Assessment of different serological assays for anti-HBs testing; results from a quality assessment program in 2013

Stijn Raven (1,2) J.L.A. Hautvast (1) J.E. van Steenbergen (3,4) R. Akkermans (1)
C. Weykamp (5,6) F. Smits (1) C.J.P.A. Hoebe (2,7) A.C.T.M. Vossen (8)

1: Academic Collaborative Centre for Public Health AMPHI, Department of Primary and Community Care, Radboud University Nijmegen Medical Centre, The Netherlands
2: Department of Medical Microbiology, School of Public Health and Primary Care (CAPHRI),Maastricht University Medical Center (MUMC+), Maastricht, The Netherlands. 3: Centre for Infectious Disease Control, Netherlands Institute of Public Health and the Environment, Bilthoven, The Netherlands. 4: Centre of Infectious Diseases, Leiden University Medical Centre, Leiden, The Netherlands. 5: MCA Laboratory, Queen Beatrix Hospital, Winterswijk, The Netherlands. 6: On behalf of the Dutch Foundation for Quality Assessment in Medical Laboratories (SKML) 7: Department of Sexual Health, Infectious Diseases and Environmental Health, South Limburg Public Health Service, The Netherlands 8: Department of Medical Microbiology, Leiden University Medical Centre, The Netherlands

Background

- Post-vaccination testing after hepatitis B vaccination is indispensable to evaluate long-term immunological protection and necessary for correct clinical management of specific risk groups.
- Using a threshold level of antibodies against hepatitis B surface antigen (anti-HBs) to define serological protection, implies reproducible and valid measurements of different diagnostic assays.
- In this study we assess the performance of different currently used anti-HBs assays.

Methods

In 2013, 42 laboratories participated in an external quality assessment (EQA) program with a set of six pooled anti-HBs serum samples around the cutoff values 10 IU/l and 100 IU/l.
Laboratories used either Axsym (Abbott Laboratories), Architect (Abbott Laboratories), Access (Beckman-Coulter), ADVIA Centaur anti-HBs2 (Siemens Healthcare Diagnostics), Elecsys, Modular or Cobas (Roche Diagnostics) or Vidas Total Quick (Biomerieux) for anti-HBs titre quantification.
All assays were calibrated against the 1st International Reference Preparation WHO 1977. We analysed covariance using mixed-model repeated measures. For the assessment of sensitivity/specificity and agreement a true positive or true negative result was defined as an anti-HBs titre respectively above or below the cutoff value by ≥ 4 of 6 assays.

Results

Different anti-HBs assays were associated with statistically significant differences in anti-HBs titres in all dilutions. Sensitivity and specificity ranged respectively from 64% - 100% and 95% -100%. Agreement between different assays around an anti-HBs titre cutoff value 10 IU/l ranged from 93%-100% and was 44% for a cutoff value of 100 IU/l.

Figure 1 Mean anti-HBs results (95% CI) of different dilutions repeatedly analysed with different test methods

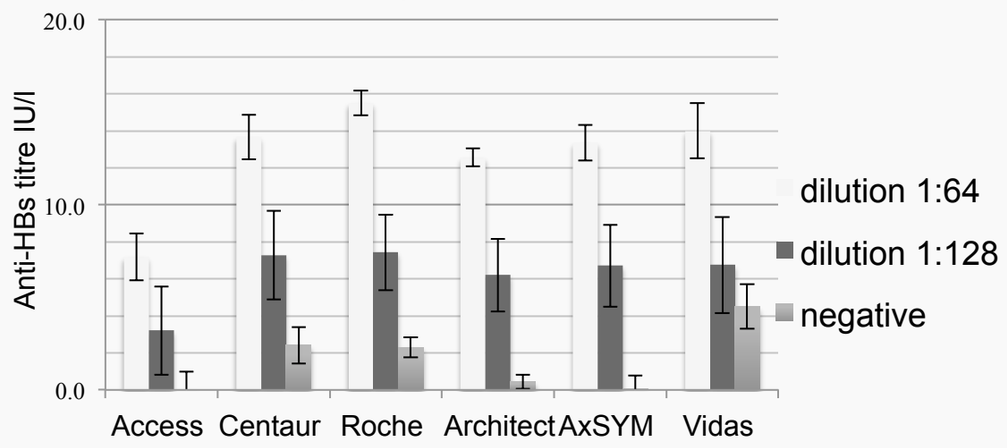

Table 1 Characteristics of 6 samples and results of agreement and the mixed model, N=494

Sample	(N)	Anti- HBs Mean (SD) (IU/l)		Coefficient of variation (%)	Agreement (%)	Fixed effect *P*- value test method	test round
Negative	(123)	1,1	(1.5)	-	100%	<0.05	0.60
1:512	(83)	2,1	(1,2)	57%	100%	<0.05	0.69
1:128	(84)	6,4	(1,9)	30%	99%	<0.05	0.81
1:64	(80)	13,2	(2,3)	17%	93%	<0.05	0.19
1:8	(39)	98,4	(17,5)	17%	44%	n.a.*	n.a.
1:4	(85)	192	(37.7)	20%	100%	<0.05	0.58

* n.a.: not applicable, measurements available of one test round and therefore not suitable for a mixed model

Table 2 Sensitivity calculated for different assays compared to an anti-HBs titre cutoff of 10 IU/l and 100 IU/l

Test assay	Sensitivity % (*) 10 IU/l	100 IU/l
Architect	99 (1/94)	69 (18/58)
Vidas	100 (0/10)	100 (0/6)
ADVIA Centaur	100 (0/15)	100 (0/9)
Roche	100 (0/48)	100 (0/28)
AxSYM	100 (0/23)	93 (1/14)
Access	64 (5/14)	67 (3/9)

*100 – (No. false-negative / total no. of true positive samples (at least 4 of 6assays anti-HBs ≥ 10IU/l or ≥ 100IU/l)) x 100

Conclusions

- EQA programs are indispensable to achieve standardisation among laboratories
- Anti-HBs assays produce different results around clinically relevant cutoff values
- Lack of agreement between assays is mostly due to false-negative results of two assays