Letters to the Editor

Tox-A Test for Clostridium difficile

In recent articles (1, 4), the results of studies comparing several of the Clostridium difficile toxin enzyme-linked immunosorbent assays (ELISAs) with tissue culture assay were presented and points were made concerning the low specificity and high rate of indeterminate results with the Tox-A Test. We point out that the Tox-A Tests used in those studies were labeled “For Investigational Use Only” and that they are not the same test that has been approved for in vitro diagnostic use.

The approved Tox-A Test exhibits higher specificity and fewer indeterminate results than those in these two articles. This statement is based on the results from a number of studies performed at various locations around the country. In our clinical trials, which were performed at six different locations and which involved the analysis of 1,130 specimens, the Tox-A Test exhibited a sensitivity and specificity of 91.9 and 97.4%, respectively, and the predictive positive and negative values were 84.5 and 98.7%, respectively, when the test was compared with tissue culture and/or toxigenic culture. The overall correlation of the Tox-A Test with tissue culture assay and/or toxigenic culture was 96.7%, and the indeterminate rate was 1.7%.

In studies presented at the 93rd ASM General Meeting last year (2, 3), the Tox-A Test was compared with other C. difficile toxin ELISAs, including the Baxter EIA, the Analytab (Cytocline) EIA, and the Premier test. The results showed that the Tox-A Test exhibited performance characteristics similar to those of the other ELISAs. Specificities of 98.3 and 100% were reported for the Tox-A Test in those studies. In one of the studies, indeterminate rates of 5.1, 0.5, and 2.6% were reported for Cytocline, Premier, and the Tox-A Test, respectively. The results from a reference laboratory that routinely uses the Tox-A Test indicated a sensitivity of 80.3% and a specificity of 98.0% compared directly with tissue culture assay when used with more than 400 specimens. In addition, the predictive positive and negative values were 87.5 and 96.7%, respectively; the overall correlation was 95.4%; and the indeterminate rate was 3.0%.

REFERENCES

Author’s Reply

Because most laboratories cannot evaluate each new product that reaches the marketplace, they are dependent upon laboratories with sufficient staff and interest to perform and report their findings of new product evaluations. These evaluations are dependent upon manufacturers supplying their products to the investigators. Most manufacturers attempt to supply investigators with products that are as close as possible to those which they will market. However, products may not perform in these evaluations as well as the manufacturer would hope. When this occurs, three choices are left to the manufacturer: (i) do nothing to the product and hope for the best, especially if it has received Food and Drug Administration approval, (ii) modify the product to improve its performance characteristics, or (iii) abandon the product. Without published, critical evaluations of the product the first strategy would be much more common than many of us would like to think. Because of critical evaluations, the second strategy is frequently followed.

Recently published abstracts indicate that improvements have been made to the TechLab Tox-A Test for detection of C. difficile toxin A. It is clear that the manufacturer deemed the second strategy to be more appropriate than the first strategy. For this, it should be commended. My coauthors and I await with interest published data in refereed journals corroborating these findings.

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Additional Data on Clinical Isolates of Campylobacter mucosalis

Figura et al. (3) reported the first isolation of Campylobacter mucosalis from children with enteritis. We disputed this identification (5), indicating that phenotypic tests were unreliable and that molecular studies must be done for positive identification of C. mucosalis. Dr. Figura (3) indicated that these presumed C. mucosalis strains had been deposited at the National Collection of Type Cultures (NCTC), London, as NCTC 12407 and NCTC 12408. We obtained strain NCTC 12408, but strain NCTC 12407 was not available from NCTC. Dr. Figura was unable to supply us with these strains (2a).

The Red Cross Hospital microbiology laboratory uses filtration and incubation in both a microaerophilic and an H2-enhanced (Oxoid BR 38; no catalyst) microaerophilic atmosphere for the isolation of bacterial pathogens (4). In a
40-month period, from October 1990 to January 1994, 1,849 strains of 12 species or subspecies of *Campylobacter*, *Helicobacter*, and *Arcobacter* were isolated from 7,680 diarrheal stool samples. Two hundred and fifty-five (13.8%) of these isolates were dependent on H2-enhanced microaerophilic growth conditions, an essential requirement of *C. concisus*, *C. mucosalis*, *C. curvis*, and *C. rectus* (7).

All 253 clinical isolates plus isolate NCTC 12408 were positive for oxidase and nitrate reductase but were negative for catalase, indoxyl acetate, hippurate, and urease. Every isolate produced abundant H2S, detectable in triple sugar iron agar and often completely blackening lead acetate strips. Isolates tolerate 1% glucose but not 3.5% NaCl. These phenotypic characteristics are shared by *C. concisus* and *C. mucosalis* (7). *C. mucosalis* can be differentiated from *C. concisus* by its ability to grow at 25°C, its cephalothin sensitivity, and its dirty yellow colony color (6). The issue of growth of *C. mucosalis* at 25°C is controversial (6, 7). Almost all the clinical isolates, plus isolate NCTC 12408, did not grow at 25°C.

Fifteen clinical isolates that were cephalothin sensitive (inhibitory zone sizes of up to 40 mm) and that had a dirty yellow colony color were selected. In other words, as defined by phenotypic criteria, these isolates were *C. mucosalis*. All 15 clinical isolates plus isolate NCTC 12408 were positive for both arylsulfatase and pyrazinamidase, which is characteristic of *C. concisus* and not *C. mucosalis* (1). High-stringency DNA-DNA hybridization studies were performed with these probes: *C. mucosalis* NCTC 11000T, *C. concisus* NCTC 11485T, *C. curvis* NCTC 11649T, and *C. rectus* NCTC 11489T. Isolate NCTC 12408 and all 15 clinical isolates reacted strongly with the *C. concisus* probe and did not react with any other probe. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (2) confirmed that the 16 isolates were *C. concisus*.

The phenotypic description of *C. mucosalis* isolates was based on only a few isolates (6). Isolate NCTC 12408 was characterized at NCTC by phenotypic, not molecular, methods as “*C. mucosalis*-like” (5a). As more and more clinical laboratories adopt filtration and H2-enhanced microaerophilic growth conditions, the problems of differentiating *C. mucosalis* and *C. concisus* and related species are becoming apparent. Molecular methods must be used for the precise identification of these underdetected pathogens.

REFERENCES
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5a. Owen, R. Personal communication.

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Author’s Reply
The genomic tests carried out by Dr. Lastovica et al. indicate that strain NCTC 12408 is *Campylobacter concisus*. The fact that NCTC 12407, the other strain phenotypically identified as *C. mucosalis*, was not available because it is nonvital (1) hampers its identification at the genomic level. However, it is highly probable that it is *C. concisus* too, since biochemical tests that we performed gave the same results obtained with NCTC 12408.

The letter by Dr. Lastovica also suggests that the results of biochemical, tolerance, and susceptibility tests, even if confirmed by a reference center, are of little usefulness with “unusual” campylobacters. I therefore wonder whether genomic assays ought to be carried out also for the most common campylobacter isolates, like *C. jejuni* and *C. coli*, which routinely are differentiated by only one test, hippurate hydrolysis.

In any case, researchers who still want to rely on phenotypic criteria for the identification of H2-dependent campylobacters have to bear in mind that susceptibility to cephalothin and production of a dirty yellow pigment are characteristics not of *C. mucosalis* but of *C. concisus* strains, as Dr. Lastovica et al. claim in their letter.

REFERENCE
1. Lastovica, A. J. Personal communication.

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Pitfalls in Immunoblot Detection of *Aspergillus* Antigens Associated with Invasive Infection

Haynes et al. published an article about the immunoblot detection of *Aspergillus* antigens associated with invasive infection in humans (2). Since invasive aspergillosis is a life-threatening disease to immunocompromised patients, fast and