LABORATORY 11: Occult Blood and Fecal Analysis

Points  Points are awarded for Admission Tickets. Points are also awarded for general lab skills, neatness, lab clean-up, and teamwork as well as successful and timely completion of study questions. Student results are expected to match the instructor's (100% accuracy). Study questions are due by the end of the next lab period or as designated by the lab instructor.

Objectives
1. According to the standards set by the instructor, the student will use the appropriate manufacturer’s instructions to analyze prepared specimen(s) for the presence of occult blood with 100% accuracy, recording results in appropriate format.
2. Evaluate the control results on the specimens and describe action to be taken if appropriate results are obtained.
3. The student will be able to in writing or verbally:
   a. Define 'occult blood'.
   b. State the principle of the Guiac occult blood test, including the appearance of positive and negative reactions and listing any interfering substances.
   c. State how the Guiac Test slide is prepared, including the length of time the slide may be used.
   d. Identify two causes of false-positive and one cause of a false-negative guaiac reaction.
   e. Describe instructions to provide a patient preparing for the Occult Blood test including diet.
   f. Explain how the immunochemical test (ICT) differs from the guaiac test for occult blood and provide at least 2 advantages that the ICT methodology has over the guaiac methodology and at least one disadvantage.
4. Use quality control results to determine the acceptability of test results
5. Answer all pre-test and study questions using related information found in the textbook, lecture guide, and this lab procedure
   • For instruction on specimen collection procedure and purpose, view: http://www.youtube.com/watch?v=kujiUjcrAd4w
   • For instruction on processing and testing of the fecal specimen for occult blood, view: http://www.youtube.com/watch?v=mWOHl3UsFos

Principles and Related Information
The hemoglobin portion of whole blood is capable of exerting a peroxidase-like activity and thus able to catalyze the oxidation of alpha-guaiaconic acid.

The reaction between alpha-guaiaconic acid in the guaiac resin (on the special absorbent paper) and hydrogen peroxide (in the developer) is catalyzed by the hemoglobin fraction of blood (if present in the specimen) producing a visible blue color.

Equipment and Supplies
1. Prepared occult blood testing slides.
2. Appropriate color developer.
3. Manufacturer’s product insert / instructions.
4. Appropriate PPE, disinfection and disposing materials.

References
Package inserts
Current UA / BF course textbook(s)
Lecture and Lab Guides & related course powerpoints.

Discussion
1. Blood may be found in stool samples as a result of various reasons including:
   a. recent gastrointestinal surgery
   b. colorectal and gastric (stomach) cancers
   c. ulcers
   d. hemorrhoids
   e. polyps
   f. inflammatory bowel disease
irritations or lesions of the gastrointestinal tract caused by medications (such as nonsteroidal anti-inflammatory drugs, also called NSAIDs)

h. irritations or lesions of the gastrointestinal tract caused by stomach acid disorders, such as reflux esophagitis

2. Patients experiencing hemorrhoid or menstrual bleeding or bleeding from the nose, gums, etc. should delay initiating testing for at least 48 hours after such bleeding has ceased.

3. To increase the chances of detecting intermittent gastrointestinal bleeding, it is recommended that stool samples be collected from three consecutive bowel movements and that two smears be made from two or more different areas of each bowel movement, especially from darkened or discolored areas of the feces. Excessive GI bleeding may result in black, tarry stools.

4. Blood present in stool samples may or may not retain its bright red color due to the digestive process. The term “occult” blood is used to describe blood that is not obvious or is hidden.

5. The occult blood is recommended for use as a diagnostic aid during routine physical examination, when hospital patients are first admitted, to monitor for bleeding in patients recuperating from surgery and other conditions, and in screening programs for colorectal cancer.

6. The occult blood test is not a test colorectal cancer or any other specific disease. It is used as a qualitative aid to the diagnosis of various gastrointestinal conditions which manifest themselves by the presence of fecal occult blood. The occult blood test detects excess blood loss which may have significance when related to certain diseases such as colorectal cancer.

7. A positive result on a properly collected and performed occult blood test should be followed up medically. A negative test usually indicates no significant blood present.

8. The accuracy of the test depends upon the status of the patient at the time the specimen is taken and may be affected by interfering substances.

9. Immunochemical testing for fecal occult blood (iFOBT). The newest methodology for detecting occult blood is based on immunochemical detection of hemoglobin in fecal specimens. The immunochemical test uses an antibody to detect the globin chain of hemoglobin to detect the blood. Since the test is specific for hemoglobin, dietary restrictions are not necessary. The test has a high degree of sensitivity (able to detect low levels of blood) and the result is clearly identified as the development of a colored line. There are some limitations however, in that it detects mainly blood from the colon, whereas the Guiac test can detect bleeding from the stomach, small and large intestine as well as the colon. In spite of this limitation and its higher cost, iFOBT test for fecal occult blood is being widely marketed.

Overview

1. A smear of a (stool) sample is applied as a very thin smear to one side of a special absorbent paper impregnated with natural guaiac resin and the window flap secured closed. NOTE: sampling should be obtained from various parts of the stool sample to increase reliability of test results.

2. Allow the testing pack to sit undisturbed for at least 3 minutes before testing (some manufacturers vary, see specific instructions). This allows any blood present to react with the guiac impregnated paper.

3. After the short incubation time, slides may be developed, or may be stored for up to 8 days after specimen application. Prepared slides must be kept away from heat and light after specimen application.

4. In the lab, testing must be performed in an area that is clean and free of blood samples to prevent accidental contamination of the slides.

5. The testing pack / paper is turned over and a special developer added.

Patient Preparation

1. If possible the patient should be placed on a red meat-free low-peroxidase diet for at least two (2) days prior to and during the testing period to reduce the possibility of positive results due to diet.

2. The patient may consume:
   a. Generous amounts of cooked and uncooked vegetables such as lettuce, corn and spinach.
b. Moderate amounts of high fiber foods such as bran cereal, peanuts, and popcorn. (Roughage that may help expose silent lesions which may bleed intermittently therefore may increase the rate of detection of true positive reactions.)

c. Well cooked pork, poultry and fish.

d. Prunes, grapes, plums and apples.

3. The patient should **not** consume:
   a. Rare and lightly cooked meats, especially beef.
   b. Cauliflower, turnips, broccoli, horseradish, radishes or cantaloupe.
   c. Iron rich supplements
   d. Vitamin C in excess of 250 mg per day. (False negative reactions have been noted with linked to vitamin C, ascorbic acid.) Patients must discontinue vitamin C supplements 2 days prior to and during testing period.
   e. For 7 days prior to and during the collection period, patients must avoid aspirin and other anti-inflammatory drugs / medications which may cause gastrointestinal irritation period.
   f. Excessive amounts of alcoholic drinks.

**Agenda**
1. Classroom discussion, overview of procedures, activities, and expectations with Q & A.
2. As directed by the instructor and following manufacturer’s directions, perform occult blood testing on specimens provided and record patient and control results.
3. Use lecture, lab and textbook materials to aid in answering study questions.
Laboratory Exercise #11: Occult Blood and Fecal Analysis
Study Questions

Student Name ______________________ Date _____________________ ___ / 20 points

Instructions: Answer the following questions using lecture notes, reading assignments, and information presented in the laboratory. Each question is worth one point unless otherwise stated. The instructor will announce when these study questions are due.

1. State four (4) reasons for performing a fecal analysis.

2. Define steatorrhea.

3. What types of conditions is the occult blood used to monitor or diagnose?

4. What is the principle of the Guiac occult blood test?

5. What could cause a false positive fecal occult blood test result using the Guiac method?

6. What could cause a false negative fecal blood test result?

7. List four foods which should be avoided two days before collecting the Guiac occult blood test.

8. List four foods which patients should be encouraged to eat three days prior to this occult blood test.

9. List at least two conditions which would require the patient to postpone / delay the sample collections for at least 48 hours.

10. What is the minimum time that the sample should be in contact with the guaiac paper before the developer is applied?

11. How long can the occult blood slides be stored after application of the specimen and before tested?

12. Briefly describe the recommended procedure for collection of stool samples for occult blood testing that would increase the chances of detecting intermittent GI bleeding? (Do not need to address dietary concerns here.)

13. In the table that follows, answer the questions about the immunochemical fecal occult blood test (iFOBT).

<table>
<thead>
<tr>
<th>How does this testing differ from guaiac?</th>
</tr>
</thead>
<tbody>
<tr>
<td>What makes this testing unique and specific to human blood?</td>
</tr>
<tr>
<td>What is the name of the commercial product?</td>
</tr>
</tbody>
</table>