Effective Use of the Clinical Microbiology Laboratory for Diagnosing Diarrheal Diseases

Janet Hines and Irving Nachamkin

Diarrheal diseases are an enormous problem worldwide and cause considerable morbidity and mortality, especially in developing nations. Four times as many diarrhea-related deaths occur worldwide in a single day than occur in an entire year in the United States [1, 2]. Individuals who are at greater risk for developing diarrheal diseases than other segments of the population, especially in developed countries, include those in daycare centers, travelers, and patients with HIV infection. During the past decade, the HIV epidemic has resulted in a relatively large number of persons at high risk for developing infectious diarrhea, particularly that due to organisms heretofore unrecognized or unheard of in immunocompetent patients.

As the number of potential etiologic agents of diarrheal disease increases, the clinical microbiology laboratory takes on an increasingly important role in identifying these agents. Of the many tests performed in the clinical microbiology laboratory, there is virtually no more labor-intensive and costly routine test than that of the stool culture and parasitological examination. The microbiological workup of stool requires a specific algorithm to isolate and identify pathogens. Factors including the number of special media required, the labor intensiveness of the process, and the generally low sensitivity of stool cultures combine to make this test one of the most expensive diagnostic tests offered; the cost ranges from $100 to more than $100,000 per positive result, depending on the tests performed, the prevalence of disease, and number of samples tested [3–5].

While stool cultures and parasitological examinations are invaluable tools for epidemiological studies, the role of these cultures in the management of acute diarrhea has not been well defined because many episodes of acute diarrhea are self limited. It is estimated that >4 million tests for bacteria and parasites are performed in the United States annually; thus, efficient use of the laboratory in an era of cost containment is critical [6]. The purpose of this paper is to review the current understanding and limitations of the clinical microbiology laboratory in the management of diarrheal illness and to dispel some common beliefs about the usefulness—or lack thereof—of certain commonly performed tests.

The Role of Empirical Treatment Without Laboratory Support

Although there are epidemiological and public health concerns about tracking enteric pathogens and identifying potential outbreaks of infection, empirical therapy in the absence of microbiological evaluation is warranted for certain patient populations with acute enteric infections. The management of traveler’s diarrhea is a good example [7]. Furthermore, several studies have shown that empirical therapy for acute diarrheal disease (particularly with fluoroquinolones), regardless of the infecting organism, reduces number of stools as well as other signs and symptoms [8–11].

The downside of empirical therapy is that treatment can prolong carriage of Salmonella species and foster recurrence of the infection [12–14]. In addition, increasing resistance to fluoroquinolones, particularly among organisms such as Campylobacter species, may reduce the efficacy of empirical treatment, necessitating a more important role for the laboratory in directing specific therapy based on susceptibility results [15]. Studies are needed to examine the cost-benefit of empirical therapy, with or without laboratory support, to better define those patients who would benefit from having specific microbiological tests performed.

For Which Patients Should Routine Stool Cultures and Ova and Parasite Examinations Be Performed?

It has been common practice to request stool cultures and/or parasitological examinations for any patient with a diarrheal illness. However, in recent years it has become increasingly apparent that such routine testing is clinically ineffective and cost ineffective. Organisms such as Salmonella, Campylobacter, and Shigella, which are the most common causes of sporadic diarrheal disease in the United States, are for the most part community acquired and rarely cause nosocomial infections. The same holds true for parasites. Clostridium difficile, on the other hand, causes both community-acquired and nosocomial diarrheal disease. Routine stool cultures are frequently performed for hospitalized patients despite the fact that the yield of such cultures is low [3, 6, 16–20].

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On the basis of studies performed at our institution [6], we recommended that routine stool cultures and parasitological examinations be limited to outpatients or inpatients who have been hospitalized for ≤3 days; we call this recommendation the "3-day rule" (Table 1). We found that routine stool cultures that are performed for patients hospitalized for >3 days accounted for >50% of the laboratory's workload, and these cultures failed to yield a single previously unrecognized organism over a 3-year period. Other studies have confirmed this recommendation [16–18, 20], and the College of American Pathologists (CAP) recently recommended that the 3-day rule be used for routine stool cultures and that a 4-day rule be used for parasitological examinations [3]. The use of such recommendations may result in savings estimated as ranging from $27 million to $73 million annually [6, 20].

What Is a Routine Stool Culture?

In an attempt to control costs and time, specimen workups in microbiology laboratories are targeted at only those pathogens important in the respective patient population. Laboratorians and clinicians should work together to establish the common enteric pathogens present in their patient population and use that information to guide routine workups. 

Campylobacter, Salmonella, and Shigella are the most common bacterial agents of diarrheal disease and should always be sought in routine stool cultures. A recent CAP survey [3] showed that most laboratories (99.3%) included Salmonella and Shigella in the routine stool workup, but only 96% routinely included Campylobacter. Thus, there are still some laboratories that do not include even the most common pathogens in the routine workup. Other organisms such as Aeromonas, Plesiomonas, Yersinia, Escherichia coli O157, and Vibrio were included in the routine culture in 30%–60% of the laboratories in the survey. For example, our laboratory does not routinely examine all stool samples for Vibrio parahaemolyticus or Yersinia enterocolitica because of the results of previous surveys of our patient population. However, if a patient presented with a syndrome consistent with appendicitis due to Yersinia or had visited an area where V. parahaemolyticus is seen more commonly (i.e., the Chesapeake Bay area), notification of the laboratory would result in the proper evaluation for these organisms.

In a recent survey of all stool specimens submitted to our laboratory during a 1-year period for detection of E. coli O157, only one sample was positive (authors' unpublished data). A survey of clinical laboratories by the Association of State and Territorial Public Health Laboratory Directors found that only 29% of surveyed laboratories screened all stool samples for E. coli O157:H7 and that 43% screened selected samples (e.g., bloody stools or in cases where the physician has made a specific request) [21]. However, E. coli O157 was reported to be the most frequently isolated organism at the University of Calgary Clinical Microbiology Laboratory (Calgary, Alberta, Canada), accounting for 34% of all positive stool cultures [18].

Microbiology laboratories do not have uniform protocols for detecting enteric pathogens. Several types of differential and selective media are used in the workup of stool samples when a particular etiologic agent is sought [3]. In general, multiple culture media are used, since there is no single medium available that is 100% sensitive. Thus, the decision to use certain culture media is usually based on both published data and personal preference [22].

### Table 1. Summary of Results of Stool Analyses for Outpatients and Inpatients, September 1987 through August 1988, at the University of Pennsylvania Medical Center (reprinted with permission from the *Journal of the American Medical Association* [6]).

<table>
<thead>
<tr>
<th>Type of test</th>
<th>Outpatients</th>
<th>Inpatients hospitalized 0–3 days</th>
<th>Inpatients hospitalized &gt;3 days</th>
<th>All patients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. tested (Percent workload)*</td>
<td>No. positive (Percent total, percent yield)†</td>
<td>No. tested (Percent workload)*</td>
<td>No. positive (Percent total, percent yield)†</td>
</tr>
<tr>
<td>Bacterial culture</td>
<td>569 (29)</td>
<td>32 (80, 6)</td>
<td>398 (20)</td>
<td>8 (20, 2)</td>
</tr>
<tr>
<td>Ova/parasite</td>
<td>622 (44)</td>
<td>33 (92, 5)</td>
<td>269 (19)</td>
<td>3 (8, 1)</td>
</tr>
<tr>
<td>C. difficile*</td>
<td>84 (3)</td>
<td>28 (5, 33)</td>
<td>516 (19)</td>
<td>88 (16, 17)</td>
</tr>
</tbody>
</table>

* Percent workload = no. of specimens tested for patient group/total no. of specimens submitted for all patient groups.
† Percent total = no. of positive results/total no. of positive results for all patient groups; percent yield = no. of positive specimens/total no. of specimens submitted for that patient group.
* Data for C. difficile assay were collected during a 3-month period and extrapolated to 1 year for comparison.
rheal diseases, although numerous articles have been published on the clinical management of diarrheal illnesses [23]. In one of the few published studies in which diagnostic strategies were addressed, three evaluations for the management of diarrheal disease in patients with AIDS were compared [24]. When treatment strategies were compared on the basis of the results of a full evaluation (stool culture for enteric pathogens, three ova and parasite examinations, two blood cultures, and endoscopy with biopsy), a limited evaluation (stool culture, three ova and parasite examinations, and two blood cultures), and a minimal evaluation (stool culture only), the clinical response rate among the patients was similar, regardless of the diagnostic strategy used. However, the overall cost differed markedly between the strategies used, and it is not surprising that the full evaluation was more than three times as expensive as the minimal and limited evaluation strategies.

Several clinical algorithms have recently been suggested for predicting C. difficile disease in hospitalized patients [25, 26]. Manabe et al. [25] identified significant variables such as the presence of fecal lactoferrin, a positive gram stain, use of cephalosporins, and the passing of semiformal stools in their model, but the ability of these variables to predict positivity for C. difficile were not assessed. Cooper et al. [26] found that peripheral leukocytosis, the presence of leukocytes in stool, and antecedent hospitalization were significant variables in their model and that these variables showed a good correlation between the number of predictors present and frequency of positive toxin results. Fecal leukocytes were present in only 24% of patients whose stool samples were positive for C. difficile toxin, which severely limited the usefulness of this predictor.

Assessment of Testing Strategies for Cost-Effective Laboratory Workup of Stool Samples

Given the forces shaping medical economics in the 1990s, various strategies have been proposed to decrease laboratory costs [5, 6, 17, 22, 27, 28]. These strategies have been based on the following factors: (1) the process of a stepwise or tiered workup based on the most likely to least likely pathogens; (2) categorization of the clinical presentation as inflammatory or noninflammatory to distinguish between patients who do and do not need cultures performed; (3) the identification of certain clinical and epidemiological circumstances under which a particular group of pathogens may be excluded; and (4) the degree to which identification of a pathogen will affect the treatment of the patient. Despite the existence of these strategies, there is no clear consensus on a uniform algorithm.

The use of a least likely to most likely strategy for workup of stool samples is a reasonable approach to the diagnostic workup of a patient with diarrheal disease. As mentioned above, common etiologies should be sought first, and when the results are negative, less common agents should be pursued, especially if the illness has not spontaneously resolved. Mild diarrhea tends to dictate its own workup and, in general, when the diarrhea stops, so do the specimens. For routine purposes, the laboratory should have a policy of rejecting samples of formed stool, since even a positive result will have a minimal impact on cases of resolving diarrhea. However, there should be exceptions to such a policy; e.g., follow-up cultures should be performed for Salmonella carriers because there is a need to demonstrate clearance of the organism. A recent CAP survey of laboratories demonstrated that fewer than 10% had any policy for limiting the number or types of samples processed for routine culture [3]. The cost-effectiveness or clinical effectiveness of a stepwise model of testing for the presence of bacterial or parasitic agents, although seemingly intuitive, has not yet been determined.

The practice of dividing infections into inflammatory vs. noninflammatory presentations is based on the supposition that a patient with bloody stools, tenesmus, fever, persistent abdominal tenderness, and the presence of fecal leukocytes is infected with an invasive bacterial pathogen and will require antimicrobial therapy [29]. This classification is limited by the fact that there is significant overlap among the syndromes caused by bacterial pathogens: some invasive bacteria cause watery diarrhea without fever, blood, or abdominal pain, as would be typical in cases of noninflammatory diarrhea. This distinction is probably most important in designating those patients most likely to benefit from treatment; however, to our knowledge, the clinical benefit of this differentiation has never been proven.

One obvious approach to limiting the workup for enteric pathogens is to consider clinical and epidemiological factors that would exclude certain pathogens. For example, requesting cultures for Vibrio species in the absence of a specific exposure (i.e., ingestion of shellfish or travel to areas of endemicity) is an excess that can no longer be afforded in the initial workup of stool samples. Requests for cultures to detect Staphylococcus aureus and yeasts that are unlikely causes of diarrhea should be limited as well.

Strategies for determining the cost-effectiveness of identifying specific pathogens and for determining how this identification affects clinical outcome have not been published, although physicians make this decision intuitively. Empirical therapy for traveler’s diarrhea is again an example of a situation in which specific identification of a pathogen will have little impact on outcome.

Is There a Role for Rapid, Nonculture Screening Tests?

Examination of stool for the presence of leukocytes began in the early twentieth century when it was common practice to examine stool for protozoa, but the clinical significance of detecting leukocytes has only recently been reevaluated. Clinicians have been routinely taught that the presence of fecal leukocytes is an indicator of “inflammatory” diarrhea and
Table 2. Detection of fecal leukocytes during infection with common bacterial enteric pathogens.

<table>
<thead>
<tr>
<th>References</th>
<th>Pathogen</th>
<th>No. of patients</th>
<th>Mean percentage positive (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>[8, 27, 32]</td>
<td><em>Campylobacter</em> species</td>
<td>194</td>
<td>58 (25–80)</td>
</tr>
<tr>
<td>[32, 33]</td>
<td><em>Escherichia coli</em> O157:H7</td>
<td>112</td>
<td>54 (42–65)</td>
</tr>
<tr>
<td>[8, 17, 32, 34]</td>
<td><em>Salmonella</em> species</td>
<td>140</td>
<td>52 (11–82)</td>
</tr>
<tr>
<td>[8, 17, 27, 32, 34, 35]</td>
<td><em>Shigella</em> species</td>
<td>252</td>
<td>73 (49–100)</td>
</tr>
<tr>
<td>[32]</td>
<td><em>Yersinia enterocolitica</em></td>
<td>27</td>
<td>48</td>
</tr>
<tr>
<td>[25, 26, 36, 37]</td>
<td><em>Clostridium difficile</em></td>
<td>160</td>
<td>42 (24–63)</td>
</tr>
</tbody>
</table>

should be used to determine whether culture of stool samples is needed [5, 29].

The algorithm proposed by Guerrant is the best example of the role of this finding [29]. In this algorithm, analysis of fecal leukocytes, and, more recently, fecal lactoferrin [30], figure prominently in the approach to the diagnosis and management of infectious diarrhea. Only those samples containing leukocytes are used, and only in such cases is the need for antimicrobial therapy evaluated. Donowitz et al. [31] also recommend fecal leukocyte analysis in the initial evaluation of patients with chronic diarrhea, although the rationale for this recommendation is not obvious. Although it is commonly believed that the fecal leukocyte test is useful for identifying patients with bacterial gastroenteritis caused by the most common agents of putatively inflammatory diarrhea (i.e., species of *Campylobacter*, *Salmonella*, and *Shigella*), the data do not necessarily support a role for this test.

Over the years, a number of studies have been performed that contain data on fecal leukocyte determinations [8, 17, 25–27, 32–37]; the data are summarized in table 2. Even for detection of the prototypical inflammatory diarrheal disease agent, *Shigella*, the sensitivity of the fecal leukocyte test is on average 73%. For the other agents, the sensitivity is ~50%. In one recent study, the positive and negative predictive values of the fecal leukocyte test were at best 45% and 93%, respectively [38]. While studies have shown that the yield of positive cultures increases for patients in whom fecal leukocytes are detected [27], the performance characteristics of this test are not sufficient for its use as a screening procedure.

Although the cost of a fecal leukocyte examination is low, the clinical effectiveness of the test is not known. To our knowledge, studies examining the impact of fecal leukocyte tests on clinical outcome have yet to be done. If one were to find that patients with fecal leukocytes were more likely to benefit from antimicrobial therapy than those without fecal leukocytes, a case might be made for doing the test.

Another test that has received some attention as an alternative to the fecal leukocyte test is the fecal lactoferrin assay. Lactoferrin is an iron-binding glycoprotein found in secondary granules in leukocytes, and it is detected with use of a commercially available latex agglutination test [30]. In a recent small study conducted by Choi et al. [30], the sensitivity of the lactoferrin assay in detecting species of *Salmonella*, *Campylobacter*, and *Shigella* varied from 83% to 93%, and its specificity varied from 61% to 100%, depending on the cutoff used for positivity. Silletti et al. [39] found that compared with culture, the fecal lactoferrin assay was more sensitive but less specific than the fecal leukocyte test: the fecal lactoferrin was 85% sensitive and 79% specific, while the fecal leukocyte test was 31% sensitive and 96% specific.

Fecal leukocyte and lactoferrin assays have also been studied as screening tests for *C. difficile*. Marx et al. [37] found that the sensitivity of fecal leukocyte analysis was only 28% among patients positive for *C. difficile* toxin. Yong et al. [36] found that fecal lactoferrin was more sensitive as a marker for *C. difficile* toxin—positive patients than was fecal leukocyte analysis (75% vs. 40%, respectively), but fecal leukocyte analysis was more specific (92% vs 46%, respectively).

In a recent study by Manaè et al. [25], both fecal leukocyte and lactoferrin assays had poor performance characteristics as markers for *C. difficile* toxin in stool samples with positive predictive values and negative predictive values ranging from 25% to 27% and from 89% to 90%, respectively. Cooper et al. [26] found that while fecal leukocytes were more often present in patients positive for *C. difficile* toxin, only 24% of toxin-positive patients had fecal leukocytes detected. Thus, even for *C. difficile* disease, these tests have little proven diagnostic efficacy, and we do not recommend performing them.

While the rapid tests described above are of limited proven usefulness, the gram stain still has a role in the diagnosis of diarrheal disease. *Campylobacter jejuni*, one of the most common causes of bacterial gastroenteritis, can be easily visualized in a stool smear, especially if the counterstain, safranin O, is replaced with basic fuchsin. The sensitivity of the gram stain has been reported as ranging from 66% to 94%, with high specificity [40]. Unfortunately, the gram stain has no value for detecting any other common enteric pathogen, since these cannot be differentiated from normal stool flora with use of the gram stain.

General Processing and Sampling Considerations

A few studies have been conducted to determine the number of specimens needed for optimal detection of enteropathogens. For detecting common enteropathogens, one or two samples appear to be adequate for episodes of acute disease. Church et al. [18] found that for children, 97% of enteropathogens were detected with one fecal sample. In a recent CAP survey of 601 institutions where both stool cultures and parasite examinations were performed, it was determined that testing of two stool specimens resulted in detection of 99% of bacteriologic agents,
and testing of three specimens resulted in detection of 99.8% of parasites [3]. In another study by Marti and Koella [41], the false-negative rate for three ova and parasite examinations varied from 0.1% (Trichuris or Ascariis species) to 5.8% (Entamoeba histolytica). Approximately 91% of parasites were detected using a single sample in a study from Duke Medical Center [16].

Valenstein and colleagues [3] recently recommended that no more than two stool specimens per patient for bacteriology and two or three samples per patient for parasitology be the standard for routine workups and that additional testing be performed only after consultation with the laboratory. The fact that the cost of detecting additional cases of disease with use of more samples is several times higher than that with a single sample (i.e., $117 for one sample vs. $116,636 for four samples) supports this recommendation.

For detection of C. difficile disease on the basis of the presence of toxin, two stool samples were shown to be more sensitive than was a single sample [25]. On the basis of our own in-house data from toxin B cytotoxicity cell culture assays, we recommend that up to three samples be tested to rule out disease (Nachamkin et al., unpublished observations).

In a recent study by Renshaw et al. [42], the sensitivity of a single assay for C. difficile toxin was 94%, with as many as four samples needed for 100% sensitivity (based on all positive tests). Repeated assays for C. difficile toxin within a 7-day period accounted for one-third of all testing performed at these authors' institution, but such assays provided clinically useful information in only 1% of cases. On the basis of this information, it would seem reasonable to send two samples to the laboratory to rule out C. difficile infection, but such samples should be spaced several days apart before testing. Additional testing (more than two specimens) for C. difficile infection should be considered only after consultation with the laboratory.

Stool samples are the most common specimens submitted to the laboratory for bacteriologic and parasitological examinations. Freshly obtained specimens that are processed soon after collection (i.e., <1 hour) are optimal for processing; however, this procedure is not practical in most situations. Therefore, all specimens should be sent to the laboratory in transport devices specifically designed for bacteriologic and parasitological examinations. Of the many transport media available, Cary-Blair medium appears to be the best medium overall for supporting the isolation of the most commonly encountered bacterial agents [40]. Recommendations for cost-effective testing for enteric pathogens are shown in table 3.

**Recent Advances**

**Bacterial Agents**

Because the variety of bacterial agents that cause diarrheal diseases is wide, little progress has been made in replacing conventional culture methods in the clinical microbiology laboratory. The most promising approaches appear to involve molecular techniques for detecting specific organisms or their toxins, but none have been commercialized or are available for routine testing [43]. A brief summary of commonly considered enteropathogens is given below.

_Aeromonas_ species and _Plesiomonas shigelloides_. The role of these organisms as enteropathogens has never been completely resolved, and the evidence for their pathogenicity is based on clinical, epidemiological, and in vitro studies. The best clinical correlates are observed when samples contain moderate-to-heavy growth of the organisms in the absence of other known enteropathogens. However, human challenge studies with these organisms have not produced disease. Three species of _Aeromonas_ are considered the major pathogenic species and include _A. hydrophila_, _A. caviae_, and _A. veronii_ biovar sobria [44]. These species can be isolated on nonselective media (i.e., sheep blood agar) if these media are used in a routine culture setup; thus, the need for special selective media is uncertain [22].

_Campylobacter_ and _Arcobacter_ species. Many advances have been made over the past 5–10 years in diagnosing campylobacter infections and describing new species that cause diarrheal diseases. Two genera, _Campylobacter_ and _Arcobacter_, are included in the recently adopted family Campylobacteraceae. There are now 19 species and subspecies within the genus _Campylobacter_ and four species in the genus _Arcobacter_, most of which have been described as causing or being associated with enteric infection [40]. Some species of _Campylobacter_ other than _C. jejuni_ and _C. coli_ that may be of particular importance include _C. fetus_ subspecies _fetus_, _C. upsaliensis_, and _Arcobacter butzleri_. _Helicobacter cinaedi_ and _Helicobacter fennelliae_, previously designated within the genus _Campylobacter_, are also important causes of proctitis and proctocolitis in immunocompromised patients.

Of particular note is the emergence of _C. jejuni_ infection as a cause of Guillain-Barré syndrome [45]. As mentioned elsewhere, routine stool cultures should always include the isolation of _Campylobacter jejuni_ and _C. coli_, since these are the most commonly recognized _Campylobacter_ species that cause diarrheal disease. A variety of culture methods such as filtration on nonselective media are needed to isolate the other species of _Campylobacter_ and should be considered for patients with persistent diarrheal disease from whom no other identifiable agent has been recovered.

_C. difficile_. In contrast to the other enteropathogens, _C. difficile_ is the most commonly recognized bacterial agent of nosocomial diarrheal disease. Several approaches to the laboratory diagnosis of _C. difficile_ disease have been published, and tests include detection of _C. difficile_ enterotoxin (toxin A or the cytotoxin, toxin B). Additional methods include culture, detection of other cellular antigens, and, more recently, molecular methods [46, 47]. At the present time, molecular methods are
still used only in the research laboratory and are not available from commercial sources. The choice to use the more common methods will be determined by issues of staffing, cost, and turnaround time (table 4).

In general, detection of C. difficile toxin B with use of a cell culture–toxin neutralization cytotoxicity assay (TCA) has been considered to be the “surrogate” criterion standard for evaluating the different test methods. It has been difficult to assess diagnostic test performance because a true criterion standard for C. difficile disease requires both clinical and laboratory criteria, which are frequently not used in most studies [46]. Commercial systems for assaying toxin B are available and appear to be essentially equivalent to those for which in-house reagents are used [48]. The format of the assays is such that experience in cell-culture techniques is not necessary, and these assays can be performed easily in most clinical microbiology laboratories.

A number of EIAs that detect C. difficile toxin A and/or toxin B are commercially available and have good performance characteristics when compared with TCA, but they are clearly less sensitive than TCA. The sensitivity and specificity of these assays have varied considerably, depending on the assay used [46, 49–51], and in some cases, positive results may need to be verified by TCA. EIAs are generally easy to perform, and the turnaround time for obtaining results is short.

Some laboratories use culture for detecting C. difficile [46]. Although culture may be a sensitive method for detecting the organism in stool samples, isolates must be tested for production of toxin, thus delaying results for several days. Therefore, we do not recommend culture as a routine method for diagnosing C. difficile disease.

Latex agglutination assays that detect a C. difficile antigen (not toxin) are commercially available, easy to perform, and rapid; however, they are not as sensitive or specific as TCA or EIA [46, 49, 51]. In addition, a method that detects toxin A or toxin B should be used for patients who are negative for antigen but clinically suspected of having C. difficile disease.

E. coli. Numerous types of E. coli that cause diarrheal disease have been described, including enterotoxigenic strains, enteropathogenic strains, enteroinvasive strains, enterohemorrhagic strains, and enteraggregative strains [52]. Of these different types, only enterohemorrhagic strains of serotype O157:H7 can be routinely detected in most clinical microbiology laboratories because a specific selective medium, sorbitol-MacConkey agar, is widely available. The geographic distribution of these strains varies, and media for detection may be available in some laboratories and not in others. Sorbitol-negative strains can be further identified with specific serotyping reagents or toxin assays [22].

The timing of cultures appears to be important: the rate of recovery of E. coli O157 is highest during the first few days after the onset of diarrhea and drops off significantly after 7 days [33]. Because serotypes other than O157 can cause disease, diagnostic alternatives such as detection of fecal verotoxin, recently proposed as Stx1 [53], may be necessary for optimal sensitivity [54, 55]. Commercial systems for detecting O157 directly in stool samples have also been recently described [56].

Y. enterocolitica. Detection of Y. enterocolitica continues to be based on conventional culture methods. The need to test for the presence of this organism in routine cultures will depend

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### Table 3. Recommendations for cost-effective testing for enteric pathogens.

- Routine cultures should always include *Campylobacter*, *Salmonella*, and *Shigella*; other agents included in the routine culture panel should be determined on the basis of in-house or regional survey data.
- Submit additional samples for less common bacteriologic agents as a second-line test after the common agents have been ruled out.
- Up to two stool samples should be submitted for bacteriologic culture, and three samples should be submitted for ova and parasite examination for optimal sensitivity. Wait for the results of the first culture and/or examination before sending additional samples, rather than ordering multiple tests.
- Two to three samples may also be needed to rule out *Clostridium difficile* disease, and samples should be spaced several days apart.
- Use the 3-day rule for routine stool cultures and/or parasitological examinations for hospitalized patients.
- Because there are new tests for *Giardia lamblia*, *Entamoeba histolytica*, and *Cryptosporidium*, consider testing specifically for these agents initially and forego the initial routine ova and parasite examination, especially when other parasitic agents are unlikely.

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### Table 4. Characteristics of assays used for detecting Clostridium difficile.

<table>
<thead>
<tr>
<th>Method</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytotoxicity assay (toxin B)</td>
<td>Sensitive and specific; bioassay; kits available</td>
<td>Not as rapid as other methods; may require tissue culture if kits not used</td>
</tr>
<tr>
<td>EIA (toxin A/B)</td>
<td>Rapid; many kits available</td>
<td>Slightly less sensitive and specific than cytotoxicity assay; positive results may need verification</td>
</tr>
<tr>
<td>Latex agglutination (nontoxin antigens)</td>
<td>Rapid</td>
<td>Does not detect toxin A or toxin B; nonspecific for toxigenic strains; least sensitive and specific; positive results should be confirmed by additional tests</td>
</tr>
<tr>
<td>Culture</td>
<td>Sensitive</td>
<td>Time-consuming; additional tests to distinguish toxigenic from nontoxigenic strains required</td>
</tr>
</tbody>
</table>
on the geographic prevalence of *Y. enterocolitica* disease. Not all strains of *Y. enterocolitica* cause diarrheal disease; certain serotypes have been identified as pathogenic (including serotypes O4, 32; O8, O13a, 13b; O18, O20; and O21) and are common in the United States. Virulence is associated with the presence of a 40- to 48-megadalton plasmid. A number of phenotypic tests including the presence of pyrazinamidase, salicycin fermentation, esculin hydrolysis, and growth characteristics on Congo red–magnesium oxalate agar appear to be helpful in identifying pathogenic strains [57].

**Vibrio species.** Twelve species are included in the genus *Vibrio*, and many are associated with gastroenteritis. In addition to the *V. cholerae* O1 serogroups, which are known to cause classic cholera, a new non-O1 serogroup, O139, has now become an important cause of epidemic cholera. In addition to *V. cholerae*, other species are associated with gastroenteritis; these species include *V. parahaemolyticus* (the most common non-*cholerae* species) as well as *V. mimicus*, *V. fluvialis*, and *V. cholisae* (which can occasionally be detected in the laboratory with use of selective media) [58].

### Parasitic Agents

Many advances have been made in the diagnosis of parasitic diseases. Because of these advances, clinicians may wish to rethink the strategy of performing ova and parasite examinations on all samples and specifically request tests for particular agents.

**Giardia lamblia**, *Cryptosporidium*, and *E. histolytica*. *G. lamblia* is usually detected during a routine ova and parasite examination by examination of concentrated stool samples and permanent smears. In the past few years, several alternative methods for detecting *G. lamblia* in stool samples have been developed and commercialized, and these methods appear to have greatly improved sensitivity when compared with conventional methods.

Direct immunofluorescence staining (DFA), which is available commercially, has been evaluated by a number of investigators; it has been shown to significantly increase the sensitivity for detecting *G. lamblia*. Garcia et al. [59] found that DFA was 100% sensitive and specific for detecting *G. lamblia* in stool samples, compared with a conventional trichrome stain, which was 93% sensitive. In a more recent study by Alles et al. [60], DFA was compared with chlorazol black E stain and was found to be more sensitive for detecting *G. lamblia*. The sensitivity of immunofluorescence was 99.2%, while that of routine histologic staining was 66.4%. Both methods were 100% specific.

For *Cryptosporidium*, the numbers were equally impressive. Garcia et al. [59] found that DFA was more sensitive than the modified acid-fast stain (100% sensitivity vs. 92% sensitivity, respectively). However, Alles et al. [60] detected 69.6% more positive samples with the reagent than with modified acid-fast stain. Immunofluorescence was 93% sensitive, while the modified acid-fast stain was 54.8% sensitive. A number of commercial antigen EIA detection systems have also been described for detecting *G. lamblia* and *Cryptosporidium* antigen in stool samples [61–63], and these also appear to be more sensitive than conventional microscopic analysis.

*E. histolytica* was recently divided into two species: the pathogenic strain retains the name *E. histolytica*, and the non-pathogenic species has been renamed *E. dispar* [64, 65]. Although the two species were formerly identified as a single organism on the basis of microscopic findings, they can be distinguished by the presence of a specific lectin in the pathogenic strains. Haque et al. [65] recently evaluated a commercial EIA specific for detection of pathogenic *E. histolytica* in stool samples. When compared with culture, microscopic examination was 60% sensitive and 79% specific, and EIA was 80% sensitive and 99% specific. When compared with zymodeme analysis to differentiate pathogenic from nonpathogenic strains, the EIA was 95% sensitive and 93% specific. Thus, EIA has the potential for improved diagnosis of *E. histolytica* infection; however, additional studies are needed to better assess this test’s performance.

The results of these studies suggest that these newer methods are superior to conventional parasitological microscopy and that they should be used more routinely for diagnosis. For patients in whom giardiasis, amebiasis, or cryptosporidiosis is suspected, specific tests as described above should be requested rather than the conventional microscopic examination for ova and parasites. An expanded menu of parasitological tests that allow more-specific options for testing should now be available to clinicians. Since conventional microscopic parasitological examinations are among the most time-consuming procedures in the laboratory, the use of some of the specific tests described above may lead to more cost-effective and clinically effective use of the parasitology laboratory.

**Microsporidia.** Microsporidia are a group of protozoa that have emerged as a cause of diarrheal disease, predominantly in patients with AIDS. However, the role of these organisms in the pathogenesis of gastrointestinal disease is unclear [66]. Four genera are in this group and include *Enteroctochozoon, Encephalitozoon, Pleistophora*, and *Nosema*; all but *Nosema* have been associated with diarrhea in patients with AIDS. A review of the associated pathology and detection of these organisms was recently published [67]. Detection of these organisms in the clinical laboratory poses significant challenges because of their small size and difficulty in distinguishing them from artifacts with use of the available staining techniques.

Several light or fluorescent microscopic stains are currently available in laboratories, most of which use Weber’s modified trichrome stain [68, 69]; more recently, Uvitex 2B, a fluorescent stain, has shown some promise [69]. For the accurate identification of microsporidia, strict adherence to the diagnostic criteria of size, shape, and morphological characteristics is critical.
Molecular biological techniques such as PCR may ultimately provide more practical and specific methods in the near future [70]. Electron microscopy is the definitive procedure for identification of microsporidia to the species level, and species identification may be important in the future if antiprotozoal therapy is found to be effective [71].

*Blastocystis hominis*. The role of *B. hominis* in causing diarrheal disease is unclear [72]. When this organism is present in large numbers in the stool, in the absence of other identifiable etiologic agents, patients have been reported to have diarrhea, abdominal pain, nausea, and vomiting that have responded to therapy with agents such as metronidazole [73–75]. The organism is readily identified with use of routine microscopic methods.

**Fungal Agents**

It has been suggested that *Candida* species may be a cause of antibiotic-associated diarrheal disease; however, only a limited number of patients have been studied [76, 77]. The role of the laboratory in detecting *Candida* in stool samples is unclear at this time. At the very least, testing should not be performed on a routine basis.

**Viral Agents**

It is estimated that 30%–40% of episodes of infectious diarrhea in the United States are caused by viral agents [78]. Rotavirus is the most important and common cause of serious gastroenteritis; this agent causes significant morbidity that results in the need for hospitalization, particularly among children [78]. In addition, rotavirus frequently causes nosocomial infections, and timely diagnosis may help limit the spread of infection. A large number of detection systems are commercially available and include EIA and rapid latex agglutination tests; all of these systems have different performance characteristics [79].

Less common causes of viral gastrointestinal infection include adenoviruses (types 40 and 41), caliciviruses (including Norwalk virus), astroviruses, and coronaviruses [80]. Commercial EIAs are available for detecting enteric adenoviruses [81]. However, at this time electron microscopy remains the only practical method for detecting the other viruses. Immunologic and molecular approaches for detecting these other viral agents have been published but remain in the developmental stages.

**References**


