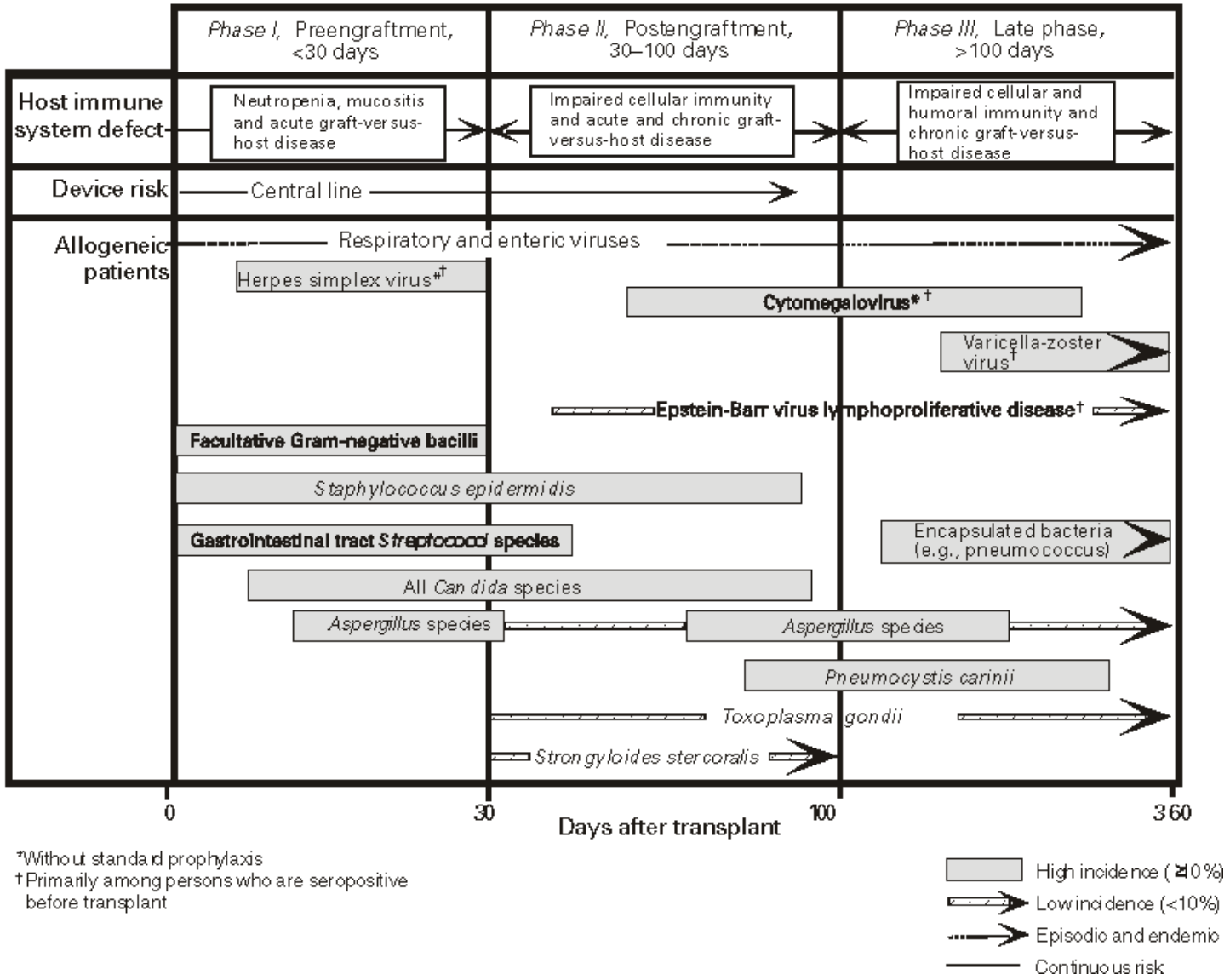
# Toxoplasmosis serology and immunosuppression

- Mandatory in all grafts
  - Donor
  - Recipients

# Toxoplasmosis serology

- Example of grafts
  - **Heart**
    - **D** Tx**+** et **R** Tx**-** → **high risk** **(no immunization in R, heart = muscle, highly infected, more than other solid organ)**
    - **D** Tx**-** et **R** Tx**+** → **low risk** **(reactivation of IgG)**
  - **Bone marrow**
    - **D** Tx**-** et **R** Tx**+** → **high risk** **(immunosuppressive treatment longer than for SOT)**
    - **D** Tx**+** et **R** Tx**-** → **low risk** **(passive transfer of lymphocytes)**

**FIGURE. Phases of opportunistic infections among allogeneic HSCT recipients**



| Type of transplant | Type of risk | |
|---|---|---|
| | Donor-transmitted disease | Reactivation |
| SOT | | |
| Heart/heart-lung transplant | High in $D^+/R^-$ | Low even in $R^+$<br>None in $R^-$ |
| Liver | Low in $D^+/R^-$ | Low in $R^+$<br>None in $R^-$ |
| Kidney | Low in $D^+/R^-$ | Low in $R^+$<br>None in $R^-$ |
| Intestine | Low in $D^+/R^-$ | None in $R^-$ |
| HSCT | | |
| Autologous | No risk | Nil in $R^-$<br>Very low in $R^+$ |
| Allogeneic | No risk | High in $R^+$<br>Risk more if donor seronegative |

$D^+$: Pretransplant donor antitoxoplasma serology positive, $R^+$: Pretransplant recipient antitoxoplasma serology positive, $R^-$: Pretransplant recipient antitoxoplasma serology negative, SOT: Solid organ transplant, HSCT: Hematopoietic stem cell transplant

From Khurana and Batra, Trop Parasitol, 2016

- Tx serology mandatory during a checkup for HIV infection

  - **Patient Tx-**
    - Tx serology at least 3 times a year
    - During the whole time of immunosuppression

  - **Patient Tx+**
    - Serology not much contributive
    - UNLESS
    - Important increase of antibodies, showing a clinical reactivation at ± long term

# Direct parasitological diagnosis

- Direct detection on biological products, biopsies: low sensitivity
- qPCR+++ (blood, bone marrow, BAL, CSF) specificity and sensitivity ++++
- Indirect techniques
  - Mouse inoculation (slow, 30 days)
  - Cellular culture (1 week)

# Treatment and prophylaxis

d' après DELFRAISSY 2004 et ANOFEL 2007

**If patient Tx-, indicate in the report the hygieno-dietetic rules to follow**

**No real consensus in other situations of immunosuppression, apart from HIV**

<table>
<tr><th>Curative treatment<br>Cerebral Tx</th><th>Primary prophylaxis<br>Tx</th><th>Secondary prophylaxis<br>Cerebral Tx<br>or maintenance treatment</th><th>Stop / Resurgence/<br>HAART</th></tr>
<tr><td>Pyrimethamine, folinic acid<br>and sulfadiazine (or clindamycine or atovaquone)<br>3 to 6 weeks</td><td>BACTRIM</td><td>Pyrimethamine and folinic acid<br>and sulfadiazine<br>(or clindamycine)<br>Reduced doses (1/2)</td><td>Stop if :<br>➢ >200 / µl CD4, analyzed twice at 3 months interval<br>➢and undetectable viral burden</td></tr>
<tr><td></td><td rowspan="3">Dapsone,<br>Pyrimethamine and<br>Folinic acid<br><br>CD4 < 100 / µl<br>(possibility to stop the prophylaxy if CD4 > 200 for at least 3 months)</td><td rowspan="3"></td><td rowspan="3">Resurgence when CD4 < 350 / µl</td></tr>
<tr><td>Association with anticonvulsant treatment</td></tr>
<tr><td>Anti-oedematous treatment<br>Corticoides ? !!!</td></tr>
</table>

- Advices for ID Tx- patients:
  - Sufficient cooking of meat before consumption
    - **All meats (white or red)**
    - **Cooking ≥ 65° C (internal temperature)**
    - **Avoid consumption of marinated, grilled or smoked meats**

- **During meals preparation**:
  - **Wash well vegetables,** especially if they are earthy and eaten raw.
  - **Wash well cooking tools** and the bench.
  - **Wash hands** after contact with vegetables, fruits or raw meat before eating.

- **During meals outside the home**:
  - Avoid consumption of raw vegetables and prefer cooked vegetables
  - Meat should be eaten well cooked and prefer consumption of fish.

- **Avoid direct contact** with objects that could have been contaminated **by cat feces** (litter, soil…) and **wear gloves** in case of their manipulation.

- Disinfect cat litter with bleach

- **Avoid direct contact with soil and wear gloves to garden. Wash hands after gardening activities** even if they were protected by gloves.