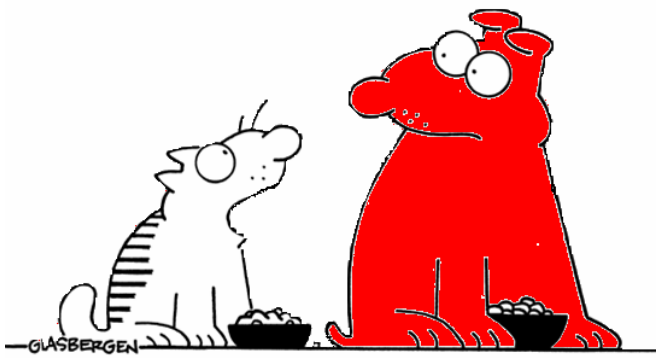
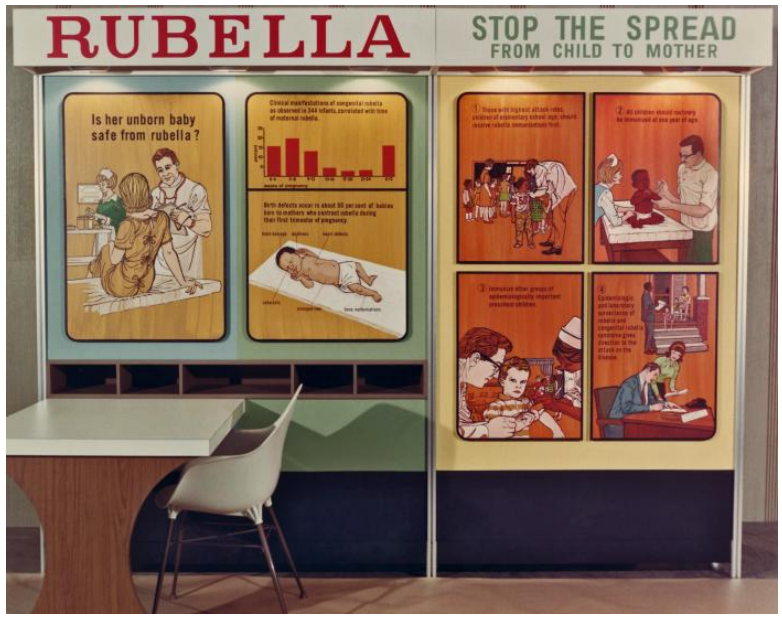
CAT RUBELLA DIAGNOSIS



"The vet says i need a hobby. I thought eating and sleeping were my hobbies!"

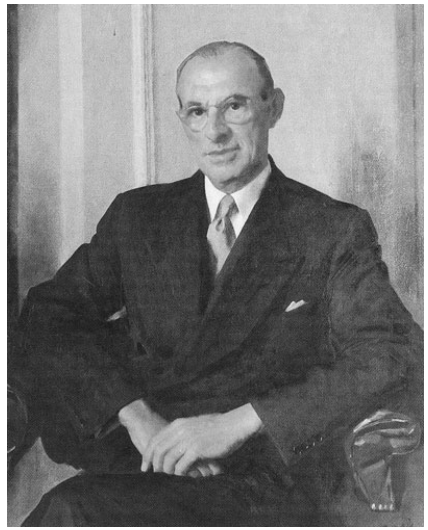
Louis Ide
24-01-2006

- History
- Rubella
- Congenital Rubella Syndrome
- Postnatally Acquired Rubella
- Differential Diagnosis
- Epidemiology
- Motivation
- Questions
- Who to diagnose
- How to diagnose
- How to confirm
- Actions



History

- Bergen (1752) and Orlow (1758): Röteln
- German Measles, 1815
- 1941 Norman McAlister Gregg. Congenital cataract following German measles in the mother. *Trans Ophthalmol Soc Aust* 1941; 3: 35-46.
- 1962 viral cell culture - serology
- 1968 electron microscopy
- 1990 full RNA sequence
- 2002 57% of the countries include rubella vaccination in their NIP



www.mja.com.au

Rubella

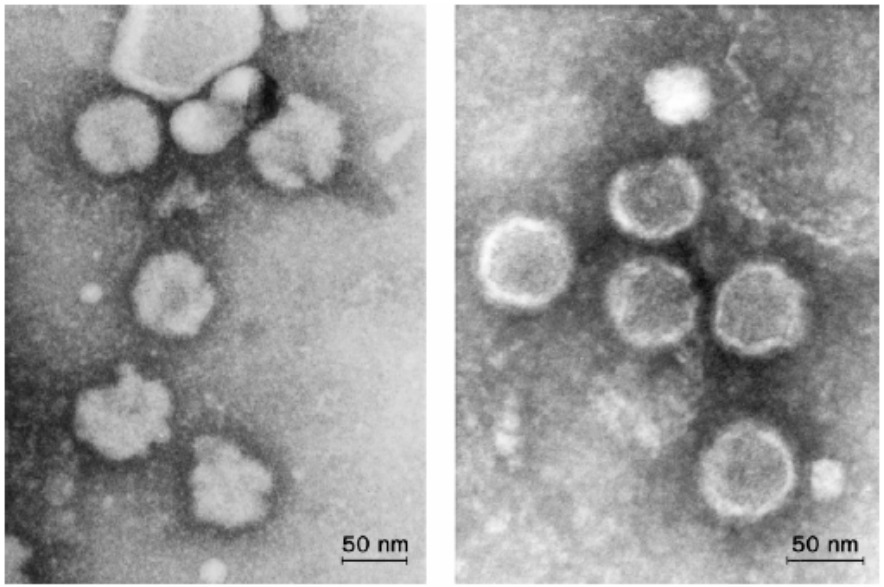


Figure 5: **Electron micrographs of rubella virus**
Courtesy of I Chrystie, St Thomas' Hospital, London, UK.

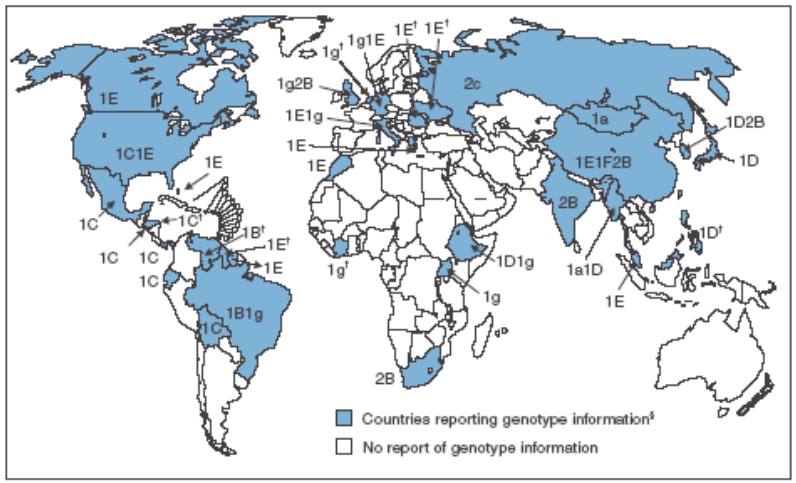


FIGURE 2. Geographic distribution of rubella virus genotypes — worldwide, 1995–2005*

* Genotype data represent a summary of information from several laboratories that was made available in July 2005.
† Viruses were characterized after importation into another country.
§ Certain countries reduced indigenous rubella to low levels or have eliminated it during this period (e.g., Canada, Cuba, the United Kingdom, and the United States).

Single-stranded RNA-virus, family of the togaviridae, genus Rubivirus, about 60 nm, surrounded by a lipoprotein envelope. Different genotypes (7+3) are described.

http://www.cdc.gov/mmwr/preview/mmwrhtml/mm5443a3.htm#fig2

Banatvala JE, Brown DW. *Lancet*. 2004 Apr 3; 363 (9415): 1127-37. Rubella. *Seminar.*

Rubella

An acute, usually benign, infectious disease caused by the rubella virus and most often affecting children and non-immune young adults, in which the virus enters the respiratory tract via droplet nuclei and spreads to the lymphatic system.

Banatvala JE, Brown DW. *Lancet*. 2004 Apr 3; 363 (9415): 1127-37. Rubella. *Seminar.*

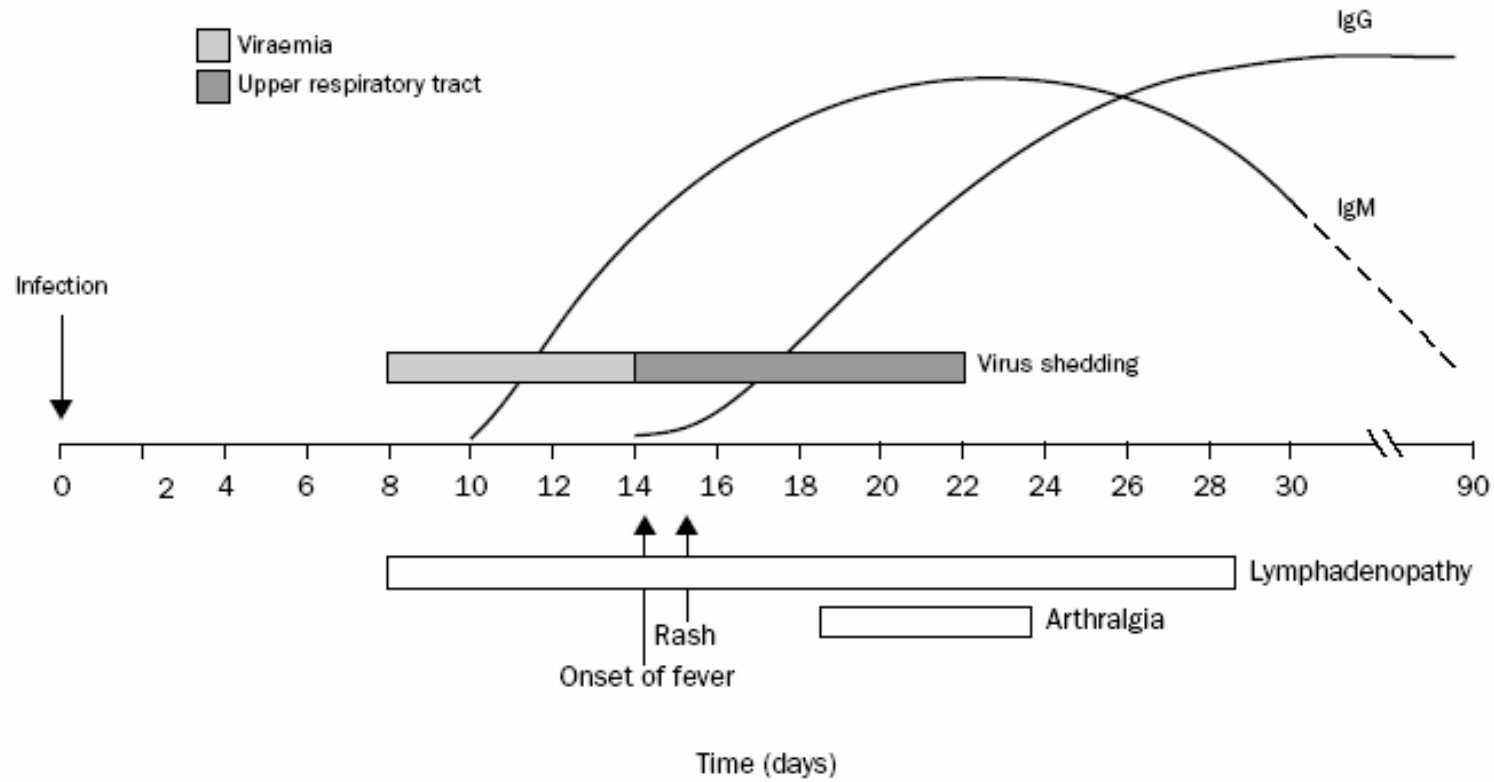


Figure 1: **Timing of key clinical, virological, and immunological features in acquired rubella infection**

Banatvala JE, Brown DW. *Lancet*. 2004 Apr 3; 363 (9415): 1127-37. Rubella. *Seminar.*

Congenital Rubella Syndrome (CRS)

- Birth defects if acquired by a pregnant woman: deafness, cataracts, heart defects, mental retardation, and liver and spleen damage (at least a **20%** chance of damage to the foetus if a woman is infected early in pregnancy), (16-18% if maternal infection was acquired between 13-20 weeks of gestation, according to The Lancet).
- After **20 weeks** the incidence is less than **2%** (Landelijke Infectieprotocollen Ndl.). No defects after 16 weeks according (The Lancet, 1982).
- The foetus produces IgM and IgG. IgM in the newborn is evidence of CRS. Differentiation of the IgG is difficult. After 1 month maternal IgG is more or less cleared.

http://www.cdc.gov/nip/ed/slides/rubella8p.ppt#314,1,Dia 1
Miller E, Cradock-Watson JE, Pollock Tm. Consequences of confirmed maternal rubella at successive stages of pregnancy. Lancet 1982; 2: 781-784.

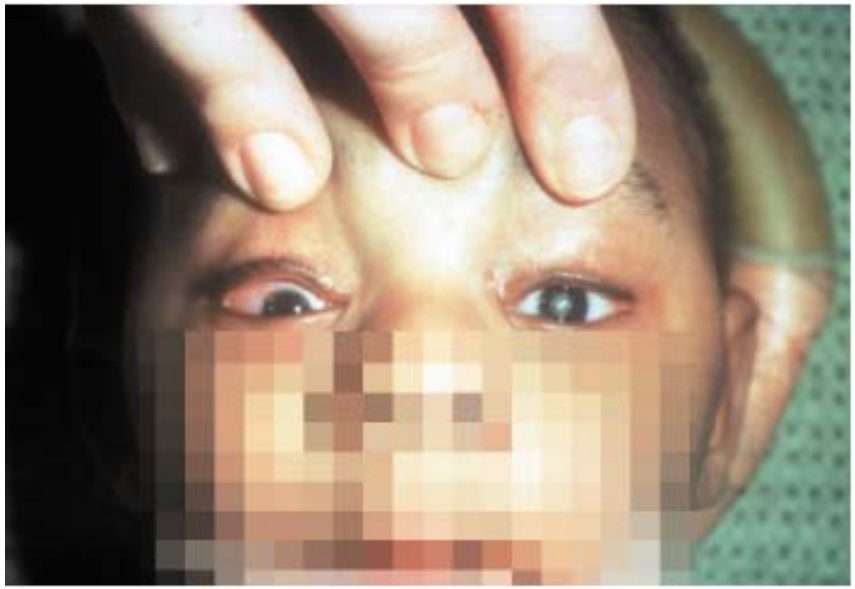
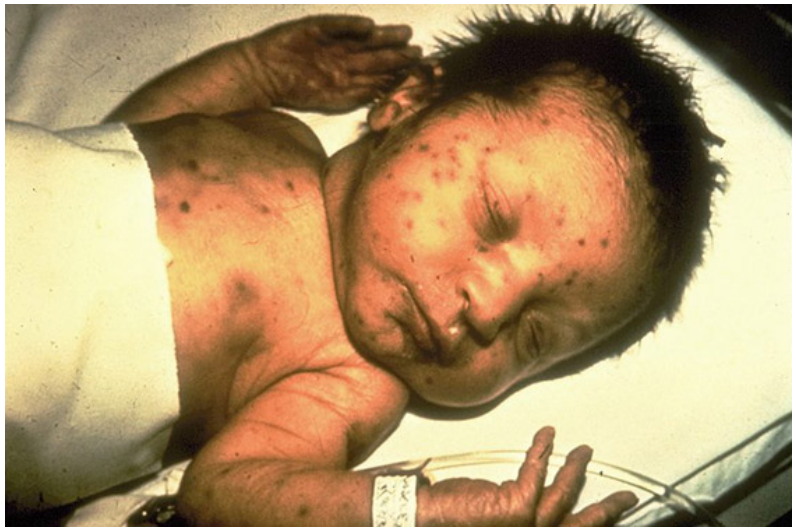


Figure 3: **Congenital rubella cataract in an infant aged 9 months**



http://www.immunizationed.org/images/infrub.jpg

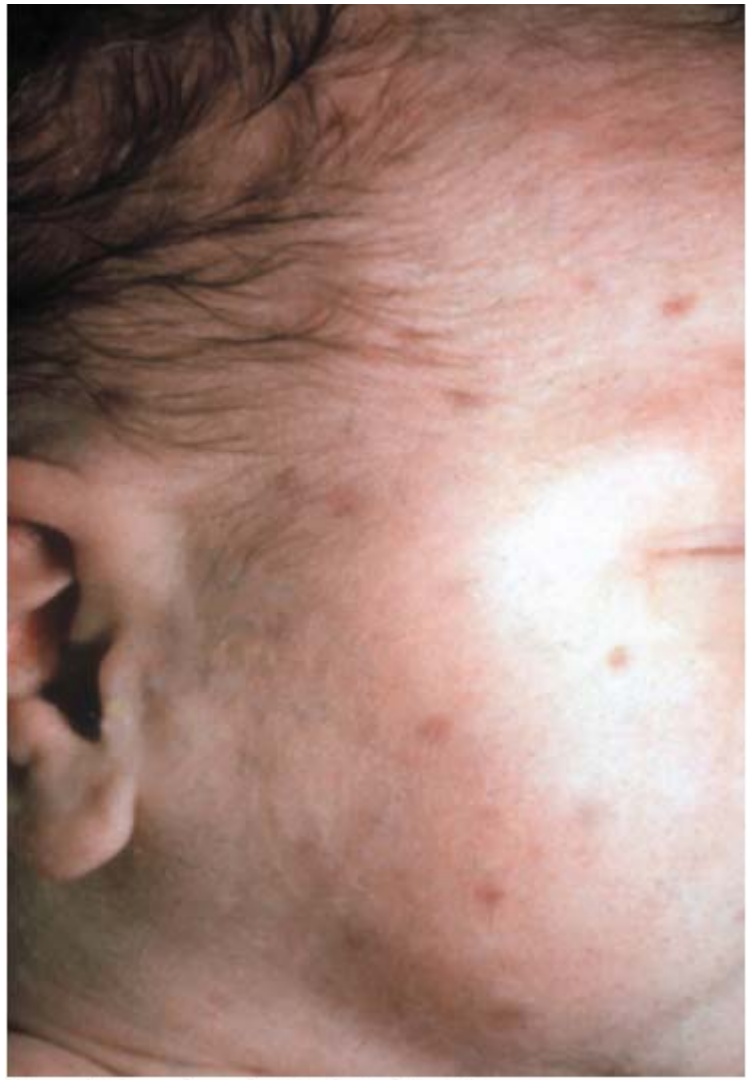


Figure 4: **Purpuric rash in infant with CRS**

Postnatally acquired rubella: extremely rare

Arthralgia - arthritis, encephalopathy, Guillain-Barré (very rare), transient thrombocytopenia, purpuric rash, haemolytic anaemia are possible complications of postnatally acquired rubella and vaccination.

Different settings: first trimester pregnancy, after first trimester, infection before conception, re-infection (less risk).

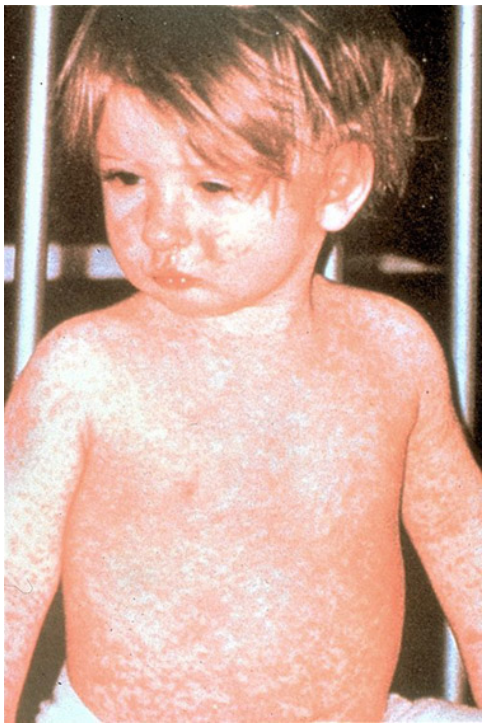
Re-infection

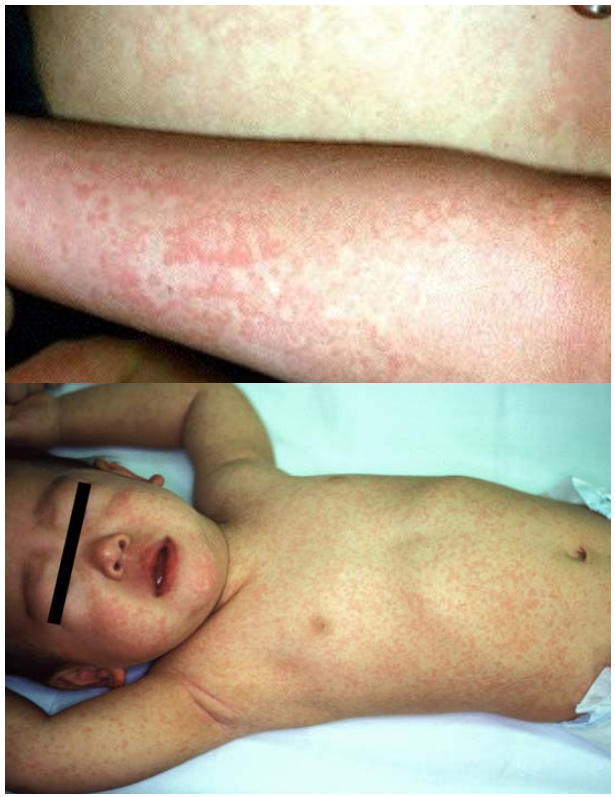
- Re-infection with rubella is almost always asymptomatic and more frequently vaccine-induced than after naturally acquired infection. It is recognised by serologic investigation. The risk of re-infection during the first trimester is low.
- **Mothers** who might have experienced re-infection **should be reassured that the risk of foetal damage is <u>extremely small</u>.**

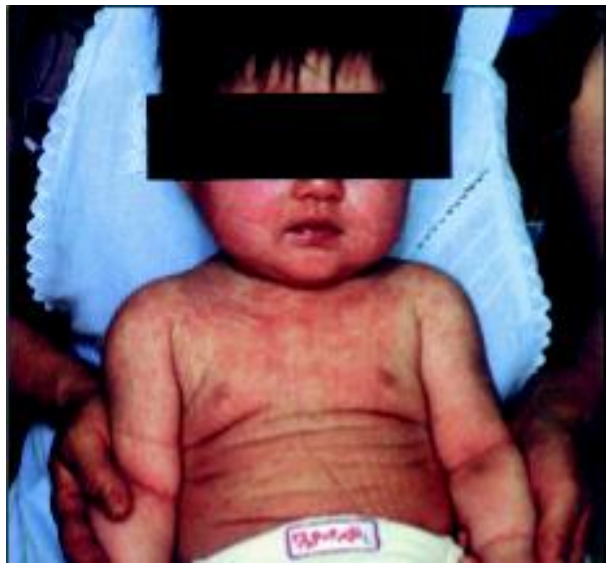
Differential Diagnosis

- Clinical diagnosis of rubella is unreliable and laboratory confirmation essential.
- DD.: Parvovirus B19, HHV 6, Dengue, measles, …

Laboratory differentiation of rubella from other rash-causing infections, such as measles, parvovirus B19, human herpesvirus 6, and enteroviruses in developed countries, and various endemic arboviruses is essential.

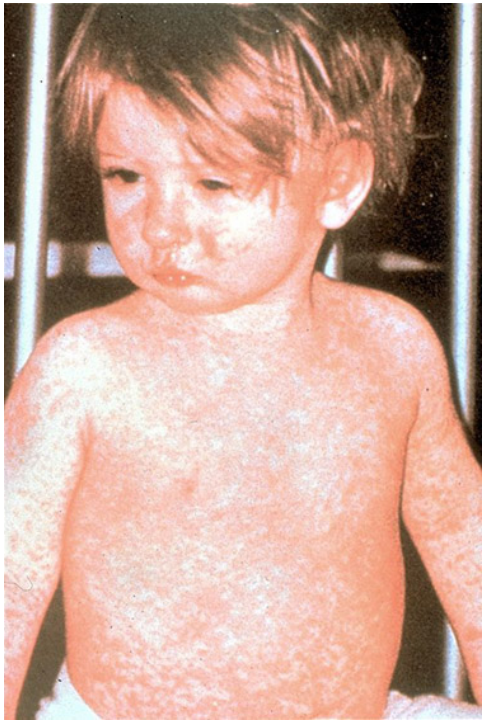


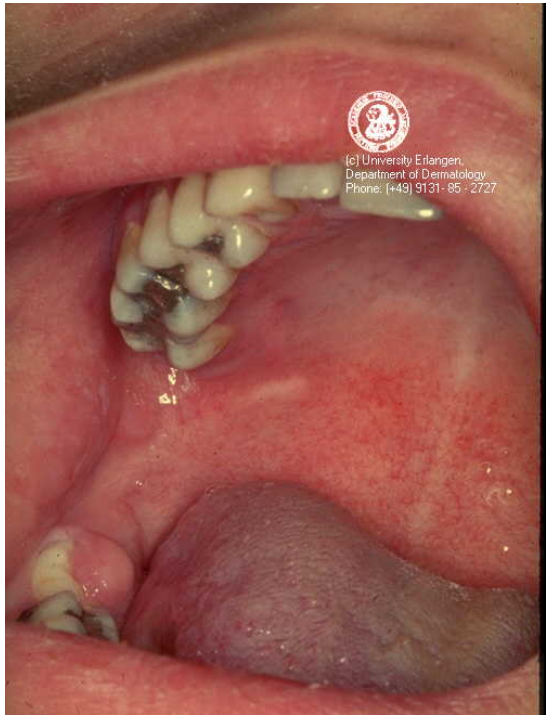




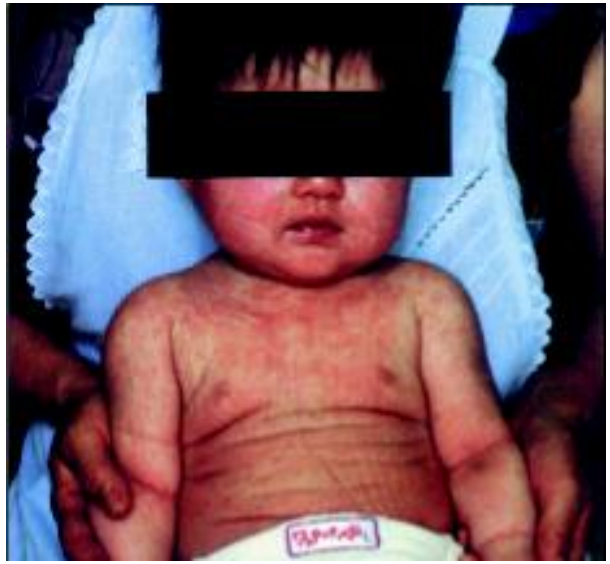
http://www.immunizationed.org/images/measbod.jpg

http://www.cdc.gov/nip/ed/slides/rubella8p.ppt#314,1,Dia 1

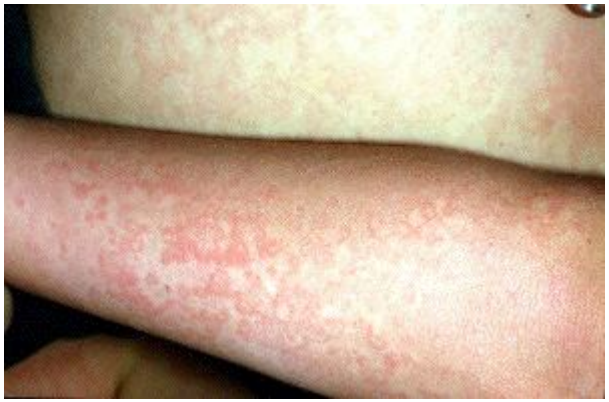




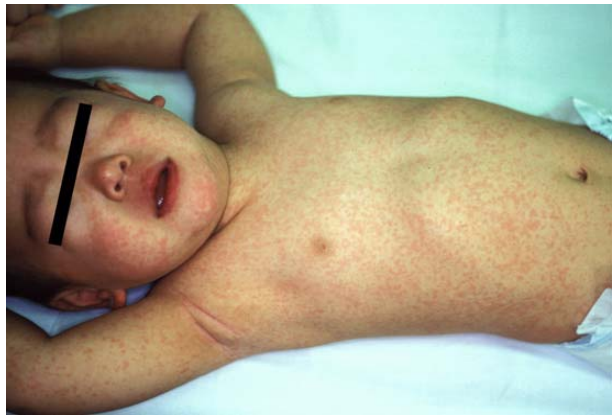
Measles



HHV 6



Parvovirus B19

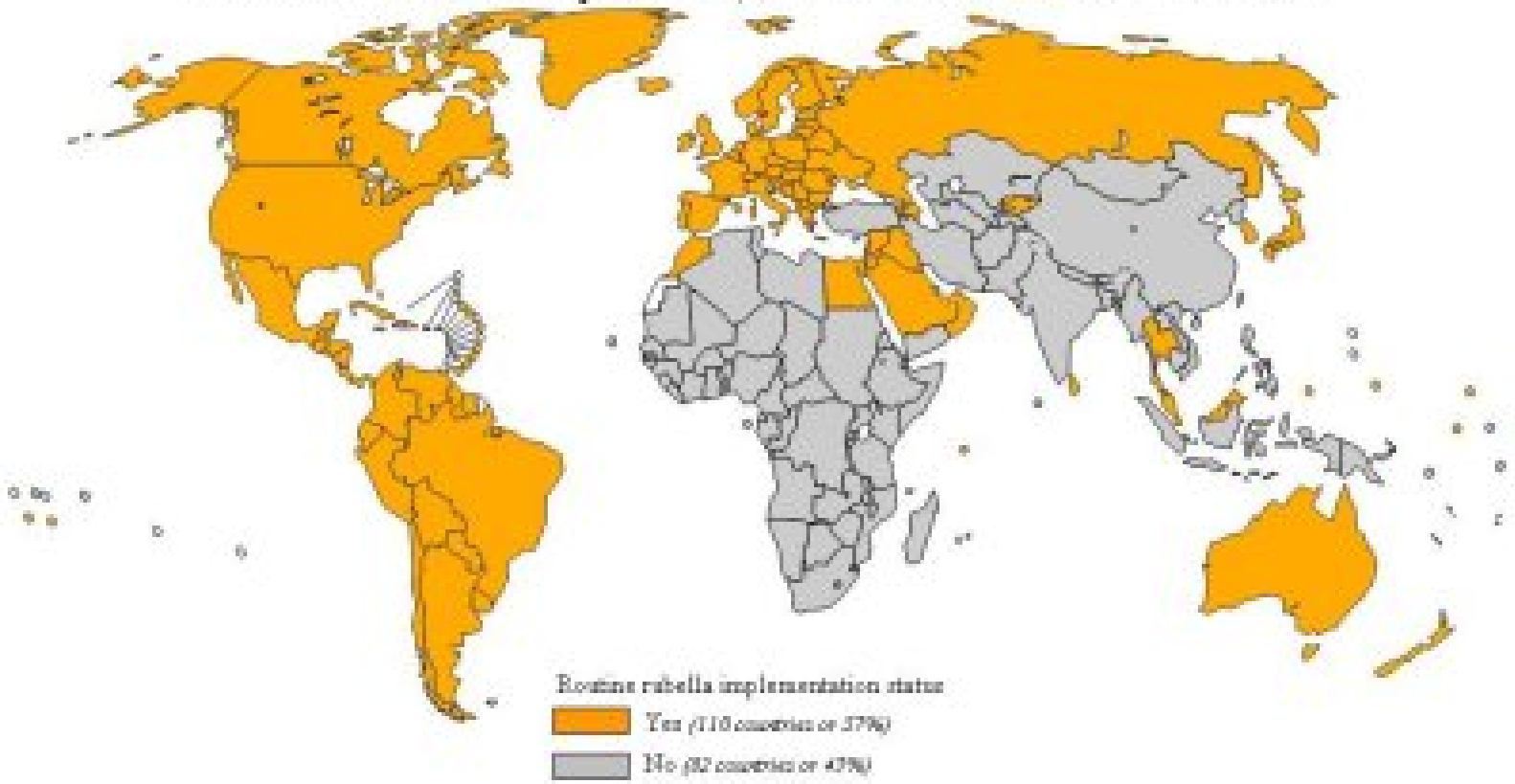


Rubella

Epidemiology

- MD’s practising in ’60-’70s will have seen cases, but others?
- Nowadays children presented with deafness also have been vaccinated: they already have rubella antibodies from MMR vaccination, how to prove?
- A changing epidemiological pattern: industrial world versus third world, migration…
- Eradication ?

Countries using rubella vaccine in their national immunization system, as of December 2003



Source: WHO/UNICEF Joint Reporting Form, 2004. Data collected from 192 WHO Member States as at 31 October 2004

Date of slide 03 October 2004

http://www.who.int/vaccines-surveillance/graphics/htmls/RubellaNationalImmun.htm

Epidemiology in Belgium

Tabel 1 : Rubivirus : evolutie van de registratiefrequentie (2001-2003)

Jaar	N	Aantal laboratoria die ten minste 1 geval diagnosticeerden	Maximum aantal gediagnosticeerde gevallen door een laboratorium	Aantal arrondissementen waarin ten minste 1 geval is gediagnosticeerd
2001	31	5	21	7
2002	21	3	19	4
2003	37	4	25	9

Tabel 2 : Rubivirus : verdeling volgens geslacht en leeftijdsgroep (N, %; 2003)

Leeftijdsgroep	Mannen		Vrouwen	
(jaar)	N	%	N	%
< 1	1	25,0	0	0,0
1 – 4	3	75,0	3	9,1
5 –14	0	0,0	2	6,1
15 - 24	0	0,0	11	33,3
25 - 44	0	0,0	16	48,5
45 - 64	0	0,0	1	3,0
≥ 65	0	0,0	0	0,0
Totaal	4	100,0	33	100,0

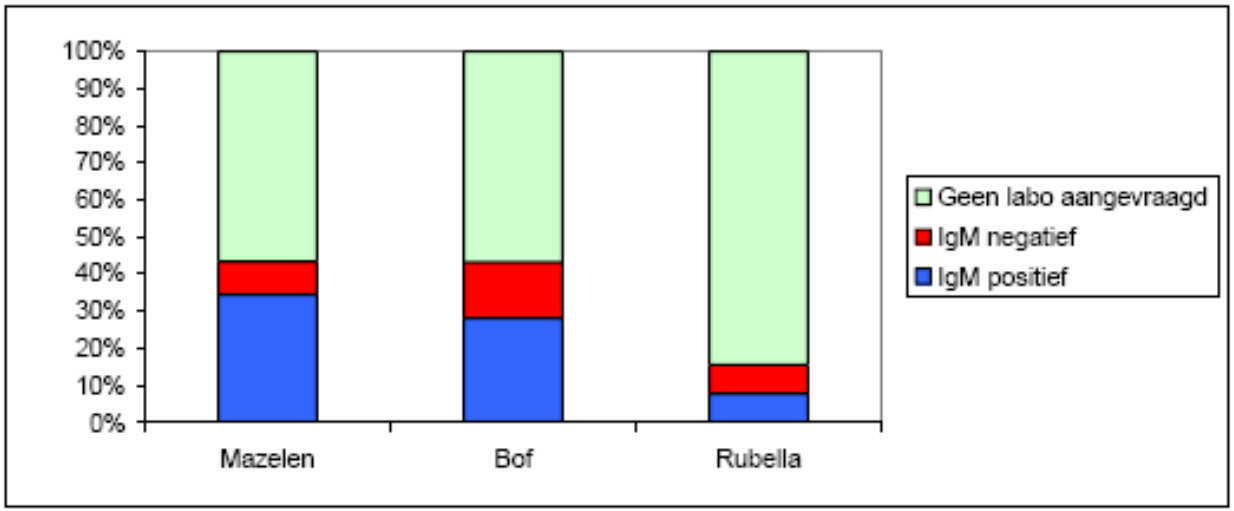
http://www.iph.fgov.be/epidemio/epinl/plabnl/plabannl/k111nllv.pdf

Epidemiology in Belgium: remarks

- The Belgian surveillance program did not separate true IgM confirmed rubella cases from positive IgM-IgG cases which were diagnosed to check vaccination status.
- For 2004 only true IgM will be taken into account.
- In 1997 6.75% of the Flemish women between 20 and 40 years was still seronegative. Immigrants and vaccine failure are likely the cause.

Epidemiology in Belgium: remarks

Surveillance of rare diseases among children in Belgium reported 58 cases of rubella in 2003.
Of those 58 **only 4** were laboratory (IgM) confirmed.



http://www.iph.fgov.be/epidemio/epinl/plabnl/eradi04nl.pdf

Rubella outbreak

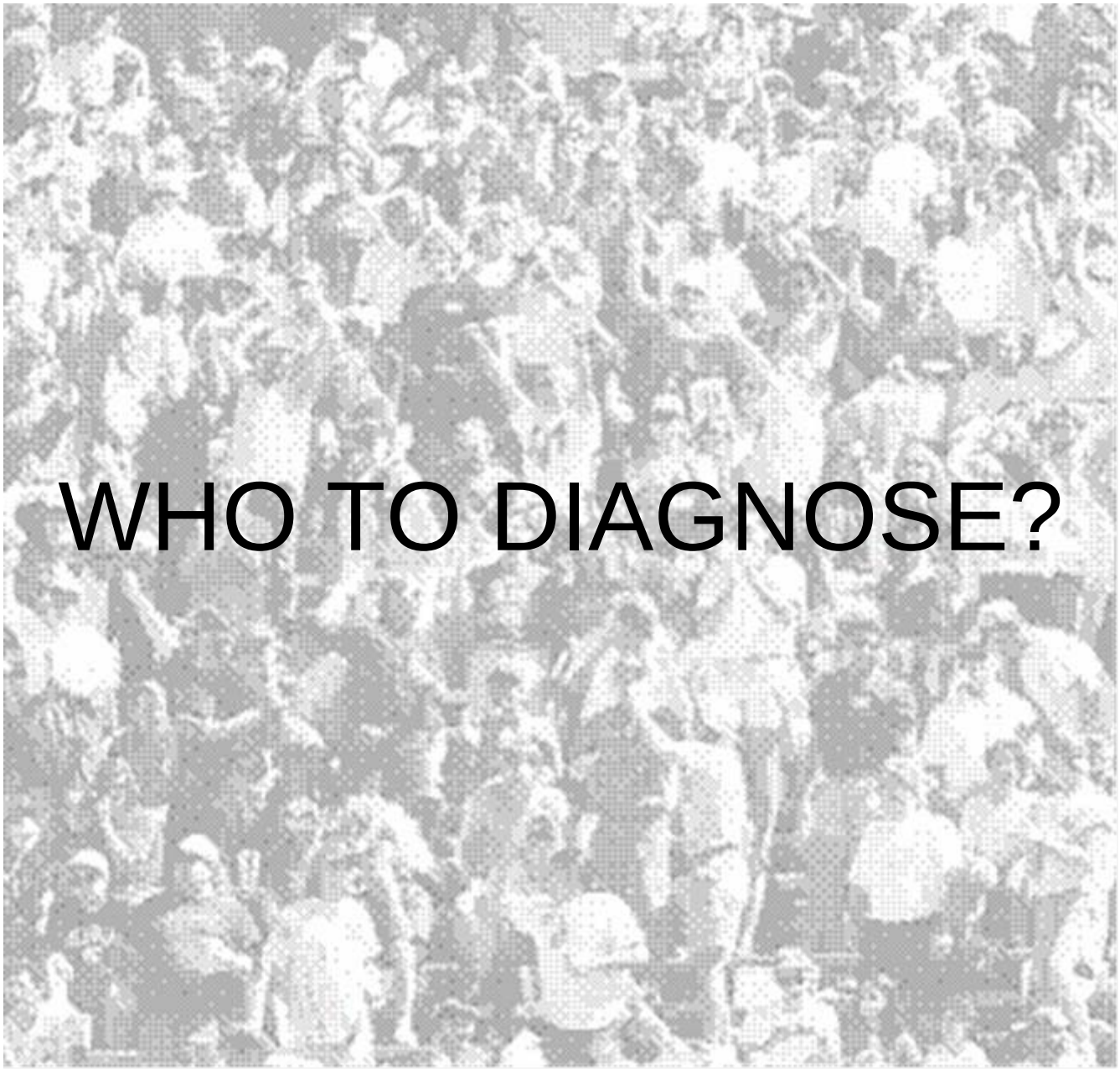
- September 2004: in the Netherlands in a **unvaccinated religious community** which spread to Canada.
- 2005: Spain, Madrid where young adults of **Latin American origin** made up a high proportion of the patients.
- **Conclusion: rubella is a rare disease in Belgium.**
- Is there a need for **target immunisation**?

Motivation of the CAT

Recently there were two Rubella **outbreaks**. One due to an unvaccinated religious community (the bible belt in the Netherlands) and another due to migration (Spain). The question arose what is the prevalence of the disease in Belgium and: "**how to diagnose rubella**", "**who to diagnose**", "**how to confirm a rubella case**". Are the current tests: the microparticle EIA (Axsym© platform) and the sucrose density test still valid? What about the efficiency, effectiveness and efficacy of both tests? What costs an EIA and a sucrose density gradient according to Activity Based Costing.

Main Questions

- Who to diagnose?
- How to diagnose Rubella?
- How to confirm a positive Rubella screening and confirm as true positive?


WHO TO DIAGNOSE?

First of all: essential anamnestic data

First of all: anamnestic data are important: **vaccination status** ? Results of **previous** antenatal screening **tests**.

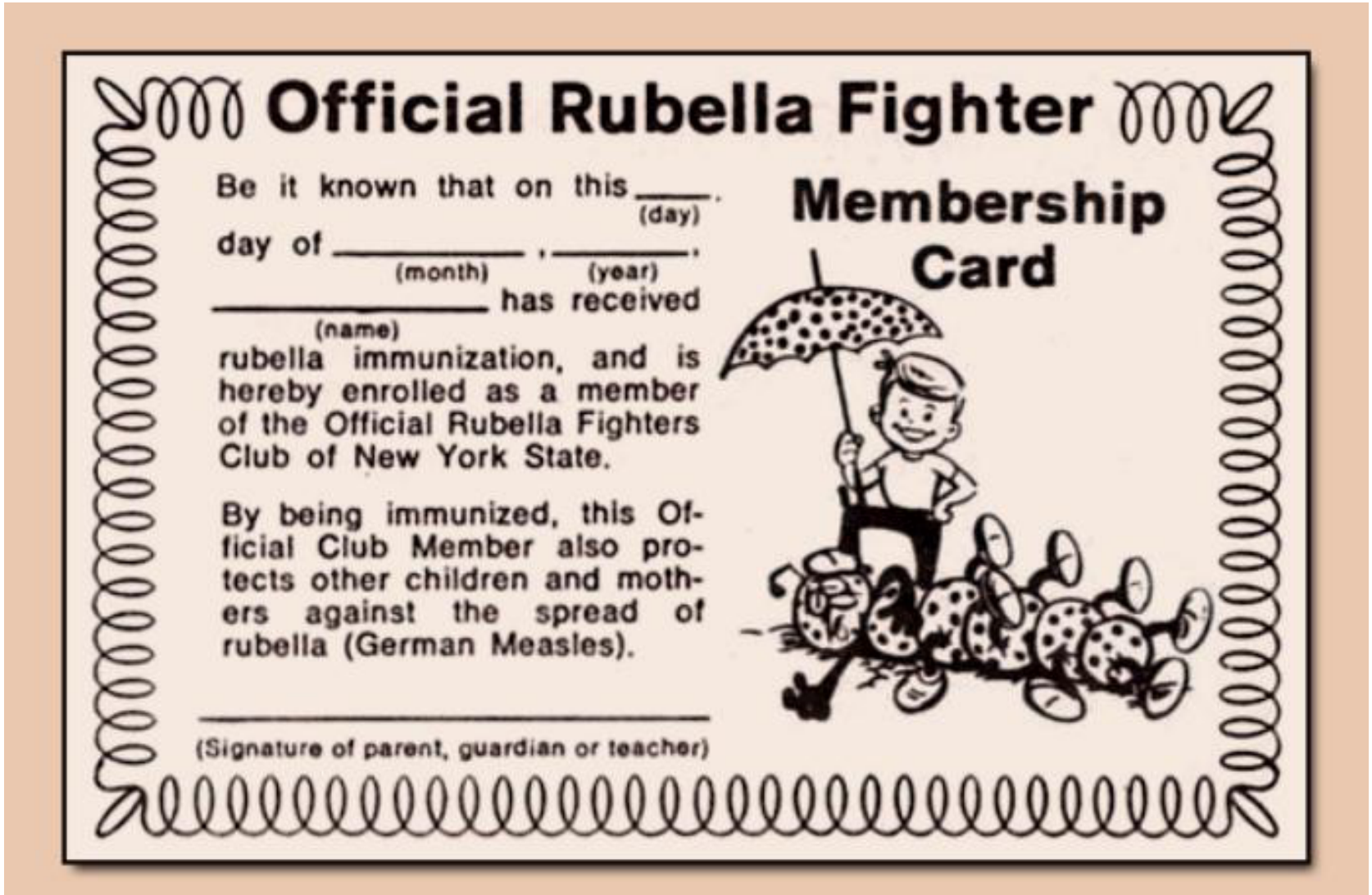
Precise details of date and duration of contact (15 minutes in one room with a rubella (suspected) case or face-to-face contact?

Is there a **suspicion** for Rubella: Is there a (non-vesicular) rash, is there a clinical syndrome compatible with CRS, was there a maternal infection? Assessing hearing loss in early infancy (K&G)?

Except during epidemics, the **clinical diagnosis of rubella** is grossly **inaccurate**.

Close collaboration between antenatal clinics and the laboratory is essential for appropriate investigation of pregnant women exposed or who have acquired rubella-like infections.

Vaccinnet?



Official Rubella Fighter

Be it known that on this ____ (day) day of _____ (month), _____ (year), _____ (name) has received rubella immunization, and is hereby enrolled as a member of the Official Rubella Fighters Club of New York State.

By being immunized, this Official Club Member also protects other children and mothers against the spread of rubella (German Measles).

_____

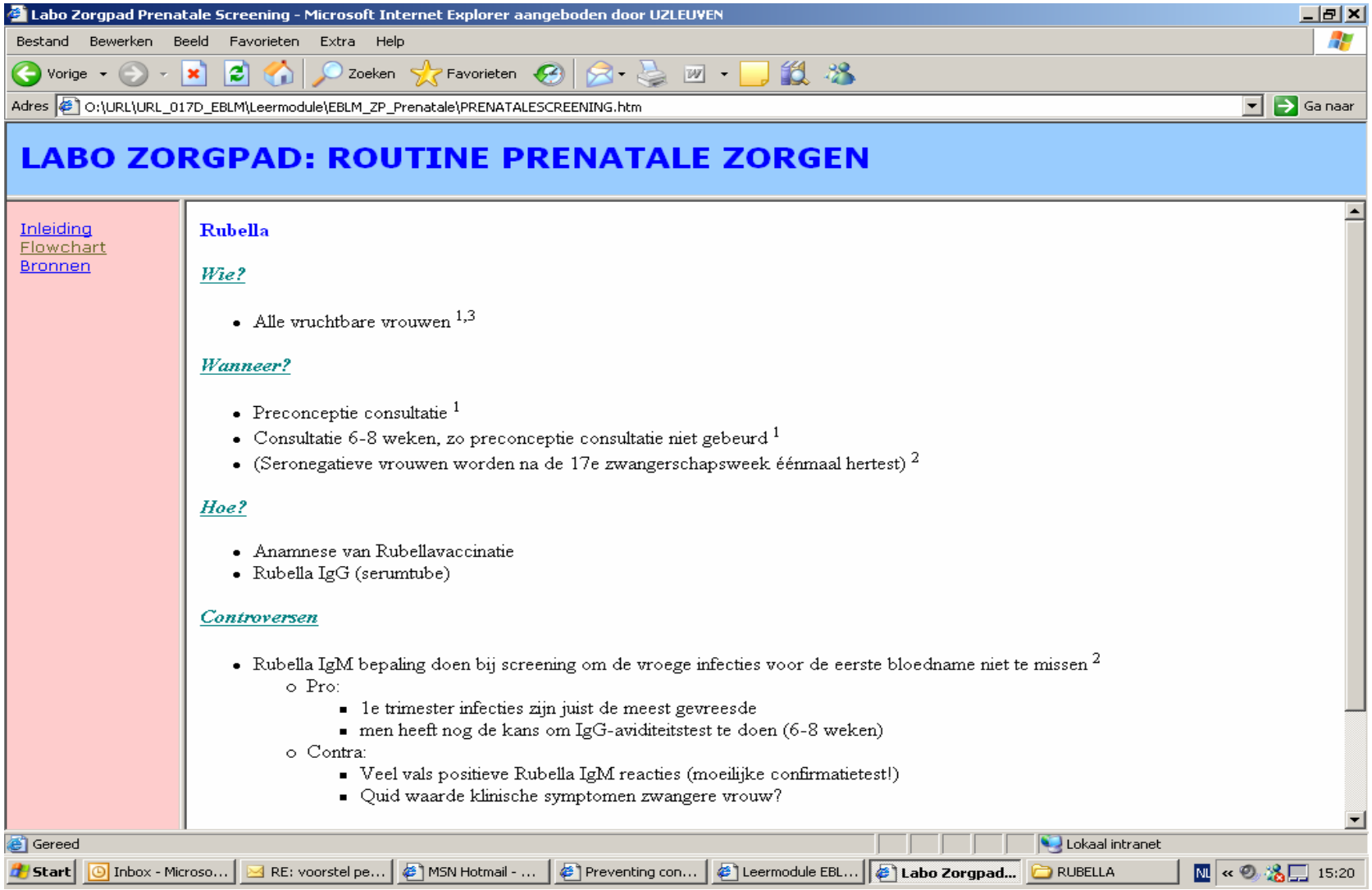
(Signature of parent, guardian or teacher)

Membership Card

Who to diagnose

- **Vaccination confirmation**: current situation IgG (clinical pathway, in house) if vaccination status is not known. Moreover with Vaccinnet one can wonder if immunisation data are known (electronically), do we have to assess immunity (cf. the Netherlands no reimbursement). Generally the cut-off is 10 IU /mL, but in pregnant women 10-15 IU /mL is considered not immune (according to experts).
- **Suspected recent or current infection** due to contact with a rubella-like syndrome, patient with a rubella-like syndrome, child whose mother had a laboratory confirmed rubella infection during pregnancy or was suspected for though which wasn't laboratory-confirmed, suspicion for a **CRS** without confirmation of maternal infection (e.g. deafness): IgG and IgM or a fourfold rise of the IgG titer (in one run in one kit).
- **Re-infection**: **no**: mother should be reassured that the risk is extremely small.

Current situation in house: clinical pathway prenatal care



eQC WIV

- «We present a young woman who wants to become pregnant. She consults her GP what to do and which examinations she needs before gestation. She cannot remember being vaccinated as a child for Rubella. The GP takes a blood sample to control the antibodies».

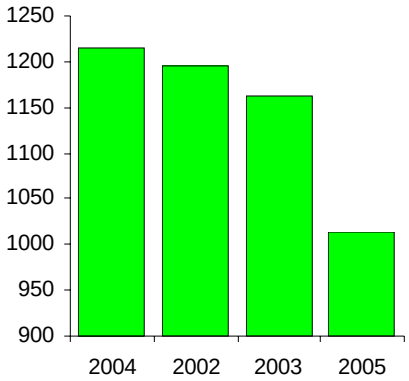
http://www.iph.fgov.be/ClinBiol/bckb33/activities/external_quality/rapports/_down/microbiologie/_nl/2005_01_GR.pdf

eQC WIV

- 188 laboratories responded. They conducted 376 tests.
- 14 laboratories performed 1 test (13 IgG en 1 IgM), 164 laboratories conducted 2 tests (IgG en IgM), 6 laboratories 3 tests (IgG, IgM, total) en 4 laboratories 4 tests (2 IgG, 2 IgM) .

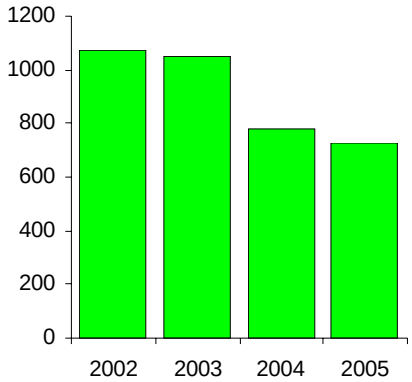
What happens in house 2002-2005

IgG rubella



Jaar	Aantal uitvoeringen (som)
2004	1214
2002	1195
2003	1162
2005	1014

IgM rubella



Jaar	Aantal uitvoeringen (som)
2002	1071
2003	1049
2004	780
2005	731

06-10-2005

MICROBIELE SEROLOGIE

VIRALE SEROLOGIE (terugbetaling beperkt tot 8 tests per voorschrift)

Serum

2235 ❑ Adenovirus IgG
2233 ❑ Adenovirus IgM
2282 ❑ Bofvirus IgG
2284 ❑ Bofvirus IgM
2242 ❑ Coxsackievirus B1 ❖
2245 ❑ Coxsackievirus B2 ❖
2248 ❑ Coxsackievirus B3 ❖
2251 ❑ Coxsackievirus B4 ❖
2254 ❑ Coxsackievirus B5 ❖
2257 ❑ Coxsackievirus B6 ❖

2082 ❑ Cytomegalovirus IgG
2084 ❑ Cytomegalovirus IgM
2085 ❑ Cytomegalovirus IgG aviditeit
2312 ❑ Dengue virus IgG
2313 ❑ Dengue virus IgM

Serum

Epstein-Barrvirus (EBV)
- Immuuncompetent
2300 ❑ EBV EBNA-1 IgG
of *(zo negatief ook EBV IgG/IgM)*
- Immuungecompromitteerd
EBV DNA zie infra bij DNA/RNA detectie - Bloed op EDTA
2002 ❑ Hepatitis A virus
2003 ❑ Hepatitis A virus IgM
Hepatitis B virus
2006 ❑ surface antigeen
2008 ❑ surface antistoffen
2009 ❑ core antistoffen
Hepatitis B virus geassocieerd
2010 ❑ e antigeen
2011 ❑ e antistoffen
2016 ❑ Hepatitis D (delta agens)
2017 ❑ Hepatitis C virus

Serum

2373 ❑ Hantavirus IgG
2374 ❑ Hantavirus IgM
2285 ❑ Herpes simplex virus IgG
2287 ❑ Herpes simplex virus IgM
2025 ❑ HIV Ag/Ab
2026 ❑ HTLV - I & II
2202 ❑ Influenzavirus type A ❖
2205 ❑ Influenzavirus type B ❖
2229 ❑ Lymfoc. choriomeningitisvirus
2288 ❑ Mazelenvirus IgG
2290 ❑ Mazelenvirus IgM
2208 ❑ Parainfluenzavirus type 1 ❖
2211 ❑ Parainfluenzavirus type 2 ❖
2214 ❑ Parainfluenzavirus type 3 ❖
2303 ❑ Parvovirus B19 IgG
2304 ❑ Parvovirus B19 IgM

Serum

2260 ❑ Poliomyelitisvirus type 1
2263 ❑ Poliomyelitisvirus type 2
2266 ❑ Poliomyelitisvirus type 3
2217 ❑ Respiratoir syncytieel virus ❖
2095 ❑ Rubellavirus IgG
2098 ❑ Rubellavirus IgM
2291 ❑ Varicella-zostervirus IgG
2293 ❑ Varicella-zostervirus IgM

Cerebrospinaal vocht ✲

2378 ❑ HTLV - I & II
2379 ❑ Lymf.choriomeningitisvirus

NIET VIRALE SEROLOGIE

Serum

1638 ❑ Aspergillus antigeen
2315 ❑ Borrelia
2220 ❑ Chlamydia (generisch) ❖
of
2222 ❑ Chlamydia trachomatis IgG (fertiliteitsonderzoek)

Serum

1731 ❑ Cryptococcus antigeen
2226 ❑ Mycoplasma pneumoniae IgG
2227 ❑ Mycoplasma pneumoniae IgM

Serum

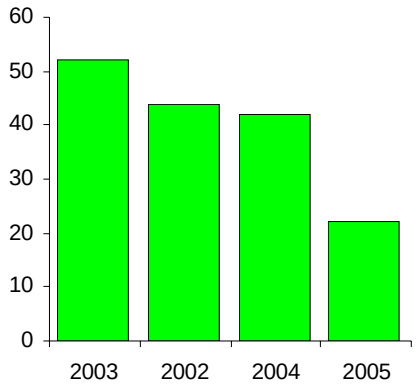
2087 ❑ Toxoplasma IgG
2089 ❑ Toxoplasma IgM
2105 ❑ Toxoplasma IgG aviditeit
2450 ❑ Treponema pallidum (syfilis)
1657 ❑ Yersinia

Cerebrospinaal vocht ✲

2434 ❑ Borrelia
1717 ❑ Cryptococcus antigeen
2440 ❑ Treponema pallidum (syfilis)

What happens in house 2002-2005

IgM gezuiverd rubella



Jaar	Aantal uitvoeringen (som)
2003	52
2002	44
2004	42
2005	22

What happens in Belgium

- IgG riziv: 159.915 ambulatory en 5.526 clinical setting
- IgM riziv: 110.778 ambulatory en 7.983 clinical setting

What happens in house 2000-2005

			63019	63020	63021
			rubella igm	rubella igg (vrouw)	rubella igm gezuiverd
Rpl. Gynae	499		2333	3240	3
Extra mure	20		401	233	156
Fertiliteits	495		176	230	
Labo fertil	497		131	149	
Bevallings	496		72	86	1
Kinderzkh	341		47	82	
Spoedgev	595		57	67	
Rpl. Intern	409		41	67	
Int.Neonat	321		61	46	
VE. Materr	430		42	52	
Rpl. Kinde	302		40	51	
Kinderzkh	343		24	28	
Dagzkh. K	305		17	24	
Neonatale	342		16	13	
Rpl. Oogzi	92		12	14	
Rpl. Consu	612		10	15	
DAGZKH.	495		11	12	

What happens extra-muros 2002-2005

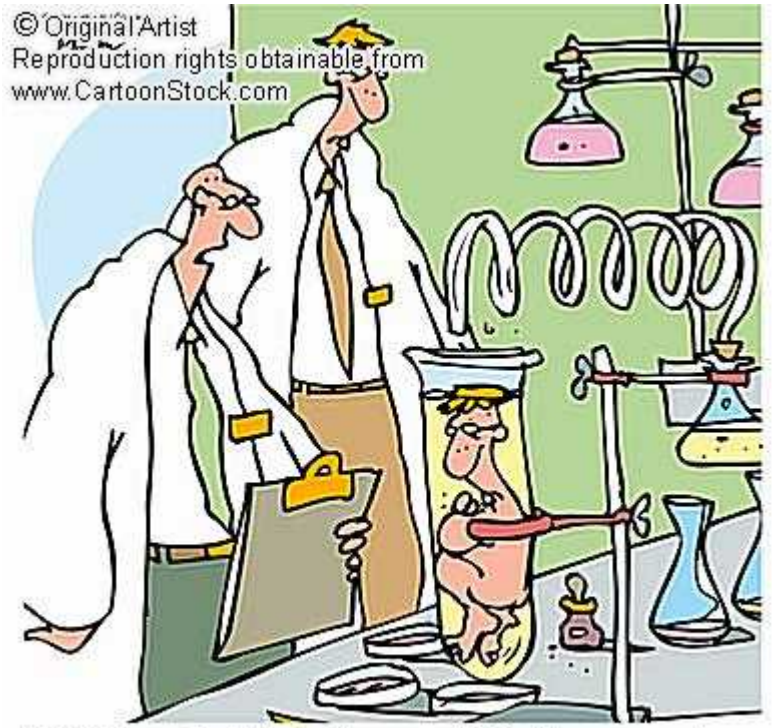
				rubella igm gezuiverd
Algemeen Sted.Ziekenhuis labo	Aalst	Niet ingevuld		24
Medisch Labo Medina BVBA	Dendermonde	Niet ingevuld		24
Centrum Medische Analyse	Herentals	Niet ingevuld		20
Klinisch labo Rigo	Genk	Niet ingevuld		14
Labo Medina BVBA	Aalter	Niet ingevuld		13
MCH	Leuven	Niet ingevuld		12
AZ Vesalius laboratorium	Tongeren	Niet ingevuld		8
Labo AZ Damiaan(H.Hart)	Oostende	Niet ingevuld		4

eQC WIV

- «We present a young woman who wants to become pregnant. She consults her GP what to do and which examinations she needs before gestation. She cannot remember being vaccinated as a child for Rubella. The GP takes a blood sample to control the antibodies».
- IgG was sufficient to determine if she was immune or not.

http://www.iph.fgov.be/ClinBiol/bckb33/activities/external_quality/rapports/_down/microbiologie/_nl/2005_01_GR.pdf

HOW TO DIAGNOSE?



"Well, it certainly looks like your DNA. How many times have I told you to wear gloves before touching anything?"

How to diagnose: possibilities in the literature

- **Hemagglutination-Inhibition** (HI) test, labour intensive, sensitive test which was considered as being the gold standard. Problems with pigeon red blood cells and inhibitors occur.
- **ELISA IgG and IgM**, most commonly used, with the advantage of international standardisation. (**IgG > 10 IU/ mL** is protective after vaccination).
- Passive hemagglutination antibody (PHA) test, lacks sensitivity, subjective reading, labour intensive.
- Latex-agglutination test (LA) lacks sensitivity, false positives, false negatives (prozone), subjective reading, labour intensive but quick in serosurveys.
- Complement fixation (CF) lacks sensitivity, subjective reading, labour intensive.
- Immunofluorescence (IFA) lacks sensitivity, subjective reading, labour intensive, cave false positives due to rheumatoid factor.
- Hemolysis in Gel (HIG), simple, sensitive and specific.
- RIA, Fluoroimmunoassay (FIA/FIAX), not commercial available and radio-active waste.
- **Immunoblotting**, western blotting.
- **Avidity** Enzyme Immunoassay (in primary rubella avidity remains 1 month low).
- Tests to detect rubella-specific neutralising antibodies, serum fractionation, haemagglutination-inhibition antibodies, single radial haemolysis.
- **IgM capture Enzyme Immunoassay (EIA)**, most widely used (infection and immunity). They are now the standard. They are cost-effective and fast.
- **PCR.**

Voor de IgG

Tabel 6.2.5.: Reagentia gebruikt voor de bepaling van Rubella IgG.

Fabrikant	Kit	S/5611
Abbott	AxSYM Rubella IgG	72
	IMx Rubella IgG	2
Bayer	ADVIA Centaur Rubella IgG	14
Beckman (verdeler Analis)	Access Rubella IgG	24
bioMérieux	VIDAS Rub IgG II	41
Dade Behring	Enzygnost anti Rubella virus IgG	3
	Hemagglutinatie in microtiterplaat	1
DiaSorin	Liaison Rubella IgG	21
	ETI-RUBEK-G Plus	6
DPC	Immulite Rubella IgG	6
Mikrogen	recomBlot Rubella IgG	1
Totaal		191

Voor de IgM

Tabel 6.2.6.: Reagentia gebruikt voor de bepaling van Rubella IgM.

Fabrikant	Kit	S/5611
Abbott	AxSYM Rubella IgM	65
	IMx Rubella IgM	2
Bayer	ADVIA Centaur Rubella IgM	14
Beckman (verdeler Analis)	Access Rubella IgM	19
bioMérieux	VIDAS Rub IgM	48
Dade Behring	Enzygnost anti Rubella virus IgM	2
DiaSorin	Liaison Rubella IgM	21
	ETI-RUBEK-M Reverse Plus	7
DPC	Immulite Rubella IgM	6
Totaal		184

http://www.iph.fgov.be/ClinBiol/bckb33/activities/external_quality/rapports/_down/microbiologie/_nl/2005_01_GR.pdf

Dutch laboratories

- RIVM: antenatal IgG is tested or nothing at all is tested (one thinks that a good vaccination coverage and herd immunity are sufficient). Due to the outbreaks screening is asked more frequently. Rubella IgG is not reimbursed in the Netherlands. Breda only offers IgG testing. IgM is sent to Tilburg. In Tilburg only one IgM assay is available.
- Vidas, AxSYM, Organon, Enzygnost is most frequently used.

How to diagnose: literature

Test **IgM**: positive result is strongly suggestive. Sensitive tests can detect low concentrations. **False-positive results** are more likely to occur if **indirect rather than antibody capture assays** are used and from other IgM antibodies which **cross-react** or **rheumatoid factor**…

IgM can **persist** a year or more after natural infection, vaccination or asymptomatic re-infection.

How to diagnose: literature

…consequently **a second** rubella-specific **IgM test** with a **different format** should be done to confirm maternal rubella in the first 20 weeks of pregnancy…

A **notable rise** in **IgG** (literature fourfold rise, one run in one kit) antibodies within 4-7 days of the onset of the symptoms, but patients frequently present after the acute phase and IgG already reached a maximum.

How to diagnose?

- IgG fourfold rise in one run: the first sample should be taken within the first week after disease and the second at least two weeks later.
- In the same run due to reproducibility. Our own data confirm these findings. Intra-run CV's are 5.244 for Rubella IgG and 5.5924 for Rubella IgM (AxSYM©). Long term CV's (4 months, different kits) for Rubella IgG are 15.27 (mean c.s. 20.62). Concerning IgM CV's are 7.09 (mean c.s. 1.3959).

How to diagnose?

In case of **CRS**:

IgM detection in foetal blood by **cordocentesis** in fetal blood (cave false-negatives)(22 weeks)

Testing **chorionic-villus** samples by **RT-PCR**, but presence of the virus doesn't always reflect infection.

Viral RNA in **amniotic fluid** (sensitivities 87-100%, RIVM: 60-80%) performed 8 weeks after maternal infection and after 15 weeks of gestation (19-23 weeks). False negative results occur.

Generally speaking physicians should be aware that false- negative results in PCR occur.

How to diagnose? IgM.

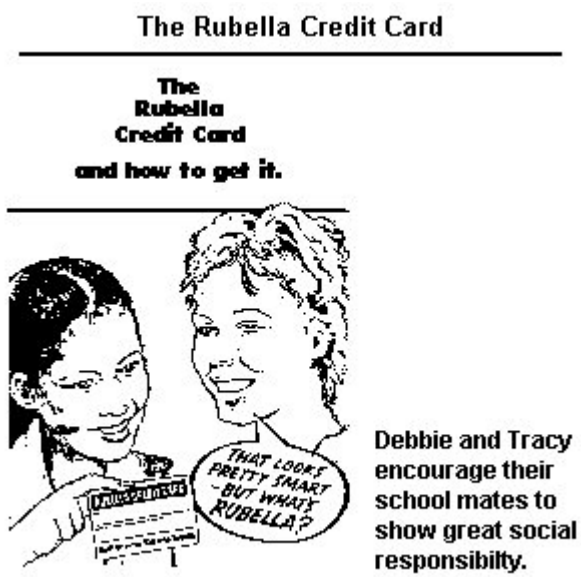
- According to several studies IgM tests should be sensitive and specific. **AxSYM©** Rubella IgM has (statistical significant) **higher sensitivities** (78.9% - 100%) but **lower specificities** (86.5% - 99.2%). Especially Rubella IgM on AxSYM© was problematic generating false positive results for measles infections.
- The AxSYM© platform is **fully automated** and **TAT is very good** (1.5 h) (>< Dade Behring© EIA's 4-4.5 h).
- The **prevalence** of rubella is extremely low, **ppv decrease** with consecutively significant risks for **false positive** results. When one chooses the most sensitive test AxSYM© is the best choice. Although AxSYM© is a microparticle EIA and not a IgM capture format which is often recommended. A **confirmation (specific) test** will be necessary.

How to diagnose? IgG.

- Concerning IgG testing on the AxSYM© platform sensitivities are 99.8% but specificities only 81.5%.
- Both are reimbursed (B250 (IgG) and B300 (IgM) cumulregel 328).

  According to ABC calculations

  IgG and IgM cost 7.34 euro.



Debbie and Tracy encourage their school mates to show great social responsibilty.

How to diagnose: Expert opinions.

- **RIVM** (Nederland) Robert van Binnendijk: In the Netherlands IgG is tested to determine immune status if testing at all. The AxSYM platform is often used and confirmation is done with Vidas (bioMérieux) or Enzygnost (Dade Behring). PCR sensitivities are not 100% but vary between 60 and 80%.
- **Tilburg** (Nederland) Marcel Peeters: should there be testing at all when there is a good vaccination? No, but when there is an outbreak you ‘re morally obliged. So the Dutch do screen for IgG (Organon)(although the Dutch government doesn’t reimburse rubella and toxoplasma screening for the pregnant woman). There is always communication with the clinician.
- **VUB**, Anne Naessens did not cooperate.

How to diagnose: Expert opinions.

- **Breda** (Nederland) Axel Jeurissen: only IgG is performed, IgM is sent to Tilburg. There is always communication with the clinician.
- **UGent** Lieve Vanrenterghem: only IgG and IgM on Access (Analis) is performed. Confirmation samples are sent to the VUB (second platform). There is post-factum communication.
- **UCL** Monique Boudéus: cut-off of 15 IU /ml (AxSYM). A pregnant woman with values between 10-15 IU /mL should be considered not immune. There is postfactum communication. Confirmation with the Vidas system.

  Monique Bodéus is not fond of avidity testing due to the quick maturation of the rubella IgG. There is no experience with immunoblot and PCR.

How to diagnose: Expert opinions.

- **Liliane Grangeot-Keros:**

  They are the reference centre for France. They use **AxSYM** and **Vidas**. AxSYM is very sensitive but according to the expert specificity is not that bad. She has experience with avidity and **immunoblot** (manual, in house). But **avidity** is a problem: besides the cut-offs and the quick maturation, it is difficult to make the difference between people who have been vaccinated and people who were infected. Immunoblotting is useful when AxSYM and Vidas don't correspond. Also **following IgM levels** is usefull. IgM after vaccination remains stable, after infection they decrease (t ½ = 3 weeks). Also here samples should be done in one run. People should test to determine immune status because vaccine failure is possible. **PCR** techniques are also used on amniotic fluid (22 weeks). PCR in saliva and urine are done to determine if CRS cases are still shedding.

How to confirm?

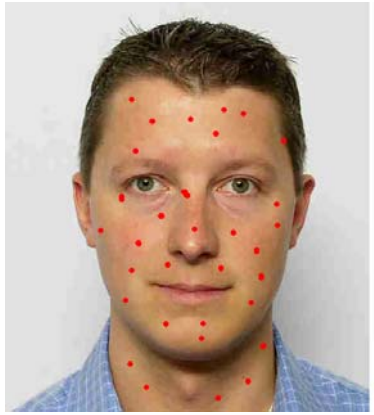
Consensus about following principle:
A very sensitive test is preferable if a confirmation test (on another platform) is possible.

How to confirm: Avidity?

- Several articles state that avidity testing can confirm a rubella case. These authors state that IgG maturation takes 8 to 12 weeks, which would be sufficient time to confirm a rubella case. But some of those studies have no references and some have small numbers of patients. Therefore it is rather difficult to estimate the maturation time of the IgG antibodies because other studies state that the avidity of IgG antibodies in primary rubella remains low for only 1 month (6 weeks).
- Dutch consensus exist not to perform avidity testing for individual diagnostics (one is always too late) only in a setting of outbreaks one can follow trends.

How to confirm: Avidity?

- Avidity testing is perhaps technical ideal, but due to very quick maturation of the IgG antibodies, patients should be tested within the first 4 to 6 weeks.
- Also there is no unanimity about the cut-off. To some authors low AI is < 30%, to some less than 40% and others 55%.
- Also, Dade Behring doesn't commercialize their Enzygnost avidity testing anymore.
- Vaccinated>< infection.



How to confirm: Immunoblot? Viral culture?

- **Western Blots** are also an option to confirm a rubella case. Some authors even recommend both avidity and immunoblot as confirmation test. Although there are not enough studies. Pustowoit et al. suggest to use avidity in combination with the immunoblot.
- **Viral culture**. Viral culture is labor intensive and difficult. It is hard to bring the rubella virus into culture. Culture is certainly not suitable for routine diagnosis, confirmation.

How to confirm: PCR?

- The detection of viral RNA in amniotic fluid, chorionic-villus sample, urine, nasal and throat swap, CSF, blood is possible, although it is difficult to pick up rubella RNA. Throat swabs give best results for surveillance.
- In case of CRS IgM determination on blood obtained by cordocentesis (cave false-negatives), can be helpful.
- Throat and urine: shedding-survey?
- But the timing (approximately 22 weeks) of sampling is too late if an abortion is considered.

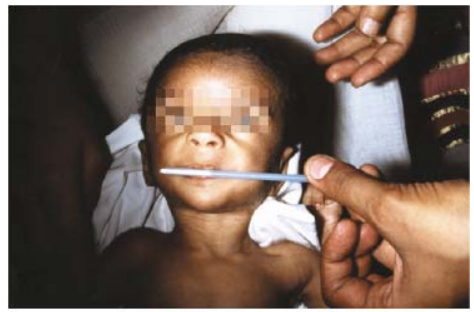


Figure 6: **Collection of oral fluid from an infant for rubella IgM detection**
Courtesy of D M Eckstein and P Vijayalakshmi, Aravind Eye Hospital, Madurai, India.

How to confirm: PCR?

- RT-PCR (nested or not) on chorionic-villus samples and amniotic fluid (in a strict time schedule: 8 weeks after maternal infection or 15-19 weeks pregnancy) can help to diagnose. Although false negative results occur and presence of the virus in chorionic-villus samples do not reflect infection of the foetus.
- More (larger) studies are necessary to determine the role of molecular tests for the diagnosis of a CRS.

How to confirm: Sucrose density gradient?

- For the moment sucrose density gradient test (a modified HI) is used in house as a confirmation test. Actually the test picks up specifically IgM. In literature only one publication sees a role in the future for a modified HI for IgM detection. The procedure is extremely labor-intensive and costs the patient 150 euro (no reimbursement, ABC: 114,58 euro).

Actions

- 1. **In house** antenatal screening is **already** incorporated **in the clinical pathway**. **Extra muros a telephone call should be made before any confirmation testing is done.** A letter to the different laboratories should explain our policy. Also professional organisations such as ‘Vlaamse Vereniging voor Obstetrie en Gynaecologie’ (VVOG) and the ‘Wetenschappelijke Vereniging Vlaamse Huisartsen’ (WVVH) should be informed and be asked to participate in the sensibilization.

Actions

- 2. Perhaps a **standard text on the protocol** of Rubella **IgM** tests should be written: *“IgM testing without relevant anamnestic data such as vaccination status, presence of a non-vesicular rash, contac with of suspected rubella case, … is useless”.*

Actions

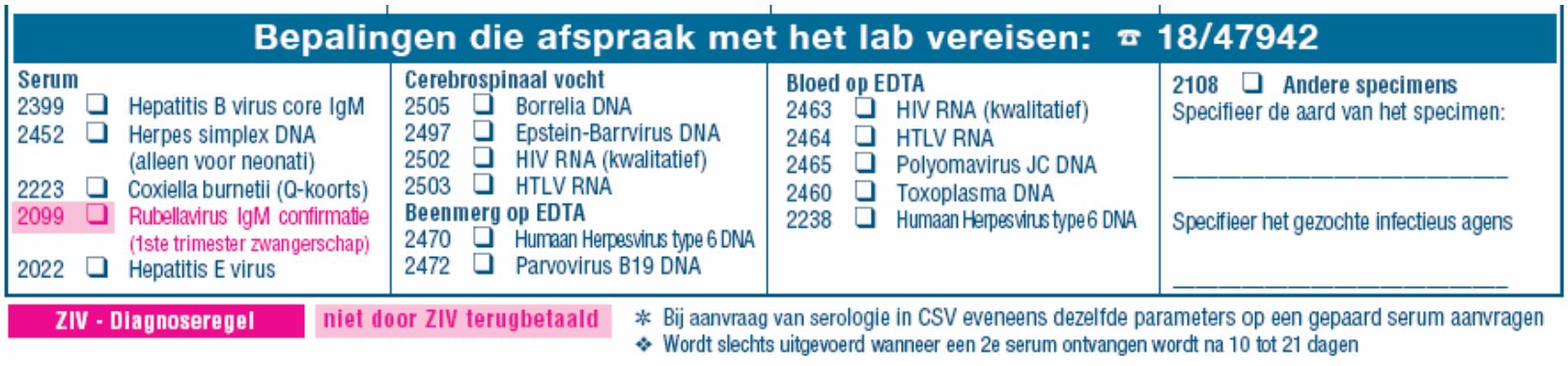
- 3. **Vaccination cards** could be provided to the patient, indicating the immune status in IU /mL. Other parameters can be indicated (blood type, etc.). The introduction should start at the moment that this **data can electronically be stocked** (Vaccinnet? SIS-card?).

Actions

- 4. **Confirmation testing** of a rubella IgM positive result is a necessity for the moment. Although the question arises if this should be a sucrose density gradient test. Considering the extremely labour intensive work, the fact that the prevalence of rubella cases is extremely low, cost-effectiveness for the test is far from optimal, the fact that IgM capture assays and PCR are available,… it would therefore be preferable to **evaluate some IgM capture EIA assays** to replace the sucrose density gradient test. At first sight Enzygnost (Dade Behring) and Vidas (bioMérieux) provide specific tests. These platforms are also optional for our laboratory considering that we use the platform already for other purposes. They should be evaluated. PCR is for the moment not an option and should be considered in an national forum, one centre who provides additional tests such as PCR and perhaps avidity testing is sufficient for this country knowing the small prevalence of rubella.

Extra action

- 5. Move rubella IgM to another place on the 3030, with a telephone call so that clinical information will be provided. And evaluation of the prenatal clinical pathway is necessary!



Bepalingen die afspraak met het lab vereisen: ☎ 18/47942

Serum
- 2399 ❑ Hepatitis B virus core IgM
- 2452 ❑ Herpes simplex DNA (alleen voor neonati)
- 2223 ❑ Coxiella burnetii (Q-koorts)
- 2099 ❑ Rubellavirus IgM confirmatie (1ste trimester zwangerschap)
- 2022 ❑ Hepatitis E virus

Cerebrospinaal vocht
- 2505 ❑ Borrelia DNA
- 2497 ❑ Epstein-Barrvirus DNA
- 2502 ❑ HIV RNA (kwalitatief)
- 2503 ❑ HTLV RNA

Beenmerg op EDTA
- 2470 ❑ Humaan Herpesvirus type 6 DNA
- 2472 ❑ Parvovirus B19 DNA

Bloed op EDTA
- 2463 ❑ HIV RNA (kwalitatief)
- 2464 ❑ HTLV RNA
- 2465 ❑ Polyomavirus JC DNA
- 2460 ❑ Toxoplasma DNA
- 2238 ❑ Humaan Herpesvirus type 6 DNA

2108 ❑ Andere specimens
Specifieer de aard van het specimen:
_____
Specifieer het gezochte infectieus agens
_____

ZIV - Diagnoseregel niet door ZIV terugbetaald

✻ Bij aanvraag van serologie in CSV eveneens dezelfde parameters op een gepaard serum aanvragen
❖ Wordt slechts uitgevoerd wanneer een 2e serum ontvangen wordt na 10 tot 21 dagen