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Mitomycin Immunoblot Colony Assay for Detection of Shiga-Like Toxin-Producing *Escherichia coli* in Fecal Samples: Comparison with DNA Probes

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We developed a direct screening immunoblot assay for the detection of Shiga-like toxin (SLT)-producing organisms in stool samples. The assay takes advantage of the phage-mediated nature of SLT production in *Escherichia coli* and the phage-inducing effects of mitomycin. The addition of mitomycin significantly enhanced the amount of toxin available for immunologic detection. By using the mitomycin-enhanced immunoblot assay, SLT-producing *E. coli* could be distinguished from non-toxin-producing *E. coli* and normal stool flora in ratios of 1:1,000 to 1:5,000. The immunoblot assay was examined in a field setting and compared with direct DNA probing for SLT-I and SLT-II. The assay was able to detect SLT-producing *E. coli* with a high level of sensitivity and specificity. Specificity was markedly improved by using a monoclonal antibody which cross-reacts with both SLT-I and SLT-II B subunits in place of the polyclonal antitoxin sera. We conclude that the mitomycin-enhanced immunoblot colony assay is a rapid and reliable alternative to DNA probing for the detection of phage-mediated SLT-producing organisms in stool samples, especially when the production and use of nucleic acid probes are not feasible. In addition, it permits isolation of positive colonies for further study and confirmation.

Many strains of *Escherichia coli* produce potent protein toxins related to Shiga toxin of *Shigella dysenteriae* type 1, termed Shiga-like toxins (SLTs) or verotoxins (19). They have been divided into two groups on the basis of their immunologic characteristics: SLT-I, which cross-reacts with antibody directed against Shiga toxin, and SLT-II, which is not immunologically cross-reactive with polyclonal anti-Shiga toxin antibody (20). *E. coli* strains that produce high levels of Shiga-like toxins have been epidemiologically linked to human disease, including hemorrhagic colitis, the hemolytic-uremic syndrome (HUS), and thrombotic thrombocytopenic purpura (9, 15, 20, 23), and are grouped together as enterohemorrhagic *E. coli*. Although *E. coli* O157:H7 has been implicated in many enterohemorrhagic *E. coli* outbreaks in the developed world, at least 50 other toxin-producing serotypes have also been associated with the development of HUS and/or hemorrhagic colitis (11, 20). Thus, SLT production, and not the O157:H7 serotype, is the better marker of the pathogenic potential of a strain.

Our understanding of the etiology, epidemiology, and pathogenesis of HUS has been hampered by the lack of a simple, rapid method for detecting SLT-producing *E. coli* in stool specimens. Current techniques to identify *E. coli* O157:H7 rely on the use of sorbitol MacConkey medium and serotyping of sorbitol-negative colonies (20). This limits the numbers of isolates tested and does not detect the *E. coli* serotypes other than O157:H7 implicated in hemorrhagic colitis and HUS. DNA probes for the genes encoding SLT-I and SLT-II have been constructed and used to screen colonies from cultures of stool samples for EHEC (3, 24). As it is currently applied, this technique is hampered by the need to isolate individual *E. coli* colonies and is limited to centers with appropriate technological abilities. Polymerase chain reaction techniques have also been used as tools for the detection of SLT-producing organisms in stool samples; however, the polymerase chain reaction is not routinely used in clinical laboratories and may not be available in developing countries (7, 22).

The genes for SLT-I and SLT-II in *E. coli* are carried on bacteriophages (18, 21). Vegetative growth of these bacteriophages can be induced by exposure to UV radiation or the cancer chemotherapeutic agent mitomycin. We have previously demonstrated that incubation of SLT-producing *E. coli* in medium containing mitomycin induces both bacteriophage replication and toxin expression (1). This method has been extended for possible diagnostic use by incorporating mitomycin into agar plates and screening the cultures grown on these plates for toxin production by immunoblotting. Our strategy was to develop an assay to screen for SLT-producing *E. coli* in stool samples. The assay takes advantage of the ability of mitomycin to induce phage and therefore increase the amount of toxin produced and available for immunologic detection (6).

Here we report the development and field testing of a mitomycin-enhanced immunoblot colony assay for detecting SLT-producing organisms in fecal specimens and compare the results with those from DNA probing of blots of stool samples for the SLT-I and SLT-II genes in fecal samples of 100 Thai children with diarrhea and 100 dairy cows (which are known to have a high prevalence of SLT-producing organisms) (25).

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MATERIALS AND METHODS

Fecal samples. Stool samples were collected from 100 children under age 5 years seen at a pediatric outpatient clinic in Bangkok, Thailand. Children were included in the study if they had diarrhea of more than 24 h in duration consisting of at least three diarrheal stools per day. Children with diarrhea were included in the study whether or not they had blood in their stool, but they were excluded if they were reported to have received antibiotic therapy in the previous month. Specimens were processed within 12 h of collection.

Rectal swabs were collected from dairy cattle at three farms outside of Bangkok. Three swabs were taken concurrently from each animal, transported to the laboratory in Cary-Blair medium, and processed within 24 h of collection.

Plating procedure. Stool homogenates (approximately 1 g) or rectal swabs were mixed with 5 ml of Luria broth, and serial dilutions (10^{-2} to 10^{-8}) were inoculated onto MacConkey agar plates (Difco, Detroit, Mich.) and the plates were incubated overnight at 37°C. Syncase agar plates containing 25 ng of mitomycin (Sigma, St. Louis, Mo.) per ml were prepared. Two 0.45-μm-pore-size nitrocellulose filters (Schleicher & Schuell, Keene, N.H.) were placed on top of each other on the syncase agar plate. By using standard replicate plating techniques, two replicate platings (one for testing for SLT-I-positive colonies and one for testing for SLT-II-positive colonies) were then made onto the top filter of the pair of filters from the MacConkey agar plates inoculated with the dilution of stool which yielded approximately 200 to 400 colonies per plate. In some experiments bacterial suspensions or stool homogenates were plated directly onto the top nitrocellulose filter. Following overnight incubation of the filter-agar plate combination at 37°C, the upper filters were used for DNA hybridization and the lower filters were used for the colony immunoblot assay.

Bacterial strains. The *E. coli* reference strains used in the present study included the food isolate O157:H7 strain 933, which produces both SLT-I and SLT-II; the lysogen C600 (933J), an SLT-I-producing strain; the lysogen C600(933W), an SLT-II-producing strain; and C600, a K-12 strain which does not produce SLTs.

Antibodies. Rabbits were inoculated with either formalin-treated purified Shiga toxin or SLT-II, and polyclonal serum was collected as described previously (5). A mouse monoclonal antibody, 4D1, directed against the B subunit of both Shiga toxin and SLT-II was obtained from mouse ascitic fluid as reported previously (4) (limited amounts of mouse ascitic fluid containing monoclonal antibody 4D1 are available upon request). To diminish the background when the assay was performed on stool specimens, all polyclonal rabbit serum (PRS) used in these experiments was preadsorbed with polymyxin B-extracted *E. coli* C600 for 2 h. Adsorbed sera were prepared as follows. A 2-ml overnight culture of *E. coli* C600 in Luria broth was spun at 5,000 rpm for 5 min. The cell pellet was resuspended in 1 ml of a 2-mg/ml solution of polymyxin B (Sigma) for 10 min at 4°C and was spun at 5,000 rpm for 3 min to remove bacterial debris. The polymyxin B extract (0.1 ml) was spotted onto nitrocellulose strips, rinsed twice in 10 mM Tris–150 mM NaCl–0.05% Tween (pH 8) (TNT) for 30 min, and blocked for 1 h with TNT containing 5% nonfat milk. The PRS was incubated with the strips (20 ml of 1:20,000-diluted sera per strip) for 30 min, and the resulting antiserum was used in the immunoblot assays.

Colony blot assay. After overnight growth, the lower nitrocellulose disks were removed from the mitomycin-containing syncase agar plate and immersed in a chloroform bath for 15 min. The filters were then blocked for 1 h with 5% nonfat milk in TNT. The filters were incubated for 1 h in a 1:20,000 dilution of preadsorbed PRS raised against Shiga toxin or SLT-II or a 1:20,000 dilution of the monoclonal antibody 4D1 (4). After incubation with PRS or monoclonal antibody, the filters were given three 5-min washes with TNT; this was followed by a 1-h incubation with alkaline phosphatase-conjugated goat anti-rabbit immunoglobulin G (Promega) for PRS or goat anti-mouse immunoglobulin G for the monoclonal antibody; this was followed by three further 5-min washes in TNT. The filters were then color developed with nitroblue tetrazolium and 5-bromo-4-chloro-3-indolylphosphate (Promega). Positive controls consisted of *E. coli* C600(933J) (SLT-I producer), C600(933W) (SLT-II producer), and O157:H7 strain 933 (SLT-I and SLT-II producer). *E. coli* C600 was used as a negative control.

DNA hybridization assays. The DNA probe for the SLT-I gene was a 1,142-bp *Bam*HI fragment isolated from recombinant plasmid pJN 37–19, and the probe for the SLT-II gene was an 842-bp *Sma*I-*Pst*I fragment isolated from recombinant plasmid pNN100-18 (17). Fragments were labeled with ^{32}P-labeled deoxynucleotide triphosphates (Dupont, NEN Research Products, Boston, Mass.) by nick translation (13). The top filters were then used for DNA probing by using standard techniques (14). Hybridization was carried out under stringent conditions (16). The controls were identical to those used for the colony blot assay.

Reprobing. All blot- or probe-positive colonies from the fecal samples were picked from a replicate plate and were grown as single colonies on filters in order to confirm the SLT genotype and phenotype by DNA probe and immunoblot techniques, respectively. When the immunoblot colony assay and the DNA probing had discordant results, we attempted to save samples for subsequent study by plating them on 10% milk agar slants.

Toxin assay. Production of Shiga toxin and SLT-II by probe- or immunoblot-positive colonies was confirmed by either an immunologic or a biochemical assay by using the P_1 glycoprotein enzyme-linked immunosorbent assay (ELISA) (2) or by measuring inhibition of protein synthesis in Vero cell monolayers (10). Briefly, the P_1 glycoprotein ELISA is based on the use of crude hydatid cyst fluid material containing toxin receptor analog glycoproteins. The hydatid cyst fluid material acts as the capture step in the assay. Toxin is released from the bacterial periplasmic space by polymyxin B, and P_1 glycoprotein-bound toxin is then detected by using a rabbit polyclonal antibody to the specific toxin being tested; this is followed by detection with a goat anti-rabbit immunoglobulin G alkaline phosphatase-conjugated antibody (Sigma) and phosphatase substrate (104; Sigma).

Cytotoxicity was assayed by measuring the effect of polymyxin B extracts from probe- or blot-positive samples on [^{3}H]leucine incorporation by Vero cells (10).

RESULTS

Optimization of mitomycin-enhanced immunoblot colony assay. When serial dilutions of toxins were spotted directly onto nitrocellulose filters, the immunoblot assay was able to detect Shiga toxin at a level of 10 pg and SLT-II at a level of 100 pg. To optimize colony assay conditions for detection of toxin producers, serial dilutions of mitomycin were added to syncase agar to achieve a final concentration of between 6.25 and 1,500 ng/ml. C600(933J), an SLT-I producer, and C600 (933W), an SLT-II producer, when grown on a nitrocellulose

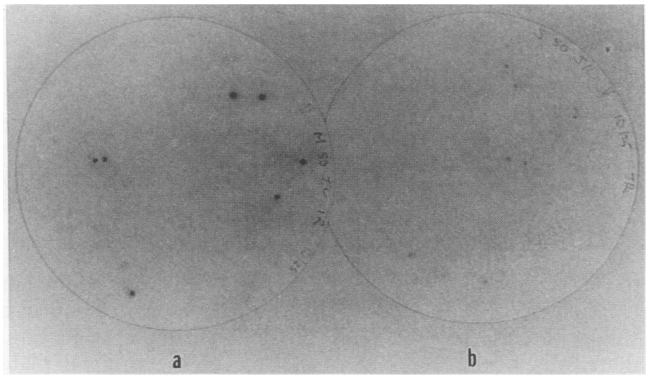

FIG. 1. Immunoblot colony assay performed with a mixture of *E. coli* C600(933J), an SLT-I-producing strain, and *E. coli* C600, a strain which does not produce toxin, in a ratio of 1:1,000. Filter A represents the assay performed with a syncase plate containing mitomycin, and filter B is the same assay performed on a plate without the addition of mitomycin.

TABLE 1. Enteric organisms other than SLT-producing E. coli identified in 100 Thai children with diarrhea

Enteric organism	% of cases
Shigella spp.[a]	3
Campylobacter jejuni[b]	11
Campylobacter coli[a]	1
Enteroinvasive *E. coli*[b]	0
Enterotoxigenic *E. coli*	5
LT[c]	1
ST[d]	1
LT[c] and ST[d]	3
Salmonella spp.[a]	16
Rotavirus[e]	18
Vibrio spp.[a]	1
Aeromonas spp.[a]	2
Plesiomonas spp.[a]	1
No pathogens identified	65

[a] Determined by standard microbiological techniques.
[b] Determined by DNA probing.
[c] LT, heat-labile toxin, determined by the Y-1 assay.
[d] ST, heat-stable toxin, determined by the suckling mouse assay.
[e] Determined by ELISA.

filter over syncase agar, both produced sufficient toxin to result in a visible immunoblot. Significant enhancement of this toxin production, resulting in larger and denser immunoblots, was noted with both C600(933J) and C600(933W) when 6.25 ng of mitomycin per ml was used. Enhancement was maximal at a concentration of 12.5 to 25 ng/ml, while colony counts fell when concentrations of 100 ng/ml were exceeded. On the basis of these findings, a concentration of 25 ng of mitomycin per ml was used for all further studies. The signal produced by *E. coli* O157:H7 (strain 933) was also enhanced at this concentration. To define the optimum shelf-life of the assay plates, syncase plates containing mitomycin were prepared and then used in the immunoblot colony assay after up to 90 days of storage at 4°C protected from light, and there was no significant loss of enhancing ability.

Studies were carried out by mixing *E. coli* C600(933J) and *E. coli* C600 in ratios ranging from 1:10 to 1:5,000. Toxin-producing colonies resulted in a visible immunoblot within a solid lawn of nontoxin producers in a ratio of as great as 1:5,000, even when they were plated over syncase agar without mitomycin. The addition of 25 ng of mitomycin per ml enhanced this reaction significantly (Fig. 1). Similar results were achieved with strain C600(933W) and *E. coli* O157:H7 strain 933, which were readily visually differentiated from non-toxin-producing *E. coli* C600 when present in a ratio of 1:1,000. Assay-to-assay variation in the strength of the immunoblot reaction was observed, but the relative difference between positive and negative strains was always easily discernible. In order to confirm the specificity of the assay, 5 immunoblot-positive and 10 immunoblot-negative colonies were picked from a replica plate of an assay plate set up with a high ratio of toxin producers to nontoxin producers and a low plating density. The selected colonies were grown overnight in syncase medium and assayed by ELISA for toxin released by polymyxin B extraction. No false-positive or false-negative results were detected.

Identifying toxin-producing organisms in stool samples. When stool samples were used in the assay, weakly positive reactions with non-SLT-producing enteric bacteria were occasionally detected. These reactions were minimized by using for the immunoblot the bottom filter from a double set of filters layered onto the same plate. Serial dilutions of stool homogenates from normal hosts, documented to be colony blot negative, were plated to determine bacterial density and were then spiked with low levels (1:1,000 to 1:5,000) of SLT-I-producing strains [O157:H7 strain 933 and C600 (933J)] and SLT-II-producing strains [O157:H7 strain 933, C600(933W)]. Toxin-producing *E. coli* was readily visually distinguishable from the normal enteric flora in the spiked stool specimens.

Field studies. Enteric pathogens were identified in 35 of 100 Thai pediatric patients with diarrhea by a variety of techniques (Table 1). Thirty-nine of the 100 children studied had bloody diarrhea.

Table 2 shows the results of the DNA probing and mitomycin-enhanced colony blot assay with polyclonal rabbit sera for SLT-I- and SLT-II-producing organisms in the stools of the 100 patients. Only 2 stool samples, both from children with bloody diarrhea and without other identifiable pathogens, were positive and 91 stool samples were negative for SLT-I by both assays. Interestingly, one of the two positive specimens was initially DNA probe negative. However, on repeat testing of the isolated immunoblot-positive colony, it was found to hybridize with the SLT-I probe. This

TABLE 2. Colony immunoblot data and DNA probing data from 100 Thai children with diarrhea and 100 cattle fecal specimens[a]

Test group and toxin	No. of samples			
	Blot positive, probe positive	Blot negative, probe negative	Blot positive, probe negative	Blot negative, probe positive
Thai children				
SLT-I	2	91	6	1[b]
SLT-II	0	99	0	1[b]
Cattle				
SLT[c]	43	41	11	5
SLT-I	35	46	16	3
SLT-II	18	64	8	10

[a] All colony blot assays described here were performed with polyclonal rabbit sera raised against SLT-I or SLT-II.
[b] Repeat probing of the isolated colony showed that it was negative.
[c] This row of data represents samples in which either toxin was identified.

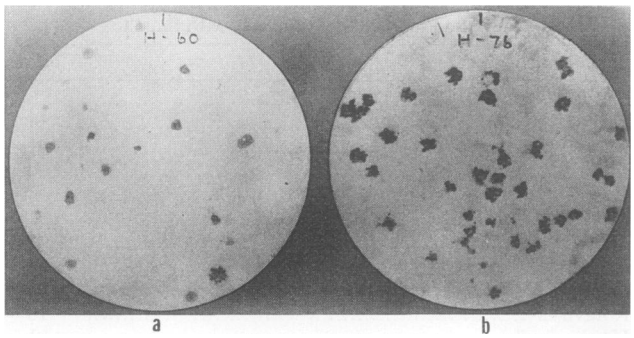

FIG. 2. Representative examples of fecal specimens from cattle which are positive for SLT-II-producing (A) and SLT-I-producing (B) organisms by the mitomycin-enhanced immunoblot colony assay.

isolate also made a neutralizable cytotoxin. Six samples were positive for SLT-I by immunoblotting but were negative by DNA probing. None of these samples produced cytotoxin, and when these colonies were reexamined by immunoblotting with a monoclonal antibody (4D1) which reacts with the B subunit of both SLT-I and SLT-II, none of the samples were positive. These data suggest that the polyclonal antibody-based assay has some degree of nonspecific reactivity.

None of the 100 samples were positive for SLT-II by either assay. One sample was positive by DNA probing but negative by the immunoblot assay. However, repeat probing of the isolated positive colony was negative for SLT-II by both techniques, suggesting that this represents either an initial false-positive DNA probe assay or inaccurate colony isolation.

Results for the cattle specimens are shown in Table 2 and Fig. 2. These data are somewhat more complex than those for the Thai children because many samples were positive for both toxins by one or the other technique. Considering SLT-I alone first, 54 samples were positive by at least one technique, including 35 that were positive by both assays, 16 that were positive only by immunoblotting, and 3 that were positive only by DNA probing. Of the 36 samples positive for SLT-II by at least one technique, 18 were identified by both assays, 8 were positive only by immunoblotting, and 10 reacted only with the DNA probe. Overall, 43 samples were positive for one or both toxins by both methods. These 43 included 5 samples that were originally probe negative but immunoblot positive and that were subsequently found to be probe positive when individually picked colonies were reprobed. These five samples therefore appear to be false negative by DNA probing.

Of the 16 available samples which had discordant results by the two techniques, 11 were immunoblot positive and probe negative for either or both toxins. Five other specimens were positive for SLT-I and/or SLT-II by DNA probing and negative for either toxin by immunoblotting. Some of the discordant results for the individual toxins may be attributable to cross-reactivity of the PRS for the SLT-I and SLT-II which were used in the initial immunoblot colony assay. For example, some samples that were positive for only SLT-II by DNA probing were positive for both SLT-I and SLT-II by the immunoblot assay. Alternatively, some colonies may have lost the SLT genes between the time of original plating and the subsequent subculture for DNA probing, as described recently (8).

With regard to further characterization of the 16 discordant samples, none of the 11 blot-positive, probe-negative colonies produced demonstrable Vero cell cytotoxins. These false-positive immunoblot reactions were eliminated when the monoclonal antibody 4D1 was substituted in the assay for the polyclonal antisera, and may therefore be classified as false-positive immunoblots by using polyclonal antibody. The five DNA probe-positive and immunoblot-negative samples failed to grow upon subculture and could not be further characterized. Since the DNA probing was performed in a single batch 2 months after the initial sample collection, there was a long interval of storage of the original plate before the subsequent probe experiments. Thus, while blot-positive colonies could be picked within 3 days for gene probing, probe-positive, blot-negative colonies had to be recovered from the stored plates after probing data were available.

Although there is no acknowledged standard for the direct detection of SLT-producing organisms in fecal specimens, we arbitrarily considered gene probing to be the measure against which to compare the sensitivity and specificity of the colony immunoblot method. Among the 200 samples evaluated, the sensitivity of the colony blot was 88% and the specificity was 89%.

DISCUSSION

Currently available methods for the detection of SLT-producing organisms directly in fecal samples lack the combination of sensitivity and simplicity necessary for the diagnostic laboratory. Because of the increasing number of patients reported to have HUS and hemorrhagic colitis secondary to infection with organisms that produce SLTs, an improved and rapid assay is needed. Detection of *E. coli* O157:H7 by screening sorbitol-negative colonies for the appropriate O and H antigens is limited by the time required and the inability to detect sorbitol-positive SLT-producing organisms of other serotypes. Assay of stool filtrates for cytotoxic activity requires the identification of neutralizable cytotoxicity, which involves tissue culture techniques and a considerable outlay of time. Use of polymerase chain reaction technology or DNA probing for individual toxins involves use of radionucleotides or expensive nonradioactive labeling techniques, is time-consuming, and is not yet routine in the diagnostic laboratory.

The mitomycin-enhanced colony blot assay has advantages over previous immunologically based assays for the detection of SLT-producing organisms in fecal samples because it incorporates a means of amplifying toxin production. Mitomycin has been shown to induce bacteriophage-mediated SLT production in *E. coli* strains (1). We demonstrated that the incorporation of mitomycin into culture plates in the colony blot assay increases the amount of toxin available for immunologic detection in several laboratory and native *E. coli* strains.

Use of the colony blot assay for screening of stool samples eliminates the need for selecting a limited number of individual colonies for screening purposes, can be completed within 24 h, and requires minimal technical expertise. In addition, the mitomycin plates have a long shelf-life, retaining their ability to enhance toxin production for at least 3 months when stored protected from light at 4°C. This would permit the use of preprepared plates and reagents for occasional screening. SLT-producing serotypes other than O157:

H7, such as *E. coli* O128, may also be detected by a mitomycin-enhanced assay (12).

Initially, when assay conditions were being optimized in the laboratory with spiked stool specimens, problems with false-positive results appeared to be eliminated with the use of a double layer of filters and chloroform treatment. However, when the polyclonal assay was then taken to the field setting for evaluation, discrepancies were encountered between the immunoblot assay and the DNA probing results. By using the polyclonal assay as performed in the field study, 6 to 11% of the fecal samples from humans and cattle were immunoblot positive and probe negative. When repeat immunoblotting of isolated positive colonies was performed with polyclonal serum, approximately 20% of these samples were immunoblot negative. While some of these may represent inaccurate colony isolation from the replica plate, when the immunoblot colony assay was later performed with a monoclonal antibody which is cross-reactive with the B subunits of SLT-I and SLT-II, all of these immunoblot-positive, probe-negative colonies were nonreactive and did not produce detectable cytotoxin. This suggests a significant degree of nonspecific reactivity with the polyclonal serum, generating false-positive results which could be eliminated by using the monoclonal antibody.

In addition, one human and five cattle blot-positive samples were initially DNA probe negative; however, when isolated immunoblot-positive colonies from these stool samples were reprobed, they were found to be DNA probe positive. Thus, DNA probing of stool cultures could be missing up to 5% of fecal samples containing SLT-producing organisms.

We were unable to further characterize the six probe-positive, immunoblot-negative isolates because of our inability to recover them on subculture. There are several possible explanations for this discordance. These organisms may contain the genes for the SLTs but express the protein only at low levels or may be poorly inducible in the presence of mitomycin. Alternatively, portions of the toxin genes may have become mutated in such a way that would allow the probe to recognize them but not allow them to produce immunologically recognizable toxin. The monoclonal antibody 4D1 recognizes only one epitope of the SLT B subunits, and therefore, any mutation of the B subunit genes or diminution in B subunit expression could affect the ability of the monoclonal antibody 4D1 immunoblot to identify an organism. A further point to bear in mind is that the two DNA probes used in the present study are primarily (or exclusively, in the case of the SLT-II probe) directed toward the gene for the A subunit. This fact could also explain the probe positivity in the context of a negative immunoblot reaction to a B subunit-directed monoclonal antibody.

The present study demonstrated that a small proportion of Thai children (approximately 2%) under age 5 years presenting with diarrhea carry SLT-producing organisms in their stools, while such strains are present in over 40% of Thai cattle. These findings confirm earlier reports of a low prevalence of SLT-producing *E. coli*-associated diarrhea in Thailand, despite the presence of SLT-producing organisms in cattle and meat products (26). It is not clear whether the SLT-producing *E. coli* found in cattle are pathogenic for humans or perhaps lack necessary attachment or colonizing factors. Although we did not perform serotyping of the SLT-producing strains identified in the present study, previous work (26) has demonstrated that organisms with a diversity of serotypes colonize these cattle. In addition, the colony blot assay was able to identify SLT-producing organisms in 2 of 39 (5%) of children presenting with bloody diarrhea, suggesting that the yield of the test may be greater if patients with bloody diarrhea were selectively screened.

In summary, the mitomycin-enhanced immunoblot colony assay is a rapid, reliable alternative to DNA probing for the detection of phage-mediated SLT-producing *E. coli*, especially when the production and use of nucleic acid probes are not feasible. We feel that the standard microbiological methods for the detection of SLT-producing *E. coli* are limited, because reliance on sorbitol MacConkey agar alone will certainly miss serotypes of SLT-producing *E. coli* other than O157:H7. Toxin assays of stool samples are fraught with difficulty and nonspecificity, and these problems are compounded by the multiplicity of immunologically distinct toxins. We used DNA probing as the best possible comparative test to the immunoblot examined in our study. What we have shown is that the colony blot assay is comparable to (and arguably better than) DNA probing and is certainly more suited to use in the field. Although there is a circularity in the argument for using DNA probing as the standard with which to compare our assay, in that there is no evidence that DNA probing is the ideal test, there was no other way for us to validate our assay. Our data also suggest that the use of the anti-B subunit monoclonal antibody in the assay dramatically improves the specificity of the test. The methodology can be used in the clinical laboratory in industrialized nations when early diagnosis of patients at risk for HUS may have therapeutic significance and for the etiologic diagnosis of those presenting with the manifestations of HUS. The simplicity of the method also makes it friendly for use in the field in developing country settings.

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