



**OneStep
 Fecal Occult Blood
 RapiCard™ InstaTest**

REF 13010-1-19

IVD See external Label 15-30°C Σ= 1 Tests

Sensitivity 50 ng hHb/ml buffer

INTENDED USE

The Cortez Diagnostics Inc. Fecal Occult Blood RapiCard™ InstaTest is an immunochemical device intended for the qualitative detection of Fecal Occult Blood by laboratories or physicians offices. It is useful in determining gastrointestinal (GI) bleeding found in a number of gastrointestinal disorders, such as: diverticulitis, colitis, polyps, and colorectal cancer. This test is recommended for use in: 1) routine physical examinations or when hospital patients are first admitted, 2) hospital monitoring for GI bleeding in patients, and 3) screening for colorectal cancer or gastrointestinal bleeding.

SUMMARY AND EXPLANATION

The American Cancer Society and Centers for Disease Control recommend an occult blood feces test annually after age 50 to aid in the early detection of colorectal cancer. Three types of assays for FOB testing are commercially available: 1) Guaiac Dye; 2) Hemoporphyrin; and, 3) Immunochemical.

- The Guaiac test is widely available but lacks high accuracy. Guaiac is a naturally occurring phenolic compound that can be oxidized to quinone by hydrogen peroxidases with a detectable color change. The sensitivity and specificity of Guaiac tests are much lower than those of Hemoporphyrin tests and Immunochemical assays. The low accuracy of the Guaiac Dye method is related to dietary peroxidases, including hemoglobin and myoglobin from meat and uncooked fruits and vegetables. Non-cancerous gastrointestinal tract bleeding and iron intake may also cause false positive results from Guaiac test.

- The Hemoporphyrin test is not affected by dietary peroxidases, but false-positive results can occur in patients with upper gastrointestinal bleeding disorders such as gastric or duodenal ulcers because porphyrins are not broken down by stomach acids.
- This immunochemical FOB rapid test is much more sensitive and has been designed to specifically detect low levels of human fecal occult blood. It is highly accurate for human hemoglobin (hHb) compared to the Guaiac and Hemoporphyrin methods. The results of immunochemical FOB rapid tests are not affected by dietary peroxidases, animal blood and ascorbic acid. A Japanese study demonstrated using immunochemical FOB tests reduced mortality by 60%.

TEST PRINCIPLE

This assay is a one-step lateral flow chromatographic immunoassay. The test strip consists of: 1) a burgundy colored conjugate pad containing mouse anti-hHb antibodies conjugated with colloidal gold and 2) a nitrocellulose membrane strip containing a Test line (T-line) and a Control line (C-line). The T-line is coated with anti-hHb antibodies, and the C-line is coated with goat anti-mouse IgG antibodies.

When an adequate volume of test specimen is dispensed into the sample well of the device, the test specimen migrates by capillary action across the test strip. If the concentration of hHb in the specimen is at or above 50 ng/ml, the T-line appears as a visible burgundy line. If the concentration of hHb in the specimen is below the detectable level, no T-line develops.

The C-line is coated with goat anti-mouse antibody, which binds to the conjugated monoclonal antibody, regardless of the presence of hHb in the sample.

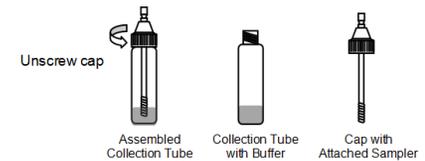
PATIENT LIMITATIONS

1. A specimen should not be collected from a patient with the following conditions that may interfere with the test results:
 - Menstrual bleeding
 - Bleeding hemorrhoids
 - Constipation bleeding
 - Urinary bleeding

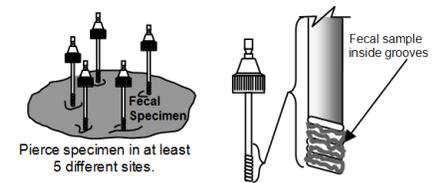
2. Alcohol and certain medications such as aspirin, indomethacin, reserpine, phenylbutazone, corticosteroids and nonsteroidal anti-inflammatory drugs may cause gastrointestinal irritation and subsequent bleeding in some patients.

SPECIMEN COLLECTION AND PREPARATION

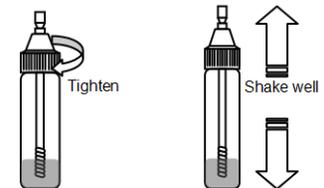
1. The specimen used in this assay is feces. It may be collected from toilet paper or caught in a clean cup. Avoid contact with toilet water.
2. Unscrew the cap (with the attached sampler) of the collection tube.



3. Randomly pierce the fecal specimen with the threaded end of the sampler in at least five (5) different sites. Wipe excess feces off the shaft and outer grooves.



4. Insert sampler in the collection tube and firmly tighten the cap.



5. Shake the tube well to mix the specimen and the FOB buffer. *NOTE:* Samples collected may be stored at least eight (8) days at ambient temperatures below 35°C (95°F), six (6) months at 2-8°C (36-46°F) and two (2) years at ≤ -20°C (≤ -4°F).



MATERIALS AND COMPONENTS

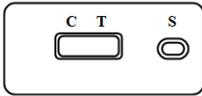
- 20 fecal collection tubes, each with 2ml FOB buffer (1x PBS with 0.02 % sodium azide)
- 20 test devices (cassettes), each sealed in a foil pouch.
- One package insert (instructions for use).

Materials required but not provided

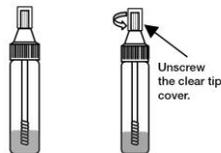
- Timer.
- An absorbent cloth or tissue (preferably disposable) or a clean disposable cup.

ASSAY PROCEDURE

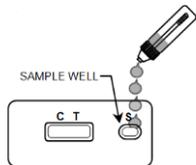
- Refrigerated specimens or other test materials, including test cassette, **must be equilibrated to room temperature before testing to avoid invalid results.**
- Remove the device from the pouch and place it on a flat surface. Label the device with specimen identification.



- Holding the collection tube upright, snap off the tip



- Squeezing the collection tube, dispense four drops of the FOB buffer in the collection tube into the sample well ("S").

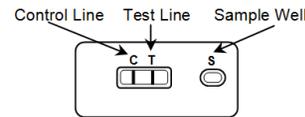


- Read the result within **5-10 minutes** after adding the FOB buffer. **IMPORTANT: Do not read the test results after ten (10) minutes.**

RESULTS

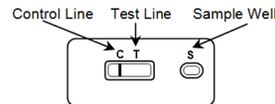
Positive:

If both **C-line** and **T-line** are present, the result is positive. A positive result indicates the level of hHb in the specimen is over 50 ng hHb/ml FOB buffer or 50 µg hHb/g feces.



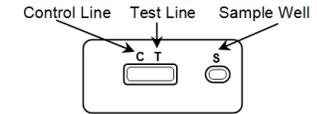
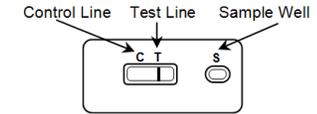
Negative:

If **only the C-line** develops in the control region of the test strip, the result is negative. A negative result indicates the hHb in the specimen is below 50 ng/ml.



INVALID:

If **no C-line** appears within 5 minutes, the result is invalid and the assay should be repeated with a new device. *NOTE:* The test line may or may not be present. However, the absence of a control line indicates an invalid test.



PERFORMANCE CHARACTERISTICS

1. Sensitivity

The sensitivity of the test is 50 ng hHb/ml buffer or 50 µg hHb/g feces.

2. Accuracy

➤ *Reference Laboratory and Physicians Office Laboratory (POL) Studies*

One hundred (100) hHb-free feces extraction specimens collected in-house were divided into 5 groups of 20 each. The five groups of extraction samples were spiked with hHb for five different concentrations, respectively: 0, 37.5 ng hHb/ml, 50 ng hHb/ml, 62.5 ng hHb/ml, and 2000 ng hHb/ml. Those specimens were blind labeled and tested with this Fecal Occult Blood Rapid Test at three (3) Physicians Office Laboratories and a Reference Laboratory.

The results obtained from the three POL sites by persons with diverse education background and work experiences agreed 97.7% (average) with the expected results. The results obtained from the Reference Laboratory agreed 99% with that expected. Overall, the accuracy of this Fecal Occult Blood Rapid Test is 98%.

➤ *Comparison studies*

Those 100 specimens were also tested in house with this Fecal Occult Blood Rapid Test and a predicate device. The correlation between this Fecal Occult Blood Test and the predicate device was over 95%.

3. Specificity

Cortez Diagnostics Fecal Occult Blood RapiCard™ InstaTest is specific to human hemoglobin. The following substances, when spiked in both positive and negative specimens, did not interfere the test results.

Substance	Concentration (µg/ml)
Beef Hemoglobin	2,000
Chicken Hemoglobin	500
Fish Hemoglobin (meat extract)	100
Horse Hemoglobin	500
Goat Hemoglobin	500
Pig Hemoglobin	500
Rabbit Hemoglobin	500
Sheep Hemoglobin (meat extract)	100
Horseradish Peroxidase	20,000
Red radish	Aqueous extract
Raw turnip	Aqueous extract
Cauliflower	Aqueous extract
Broccoli	Aqueous extract
Parsnip	Aqueous extract
Cantaloupe	Aqueous extract
Vitamin C (ascorbic acid)	Dietary supplement
Iron	Dietary supplement

Operators should always follow the appropriate federal, state, and local guidelines concerning the running of external quality controls, including positive and negative, to assure the proper performance of the device.

- Allison JB, Takawa IS, Ransom LJ, Adrian AL. A comparison of fecal occult blood tests for colorectal-cancer screening. *N Engl J Med* 1996; 334:155-159.
- Saito H. Screening for colorectal cancer by immunochemical fecal occult blood testing (Review). *Jpn J Cancer Res* 1996; 87:1011-1024.

LIMITATIONS OF PROCEDURE

- Results cannot be considered conclusive evidence of the presence or absence of gastrointestinal bleeding or pathology. A positive result should be followed up with additional diagnostic procedures to determine the exact cause and source of the occult blood in the feces.
- A negative result can be obtained even when a gastrointestinal disorder is present. For example, some polyps and colorectal cancers may bleed intermittently or not at all during certain stages of the disease.
- False negative results may occur when occult blood is not uniformly distributed throughout the bowel movement and the formation of a fecal sample. Repeat testing is recommended if a pathological condition is suspected.

PRECAUTION

- For in vitro diagnostic use only
- Do not use expired kit components.
- Treat all specimens and used assay materials as if they are infectious.
- Dispose of all used test components in a biohazard container, per clinical lab procedures.

Storage

The test device is stable when stored in a controlled environment at 15-30°C (59-86°F) for up to 2 years or until the expiration date printed on the label, whichever comes first. Do not expose the kit components to temperatures over 30°C (86°F).

REFERENCES

- American Cancer Society, Inc. Cancer Reference Information: Can Colon and Rectum Cancer Be Found Early? [Online] Available: <http://www.cancer.org>

 ISO 13485 ISO 9001	
 Diagnostic Automation/ Cortez Diagnostics, Inc. 21250 Califa Street , Suite 102 and 116, Woodland Hills, California 91367 USA	
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CORTEZ- OneStep Fecal Occult Blood RapiCard™ InstaTest -2016	
EC	REP
CEpartner4U, Esdoornlaan 13, 3951DB Maarn, The Netherlands. www.cepartner4u.eu	
Revision D 2013-07-22	

QUALITY CONTROL

Internal Quality Control

Cortez Diagnostics Fecal Occult Blood RapiCard™ InstaTest contains a built-in quality control feature, the Control line (C line). The presence of this C-line indicates that an adequate sample volume was used and that the reagents migrated properly. If a C-line does not form, the test is considered invalid. In this case, review the entire procedure and repeat the testing with a new device.

External Quality Control