

Quality Assurance Considerations in *Cryptosporidium* Antibody Tests

Priest et al. (12) reported a comparison of serological techniques to detect responses to two *Cryptosporidium* antigens. They compared a low-cost minigel format blot assay that we developed with a Centers for Disease Control and Prevention (CDC)-developed enzyme-linked immunosorbent assay (ELISA) and a large-format gel. The minigel analysis was performed in a British Columbia laboratory. The minigel and large-format gel detected similar positive and negative responses to the 15- and 17-kDa antigens; however, the CDC ELISA had 23% false-positive responses and 12% false-negative responses to the 15- to 17-kDa antigen. For the 27-kDa antigen, the large-format gel detected more positive responses than did either the minigel or ELISA.

In 1996, we trained the British Columbia staff in Albuquerque to conduct our minigel assay and provided copies of our quality assurance (QA) procedures. These procedures require computer imaging of the blots and rejecting blots with a weak positive control (PC) or when the coefficient of variation (CV) for duplicate PCs is greater than 35% (3). When contacted, Dr. Priest stated that the blots used in their publication were not computer imaged and that QA procedures were informally conducted by manual inspection. Although we requested computer images of these blots for additional QA evaluation, they were not made available to us.

To reduce costs, the minigel uses only a small fraction of the antigen needed for either the ELISA or large-format blot. As a result, considerable care must be taken to minimize sources of variance and to insure that the antigen is uniformly applied across the blot. In addition, since we are interested in both detecting a response and measuring the intensity of that response, it is critical that blot performance be continuously monitored. Since responses tend to fade over time, the imaging must be done shortly after the blots are completed to avoid underestimating the CV.

Because the cost of the minigel assay is dramatically lower than the cost of the large-format gel assay and, we believe, lower than the cost of the ELISA, we compared performance of the minigel to that of the large-format gel. The large-format gel assay was used as the standard of comparison by Priest et al. We compared detection of a response to each antigen for 42 samples from two prior studies (3, 4). Concordance in detection was 79% for the 15- to 17-kDa antigen and 86% for the 27-kDa antigen. The minigel detected six responses to the 15- to 17-kDa antigen and three to the 27-kDa antigen that the large gel missed. The large gel detected three responses to the 15- to 17-kDa antigen and three to the 27-kDa antigen that the minigel missed. These data indicate that the minigel assay, with appropriate quality control procedures, can generate findings comparable to those for the large-format gel assay at a small fraction of the cost.

Most importantly, we have used the miniblotted assay to repeatedly relate a consistent set of drinking water and recreational water exposures as well as individual risk factors to increased occurrence and intensity of serological responses and seroconversions (1, 2, 5–11; F. J. Frost and F. G. Graun, Letter, *Infect. Immun.* **66**:4008–4009, 1999). Had the miniblotted assay inaccurately detected responses or estimated the intensities of the responses, we would not have replicated a consistent set of interpretable findings in different settings, in different coun-

tries, and on different continents in both cross-sectional and longitudinal studies. The miniblotted assay also generated a very detailed description of increasing and declining intensity of serological responses to an accidental *Cryptosporidium* infection (T. B. Muller, F. J. Frost, G. F. Craun, and R. L. Calderon, Letter, *Infect. Immun.* **66**:4008–4009, 2001). This could not have occurred with the minigel blot performance described by Priest et al.

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Floyd Frost*
Tim Muller
Twila Kunde
Lovelace Clinic Foundation
2425 Ridgecrest Drive S.E.
Albuquerque, NM 87108

Gunther Craun
Gunther F. Craun and Associates
101 West Frederick St., Suite 205
Staunton, VA 24401

*Phone: (505) 348-8776
Fax: (505) 348-4802
E-mail: ffrost@lrri.org

Authors' Reply

The main purpose of our recently published study (3) was to document the ability of two newly developed ELISAs to detect antigen-specific serum immunoglobulin G antibody responses

and to describe the course of these antibody responses in serial samples from laboratory-confirmed cryptosporidiosis patients. We ran both large-gel format and minigel format Western blots in an effort to determine which was better suited as a "gold standard" for comparison with the ELISAs. We found that, although the minigel format blot did detect most of the 17-kDa antigen responses, it was less able to detect responses to the 27-kDa antigen (29% false-negative rate). We attributed this finding to the fact that the minigel format Western blot, as described by Frost et al. (1, 2), uses less than 10% of the antigen per mm of membrane used in the large-format blot developed in our laboratory (3). We concluded that, although neither the Triton antigen ELISA nor the recombinant 27-kDa antigen ELISA were 100% sensitive or specific relative to the large-format Western blot, these assays were better suited for use in large-scale epidemiologic studies than were either of the blot format assays.

We have reviewed the 18 minigel format Western blots that were used in our recent study on the kinetics of the antibody response to *Cryptosporidium parvum* and have compared the results from our blots to those reported by Frost et al. in their recent study (1). Although we did not feel it necessary to repeat the details of the Frost et al. quality control protocol in our publication, we did indeed scan each of the blots immediately after the development was complete, and we recorded a digital image for later analysis. The "volumes" (a function of the area and of the pixel intensity in the selected area) of the bands corresponding to the 27- and 17-kDa antigens in each of the sample and control lanes were determined by using commercially available integration software. From an analysis of the 27- and 17-kDa antigen volume results in the PC lanes we have concluded that none of the blots could be rejected on the basis of a weak PC response (values within 2 standard deviations of the mean response) (1) or on the basis of an uneven antigen distribution across the blot (CV below 35% for replicates on same blot) (1). The results of the comparison of 42 samples by large-gel format and minigel format Western blots described by Frost et al. in the above letter are at variance with our own results obtained from 233 samples, but we are unable to evaluate either their methods or the quality of their results from the description provided in their letter.

The lower sensitivity of the minigel format blot assay for the detection of responses to the 27-kDa antigen does not detract from its potential utility as an epidemiologic tool, nor does the lower sensitivity imply that the proper use of such a tool will generate inconsistent results. In our opinion, however, we would suggest that the Western blotting approach described by

Frost et al. (1, 2), even with their considerable efforts and expertise in quality control, is only semiquantitative. We would further suggest that the digital integration of the blot responses is valid only within a narrow range and that these results are subject to considerable variability; for instance, our 27-kDa antigen PC values were distributed over a 2.5-fold range, whereas those described by Frost et al. (1) were distributed over a 7.5-fold range (from 143 to 1,078 integration units). The main intent of our publication was to demonstrate that an ELISA format could be used to circumvent the issues related to the quantitation of bands on blots and that the quantitative results obtained by ELISA compared favorably with the qualitative results obtained by Western blotting. Furthermore, the ELISA format uses less antigen per sample than the minigel format assay and is considerably cheaper in terms of time and reagents. We believe that, with the proper control sera, the ELISA is an attractive alternative to the Western blot assay.

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Jeffrey W. Priest*
Patrick J. Lammie
Division of Parasitic Diseases
Centers for Disease Control and Prevention
Atlanta, GA 30341

Anna Li
Mohamad Khan
Corinne S. Ong
Judith Isaac-Renton
Department of Laboratory Pathology and
Laboratory Medicine
University of British Columbia and British
Columbia Centre for Disease Control
Laboratory Services
Vancouver, British Columbia V5Z 4R4, Canada

*Phone: (770) 488-4587
 Fax: (770) 488-4108
 E-mail: jpriest@cdc.gov