



Rijksinstituut voor Volksgezondheid
en Milieu
*Ministerie van Volksgezondheid,
Welzijn en Sport*



B(of)
M(azelen)
R(ubella)

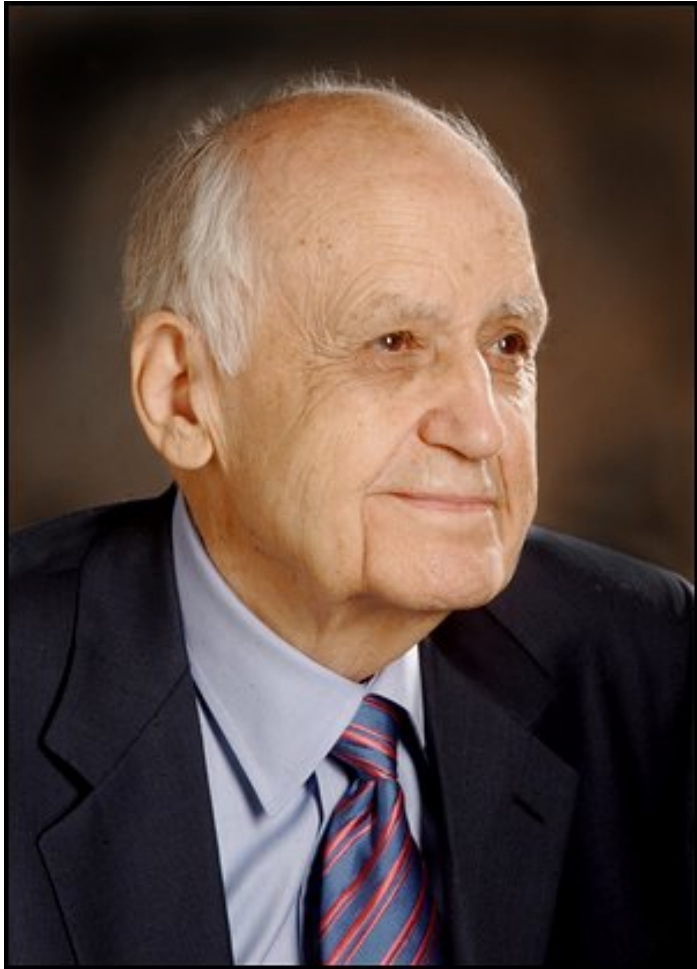
IgG & immuniteit

Rob van Binnendijk
SKML, 9 juni 2015



The New York Times
may 6, 2013

**Jeryl Lynn Hilleman with her sister, Kirsten, in 1966,
as a doctor gave her the mumps vaccine developed by their father**



Maurice R. Hilleman
The man behind childhood immunizations as we know it today

Measles
Mumps
Chickenpox
Rubella
hepatitis A/ B
Haemophilus Influenzae

Nature Medicine **11**, S2 (2005)
doi: 10.1038/nm1223

"Maurice should be recognized as the most successful vaccinologist in history"

"His vaccines save nearly 8 million lives a year"

Robert Gallo

Impact van mazelen vaccinatie. Veel meer dan alleen mazelen !!



Mina et al. 2015. Science vol.348, pp694-699

Measles is known to suppress the immune system for a few weeks after an infection. After an infection, a child's immune system has to almost start over, rebuilding its immune protection against diseases it has already seen before.

It erases immune protection to other diseases.

The new study provides "compelling evidence" that measles affects the immune system for two to three years. That's much longer than previously thought.



BMR vaccinatie in Nederland

- M+R vaccinatie in het RVP: 1976
- *R voor meisjes; 1970-1977*
- B toegevoegd aan het RVP in 1987 > BMR vaccin
- *BMR 'catch-up' voor kleuters; 1983-1987*

Huidige vaccinatie schema:

- BMR-1 op 14 mnd
- BMR-2 op 9 jarige leeftijd

Vaccinatie dekking:

- 1ste BMR: 96 %
- 2de BMR: 93 %, gemiddelde coverage ~95%

Bof, mazelen en rubella in Nederland

Mazelen komt nog geregeld voor in Nederland

- hoge infectiedruk vanuit Europa, meeste cases gelinkt aan import
- opbouw van “vatbaren”
 - antroposofen (2008); religieus (epidemie 2013/2014)
 - andere niet-gevaccineerden
 - Gevaccineerde volwassenen > minder bescherming (?); gemiste vaccinaties

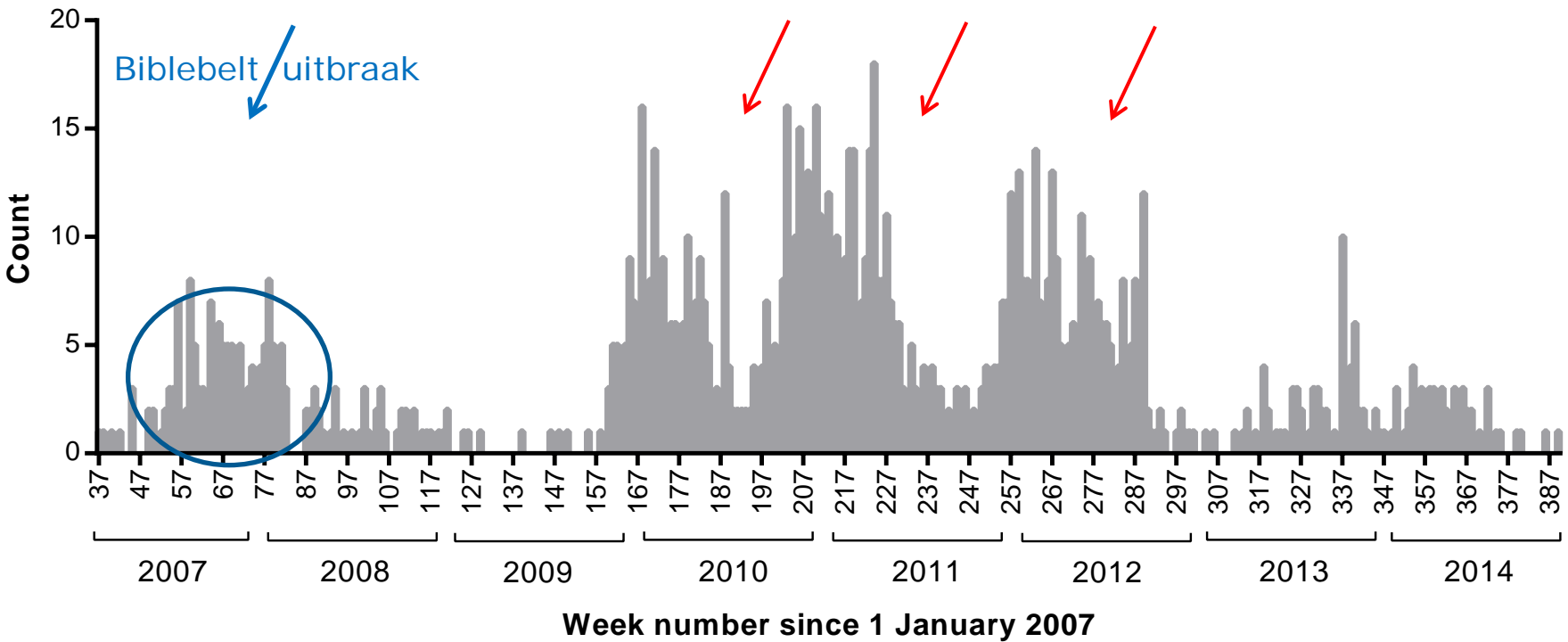
Rubella komt bijna niet meer voor in Nederland

- religieus (epidemie 2004/2005)
- rubellavirus minder besmettelijk; bronnen moeilijk te achterhalen (CRS/uveïtis)
- risico's tijdens zwangerschap (CRI/CRS) > voldoende gevaccineerd ?/ beschermd?
-> onduidelijke vaccinatie status niet-autochtone populatie

Bof zal blijven terugkomen in Nederland

- religieus (epidemie 2007/2008)
- Gevaccineerde volwassenen > falende immuniteit
-> personen > 17 jaar, na verlaten van middelbare school (o.a. studenten)

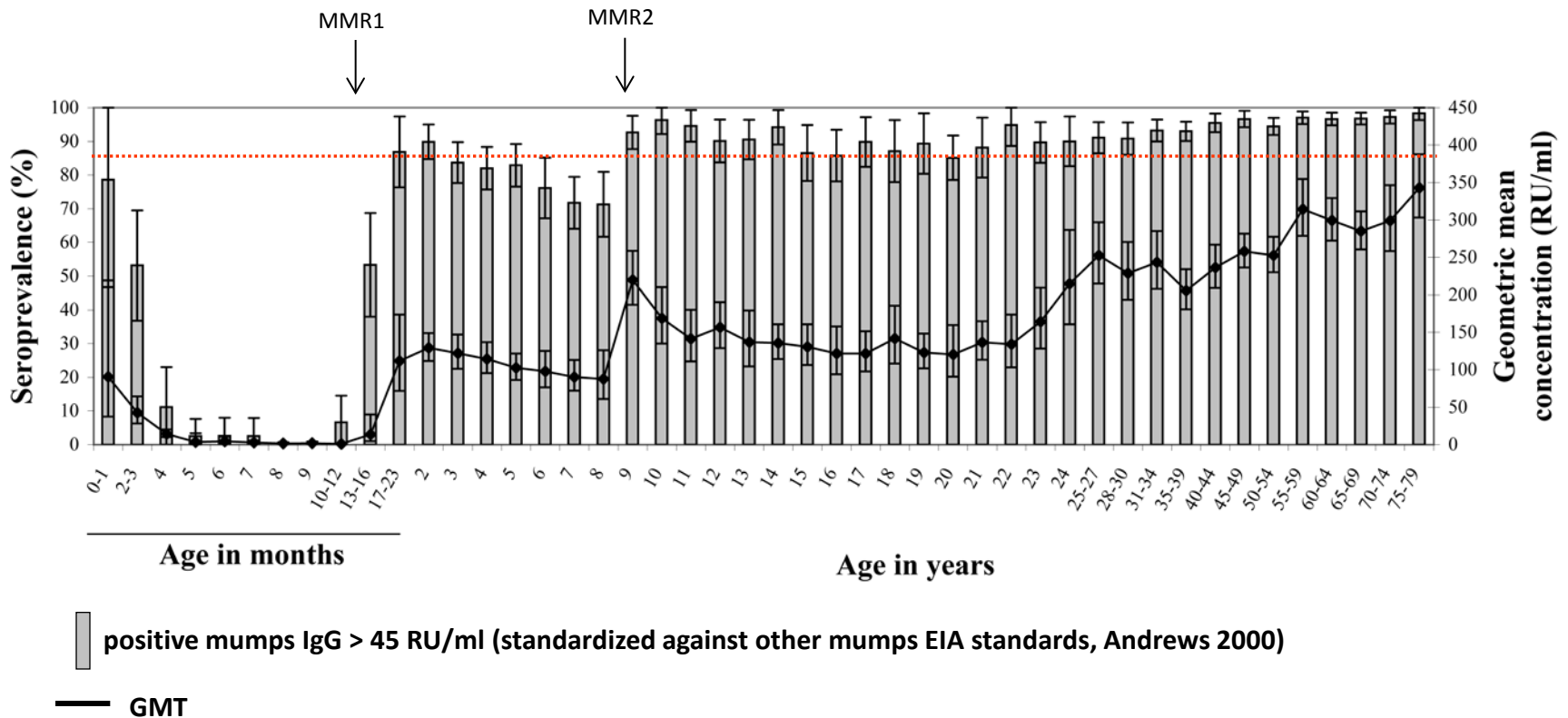
Mumps patients tested positive at the RIVM





- Kan de dominantie van bof worden verklaard door epidemiologische factoren?
- Hebben bof wildtypes een virologisch voordeel in pathogenese en in transmissie ?
- Hebben bof wildtypes een immunologisch voordeel ?
- **Is er sprake van falende vaccinatie-verkregen immuniteit ?**

Bof immuunstatus onderzoek (Pienter 2, 2006)



Smits et al., 2013



4 15

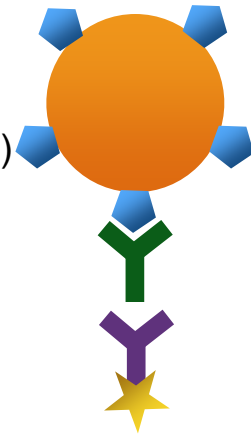
arbitrary units (). Titres were used in the regression analysis) of the EIAs used in the reference laboratory and in the participating countries. Comparison of the local non-standardized (Nstd) cut-off with the reference laboratory cut-off expressed in the local units (Std)*

		Reference laboratory: Germany (Behring)		
		Negative < 230# < 8*	Equivocal 230–500# 8–14*	Positive > 500# > 14*
Australia	Nstd#	< 230	230–485	> 485
(Behring)	Std#	< 294	294–710	> 710
Denmark	Nstd#	< 250	250–450	> 450
(Behring)	Std#	< 251	251–500	> 500
Finland	Nstd*	< 10	10–15	> 15
(in-house)	Std*	< 13	13–18	> 18
France	Nstd#	< 230	230–500	> 500
(Behring)	Std#	< 192	192–377	> 377
Italy	Nstd*	< 10	–	> 10
(Behring)	Std*	< 15	15–23	> 23
The Netherlands	Nstd#	< 45	45–60	> 60
(in-house)	Std#	< 50	50–72	> 72
UK	Nstd*	< 8	8–14	> 14
(biostat)	Std*	< 15	15–28	> 28

Luminex assay: measuring IgG concentrations



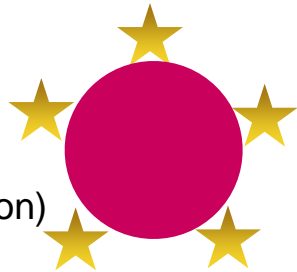
bead
+
mumps ag (Jeryl Lynn)



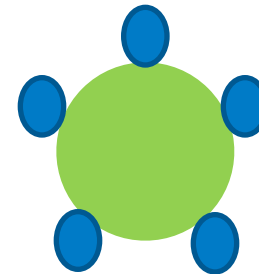
IgG in sample

detection antibody

bead
+
measles ag (Edmonston)



bead
+
rubella ag (HPV-77)



Identification of mumps virus infections

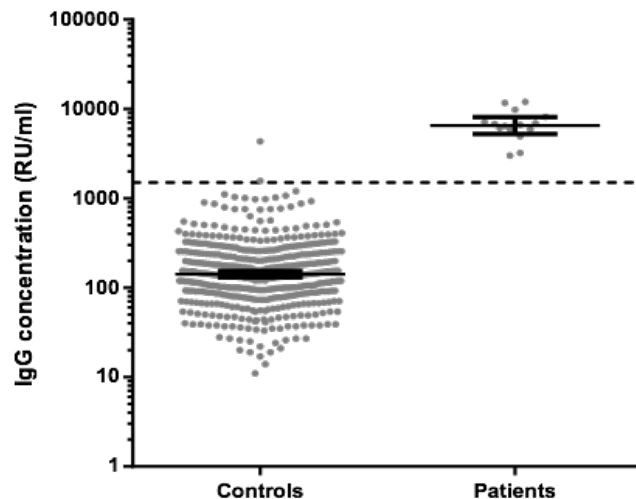


1
fourfold or more
increase in IgG

→



2
IgG ≥ 1500 RU/ml



Attack rates (on the basis of IgG seroconversion)

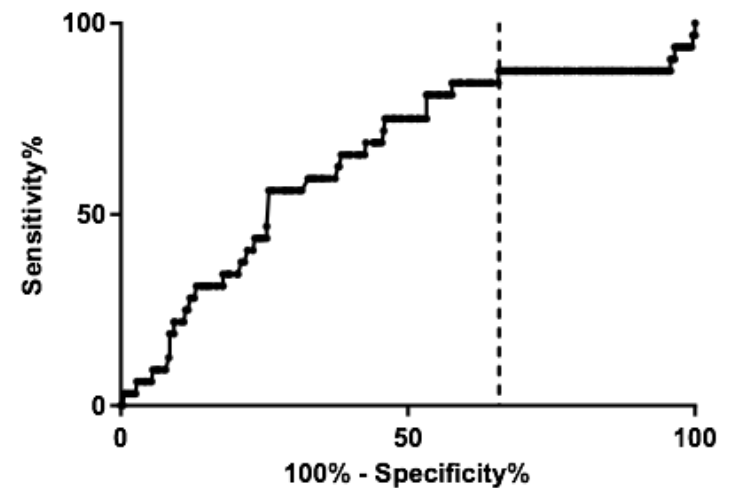
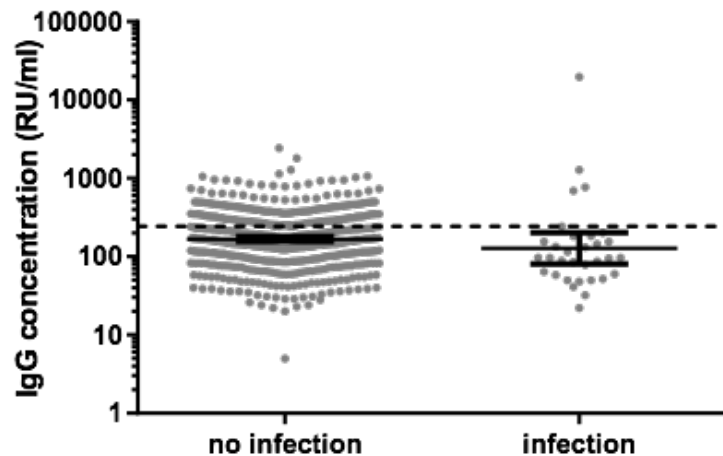
- About 1/3 of the mumps infections is symptomatic
- Serology is an appropriate tool to identify mumps infections

Cohort	Attack rates in vaccinated students (2x MMR; n=533)	
	<i>All infections</i>	<i>Symptomatic infections</i>
2007	4 / 98 (4.1%)	2 (2.0%)
2008	6 / 90 (6.7%)	5 (5.6%)
2009	9 / 171 (5.3%)	1 (0.6%)
2010	13 / 174 (7.5%)	3 (1.7%)
Total	32 (6.0%)	11 (2.1%)

Correlate of protection?

Cutoff at 243 RU/ml:

- Sensitivity 87.5% (95% CI 71.0-96.5%)
- Specificity 34.1% (95% CI 30.1-38.3%)



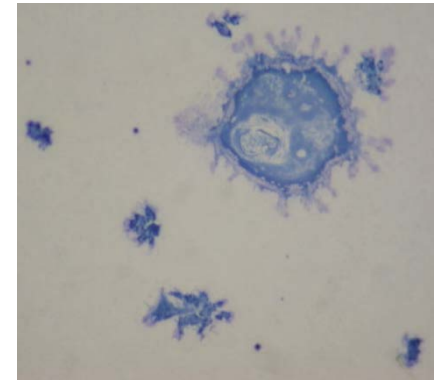
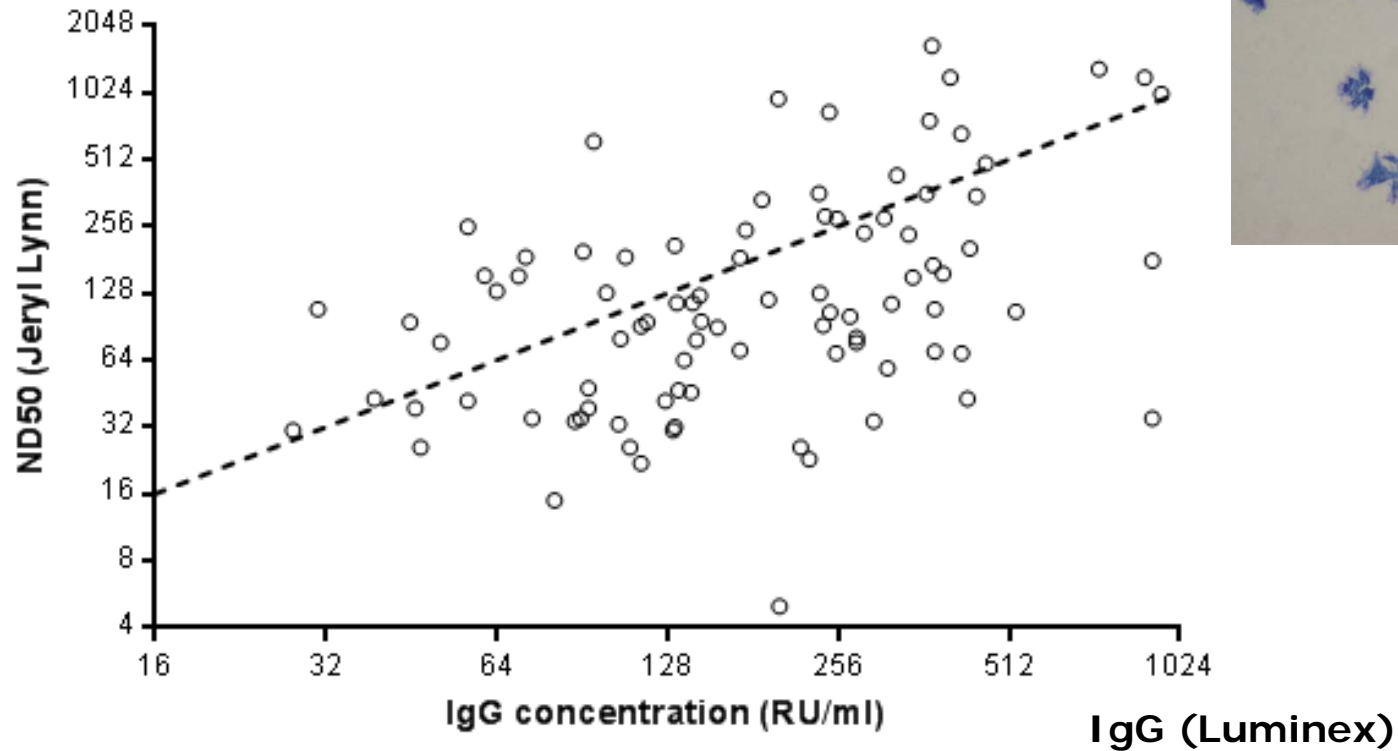
Gouma 2014

Detection of neutralizing antibodies

focal reduction neutralization test



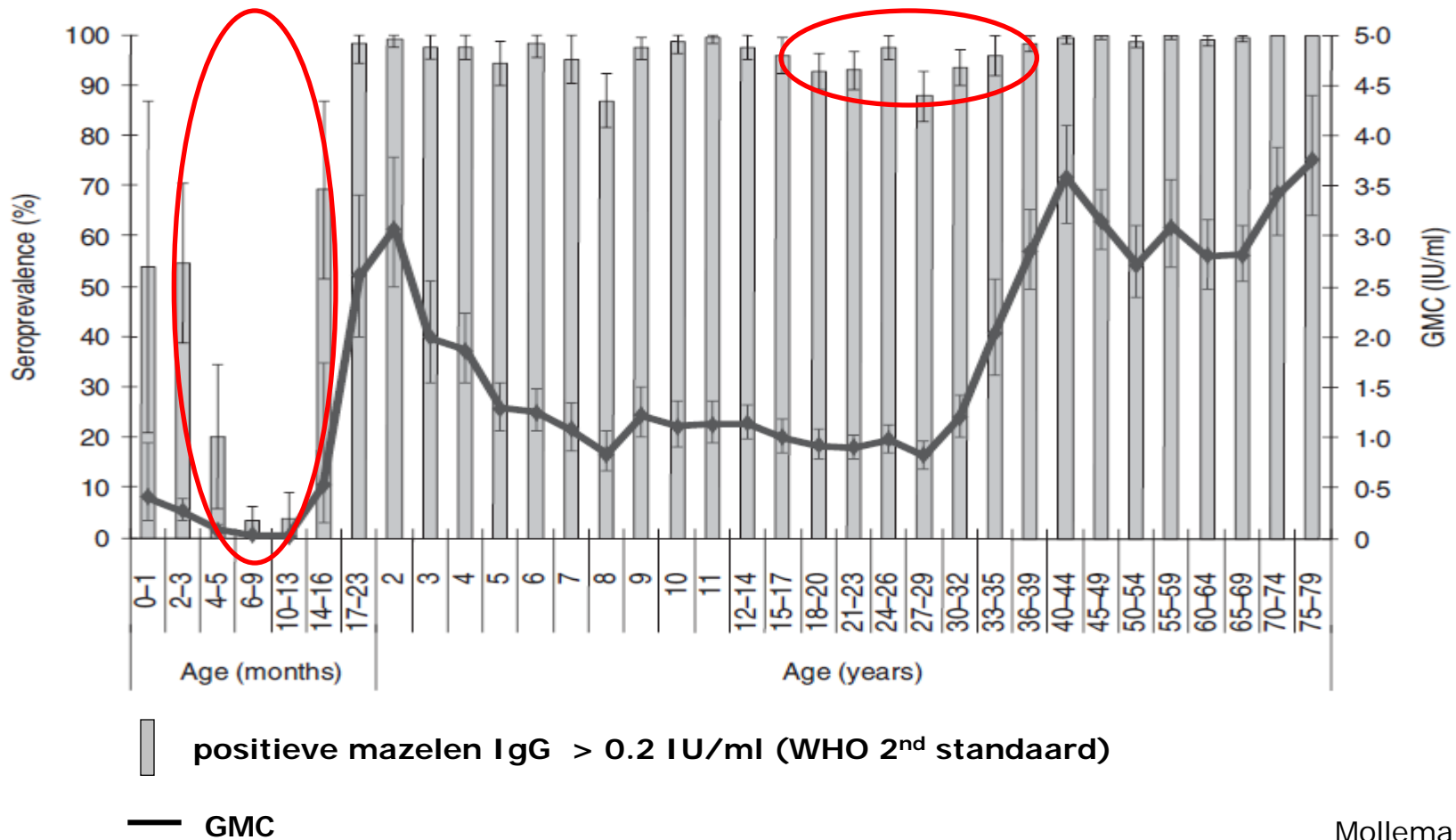
Neutralization



conclusies (bof)

- Bof serum IgG
 - adequate tool om bof infectie (en attack rates) aan te tonen onder gevaccineerden
 - slechte indicator voor bof immuniteit
- Bofvirus 'exposure' complex in studie populatie (onderschatting specificiteit)
- Bof immuniteit bepaald door andere immuunfactoren

Mazelen immuunstatus onderzoek > parallelen met bof (Pienter 2, 2006)

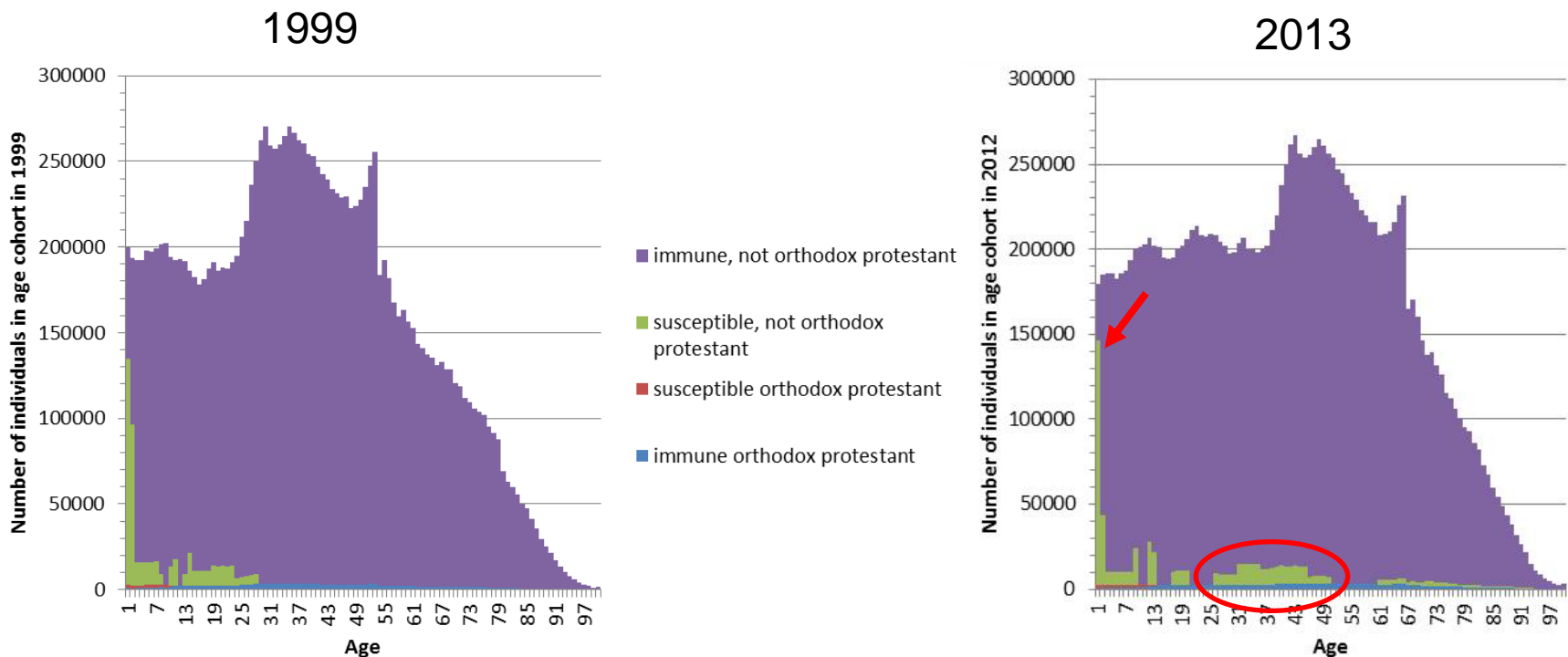


Mollema 2014

Evaluatie mazelen infectie risico bij aanvang epidemie 2013

Risico-analyse op basis van gestratificeerde (IgG) antistoffen in serum (Pienter data)

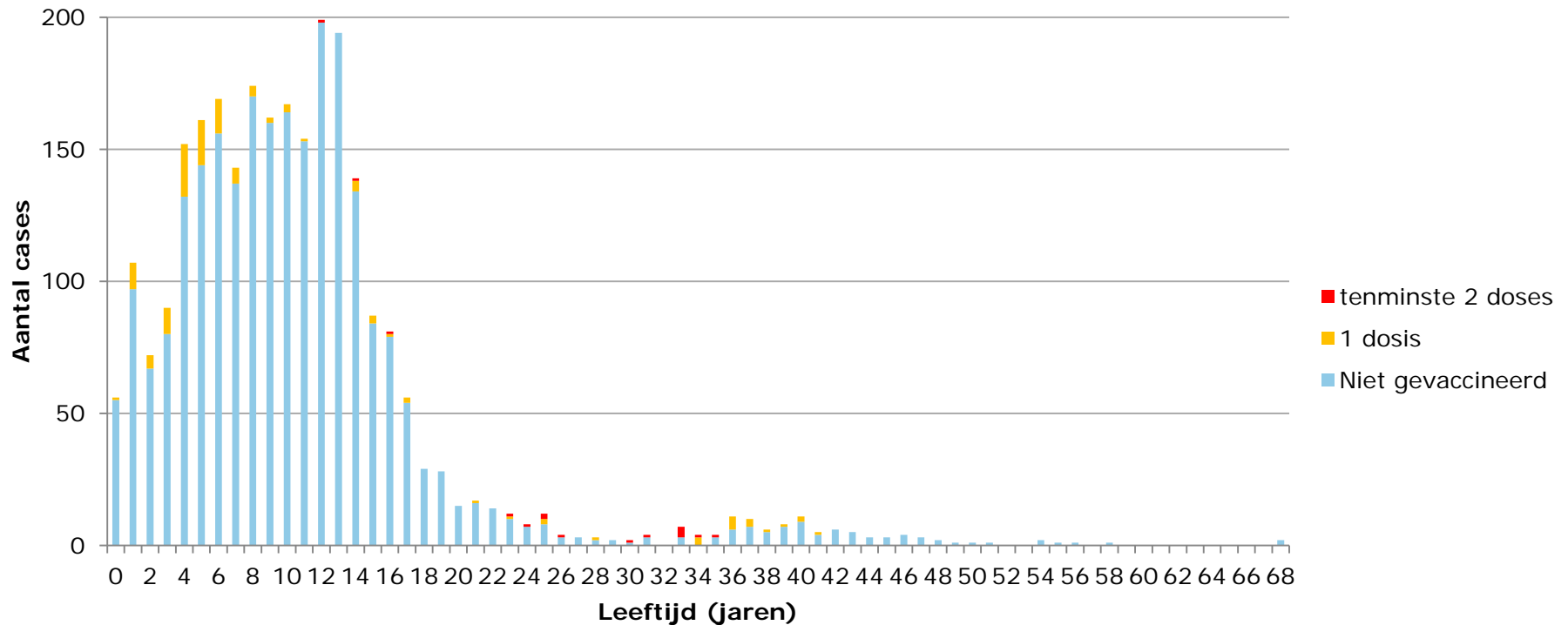
- hoger percentage seronegatieven onder volwassenen
 - * mogelijk meer mazelen in deze groep dan in 1999; zorgpersoneel
- hogere infectie druk onder kinderen < 14 maanden



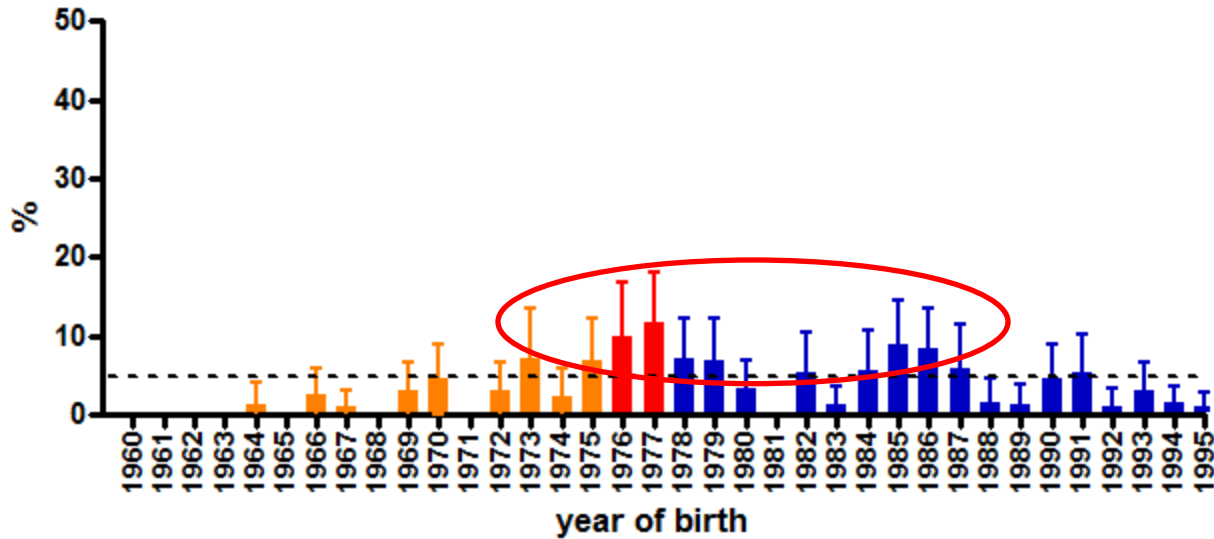
Mazelen epidemie 2013: de cijfers



Leeftijdsverdeling mazelen cases – alle leeftijden

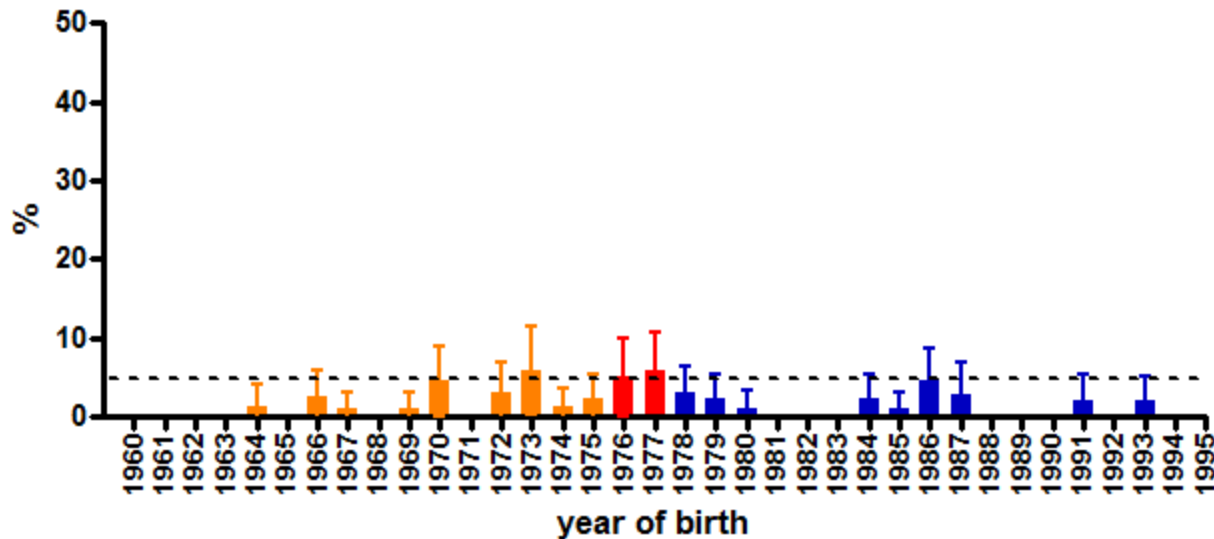


IgG concentrations < 200 mIU/ml
(Pienter 2006, selection 1960-1995, n= 3093)

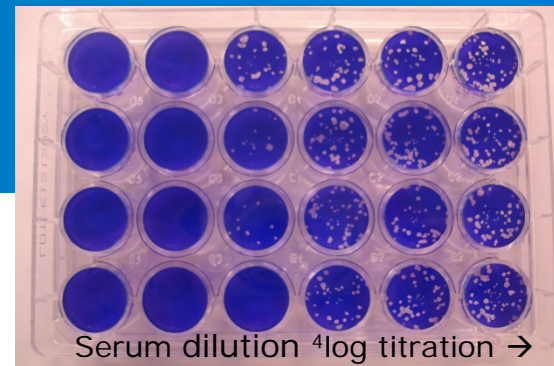


Mollema 2014

IgG concentrations < 120 mIU/ml
(Pienter 2006, selection 1960-1995, n= 3093)



Plaque-reduction neutralization (PRN)



- Antibody concentration resulting in 50% reduction of virus titer/plaques
- Currently the best standard for measuring humoral protection in measles
- Antibody levels > 120 mIU/ml is the estimated threshold for immune protection
- However: Few data from clinical surveys supporting the protective threshold; extrapolation from older studies (Chen et al.1990, 8 clinical cases only).
- PRN is labor intensive, when compared to enzyme immunoassay (EIA)
 - Test is prone to biological variation, log-2 value range
 - Good SOP (NIBSC/FDA/HPE 2005; Cohen et al. 2007/2008)
 - Use of WHO (2nd/3rd) serum-standard to express PRN in international units



Many (clinical) laboratories use commercial EIA (IgG) testing in measles

- Less labor intensive, well-standardized
- Last EIA evaluation: Ratnam et al. 1995

Applicability:

- disease confirmation (response to infection, titer rise): yes
- seropositivity acquired through infection: yes
- seropositivity acquired through vaccination: depends/questionable
- correlate of immune protection: questionable/no

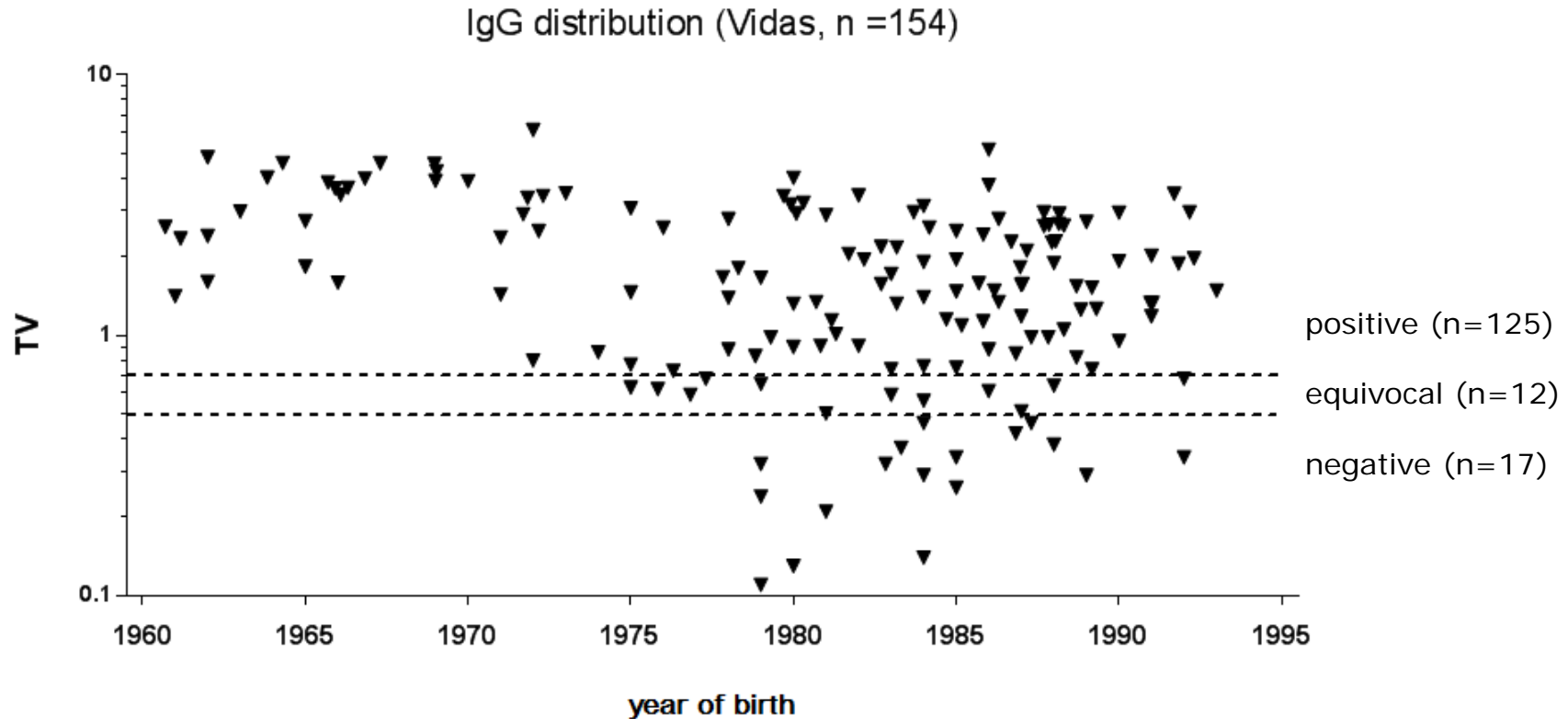


- Kind met mazelen opgenomen in ziekenhuis
 - 2 volledig gevaccineerde medewerkers kregen mazelen
- Vragen van/aan de "expert":
 - Hoe goed is de mazelen immuniteit na 2 (volledige) mazelen vaccinaties
 - Hoe betrouwbaar is "ik heb de mazelen gehad" of de opgegeven vaccinatie?
 - Zorgmedewerkers screenen op antistoffen? Vaccineren van seronegatieven?

Praktijk:

- Ziekenhuis geconfronteerd met hoog % "IgG negatieven"
- LUMC/ RIVM geconsulteerd bij interpretatie van serologische uitkomsten

Ziekenhuis setting 1 (2013)

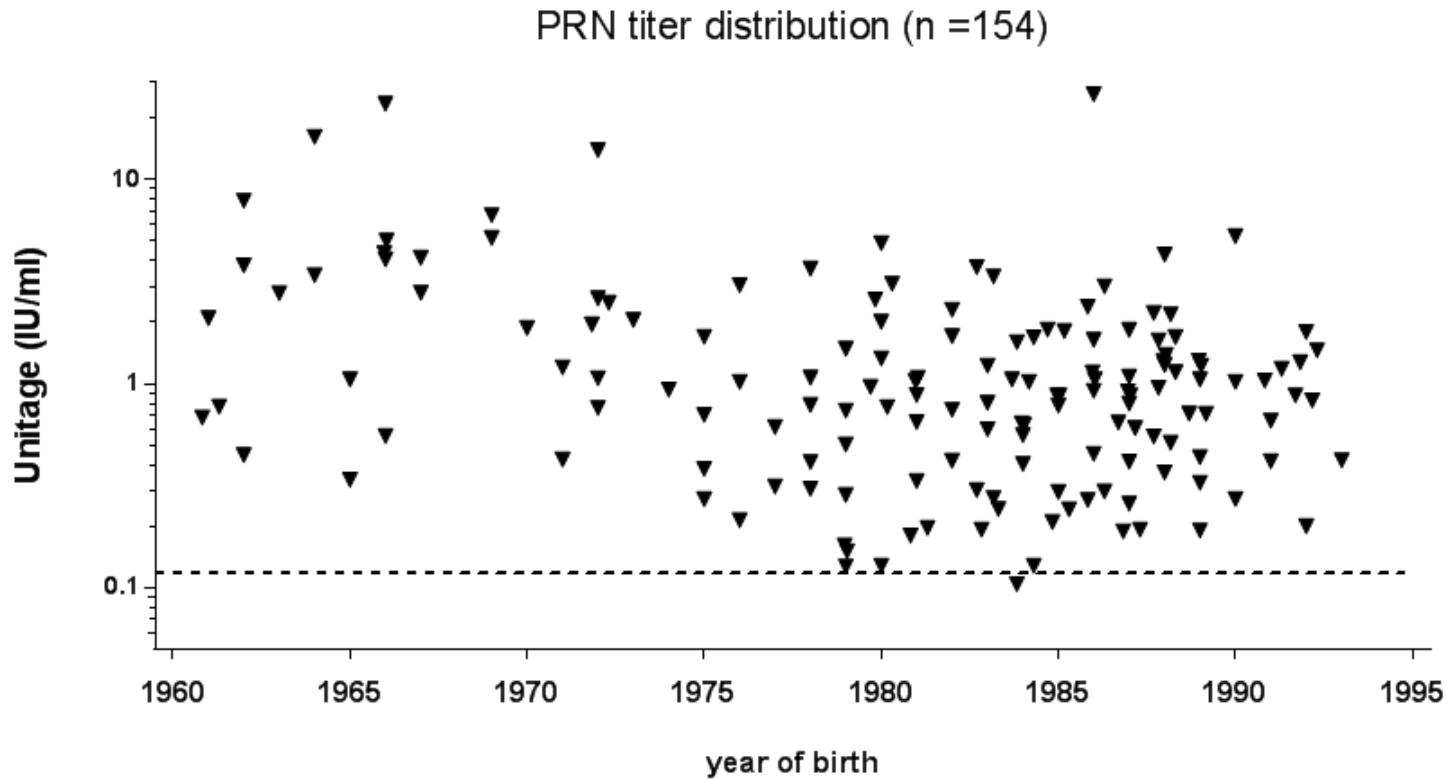


↑
M1/BMR2, catch-up

↑
BMR1/BMR2

Dorigo et al. in press

Ziekenhuis setting 1 (2013)



↑
M1/BMR2, catch-up

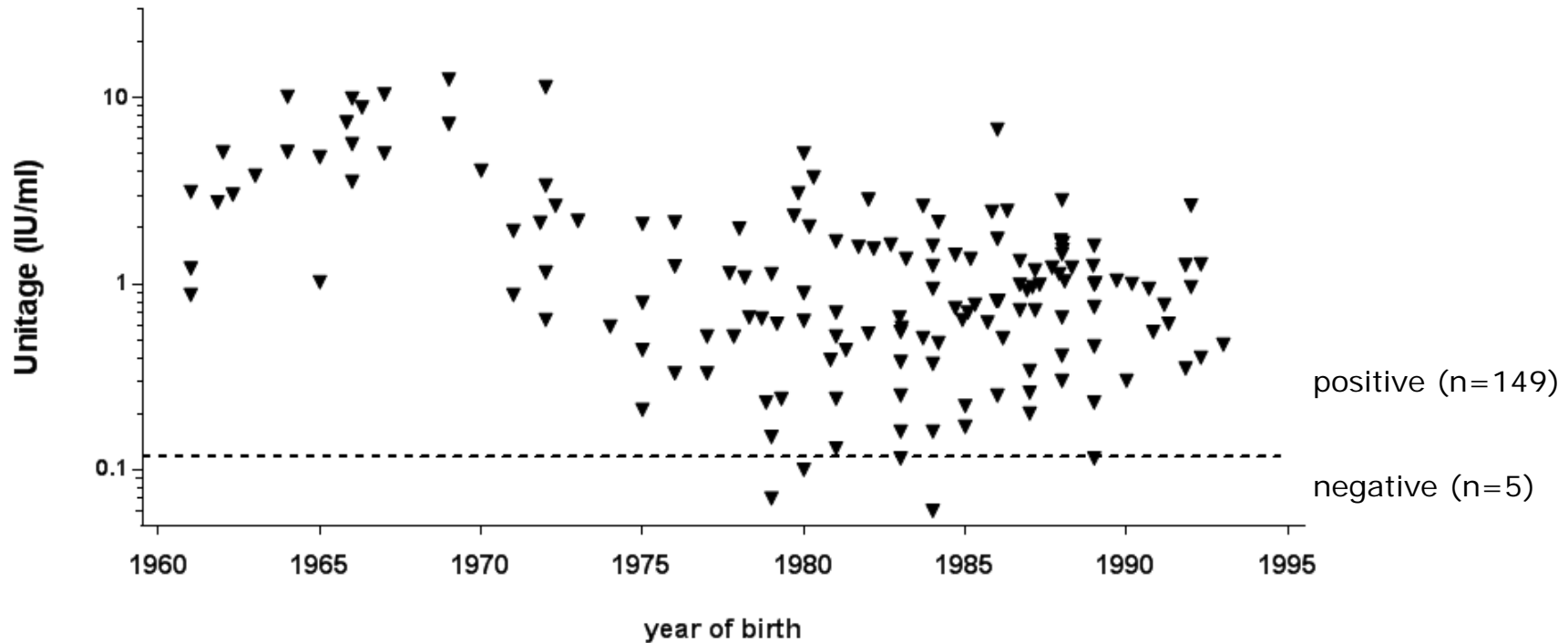
↑
BMR1/BMR2

Dorigo et al. in press

Ziekenhuis setting 1 (2013)



IgG titer distribution (MIA, n =154)



↑
M1/BMR2, catch-up

↑
BMR1/BMR2

Dorigo et al. in press

Ziekenhuis setting 1 (2013)



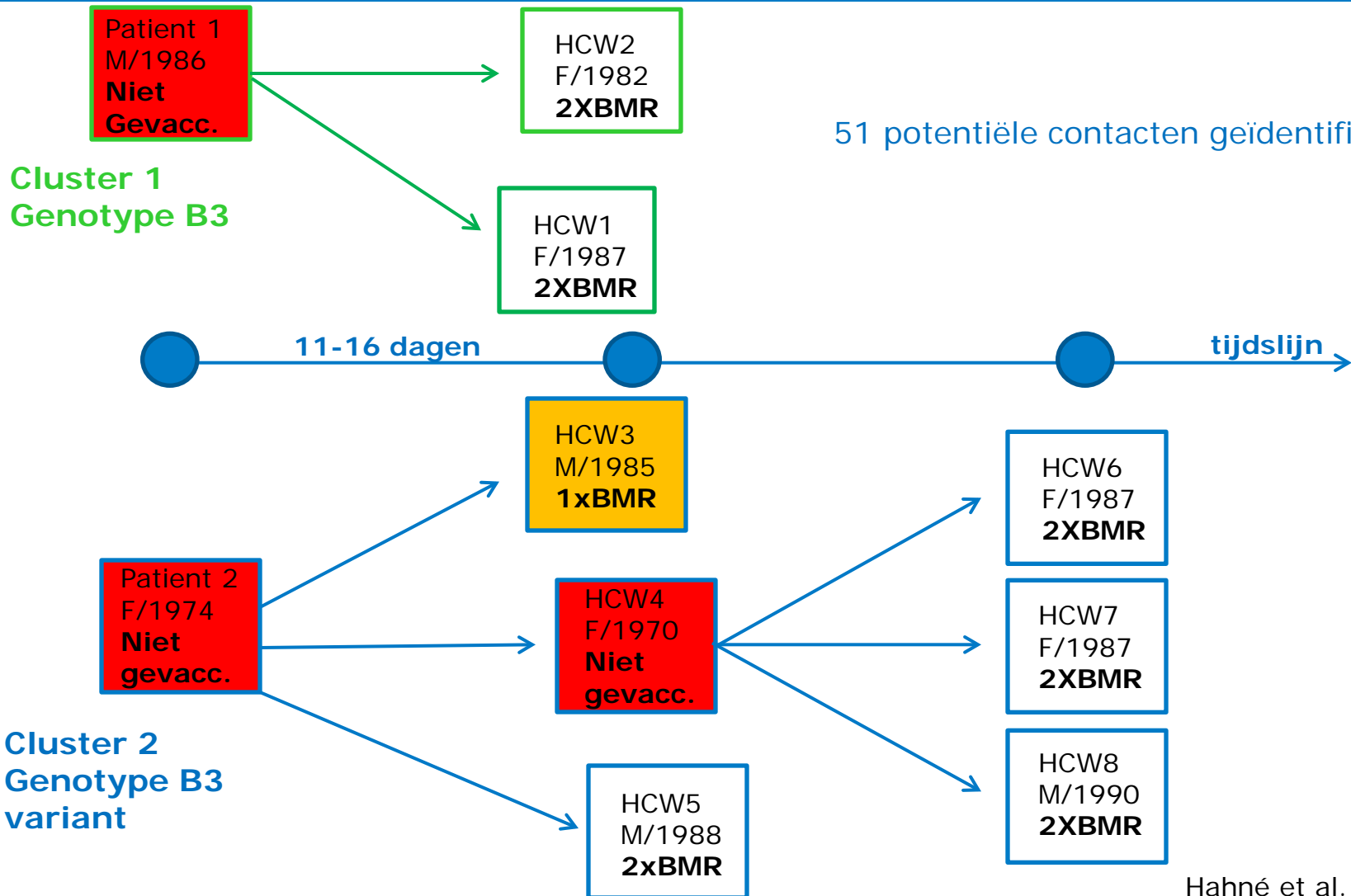
EIA brand (manufacturer)	# tested	result	PRN <120	PRN ≥120	all	% seropositive*		
						1960-1974	1975-1985	1986-1995
Enzygnost (Siemens)	154	positive	0	139	90.8	31/31 (100%)	56/67 (84%)	52/56 (93%)
		negative	1	14				
Vidas (bioMerieux)	154	positive	0	137	89.5	31/31 (100%)	55/67 (82%)	51/56 (91%)
		negative	1	16				
Liaison (Diasorin)	154	positive	0	136	88.9	31/31 (100%)	54/67 (81%)	51/56 (91%)
		negative	1	17				
MIA (Luminex, inhouse)	154	positive	0	149	97.4	31/31 (100%)	63/67 (94%)	55/56 (98%)
		negative	1	4				

Dorigo et al. in press

Ziekenhuis setting 2 (2014)



51 potentiële contacten geïdentificeerd



Hahné et al. in prep.

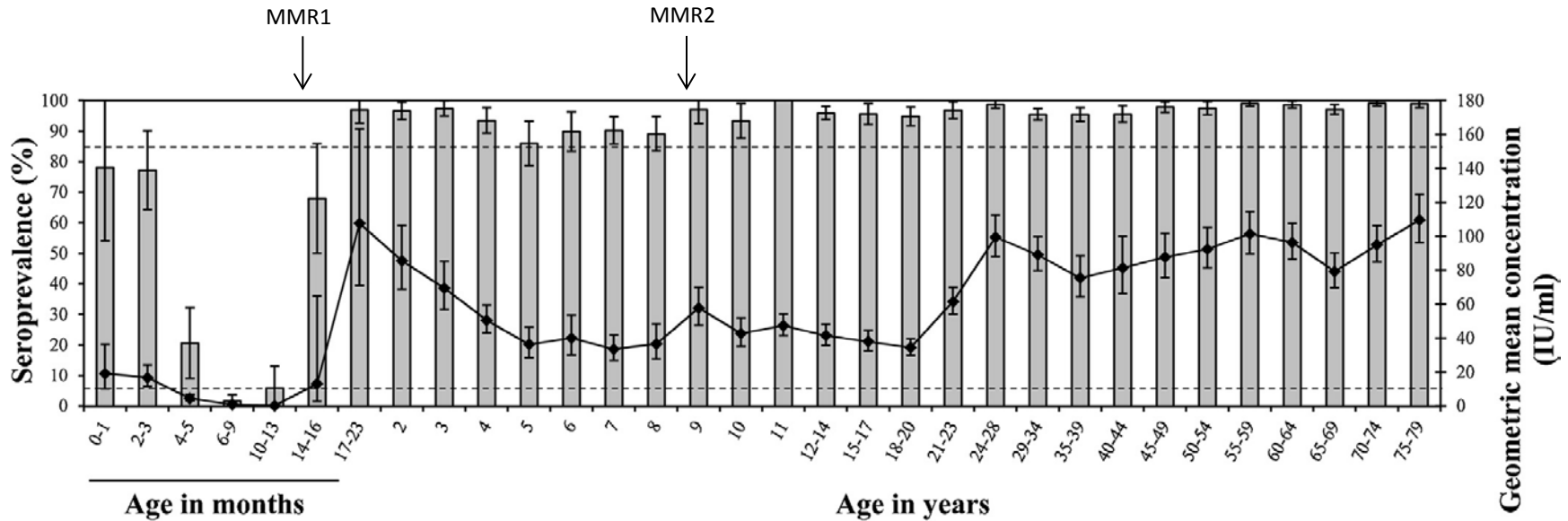


- 6 volledig gevaccineerde medewerkers met mazelen
 - Vaccin-effectiviteit (VE); relatief laag (maar ook onbetrouwbaar)
 - Transmissie > via ongevaccineerde personen
 - 3 gevaccineerde personen besmet door 1 ongevaccineerd persoon
 - > veel contacten, hoge virale load (PCR)
- (serologisch) profiel van de vaccinfalers:
 - secundair vaccinfalen: pre-mazelen IgG & PRN aantoonbaar vóór expositie
 - snelle/hoge antistofontwikkeling kort na infectie > secundaire respons
 - immunrespons impliceert snelle “clearance” van infectie
 - > milder ziektebeloop, kortere/lagere virale uitscheiding

Conclusies (mazelen)

- Meerderheid van personen uit geboortecohort 1965-1995 hebben neutraliserende (beschermende) antistoffen tegen mazelen (97-99%)
- Gebruikelijke EIA testen zijn onvoldoende sensitief (en specifiek), te weinig geschikt voor seroprevalentie, met name bij gevaccineerden (>10%)
- Goede correlatie tussen PRN en MIA IgG Luminex (populatiestudies, Pienter)
- Virus neutralisatie: belangrijke indicator voor bescherming, niet absoluut
- Niet-/onvolledig gevaccineerd zorgpersoneel vaccineren

Rubella (rodehond) immuunstatus onderzoek (Pienter 2, 2006)



positive rubella IgG > 10 IU/ml < Vraag van/aan de " expert" 10 IU/ml beschermend ?
 GMT

Smits et al., 2013

Lack of standardization of rubella IgG assays Is there any solution?

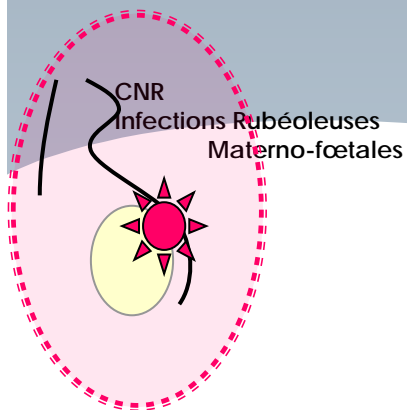
Report from Rubella IgG standardization working group

Liliane GRANGEOT-KEROS, Christelle VAULOUP-FELLOUS

National Reference Laboratory for Rubella

Virology department, Groupe Hospitalier Paris-Sud

Faculty of medicine Paris-Sud 11 University, France



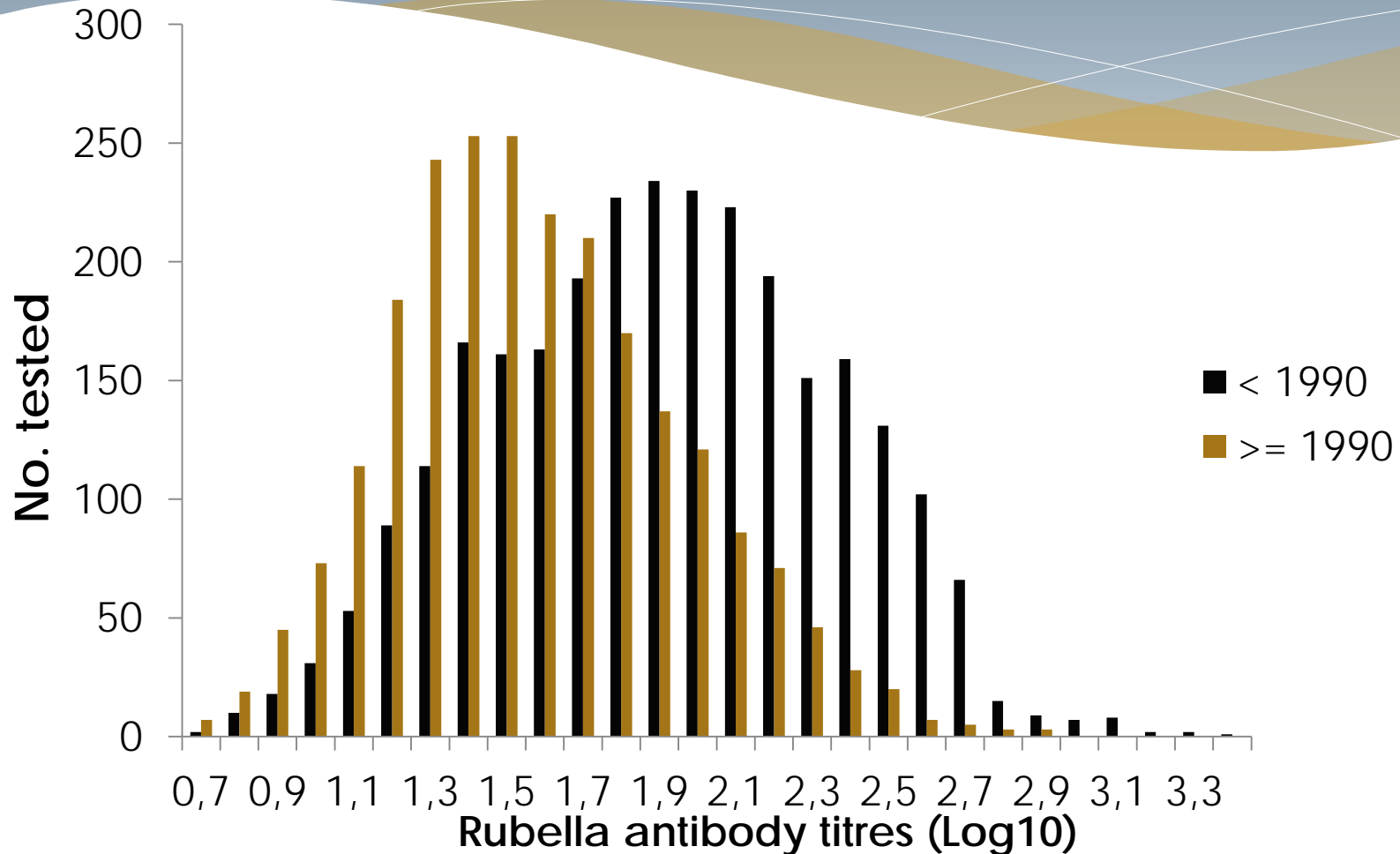
EQA: results

Assays	Cut-off IU/mL	IgG (mean) IU/mL		
		EQ1	EQ2	EQ3
Abbott Architect	10	46.9	44.8	21.6
Abbott AxSYM	10	76.7	75.7	44.5
Beckman Access/DXI	15	115	63.2	56.7
DiaSorin Liaison	11	117	31.2	62.3
Siemens Immulite	10	130	54.4	56
Biomérieux Vidas	15	131.5	93.9	57.9
Ortho Vitros	10	326	49.3	143.3
Roche: Cobas 6000/Elecsys/Modular	10	383	255.8	89.9
Siemens Advia Centaur	10	> 500	110.8	180.7

Antenatal screening for rubella in England (2005–2011)

Year	No. Tested	% Negative
2005	518,302	2.59
2006	598,243	2.89
2007	647,820	3.06
2008	659,256	3.29
2009	683,992	4.28
2010	696,137	4.46
2011	675,204	5.35

RV-IgG level distribution according to the year of birth in France (2009–2010)



RV-IgG evaluation 2013

Rubella Standardization Committee

- * **325 samples pretested-negative RV-IgG** (from France, Italy and Germany) were tested with 8 ELISA assays, 1 Immuno-blot, and 1 Neutralization assay ¹
- * E1 alone is sufficient enough to elicit neutralizing anti-rubella virus antibodies ²



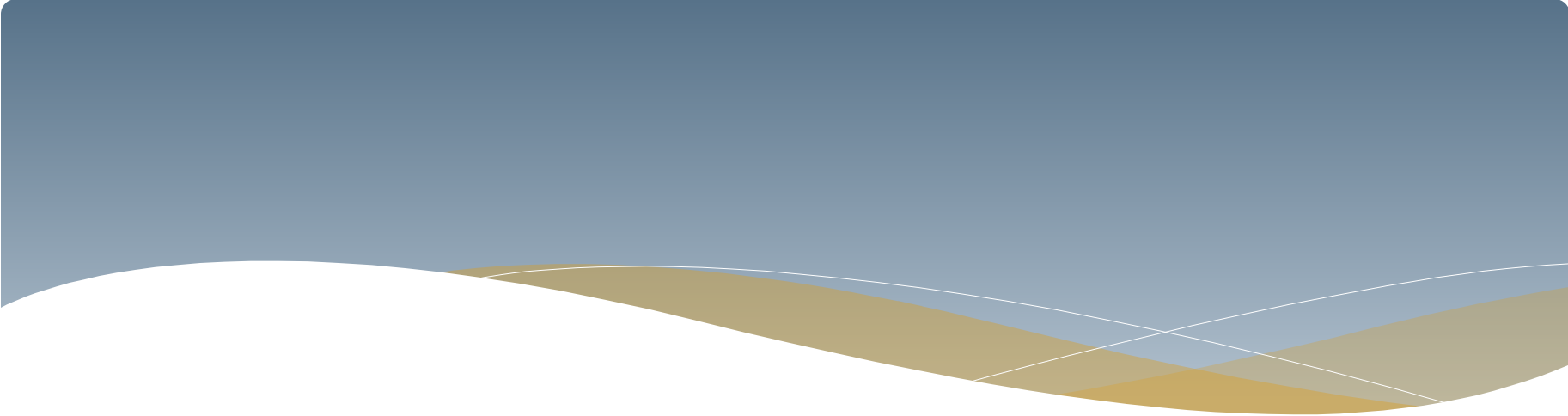
1. Kindly performed by Joe Icenogle group, CDC, Atlanta, USA

2. Perrenoud G, et al. *Vaccine* 2004;23:480-8

Rubella negatief geteste IgG serum samples (Frankrijk, Italië, Duitsland); correlatie met functionele serologie (Immunoblot, virus neutralisatie)

	Immuno- blot	Netrali- zation assay	Dxi Beckman -Coulter	Architect Abbott	Vidas bio Mérieux	Enzygnost Siemens	LXL DiaSorin	Cobas Roche	Centaur Siemens	Serion
Negative	134/325 41%	94/322 29%	196/325 60%	207/325 64%	202/325 62%	152/325 47%	209/325 64%	135/325 42%	158/325 48%	215/325 66%
Equiv	-		113/325 35%	107/325 33%	58/325 18%	49/325 15%	84/325 26%	-	51/325 16%	88/325 27%
Positive	191/325 59%	228/322 71%	16/325 5%	11/325 3%	65/325 20%	124/325 38%	32/325 10%	190/325 58%	116/325 36%	22/325 7%

59% women considered susceptible have specific anti-E1 Ab
71% women considered susceptible have neutralizing Ab

- 
- * 52 % women considered susceptible with an ELISA assay have specific anti-E1 Ab => **97-100 % experience a secondary immune response after vaccination**
 - * Negative WB => RV-IgG are always < half the current cut-off of ELISA assays => **91-98 % patients experience primary immune response after vaccination**

Immuno blot = reliable standard

Perspectives

Ongoing action:

Preparation of a panel* of true rubella seronegative samples
=> will be available for manufacturers to improve accuracy of their RV-IgG assays (Availability of the panel will have to be proposed to **all** manufacturers !).

* 100 blood packs from Australia, France, the Netherlands, South Africa, Turkey; aliquots: 0.5 mL; RV-IgG titer: ≤ 3 IU/mL, confirmed with 3 different IgG assays and one immunoblot

Key points still to be discussed:

- ✓ Is the use of IU/mL necessary ?
- ✓ Should the results be qualitatively or quantitatively reported ?

LUMC

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UMC Utrecht

Greet Boland

Tergooi

Wendelien Dorigo

Haga

Nathalie van Burgel

Bronovo

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Clinicians, Participants, Patients

WHO Global Measles/Rubella Lab Network Group

RIVM

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Jeroen Cremer

Gaby Smits

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Tessa van 't Klooster-Schuring

Hester de Melker

Susan Hahné

Rob van Binnendijk





Table 1. *Composition of reference serum panels for measles, mumps and rubella*

	Antigen		
	Measles	Mumps	Rubella
Reference laboratory	Denmark SSI, Copenhagen	Germany RKI, Berlin	UK PHL, Preston
Panel size	137	150	152*
Serum type			
Positive	91	72	78
Equivocal	10	18	11
Negative	36	60	63
Standard sera	2nd International Reference Preparation	Working standard, RKI 5/96	International Reference Preparation*
Units	5000 mIU/ml	1000 arbitrary units/ml	

* Dilution series of the International Standard in PBS and negative serum included (12 samples).