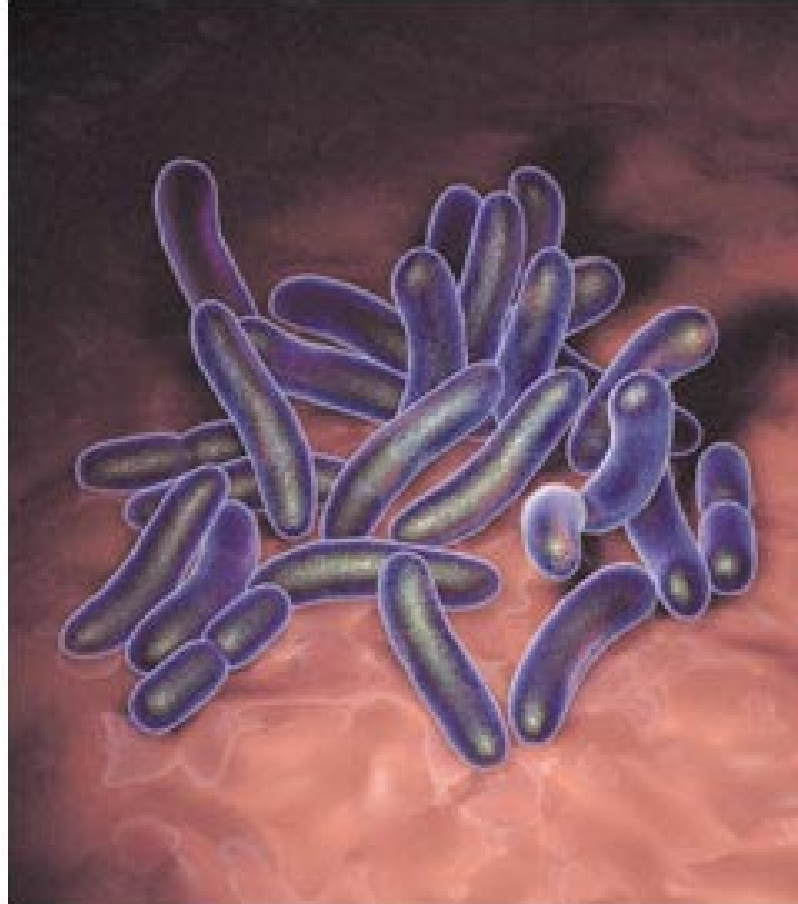
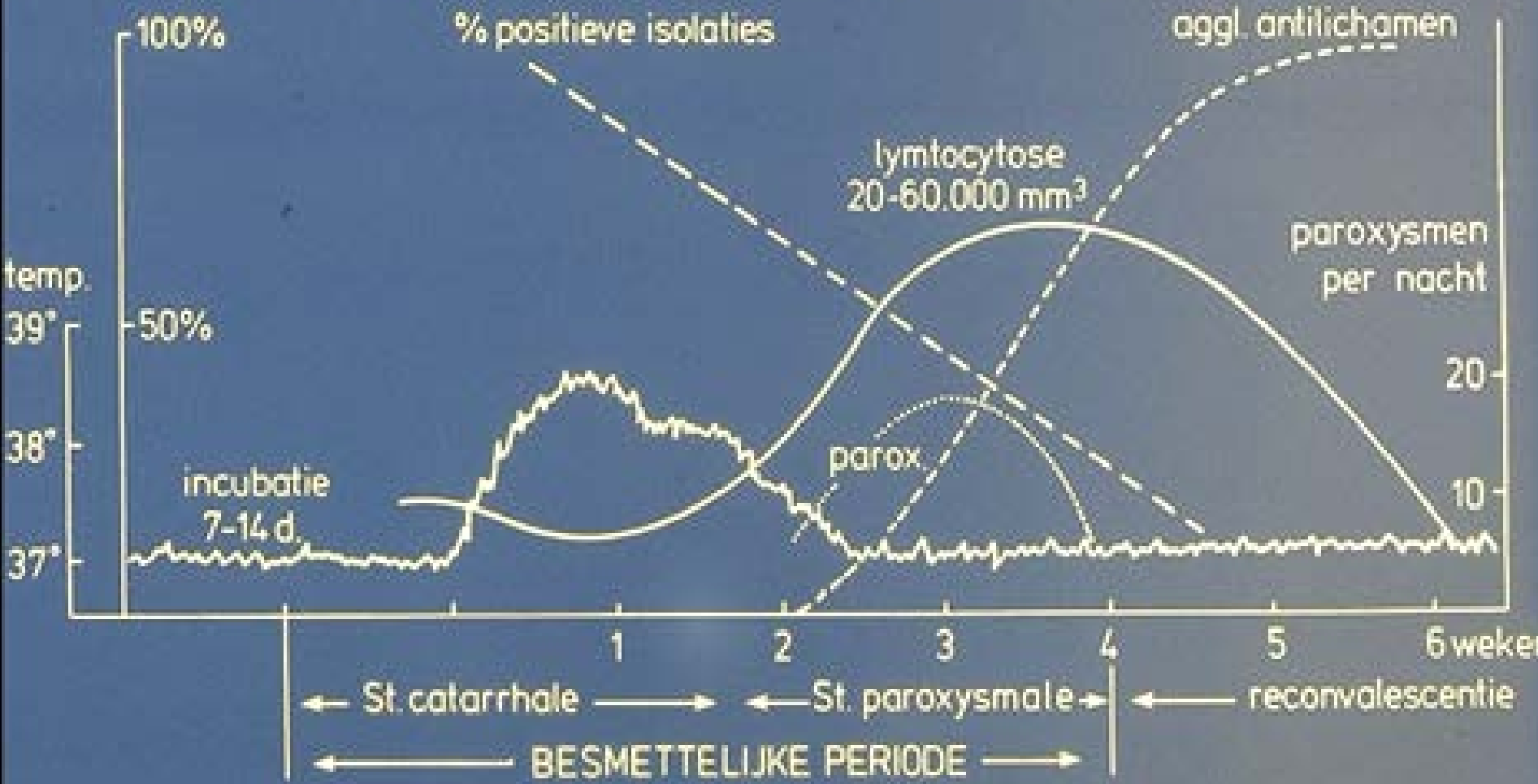


*Bordetella pertussis*



# SCHEMATISCHE WEERGAVE VAN HET ZIEKTEBEELD KINKHOEST



# Antigenen voor serodiagnostiek van luchtweginfectie met *Bordetella pertussis*

- Whole cell sonicaat
- OMP extract
  
- Antigenen vervat in acellulaire kinkhoestvaccins
  - **Pertussis toxine (Ptx)**
  - Filamenteus haemagglutinine (FHA)
  - Pertactin (Prn)
  - Fimbriae (Fim)
  
- Antigenen NIET vervat in acellulaire kinkhoestvaccins
  - Adenylate cyclase toxine (ACT)
  - Catalytic domain of ACT (CatACT)
  - Bordetella resistance to killing protein A (BrkA)
  - Peptidoglycan associated lipoprotein (PAL, BP3352)
  - Lipo-oligosaccharide of *B.pertussis* (LOS-Bp)

## Immuunglobuline subklassen voor serodiagnostiek van luchtweginfectie met *Bordetella pertussis*

- IgM: alleen gevoelig in immuun-naïeve individuen, maar ook in die situatie niet gevoeliger dan IgG. Inductie door (eerste) vaccinatie.
- IgA: matig gevoelig; minder gevoelig dan IgG; hoe jonger het kind hoe vaker verminderde of afwezige IgA-respons; wordt niet of gering geïnduceerd door vaccinatie.
- IgG: meest gevoelig. Wordt geïnduceerd door vaccinatie.

Reproductive rate of pertussis in  
immune-naive population

$$R_0 = 17$$

# Waning immunity

- Waning immunity na vaccinatie (3 tot 8 jaar?)
  - Na vaccinaties op leeftijd 2, 3, 4 en 11 maanden en 4 jaar dalen de vaccin-geïnduceerde matig hoge piek-concentraties van IgG antistoffen tegen Ptx binnen 1 jaar weer naar lage of onmeetbare waarden.
- Waning immunity na infectie (5 tot 25 jaar?)
  - Na natuurlijke infectie dalen de door infectie geïnduceerde hoge concentraties van IgG antistoffen tegen Ptx binnen 2 jaar weer naar lage of matig hoge waarden.
- In populaties met zeer hoge vaccinatiegraad is er persisterende circulatie van *Bordetella pertussis*

# Pertussis in the Netherlands 1996-2000

Incidence per 100 000 population per year  
(peak-incidence)

- Mortality: 0.01 (< 3 months)
- Hospital admissions: 2.5 (< 4 months)
- Notifications: 26 (3 – 8 yr)
- Infections in population: 6600 (10 – 55 yr)

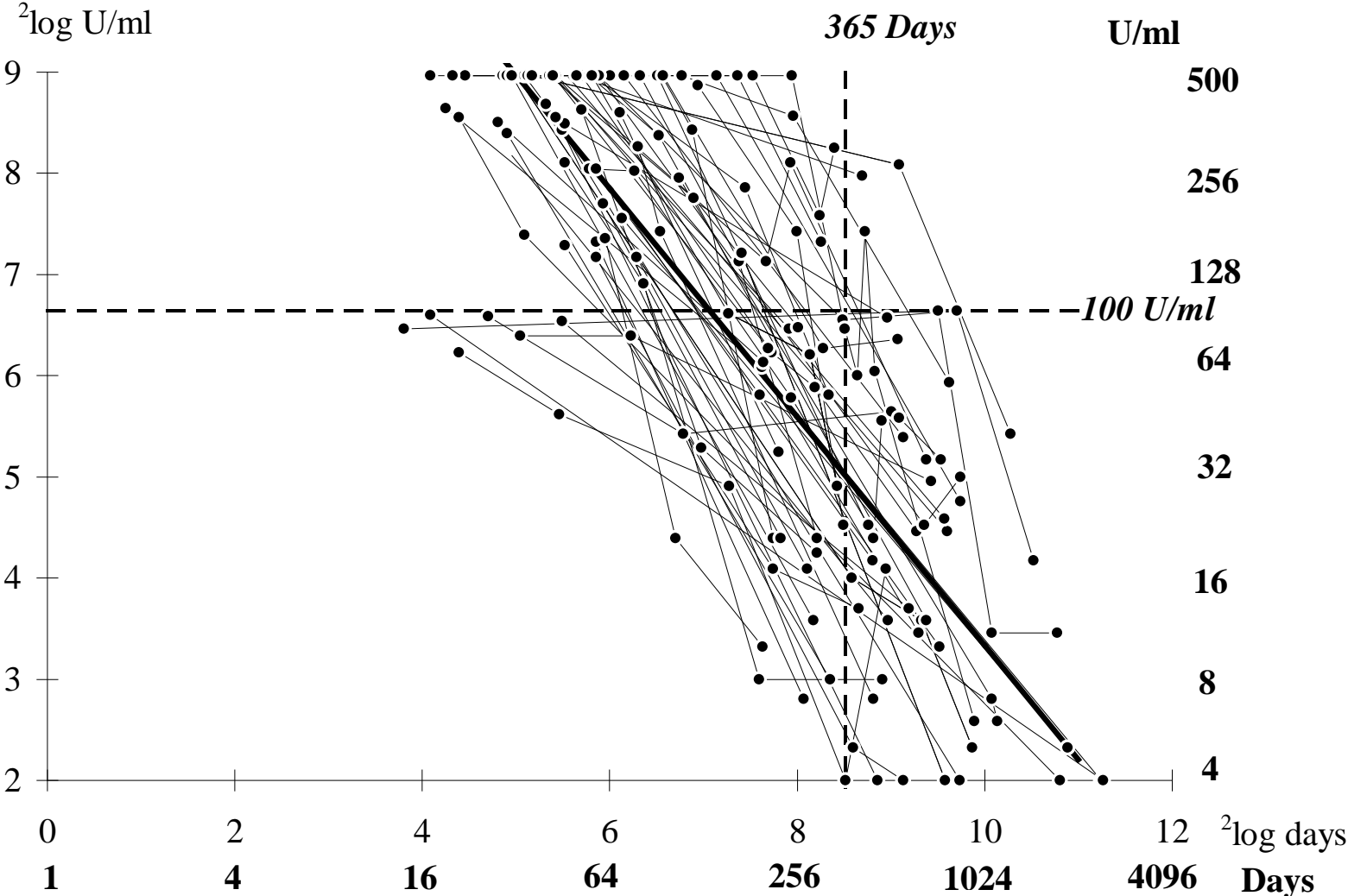
# Technieken voor serodiagnostiek van luchtweginfectie met *Bordetella pertussis*

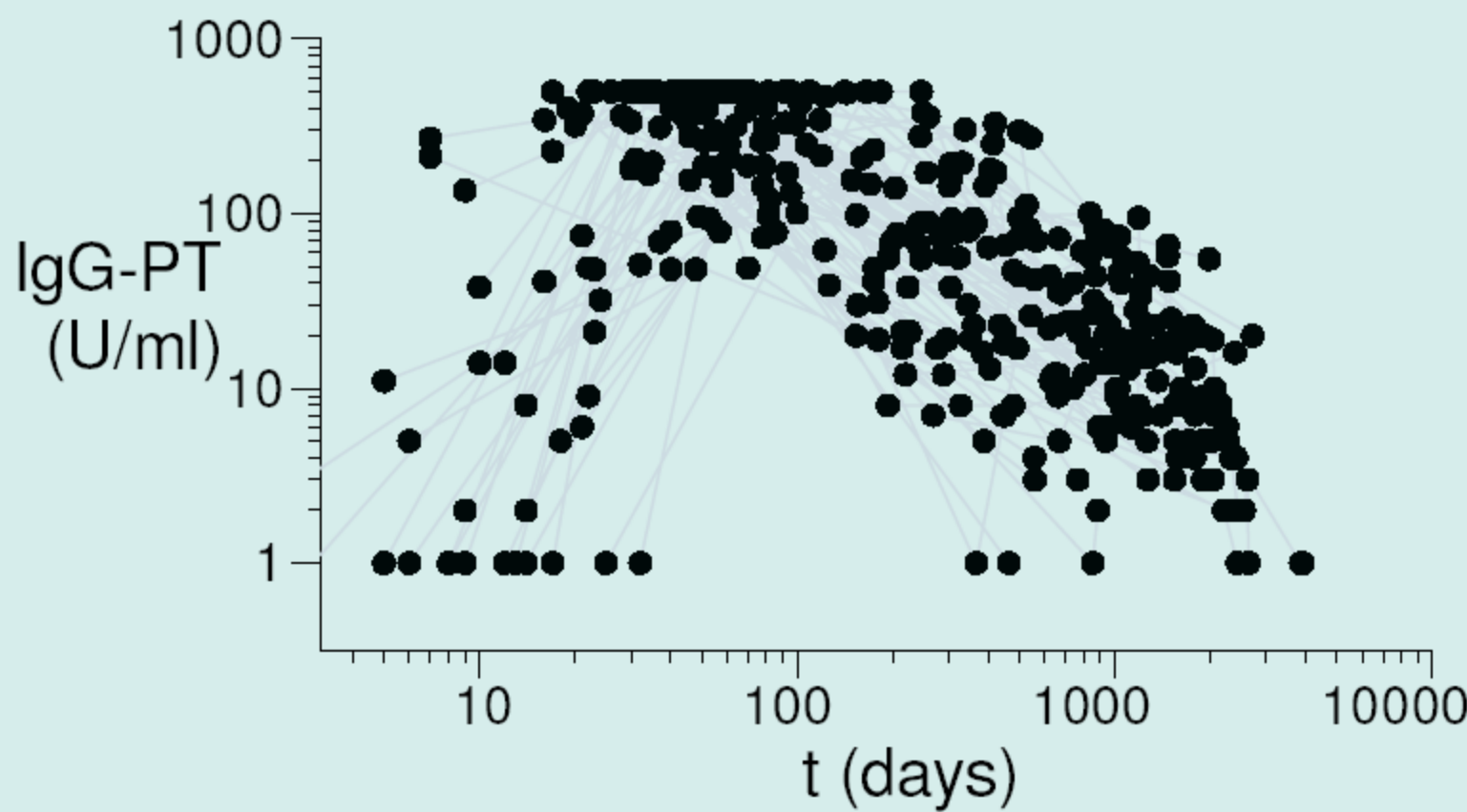
- Complement fixatie
- Bacteriële agglutinatie
- Immunoblot
- Elisa

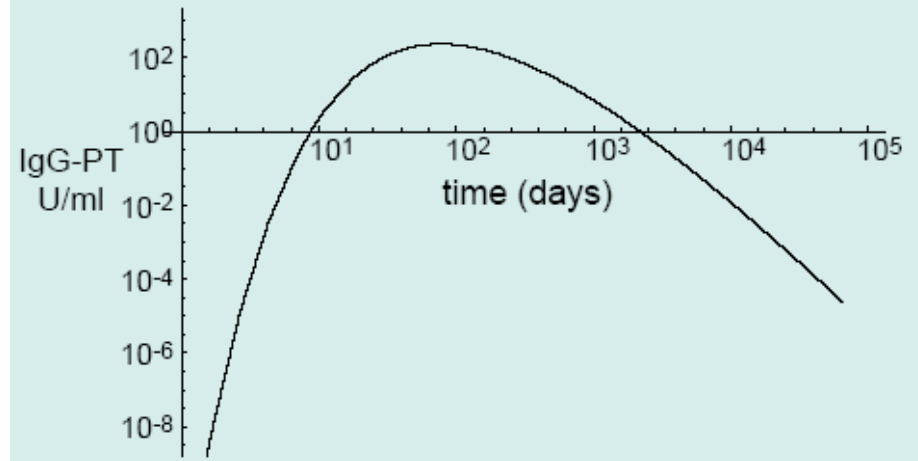
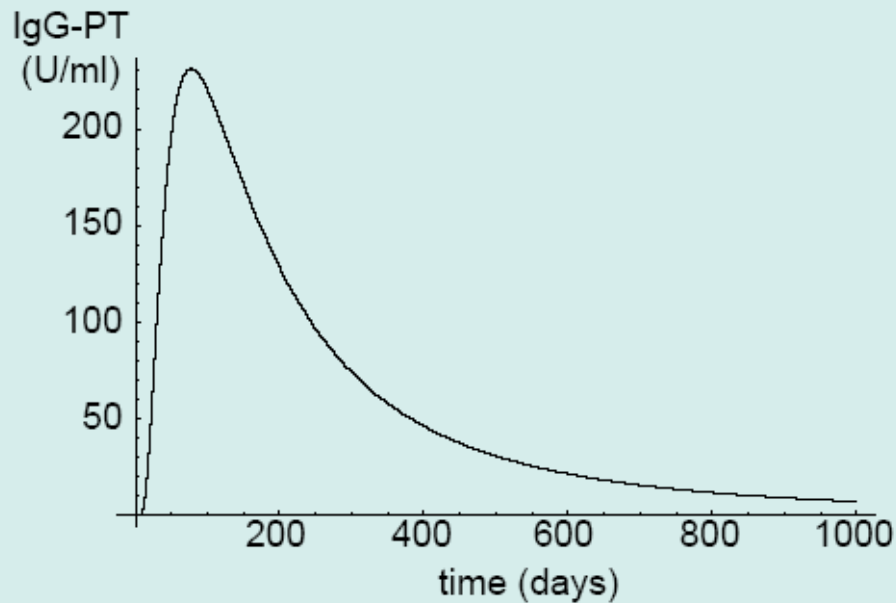


IgG-Ptx / IgG-PT

# IgG-PT levels versus time elapsed since date of onset of pertussis (n=57)







$$f(x; \theta) = d + c \left[ x + b \left( 1 - \sqrt{1 + \frac{x^2}{a}} \right) \right]$$

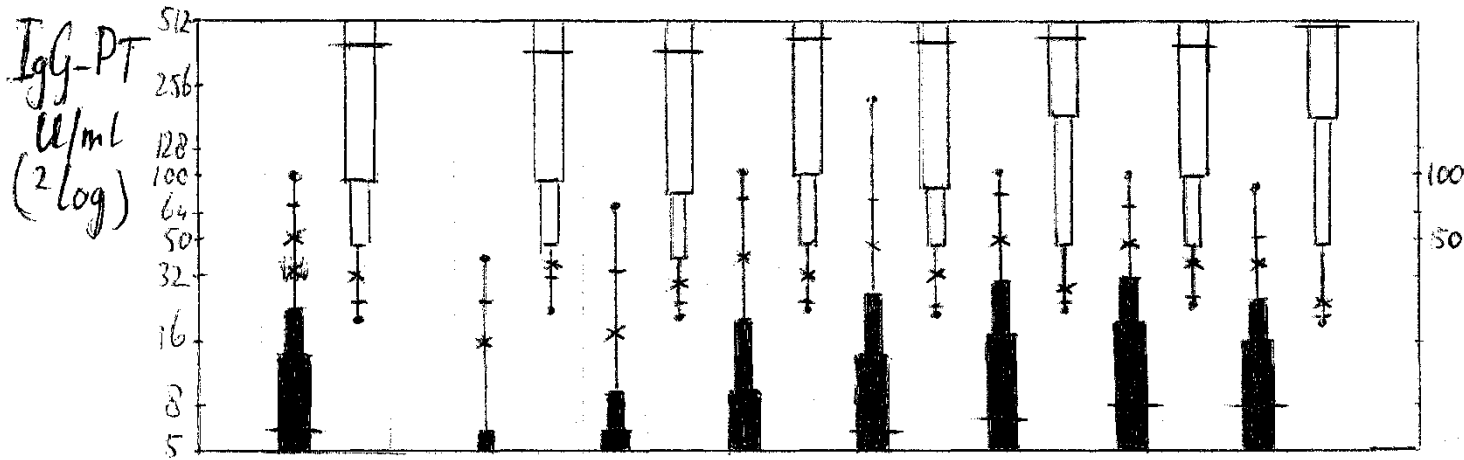
$(a, c, \text{ and } d > 0; b > \sqrt{a}).$

$$\text{asymptotes: } \lim_{x \rightarrow \pm\infty} f(x; \theta) - \left[ d + bc + cx \left( 1 \mp \frac{b}{\sqrt{a}} \right) \right] = 0$$

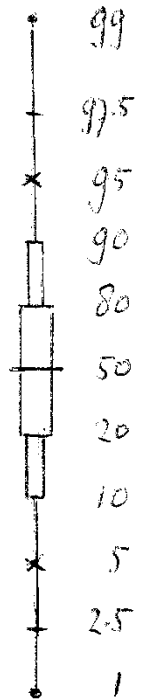
## 7756 populatie sera (PIENTER) 1995-1996

- De gevaccineerden binnen de populatie van die tijd waren gevaccineerd met het RIVM whole cell pertussis vaccin welk vaccin niet of nauwelijks Ptx bevatte en dus niet of nauwelijks IgG-Ptx induceerde.

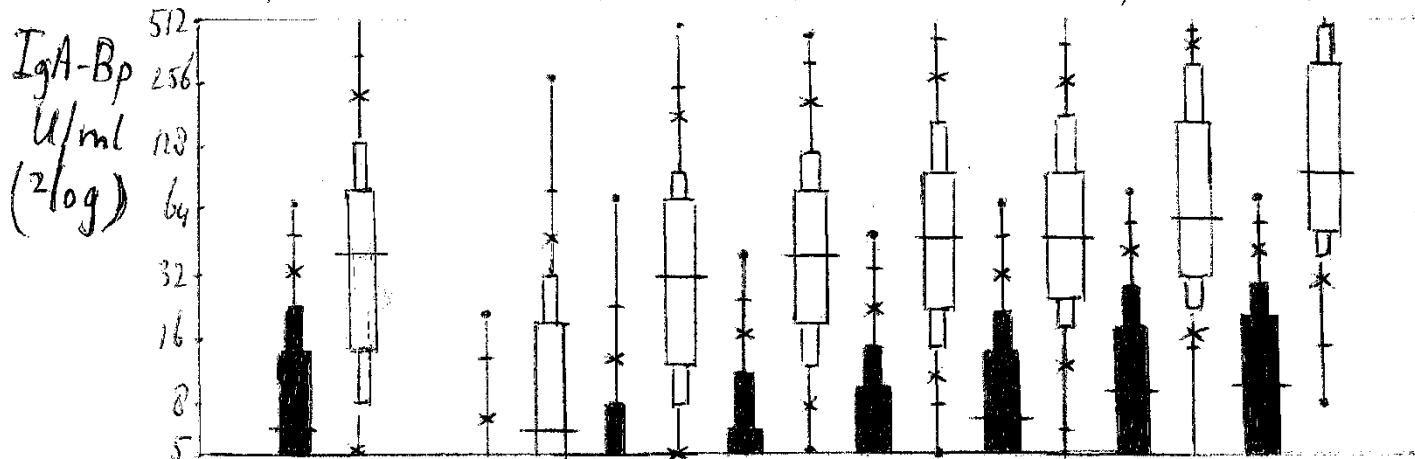
□ 2<sup>e</sup> sera van gepaarde sera met > 4-voudige stijging tot > 20 U/ml  
 ■ populatie sera (PIENTER) van IgG-PT



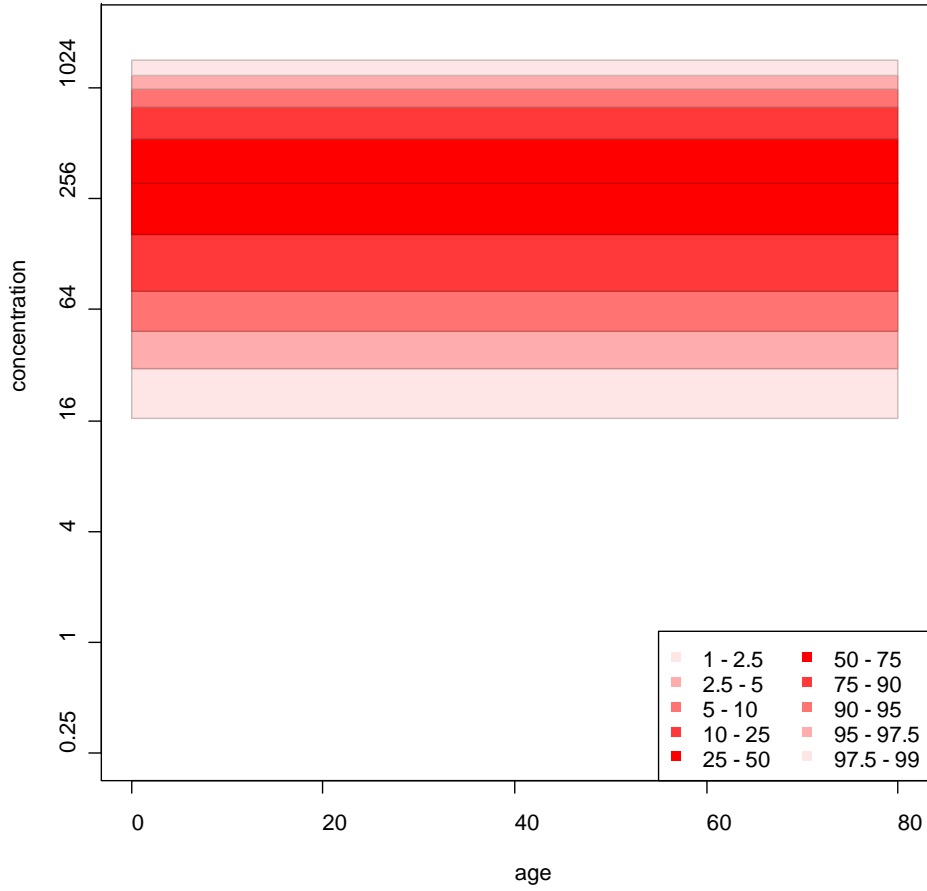
Distributie PerCentielen



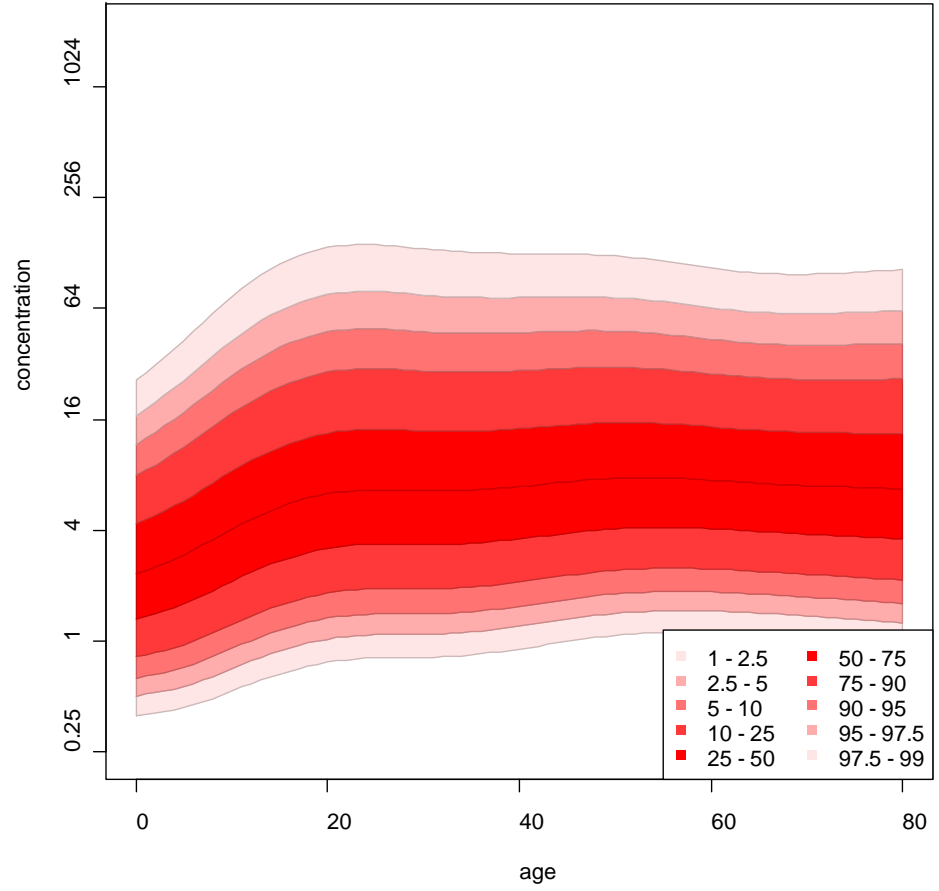
Totaal 7756 4065  
 <1 203 268  
 1-4 721 1134  
 5-9 550 1934  
 10-19 1031 538  
 20-39 1657 745  
 40-59 1942 165  
 >60 1658 72



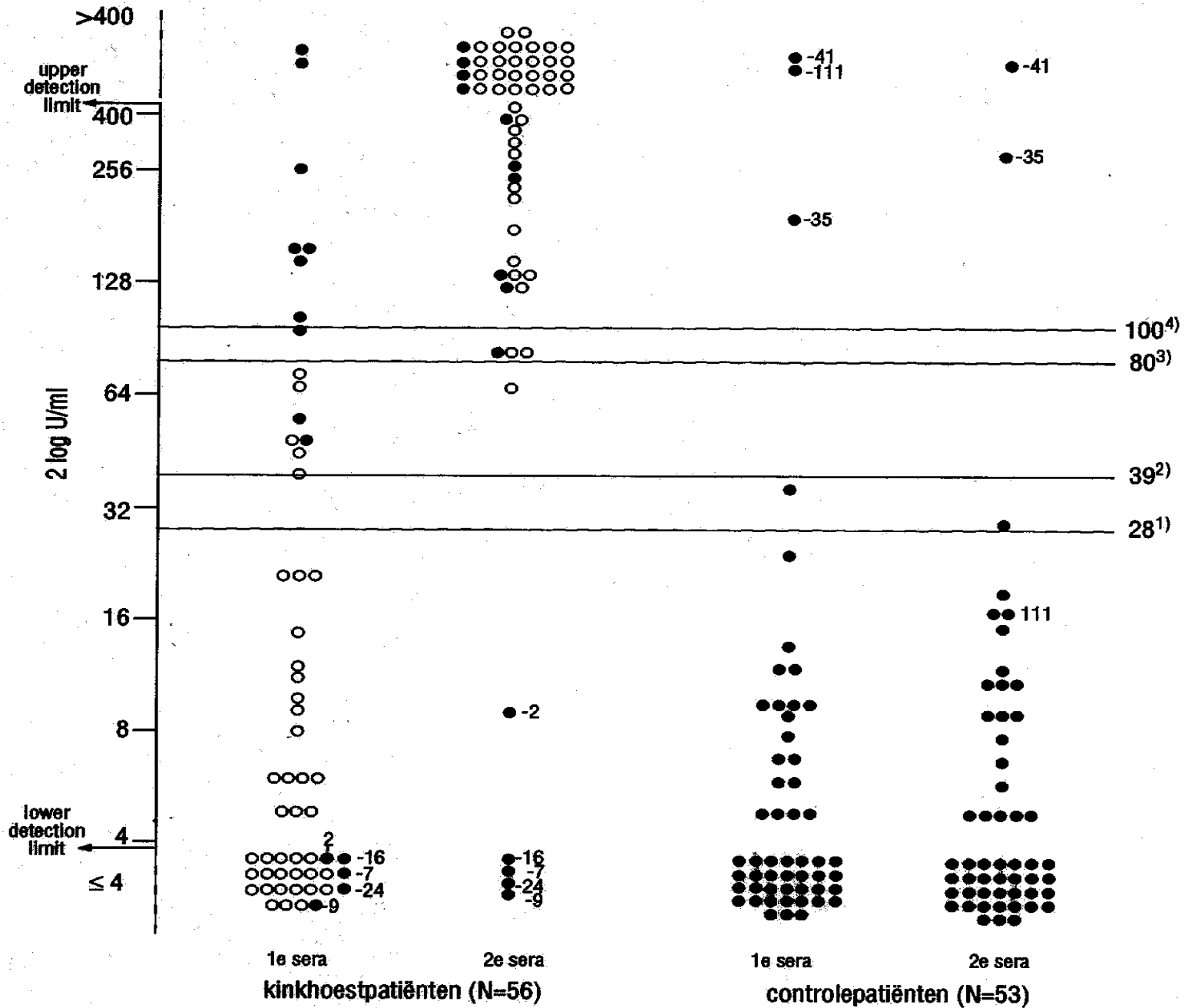
**IgG patients**



**IgG controls**

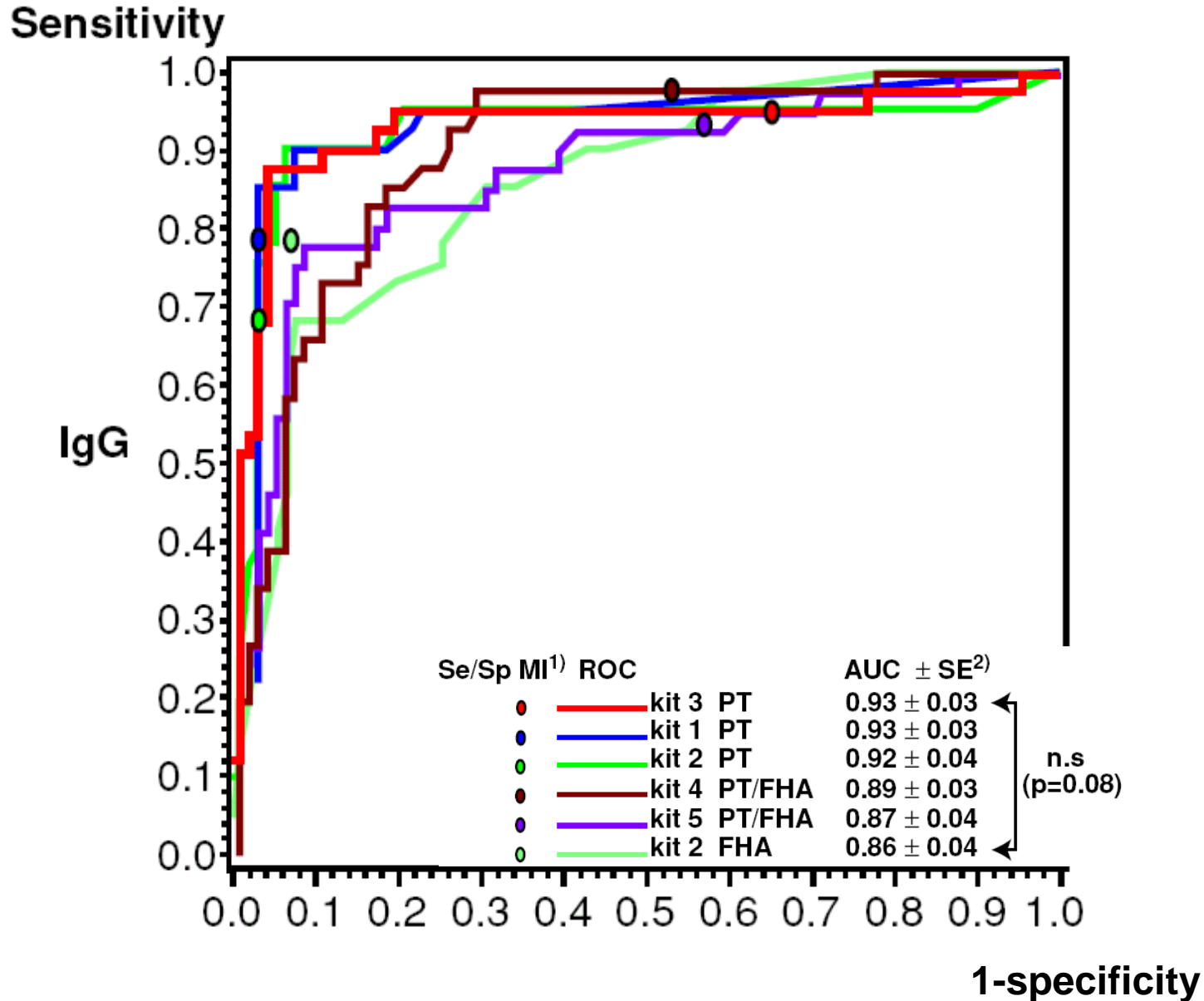


# IgG-PT - RIVM





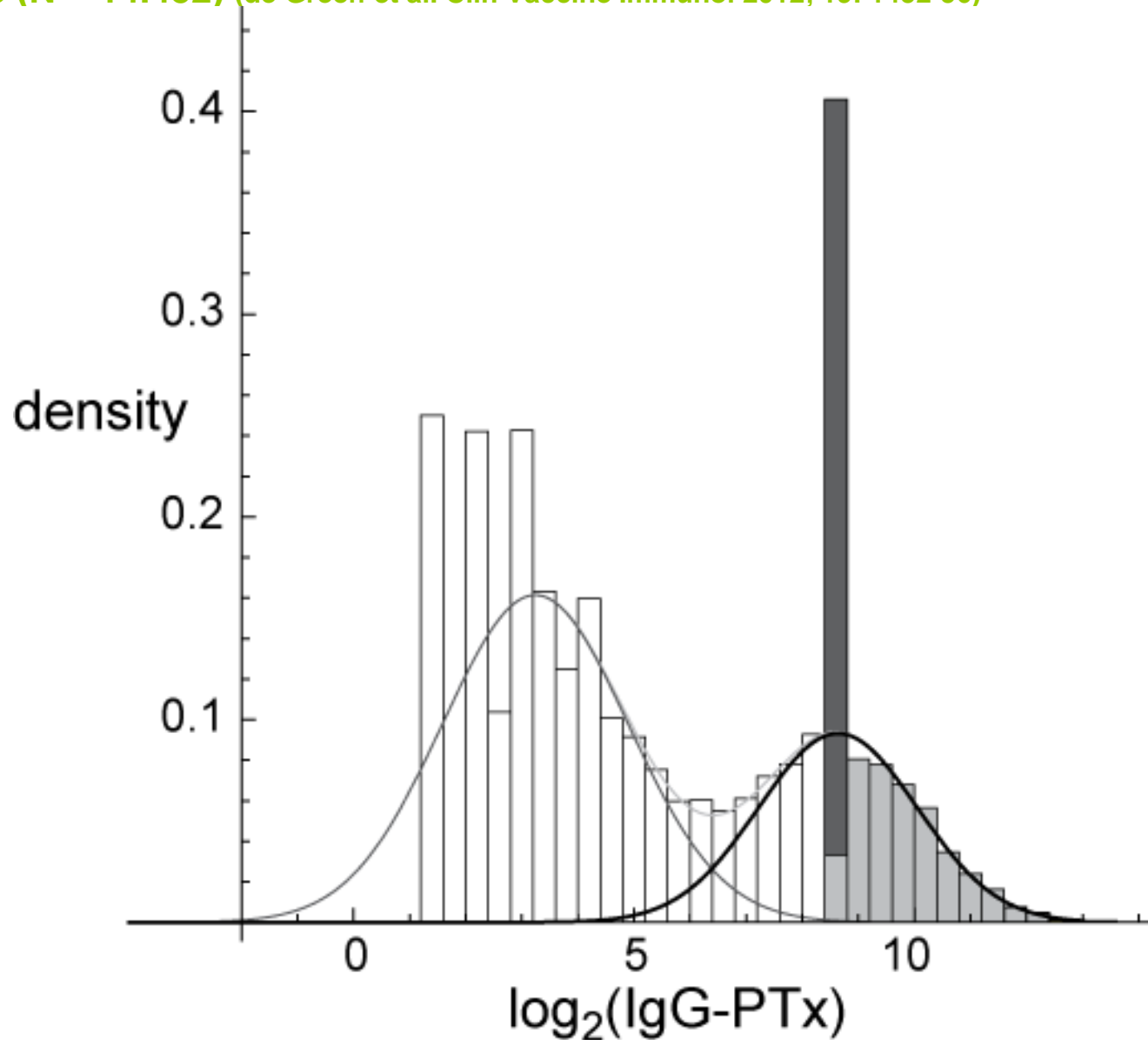
**ROC curves of IgG-antibody parameters (PT, PT/FHA, FHA) in late sera of patients with PCR confirmed pertussis (n=41) and sera of controls (n=91)**



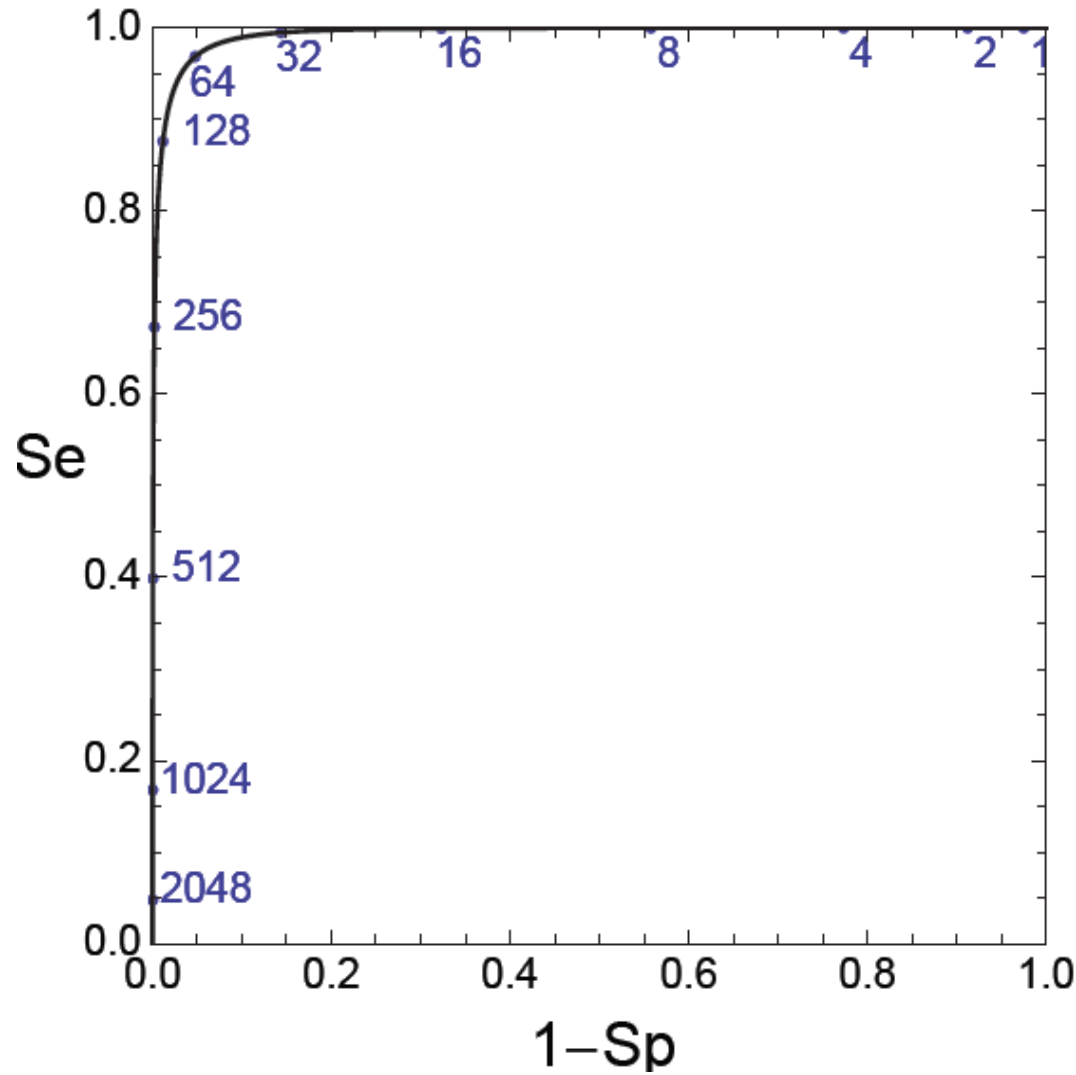
# Ontwikkelingen

- Giammanco et al, 2003:
  - 100 RIVM-IgG-PT EU/ml = 125 FDA-lot-3 EU/ml
- RIVM:
  - Sinds oktober 2003 wordt in de IgG-PT EIA van RIVM als referentieserum lot-3 FDA/USA gebruikt: uitdrukking in “FDA-lot-3 EU/ml”.
- Xing at al, 2009:
  - Internationaal IgG-PT standaardserum waarvan de IgG-Ptx IU/ml equivalent zij aan “lot-3 FDA EU/ml”.
  - Deze WHO standaard IS 06/140 is verkrijgbaar bij The National Institute for Biological Standards and Control (NIBSC), Londen, UK.

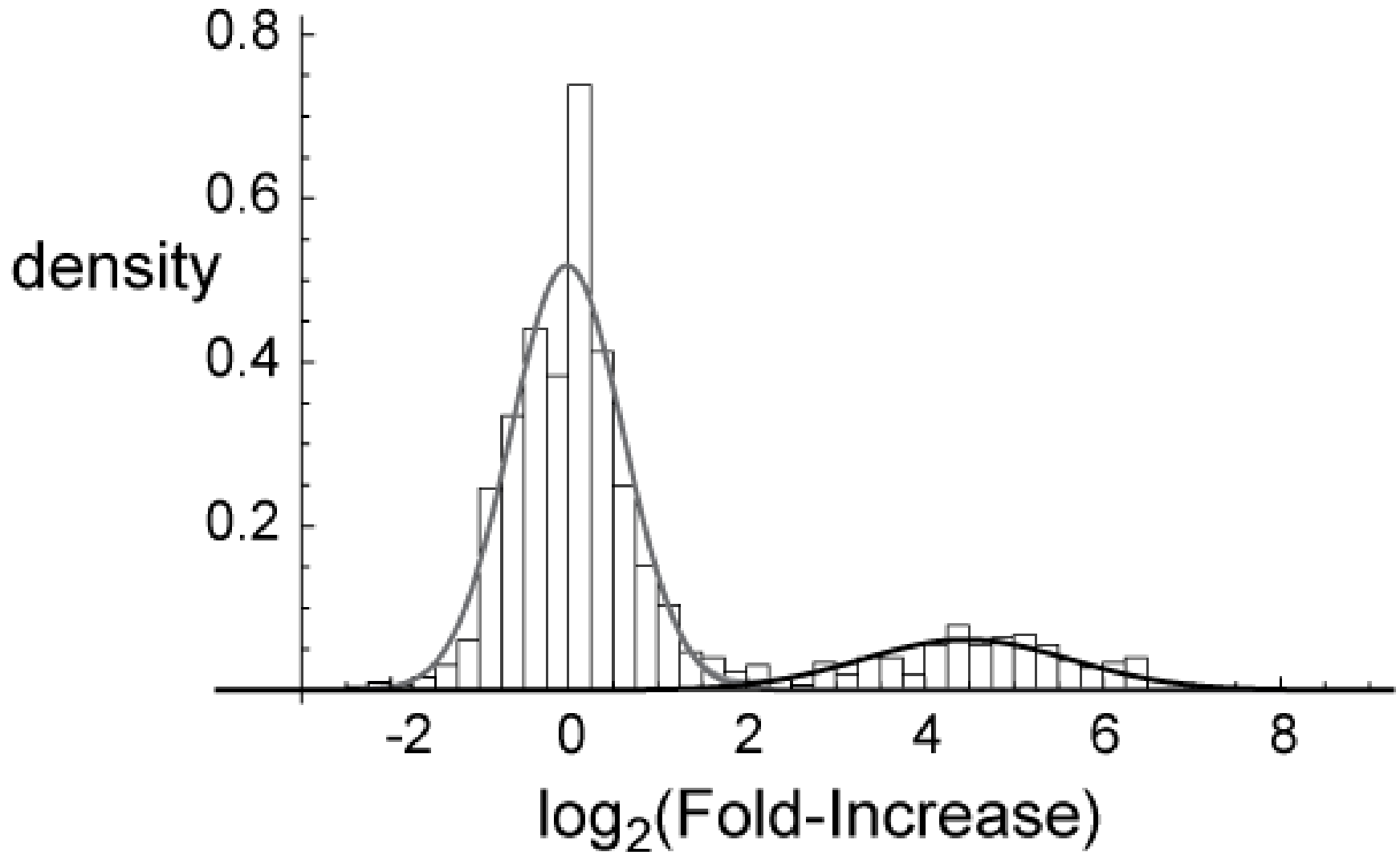
**Serodiagnostic database of RIVM: two-component cluster analysis of single sera of patients suspected of pertussis, obtained < 100 days after onset of disease (N = 14.452)** (de Greeff et al. Clin Vaccine Immunol 2012; 19: 1452-56)



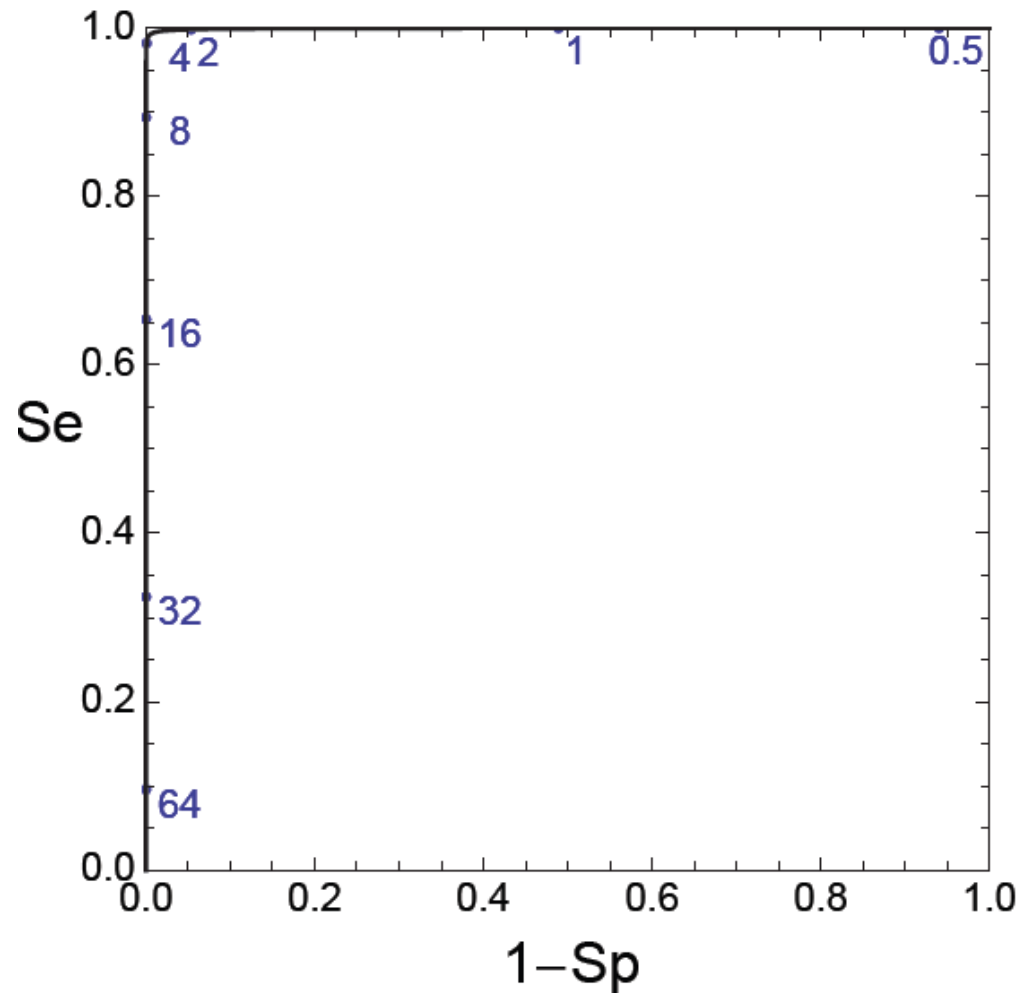
**ROC curve of two-component cluster analysis of single sera of patients suspected of pertussis, obtained < 100 days after onset of disease. (N = 14.452)** (de Greeff et al. Clin Vaccine Immunol 2012; 19: 1452-56)



**Two component cluster analysis of serumpairs of patients suspected of pertussis who had IgG-PT in first serum of 5 – 25 IU/ml (N = 1.316).** (de Greeff et al. Clin Vaccine Immunol 2012; 19: 1452-56)



**ROC curve of two component cluster analysis of serumpairs of patients suspected of pertussis who had IgG-PT in first serum of 5 – 25 IU/ml (N = 1.316)** (de Greeff et al. Clin Vaccine Immunol 2012; 19: 1452-56)



**Single sera obtained < 100 days after onset of disease.**

**N = 14.452** (de Greeff et al 2012)

**TABLE 1 Cutoffs and sensitivities in single serum samples obtained within 100 days after onset of illness for different levels of specificity**

<b>Specificity (%)</b>	<b>Estimated cutoff (IU/ml)</b>	<b>Sensitivity (%)</b>
90	41.1	99.0
95	62.3	97.1
97.5	89.4	93.6
98	99.6	92.1
99	136.1	86.3

# Serumpairs

N = 2.455 (de Greeff 2012)

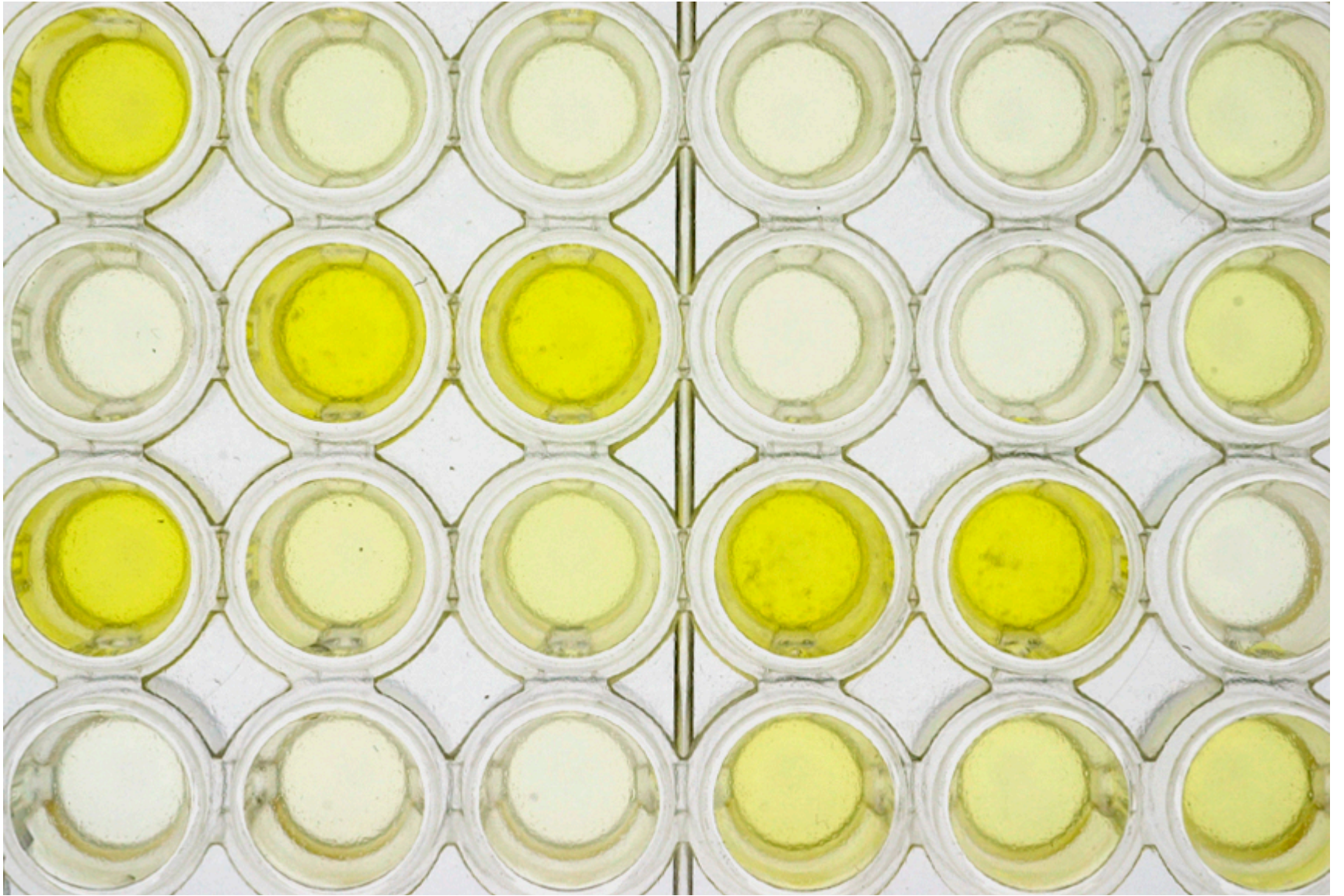
TABLE 2 Optimum cutoffs (fold increase), sensitivities, and specificities estimated with a mixture model for paired sera, stratified by IgG-Ptx level in the first serum sample

IgG-Ptx concn (IU/ml) in first serum sample	No. of samples	AUC	Optimum cutoff (fold increase)	Specificity (%)	Sensitivity (%)
<5	473	0.999	3.3	99.4	99.8
5–24	1,316	0.999	3.1	99.2	99.6
25–49	339	0.999	2.8	99.3	99.2
50–99	253	0.999	2.3	98.9	98.4
100–199	74	0.989	1.5	94.9	94.7
<5–199	2,455	0.924	2.1	84.4	97.7



# (Mijn) conclusies

- IgA-component in commerciële EIA's
  - Interpretatie-criteria onvoldoende gevalideerd (?)
  - Leeftijdsafhankelijkheid in interpretatie-criteria ontbreekt
  - IgA draagt niet (nauwelijks) bij aan IgG en verhoogt complexiteit
  - Niet gebruiken ....
- IgG-PT in enkelvoudige sera
  - $\text{IgG-PT} \geq 125 \text{ IU/ml}$  = diagnostisch
  - $\text{IgG-PT} < 125$  en  $> 62 \text{ IU/ml}$  = suspect, svp 2e serum
  - $\text{IgG-PT} \leq 62 \text{ IU/ml}$  = binnen normale spreiding, svp 2e serum
  - Niet (zeker) toepasbaar binnen 1 jaar na 3e, 4e of 5e vaccinatie tegen kinkhoest
- IgG-PT in gepaarde sera
  - Toename  $\text{IgG-PT} \geq 3$ -voudig tot minstens  $20 \text{ IU/ml}$ : diagnostisch



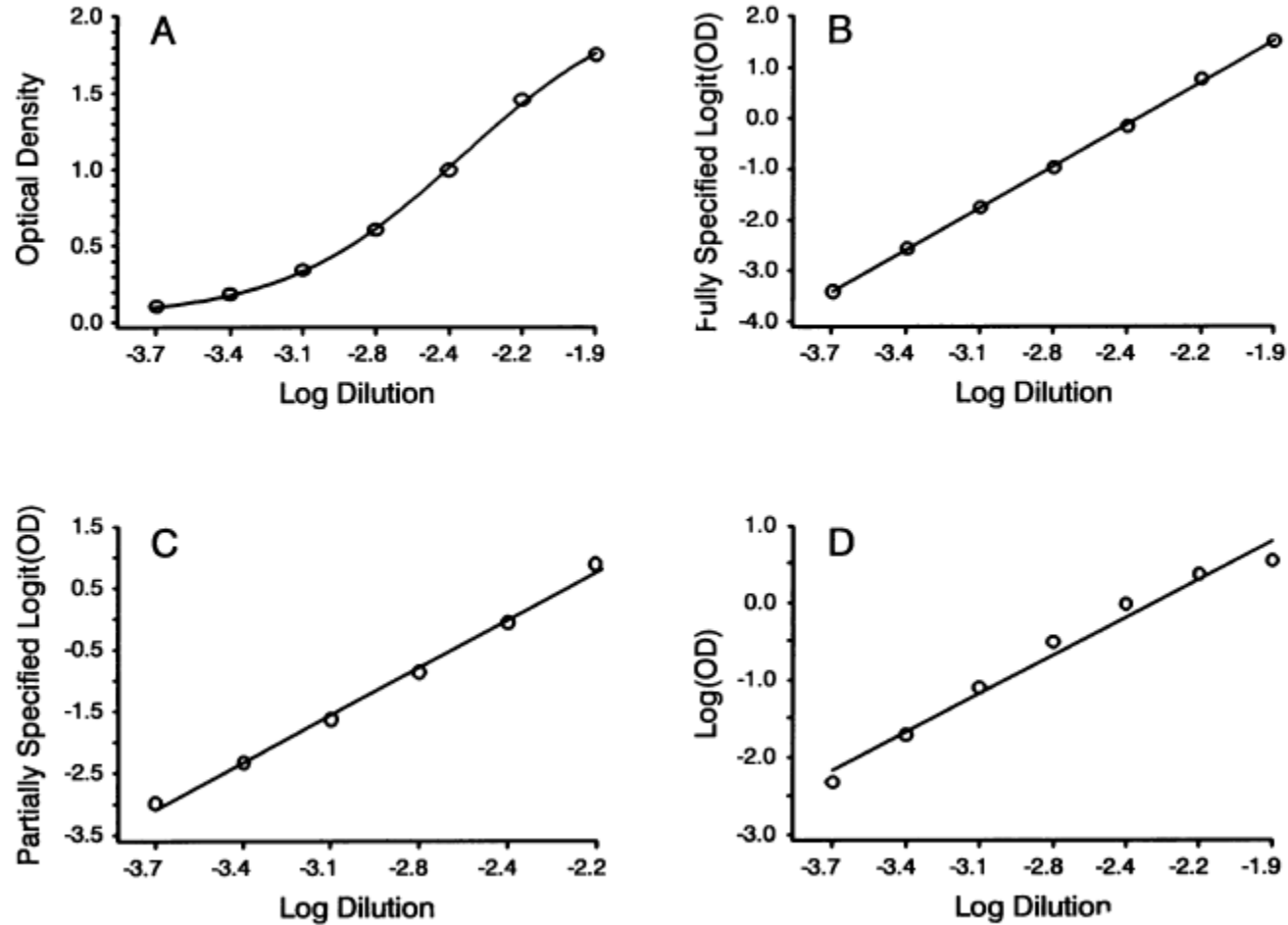


FIG. 1. Graphs displaying four standard curves fit to a typical set of calibration data from a serially diluted standard reference serum (PB-2; 4,800 U/ml). Mathematical models corresponding to each curve are the four-parameter logistic-log (A), the fully specified logit-log (B), the partially specified logit-log (C), and the log-log (D) models.

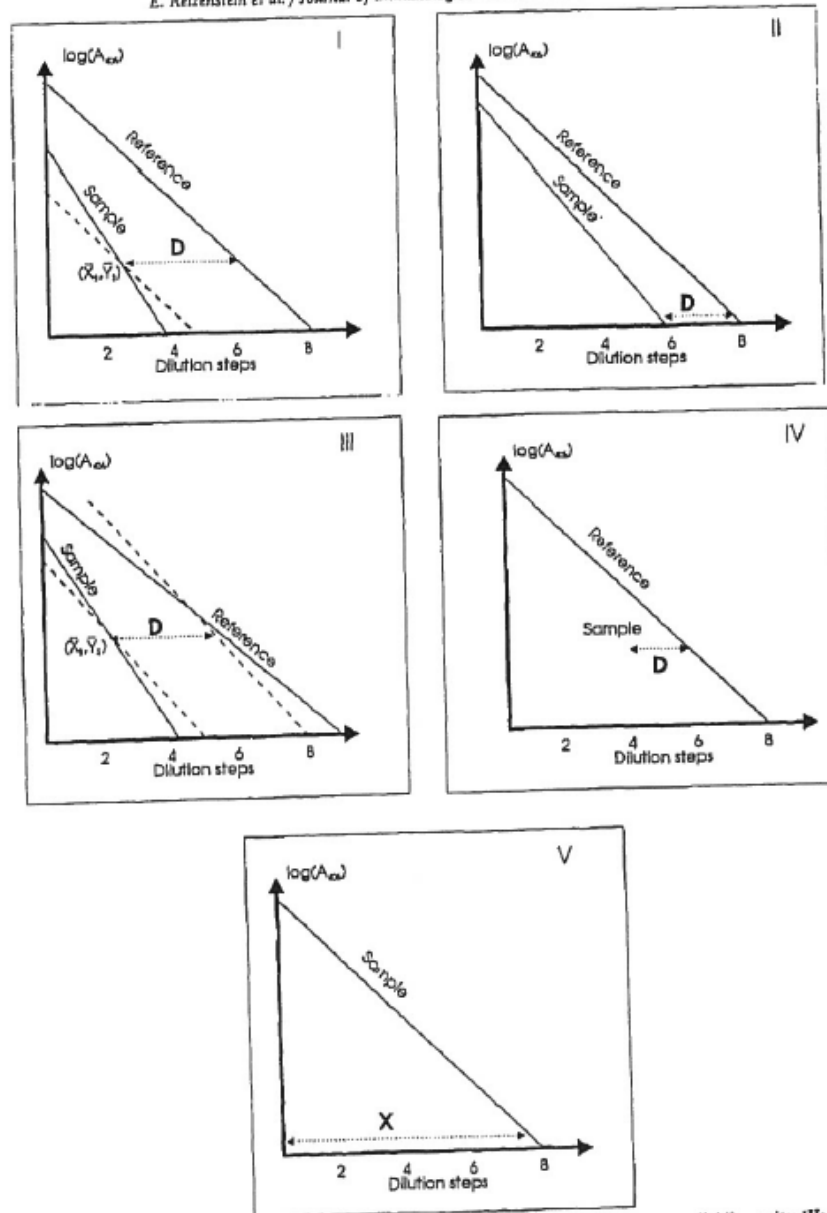
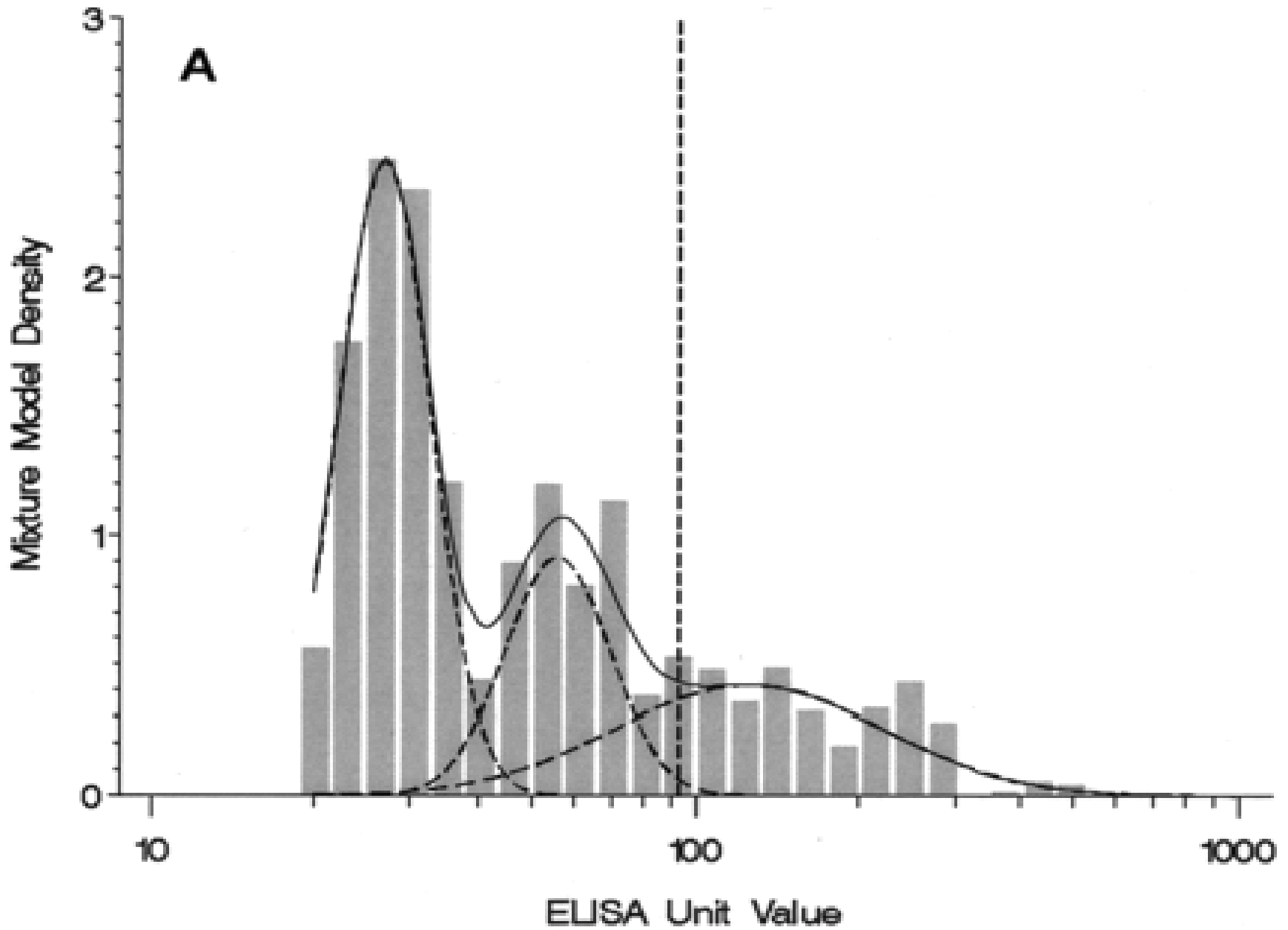


Fig. 1. Calculation modes for the expression of ELISA results by I: reference line units; II: non-parallel line units; III: parallel line units; IV: single point reference line units; and V endpoint titres.  $\bar{X}_s$  and  $\bar{Y}_s$  indicate the mean value of the dilution steps and  $\log A_{500}$  respectively, and D the distance in dilution steps between the serum and the reference line.



# Similarity of IgG-PT distribution in populations of USA (NHANES) and the Netherlands

<b>IgG-PT</b>	<b>spec (%) USA pop.sera* 10 – 49 years n=5049</b>	<b>spec (%) dutch pop.sera** 10 – 49 years n=3641</b>
<b>FDA IU/ml #</b>		
<b>5</b>	<b>..</b>	<b>50.1</b>
<b>8.4</b>	<b>..</b>	<b>67.5</b>
<b>10</b>	<b>..</b>	<b>73.4</b>
<b>20</b>	<b>84.6</b>	<b>85.3</b>
<b>50</b>	<b>..</b>	<b>94,9</b>
<b>79</b>	<b>..</b>	<b>97.0</b>
<b>88</b>	<b>..</b>	<b>97.5</b>
<b>94</b>	<b>97.5</b>	<b>97.8</b>
<b>99</b>	<b>..</b>	<b>98.0</b>
<b>125</b>	<b>..</b>	<b>99.0</b>
<b>198</b>	<b>99.0</b>	<b>99.1</b>

\* Baughman AL et al. Clin Diagn Lab Immunol 2004; 11 (6): 1045-1053.

\*\* Melker HE de et al. J Clin Microbiol 2000; 38 (2): 800-806

# Giammanco A et al. Vaccine 2003; 22(1): 112-20.

# Resultaten door samenwerking

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Guy Berbers	Marcel Peeters
Anna Giammanco	Arend Jan van Houte
Jan van de Kasstele	Harold Verbakel
Joop Schellekens	

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