CAT
Critically Appraised Topic

RUBELLA DIAGNOSIS

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CLINICAL BOTTOM LINE

There are different settings in which laboratory rubella diagnosis is indicated. In a antenatal setting an immunisation card can indicate a vaccination status (preferably two tests on different blood samples). Often the patient does not have an immunization card and anamnestic data are unreliable, therefore IgG rubella (and IgG rubella only) testing is indicated. In the UK testing happens irrespective of a immunisation history, report, … Most of the tests performed in our country should be specific rubella IgG antibodies. In the Netherlands discussion arises if any testing is necessary if there is a good vaccination coverage. Due to the outbreaks the Dutch continue to screen. Rubella IgG > 10 IU/ mL is considered 'immune' according to epidemiological studies (CDC, Skendzel et al.)(some experts take > 15 IU/ mL for pregnant women as a cut-off). Rubella immunization programs are beneficial economically in both developed and developing countries. In Belgium very few cases of rubella are reported but outbreaks can occur due to migration and due to people who refuse vaccination (although in Belgium there is no bible belt like there is one in the Netherlands).

As the clinical diagnosis of rubella is difficult and unreliable, the laboratory confirmation is essential. One should realize that in our setting where the prevalence of rubella is extremely low, positive predictive values decrease and that there are significant risks for false positive results. Specific rubella IgM is indicated when a recent or current rubella infection is suspected. Interpretation of the test is necessary by a close communication between clinicians and the laboratory to exclude false positive IgM results. The AxSYM© platform (microparticle EIA) is the most sensitive IgM assay at the expenses of a low specificity.

Also a fourfold rise of the IgG antibodies is indicative for a recent infection if the first sample is taken within the first week after the disease and the second after two weeks. Both samples should be tested in the same test run due to the low reproducibility of the test.

In patients with a non-vesicular rash illness other possible causes (such as parvovirus B19) of such a rash should be excluded. Following the literature, a sensitive IgM test and annex confirmation of IgM rubella (with a different platform) should be done in case of suspicion of recent rubella infection. The sucrose density gradient test is considered extremely labour-intensive, costs a lot and is now in literature replaced by other techniques. An IgM capture EIA is an option (bioMérieux©, Dade Behring©, …).

Avidity testing is technical ideal, but due to very quick maturation of the IgG antibodies, patients should be tested within the first 4 to 6 weeks. Except in case of CRS were maturation is slower. More studies are warranted with larger study populations which calculate the maturation time of the IgG antibodies.
Some authors state that immunoblotting is a worthy alternative for confirmation, but only one large study suggests the use of immunoblotting if done in combination with avidity testing. There are insufficient studies to determine if immunoblotting can be used as a confirmation test solely.

In case of CRS IgM testing of the foetus through cordocentesis, can be helpful. But the timing (approximately 22 weeks) of sampling is too late if an abortion is considered. Also RT-PCR (nested or not) on chorionic-villus samples and amniotic fluid (in a strict time schedule, also 19 weeks) 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 studies with larger study populations are also necessary.

CLINICAL/DIAGNOSTIC SCENARIO

Rubella, also known as German measles or in Dutch as ‘rode hond’, was first described by two German physicians Bergen (1752) and Orlow (1758). In 1941 Norman McAlister Gregg, an Australian ophthalmologist, recognised a group of infants born with congenital cataract. Most of the mothers had a history of rubella in early pregnancy. His findings were confirmed by others. In 1962 the rubella virus was isolated in cell culture. In 1969-1970 attenuated rubella vaccines were licensed. And in 2002 out of 212 countries 123 (57%) countries included rubella vaccination in their national immunisation programmes.

Rubella is a single-stranded RNA-virus of the family of the *Togaviridae* (genus *Rubivirus*). The virus particle is about 60 nm, surrounded by a lipoprotein envelope. Different genotypes (7+3) are described.
Rubella is an acute, usually benign, infectious disease most often affecting children and non-immune young adults. The virus enters the respiratory tract via droplet nuclei and spreads to the lymphatic system (Figure 1). Nevertheless Congenital Rubella Syndrome is characterised by birth defects if the disease is acquired by a pregnant woman: deafness, cataracts, heart defects, mental retardation, and liver and spleen damage are the most frequently seen symptoms (at least a 20% chance of damage to the foetus if a woman is infected early in pregnancy). After 20 weeks the incidence of CRS is less than 2% according to literature. Arthralgia - arthritis, encephalopathy, Guillain-Barré (very rare),

transient thrombocytopenia, purpuric rash, haemolytic anaemia are possible complications of postnatally acquired rubella and vaccination. Re-infection with rubella is almost always asymptomatic and more frequently vaccine-induced than due to a naturally acquired infection. Re-infection 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 extremely small.

Worldwide rubella remains a major problem. The current WHO position is that there are more than 100000 CRS cases occurring every year. Europe has set target for the elimination of measles by 2007, and the reduction of CRS to fewer than one per 100000 livebirths by 2010. Unfortunately, rubella prevalence has increased strikingly in central and eastern Europe. Also, despite vaccination (especially in industrialised countries) outbreaks still occur. In Spain (Madrid 2005) 431 suspected cases of rubella were notified. Young adults of Latin American origin made up a high proportion of patients. In the Netherlands a rubella outbreak occurred in 2004. Up to 17 May 2005 309 laboratory confirmed cases have been reported among a religious community in the Netherlands.
In Belgium rubella is rare. In 2003 58 rubella cases were registered. The diagnosis was made clinically. Only 4 cases were laboratory confirmed (IgM specific antibodies) (Figure 2).

QUESTION(S)

The questions arose what is the prevalence of the disease in Belgium and consecutively "how to diagnose rubella", "who to diagnose", and "how to confirm a rubella case". Are the current tests: the microparticle EIA (Axsym© platform) and the sucrose density gradient 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.

SEARCH TERMS

- Pitfall: MeSH term German Measles >< Rubella.
- Clinical query: 'rubella, systematic review', 'rubella, diagnosis, specific/narrow search', 'rubella, diagnosis, broad/sensitive search', 'German Measles, specific/narrow search', 'German Measles, broad/sensitive search'
- MeSH database: 'rubella', 'rubella/diagnosis', 'German Measles'
- Pubmed: 'rubella, diagnosis' (with limits: review, human), 'rubella, avidity, maturation', 'rubella, immigrant', 'interpretation, rubella, serology', 'maternal, reinfection, rubella, outcome'

Databases: Pubmed, Pubmed Clinical Queries, Pubmed Systematic Reviews, SUMsearch, National Guideline Clearinghouse, Cochrane Library, UpToDate, Institute for Clinical Systems Improvement

Professional organisations: World Health Organization (www.who.int), Centers for Disease Control and Prevention (www.cdc.gov), National Committee for Clinical Laboratory Standards (NCCLS; http://www.nccls.org/)

RELEVANT EVIDENCE/REFERENCES

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APPRAISAL

Who to diagnose.

1. To asses immunity: according to literature, rubella IgG detection is indicated. In the UK a national standard serum is available and an antibody concentration of 10 IU/ mL (The Lancet, Guidelines for surveillance of CRS and rubella, …) is generally accepted as indicative of immunity. Some experts take 10 – 15 IU/ mL as a grey zone, which means that for pregnant women this should be considered as not immune. Most authors use 10 IU/ mL as a standard. Reference sera are available from the CDC and NIBSC (USA, UK).
2. To determine current or recent infection: rubella specific IgM is used. However vaccination status, previous results, information about possible contacts, recnt vaccination … should be obtained. Close collaboration between laboratory and clinicians are mandatory because of possible false positive results. Also IgM can remain positive during one year. A considerably rise (fourfold) in IgG titer is also indicative of a current or recent infection. A rise in titer is relevant if noticed in one run. The first sample should be taken within the first week after disease and the second at least two weeks later. Both samples should be tested in the same test run due to the low reproducibility of the test according to literature. 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 of the control sample 20.62). Concerning IgM CV's are 7.09 (mean of the control sample 1.3959). Finally one should realize that in our setting where the prevalence of rubella is extremely low, positive predictive values decreases and that there are significant risks for false positive results. Confirmation testing will be warranted.
3. In case of CRS IgM detection in fetal blood by cordocentesis (cave false-negatives) (approximately 22 weeks), testing chorionic-villus samples by RT-PCR, viral RNA in amniotic fluid (sensitivities 87-100% >< 60-80% RIVM) performed 8 weeks after maternal infection and after 15 weeks of gestation (19-23 weeks) can lead to diagnosis. Generally clinicians should be aware that false negative results do occur with PCR . And the presence of virus in chorionic-villus biopsy samples might not reflect fetal infection. In perspective of 'therapeutic' options (abortion) one should consider timing of testing.

Conclusion:

In a antenatal setting rubella IgG is sufficient and indicated if there is no conclusive anamnestic data about vaccination status (vaccination card, two tests on different blood samples). Rubella IgG > 10 IU/ mL is considered as an immune status. If a woman is pregnant and there is a history of rash or contact (in the same room for over 15 minutes or face-to-face contact) the cut-off is 15 IU/ mL. Rubella IgM testing is indicated when a recent or current rubella infection is suspected. Fourfold rise of IgG antibodies in one run of which the first sample is taken within one week after symptoms and a second after two weeks is indicative. In case of CRS, serology of the mother as well as the fetus is helpful. PCR is additional, but false negative results, presence of the virus without infection, … should be considered. Communication between laboratory and clinics is mandatory.

How to diagnose.

1. ELISA techniques are most commonly used: it is the fastest and most cost-effective method. Haemagglutination Inhibition test was once the 'standard'. It allowed to screen and diagnose. For rubella IgG, false positive results may be obtained. If indicator erythrocytes are obtained easily, inhibitors can be removed easily and there is an internal standardization, HI can be used. Nowadays it may be modified to detect rubella specific IgM, but several authors consider the IgM capture EIA as today's standard and don't utilize the HI test anymore. Immunofluorescent antibody assay is less used (due to false positives with rheumatoid factor). Latex agglutination appears to be sensitive and specific if performed by experienced laboratory personnel. Its interpretation is nevertheless subjective (especially in serosurveys) but fast and easy. It is not considered use-full in our purpose. Single radial haemolysis is not commercial available.
   Thus most commonly enzyme immuno-assays are used for IgG and IgM. Concerning IgM, false positive results occur (cross-reaction, rheumatoid factor). Antibody capture assay are preferable and give less false positives (Figure 5,7). Those EIA's are fully automated, without pretreatment and are now the standard.

2. What do the Belgian laboratories use:

   Abbott, AxSYM IgM (43%)
   Abbott, IMx Rubella IgM (0.5%)
   Abbott, IgM EIA (1%)
   bioMérieux, VIDAS Rub IgM II (28%)
   bioMérieux, IgM EIA (1.5%)
   DPC, Immulite Rubella IgM (2%)
   Dia Sorin, ETI-RUBEK-M reverse plus (10%)
   Biorad IgM rubella (1.5%)

Abbott, AxSYM IgG (38%)
Abbott, IMx Rubella IgG (1%)
Bayer, ADVIA Centaur Rubella IgG (7%)
Beckman (Analis), Access Rubella IgG (13%)
bioMérieux, VIDAS Rub IgG II (21%)
Dade Behring, Enzygnost anti Rubella virus IgG (2%)
Hemagglutination in microtiter plates (1%)
Dia Sorin, Liaison rubella IgG (11%)
Dia Sorin, ETI-RUBEK-G plus (3%)
DPC, Immulite Rubella IgG (3%)
Mikrogen recomBlot Rubella IgG (1%)

What do the Dutch laboratories use:

Most frequently IgM – IgG capture ELISA is used. Following platforms are most popular: VIDAS and Enzygnost. Also AxSYM© micropaticle EIA is quite popular.

3. Literature.

Several studies have compared several methods to diagnose rubella. Especially we are interested in IgM testing as IgM indicates presence or absence of disease. 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 (Figures 5,7).
The AxSYM© platform is fully automated and TAT is very good (1.5 h) (compared to Dade Behring© EIA's 4-4.5 h). (Figure 6) When one chooses the most sensitive test AxSYM© is the best choice. Although AxSYM© is a microparticle EIA and not really a IgM capture format which is often recommended. A confirmation test will be necessary, because very low levels of IgM can be detected (after re-infection and one year after infection). Also Corcoran et al. reported three cases of CRS with false negative AxSYM© results.
The hypothesis is that the high amount of fetal and maternal IgG compete with the IgM antibodies. The authors suggest a IgM capture assay to exclude CRS if AxSYM results are negative. Finally absence of IgM does not exclude a rubella infection. In conclusion a very sensitive test is preferable if a confirmation test (on another platform) is possible (with a lower sensitivity), preferably an IgM-capture assay. Although the IgM antibody capture radiommunoassay (MACRIA) is perhaps slightly more sensitive, the greater stability of the enzyme label, no radioactive waste, visual and quantitative assessment make IgM antibody capture EIA (MACEIA) preferable.
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 Activity Based Costing calculations IgG and IgM cost 7.34 euro.

4. Expert opinion.

   Rubella diagnostics, 10 questions for the experts.

   Vaccination cards?
   Systematical contact with the clinic??
   Antenatal setting?
   Which platform?
   What if IgG én IgM is asked: contact?
   What if IgM is positive, confirmation?
   If yes, which?
   What about avidity?
   What about PCR?
   What about immunoblot?

   VUB Anne Naessens. Did not cooperate.
   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.
   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.
   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.

No-one used vaccination card except in UGent there is a system that gynaecology uses. The lab is not involved.

Reference center in Belgium: for the moment none, unofficially VUB.

Conclusion: All together the AxSYM© EIA platform for Rubella testing is a very good choice. It is the most sensitive test (IgG and IgM). It is a fully automated test. The TAT is 1.5h. But this test warrants a second line confirmation test, because very low levels of IgM can be detected (after re-infection and one year after infection). Tests for rubella IgM are not indicated unless there is a history of rash in a pregnant woman or contact with a rubella-like rash.

How to confirm a rubella case.

Consensus about the principle: A second rubella-specific IgM test with a different format (with a alternative test principle) should be done to confirm rubella.

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). One article gathered more or less a representative group of patients (n=81, confirmed rubella and n=46, vaccinated people) (Figure 8). For the confirmed rubella cases the avidity index (AI) was more than 30% after six weeks after the onset of rash. For the vaccinated group AI was over 20% during the first day of their rash fever illness. Based on these data the period over which IgG antibodies mature is for the moment perhaps too short in our setting (not for survey settings during an outbreak). 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. 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. Except in case of CRS were maturation is slower. Maternal antibodies in the child disappear after one month and the child's own antibodies remain. Those infant antibodies maturate extremely slow. Studies which claim a longer maturation period are studies with small study groups. Also there is no unanimity about the cut-off. To some authors low AI is < 30%, to some less than 40% and others 55%. In one article the differences between the cut-offs are attributed to the small numbers of defined sera used in the studies. Differentiation between vaccinated and infected people is difficult. Also, Dade Behring

doesn't commercialize their Enzygnost avidity testing anymore. One should introduce a in house avidity testing if one choose to perform avidity testing.

Immunoblots. Western Blots are also an option to confirm a rubella case. Some authors even recommend both avidity and immunoblot as confirmation test. While E2-specific antibodies appear with a delay (3-4 months), E1-antibodies appear as early as 4-6 days post-infection. Although there are not enough studies. Pustowoit et al. suggest to use avidity in combination with the immunoblot. Professor Grangeot-Keros uses a manual in-house method.

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.

RT-PCR (nested). 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. But the timing (approximately 22 weeks) of sampling is too late if an abortion is considered.
Also 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.

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).

ACTION

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.
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, contact with of suspected rubella case, … is useless"*.

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?).
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.

ATTACHMENTS

FIGURE 1.

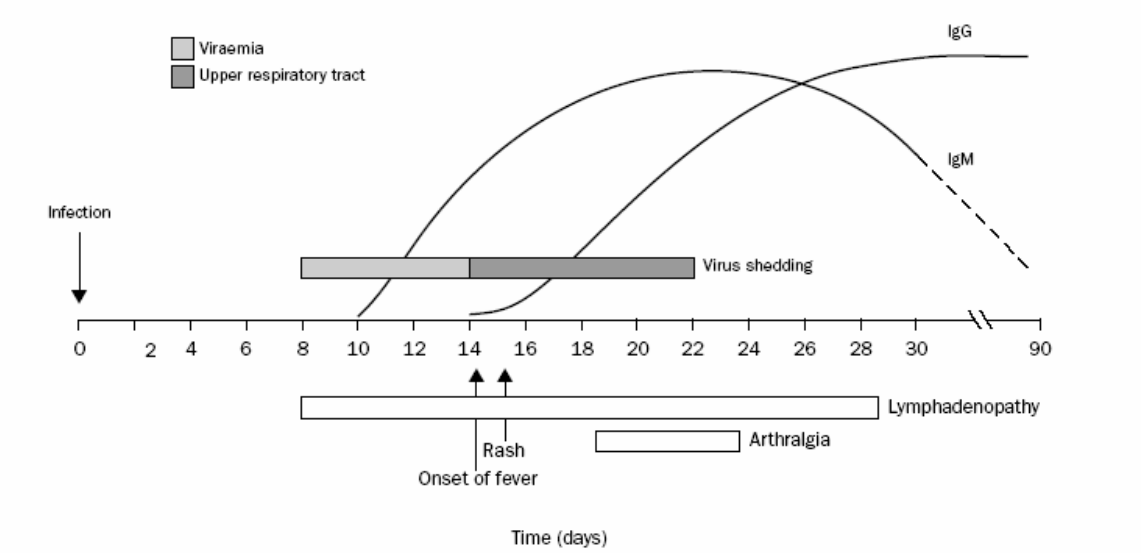

Figure 1: **Timing of key clinical, virological, and immunological features in acquired rubella infection**

FIGURE 2.

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 (jaar)	Mannen		Vrouwen	
	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

Slechts 4/58 aangegeven gevallen (7%) zijn door het laboratorium bevestigd op basis van een positieve serologie van specifieke IgM antilichamen. De klinische diagnose van exantheemziektes is niet zo specifiek en dus zijn heel wat meldingsfouten mogelijk.

FIGURE 3.

Rubella 2002-2005

V

			63019	63020	63021
			rubella igm	rubella igg (vrouw)	rubella igm gezuiverd
Rpl. Gynaeco-Verlosk	499		2333	3240	3
Extra muros staal	20		401	233	156
Fertiliteitscentrum	495		176	230	
Labo fertiliteit	497		131	149	
Bevallingskwartier	496		72	86	1
Kinderzkh E341	341		47	82	
Spoedgevallen	595		57	67	
Rpl. Interne geneesk	409		41	67	
Int.Neonatale zorgen	321		61	46	
VE. Matern. z.wiegen	430		42	52	
Rpl. Kindergen.	302		40	51	
Kinderzkh E343	343		24	28	
Dagzkh. Kinderen	305		17	24	
Neonatale zorg N*	342		16	13	
Rpl. Oogziekten	92		12	14	
Rpl. Consultatie B	612		10	15	
DAGZKH. GYNAECOLOGIE	495		11	12	
Rpl. UMC Lubbeek	690		8	15	
DAGZKH. KINDEREN	305		9	13	

FIGURE 4.

Table 6: Selected rubella IgM assays based on the IgM-capture method (adapted from *Hudson & Morgan-Capner 1996*)

Product name	Country of manufacture	Format	Duration (hours)[1]
Centocor Rubella M	United Kingdom	8 well strips	2.50
Eurogenetics Rubella IgM ELISA	Belgium	individual wells	2.50
HUMAN Rubella-Virus Direct IgM ELISA	Germany	8 well strips	1.25
Kodak Amerlite Rubella IgM Assay	United Kingdom	individual wells	2.10
Organon Rubenostika IgM	Holland	12 well strips	2.50
Sigma Rubella IgM (Capture)	United States	8 well strips	2.50
Sorin ETI-Rubek M Reverse	Italy	8 well strips	2.50

[1] Incubation times only

FIGURE 5.

Table 1
Relative sensitivity and specificity of rubella virus IgM antibody tests (95% confidence intervals)

	Sensitivity				Specificity	
	Number of positive/total tested[a]	Overall (%)[a]	Acute sera (%)[b]	Convalescent sera (%)[c]	Number of negative/total tested	Overall (%)
Meddens	248/323	76.8 (72.2, 81.4)	54.9	97.4	461/491	93.9 (91.8, 96.0)
Denka Seiken	235/312	75.3 (70.5, 80.1)	51.8	97.3	468/487	96.1 (94.4, 97.8)
Behring	246/324	75.9 (71.3, 80.6)	51.4	98.7	473/499	94.8 (92.8, 96.7)
Wampole	240/324	74.1 (69.3, 78.8)	52.1	96.1	469/497	94.4 (92.3, 96.4)
Captia	174/262	66.4 (60.7, 72.1)	40.0	94.3	385/396	97.2 (95.6, 98.8)
Sigma	183/262	69.8 (64.3, 75.4)	40.9	97.5	340/397	85.6 (82.2, 89.1)
Axsym	138/175	78.9 (72.8, 84.9)	57.5	98.8	135/156	86.5 (81.2, 91.9)

[a] Column 2 data used to calculate the overall % sensitivity shown in Column 3 (mean 11 days post-rash onset).
[b] Sensitivity was calculated only using acute samples collected on or prior to 10 days post-rash onset (mean 2 days post-rash onset).
[c] Sensitivity was calculated only using convalescent samples collected greater than 10 days post-rash onset (mean 19 days post-rash onset).

FIGURE 6.

Table 2
Assays evaluated

Product name (country of manufacture)	IgM capture (Mcap) or antiglobulin solid-phase antigen assay (antiglob)	Format[a]	Duration of test (incubation times only) (h)
Abbott Rubazyme-M (USA)	Antiglob	Beads	4.5
Behring Enzygnost Anti-Rubella-Virus/IgM (Germany)	Antiglob	16 well strips[b]	2.75
BioWhittaker Rubestat-M (USA)	Antiglob	8 well strips[b]	2.0
Centocor Rubella M (UK)	Mcap	8 well strips	2.5
Diamedix Rubella IgM Microassay (USA)	Antiglob	Individual wells	1.5
Eurogenetics Rubella IgM ELISA (Belgium)	Mcap	Individual wells	2.5
Gull Rubella IgM ELISA (USA)	Antiglob	8 well strips	1.5
HUMAN Rubella-Virus direct IgM ELISA (Germany)	Mcap	8 well strips	1.25
Kodak Amerlite Rubella IgM Assay (UK)	Mcap	Individual wells	2.1
Organon Rubenostika IgM (Holland)	Mcap	12 well strips	2.5
Platest Rubella IgM (Spain)	Antiglob	8 well strips[b]	1.2
Sigma Rubella IgM (USA)	Antiglob	4 well strips	1.5
Sigma Rubella IgM (Capture) (USA)	Mcap	8 well strips	2.5
Sorin ETI-Rubek M Reverse (Italy)	Mcap	8 well strips	2.5
Zeus Rubella IgM ELISA (USA)	Antiglob	8 well strips	1.2

[a] Individual wells = strips with break-off wells.
[b] For antiglobulin assays, includes control antigen coated wells.

FIGURE 7.

Table 3
Summary of sensitivity and specificity

	Sensitivity[a] (%)			Specificity[a] (%)		
	A	B	C	A	B	C
Abbott	75	75	86	92	97	97
Behring	79	79	93	90	96	96
BioWhittaker	67	67	77	87	88	88
Centocor	89	89	98	90	96	96
Diamedix	67	67	82	94	96	96
Eurogenetics	64	64	76	94	99	99
Gull	75	75	89	92	98	98
Human	85	85	93	93	98	98
Kodak	90	90	98	93	99	99
Organon	75	75	88	98	100	100
Platest	92	92	95	82	88	88
Sigma (antiglob)	63	63	79	95	96	96
Sigma (Mcap)	88	88	95	93	95	95
Sorin	78	78	91	96	100	100
Zeus	74	74	88	91	96	96
MACRIA	100	100	100	84	90	90

[a] A, all categories of sera; B, excluding category of sera giving non-specific reactivity by MACRIA; C, as B except re-infection category also excluded.

FIGURE 8.

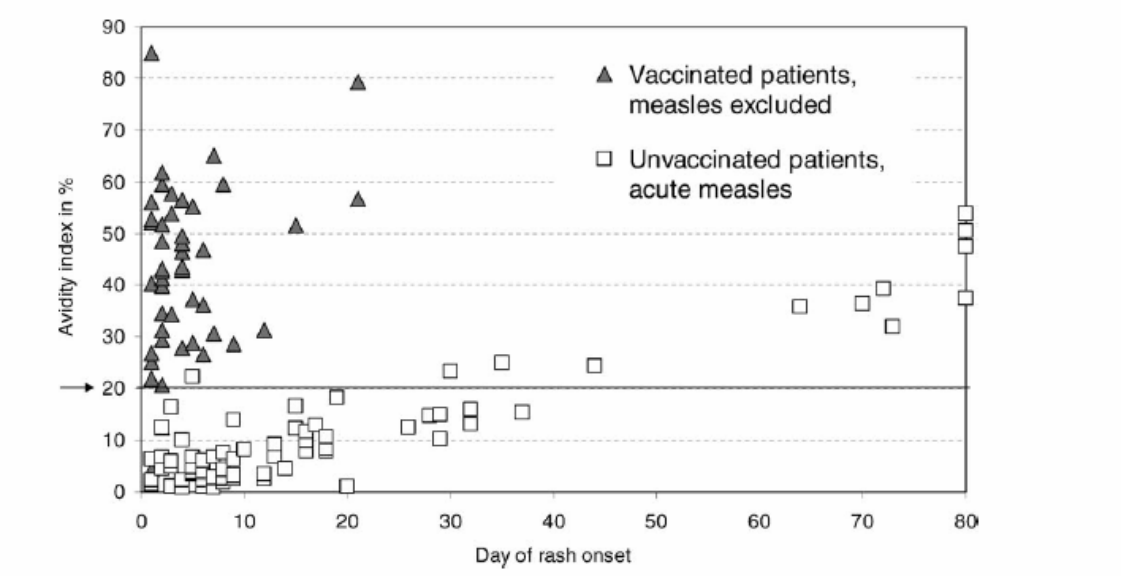

FIGURE 9.

				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