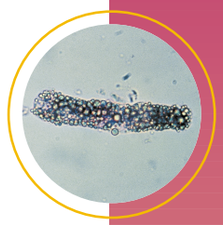
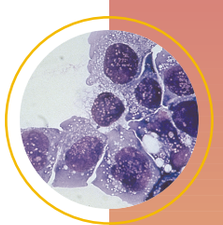
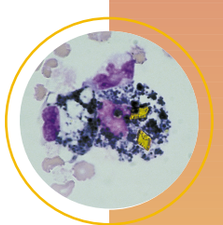
CHAPTER 15







Fecal Analysis

LEARNING OBJECTIVES

Upon completion of this chapter, the reader will be able to:

1. Describe the normal composition of feces.
2. Differentiate between secretory and osmotic diarrhea.
3. Instruct patients in the collection of random and quantitative stool specimens.
4. State a pathogenic and a nonpathogenic cause for stools colored red, black, and pale yellow.
5. State the significance of bulky, ribbon-like, and mucus-containing stools.
6. State the significance of increased neutrophils in a stool specimen.
7. Describe a positive microscopic examination for muscle fibers.
8. Name the fecal fats stained by Sudan III, and give the conditions under which they will stain.
9. Describe and interpret the microscopic results that will be seen when a specimen from a patient with steatorrhea is stained with Sudan III.
10. Explain the principle of the guaiac test for occult blood and the reasons that guaiac is the reagent of choice.
11. Instruct a patient in the collection of specimens for occult blood, including providing an explanation of dietary restrictions.
12. Briefly describe a chemical screening test performed on feces for each of the following: fetal hemoglobin, pancreatic insufficiency, and carbohydrate intolerance.

KEY TERMS

malabsorption
maldigestion
occult blood
osmotic diarrhea
pancreatic insufficiency
secretory diarrhea
steatorrhea

In the minds of most laboratory personnel, analysis of fecal specimens fits into the category of a "necessary evil." However, as an end product of body metabolism, feces do provide valuable diagnostic information. Routine fecal examination includes macroscopic, microscopic, and chemical analyses for the early detection of gastrointestinal bleeding, liver and biliary duct disorders, ***maldigestion/malabsorption*** syndromes, and inflammation. Of equal diagnostic value is the detection and identification of pathogenic bacteria and parasites; however, these procedures are best covered in a microbiology textbook and will not be discussed here.

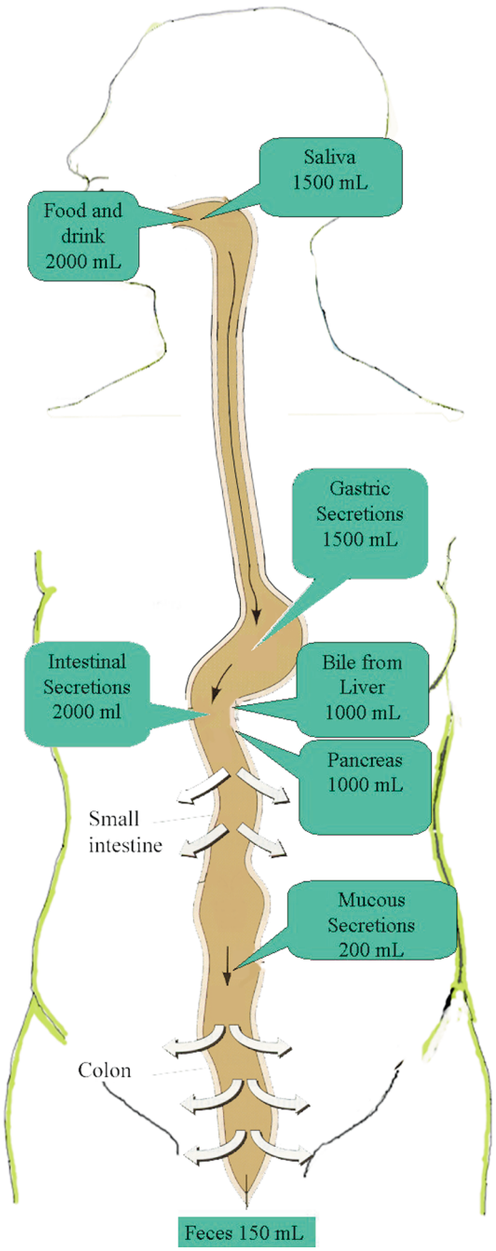


FIGURE 15–1 Fluid regulation in the gastrointestinal tract. (Adapted from Martini, FH: Fundamentals of Anatomy and Physiology. Prentice Hall, New Jersey, 1998.)

Physiology

The normal fecal specimen contains bacteria, cellulose and other undigested foodstuffs, gastrointestinal secretions, bile pigments, cells from the intestinal walls, electrolytes, and water. Many species of bacteria make up the normal flora of the intestines. Bacterial metabolism produces the strong odor associated with feces and intestinal gas or **flatus**.

Although digestion of ingested proteins, carbohydrates, and fats takes place throughout the **alimentary tract**, the small intestine is the primary site for the final breakdown and reabsorption of these compounds. Digestive enzymes secreted into the small intestine by the pancreas include trypsin, chymotrypsin, amino peptidase, and lipase. Bile salts provided by the liver aid in the digestion of fats. A deficiency in any of these substances causes the inability to digest and, therefore, reabsorb certain foods. Excess undigested or unreabsorbed material will then appear in the feces, and patients exhibit symptoms of maldigestion and malabsorption. As shown in Figure 15–1, approximately 9000 mL of ingested fluid, saliva, gastric, liver, pancreatic, and intestinal secretions enter the digestive tract each day. Under normal conditions, only between 500 to 1500 mL of this fluid reaches the large intestine, and only about 150 mL is excreted in the feces. Water and electrolytes are readily absorbed in both the small and large intestines, resulting in a fecal electrolyte content that is similar to that of plasma.

The large intestine is capable of absorbing approximately 3000 mL of water. When the amount of water reaching the large intestine exceeds this amount, it is excreted with the solid fecal material, producing **diarrhea**. **Constipation**, on the other hand, provides time for additional water to be reabsorbed from the fecal material, producing small, hard **stools**.

Bacterial, viral, and protozoan infections produce increased secretion of water and electrolytes, which override the reabsorptive ability of the large intestine (***secretory diarrhea***). Incomplete breakdown or reabsorption of foodstuffs presents increased fecal material to the large intestine resulting in the retention of water and electrolytes in the large intestine (***osmotic diarrhea***). Laboratory testing of feces is frequently performed to aid in determining the cause of diarrhea (Table 15–1).

Specimen Collection

Collection of a fecal specimen, frequently called a stool specimen, is not an easy task for patients. Detailed instructions and appropriate containers should be provided.

Patients should be instructed to collect the specimen in a clean container, such as a bedpan or disposable container, and transfer the specimen to the laboratory container. Patients should understand that the specimen must not be contaminated with urine or toilet water that may contain chemical disinfectants. Some kits provided for the collec-

TABLE 15–1 **Common Fecal Tests for Diarrhea**

Secretory	Osmotic
Stool cultures	Microscopic fecal fats
Ova and parasite examinations	Muscle fiber detection
Rotavirus immunoassay	Qualitative fecal fats
Fecal leukocytes	Trypsin screening
	Microscopic fecal fats
	Muscle fiber detection
	Quantitative fecal fats
	Clinitest
	D-xylose tolerance test
	Lactose tolerance test

tion of specimens for ***occult blood*** contain paper that can be floated in the toilet bowl to collect the specimen. This method should only be used when collecting specimens to be tested using the kit in which they are included. Containers containing preservatives for ova and parasites must not be used to collect specimens for other tests.

Random specimens suitable for qualitative testing for blood and microscopic examination for leukocytes, muscle fibers, and fecal fats are usually collected in plastic or glass containers with screw-capped tops similar to those used for urine specimens. Material collected on a physician's glove and samples applied to filter paper in occult blood testing kits are also received.

For quantitative testing, such as for fecal fats, timed specimens are required. Because of the variability of bowel habits and the transit time required for food to pass through the digestive tract, the most representative sample is a 3-day collection. These specimens are frequently collected in paint cans to accommodate the specimen quantity and facilitate emulsification prior to testing. Care must be taken when opening any fecal specimen to slowly release gas that has accumulated within the container. Also, patients must be cautioned not to contaminate the outside of the container.

Macroscopic Screening

The first indication of gastrointestinal disturbances can often be provided by changes in the brown color and formed consistency of the normal stool. Of course, the appearance of abnormal fecal color may also be caused by the ingestion of highly pigmented foods and medications, so a differentiation must be made between this and a possible pathologic cause.

COLOR

The brown color of the feces results from intestinal oxidation of urobilinogen to urobilin. As discussed in Chapter 5, urobilinogen formed in the degradation of hemoglobin passes through the bile duct to the small intestine. Therefore, stools appearing pale in color may signify a blockage of the bile duct. Pale stools are also associated with diagnostic procedures using barium sulfate.

A primary concern is the presence of blood in a stool specimen. Depending on the area of the intestinal tract from which bleeding is occurring, the color can range from bright to dark red to black. Blood originating from the esophagus, stomach, or duodenum takes approximately 3 days to appear in the stool; during this time, degradation of hemoglobin produces the characteristic black, tarry stool. Likewise, blood from the lower gastrointestinal tract requires less time to appear and will retain its original red color. Both black and red stools should be chemically tested for the presence of blood, because ingestion of iron, charcoal, or bismuth will often produce a black stool, and medications and foods, including beets, will produce a red stool.

Green stools may be observed in patients taking oral antibiotics owing to oxidation of fecal bilirubin to biliverdin. Ingestion of increased amounts of green vegetables or food coloring also will produce green stools.

APPEARANCE

Besides variations in color, additional abnormalities that may be observed during the macroscopic examination include the watery consistency present in diarrhea and the small, hard stools seen with constipation. Slender, ribbon-like stools suggest an obstruction of the normal passage of material through the intestine.

Pale stools associated with biliary obstruction will appear bulky and frothy and frequently have a foul odor. Absence of bile salts that assist pancreatic lipase in the breakdown and subsequent reabsorption of triglycerides produces an increase in stool fat termed ***steatorrhea***. Likewise, pan-

TABLE 15–2 **Macroscopic Stool Characteristics**[1,5]

Color/Appearance	Possible Cause
Black	Upper gastrointestinal bleeding
	Iron therapy
	Charcoal
	Bismuth (antacids)
Red	Lower gastrointestinal bleeding
	Beets and food coloring
	Rifampin
Pale yellow, white, gray	Bile-duct obstruction
	Barium sulfate
Green	Biliverdin/oral antibiotics
	Green vegetables
Bulky/frothy	Bile-duct obstruction
	Pancreatic disorders
Ribbon-like	Intestinal constriction
Mucus/blood-streaked mucus	Colitis
	Dysentery
	Malignancy
	Constipation

creatic disorders, including cystic fibrosis, chronic pancreatitis, and carcinoma that decrease the production of pancreatic enzymes, are also associated with steatorrhea.

The presence of mucus-coated stools is indicative of intestinal inflammation or irritation. Mucus-coated stools may be caused by pathologic colitis or excessive straining during elimination. Blood-streaked mucus suggests damage to the intestinal walls, possibly caused by bacterial or amebic **dysentery** or malignancy. The presence of mucus should be reported (Table 15–2).

Microscopic Examination of Feces

Microscopic screening of fecal smears is performed to detect the presence of leukocytes associated with microbial diarrhea and undigested muscle fibers and fats associated with steatorrhea.

FECAL LEUKOCYTES

Leukocytes, primarily neutrophils, are seen in the feces in conditions that affect the intestinal mucosa, such as ulcerative colitis and bacterial dysentery. Microscopic screening is performed as a preliminary test to determine whether diarrhea is being caused by invasive bacterial pathogens including *Salmonella*, *Shigella*, *Campylobacter*, *Yersinia*, and enteroinvasive *Escherichia coli*. Bacteria that cause diarrhea by toxin production, such as *Staphylococcus aureus* and *Vibrio* sp., viruses, and parasites usually do not cause the appearance of fecal leukocytes. Therefore, the presence or absence of fecal neutrophils can provide the physician with diagnostic information prior to the receiving of a culture report.

Specimens can be examined as wet preparations stained with methylene blue or as dried smears stained with Wright's or Gram stain. Methylene blue staining is the faster procedure but may be more difficult to interpret. Dried preparations stained with either Wright's or Gram stains provide permanent slides for evaluation. An additional advantage of the Gram stain is the observation of gram-positive and gram-negative bacteria, which could aid in the initial treatment.[8] All slide preparations must be performed on fresh specimens. When examining preparations under high power, as few as three neutrophils per high power field can be indicative of an invasive condition.[1] Using oil immersion, the finding of any neutrophils has approximately 70 percent sensitivity for the presence of invasive bacteria.[10]

A lactoferrin latex agglutination test is available for the detection of fecal leukocytes and remains sensitive in refrigerated and frozen specimens. The presence of lactoferrin, a component of granulocyte secondary granules, is indicative of an invasive bacterial pathogen.[9]

MUSCLE FIBERS

Microscopic examination of the feces for the presence of undigested striated muscle fibers can be helpful in the diagnosis and monitoring of patients with ***pancreatic insufficiency***, such as in cases of cystic fibrosis. It is frequently ordered in conjunction with microscopic examinations for fecal fats. Increased amounts of striated fibers may also be seen in biliary obstruction and **gastrocolic fistulas**.

Slides for muscle fiber detection are prepared by emulsifying a small amount of stool in 10 percent alcoholic eosin, which enhances the muscle fiber striations. The entire slide is examined for exactly 5 minutes, and the number of red-stained fibers with well-preserved striations is counted. Care must be taken to correctly classify the fibers observed. Undigested fibers have visible striations running both vertically and horizontally. Partially digested fibers exhibit striations in only one direction, and digested fibers have no visible striations. Only undigested fibers are counted, and the presence of more than 10 is reported as increased.

To produce a representative sample, patients should be instructed to include red meat in their diet prior to collecting the specimen. Specimens should be examined within 24 hours of collection.

PROCEDURE

Methylene Blue Stain Procedure for Fecal Leukocytes

1. Place mucus or a drop of liquid stool on a slide.
2. Add two drops Löffler methylene blue.
3. Mix with a wooden applicator stick.
4. Allow to stand 2–3 minutes.
5. Examine for neutrophils under high power.

QUALITATIVE FECAL FATS

Specimens from suspected cases of steatorrhea can be screened microscopically for the presence of excess fecal fat. The procedure can also be used to monitor patients undergoing treatment for malabsorption disorders.[15] In general, correlation between the qualitative and quantitative fecal fat procedures is good; however, additional unstained phospholipids and cholesterol esters are measured by the quantitative procedure.[6,12] Lipids included in the microscopic examination of feces are neutral fats (triglycerides), fatty acid salts (soaps), fatty acids, and cholesterol. Their presence can be observed microscopically by staining with

PROCEDURE

Muscle Fiber Procedure

1. Emulsify a small amount of stool in two drops of 10% eosin in alcohol.
2. Coverslip and let stand 3 minutes.
3. Examine under high power for 5 minutes.
4. Count the number of undigested fibers.

PROCEDURE

Neutral Fat Stain Procedure

1. Homogenize one part stool with two parts water.
2. Mix emulsified stool with one drop 95% ethyl alcohol on slide.
3. Add two drops saturated Sudan III in 95% ethanol.
4. Mix and coverslip.
5. Examine under high power.
6. Count orange droplets per high-power field.

the dyes Sudan III, Sudan IV, or oil red O, of which Sudan III is the most routinely used. The staining procedure consists of two parts, the neutral fat stain and the split fat stain.

Neutral fats are readily stained by Sudan III and appear as large orange-red droplets, often located near the edge of the coverslip.[14] Observation of more than 60 droplets/hpf can be indicative of steatorrhea; however, the split fat stain representing total fat content can provide a better indication.[4] The breakdown of neutral fats by bacterial lipase and the spontaneous hydrolysis of neutral fats may lower the neutral fat count. This also prevents using comparison of the two slide tests to determine whether maldigestion or malabsorption is causing steatorrhea.

Soaps and fatty acids do not stain directly with Sudan III. Therefore, a second slide must be examined after the specimen has been mixed with acetic acid and heated. Examination of this slide will reveal stained droplets that represent not only the free fatty acids but also the fatty acids produced by hydrolysis of the soaps and the neutral fats. When examining this split fat slide, both the number and size of the fat droplets must be considered. Normal specimens may contain as many as 100 small droplets, less than 4 μm in size, per hpf. The same number of droplets measuring 1 to 8 μm is considered slightly increased, and 100 droplets measuring 6 to 75 μm is increased.[3]

Cholesterol is stained by Sudan III after heating and as the specimen cools forms crystals that can be identified microscopically.

PROCEDURE

Split Fat Stain Procedure

1. Mix emulsified stool with one drop of 36% acetic acid.
2. Add two drops saturated Sudan III.
3. Mix and coverslip.
4. Heat gently almost to boiling.
5. Examine under high power.
6. Count and measure the orange droplets per high-power field.

Chemical Testing of Feces

OCCULT BLOOD

By far the most frequently performed fecal analysis is the chemical screening test for the detection of occult blood. As discussed previously, bleeding in the upper gastrointestinal tract may produce a black, tarry stool, and bleeding in the lower gastrointestinal tract may result in an overtly bloody stool. However, because any bleeding in excess of 2.5 mL/150 g of stool is considered pathologically significant, and no visible signs of bleeding may be present with this amount of blood, fecal occult blood testing (**FOBT**) is necessary. Originally used primarily to test suspected cases of gastrointestinal disease, FOBT has currently become widely used as a mass screening procedure for the early detection of colorectal cancer. Testing for occult blood has a high positive predictive value for detection of colorectal cancer in the early stages and is recommended by the American Cancer Society, particularly for persons older than age 50.

The most frequently encountered screening tests for occult blood are based on detection of the pseudoperoxidase activity of hemoglobin. This is the same principle as the reagent strip test for urinary blood, but uses a different indicator chromogen. The reaction uses the pseudoperoxidase activity of hemoglobin reacting with hydrogen peroxide to oxidize a colorless compound to a colored compound:

$$\text{Hemoglobin} \xrightarrow[\text{Peroxidase}]{\text{Pseudo}} H_2O_2 \xrightarrow{(0)} \text{Guaiac} \rightarrow \underset{\text{(Blue color)}}{\text{Oxidized guaiac}} + H_2O$$

Several different indicator chromagens have been used to detect occult blood. All react in the same chemical manner but vary in their sensitivity. Listed in order of decreasing sensitivity, these compounds include benzidine, ortho-tolidine, and gum guaiac. Contrary to most chemical testing, the least sensitive reagent, guaiac, is preferred for routine testing. Considering that a normal stool can contain up to 2.5 mL of blood, a less sensitive chemical reactant is understandably more desirable. In addition, pseudoperoxidase activity is present from hemoglobin and myoglobin in ingested meat and fish, certain vegetables and fruits, and some intestinal bacteria. Therefore, to prevent false-positive reactions, the sensitivity of the test must be decreased. This can be accomplished by varying the amount and purity of the guaiac reagent used in the test.

Many commercial testing kits are available for occult blood testing with guaiac reagent. The kits contain guaiac-impregnated filter paper, to which the fecal specimen and hydrogen peroxide are added. Two or three filter paper areas are provided for application of material taken from different areas of the stool, and positive and negative controls are also included. Obtaining samples from the center of the stool will avoid false-positives from external contamination. Addition of hydrogen peroxide to the back of the filter paper that contains stool produces a blue color with guaiac reagent when pseudoperoxidase activity is present.

Packaging of the guaiac-impregnated filter paper in individually sealed containers has facilitated the screening pro-

Summary of Occult Blood Testing Interference

False-Positive

Aspirin and anti-inflammatory medications
Red meat
Horseradish
Raw broccoli, cauliflower, radishes, turnips
Melons
Menstrual and hemorrhoid contamination

False-Negative

Vitamin C >250 mg/d
Iron supplements containing vitamin C

gram for colorectal cancer by allowing persons at home to place the specimen on the filter paper and bring or mail it to the laboratory for testing. To prevent false-positive reactions, specimens mailed to the laboratory should not be rehydrated prior to adding the hydrogen peroxide, unless specifically instructed by the kit manufacturer (Hemocult II Sansa, Smith Kline Diagnostics, Sunnyvale, CA). Specimens applied to the paper in the laboratory should be allowed to dry prior to testing. The specimens should be tested within 6 days of collection. Two samples from three different stools should be tested before a negative result is confirmed. Patients should be instructed to avoid eating red meats, horseradish, melons, raw broccoli, cauliflower, radishes, and turnips for 3 days prior to specimen collection. This will prevent the presence of dietary pseudoperoxidases in the stool. Aspirin and nonsteroidal anti-inflammatory agents, other than acetaminophen, should not be taken for 7 days prior to specimen collection to prevent possible gastrointestinal irritation. Vitamin C and iron supplements containing vitamin C should be avoided for 3 days prior to collections, because ascorbic acid is a strong reducing agent that will interfere with the peroxidase reaction.[7]

Additional more sensitive and specific methods for the detection of occult blood have been developed. Hemoquant (Smith Kline Diagnostics, Sunnyvale, CA) provides a fluorometric test for hemoglobin and porphyrin. As hemoglobin progresses through the intestinal tract, bacterial actions degrade it to porphyrin that the guaiac test cannot detect. This can result in some false-negative results from upper gastrointestinal bleeding when using the guaiac test. The immunologic tests, HemeSelect and FlexSure OBT (Smith Kline Diagnostics, Sunnyvale, CA) are specific for human hemoglobin and do not require dietary restrictions. They are more sensitive to lower gastrointestinal bleeding and can be used for patients who are taking aspirin and other anti-inflammatory medications. Collection kits are similar to those used for guaiac testing and can be provided to patients for home collection.

QUANTITATIVE FECAL FAT TESTING

Quantitative fecal fat analysis is used as a confirmatory test for steatorrhea. As discussed previously, quantitative fecal analysis requires the collection of at least a 3-day specimen.

PROCEDURE

APT TEST PROCEDURE

1. Emulsify specimen in water.
2. Centrifuge.
3. Divide pink supernatant into two tubes.
4. Add 1% sodium hydroxide to one tube.
5. Wait 2 minutes.
6. Compare color with that in the control tube.
7. Prepare controls using cord blood and adult blood.

The patient must also maintain a regulated intake of fat (100 g/d) prior to and during the collection period. Paint cans make excellent collection containers because the specimen must be homogenized prior to analysis, and this can be accomplished by placing the container on a conventional paint-can shaker. The method routinely used for fecal fat measurement is the Van de Kamer titration, although gravimetric methods are available.[14] Fecal lipids are converted to fatty acids and titrated to a neutral end point with sodium hydroxide. The fat content is reported as grams of fat or the coefficient of fat retention per 24 hours. Normal values based on a 100 g/d intake are 1 to 6 g/d or a coefficient of fat retention of at least 95 percent. The coefficient of fat retention is calculated as follows:

$$\frac{(\text{dietary fat} - \text{fecal fat})}{(\text{dietary fat})} \times 100$$

APT TEST (FETAL HEMOGLOBIN)

Grossly bloody stools and vomitus are sometimes seen in neonates as the result of swallowing maternal blood during delivery. Should it be necessary to distinguish between the presence of fetal blood or maternal blood in an infant's stool or vomitus, the Apt test may be requested.

The material to be tested is emulsified in water to release hemoglobin, and after centrifugation, 1 percent sodium hydroxide is added to the pink hemoglobin-containing supernatant. In the presence of alkali-resistant fetal hemoglobin, the solution will remain pink, whereas denaturation of the maternal hemoglobin will produce a yellow-brown supernatant after standing for 2 minutes. The Apt test distinguishes not only between fetal hemoglobin and hemoglobin A but also between maternal hemoglobins AS, CS, and SS and fetal hemoglobin. The presence of maternal thalassemia major would produce erroneous results owing to the high concentration of hemoglobin F. Stool specimens should be tested when fresh. They may appear bloody but should not be black and tarry, because this would indicate already denatured hemoglobin.[2]

FECAL ENZYMES

Enzymes supplied to the gastrointestinal tract by the pancreas are essential for the digestion of dietary proteins,

TABLE 15–3 **Summary of Fecal Screening Tests**

Test	Methodology/Principle	Interpretation
Examination for neutrophils	Microscopic count of neutrophils in smear stained with methylene blue, Gram stain, or Wright's stain	Three per high-power field indicates condition affecting intestinal wall
Qualitative fecal fats	Microscopic examination of direct smear stained with Sudan III	60 large orange-red droplets indicates malabsorption
	Microscopic examination of smear heated with acetic acid and Sudan III	100 orange-red droplets measuring 6–75 μm indicates malabsorption
Occult blood	Pseudoperoxidase activity of hemoglobin liberates oxygen from hydrogen peroxide to oxidize guaiac reagent	Blue color indicates gastrointestinal bleeding
Apt test	Addition of sodium hydroxide to hemoglobin-containing emulsion determines presence of maternal or fetal blood	Pink color indicates presence of fetal blood
Trypsin	Emulsified specimen placed on x-ray paper determines ability to digest gelatin	Inability to digest gelatin indicates lack of trypsin
Clinitest	Addition of Clinitest tablet to emulsified stool detects presence of reducing substances	Reaction of 0.5 g/dL reducing substances suggests carbohydrate intolerance

carbohydrates, and fats. A decrease in production of these enzymes (pancreatic insufficiency) is associated with disorders such as chronic pancreatitis and cystic fibrosis. Steatorrhea and the presence of undigested foodstuffs are present in the feces.

Analysis of the feces focuses primarily on the proteolytic enzymes, trypsin, chymotrypsin, and elastase I. Historically, absence of trypsin has been screened for by exposing x-ray paper to stool emulsified in water. When trypsin is present in the stool, it will digest the gelatin on the paper, leaving a clear area. Inability to digest the gelatin indicates a deficiency in trypsin production. The gelatin test is an insensitive procedure that detects only severe cases of pancreatic insufficiency. In addition, false-negative results may occur as the result of intestinal degradation of trypsin and the possible presence of trypsin inhibitors in the feces. The proteolytic activity of bacteria enzymes may produce false-positive results in old specimens.

Fecal chymotrypsin is more resistant to intestinal degradation and is a more sensitive indicator of less severe cases of pancreatic insufficiency. It also will remain stable in fecal specimens for up to 10 days at room temperature. Chymotrypsin is capable of gelatin hydrolysis but is most frequently measured by spectrophotometric methods.

Elastase I is an isoenzyme of the enzyme elastase and is the enzyme form that the pancreas produces. It is present in high concentrations in pancreatic secretions and is strongly resistant to degradation. Elastase I can be measured by immunoassay and provides a very sensitive indicator of pancreatic insufficiency.[11,13]

CARBOHYDRATES

The presence of increased carbohydrates in the stool will produce an osmotic diarrhea. Carbohydrates in the feces may be present as a result of intestinal inability to reabsorb carbohydrates, as is seen in celiac disease, or caused by lack of digestive enzymes such as lactase resulting in lactose intolerance. Carbohydrate malabsorption or intolerance (maldigestion) is primarily analyzed by serum and urine tests; however, an increased concentration of carbohydrate can be detected by performing a copper reduction test on the fecal specimen. Fecal carbohydrate testing is most valuable in assessing cases of infant diarrhea and may be accompanied by a pH determination. Normal stool pH is between 7 and 8; however, increased use of carbohydrates by intestinal bacteria will lower the pH to below 5.5 in cases of carbohydrate disorders.

The copper reduction test is performed using a Clinitest tablet (Bayer Diagnostics, Elkhart, IN) and one part stool emulsified in two parts water. A result of 0.5 g/dL is considered indicative of carbohydrate intolerance. As discussed in Chapter 5, this is a general test for the presence of reducing substances, and a positive result would be followed by more specific serum carbohydrate tolerance tests, the most common being the D-xylose test for malabsorption and the lactose tolerance test for maldigestion.

A summary of fecal screening tests is presented in Table 15–3.

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STUDY QUESTIONS

1. In what part of the digestive tract do pancreatic enzymes and bile salts contribute to digestion?
2. What is the primary digestive process taking place in the large intestine?
3. State whether the following tests are performed to detect secretory or osmotic diarrhea: fecal fats, Clinitest, fecal neutrophils, and muscle fiber examination.
4. State three methods of collection by which the laboratory might receive specimens for occult blood testing.
5. Why is a 3-day specimen recommended for quantitative fecal testing?
6. How can a laboratory accident be avoided when a quantitative fecal collection is received?
7. How does blockage of the bile duct affect the color and appearance of a stool? Why do these changes occur?
8. How does the significance of a bloody stool differ from that of a black, tarry stool?
9. A patient being treated for a sinus infection produces a green-colored stool. Is this significant? Why or why not?
10. State a pathologic and nonpathologic cause of mucus-containing stools.
11. Why does constipation cause production of small, hard stools?
12. What is the significance of fecal neutrophils?
13. Would the presence of fecal neutrophils be expected with diarrhea caused by a rotavirus? *Salmonella*?
14. How can a fecal specimen that has been refrigerated overnight be tested for the presence of neutrophils?
15. Describe the appearance of a microscopic slide that is positive for muscle fibers. What other microscopic test might be requested?
16. How does performance of microscopic examination for triglycerides differ from that performed to detect fatty acids?
17. Describe a slide that is positive for neutral fats and one that is positive for fatty acids.
18. Why is guaiac the reagent of choice for fecal occult blood testing? What is the principle of this test?
19. What is the recommended number of samples that should be tested to confirm a negative occult blood result? From what part of the stool should the samples be taken? Why?
20. What are the advantages of the Hemoquant and the FlexSure FOBT tests over the guaiac test? Which is more sensitive to upper gastrointestinal bleeding? Why?
21. Define steatorrhea. Would a coefficient of fat retention of 85 percent be considered indicative of steatorrhea? Why or why not?
22. What is the significance of an Apt test that remains pink after addition of sodium hydroxide?
23. Is failure of a stool sample to digest the gelatin on x-ray film associated with pancreatic insufficiency? How could you confirm that this is not a false-negative result?
24. What would cause the pH of a specimen collected in a case of infant diarrhea to be low?
25. State two reasons why increased carbohydrates may be present in a stool.

CASE STUDIES AND CLINICAL SITUATIONS

1. Microscopic screening of a stool from a patient exhibiting prolonged diarrhea shows increased fecal neutrophils and normal qualitative fecal fats and meat fibers.
   a. What type of diarrhea do these results suggest?
   b. Name an additional test that could provide more diagnostic information.
   c. Name one probable result for this test and one improbable result.
   d. If the test for fecal neutrophils was negative and the fecal fat concentration increased, what type of diarrhea is suggested?
2. Laboratory studies are being performed on a 5-year-old boy to determine whether there is a metabolic reason for his continued failure to gain weight. In addition to having blood drawn, the patient has a sweat chloride collected, provides a random stool sample, and is asked to collect a 72-hour stool sample.
   a. How can the presence of steatorrhea be screened for by testing the random stool sample?
   b. How does this test distinguish among neutral fats, soaps, and fatty acids?

c. What confirmatory test should be performed?
d. Describe the appearance of the stool specimens if steatorrhea is present.
e. If a diagnosis of cystic fibrosis is suspected, state two screening tests that could be performed on a stool specimen to aid in the diagnosis.
f. State a possible reason for a false-negative reaction in each of these tests.
g. What confirmatory test could be performed?

3. A physician's office laboratory is experiencing inconsistencies in the results of patient-collected specimens for FOBT. Patients are instructed to submit samples from two areas of three different stools. Positive and negative controls are producing satisfactory results. Patient #1 is a 30-year-old woman taking over-the-counter medications for gastric reflux who has reported passing frequent black stools. The results of all three specimens are negative for occult blood. Patient #2 is a 70-year-old woman suffering from arthritis. She is taking the test as part of a routine physical. The results of all three specimens are positive for occult blood. Patient #3 is a 50-year-old man advised by the doctor to lose 30 lb. He has been doing well on a high-protein, low-carbohydrate diet. Two of his three specimens are positive for occult blood.
a. What is the possible nonpathologic cause of the unexpected results for patient #1? Patient #2? Patient #3?
b. How could the physician's office staff avoid these discrepancies?
c. What testing methodology could be used for patients #2 and #3?

4. A watery black stool from a neonate is received in the laboratory with requests for an Apt test, fecal pH, and a Clinitest.
a. Can all three tests be performed on this specimen? Why?
b. If the Clinitest is positive, what pH reading can be expected? Why?
c. The infant's hemoglobin remains constant at 18 g/dL. What was the significance of the black stool?
d. Would this infant be expected to have ketonuria? Why or why not?