

Short communication

Evaluation of two enzyme immunoassays for detection of *Clostridium difficile* toxins A and B in swine

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Abstract

Diagnosis of *Clostridium difficile*-associated disease (CDAD) in neonatal pigs is accomplished, in part, by detection of toxins A (TcdA) and B (TcdB) in feces or colonic contents. Samples ($n = 115$) were tested simultaneously with two toxin assays (*Clostridium difficile* Tox A/B IITM, TechLab, Blacksburg, VA; Gastro-tect *Clostridium difficile* Toxin A + B, Medical Chemical Corporation). Previous comparison of the Tox A/B II assay to the reference method (toxicity in CHO cell monolayers) revealed an overall correlation of 88%, with 91% sensitivity and 86% specificity, a positive predictive value of 86 and a negative predictive value of 84. In comparing the two EIAs, a group of nine samples were positive in both assays and a group of 92 were negative in both. However, 14 samples positive in the Tox A/B II were negative in the Gastro-tect assay. Thus, in comparison to the Tox A/B II assay, the Gastro-tect assay was 100% specific but only 39% sensitive. Its negative predictive value was 87, but its positive predictive value was 100. Thus, the Tox A/B II kit is apparently superior to the Gastro-tect Toxin A + B test for diagnosis of CDAD in neonatal pigs.

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1. Introduction

Clostridium difficile is well accepted as the etiologic agent of *C. difficile*-associated disease (CDAD) in neonatal pigs (Songer, 2004; Songer and Anderson, 2006; Songer et al., 2000a,b; Waters et al., 1998; Yaeger et al., 2002). Infected herds may

experience morbidity rates as high as 97% and, while most piglets recover, the mortality rate in some outbreaks may be as high as 16%. Affected pigs present with pasty, yellow diarrhea, but some may be constipated or even obstipated. Small intestine is not affected. Mesocolonic edema is common, though not pathognomonic. Focal neutrophil infiltration into colonic mucosa, and mucosal epithelial erosion or ulceration are characteristic. Exudation of neutrophils into colonic lumen forms the hallmark “volcano lesion”. Like human strains of *C. difficile*, most porcine strains produce both TcdA and TcdB.

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Comprehensive diagnosis of porcine CDAD includes clinical history, gross and microscopic pathology, bacteriologic culture, detection of TcdA and/or TcdB in feces or colonic contents. Toxin detection is the primary confirmatory CDAD diagnostic test for specimens obtained antemortem or postmortem. Toxicity for Chinese hamster ovary (CHO) cells is the reference method, but earlier we compared results of the cytotoxicity assay with those in a commercial enzyme immunoassay (Tox A/B; TechLab, Blacksburg, VA, USA). This EIA compared favorably to the cytotoxicity assay (Table 1), with sensitivity of 91%, specificity of 86%, positive predictive value of 84%, and negative predictive value of 86% (Post et al., 2002). In this work, we compared the Tox A/B assay to another commercial EIA for detection of toxins in fecal samples from neonatal pigs.

2. Materials and methods

2.1. Assays

In previous work, results from testing the *C. difficile* Tox A/B IITM (TechLab, Blacksburg, VA) had an overall correlation of 88% with those derived from the reference method, with 91% sensitivity, 86% specificity, 86% positive predictive value, and 84% negative predictive value (Table 1). In this work, we used the Tox A/B as the standard and compared the Gastro-tect *C. difficile* Toxin A + B Antigen Detection Kit (Medical Chemical Corporation, Torrance, CA) to it. Tox A/B II uses affinity-purified goat polyclonal antibodies for capture of TcdA and TcdB, while Gastro-tect Toxin A + B uses similar antibodies of rabbit origin. Detection antibodies in the Tox A/B kit are HRP-labelled mouse monoclonal (for TcdA) and goat polyclonal (for TcdB); those in the Toxin A + B kit are unlabelled chicken polyclonal for both toxins, with detection by a peroxidase-labelled anti-chicken

conjugate. The detection system in both is horseradish peroxidase, with tetra-methyl-benzidine as substrate.

2.2. Specimens

Fecal (collected by rectal swab) or intestinal content (collected at necropsy) samples ($n = 115$) were obtained in duplicate, using respective manufacturers' collection systems (Stool-PrepTM, TechLab; Gastro-Tect Quick 'N' Easy Fecal Sampling Device, Medical Chemical Corporation) and stored at 4 °C for ≤ 24 h prior to testing. EIAs were performed according to manufacturers' instructions. Color change was rated 0–4 per product specifications.

3. Results and discussion

Nine samples were positive by both tests and 92 were negative by both (Table 2). However, 14 samples which were positive by Tox A/B II were negative by Gastro-tect Toxin A + B. Thus, by comparison to the Tox A/B II, the Gastro-tect Toxin A + B was 100% specific but only 39% sensitive. The positive predictive value was 100 and the negative predictive value was 87 (Table 1). Furthermore, reaction scores on positive samples averaged 2.6 in the Tox A/B II and only 1.1 in Gastro-tect Toxin A + B.

Detection of *C. difficile* toxins in feces is a key element to CDAD diagnosis in both humans and domestic animals, and EIA is a desirable testing format due to the savings it affords in both time and labor. Tests with both kits examined here were completed in ≤ 1 h. Based upon the previously determined sensitivity and specificity of the Tox A/B II (Post et al., 2002), the Gastro-tect Toxin A + B failed to detect $>60\%$ of the positive samples. On the basis of these results, the Tox A/B II is clearly superior for use in diagnosis of porcine CDAD. These results

Table 1
Correlation of results of Tox A/B and cytotoxicity (Post et al., 2002)

| Tox A/B | Cytotoxicity assay | |
|----------|--------------------|----------|
| | Positive | Negative |
| Positive | 20 | 4 |
| Negative | 2 | 24 |

Table 2
Results of examination of 115 porcine fecal samples by two commercial enzyme immunoassays for detection of TcdA and TcdB

| Tox A/B II | Gastro-tect Toxin A + B | | |
|------------|-------------------------|----------|-------|
| | Positive | Negative | Total |
| Positive | 9 | 14 | 23 |
| Negative | 0 | 92 | 92 |
| Total | 9 | 106 | 115 |

say nothing about the utility of the Gastro-tect Toxin A + B for its intended purpose, namely diagnosis of human CDAD. Porcine specimens may contain substances which compromise its accuracy.

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