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LABORATORY APPROACHES TO INFECTIOUS DIARRHEA

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This article summarizes the commonest causes of infectious diarrheal disease and focuses on diagnostic tests that are, for the most part, readily available from clinical laboratories in developed countries. Because laboratory tests for fecal specimens are time-consuming and expensive, their inclusion in routine patient management usually is limited in nonhospitalized patients to those with severe or protracted diarrhea and in patients whose stool is shown to contain fecal leukocytes. Young children, hospitalized patients, debilitated individuals, and immunocompromised hosts are at particular risk for severe diarrheal disease and can benefit from appropriate laboratory support. The presence of a community outbreak is grounds for performing directed laboratory testing for diagnostic and epidemiologic purposes.

BACKGROUND

Infectious diarrhea is a common disease syndrome that continues to be a significant cause of morbidity and mortality through much of the world despite a better understanding of causative agents and advances in diagnostic and therapeutic approaches. Estimates of the number of cases of infectious diarrhea each year in the United States range from 25 million to 99 million, resulting in 2.2 million hospital visits.[24, 29, 47, 53, 69] Children less than 5 years old are particularly susceptible to infectious diarrheal disease for a variety of epidemiologic and immunologic reasons, producing greater than 20 million cases that result in approximately 200,000 hospitalizations and 400 deaths.[33, 46, 69, 72] Much of the childhood mortality is related directly to the dehydration associated with infectious diarrheal disease.[48]

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Table 1. VARIOUS INFECTIOUS AGENTS PRODUCING DIARRHEAL DISEASE IN ADULTS AND CHILDREN

Bacteria	Parasites	Viruses
Gram-positive	*Balantidium coli*	Astroviruses*
Bacillus cereus	*Blastocystis hominis*	Enteric adenoviruses*
*Clostridium difficile**	*Cryptosporidium parvum**	(serotypes 40, 41)
*Clostridium perfringens**	*Cyclospora cayetanensis*	Calicivirus*
Staphylococcus aureus	*Dientamoeba fragilis**	Norwalk viruses
Gram-negative	*Entamoeba polecki*	Norwalk-like viruses
Aeromonas hydrophila	*Entamoeba histolytica**	Coronavirus*
*Campylobacter jejuni**	*Giardia lamblia**	Rotavirus*
Enterohemorrhagic *E. coli**	*Isospora belli*	
Enterotoxigenic *E. coli**	*Strongyloides stercoralis*	
Plesiomonas shigelloides		
*Salmonella**		
*Shigella**		
Vibrio cholerae 01 and 139*		
Other *Vibrio**		
Yersinia enterocolytica		

*Those for which children are at particular risk.

Although many noninfectious disease states and therapeutic interventions may produce acute or chronic diarrheal disease, bacterial, parasitic, and viral agents are among the commonest causes. Table 1 summarizes the various microorganisms producing diarrheal diseases in adults and children. Causative agents having a greater likelihood to produce diarrhea in patients infected with human immunodeficiency virus type 1 (HIV-1) in particular and immunocompromised patients in general are summarized in Table 2.

Infectious diarrhea can be classified into 2 distinct clinical syndromes for which patient management and treatment vary: inflammatory or bloody diarrhea and noninflammatory, nonbloody diarrhea.[35, 43, 84] Inflammatory diarrheas usually present with fever, tenesmus, and severe abdominal pain; large numbers of fecal leukocytes often are detectable in the stool, and inflammatory lesions may be seen on intestinal mucosal biopsy. Inflammatory diarrheas produce a severer form of acute diarrhea and require additional medical and laboratory evaluation. In contrast, noninflammatory diarrheas generally are milder in immunocompetent hosts, although severe fluid loss with attendant morbidity and mortality can occur, especially in malnourished individuals.[66]

Inflammatory diarrheas are caused primarily by invasive or toxin-producing organisms, including *Campylobacter jejuni*, *Clostridium difficile*, enterohemorrhagic

Table 2. AGENTS PLACING HIV-1–INFECTED PATIENTS AT PARTICULAR RISK FOR POTENTIALLY SEVERE DIARRHEAL DISEASE

Bacteria	Parasites	Viruses
Mycobacterium avium complex	*Cryptosporidium parvum*	Astrovirus
Campylobacter jejuni	*Cyclospora cayetanensis*	Cytomegalovirus
Salmonella	*Enterocytozoon bieneusi*	Herpes simplex virus
Shigella	*Encephalitozoon intestinalis*	
	Isospora belli	

and enteroinvasive *Escherichia coli* (EHEC and EIEC), *Shigella*, nontyphi *Salmonella*, and *Entamoeba histolytica*. These organisms can produce obvious macroscopic and histologic alterations of the mucosal lining of the colon.[1, 6, 22, 66] *Salmonella typhi*, *Salmonella paratyphi* A, B, C, *Yersinia enterocolytica* and *Yersinia pseudotuberculosis* are the usual agents of enteric fever or enteric fever-like syndromes but produce diarrhea much less frequently.

Rotavirus, Norwalk virus, enteric adenovirus, *Giardia lamblia*, *Cryptosporidium parvum*, *Vibrio cholerae*, and enterotoxigenic *E. coli* (ETEC) are among the commonest pathogens implicated in noninflammatory diarrhea.[19, 33] In addition to these agents, food-borne outbreaks of noninflammatory diarrhea may be caused by preformed toxins of *Staphylococcus aureus* and *Bacillus cereus* and by in vivo production of *Clostridium perfringens* toxins. These organisms or their toxins primarily affect the small intestine rather than the colon. In contrast to invasive or toxigenic inflammatory diarrheal agents, these organisms may adhere to the small intestine but fail to produce significant disruption of normal mucosal architecture. Noninflammatory diarrheas can present with symptoms of nausea, vomiting, and abdominal cramping. Diarrhea is watery, but blood and polymorphonuclear leukocytes generally are absent from the stool.

Numerous vehicles for transmission of infectious diarrhea agents have been identified in outbreaks, with most being acquired after ingestion of contaminated foods or water. Water has been the common vehicle for outbreak transmission of *G. lamblia*, *C. jejuni*, *C. parvum*, and Norwalk virus. Craun[31] and La Via[65] reported that *G. lamblia* is the most commonly isolated parasitic agent associated with waterborne outbreaks in the United States. Ice has been identified as a source of infection with ETEC, Norwalk virus, and *Vibrio cholerae*.[21, 47] Unpasteurized milk has been another vehicle for transmitting *Salmonella*, *C. jejuni*, and *Y. enterocolytica*.[48] Many studies have shown that contaminated chickens and eggs are the main culprit for outbreaks of salmonellosis.[96] Fish and contaminated seafood are the common vehicle for outbreaks of vibriosis and Norwalk virus disease.[73] During July and August 1997, *Vibrio parahaemolyticus* was responsible for the largest reported outbreak of diarrheal disease associated with consumption of raw shellfish in North America, with 209 persons affected in the Pacific Northwest.[26] Uncooked or undercooked beef, poultry and pork are recognized as potential sources of infection caused by EHEC (see the article by Tarr and Neill, page 735).

GUIDELINES FOR LABORATORY EVALUATION OF DIARRHEA

Most cases of acute diarrhea are self-limited and do not come to the attention of health care practitioners. Cases that are protracted or severe (producing dehydration and weakness), especially when accompanied by overt fecal blood, do come to the attention of physicians and often require diagnostic laboratory evaluation. In this setting, diagnostic laboratory tests are an important adjunct for identifying the offending pathogen or for ruling out more serious or potentially treatable conditions.[2, 6, 16, 39, 48]

A systematic approach to the evaluation of diarrheal disease before submission of specimens for laboratory evaluation includes documenting disease characteristics (nature of onset, frequency, duration, and qualitative description of stool), obtaining an adequate patient or family history (patient characteristics, activities, diet, medication, travel history, family and community contacts, and hospitalizations), and conducting a thorough physical examination.[8, 16] Talal and Murray[95] described a more detailed systematic approach to diagnose common

causes of diarrhea. Aranda-Michel and Giannella[6] suggested that laboratory diagnostic tests should be reserved for patients with inflammatory bloody diarrhea with fever greater than 38.3°C and for immunocompromised patients.

The reader is referred to detailed consensus guidelines published by the American College of Gastroenterology and the Infectious Diseases Society of America that outline the application of diagnostic methods for identifying enteric infections that require specific therapy or that are responsive to control measures.[35, 51] Factors leading to the development of such guidelines include the increasing array of enteric pathogens associated with diarrheal illness and the need to develop cost-effective approaches when targeting patient evaluation and management. Briefly, laboratory approaches recommended in these guidelines to improve cost-effectiveness of stool culture include selective testing for the most likely pathogen; discouraging the submission of stool specimens from patients who have been hospitalized for greater than 3 days, unless overriding circumstances prevail (exceptions include hospital outbreaks, advanced age, comorbid disease, neutropenia, and HIV infection); specific examination for C. *difficile* or its toxins in appropriate settings for patients who have been hospitalized for greater than 3 days; and use of screening tests, such as fecal leukocytes or fecal lactoferrin, for possible inflammatory or invasive diarrhea. Table 3 is a summary of common laboratory tests used in evaluating acute infectious diarrhea.

TRANSPORT AND EXAMINATION OF FECAL SPECIMENS

Diarrheic stool specimens obtained during the acute stage of the disease are recommended for bacteriologic studies. Cary-Blair transport medium should be used when transporting or anticipating a delay of more than 2 hours in processing the specimen for culture.[89] Intestinal parasites usually are detected and identified with direct microscopy of fresh or formalin-preserved and polyvinyl

Table 3. ROUTINELY AVAILABLE FECAL LABORATORY TESTS USED IN THE EVALUATION OF INFECTIOUS DIARRHEA

Culture-Based Tests	Tests for Parasites	Other Tests
Routine stool culture	Ova and parasite	*Clostridium difficile*
Salmonella	examination	Toxin B cytotoxicity
Shigella	Direct wet mount	assay
Campylobacter jejuni	Concentration procedure	Toxins A and B by
Aeromonas/Plesiomonas	Permanent stain	immunoassay
	Giardia lamblia	Common antigen by
Special stool culture	Immunoassay	immunoassay
E. coli O157:H7	*Entamoeba histolytica*	Fecal leukocytes
Yersinia enterocolytica	Immunoassay	Fecal lactoferrin
Vibrio cholerae	*Cryptosporidium parvum*	Shiga and Shiga-like toxins
Vibrio parahaemolyticus	Modified acid-fast stain	by immunoassay
Clostridium difficile	Immunoassay	Rotavirus by
Mycobacterium avium	*Isospora belli*	immunoassay
	Modified acid-fast stain	Electron microscopy for
	Cyclospora cayetanensis	viruses
	Modified acid-fast stain	
	Microsporida	
	Modified trichrome stain	

alcohol (PVA)–preserved stool specimens.[92] Many viruses, including rotaviruses, adenoviruses, astroviruses, caliciviruses, Norwalk virus, and other small, round viruses can be detected with electron microscopy, but stool specimens must be collected as close to the onset of the disease as possible, generally within the first 48 to 72 hours.[67]

The stool should be examined for color, volume, consistency, and presence of blood and leukocytes. A simple, rapid, and useful test for acute infectious diarrhea is microscopic examination for fecal leukocytes in stool. Loeffler's methylene blue and Gram stain may be used for direct microscopic examination of fecal specimens for the presence of fecal leukocytes. Fecal leukocytes may be observed after mixing equal amounts of methylene blue and fecal suspension on a slide and examining using a light microscope at 400 times magnification.[46] Fecal leukocyte evaluation is considered positive if 3 or more leukocytes are seen per high-power field in 4 or more fields.[46, 89] A positive examination indicates an inflammatory condition in the colon, not necessarily associated with an infection. Fecal leukocytes generally are observed in patients with salmonellosis, shigellosis, campylobacteriosis, EIEC, EHEC, staphylococcal enterocolitis, *Entamoeba histolytica*, ulcerative colitis, Crohn's disease, and pseudomembranous colitis, among others.[69, 72] Gram-stained smears of stool may be useful in determining the presence of fecal leukocytes but offer some disadvantages. Slides can be difficult to interpret, smear thickness and staining are difficult to standardize, cells deteriorate quickly, and specimens must be examined within minutes of collection to be accurate. Because the sensitivity of direct microscopic examination is 60% to 70%, the absence of fecal leukocytes does not exclude enteric pathogens.[93]

Detection of lactoferrin is an indirect measure of the presence of leukocytes, from which it is released, and may offer advantages of increased sensitivity and rapidity of test performance.[50] A commercially available latex agglutination test (Leuko-Test, TechLab Inc, Blacksburg, VA) for the detection of elevated levels of fecal lactoferrin[55, 56] shows promise as a screening test for inflammatory diarrhea. This assay can measure elevated levels of lactoferrin released from deteriorated or damaged leukocytes in stool specimens, an advantage over the traditional fecal leutocyte assay. Yong et al[100] showed that the fecal lactoferrin test was more sensitive (75%) than methylene blue microscopy (40%) for the detection of leukocytes in *C. difficile*–associated disease.

DIAGNOSTIC APPROACHES

Numerous diagnostic laboratory techniques have been used to identify viral, bacterial, and parasitic agents for acute infectious diarrhea, including routine stool cultures for the common enteric pathogens (*Salmonella, Shigella, Campylobacter*), special cultures (*Yersinia enterocolytica, E. coli* O157:H7, *C. difficile*, others), phase-contrast microscopy for motile *Campylobacter*,[60, 91] microscopic examination for ova and parasites, *C. difficile* toxin and Shiga toxin assays, detection of virus and parasites by enzyme immunoassay (EIA), serologic techniques, electron microscopy for viruses, flexible sigmoidoscopy with biopsy and histology, examination of duodenal aspirates, and differential fecal stains for parasites and mycobacteria.[84] The use of immunoelectron microscopy, EIA, tissue culture, molecular probes, and polymerase chain reaction[20, 38, 63, 78] has improved significantly the diagnosis of infectious diarrhea caused by bacteria, parasites and viruses, although many of these techniques are not routinely available. Use of special concentration and staining techniques has improved the detection of

intestinal parasites, such as *Cryptosporidium parvum, Isospora belli, Cyclospora cayetanensis,* and microsporidia.[46, 65, 92] With currently available techniques, bacteriologic diarrheal agents are recovered in about 10% of stool cultures[54]; 20% to 53% of all diarrheal disease remains undiagnosed.[88]

SALMONELLA, SHIGELLA, AND CAMPYLOBACTER

All patients with fever and evidence of inflammatory diarrhea should be cultured for *Salmonella, Shigella,* and *Campylobacter;* these organisms are the commonest bacterial agents of infectious diarrhea in children.[1, 34, 93] These organisms produce enterotoxins that may play a role in the pathogenesis of diarrheal disease. Media such as MacConkey (MAC), xylose-lysine-deoxycholate (XLD), eosin–methylene blue (EMB), and *Salmonella-Shigella* (SS) agars are used routinely to isolate these organisms. Novobiocin-brilliant green-glucose agar (NBG), and novobiocin-brilliant green-glucose-lactose agar (NBGL) have been recommended for this purpose. Ramach agar, SM-ID medium, xylose-lysine-tergitol (XLT4) medium, and modified semisolid Rappaport Vassiliadis medium (MSRV) have been used to improve isolation of *Salmonella* from stool specimens.[7, 37, 77] Most of the commercially available identification systems adequately identify *Salmonella.* The 4-methylumbelliferyl-caprilate (MUCAP) test is used for rapid identification of *Salmonella* strains from agar plates.[81] The latex agglutination (LA) test is helpful in the direct detection of *Salmonella* or *Shigella* serotypes from enrichment broth with an overall sensitivity of 86% for *Salmonella* and 85% for *Shigella* when compared with conventional methods.[9, 75, 76]

Four species—*Shigella dysenteriae, Shigella flexneri, Shigella boydii,* and *Shigella sonnei*—produce shigellosis.[36] An estimated 18,000 cases of shigellosis occur annually in the United States; in an outbreak of at least 30 culture-confirmed cases, *S. sonnei* infections were documented and linked to eating a nationally distributed dip.[27] *Shigella* usually elaborate cytotoxins that cause destruction of mucosal cells. *S. dysenteriae* type I produces an exotoxin that has enterotoxic and neurotoxic actions. Direct examination of fresh dysenteric stools may show a cellular exudate with erythrocytes and numerous leukocytes.[27, 46] The definitive diagnosis of shigellosis is made by stool culture using selective and nonselective media. Selective differential media, such as XLD, SS, deoxycholate-citrate, Hektoen enteric (HE), and MAC agars, are well suited for the isolation of *Shigella* from stool specimens. DNA hybridization assays and DNA probes specific for invasion genes have been used for the diagnosis of shigellosis,[14, 38, 41] but these assays are not readily available in clinical laboratories. Commercial products can be used for the identification of *Shigella* in conjunction with serologic typing.

C. jejuni is the commonest bacterial cause of infectious diarrhea in the United States, with an estimated 2.2 to 2.4 million cases occuring annually.[5, 97] Contaminated food (poultry), contaminated water, or contacts with infected animals are the usual modes of transmission. *Campylobacter* and related organisms are microaerophilic and grow best in an atmosphere containing 5% to 10% oxygen. All campylobacters grow at 37°C except for *C. jejuni,* which grows best at 42°C. The commonest media used for isolation are blood-based, antibiotic-containing media, such as Skirrow's, Butzler's and Campy-BAP.

Direct examination of fecal specimens by dark-field or phase-contrast microscopy may provide a presumptive diagnosis of campylobacteriosis because the organisms exhibit a typical *darting* motility, although the test lacks sensitivity (54% to 75%). Definitive diagnosis of *C. jejuni* infection is based on a positive stool culture. Latex agglutination tests have been used for confirmation of *C.*

jejuni grown on plates and show a high degree of sensitivity and specificity. Olive et al[81] used an alkaline phosphatase–labeled synthetic oligonucleotide probe for detection of *C. jejuni* and *Campylobacter coli* in fecal specimens, achieving a sensitivity of 82.6% and specificity of 100%.

AEROMONAS AND *PLESIOMONAS*

Aeromonads produce enterotoxins, cytotoxins, and hemolysins and are recognized as an important cause of acute diarrheal disease in young children, with *Aeromonas caviae* being the most commonly isolated species from stool specimens.[74, 79] Most outbreaks have been associated with the ingestion of contaminated water and shellfish. Diarrhea is seen more commonly in children less than 3 years old and in day care centers. Although disease is self-limited in most patients, some may develop fever, abdominal pain, and bloody diarrhea with the presence of fecal leukocytes. The organisms are isolated from freshly collected stool specimens early after onset of the disease. Aeromonads grow well on sheep blood agar (BA) and MAC agar; CIN or BA containing ampicillin may be used to isolate the organism from mixed cultures.[90] The organisms are identified based on oxidase, catalase, nitrate, esculin hydrolysis, and gas from glucose reactions.[57]

Plesiomonas shigelloides has been incriminated as a cause of acute diarrhea and is transmitted most commonly through water and seafood (shrimps, oysters) and also chickens.[74] Investigators have identified some potential virulence factors, such as β-hemolysin and a cholera-like toxin, although the precise role of these factors in pathogenesis has not been clarified. The organism is isolated easily using common enteric media (BA and MAC) but does not grow well on thiosulfate citrate bile salt (TCBS) agar. *P. shigelloides* is identified based on positive reactions with ornithine decarboxylase, lysine decarboxylase, and arginine dihydrolase tests.[74]

VIBRIO CHOLERAE AND *VIBRIO PARAHAEMOLYTICUS*

Vibrio cholerae is a major cause of epidemic diarrhea in Asia, Africa, and Latin America; only serogroups 01 and serogroup 0139 are associated with cholera. *V. cholerae* primarily affects the small intestine by the production of an enterotoxin that produces profuse watery diarrhea, vomiting, circulatory collapse, and shock.[49] Left untreated, a patient may become hypotensive within 1 hour of the onset of symptoms and be dead within 2 to 3 hours.[49] Phase-contrast or dark-field microscopy is useful in detection of vibrios from stool specimens, in which large numbers of bacilli display a characteristic *shooting stars* motility.[12] The rapid diagnosis of vibrios can be made by immobilizing motile vibrios with serotype-specific Ogawa or Inaba antisera.[49] Ogawa or Inaba antisera do not immobilize the new epidemic strain of *V. cholerae* 0139 Bengal, however. Diagnosis of vibrios is accomplished best by culturing stool or a rectal swab on TCBS selective agar. Conventional biochemical and serologic tests permit definitive identification. With advances in molecular diagnostics, a rapid diagnosis of cholera can be achieved by using an AP-labeled, cytotoxin-specific oligonucleotide to identify *V. cholerae* from the stool specimens.[99]

Vibrio parahaemolyticus has been recognized as the major cause of acute diarrheal disease in Japan. The organisms cause watery diarrhea with abdominal pain, nausea, chills, and fever, although the disease usually is self-limited and

lasts only a few days. Approximately 30 to 40 cases of *V. parahaemolyticus* infection are seen in the Gulf Coast states of Alabama, Florida, Louisiana, and Texas annually.[23] Direct microscopic examination of the stool may reveal a few leukocytes and erythrocytes. The organism is isolated from stool by using TCBS agar as for *V. cholerae*, in which it appears as an opaque colony. Transmission usually occurs through the ingestion of raw or undercooked shellfish, particularly oysters.

ESCHERICHIA COLI

Five different types of diarrheagenic *E. coli* have been described on the basis of clinical and epidemiologic features: ETEC, enteropathogenic *E. coli* (EPEC), EHEC, EIEC, and enteroadherent *E. coli* (EAEC). ETEC and EPEC are important causes of diarrhea in infants, and ETEC is a primary cause of traveler's diarrhea.[22, 28, 61] Children less than 2 years old have a much higher risk of acquiring traveler's diarrhea. Diarrheagenic *E. coli* can produce watery, cholera-like diarrhea (ETEC), dysentery-like symptoms (EIEC), and grossly bloody diarrhea (EHEC). *E. coli* O157:H7, one of the common EHEC serotypes, was described first in 1982 and produces a Shiga-like toxin that causes sporadic cases and outbreaks of hemorrhagic colitis.[85] The organism is associated with severe hemolytic-uremic syndrome, especially in children (see articles by Ramaswamy and Jacobson, page 611, and Tarr and Neill, page 735). One of the major distinguishing cultural characteristics of this serotype is that about 80% of isolates do not ferment D-sorbitol. The medium of choice for isolation of this organism is sorbitol-MacConkey (SMAC) agar. Agar or broth medium containing the substrate 4-methylumbellifery1-β-D-glucuronide (MUG) can be used for detection of glucuronidase produced by *E. coli*. About 92% of *E. coli* O157:H7 isolates lack the enzyme β-glucuronidase and do not produce a fluorescent product when observed with long-wave UV light. Latex agglutination can be used as a rapid test for *E. coli* serogroup O157 detection in primary cultures. The MUG assay, sorbitol-fermentation testing, and agglutination in *E. coli* O157 antiserum are useful tests for screening for cytotoxigenic strains of O157.[68] Methods for the recovery and detection of other pathogenic *E. coli* are not routinely available.

CLOSTRIDIUM DIFFICILE

C. difficile is one of the most frequently identified causes of nosocomial diarrhea.[10, 64] *C. difficile* has been recognized as a causative agent in antibiotic-associated diarrhea,[61] antibiotic-associated colitis,[42] and pseudomembranous colitis.[17, 18, 30, 62, 70, 94] Severe or fatal *C. difficile*–associated disease can occur more frequently in some immunocompromised hosts, such as patients infected with HIV or patients who have received bone marrow transplants.[40, 45, 58] Peterson[86] found that infants and cystic fibrosis patients are asymptomatic carriers of toxigenic *C. difficile* with colonization rates of 50% in infants and 32% in cystic fibrosis patients.

Many laboratory tests are available to assist in diagnosis, and each has strengths and drawbacks that vary depending on the incidence of disease in the patient population being tested and the particular underlying medical condition. Culture of stool for *C. difficile* using selective agar techniques has long been a mainstay in detecting the presence of the organism. George et al[44] was the first to describe a selective medium (cycloserine-cefoxitin-fructose agar [CCFA]) for

recovery of *C. difficile* from stool; this formulation is in common use today. Direct detection of a *C. difficile*–associated antigen, glutamate dehydrogenase, in stool is another approach in documenting presence of the organism. Direct detection of *C. difficile* toxins responsible for clinical disease has become more popular because neither the culture method nor the marker antigen test distinguishes toxigenic from nontoxigenic strains, and cultures require at least 48 hours.[3, 71]

    C. difficile produces 2 protein exotoxins, toxin A and toxin B, that are responsible for clinical disease: toxin A is an enterotoxin that causes fluid secretion, mucosal injury, and inflammation, whereas toxin B is a potent cytotoxin primarily mediating damage to colonic mucosa.[15] Because *C. difficile* disease is a treatable condition that must be differentiated from other causes of infectious and noninfectious diarrheal disease, considerable efforts have been made to develop rapid, sensitive, and specific tests for the detection of *C. difficile* toxins and the common antigen. Many EIA kits detecting toxin A, toxin B, or both are commercially available and vary considerably in sensitivity (34% to 100%) and specificity (88% to 100%). EIA and LA kits are available for detection of *C. difficile* glutamate dehydrogenase, although positive results do not differentiate toxigenic from nontoxigenic (i.e., colonizing) strains. Negative results with simultaneously performed glutamate dehydrogenase and toxin A tests reliably rule out the possibility of *C. difficile*–associated disease (negative predictive value, 99.6%).[11] Despite availability and rapidity of EIA *C. difficile* antigen and toxin tests, the cytotoxin B tissue culture assay is the most specific test for detection of toxigenic *C. difficile*.

VIRAL ENTERITIDES

    Many viruses are known to cause diarrheal disease, including members of the families Reoviridae (rotavirus), Adenoviridae (enteric adenoviruses), Calicivaridae (Norwalk group viruses and caliciviruses), Astroviridae (astrovirus), and Coronaviridae (coronavirus).[25, 87] Most viral enteric infections remain undiagnosed; commonly available laboratory tests are directed primarily at rotavirus, adenovirus, and Norwalk virus.

    Rotavirus is recognized increasingly as the major cause of severe diarrhea in young children, producing an estimated 3.5 million cases of diarrheal illness yearly.[32, 52, 59, 69, 83] Transmission of rotavirus occurs primarily through the oralfecal route. The classification of antigenic properties of rotavirus is based primarily on the viral capsid proteins and includes 7 major groups—group A through G.[80, 83] Group A strains are encountered most commonly. Rotaviruses are shed continuously during the course of the disease, and viral antigens can be detected by EIA 1 week after infection.[68, 98] Laboratory methods currently available for detection of rotavirus in stool include LA, EIA (standard and membrane format), and electron microscopy, with the last-mentioned regarded as the gold standard by virtue of being able to recognize all rotaviruses and other enteric viruses. Rotavirus LA and membrane EIA are simple, rapid, and generally accurate tests, especially when applied early in an infection, although standard EIA methods may be expected to be more sensitive. EIA methods are more sensitive than electron microscopy.

    Adenovirus serotypes 40 and 41 are common viral causes of diarrhea in children less than 2 years old, being responsible for 5% to 20% of hospitalizations for childhood diarrhea.[68, 87] These serotypes can be cultured in Graham 293 cells or Chang conjunctival cells. Clinical features of enteric adenovirus infection are watery diarrhea lasting 5 to 14 days, vomiting, abdominal pain, and low-

grade fever; secondary spread among children is common. Diagnosis of enteric adenovirus infection can be established by electron microscopy detection of adenoviruses or EIA tests specific for serotypes 40 and 41. Adenovirus serotype 31 has been associated with infantile diarrhea.

Norwalk virus was described first after an outbreak of acute gastroenteritis in Norwalk, Ohio, in 1972.[69, 87] Clinical features of Norwalk virus and Norwalk-like virus infections include nausea, vomiting, diarrhea, abdominal pain, headache, and fever, which usually last 24 to 48 hours with a mean incubation period of 24 hours.[87] The disease may be transmitted through contaminated food or water and by secondary contact. Norwalk and other caliciviruses can be detected with electron microscopy; immunoelectron microscopy has been used for typing of these organisms.[32] Although EIAs for detection of caliciviruses have been developed, they are not routinely available; electron microscopy and immuno-electron microscopy remain as the fundamental tools for detection of viral diarrheal agents.

PARASITES

As a general rule, diarrheal disease produced by parasites is seen primarily with protozoal infections (see Table 1); helminthic diarrheal disease occurs infre-·quently and, when present, results from unusually heavy worm burdens, primarily caused by infections with *Strongyloides stercoralis*, *Trichuris trichiura*, and the hookworms *Ancylostoma duodenale* and *Necator americanus*. Gastrointestinal parasite infections vary considerably in their ability to produce symptoms from asymptomatic carriage (*E. histolytica/E. dispar*, *G. lamblia*, microsporidia, *S. stercoralis*) to nonspecific diarrhea (*G. lamblia*, *C. parvum*, *I. belli*, *C. cayetanensis*, microsporidia, others) to life-threatening colitis or fluid loss (*E. histolytica*, *C. parvum*, *S. stercoralis*),

The traditional ova and parasite stool examination has changed little over many decades and includes macroscopic examination for adult worms and proglottids; a direct wet mount examination to detect parasite motility (if fresh diarrheic stool is submitted); a concentration technique to aid in recovery of protozoan cysts and helminth eggs and larvae; and a thin fecal smear, which is fixed and stained with a permanent stain such as trichrome or iron hematoxylin to detect protozoal trophozoites and cysts. Historic recommendations for outpatient testing call for the submission of 3 specimens collected on alternate days to improve yield.[92] This approach, although labor intensive, relatively expensive, and modestly insensitive, offers an exceptional opportunity to recover and identify most offending parasitic agents and does not require expensive or unusual laboratory equipment. Most of the more recently described protozoal agents producing diarrhea are not detectable by these methods, requiring additional specific stains or EIA methods. Agents not routinely recovered by the ova and parasite test include species for which HIV-infected individuals are at particular risk: the coccidians *C. parvum*, *C. cayetanensis*, and *I. belli* and the microsporidians *E. bieneusi* and *E. intestinalis*. For these species, specific requests must be made of the laboratory to perform the indicated stain or EIA (see Table 3).

Newer EIA, membrane-EIA, and immunofluorescence methods are available for routine testing for *E. histolytica*, *G. lamblia*, and *C. parvum*. When directed testing is preferable because of suggestive patient symptoms and history or during an outbreak situation, these newer tests offer advantages of improved sensitivity, specificity, and rapidity over the ova and parasite test. They are

organism specific, however, and quickly add extra cost to the patient laboratory workup.[92]

SUMMARY

Future applications of advanced molecular diagnostics in clinical laboratories will enhance significantly capabilities to diagnose bacterial, parasitic, and viral agents in the early course of disease through enhanced assay sensitivities and specificities and improved turnaround times, theoretically leading to more timely and directed therapeutic intervention. Until such time, clinicians must continue to rely on clinical judgment and the diverse battery of traditional culture techniques, direct examination (including light microscopy and electron microscopy), and immunoassays that are available. Cost considerations and the ever-increasing array of infectious agents responsible for infectious gastroenteritis will continue to drive the development of practice guidelines to assist practitioners with reasoned and reasonable approaches to management of diarrheal illnesses.

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