EXERCISE 12: FECAL OCCULT BLOOD TEST

Points: 20

Objectives:

1. State the primary and secondary uses of the fecal occult blood test (FOBT).
2. Define “occult” blood.
3. Describe the diet and state the foods and medications which should be avoided prior to performance of the guaiac FOBT.
4. List the foods which patients are encouraged to eat prior to guaiac FOBT.
5. State how the guaiac slide is prepared, including the length of time the slide may be used after specimen application.
6. State the principle of both the guaiac FOBT and immunochemical FOBT.
7. State the advantages that the immunochemical FOBT has over the traditional guaiac test.
8. Describe the quality control which must be performed and action which must be taken if accurate results are not obtained.
9. Following the manufacturer’s directions to preform occult blood testing on samples provided by the instructor and correctly record the results of the patient and controls.
10. Evaluate the results of the test controls and patient results to correctly interpret both guaiac and immunochemical FOBT.
11. State 3 conditions during which patients should not preform the guaiac FOBT, and 4 conditions during which patients should not preform the immunochemical FOBT.

Discussion

The fecal occult blood test (FOBT) is a rapid, convenient, and virtually odorless qualitative method for detecting fecal occult blood. Occult is a word meaning “hidden”. Blood can be present in a stool sample, but due to the digestive process, will not retain its bright red color.

Many diseases can cause hidden blood in the stool. The primary use of the FOBT is to screen for early colon cancer. A secondary use of the FOBT is determining the cause of anemia, such as in blood loss from a bleeding ulcer. The test is also used to detect the early stages of other gastrointestinal problems such as polyps, colitis, diverticulitis, and fissures which may not show any visible symptoms, only the presence of occult blood.

This test is a qualitative aid to the diagnosis of various gastrointestinal conditions which manifest themselves by the presence of fecal occult blood.

The fecal occult blood test detects excess blood loss which may have significance when related to certain diseases such as colorectal cancer. A positive test usually indicates blood loss more than normal and should be followed up medically. A negative test usually indicates that no blood loss more than normal is apparent in the fecal specimen tested. 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.

Traditionally, the most widely used occult blood test is based on the guaiac procedure. More recently, an immunochemical test that is specific for human blood has been developed. Both methods will be discussed.
GUAIAC BASED OCCULT BLOOD TEST

Most tests of this nature use a special guaiac impregnated paper to which the patient applies a smear from a stool sample. When the sample is submitted to the lab, a few drops of a special developer is added to the back of the paper, and the paper is observed for the development of a blue color. Any blue color is a positive test.

**Principle**

This test is based on the oxidation of guaiac by a hydrogen peroxide developer to form a blue-colored compound if the **heme** portion of blood is present in the test sample. The hydrogen peroxide catalyzes the oxidation of alpha guaiaconic acid, the active ingredient of the guaiac paper, if the heme portion of hemoglobin is present in the fecal specimen, to form a highly conjugated blue quinine compound.

**Quality Control**

Most test cards come with built in positive and negative controls. The developer is added to the areas with the patient sample as well as the control areas on the slide. The positive control must turn blue and the negative control area must remain colorless. If the appropriate reaction does not occur the results of the patient testing CANNOT be reported out. The patient will need to be called and new cards distributed. Trouble shooting on the inaccurate cards should include calling the manufacturer to determine if these particular lot numbers of cards are defective.

**Patient Preparation**

If possible the patient instructions should followed at least 7 days prior to the test and should continue through the test period. Patient instructions include both drug and diet guidelines. Patient should also be encourage to increase roughage in their diet prior to the test, as that may help uncover silent lesions which may bleed intermittently and may increase the rate of true positive reactions.

**Suggested Drug and Diet Guidelines**

**Avoid:**

*Seven days* prior to and during the test:
1. Non-steroidal anti-inflammatory drugs such as ibuprofen (Motrin*, Advil**) or naproxen
2. Aspirin (more than one adult aspirin a day). (Acetaminophen (Tylenol*) can be taken as needed.

*Three days* prior to and during the test patients should avoid:
1. Vitamin C in excess of 250 mg a day from supplements, citrus fruits and juices.
2. Red or rare meat, including processed meats, beef, lamb and liver.
3. Raw vegetables and fruits such as: broccoli, turnips, horseradish, cauliflower, red radishes, parsnips and cantaloupe.

**Foods to eat:**
1. Any cooked vegetables and fruits, especially lettuce, spinach, and corn.
2. Prunes, grapes, plums and apples.
3. High fiber foods: Peanuts, popcorn and bran cereals. Roughage in the diet can increase test accuracy by helping to uncover “silent” lesions.

If any of the above dietary restrictions and recommendations are known to cause discomfort, patients should be instructed to inform their physician. The patient should always consult the physician before discontinuing or interrupting any prescription medications. Patients should talk to their doctor or pharmacist if they have any questions about medications they take regularly.
Specimen Collection and Testing

The specimen required is a small stool sample. Patients are to collect a small sample from 2 different areas of the stool and apply them as a very thin smear to the appropriate area of the collection card. Slides may be developed immediately after specimen application, but are best developed no sooner than 3 days after sample application. Waiting 3 days allows any fruit and vegetable peroxidases present in the sample to degrade.

Slides are stable when stored at room temperature for up to 14 days after specimen application. Once they have been prepared with a specimen, keep the slides away from heat and light. The work area should be kept clean and free of blood to avoid accidental contact of blood with the slides.

Patients experiencing hemorrhoidal bleeding, having a menstrual period, or bleeding from the nose, gums, etc. should delay testing for at least 48 hours from the time that all such bleeding has stopped. To increase the chances of detecting intermittent gastrointestinal bleeding, it is recommended that stool samples be collected from bowel movements on three (3) different days and that two smears be made from two different areas of each bowel movement, especially from darkened or discolored areas of the feces. Excessive GI bleeding may result in black, tarry stools.

Interfering Substances

False positive test is one where the test is positive but the patient is not experiencing gastrointestinal disease or bleeding. Substances that can cause false positives on a guaiac based fecal occult blood test include:

- Red meat (beef, lamb and liver)
- Aspirin (greater than 325 mg/day) and other non-steroidal anti-inflammatory drugs such as ibuprofen, indomethacin and naproxen
- Corticosteroids, phenylbutazone, reserpine, anticoagulants, antimetabolites, and cancer chemotherapeutic drugs
- Alcohol in excess
- The application of antiseptic preparations containing iodine (povidone/iodine mixture)

False negative test is one where the test is negative but the patient is experiencing some type of gastrointestinal disease or bleeding. Substances that may cause a false negative include:

- Ascorbic acid (vitamin C) in excess of 250 mg per day
- Excessive amounts of vitamin C enriched foods (citrus fruits and juices)
- Iron supplements which contain quantities of vitamin C in excess of 250 mg per day

Test Interpretation

Positive Result: Blue color seen in both the Patient Specimen area and Positive Control area

No blue color in the Negative Control area

Negative Results: Blue color seen only in the Positive Control area

No blue color seen in the Patient Specimen area, and Negative Control area

Invalid Results: No color development in the Positive Control area regardless of color development in the Patient Test area and Negative Control area

OR

Blue color seen in the Negative Control area regardless of color development in the Patient Test area or Positive Control area
IMMUNOCHEMICAL FECAL OCCULT BLOOD TEST

Two advantages the Immunochemical Fecal Occult Blood (FOBT) test has over the traditional guaiac-based method to detect occult blood include:

- The immunochemical test has no dietary restrictions prior to testing.
- Guaiac tests lack sensitivity and specificity whereas the immunochemical test is specific for human hemoglobin.

Principle

This test utilizes a qualitative, sandwich dye conjugate immunoassay to selectively identify the globulin component of human hemoglobin in fecal specimens. The immunoassay uses a combination of monoclonal and polyclonal antibodies, utilizes an immunochemical chromatographic method for detection and has a high degree of analytical sensitivity.

Specimen Collection

The patient is provided with a sample collection vial which contains a specimen sampling applicator. After the patient has the stool specimen, the patient uses the sampling applicator from the vial to collect samples from multiple areas of the stool. The applicator is then returned to the collection vial where the stool sample mixes with buffer solution. The patient labels the vial with their name and returns it to the lab for testing. The patient must be provided with the necessary information for proper handling and transport of the vial, which may vary with different manufactures.

Testing Procedure

Once it is received in the lab, the buffer solution is properly mixed and a few drops of the buffer solution is introduced into a test device. The sample will migrate through the test device for a specific amount of time, usually about 5 minutes. The development of a colored line at the “T” (test) area indicates the presence of human hemoglobin.

Quality Control and Interpretation of Test Results

The test devices have built in procedural controls. At the “C” (control) area of the device, a colored line will develop when the buffer solution reacts with the conjugate specific polyclonal antibodies. This serves as a positive control for that device and indicates that the antibodies are working properly. A clear background of the test device is an internal negative procedural control.

Best practices for laboratories recommends performing positive and negative external controls with every lot of test cassettes received or as often as necessary to meet the laboratory quality control standards.

Results are to be interpreted as follows:

<table>
<thead>
<tr>
<th>Results</th>
<th>Appearance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>One color line appears at the “C” area and the “T” area while the background is white.</td>
</tr>
<tr>
<td>Negative</td>
<td>One color line appears at the “C” area while the background is white. There is NO colored line in the “T” area.</td>
</tr>
</tbody>
</table>
| Invalid  | • No colored line appears at either the “C” or the “T” areas  
|          | • A colored line appears only at the “T” area.  
|          | • The background of the test device is not white. |
If the internal device controls do not give the expected results the test must be repeated. If the expected results are still not obtained the test is invalid and the patient results cannot be reported out. One option is to select a kit with a different lot number, if available, and repeat the test.

**Patient Preparation**

Because the Immunochemical FOB test is specific for human blood, no special drug or dietary restrictions are required. However, since the test is very sensitive, patients should not collect or test their stool sample if they are having any of the following conditions:

1) Three days before, during or three days after their menstrual period
2) Bleeding hemorrhoids
3) Blood in their urine
4) Open cuts on their hands
5) Straining during their bowel movement

This method detects the globin portion of undegraded hemoglobin so it has increased specificity for lower G.I. bleeding. Because hemoglobin from upper G.I. bleeding is generally degraded before reaching the large intestine it is usually immunochemically nonreactive.
**Procedure: Fecal Occult Blood Test**

**Guaiac Based FOB Testing**

1. Write the patient information from the front flap of the slide onto your report form.

2. Turn the slide over and open the flap to expose the test area. **NOTE:** The slides have fecal material on them, handle with care.

3. Apply drops of developer solution to each smear in the Specimen Test Area AND control areas as directed on the test slide, package insert or instructor directions.

4. *Read results within 30 to 60 seconds.* Any trace of blue color is positive for occult blood. Color begins to fade after 2 to 4 minutes.

5. Record the results of the patient and both controls on the report form.

6. Record the test interpretation for each sample under “Results”.

**Immunochemical FOB Testing**

1. Write the patient information from the specimen vial onto your report form.

2. Open the test cassette and write the patient’s last name on the top of the cassette.

3. Follow your instructor’s directions or the manufacture’s procedure card to mix the sample with the buffer in the vial.

4. Holding the vial upright, carefully unscrew the clear tip cover to reveal the dropper.

5. Dispense the appropriate number of drops from the vial into the sample well of the cassette. The number of drops will vary with the manufacturer.

6. Note the time that the drops were applied. Observe the cassette at the time specified by the manufacturer for the reading of test results.

7. Record the presence or absence of color development at the “T” (test) and “C” (control) lines and the color of the background in the appropriate columns on your report form.

8. Record the acceptability of test results based on the development of color at the Control line and the color of the background.

9. Record the test interpretation for each sample based on the development of color at the Test line and the results of the Test Acceptability.
EXERCISE 12: FECAL OCCULT BLOOD- STUDY QUESTIONS

Name ___________________________ Date _______________ Points ________/18

1. State the primary and secondary uses of the fecal occult blood test (FOBT)? (2 points)
   
a. 

   b. 

2. What is the principle of the guaiac FOBT? (i.e., what reacts with what to give a positive reaction? Describe the appearance of a positive test reaction) (2 points)
   
a. 

   b. 

3. List three SPECIFIC FOODS which should be avoided two days before performing the guaiac FOBT. (1.5 points)
   
a. 

   b. 

   c. 

4. List three SPECIFIC FOODS which patients should be encouraged to eat three days prior to the guaiac FOBT. (1.5 points)
   
a. 

   b. 

   c. 

5. List three conditions which would result in a delay of 48 hours from the time condition ceases in the collection of the guaiac FOBT. (3 points)
   
a. 

   b. 

   c. 

6. How long may the guaiac FOBT slides be stored after application of the specimen? (1 point)
7. How many stool samples should be tested to increase the chances of detecting intermittent GI bleeding using the **guaiac** testing method? (1 point)

8. What is the principle of the **immunochemical** FOBT? (i.e., what reacts with what to give a positive reaction? Describe the appearance of a positive test reaction) (2 points)
   a. 
   b. 

9. State two advantages that the **immunochemical** FOBT has over the **guaiac** FOBT. (2 points)
   a. 
   b. 

10. List 4 conditions for which a patient should delay testing of the **immunochemical** FOB test. (2 points)
    a. 
    b. 
    c. 
    d.
Exercise 12: Fecal Occult Blood Test - Recording Results

Name _________________________________ Date _________________ Points _____/20

Instructions
• Your instructor will demonstrate the procedures. Record those results in the appropriate area.
• For samples given to you, record the name and ID or DOB in the appropriate area below.

For the Guaiac Test
• Observe the “control” area(s) and patient area of the slide for color development.
• Record any blue color seen as ‘Yes’. If no blue color is seen, report as “No”.
• If the cards you are given do not have a negative control area, report as NA (Not Applicable).
• Record Interpretation of Results as “Positive” or “Negative” or “Invalid”.
• When finished, have your instructor verify your test results.

Guaiac Test – Record Observations and Interpretation of Results

| Color Development – any blue color: | Interpretation of Results: |
| Positive | Negative | Patient | Control | Control | Negative | Patient | Area | Area | Specimen | Area | or | or | Invalid |
| Control | Control | SPECIMEN | Area | Area | Area | Area | Area | Area | Area | Area | Area | Area |

| Demonstration Slide #1 | | | | | | | |
| Patient Name: | | |
| ID or DOB: | | |
| Demonstration Slide #2 | | | | | | | |
| Patient Name: | | |
| ID or DOB: | | |

For the Immunochemical FOB test
• Observe the appropriate areas of the test cassette at the correct time and record them below.
• Determine and record the acceptability and interpretation of test results
• When finished, have your instructor verify your test results.

Immunochemical FOB – Record Observations and Interpretation of Results

| Color Development: | Background: | Results | Test Interpretation: |
| YES or NO | CLEAR or COLORED | Acceptable: | POSITIVE, NEGATIVE, or INVALID |
| Control Line | Test Line | YES or NO | |

| Positive Control Lot # | | | |
| Expiration Date: | | | |
| Negative Control Lot # | | | |
| Expiration Date: | | | |
| Patient Name: | | |
| ID or DOB: | | |

FOR INSTRUCTOR USE ONLY
a. For Guaiac Test: Demonstration slides color development and test interpretation worth 0.5 points each (2 points each line)
b. For Patient Test: Name, ID or DOB, color development and test interpretation worth 0.5 points each (3 points each line)
c. For IFOB: Lot number, expiration date, Patient Name, ID or DOB, Control Line, Test Line, Background, Acceptability and Interpretation :0.5 point each (3 points each line)
d. Count off 0.25 point for each instance where symbols are used in place of Yes, No, NA, Positive, Negative or Invalid.