

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

KEY TERMS

constipation

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 (GI) bleeding, liver and biliary duct disorders, **maldigestion/malabsorption** syndromes, inflammation, and causes of diarrhea and steatorrhea. 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 are not discussed here.

■ ■ ● 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 (*flatus*). Carbohydrates, especially oligosaccharides, that are resistant to digestion pass through the upper intestine unchanged but are metabolized by bacteria in the lower intestine, producing large amounts of flatus. Excessive gas production also occurs in lactose-intolerant individuals when the intestinal bacteria metabolize the lactose from consumed milk or lactose-containing substances.

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, to reabsorb certain foods. Excess undigested or unreabsorbed material then appear in the feces, and the patient exhibits 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*.

Diarrhea

Diarrhea is defined as an increase in daily stool weight above 200 g with increased liquidity and frequency of more than three times per day. Diarrhea classification can be based on four factors: duration of the illness, mechanism, severity, and stool characteristics. Diarrhea lasting less than 4 weeks is defined as acute, and diarrhea persisting for more than 4 weeks is termed chronic diarrhea.

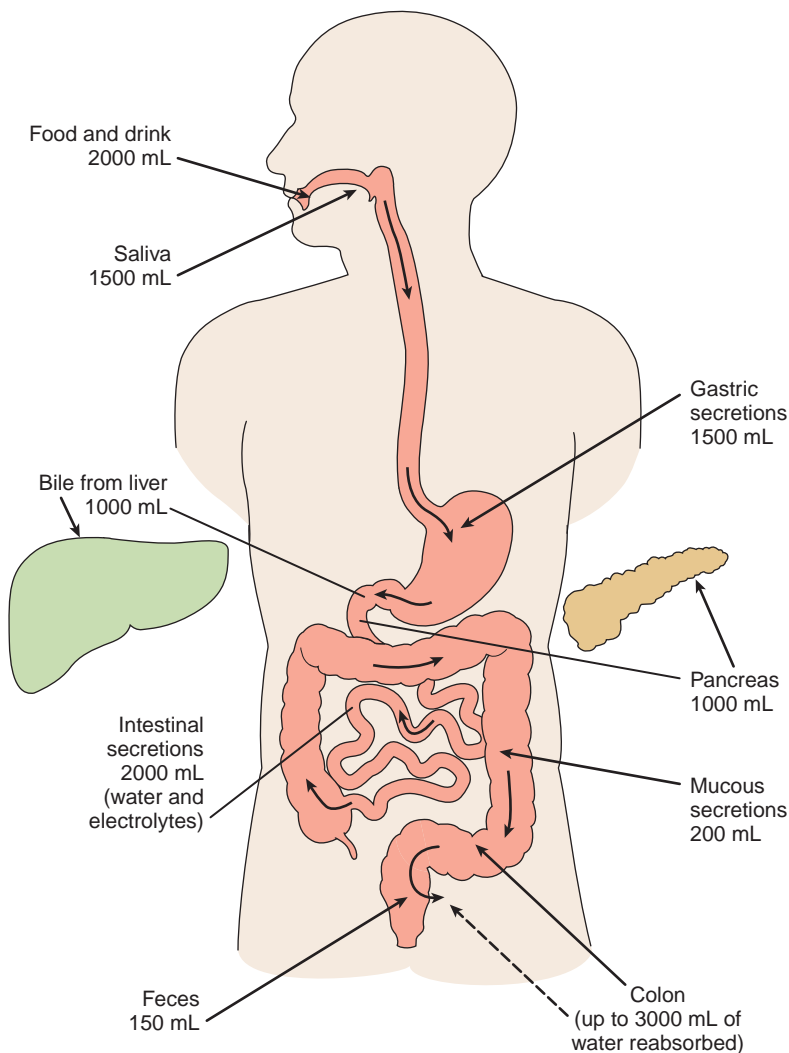


Figure 15-1 Fluid regulation in the gastrointestinal tract.

The major mechanisms of diarrhea are secretory, osmotic, and altered motility. The laboratory tests used to differentiate these mechanisms are fecal electrolytes (fecal sodium, fecal potassium), fecal osmolality, and stool pH. The total fecal osmolarity is close to the serum osmolality (290 mOsm/kg). The fecal sodium and fecal potassium results are used to calculate the fecal osmotic gap. The fecal osmotic gap is calculated as follows:

$$\text{Osmotic gap} = 290 - [2 (\text{fecal sodium} + \text{fecal potassium})]$$

The osmotic gap in all forms of osmotic diarrhea is greater than 50 mOsm/kg and less than 50 mOsm/kg in secretory diarrhea. Electrolytes are increased in secretory diarrhea and negligible in osmotic diarrhea. A fecal fluid pH of less than 5.6 indicates a malabsorption of sugars, causing an osmotic diarrhea.

Secretory Diarrhea

Bacterial, viral, and protozoan infections produce increased secretion of water and electrolytes, which override the reabsorptive ability of the large intestine (*secretory diarrhea*). Enterotoxin-producing organisms such as *Escherichia coli*, *Clostridium*, *Vibrio cholerae*, *Salmonella*, *Shigella*, *Staphylococcus*, *Campylobacter*, protozoa, and parasites such as *Cryptosporidium* can stimulate these water and electrolyte secretions. Other causes of secretory diarrhea are drugs, stimulant laxatives, hormones, inflammatory bowel disease (Crohn disease, ulcerative colitis, lymphocytic colitis, diverticulitis), endocrine disorders (hyperthyroidism, Zollinger-Ellison syndrome, vipoma), neoplasms, and collagen vascular disease.

Osmotic Diarrhea

Incomplete breakdown or reabsorption of food presents increased fecal material to the large intestine, resulting in the retention of water and electrolytes in the large intestine (*osmotic diarrhea*), which in turn results in excessive watery stool. Maldigestion (the impaired digestion of food) and malabsorption (the impaired absorption of nutrients by the intestine) contribute to osmotic diarrhea. The presence of unabsorbable solute increases the stool osmolality and the concentration of electrolytes is lower, resulting in an increased osmotic gap. Causes of osmotic diarrhea include disaccharidase deficiency (lactose intolerance), malabsorption (celiac sprue), poorly absorbed sugars (lactose, sorbitol, mannitol), laxatives, magnesium-containing antacids, amebiasis, and antibiotic administration. Laboratory testing of feces is frequently performed to aid in determining the cause of diarrhea (Table 15–1).

Altered Motility

Altered motility describes conditions of enhanced motility (hypermotility) or slow motility (constipation). Both can be seen in irritable bowel syndrome (*IBS*), which is a functional

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
	Fecal electrolytes
	Stool pH
	Fecal osmolality

disorder in which the nerves and muscles of the bowel are extra sensitive, causing cramping, bloating, flatus, diarrhea, and constipation. IBS can be triggered by food, chemicals, emotional stress, and exercise.

Rapid (accelerated) gastric emptying (*RGE*) dumping syndrome describes hypermotility of the stomach and the shortened gastric emptying half-time, which causes the small intestine to fill too quickly with undigested food from the stomach. It is the hallmark of early dumping syndrome (*EDS*).¹ Healthy individuals have a gastric emptying half-time range of 35 to 100 minutes, which varies with age and gender. A gastric emptying time of less than 35 minutes is considered RGE.¹ This can be caused by disturbances in the gastric reservoir or in the transporting function. Alterations in the motor functions of the stomach result in the accumulation of large amounts of osmotically active solids and liquids to be transported into the small intestine. Normal gastric emptying is controlled by fundic tone, duodenal feedback, and GI hormones. These are altered after gastric surgery, resulting in clinically significant dumping syndrome in approximately 10% of patients.²

RGE can be divided into early dumping and late dumping depending upon how soon after a meal the symptoms occur. EDS symptoms begin 10 to 30 minutes following meal ingestion.² Symptoms include nausea, vomiting, bloating, cramping, diarrhea, dizziness, and fatigue. Late dumping occurs 2 to 3 hours after a meal and is characterized by weakness, sweating, and dizziness.¹ Hypoglycemia is often a complication of dumping syndrome. The main causes of dumping syndrome include gastrectomy, gastric bypass surgery, post-vagotomy status, Zollinger-Ellison syndrome, duodenal ulcer disease, and diabetes mellitus.¹

Steatorrhea

Detection of steatorrhea is useful for the diagnosis of pancreatic insufficiency and small bowel disorders that cause malabsorption. Absence of bile salts that assist pancreatic lipase in the breakdown and subsequent reabsorption of triglycerides produces an increase in stool fat (*steatorrhea*) that exceeds 6 g per day. Likewise, pancreatic disorders, including cystic fibrosis, chronic pancreatitis, and carcinoma that decrease the production of pancreatic enzymes, are also associated with steatorrhea. Steatorrhea may be present in both maldigestion and malabsorption conditions and can be distinguished by the D-xylose test. D-xylose is a sugar that does not need to be digested but does need to be absorbed to be present in the urine. If urine D-xylose is low, the resulting steatorrhea would indicate a malabsorption condition. Malabsorption causes include bacterial overgrowth, intestinal resection, celiac disease, tropical sprue, lymphoma, Whipple disease, *Giardia lamblia* infestation, Crohn disease, and intestinal ischemia. A normal D-xylose test indicates pancreatitis.

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, which may contain chemical *disinfectants*. Some kits provided for the collection of specimens to be screened 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 that contain 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 stercobilinogen to urobilin. As discussed in Chapter 5, conjugated bilirubin formed in the degradation of hemoglobin passes through the bile duct to the small intestine where intestinal bacteria convert it to urobilinogen and stercobilinogen. Therefore, stools that appear pale may signify a blockage of the bile duct. Pale stools are also associated with diagnostic procedures that use 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 occurs, the color can range from bright red to dark red to black. Blood that originates 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 retains 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 often produces a black stool, and medications and foods, including beets, produce a red stool.

Green stools may be observed in patients taking oral antibiotics, because of the oxidation of fecal bilirubin to biliverdin. Ingestion of increased amounts of green vegetables or food coloring also produces 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 and steatorrhea appear bulky and frothy and frequently have a foul odor. Stools may appear greasy and may float.

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).

Table 15–2 Macroscopic Stool Characteristics^{12,26}

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

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 *E. coli*. Bacteria that cause diarrhea by toxin production, such as *Staphylococcus aureus* and *Vibrio* spp., 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.³ All slide preparations must be performed on fresh specimens. In an examination of preparations under high power, as few as three neutrophils per high-power field can be indicative of an invasive condition.⁴ Using oil immersion, the finding of any neutrophils has approximately 70% sensitivity for the presence of invasive bacteria.⁵

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.⁶

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% 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.

Qualitative Fecal Fats

Specimens from suspected cases of steatorrhea can be screened microscopically for the presence of excess fecal fat.

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.

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.

The procedure can also be used to monitor patients undergoing treatment for malabsorption disorders.⁷ 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.^{8,9} 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 the dyes Sudan III, Sudan IV, or oil red O; 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.¹⁰ Observation of more than 60 droplets/high-power field can be indicative of steatorrhea; however, the split fat stain representing total fat content can provide a better indication.¹¹ The breakdown of neutral fats by bacterial lipase and the spontaneous hydrolysis of neutral fats may lower the neutral fat count. This also precludes the 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 reveals 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. In an examination of 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 high-power field. The same number of droplets measuring 1 to 8 μm is considered slightly increased, and 100 droplets measuring 6 to 75 μm is increased.¹²

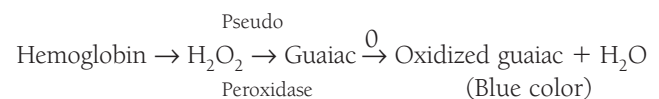
Cholesterol is stained by Sudan III after heating and as the specimen cools forms crystals that can be identified microscopically.

Chemical Testing of Feces

Occult Blood

By far the most frequently performed fecal analysis is the chemical screening test for the detection of occult (hidden) 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. Annual 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 50.¹³

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:



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 sen-

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.

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.

sitivity, 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 avoids 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 program 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 SENSEA, Beckman Coulter, Fullerton, Calif.). 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 prevents the presence of dietary pseudoperoxidases in the stool. Aspirin and NSAIDs 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 interferes with the peroxidase reaction.¹⁴

Additional, more sensitive and specific methods, for the detection of occult blood have been developed. Hemoquant (SmithKline Diagnostics, Sunnyvale, Calif.) 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 immunochemical fecal occult blood test (iFOBT), Hemocult ICT (Beckman Coulter, Fullerton, Calif.) is specific for the globin portion of human hemoglobin and uses

anti-human hemoglobin antibodies. Because Hemocult ICT is specific for human blood in feces, it does not require dietary or drug restrictions. It is more sensitive to lower GI bleeding that could be an indicator of colon cancer or other gastrointestinal disease and can be used for patients who are taking aspirin and other anti-inflammatory medications. The Hemocult ICT does not detect bleeding from other sources such as a bleeding ulcer; thus decreasing the chance for false positives. Hemoglobin from upper GI bleeding is degraded by bacterial and digestive enzymes before reaching the large intestine and is immunochemically nonreactive. In contrast, there is little hemoglobin degradation in lower GI bleeding; therefore, the blood is immunochemically active.¹⁵ 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, quantitative fecal analysis requires the collection of at least a 3-day specimen. 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. Refrigerating the specimen prevents any bacterial degradation. The method routinely used for fecal fat measurement is the Van de Kamer titration, although gravimetric methods are available.¹⁰ Fecal lipids are converted to fatty acids and titrated to a neutral endpoint with sodium hydroxide. The fat

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

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%. The coefficient of fat retention is calculated as follows:

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

Although the Van de Kamer titration is the gold standard for fecal fat, the acid steatocrit is a rapid test to estimate the amount of fat excretion. It is similar to the microhematocrit test and is more convenient than a 72-hour stool collection. The acid steatocrit is a reliable tool to monitor a patient's response to therapy and screen for steatorrhea in pediatric populations.^{16,17}

PROCEDURE

Acid Steatocrit Procedure

- 0.5 g of feces from a spot collection is diluted 1 to 4 with deionized water.
- Vortex for 2 minutes to homogenize the specimen.
- A volume of 5 N perchloric acid equal to 20% of the homogenate volume is added and the mixture is then vortexed for 30 seconds. Confirm the pH to be <1.
- Place the acid-homogenate mixture in 75 microliter plain hematocrit capillary tube. Seal the end with wax.
- The capillary tube is centrifuged horizontally at 13,000 rpm for 15 minutes in a microhematocrit centrifuge. This separates fat as an upper layer overlying a solid fecal layer.
- The length of the fat and solid layers are measured using a magnifying lens.
- Calculate the acid steatocrit in percent.
- Calculate the fecal fat in grams per 24 hours.

The acid steatocrit in percent = (fatty layer length in cm) / [(fatty layer length in cm) + (solid layer length)] × 100

The fecal fat for adults is quantitated as follows:

Fecal fat in grams per 24 hours = [0.45 × (acid steatocrit in percent as a whole number)] – 0.43

An acid steatocrit value <31% was considered normal while a value >31% indicated steatorrhea in adults.

The fecal fat for children up to the age of 15 years is as follows:

Fecal fat in grams per 24 hours = [0.1939 × (acid steatocrit in percent as a whole number)] – 0.2174

Acid steatocrit is higher in infants and dropped with age.¹⁸ An acid steatocrit of <10% is indicative of steatorrhea in children.¹⁷

Near-infrared reflectance spectroscopy (NIRA) is a rapid procedure for fecal fat that requires less stool handling by laboratory personnel. The test requires a 48-to-72-hour stool collection to exclude day-to-day variability, but it does not require reagents after homogenization of the sample. The result is based on the measurement and computed processing of signal data from reflectance of fecal surface, which is scanned with infrared light between 1400 nM and 2600 nM wavelength. The results are calculated from calibration derived from known samples. The technique quantitates water, fat, and nitrogen in grams per 24 hours.¹⁹ A summary of tests and current instrumentation for fecal fat analysis is presented in Table 15–3.

■ ■ ● 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 (Hb) and, after centrifugation, 1% sodium hydroxide is added to the pink hemoglobin-containing supernatant. In the presence of alkali-resistant fetal hemoglobin, the solution remains pink (Hb F), whereas denaturation of the maternal hemoglobin (Hb A) produces 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

Table 15–3 Tests, Materials, and Instrumentation for Fecal Fat Analysis¹⁹

Procedure	Materials, Instrumentation
Sudan III	Sudan stain, microscopy
Steatocrit and acid steatocrit	Hematocrit centrifuge, gravimetric assay
Fecal elastase-I	Immunoassay ELISA technique
Near-infrared reflectance spectroscopy (NIRA)	NIRA spectrophotometer. Wavelengths Range 1400–2600 nM. Computer Software for processing spectra
Van de Kamer	Fecal fat extraction and titration of long chain fatty acid by sodium hydroxide

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 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.²⁰

Fecal Enzymes

Enzymes supplied to the gastrointestinal tract by the pancreas are essential for the digestion of dietary proteins, carbohydrates, and fats. A decrease in production of these enzymes (pancreatic insufficiency) is associated with disorders such as chronic pancreatitis and cystic fibrosis. Steatorrhea occurs, and there is the presence of undigested food 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 digests 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 remains 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 produced by the pancreas. It is present in high concentrations in pancreatic secretions and is strongly resistant to degradation. It accounts for about 6% of all secreted pancreatic enzymes.²¹ Fecal elastase I is pancreas specific and its concentration is about five times higher than in pancreatic juice. It is not affected by motility disorders or mucosal defects.²² Elastase I can be measured by immunoassay using the ELISA kit and provides a very

sensitive indicator of exocrine pancreatic insufficiency.^{23,24} It is easy to perform and requires only a single stool sample. The ELISA test uses monoclonal antibodies against human pancreatic elastase-1; therefore, the result is specific for human enzyme and not affected by pancreatic enzyme replacement therapy.²¹ The test is specific in differentiating pancreatic from nonpancreatic causes in patients with steatorrhea.²²

Carbohydrates

The presence of increased carbohydrates in the stool produces an osmotic diarrhea by the osmotic pressure of the unabsorbed sugar in the intestine drawing in fluid and electrolytes. 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. Idiopathic lactase deficiency is common, predominantly occurring in the African, Asian, and Southern European Greek populations. 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. Testing for fecal reducing substances detects congenital disaccharidase deficiencies as well as enzyme deficiencies due to nonspecific mucosal injury. 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 bacterial fermentation increases the lactic acid level and lowers the pH to below 5.5 in cases of carbohydrate disorders.

The copper reduction test is performed using a Clinitest tablet (Siemens Medical Solutions Diagnostics, Tarrytown, N.Y.) and one part stool emulsified in two parts water. A result of 0.5 g/dL is considered indicative of carbohydrate intolerance. The Clinitest on stools can distinguish between diarrhea caused by abnormal excretion of reducing sugars and those caused by various viruses and parasites. Sucrose is not detected by the Clinitest method because it is not a reducing sugar. In premature infants there is correlation between a positive Clinitest and inflammatory necrotizing enterocolitis. 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. Stool chromatography to identify the malabsorbed carbohydrate is available but rarely necessary for the diagnosis of sugar intolerance. Small-bowel biopsy specimens for histologic examination and the assay of disaccharidase enzyme activity differentiate primary from secondary disaccharidase intolerance.²⁵

A summary of fecal screening tests is presented in Table 15-4.

Table 15–4 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 Microscopic examination of smear heated with acetic acid and Sudan III	60 large orange-red droplets indicates malabsorption 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

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STUDY QUESTIONS

- In what part of the digestive tract do pancreatic enzymes and bile salts contribute to digestion?
 - Large intestine
 - Liver
 - Small intestine
 - Stomach
- Where does the reabsorption of water take place in the primary digestive process?
 - Large intestine
 - Pancreas
 - Small intestine
 - Stomach
- Which of the following tests is *not* performed to detect osmotic diarrhea?
 - Clinitest
 - Fecal fats
 - Fecal neutrophils
 - Muscle fibers
- The normal composition of feces includes all of the following *except*:
 - Bacteria
 - Blood
 - Electrolytes
 - Water
- What is the fecal test that requires a 3-day specimen?
 - Fecal occult blood
 - APT test
 - Elastase I
 - Quantitative fecal fat testing
- The normal brown color of the feces is produced by:
 - Cellulose
 - Pancreatic enzyme
 - Undigested foodstuffs
 - Urobilin
- Diarrhea can result from all of the following *except*:
 - Addition of pathogenic organisms to the normal intestinal flora
 - Disruption of the normal intestinal bacterial flora
 - Increased concentration of fecal electrolytes
 - Increased reabsorption of intestinal water and electrolytes
- Stools from persons with steatorrhea will contain excess amounts of:
 - Barium sulfate
 - Blood
 - Fat
 - Mucus
- Which of the following pairings of stool appearance and cause does *not* match?
 - Black, tarry: blood
 - Pale, frothy: steatorrhea
 - Yellow-gray: bile duct obstruction
 - Yellow-green: barium sulfate
- Stool specimens that appear ribbon-like are indicative of which condition?
 - Bile-duct obstruction
 - Colitis
 - Intestinal constriction
 - Malignancy
- A black tarry stool is indicative of:
 - Upper GI bleeding
 - Lower GI bleeding
 - Excess fat
 - Excess carbohydrates
- Chemical screening tests performed on feces include all of the following *except*:
 - APT test
 - Clinitest
 - Pilocarpine iontophoresis
 - Trypsin digestion
- Secretory diarrhea is caused by:
 - Antibiotic administration
 - Lactose intolerance
 - Celiac sprue
 - Vibrio cholerae*
- The fecal osmotic gap is elevated in which disorder?
 - Dumping syndrome
 - Osmotic diarrhea
 - Secretory diarrhea
 - Steatorrhea
- Microscopic examination of stools provides preliminary information as to the cause of diarrhea because:
 - Neutrophils are present in conditions caused by toxin-producing bacteria
 - Neutrophils are present in conditions that affect the intestinal wall
 - Red and white blood cells are present if the cause is bacterial
 - Neutrophils are present if the condition is of non-bacterial etiology
- True or False*: The presence of fecal neutrophils would be expected with diarrhea caused by a rotavirus.
- Large orange-red droplets seen on direct microscopic examination of stools mixed with Sudan III represent:
 - Cholesterol
 - Fatty acids
 - Neutral fats
 - Soaps

Continued on following page

Continued

18. Microscopic examination of stools mixed with Sudan III and glacial acetic acid and then heated will show small orange-red droplets that represent:
- Fatty acids and soaps
 - Fatty acids and neutral fats
 - Fatty acids, soaps, and neutral fats
 - Soaps
19. When performing a microscopic stool examination for muscle fibers, the structures that should be counted:
- Are coiled and stain blue
 - Contain no visible striations
 - Have two-dimensional striations
 - Have vertical striations and stain red
20. A value of 85% fat retention would indicate:
- Dumping syndrome
 - Osmotic diarrhea
 - Secretory diarrhea
 - Steatorrhea
21. Which of the following tests would *not* be indicative of steatorrhea?
- Fecal elastase-I
 - Fecal occult blood
 - Sudan III
 - Van de Kamer
22. Gum guaiac is preferred over ortho-tolidine for “occult” blood in mass screening tests because:
- There is less interference from dietary hemoglobin
 - Ortho-tolidine is less sensitive
 - Gum guaiac reacts equally with formed and watery stools
 - Filter paper is more easily impregnated with gum guaiac
23. The term “occult” blood describes blood that:
- Is produced in the lower GI tract
 - Is produced in the upper GI tract
 - Is not visibly apparent in the stool specimen
 - Produces a black, tarry stool
24. What is the recommended number of samples that should be tested to confirm a negative occult blood result?
- One random specimen
 - Two samples taken from different parts of three stools
 - Three samples taken from the outermost portion of the stool
 - Three samples taken from different parts of two stools
25. Which test is more sensitive to upper GI bleeding?
- Guaic fecal occult blood
 - Hemoquant
 - Immunochemical fecal occult blood
 - Sudan III
26. Annual testing for fecal occult blood has a high predictive value for the detection of:
- Colorectal cancer
 - Malabsorption syndromes
 - Pancreatic deficiencies
 - Ulcers
27. Tests for the detection of “occult” blood rely on the:
- Reaction of hemoglobin with hydrogen peroxide
 - Pseudoperoxidase activity of hemoglobin
 - Reaction of hemoglobin with ortho-tolidine
 - Pseudoperoxidase activity of hydrogen peroxide
28. What is the significance of an APT test that remains pink after addition of sodium hydroxide?
- Fecal fat is present.
 - Fetal hemoglobin is present.
 - Fecal trypsin is present.
 - Vitamin C is present.
29. In the Van de Kamer method for quantitative fecal fat determinations, fecal lipids are:
- Converted to fatty acids prior to titrating with sodium hydroxide
 - Homogenized and titrated to a neutral endpoint with sodium hydroxide
 - Measured gravimetrically after washing
 - Measured by spectrophotometer after addition of Sudan III
30. A patient whose stool exhibits increased fats, undigested muscle fibers, and the inability to digest gelatin may have:
- Bacterial dysentery
 - A duodenal ulcer
 - Cystic fibrosis
 - Lactose intolerance
31. A stool specimen collected from an infant with diarrhea has a pH of 5.0. This result correlates with a:
- Positive APT test
 - Negative trypsin test
 - Positive Clinitest
 - Negative occult blood test
32. Which of the following tests differentiates a malabsorption cause from a maldigestion cause in steatorrhea?
- APT test
 - D-xylose test
 - Lactose tolerance test
 - Occult blood test

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 Number One 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 Number Two 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 Number Three 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 Number One? Patient Number Two? Patient Number Three?
 - b. How could the physician's office staff avoid these discrepancies?
 - c. What testing methodology could be used for Patients Number Two and Number Three?
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?