

Interlaboratory Reproducibility of an Enzyme-Linked Immunosorbent Assay for Quantitation of Antibodies for *Haemophilus influenzae* Type b Polysaccharide

In a recent article, Mariani and collaborators (2) described a competitive enzyme-linked immunosorbent assay (ELISA) for the quantitation of serum antibodies to *Haemophilus influenzae* type b (Hib) which correlated very well with the traditional radioantigen binding assay (RABA) and a previously described direct ELISA (3).

While their analyses were very comprehensive, the basis for their comparison of methods was data generated in the same laboratory. Mariani et al. did not reference an interlaboratory study evaluating the direct ELISA which indicated that the reproducibility of the HbO-HA assay is laboratory dependent (1). In the HbO-HA direct ELISA interlaboratory study, 7 of 11 laboratories reported higher antibody concentrations for low-titered sera than expected based on the RABA. Based on the data generated by Mariani et al., it appears that their laboratory encountered this previously described difficulty. The fact that four laboratories in the previous interlaboratory study generated data by the HbO-HA direct ELISA that were consistent with the RABA indicates that the variation is a laboratory-specific phenomenon.

The reasons why some laboratories encounter difficulty with low-titered sera in the HbO-HA ELISA are unclear. We speculate that direct ELISAs are sensitive to low levels of endotoxin contamination, which are introduced via buffers and glassware used during the antigen-coating steps; human sera contain antibodies which can bind to these contaminants.

Another possibility is that the Mariani et al. study used sera which were more concentrated (1:20 dilution) than the 1:50 dilution recommended by Phipps et al., which may enhance the nonspecific binding of human immunoglobulins (2). In our experience, a starting dilution of 1:50 provides sufficient sensitivity in the HbO-HA ELISA to quantitate to 0.1 µg/ml. Thirdly, we have noted that plates vary by lot and by manufacturer in their performance characteristics, requiring prescreening for optimal and specific antigen-binding capacity.

For those laboratories that are unable to correct this background binding which affects low-titered specimens, the Mariani et al. competitive ELISA appears to provide a sensitive alternative method for quantitation of human antibodies to Hib polysaccharide.

REFERENCES

1. Madore, D. V., P. Anderson, B. D. Baxter, G. M. Carlone, K. M. Edwards, R. G. Hamilton, P. Holder, H. Kayhty, D. C. Phipps, C. C. A. Peeters, R. Schneerson, G. R. Siber, J. I. Ward, and C. E. Frasch. 1996. Interlaboratory study evaluating quantitation of antibodies to *Haemophilus influenzae* type b polysaccharide by enzyme-linked immunosorbent assay. *Clin. Diagn. Lab. Immunol.* 3:84-88.
2. Mariani, M., E. Luzzi, D. Proietti, S. Mancianti, D. Casini, P. Costantino, P. van Gageldonk, and G. Berbers. 1998. A competitive enzyme-linked immunosorbent assay for measuring the levels of serum antibody to *Haemophilus influenzae* type b. *Clin. Diagn. Lab. Immunol.* 5:667-674.
3. Phipps, D. C., J. West, R. Eby, M. Koster, D. V. Madore, and S. A. Quataert.

1990. An ELISA employing a *Haemophilus influenzae* type b oligosaccharide-human serum albumin conjugate correlates with the radioantigen binding assay. *J. Immunol. Methods* 135:121-128.

Dace V. Madore
Sally A. Quataert
Wyeth-Lederle Vaccines and Pediatrics
West Henrietta, New York 14586-9728

Author's Reply

Dr. Madore and Dr. Quataert in their interlaboratory study conducted with an indirect ELISA evidenced and confirmed the laboratory dependence of the assay and therefore the variation of the assay as we also found and attempted to solve. The fact that 7 of 11 participating laboratories (63.6%) reported higher antibody concentrations for low-titered sera than was expected on the basis of the RABA provides clear evidence that the problem does exist. We cannot agree with Dr. Madore's and Quataert's conclusion that such a high percentage of well-referenced laboratories is not able to correct for background binding. Of course the assumption that indirect ELISAs may be sensitive to low levels of endotoxin contamination can be a valuable hypothesis that should be demonstrated. In our case, we have performed the assays in a sterile and pyrogen-free environment. Perhaps in some laboratories it would be more cumbersome to work in a sterile and pyrogen-free environment than to perform a competitive assay. The background problem, which varied from serum to serum, was encountered in both our laboratories (Chiron and RIVM), thus increasing the percentage of laboratories with the problem. We encountered the background problem both with 1:20 and 1:50 starting dilutions as well as with high-titered samples, where the impact on the result was less profound. As the background binding was not observed with all serum samples, the problem also seems to be related to the quality of the serum.

The background problem might not really be so important indeed, but we wanted to provide a solution to this phenomenon since the results we obtained were used to evaluate the effectiveness of anti-Hib vaccines. Therefore we preferred to exclude doubtful or borderline results from the percentages of vaccinees. The more restrictive the assay the more reliable the effectiveness of the vaccine will be.

In conclusion, we think that a more stringent assay may be useful and, perhaps, can avoid the laboratory dependence of the assay evidenced in the interlaboratory study as well as the dependence of the assay on the quality of the serum samples used.

M. Mariani
Immunology Department
Chiron Research Centre
Siena, Italy

G. A. M. Berbers
Laboratory for Clinical Vaccine Research
RIVM
Bilthoven, The Netherlands