Testing for Toxoplasmosis and Rubella Infections

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NRL, Melbourne, Australia

Roche Symposium
Dubai
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Toxoplasma: Parasite

- World-wide distribution
- Obligate intracellular parasite
- Infects most warm blooded animals

Toxoplasma: Life Cycle

- Three infective parasitic phases
  - Rapidly dividing, invasive tachyzoite
  - Slowly dividing bradyzoite (tissue cysts)
  - Sporozoite (within the oozyte)
- Both sexual and asexual replication
- Transmission between both intermediate and definitive hosts (sexual cycle) and between intermediate hosts (asexual cycle) and even between definitive hosts

Life cycle of Toxoplasma gondii. Shown are the biology, infection, and replication of the three infective stages of the parasites in their respective hosts.
Toxoplasma: Prevalence

- Assumed global prevalence of 25-30%
- Prevalence varies widely between countries (10 – 80%)
  - Low prevalence (10-30%) – North America, SE Asia, Northern Europe
  - Medium prevalence (30-50%) Central and Southern Europe
  - High prevalence (>50%) in Latin America and tropical Africa
- Higher prevalence in humid, warm countries
- Linked to dietary habits, methods of cooking, hand washing, types of meat and vegetables eaten
- Humans infected by ingestion of
  - tissue infected with cysts
  - Infected soil or water
- Meat consumption estimated to be responsible for 30-60% of infections, soil contact 6-17%
# Toxoplasma: Prevalence

## The global burden of congenital toxoplasmosis: a systematic review

Paul R Torgerson & Pierpaolo Mastroiacovo  
Volume 91, Number 7, July 2013, 501-508

### Table 2. Global incidence and burden of congenital toxoplasmosis, by region of the World Health Organization

<table>
<thead>
<tr>
<th>Region</th>
<th>Incident cases (95% CI)</th>
<th>Incidence* (95% CI)</th>
<th>DALYs (95% CI)</th>
<th>DALYs* (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AFR D</td>
<td>26 500 (24 300–30 100)</td>
<td>2.0 (1.8–2.3)</td>
<td>171 500 (92 300–294 500)</td>
<td>13 (6.9–22)</td>
</tr>
<tr>
<td>AFR E</td>
<td>37 000 (33 900–41 000)</td>
<td>2.4 (2.2–2.5)</td>
<td>235 900 (129 600–379 000)</td>
<td>15 (8.3–24)</td>
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<tr>
<td>AMR A</td>
<td>2940 (2360–3540)</td>
<td>0.6 (0.5–0.8)</td>
<td>19 700 (14 100–26 700)</td>
<td>4.2 (3.0–5.7)</td>
</tr>
<tr>
<td>AMR B</td>
<td>15 300 (13 100–17 800)</td>
<td>1.8 (1.5–2.0)</td>
<td>105 300 (82 500–127 500)</td>
<td>12 (9.4–15)</td>
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<tr>
<td>AMR C</td>
<td>5077 (4225–6792)</td>
<td>3.4 (2.5–4.1)</td>
<td>35 000 (24 400–41 200)</td>
<td>19 (13–22)</td>
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<tr>
<td><strong>EMR B</strong></td>
<td><strong>8450 (6950–9530)</strong></td>
<td><strong>2.5 (2.1–2.9)</strong></td>
<td><strong>53 900 (27 800–84 800)</strong></td>
<td><strong>17 (8.5–26)</strong></td>
</tr>
<tr>
<td><strong>EMR D</strong></td>
<td><strong>26 300 (21 200–31 200)</strong></td>
<td><strong>2.2 (1.7–2.6)</strong></td>
<td><strong>164 900 (84 600–277 800)</strong></td>
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<tr>
<td>EUR A</td>
<td>2170 (1900–2896)</td>
<td>0.5 (0.4–0.6)</td>
<td>13 600 (7 508–23 400)</td>
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<td>EUR B</td>
<td>5200 (4500–6090)</td>
<td>1.5 (1.3–1.7)</td>
<td>32 200 (17 500–54 700)</td>
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<td>EUR C</td>
<td>4200 (3700–4800)</td>
<td>1.6 (1.4–1.8)</td>
<td>26 400 (14 400–42 700)</td>
<td>10 (5.4–16)</td>
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<tr>
<td>SEAR B</td>
<td>6430 (4240–8600)</td>
<td>1.3 (0.9–1.7)</td>
<td>40 300 (18 700–71 800)</td>
<td>8.1 (3.8–14)</td>
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<tr>
<td>SEAR D</td>
<td>25 400 (20 700–30 700)</td>
<td>0.8 (0.7–1.0)</td>
<td>158 300 (85 900–275 400)</td>
<td>5.1 (2.8–8.9)</td>
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<tr>
<td>WPR A</td>
<td>960 (720–1200)</td>
<td>0.6 (0.5–0.8)</td>
<td>5950 (2000–10 100)</td>
<td>3.9 (1.9–6.6)</td>
</tr>
<tr>
<td>WPR B</td>
<td>24 200 (20 500–28100)</td>
<td>1.1 (0.9–1.3)</td>
<td>154 700 (81 200–253 000)</td>
<td>7.1 (3.7–12)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>190 100 (179 300–206 300)</strong></td>
<td><strong>1.5 (1.4–1.6)</strong></td>
<td><strong>1 200 000 (760 000–1 900 000)</strong></td>
<td><strong>9.6 (5.8–15)</strong></td>
</tr>
</tbody>
</table>

AFR, African Region; AMR, Region of the Americas; CI, credible interval; DALY, disability-adjusted life year; EMR, Eastern Mediterranean Region; EUR, European Region; SEAR, South-East Asia Region; WPR, Western Pacific Region.

* Per 1000 live births.
Toxoplasma: Prevalence

Prevalence in Middle East
- Varies considerably but is generally high
- High incidence of about 2%

Prevalence
- Middle East - 30 – 50%
- Saudi Arabia – 27.8% (95% CI = 20.6 – 36.3%)
- Iran* - 50.0% (95% CI = 43.85 to 56.17)
- Iran# - 43% (95% CI = 38 – 48%)
- Yemen# - 46.2%

Is a significant public health issue, esp antenatal

* Immunocompromised # Antenatal

Sources of *T. gondii* infection in humans. The various sources of food-borne and environmental contamination of humans are represented.
Toxoplasma: Clinical Disease

- Immunocompetent host
  - fever
  - lymphadenopathy
  - myalgia
  - chorioretinitis

- Immunocompromised host
  - reactivation resulting from cyst rupture
  - encephalitis – headache, lethargy, memory loss, ataxia
  - multi-organ – lung, heart, bone marrow, kidney, spleen

- Congenital
  - Mental retardation, seizures, microcephalus, deafness
  - Eye lesions – cataracts, microphthalmia, optical neuritis
  - Epilepsy, anaemia, TCP, pneumonitis
Toxoplasma: Diagnosis

- Mainly relies on retrospective serology
- Pre-natal protective immunity screening
- Serology tests:
  - Sabin-Feldman dye test
  - Indirect immunofluorescence
  - EIA (MTP and automated)
- Usually IgG (immunity) and IgM (acute)
- Avidity assays
- Toxoplasma DNA
Toxoplasma: Antibody Response

IgM titer reaches a plateau within 1 month

IgG titer reaches a plateau within 2–3 months in the absence of treatment

Ab titers

IgG (ELISA)

IgG (dye-test, IFAT)

IgA

IgM

Months

0 1 2 3 4 5 6 7 8 9 10
Toxoplasma: Antibody Response

- Often have low-level IgG results
  - May require confirmation with second assay or Western blot, esp in organ donors
- IgM positive results may require confirmation
- Assay kinetics vary widely – must validate
- Persistence of IgM for > 2 years is documented
- Interpret of IgM positive result with caution
- Incorrect interpretation may lead to unnecessary abortion
Toxoplasma: Testing

- Commercial avidity assays available
- Assess the maturity of IgG antibody
- Uses a wash step with urea to dissociate immature (recent) antibodies
- Antibody maturity may be delayed with treatment
Toxoplasma: Testing

**Prenatal diagnosis**
- Detection of DNA in amniotic fluid
- Assays vary considerably
- Quantitative PCR correlates with clinical symptoms in foetus
- +/- cell culture

**Post natal diagnosis**
- Detection of parasite in cord blood
- Neonatal serology – IgM or IgA in neonate
- Assays not validated for cord blood
- Both IgA and IgM detection increases PPV
Toxoplasma: Testing

Diagnosis of immunocompromised
- BAL, blood, CSF or biopsy PCR
- Varying sensitivities of assays
- Serology less useful
  - May exclude infection in symptomatic patients
  - Detection of rise in titre
  - IgM may reappear in reactivation
Rubella

From: Fenner and White, courtesy Kath Hayes
Rubella virus

- Single stranded RNA;
- Genus: rubivirus;
- **Family: Togaviridae**

Three structural polypeptides

- Nucleocapsid, (C polypeptide chain)
- E1 glycopolypeptide (predominant reactivity)
- E2a glycopolypeptide
- E2b glycopolypeptide
Rubella: Clinical Disease

- Human disease
- Rubella is a vaccine-preventable disease
- Before the introduction of vaccination programmes, rubella caused a mild childhood disease
- Wild-type infection of children is self-limiting and results in a life-long immunity
Rubella: Clinical Disease

- Infection during pregnancy can result in congenital rubella syndrome (CRS)
- CRS results in a range of neurological, ophthalmic, and auditory complications
- Estimated lifetime cost of CRS was USD 300,000 in 1980s
- 1962-5 US epidemic cost est. $1.5b
Rubella: Immune Response
Rubella IgG Assays

<table>
<thead>
<tr>
<th>Assay</th>
<th>Units</th>
</tr>
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<tbody>
<tr>
<td>Viral neutralisation</td>
<td>titre</td>
</tr>
<tr>
<td>Haemagglutination inhibition</td>
<td>titre</td>
</tr>
<tr>
<td>Latex agglutination</td>
<td>titre</td>
</tr>
<tr>
<td>Immunofluorescence</td>
<td>titre</td>
</tr>
<tr>
<td>Single radial diffusion</td>
<td>IU/mL</td>
</tr>
<tr>
<td>Microtire plate EIA</td>
<td>IU/mL</td>
</tr>
<tr>
<td>Automated EIA (viral lysate)</td>
<td>IU/mL</td>
</tr>
<tr>
<td>Automated EIA (recombinant)</td>
<td>IU/mL</td>
</tr>
</tbody>
</table>
Rubella: Testing

- Recent infection in adults and children
  - Rubella IgM detection
  - Seroconversion of rubella IgG
  - Rise in titre (paired sera 10-14 days)
  - Avidity testing

- Problems in Rubella IgM test interpretation
  - False positive occur due to cross reactivity with infections with other organisms, autoimmunity and biological factors
  - Persistence of IgM
  - Low prevalence of infection
Rubella: Issues with Quantification

- Vaccination
- Poor International Standard
- Establishing cut-off
- Lack of standardisation
- Resolution of issue
Rubella: Vaccination

- Vaccination of 10-14 year-old girls started in 1971
- MMR vaccination of infants was introduced in 1989
- Vaccination of both boys and girls (10 – 16 years) was started in 1994
- Immune response to vaccination is often weaker than that found in wild type infection
Rubella: Vaccination

Rubella: Vaccination

BMR vaccinaties 1-1-2004
per gemeente, cohort 2001, eerste vaccinatie zuigelingen (14 maanden)

Percentage
- < 80
- 80 - 90
- 90 - 95
- ≥ 95

provincies

 Courtesy of Hans Zaaijer, Sanquin
Rubella: International Standard

- Second International Standard established in 1970
- Based on BS/94.1762 standard
- Normal human immunoglobulin with equal volume of saline (lyophilised) – polyclonal antibodies
- IFU states - “Use of immunoglobulin preparations as a reference material for immunoassays is not an ideal solution”. 

NRL Science of Quality
Determination of Assay Cut-off

- Initial studies on HAI and neutralization assays
- Bradstreet (1978) suggested minimum titre be 24-48 IU (HAI -1:16-1:20)
- Original recommendation from Rubella Subcommittee on Rubella Serology suggested cut-off of 15 IU/mL (NCCSL/CSLI)
- IMx cut-off 10 IU/mL (Abbott, 1987)
- Reviewed cut-off was 10 IU/mL (CDC, 1988)
- All reports acknowledge false positive and negative results associated with cut-off
<table>
<thead>
<tr>
<th>Solid Phase</th>
<th>ARCHITECT</th>
<th>AxsYM</th>
<th>Elecsys</th>
<th>VIDAS</th>
<th>Vitros</th>
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<tbody>
<tr>
<td>Antigen</td>
<td>Microparticles</td>
<td>Microparticles</td>
<td>Magnetic beads</td>
<td>Solid Phase Receptacles (SPR)</td>
<td>Wells</td>
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<tr>
<td>Detection system</td>
<td>Chemiluminescence</td>
<td>Methylumbelliferyl immunofluorescence</td>
<td>Chemiluminescence</td>
<td>Methylumbelliferyl immunofluorescence</td>
<td>Luminescence</td>
</tr>
<tr>
<td>Number of calibrators</td>
<td>6</td>
<td>6</td>
<td>2</td>
<td>1*</td>
<td>Four parameter logistic curve</td>
</tr>
<tr>
<td>Calibration range (IU/mL)</td>
<td>0 - 500</td>
<td>0 - 500</td>
<td>0.17 - 500</td>
<td>0 - 250</td>
<td>0 - 350</td>
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<tr>
<td>Negative range (IU/mL)</td>
<td>&lt;4.9</td>
<td>&lt;5.0</td>
<td>&lt;10</td>
<td>&lt;5.0</td>
<td>&lt;9.99</td>
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<tr>
<td>Equivocal range (IU/mL) (grey zone)</td>
<td>5.0-9.9</td>
<td>5.0-9.9</td>
<td>NA</td>
<td>5.0-10.0</td>
<td>10.0 - 14.9 **</td>
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<tr>
<td>Positive range (IU/mL)</td>
<td>&gt;10.0</td>
<td>&gt;10.0</td>
<td>&gt;10</td>
<td>&gt;10.0</td>
<td>&gt;15.0</td>
</tr>
</tbody>
</table>

* In addition to Master calibration; ** Low positive
325 pretetsted-negative RV-IgG samples (from France, Italy and Germany) were tested with 9 assays:

- Immuno-blot Mikrogen
- DxI Beckmann-Coulter
- Architect Abbott
- VIDAS bioMérieux
- Enzygnost Siemens
- LXL Diasorin
- Cobas 6000 Roche
- Centaur Siemens
- Serion

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National Reference Laboratory for Rubella
Virology department, Groupe Hospitalier
Paris-Sud
Medecine Faculty Paris-Sud 11 University, France
<table>
<thead>
<tr>
<th></th>
<th>Immuno-blot Mikrogen</th>
<th>Dxl Beckmann-Coulter</th>
<th>Architect Abbott</th>
<th>VIDAS bioMérieux</th>
<th>Enzygnost Siemens</th>
<th>LXL Diasorin</th>
<th>Cobas 6000 Roche</th>
<th>Centaur Siemens</th>
<th>Serion</th>
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</thead>
<tbody>
<tr>
<td><strong>Negative</strong></td>
<td>134/325</td>
<td>-</td>
<td>207/325</td>
<td>202/325</td>
<td>152/325</td>
<td>209/325</td>
<td>135/325</td>
<td>158/325</td>
<td>215/325</td>
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<tr>
<td></td>
<td>41%</td>
<td>-</td>
<td>64%</td>
<td>62%</td>
<td>47%</td>
<td>64%</td>
<td>48%</td>
<td>66%</td>
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<tr>
<td><strong>Equivocal</strong></td>
<td>-</td>
<td>-</td>
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<td>49/325</td>
<td>84/325</td>
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<tr>
<td></td>
<td>-</td>
<td>-</td>
<td>33%</td>
<td>18%</td>
<td>15%</td>
<td>26%</td>
<td>-</td>
<td>16%</td>
<td>27%</td>
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<tr>
<td><strong>Positive</strong></td>
<td>191/325</td>
<td>-</td>
<td>11/325</td>
<td>65/325</td>
<td>124/325</td>
<td>32/325</td>
<td>190/325</td>
<td>116/325</td>
<td>22/325</td>
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<tr>
<td></td>
<td>59%</td>
<td>-</td>
<td>3%</td>
<td>20%</td>
<td>38%</td>
<td>10%</td>
<td>58%</td>
<td>36%</td>
<td>7%</td>
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## Results (2)

<table>
<thead>
<tr>
<th>IBlot</th>
<th>DxI</th>
<th>Architect</th>
<th>VIDAS</th>
<th>Enzygnost</th>
<th>LXL</th>
<th>Cobas 6000</th>
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<tr>
<td></td>
<td>Beckmann-Coulter</td>
<td>Abbott</td>
<td>bioMérieux</td>
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<td>Diasorin</td>
<td>Roche</td>
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<tr>
<td></td>
<td>E: 10-14</td>
<td>E: 5-9</td>
<td>E: 10-15</td>
<td>E: 5-6</td>
<td>E: 5-9</td>
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<td>P</td>
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<td>N 13</td>
<td>E 16</td>
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<td>N 42,1</td>
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<td>E 5,4</td>
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<td>E 5</td>
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<td>E 10</td>
<td>E 6</td>
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<td>E 13</td>
<td>E 8</td>
<td>P 5,5</td>
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<td>N 9</td>
<td>N 5</td>
<td>E 6,3</td>
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<td>N 4,2</td>
<td>N 7</td>
<td>N 5</td>
<td>E &lt;3</td>
<td>N 11,8</td>
<td>P 9,3</td>
<td>E 6,1</td>
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<tr>
<td>P</td>
<td>8,9</td>
<td>N 5</td>
<td>E 14</td>
<td>E 8</td>
<td>P 5,7</td>
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<td>N 11</td>
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<td>P 8,8</td>
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<td>E 7</td>
<td>P 8,6</td>
<td>E 7,7</td>
<td>N 23,5</td>
<td>P 12,5</td>
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<td>12,2</td>
<td>E 7</td>
<td>E 10</td>
<td>E 13</td>
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<td>N &gt;500</td>
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<td>P 10,8</td>
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<tr>
<td>P</td>
<td>9,5</td>
<td>N 6,1</td>
<td>E 12</td>
<td>E 8</td>
<td>P 4,4</td>
<td>N 19,2</td>
<td>P 7,4</td>
<td>E 11,4</td>
</tr>
</tbody>
</table>
Resolution of Issue

- Developed a panel of highly characterised samples negative for rubella-IgG
- WHO convened a consultation on 30th June 2017
- Adopted by the WHO Expert Committee on Biological Standardization (ECBS) in October 2017
- Comprised of representatives from WHO, Paul Ehrlich, CDC, FDA, National Institute of Biological Standards and Controls, NRL and other interested parties including manufacturers

Recommendations were:

- RUBI-1-94 should continue to be available
- Noted lack of commutability
- Reconsider appropriateness of 10 IU/mL and as a cut-off
- Consider highly specific qualitative assays
Quality Assurance

- NRL provides external quality assessment schemes (EQAS)

- Run control (QC) program for rubella and toxoplasma testing
Quality Assurance

- Peer comparison realtime software
- NRL QConnect limits – superior to Westgard rules
- QC optimised for test platforms
- NRL scientific and technical support
- Interfacing available
Thank You

WHAT WE OFFER

- **Evaluations**
  Independent assessment of EQA and provision of customized validation and verification panels, analysis and reporting

- **EQAS**
  Proficiency programmes designed to assess the accuracy of tests and testing processes

- **Testing**
  TQA licensed screening of blood and tissue donors, reference testing, and contract testing for projects

- **Training**
  Customised and sustainable training to enhance quality of infectious disease testing through education, advocacy and membership

- **QConnect**
  Comprehensive CC programme providing CC samples, software and associated services to monitor the precision and accuracy of test results

- **Events**
  Annual educational events allowing participants to expand their knowledge in a forum of open discussion

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Learning Objectives

Toxoplasma
- Natural history of the parasite
- Clinical diseases immune response
- Diagnosis of disease
- Considerations interpreting test results

Rubella
- Virus
- Clinical disease
- Immune response
- Laboratory tests
- Issues with quantification
References: Toxoplasmosis

References: Rubella

- Bouthry E, et al. 2014. An evaluation of nine rubella IgG assays highlights many discrepancies in the interpretation of the results. 24th European Congress of Clinical Microbiology and Infectious Diseases, Barcelona, Spain