HEMAPROMPT

A Guaiac Test for Fecal Occult Blood
For use by Medical Professionals

INTENDED USE
HemaPrompt is a guaiac-based in-vitro slide method for the qualitative detection of fecal occult blood. It is a useful aid in the diagnosis of a number of gastro-intestinal disorders, and is recommended for use in [1] routine physical examination [2] routine hospital testing [3] mass screening for colorectal cancer or gastrointestinal bleeding from any other source. It is recommended that at least three consecutive bowel movements are used for adequate screening of each individual.

SUMMARY and EXPLANATION
The guaiac-peroxidase reaction, initially described by Van Deen [1], when adapted to the slide format, has been a widely used method for the detection of fecal occult blood, which is a sign of many gastro-intestinal disorders, and signals the necessity of follow-up by other diagnostic methods. Guaiac slide tests overcome the instability of guaiac solution and the hypersensitivity of benzidine and ortho-tolldine. It provides a method of testing for the presence of occult blood in which a thin smear of feces to be tested is applied to the guaiac paper window. The convenience of HemaPrompt is that the developing solution is contained on each individual test card in a premeasured quantity, and is applied automatically to the guaiac paper by pulling the tab on the card. The application of developer solution from a separate dispenser bottle is thus avoided. The monitor printed on the guaiac paper indicates the chemicals are functioning correctly. HemaPrompt results cannot be considered conclusive evidence of the presence or absence of GI bleeding or pathology. False positive/negative reactions are known to be caused by a person's particular diet or medications (see Patient Preparation below). The test is intended as a preliminary screen and not as a replacement for other diagnostic procedures such as sigmoidoscopy, barium enema, and x-ray studies.

PRINCIPLE
The use of guaiac as a test for the presence of blood is based on the oxidation of phenolic compounds present in guaiac to quinones, resulting in the production of a blue color [2]. If blood is present, the heme portion of the hemoglobin molecule can function in a pseudoenzymatic manner, catalyzing the release of oxygen from hydrogen peroxide which in turn causes the oxidation of guaiac. HemaPrompt is a version of the laboratory guaiac slide test for fecal occult blood, and is composed of guaiac-impregnated paper mounted on a cardboard frame which permits sample applications to one side with development and interpretation from the reverse side. Feces containing occult blood contacts the guaiac impregnated paper and a pseudoperoxidase reaction occurs when developing solution is brought into contact with the guaiac paper, by pulling the tab. The test paper will turn blue after thirty seconds in the presence of more than 2mg hemoglobin/gram feces, but will remain uncolored in the absence of at least this quantity of hemoglobin and in this regard is equivalent to other guaiac based fecal occult blood tests available [16], (see Performance Characteristics below). The monitor on the guaiac slide indicates the chemicals are functioning correctly.

REAGENTS and MATERIALS SUPPLIED
Guaiac impregnated paper and developing solution are both mounted on each slide, as is the positive control. The developing solution, a mixture of 60-70% denatured ethyl alcohol and approximately 6% hydrogen peroxide, is contained within a developing pad and is exposed after pulling the tab. The positive monitor contains a hematoid. The specimen can be applied directly by the gloved finger, toilet paper or a spatula. There are 50 slides in each box (Catalogue Number HP50).
Important Note: Current U.S. Postal Regulations prohibit mailing completed test slides in standard envelopes.

STORAGE and STABILITY
Each slide is equipped with a control to monitor the effectiveness of the chemicals and hence the test itself; the positive control must turn blue to indicate a properly functioning test. Failure of this control to produce the appropriate color reactions is indicative of product deterioration and the test results are invalid.
HemaPrompt slides should be stored at room temperature 10-24°C (50-75°F) and should be protected from heat, sunlight, fluorescent light, U.V. radiation, humidity, volatile chemicals and gases. Do not refrigerate or freeze. They are stable until the expiration date indicated on each slide, after which time the slide should not be used.

WARNING and PRECAUTIONS
HemaPrompt is intended for in-vitro diagnostic use only. Skin or eye contact with the developing pad which is exposed after pulling the tab, should be avoided; flush the affected area with water should contact occur. Ingestion may be fatal or cause blindness. Keep away from heat sparks or open flame.

QUALITY CONTROL
Each slide is equipped with internal controls to monitor the effectiveness of the chemicals and hence the test itself. The positive internal control monitor appears as a blue cross and must turn blue after application of developer. The negative internal control is the background behind the positive control, which should remain unchanged in color. Failure of the controls to produce the appropriate reactions is indicative of product deterioration and the test results are invalid.

PATIENT PREPARATION
A special diet as described below is recommended to decrease the possibility of false positive results. On the other hand, dispensing with such a diet for initial screening purpose may increase patient compliance but a positive result under these circumstances would indicate the need to repeat the test in which a special diet two days prior to and during the three day test period is followed. This diet should EXCLUDE red and rare meats, horseradish, raw fruits and vegetables like broccoli, cauliflower, red radish, cantaloupe, parsnips and turnips, or other high peroxidase containing vegetables, which can cause false positive results. An acceptable diet could include cooked fruit and vegetable such as spinach and corn as well as lettuce, prunes, grapes, and apples. Cereal, and well cooked fish and fowl are also acceptable. If any of the recommended foods are known to cause discomfort, the patient should consult his or her physician.
Because gastro-intestinal lesions may bleed intermittently and blood in feces is not distributed uniformly all patients who test positive regardless of diet should be followed up with additional diagnostic procedures. Certain medications such as aspirin, [3] indomethacin, phenylbutazone, reserpine, corticosteroids and nonsteroidal anti-inflammatory drugs can cause gastro-intestinal bleeding and thus give positive reactions; dosages of greater than 250mg. of Vitamin C per day have been shown to cause false negative results [4], while iron containing compounds have been mentioned as a cause of false positive reactions [5]. On the advice of the physician, these medications might be temporarily discontinued for 7 days prior to and during the test period.

SPECIMEN COLLECTION
Stool samples should not be collected if the patient is experiencing menstrual bleeding, constipation bleeding, bleeding hemorrhoids, or when rectal suppositories or medication is being used.
It is recommended that smears be collected from two different areas of each stool from three consecutive bowel movements as closely spaced in time as possible, [6][7], or by the physician following a rectal exam. Using toilet paper, gloved finger or spatula, a specimen is taken from stool smeared on the bowl and above the toilet water level, from the toilet paper used following defecation, or from a specimen caught in a clean cup. Application to the slide may be performed from the gloved finger (as after a rectal exam), spatula, or by use of the toilet paper described above. It is important that the stool specimen is applied as a very thin smear to each of the slide windows and that no more than 6 days should elapse between preparation and testing, with patients being instructed to return all slides to the physician as soon as possible. Rehydration of the specimen is neither necessary nor recommended. Patient specimens and all materials that come in contact with them should be handled as potentially infectious and disposed of with proper precaution. Do not allow contact with skin or mucous membranes.

TESTING PROCEDURE
After the fecal specimen has been collected, open the test card, so that both specimen windows are visible. Apply a **very thin smear** of fecal specimen to each specimen window on the test card. The specimen windows should not be completely covered with the specimen: an outer periphery of white should be left for contrasting background color. The test card may be developed immediately (**fecal specimens** may be developed for up to 5 days after specimen application to the test card when stored at room temperature 10-24°C (50-75°F). Close the card and turn the card over to the reverse side. Lift the silver tab, exposing the developer pad, and grip the card where indicated. **Slowly** pull the silver tab all the way to the right and completely remove it from the test card. Results should be interpreted after 60 seconds, but before 3 minutes (weak positive results may fade after that time).

INTERPRETATION of RESULTS
After pulling the tab out to its limit, results are to be read from the reverse side of the card and through the clear plastic window. ANY trace of blue coloration is to be regarded as a positive for occult blood. An absence of blue indicates no detectable occult blood. These results should be read at room temperature 10-24°C (50-75°F) at 60 seconds and before 3 minutes of pulling the tab. Within this time period, the proper functioning of the reagents is indicated by the positive monitor turning blue: Should the monitor reaction be different, the test results are invalid. Contact Aerscher at 401-778-2957 for assistance if an invalid test result occurs. Color blind persons should not interpret the results. Neither the intensity nor the shade of blue from the positive performance monitor should be used as a reference for the appearance of positive test results.

LIMITATIONS of the TEST
Gastro-intestinal cancers, adenomas and ulcerations do not always bleed. Results cannot be considered conclusive evidence of the presence or absence of GI bleeding or pathology, and false positive/negative reactions are known to occur under certain circumstances such as a person's diet and medications (see Patient Preparation above). The test is not intended as a replacement for other diagnostic procedures and further testing and examination by the physician such as sigmoidoscopy, barium enema, and x-ray studies, need to be performed to determine the exact cause and source of the occult blood in the stool.

EXPECTED RESULTS
Guaiac impregnated paper has been extensively studied, and these clinical studies indicate that guaiac impregnated slide test yield a positive result 3-5% of the time in screening programs and the percent of false positive results lies in the range of 1-2% under controlled conditions [8][9][10][11]. Sensitivity (% of subjects with the condition being sought who test positive) is difficult to estimate, but in series of patients with known colorectal cancer, 50-87% have been reported to yield positive reactions [12][13][14]. Estimates of positive reactions with adenomatous bleeding have varied widely, and appear dependant to a degree on the size of polyp, with polyps less than 2 cm yielding less than 5% positive reactions.

PERFORMANCE CHARACTERISTICS
Hemoglobin was diluted with distilled water to the following concentrations: 1mg/ml, 2mg/ml, 4mg/ml, and 6mg/ml, equivalent to 0.1, 0.2, 0.4, and 0.6 grams hemoglobin per 100 grams of stool respectively, and approximately 1, 2, 4 and 6 ml. of blood per 100 grams of stool. These dilutions were used to test the sensitivity of HemaPrompt as well as to compare the reactions of several other commercially available guaiac slide tests concurrently with HemaPrompt. In testing, HemaPrompt reacted positively at hemoglobin concentrations of 4mg/ml or greater, in less than 1 minute. At 2mg/ml, HemaPrompt gave a positive reaction in less than 3 minutes when a lesser degree of blue was evident. Below 2mg/ml no positive reactions were observed. Furthermore all positive monitors reacted in the expected manner (+ve turned blue). Exposing the guaiac paper to UV light for ten minutes inactivated the expected reaction.
It was concluded that HemaPrompt reacted positively to all hemoglobin levels above 2mg/ml, at which level several other commercially available guaiac slide tests reacted comparatively and similarly to HemaPrompt [16].

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