Testing for Toxoplasmosis and Rubella Infections

Dimech W
NRL, Melbourne, Australia

Roche Symposium
Dubai
April 15th 2019

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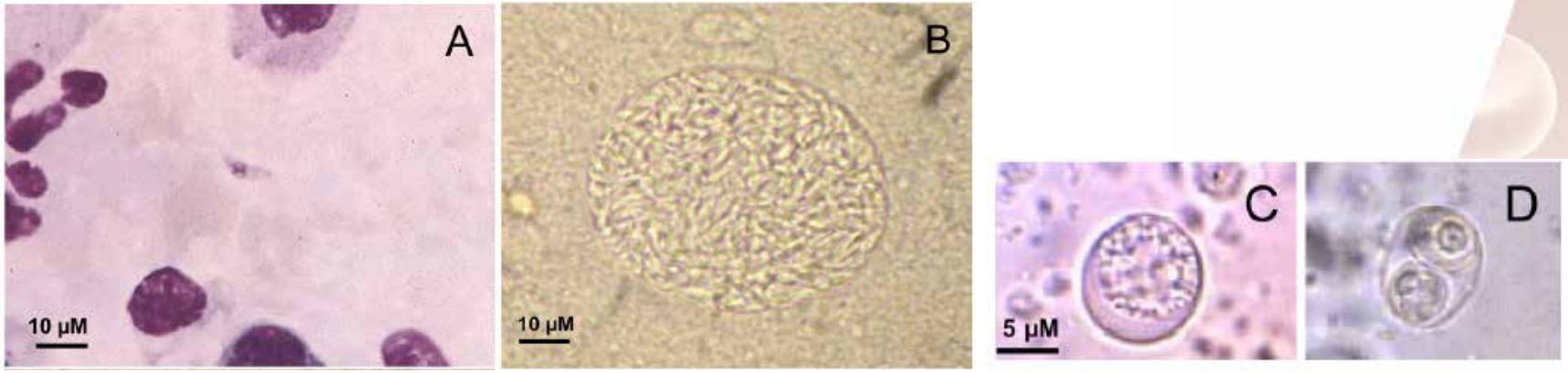
Toxoplasma: Parasite

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



Robert-Gangneux F. and Darde M-L. Epidemiology of and Diagnostic Strategies for Toxoplasmosis. *Clin Micro Rev*. 2012 25(2):264

Toxoplasma: Life Cycle

- Three infective parasitic phases
  - Rapidly dividing, invasive tachyzoite
  - Slowly dividing bradyzoite (tissue cysts)
  - Sporozoite (within the ooztye)
- 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

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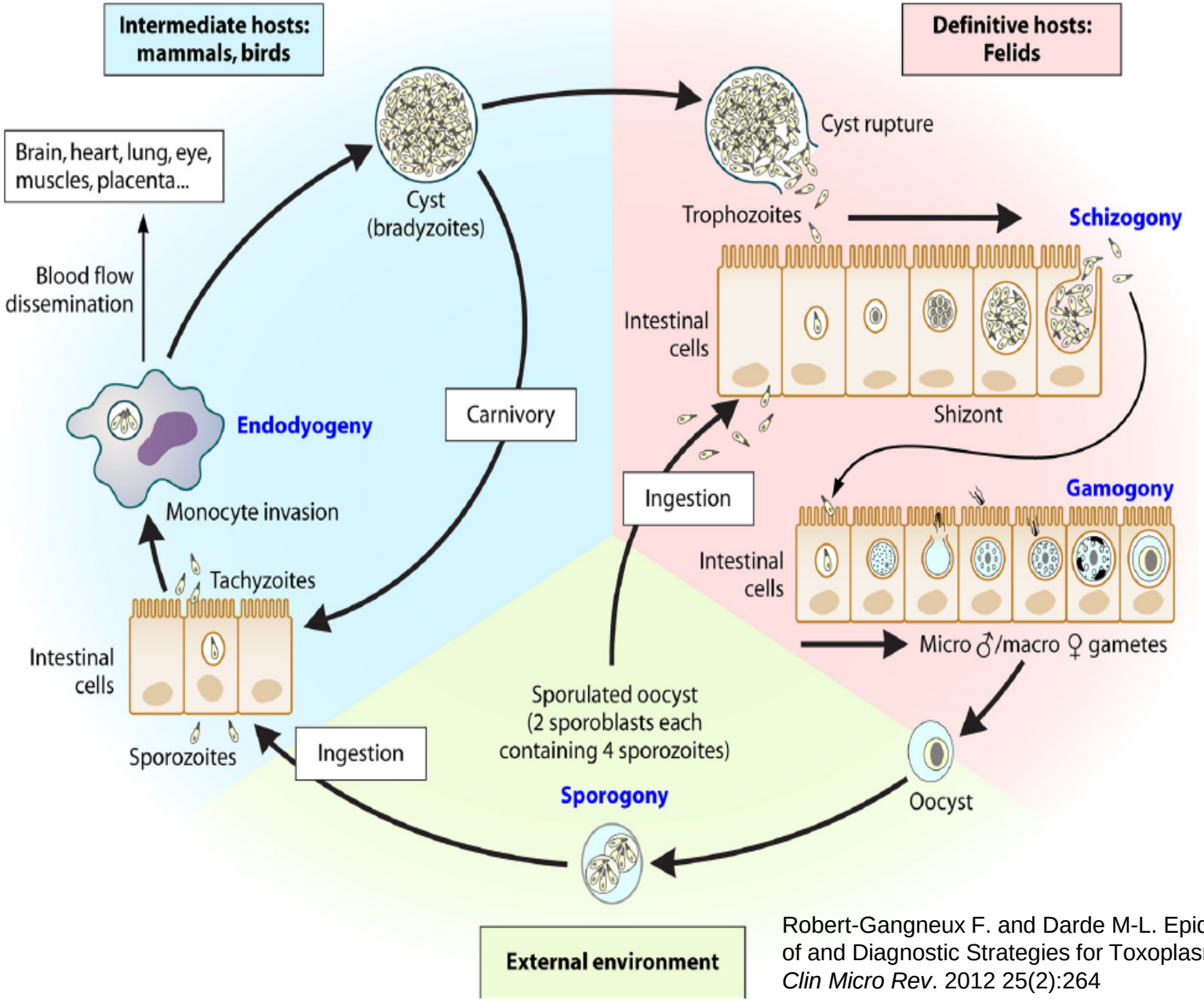

Robert-Gangneux F. and Darde M-L. Epidemiology of and Diagnostic Strategies for Toxoplasmosis. *Clin Micro Rev*. 2012 25(2):264

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%

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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

Region	Incident cases (95% CI)	Incidence[a] (95% CI)	DALYs (95% CI)	DALYs[a] (95% CI)
AFR D	26 500 (24 300–30 100)	2.0 (1.8–2.3)	171 500 (92 300–294 500)	13 (6.9–22)
AFR E	37 000 (33 900–41 000)	2.4 (2.2–2.5)	235 900 (129 600–379 000)	15 (8.3–24)
AMR A	2940 (2360–3540)	0.6 (0.5–0.8)	19 700 (14 100–26 700)	4.2 (3.0–5.7)
AMR B	15 300 (13 100–17 800)	1.8 (1.5–2.0)	105 300 (82 500–127 500)	12 (9.4–15)
AMR C	5077 (4225–6792)	3.4 (2.5–4.1)	35 000 (24 400–41 200)	19 (13–22)
EMR B	8450 (6950–9530)	2.5 (2.1–2.9)	53 900 (27 800–84 800)	17 (8.5–26)
EMR D	26 300 (21 200–31 200)	2.2 (1.7–2.6)	164 900 (84 600–277 800)	14 (6.9–23)
EUR A	2170 (1900–2896)	0.5 (0.4–0.6)	13 600 (7 508–23 400)	2.8 (1.3–4.3)
EUR B	5200 (4500–6090)	1.5 (1.3–1.7)	32 200 (17 500–54 700)	9.2 (5.0–16)
EUR C	4200 (3700–4800)	1.6 (1.4–1.8)	26 400 (14 400–42 700)	10 (5.4–16)
SEAR B	6430 (4240–8600)	1.3 (0.9–1.7)	40 300 (18 700–71 800)	8.1 (3.8–14)
SEAR D	25 400 (20 700–30 700)	0.8 (0.7–1.0)	158 300 (85 900–275 400)	5.1 (2.8–8.9)
WPR A	960 (720–1200)	0.6 (0.5–0.8)	5950 (2900–10 100)	3.9 (1.9–6.6)
WPR B	24 200 (20 500–28100)	1.1 (0.9–1.3)	154 700 (81 200–253 000)	7.1 (3.7–12)
Total	**190 100 (179 300–206 300)**	**1.5 (1.4–1.6)**	**1 200 000 (760 000–1 900 000)**	**9.6 (5.8–15)**

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.

[a] Per 1000 live births.

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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 heath issue, esp antenatal

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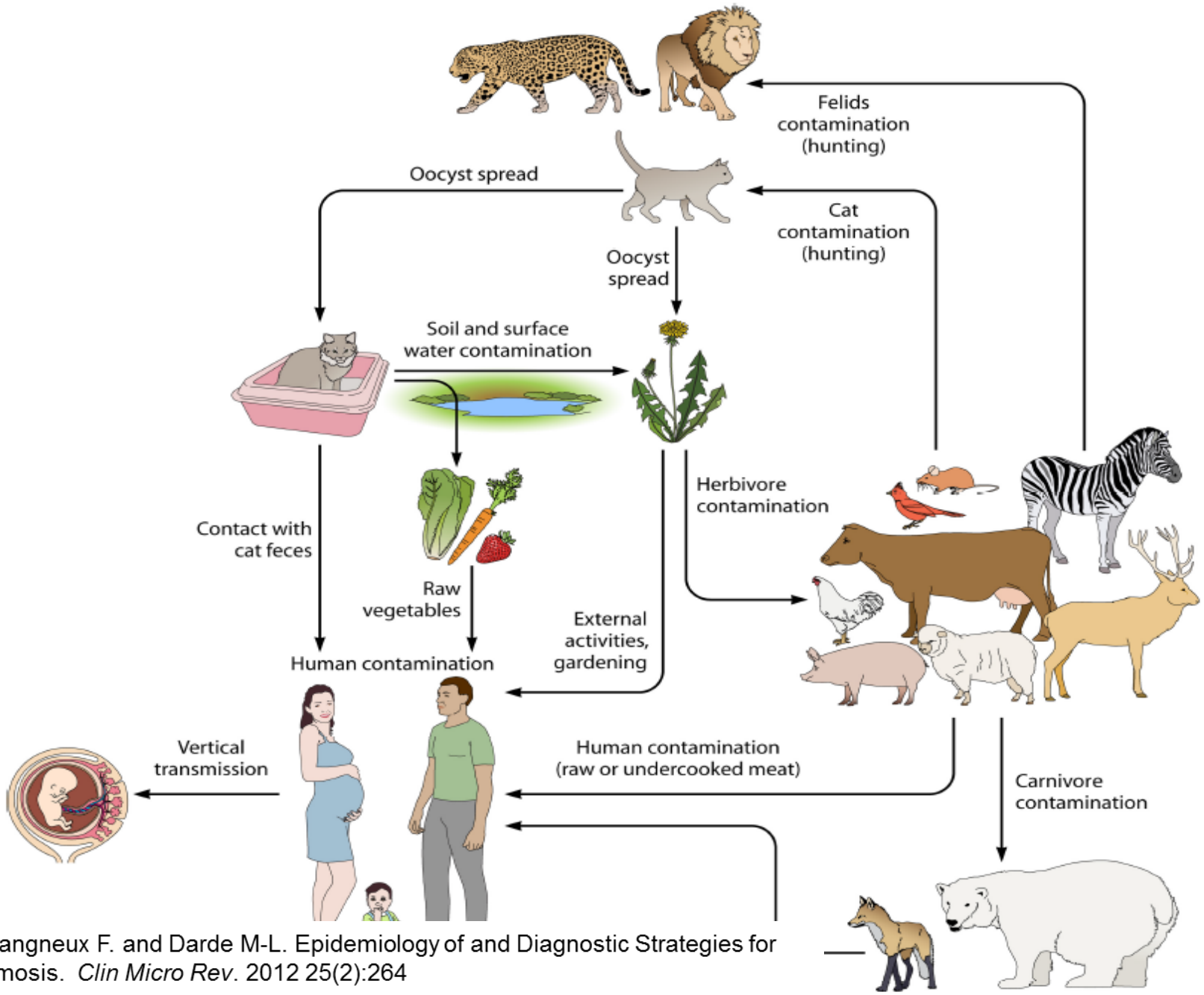
* Immunocompromised # Antenatal



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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

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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

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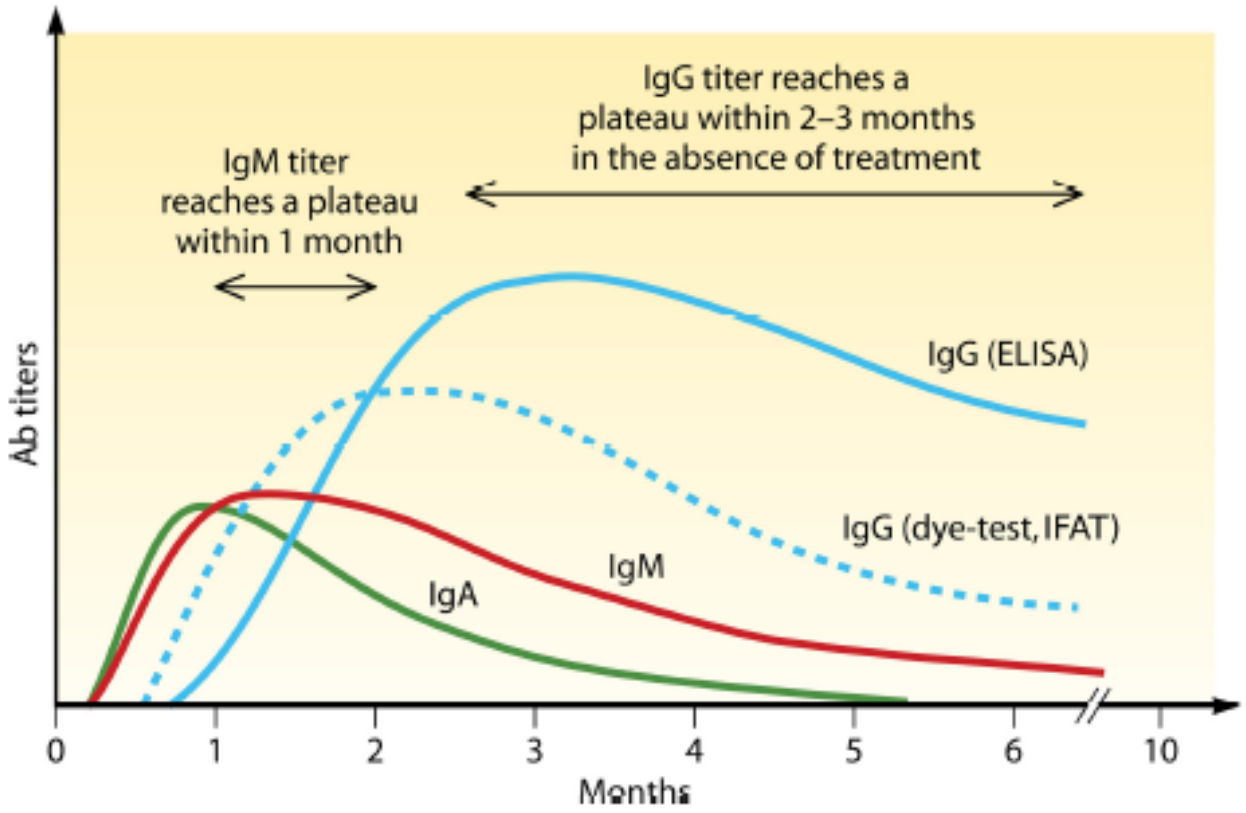
Toxoplasma: Antibody Response



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

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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

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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

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Toxoplasma: Testing

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

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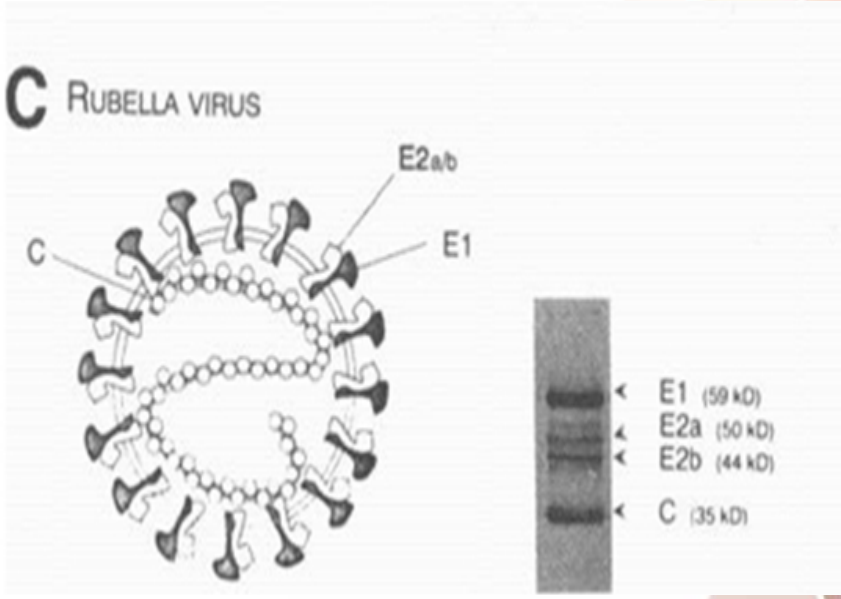
Rubella

From: Fenner and White, courtesy Kath Hayes

Rubella virus



- Single stranded RNA;
- Genus: rubivirus;
- *Family: Togaviridae*
- Three structural polypeptides
  - Nucleocapsid, (C polypeptide chain)
  - El glycopolypeptide (predominant reactivity)
  - E2a glycopolypeptide
  - E2b glycopolypeptide

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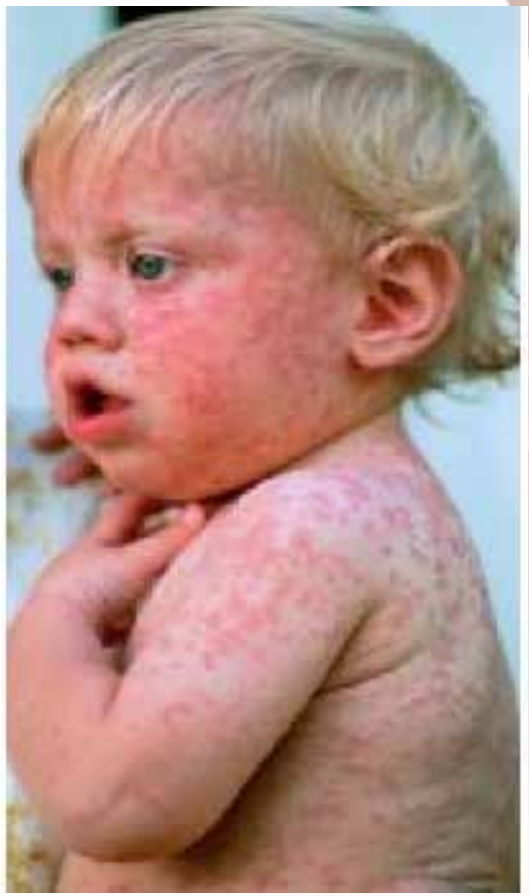
Diagnostic Virology Protocols
Methods in Molecular Medicine™ Volume 12, 1998, pp 143-157

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



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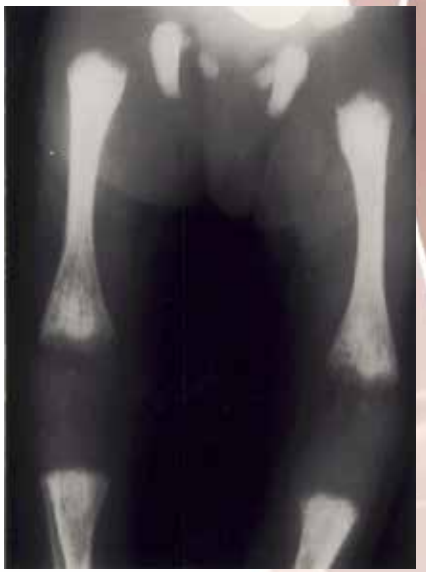
Rubella: Clinical Disease

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



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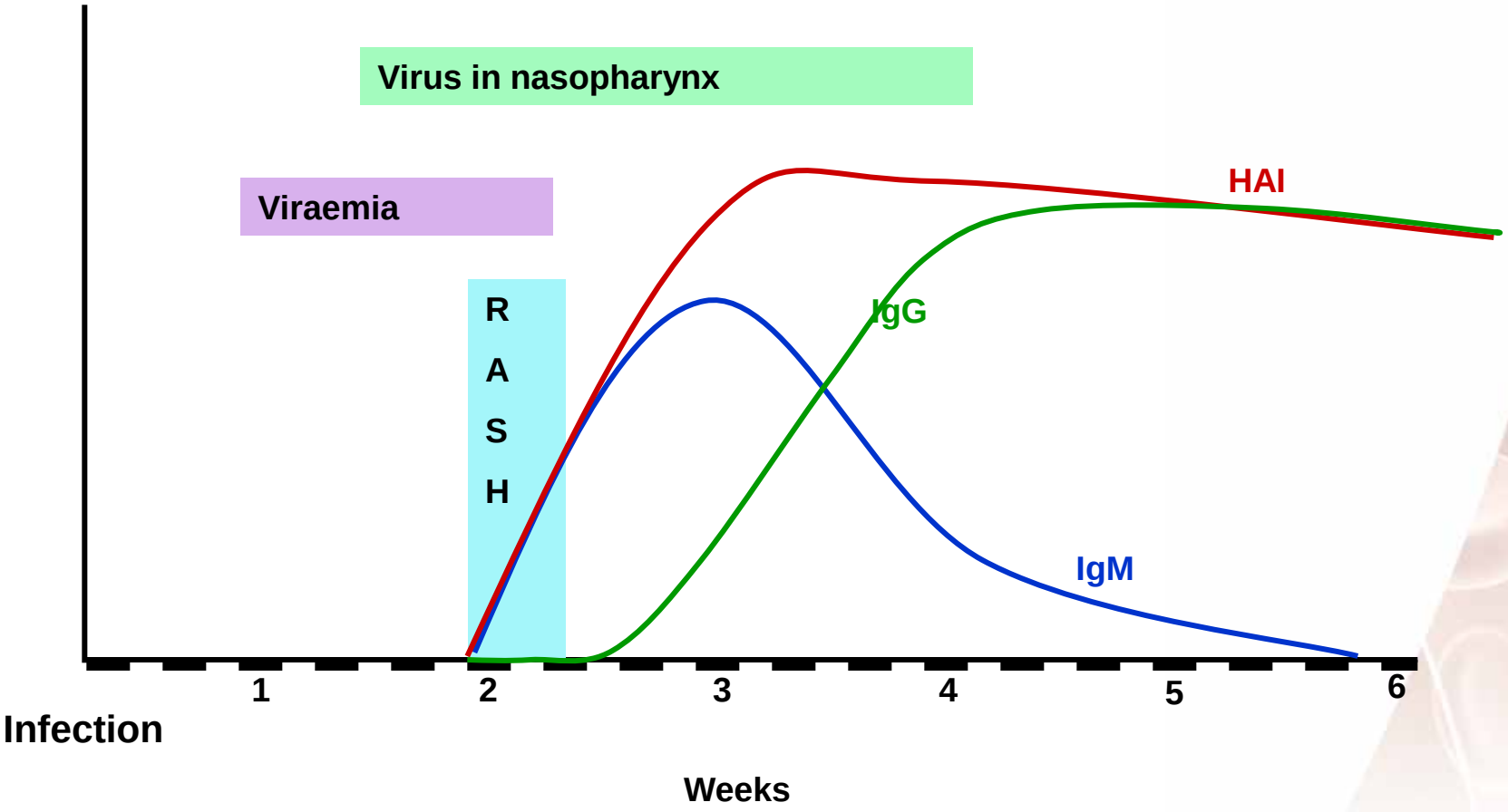
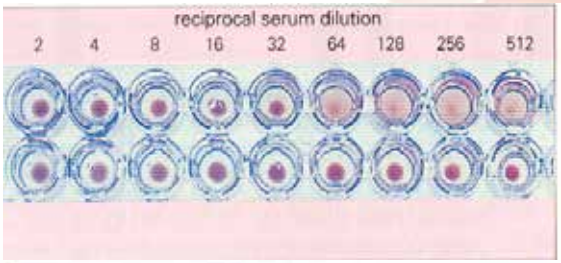
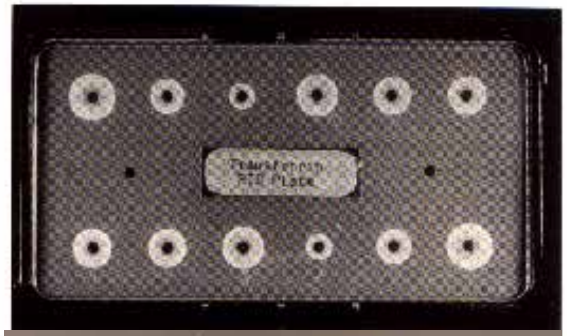
Rubella: Immune Response



Rubella IgG Assays

Assay	Units
Viral neutralisation	titre
Haemagglutination inhibition	titre
Latex agglutination	titre
Immunofluorescence	titre
Single radial diffusion	IU/mL
Microtire plate EIA	IU/mL
Automated EIA (viral lysate)	IU/mL
Automated EIA (recombinant)	IU/mL





Rubella: Testing

- Recent infection in adults and children
  - Rubella IgM detection
  - Seroconversion of rubella IgG
  - Rise in titre (paired sera10-14 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

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Rubella: Issues with Quantification

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

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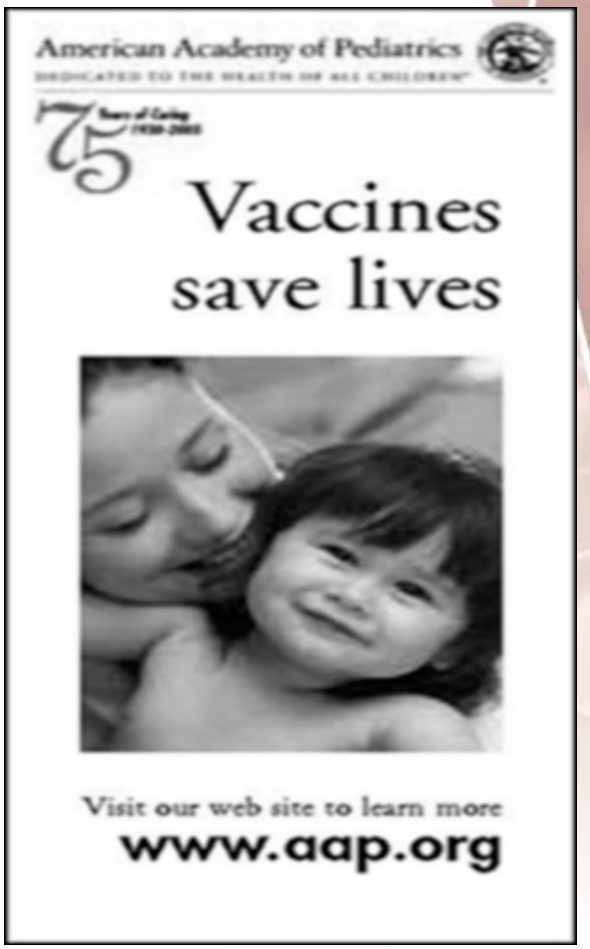
Rubella: Vaccination

- 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



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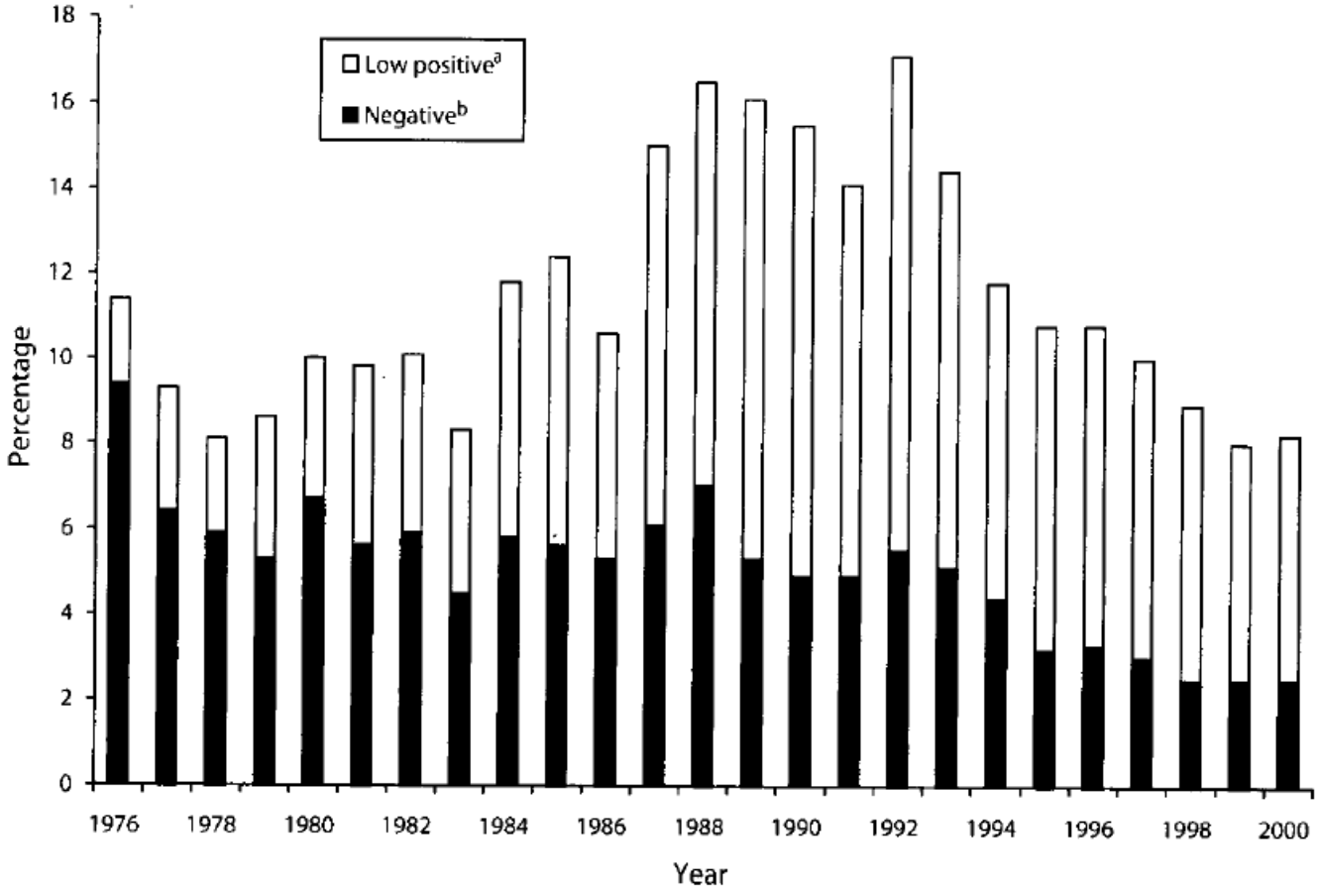
Rubella: Vaccination



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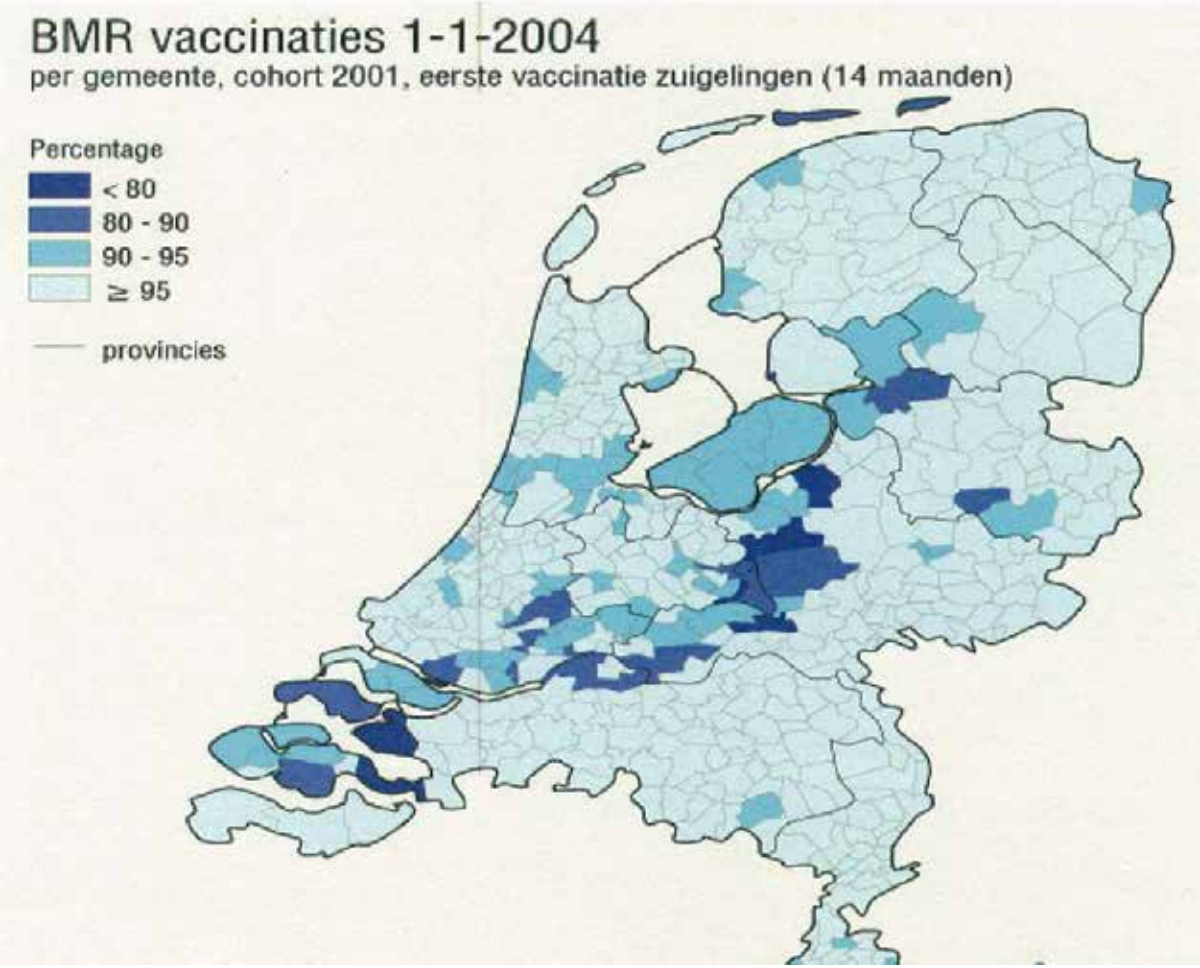
Rubella: Vaccination



Courtesy of Hans Zaaijer, Sanquin

Rubella: International Standard

- Second International Standard established in 1970
- Third International Standard (proposed) (RUBI-1-94): prepared by Statens Serum Institute in 1995
- 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”.

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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

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	ARCHITECT	AxSYM	Elecsys	VIDAS	Vitros
Solid Phase	Microparticles	Microparticles	Magnetic beads	Solid Phase Receptacles (SPR)	Wells
Antigen	Partially purified rubella virus	Partially purified rubella virus (strain HPV77)	Rubella-like particles and recombinant E1 antigen	Rubella antigen (strain MR 383)	UV-treated rubella antigen from cell culture
Detection system	Chemiluminescence	Methylumbelliferyl immunofluorescence	Chemiluminescence	Methylumbelliferyl immunofluorescence	Luminescence
Number of calibrators	6	6	2	1*	Four parameter logistic curve
Calibration range (IU/mL)	0 - 500	0 - 500	0.17 - 500	0 - 250	0 - 350
Standard	WHO standard 1st International Standard (RUB-1-94)	WHO standard (not specified)	WHO standard 1st International Standard (RUB-1-94)	WHO standard 1st International Standard (RUB-1-94)	WHO standard 1st International Standard (RUB-1-94)
Negative range (IU/mL)	<4.9	<5.0	<10	<5.0	<9.99
Equivocal range (IU/mL) (grey zone)	5.0-9.9	5.0-9.9	NA	5.0-10.0	10.0 - 14.9 **
Positive range (IU/mL)	>10.0	>10.0	>10	≥10.0	>15.0

* In addition to Master calibration; ** Low positive

RV-IgG evaluation 2013

* **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

Christelle VAULOUP-FELLOUS
National Reference Laboratory for Rubella
Virology department, Groupe Hospitalier Paris-Sud
Medecine Faculty Paris-Sud 11 University, France

Results (1)

	Immuno-blot Mikrogen	DxI Beckmann-Coulter	Architect Abbott	VIDAS bioMérieux	Enzygnost Siemens	LXL Diasorin	Cobas 6000 Roche	Centaur Siemens	Serion
Negative	134/325 **41%**	-	207/325 **64%**	202/325 **62%**	152/325 **47%**	209/325 **64%**	*135/325*	158/325 **48%**	215/325 **66%**
Equivocal	-	-	107/325 **33%**	58/325 **18%**	49/325 **15%**	84/325 **26%**	-	51/325 **16%**	88/325 **27%**
Positive	191/325 **59%**	-	11/325 **3%**	65/325 **20%**	124/325 **38%**	32/325 **10%**	*190/325 58%*	116/325 **36%**	22/325 **7%**

Results (2)

IBlot	DxI Beckmann-Coulter E: 10-14		Architect Abbott E: 5-9		VIDAS bioMérieux E: 10-15		Enzygnost Siemens E: 5-6		LXL Diasorin E: 5-9		Cobas 6000 Roche N<10		Centaur Siemens E: 5-10		Serion E: 10-20	
P	11,1	E	1,8	N	13	E	16	P	21,9	P	4,3	N	42,1	P	28,4	P
P	12,8	E	4,3	N	13	E	6	E	5,4	E	11,6	P	11,1	P	7,36	N
P	12,2	E	4,1	N	11	E	5	E	8,8	E	10,5	P	25,1	P	14,5	E
P	9,4	N	5	E	10	E	6	E	3,5	N	60,4	P	10,7	P	8,11	N
P	9,8	N	7,6	E	13	E	8	P	5,5	E	5	N	11,7	P	10,8	E
P	7,7	N	4,8	N	9	N	5	E	6,3	E	61,1	P	13,3	P	9,35	N
P	6,8	N	4,2	N	7	N	5	E	<3	N	11,8	P	9,3	E	6,1	N
P	8,9	N	5	E	14	E	8	P	5,7	E	41,2	P	17,1	P	10,6	E
P	8,3	N	4,8	N	11	E	8	P	8,8	E	11,4	P	13,6	P	12,1	E
P	12	E	4,1	N	12	E	7	P	8,6	E	7,7	N	23,5	P	12,5	E
P	12,2	E	7	E	10	E	13	P	4,9	N	>500	P	14,1	P	10,8	E
P	9,5	N	6,1	E	12	E	8	P	4,4	N	19,2	P	7,4	E	11,4	E

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

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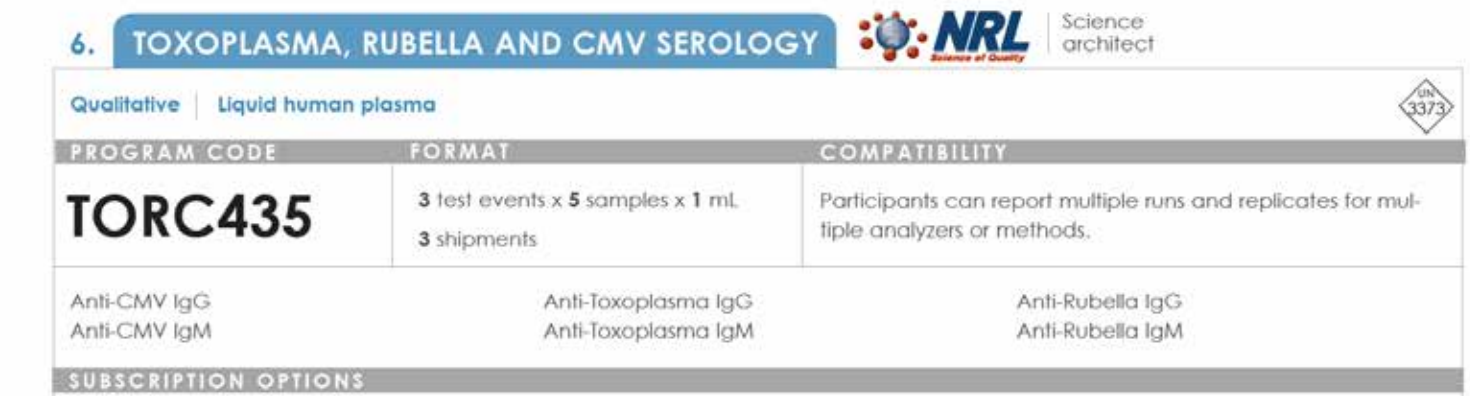
Quality Assurance

- NRL provides external quality assessment schemes (EQAS)



6. TOXOPLASMA, RUBELLA AND CMV SEROLOGY

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Qualitative | Liquid human plasma

UN 3373

PROGRAM CODE	FORMAT	COMPATIBILITY
TORC435	**3** test events x **5** samples x **1** mL **3** shipments	Participants can report multiple runs and replicates for multiple analyzers or methods.

Anti-CMV IgG Anti-CMV IgM	Anti-Toxoplasma IgG Anti-Toxoplasma IgM	Anti-Rubella IgG Anti-Rubella IgM

SUBSCRIPTION OPTIONS

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

QConnect
Follow the trend

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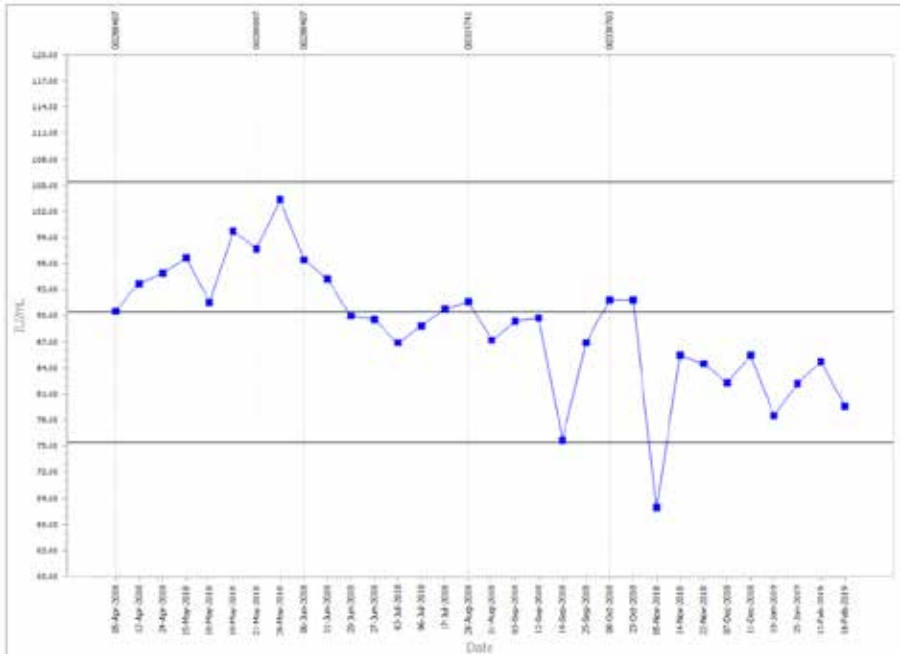
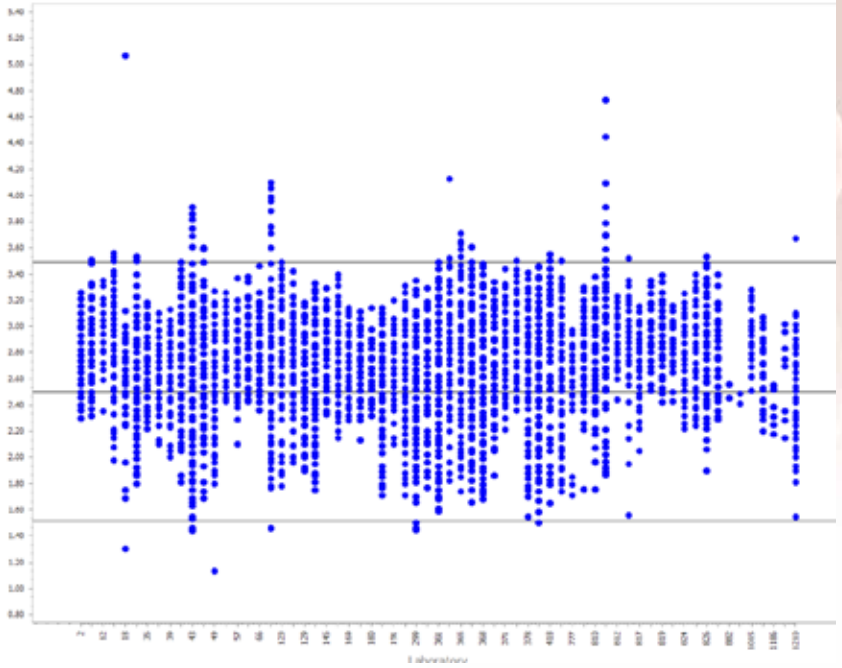
Quality Assurance

- 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 IVDs and provision of customised validation and verification panels, analysis and reporting

EQAS

Proficiency programmes designed to assess the integrity of tests and testing processes

Testing

TGA 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 mentorship

QConnect
Follow the trend

QConnect

Comprehensive QC programme providing QC samples, software and associated services to monitor the precision and accuracy of test results

Events

Annual educational events allowing delegates 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

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