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PRODUCT INFORMATION

InSure® ONE™ is CLIA Waived

Product Name

InSure® ONE™ Patient Collection Kits (available in 10, 25 and 50 packs)
Each InSure® ONE™ Patient Collection Kit contains (may vary):
1 x InSure® ONE™ Test Card
1 x InSure® Brush Kit
1 x InSure® ONE™ Instructions for Use – Patient
1 x Reply Form
1 x Reply Envelope

InSure® Developer Components:
To run each test requires an Instructions for Use, Run Buffer and a Test Strip. Materials are available in the following configurations:

Bulk Items:
InSure® ONE™ or InSure® FIT™ Test Strips - 25 per vial
InSure® ONE™ or InSure® FIT™ Run Buffer - 1 Liter
InSure® Dropper Bottles - (4 pack)

Convenience Kits:
InSure® ONE™ Developer Kit
Each Developer Kit contains:
1 x InSure® ONE™ Instructions for Use – Professional
1 x InSure® Run Buffer (9mL)
25 x InSure® ONE™ Test Strips

Materials for QC:
InSure® ONE™ Test Cards for QC contains:
50 x InSure® ONE™ Test Cards

InSure® / InSure® FIT™ FOBT Controls contains:
1 Instructions for Use, 1 Positive Control, 1 Negative Control

Within the USA, for more information, call Enterix Inc. at 1-800-531-3681 or visit our website at www.insuretest.com. Within all other countries please contact your local distributor.

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SYMBOL KEY

Keep away from heat	Biohazard
Attention, see instructions for use	CE Mark
IVD In vitro diagnostic medical device	REF Catalog number
Do not reuse	LOT Batch code
Manufacturer	Temperature limitation
EC REP European Community Authorized Representative	Use by (Year Month Day)
CONTENTS Contents	PATENTED This product is covered by one or more patents
Contains sufficient for (n) tests	

Enterix Inc., A Clinical Genomics Inc. Company
236 Fernwood Ave
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EC REP
mdi Europa GmbH
Langenhagener Straße 71
D-30855 Langenhagen
Germany

Part Number: ***13085.01***

CE

InSure® ONE™
One Day Fecal Immunochemical Test

One Day Fecal Immunochemical Test
Instructions for Use

InSure® ONE™ is CLIA Waived

INTENDED USE/INDICATIONS FOR USE

InSure® ONE™ is a fecal immunochemical test (FIT) that qualitatively detects human hemoglobin from blood in fecal samples. The samples will generally be collected by the test subject at home and the test developed at laboratories or professional offices. The InSure® ONE™ test is used to aid in the detection of lower gastrointestinal bleeding.

IVD MEDICAL DEVICE DIRECTIVE

InSure® ONE™ Test complies with the IVD Medical Device Directive 98/79/EC and carries the CE mark.

SUMMARY AND EXPLANATION OF THE TEST

The fecal occult blood test (FOBT) was described for general medical use in 1942.[1] The active ingredients, guaiac impregnated filter paper and hydrogen peroxide, react with hemoglobin or other substances (e.g., hematin, heme as well as peroxidases from fruits and vegetables) to give a visible blue color. The principal use of these tests is to aid in the detection of lower gastrointestinal bleeding.[3-7]
There are many dietary sources of peroxidase activity, most notably heme from red meat, and these dietary sources of peroxidase activity can cause false positive test results in guaiac FOBTs.
Another source of false positives with guaiac tests is from common medications, such as aspirin, that may cause low grade, clinically insignificant amounts of gastric bleeding. Conversely, vitamin C may cause false negative test results in guaiac FOBTs.[8] Because these substances may interfere with the test result, patients are required to follow a special restricted diet prior to and during sample collection for guaiac FOBTs[8].
InSure® ONE™ is an immunochemical test specifically designed to detect human hemoglobin from lower gastrointestinal bleeding.
InSure® ONE™ uses anti-human hemoglobin antibodies (monoclonal and polyclonal) that react with the globin portion of the hemoglobin. Hemoglobin from upper gastrointestinal bleeding (i.e., oral cavity, esophagus, stomach or small intestine) is generally degraded by the body's normal digestion and by the action of bacterial enzymes. It is therefore rendered immunochemically non-reactive before reaching the large intestine.[2-9, 11-15] Conversely, hemoglobin from lower gastrointestinal bleeding (i.e., cecum, colon or rectum) undergoes less degradation, remaining at least partially immunochemically reactive. Thus, immunochemical fecal occult blood tests that detect undegraded hemoglobin have increased biological specificity for lower gastrointestinal bleeding and any associated pathology.[2-9, 11-15] Because InSure® ONE™ is specific for human blood, no special dietary restrictions are required.
The American Cancer Society's (ACS) Recommendations for Screening and Surveillance for the Early Detection of Adenomatous Polyps and Colorectal Cancer[16] states "…in comparison with guaiac based tests for the detection of occult blood, immunochemical tests are more patient-friendly, and are likely to be equal or better in sensitivity and specificity."
The InSure® ONE™ has a simplified sampling method which combines patented brush technology shown to improve participation rates with a single bowel-movement format, without a reduction in performance.[17, 18]
All fecal blood tests are subject to certain general limitations. These include lower gastrointestinal lesions that may bleed intermittently, blood may be non-uniformly distributed in feces and detection of blood may not always be an indication of gastrointestinal pathology (see LIMITATIONS OF PROCEDURE).

PRINCIPLES OF THE PROCEDURE

The InSure® ONE™ Test uses the principle of immunochromatography to detect human hemoglobin from blood in samples of toilet bowl water collected from around the stool after defecation. The test result is obtained from *one* stool sample. The toilet bowl water is collected after brushing the surface of the stool to release any blood into the surrounding water. Long handled brushes are used to collect and transfer two samples of toilet bowl water from *one* bowel movement to the InSure®ONE™ Test Card. The Test Card serves as a means to transport the dried sample to the testing site. At the testing site, an immunochromatographic Test Strip is inserted into the Test Card and Run Buffer is added to rehydrate the samples and to extract the hemoglobin, if present, from the samples. The sample flows along the Test Strip, rehydrates the colloidal gold anti-human hemoglobin conjugate and, if hemoglobin is present in the sample, forms a hemoglobin-conjugate immune complex. The complex is then captured on the Test Strip, in a zone containing immobilized anti-human hemoglobin antibodies, to form a visible test line – a positive test. No Test Line forms in the absence of human hemoglobin in the sample – a negative test. Unbound conjugate continues to migrate along the Test Strip and binds to the Control Line, which contains conjugate-specific antibodies. The Control Line therefore serves to confirm that reagent flow has been completed and that the test is functional.

MATERIALS

InSure® Test Strips

The Test Strips contain a mouse monoclonal anti-human hemoglobin Test Line and a conjugate-specific polyclonal (donkey anti-goat) antibody Control Line and a conjugate of anti-human hemoglobin polyclonal antibodies bound to colored (colloidal gold) particles; all antibodies are from a U.S. source.

InSure® Run Buffer

Contains borate salts, ethanol (10%), bovine serum albumin (from a U.S. source) and 0.09% sodium azide as preservative.

Materials Required But Not Provided

- Timer: To 5 minutes,with seconds
- Disposable latex gloves
- Protective facewear including eyewear

WARNINGS AND PRECAUTIONS

- *In vitro* diagnostic use only. IVD

CAUTION: Observe universal safety precautions and other appropriate laboratory procedures when collecting and handling patient samples. All samples and materials that come in contact with them should be handled as potentially infectious.

- The areas for running External Quality Controls and the Test Procedure should be wiped down and cleaned to avoid contamination.
- Change gloves after running controls to avoid contamination.
- Gloved hands and the test area should be kept clean and free of blood to avoid contamination of the Test Cards and Test Strips.
- When handling the Test Strip avoid the middle area of the Test Strip (nitrocellulose portion). Handle the end opposite of the arrows at all times when holding the Test Strip.
- Use only InSure® ONE™ Test Cards for QC (50 Test Cards) for preparing control test samples.
- DO NOT remove Test Strips from their foil pack or vial until ready for use.
- DO NOT use Test Cards, Test Strips and Run Buffer after their labeled expiration dates.
- Use only InSure® Run Buffer to develop the Test Cards.
- DO NOT use any Run Buffer from a container that appears to have leaked.
- Avoid skin and eye contact. Refer to the SDS.
- The Run Buffer contains sodium azide. Sodium azide may react with lead copper plumbing to form highly explosive metalazides. Upon disposal, flush with large volumes of water to prevent azide buildup. Avoid reagent contact with eyes, mucous membranes or skin lesions. If contact occurs flush affected area with water for 15 minutes and consult a physician.

STORAGE AND STABILITY

Store Test Strips in their unopened foil pack or vial at 2 - 25ºC (36 - 77ºF).
DO NOT FREEZE. When stored as directed, InSure® ONE™ Test Strips are stable until their labeled expiration date. The InSure® ONE™ Collection Kits must be protected from heat (>37ºC/>99ºF) and direct sunlight, and used before their expiration date.
InSure® Run Buffer should be stored at 2 - 25ºC (36 - 77ºF) until its labeled expiration date. (Note warnings and precautions on handling and storage of Test Strips.)

PATIENT PREPARATION

No special dietary or medicinal restrictions are required for this test; however patients should closely follow the Instructions for Use included in the InSure® ONE™ Collection Kit. Roughage in the diet can increase test accuracy by helping uncover "silent" lesions that bleed intermittently.[19] Patients should not collect samples three days before, during, or three days after their menstrual period, if they have bleeding hemorrhoids, if there is visible blood in their urine or in the toilet bowl, or if they have bleeding cuts on their hands.

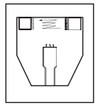
SAMPLE COLLECTION AND STORAGE

Instructions for Use for sample collection and handling are included in the InSure® ONE™ Collection Kit. The dried samples when collected and stored as directed are stable for up to 14 days at room temperature, below 99ºF (37ºC).[20]

TEST PROCEDURE/DIRECTIONS FOR USE

IMPORTANT **Do not touch Test Strip or Test Card with reagent bottles**

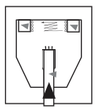
1.Remove the back panel of the Test Card.



2.When handling the Test Strip avoid the middle area of the Test Strip (nitrocellulose portion). Use the end opposite of the arrows at all times while handling the Test Strip.

AVOID HANDLING THIS AREA OF THE TEST STRIP

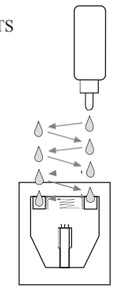
3.Insert the Test Strip so that it securely locks into cut-out. (Note precautions on handling and storage of Test Strips).



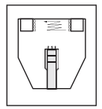
REAGENT PORTS

TEST STRIP

4.Add Run Buffer, one drop at a time alternately to each reagent port, until each port has received four drops (~150 µL).

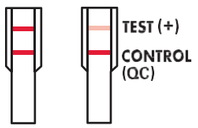


5.Wait for 5 min. and read the result.

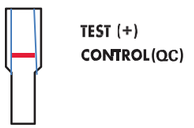


Note: If the Run Buffer does not flow onto the Test Strip due to excess specimen, add two more drops of Run Buffer to each reagent port and wait an additional 5 minutes.

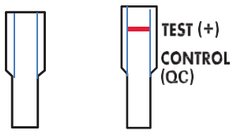
INTERPRETATION OF TEST RESULTS



Positive Test (DETECTED)
The test is positive, indicating the presence of blood, if two lines (Test and Control) are visible on the Test Strip. Any trace of a line in the Test Line area is a positive test result.



Negative Test (UNDETECTED)
The test is negative, indicating no blood was detected, if only the Control Line is visible and there are no traces of a line in the Test Line area.



Invalid Test:
The test is invalid if the Control Line does not appear. If this occurs, the test should be repeated with a new specimen Test Card.

Notes:

- Carefully look for the appearance of a Test Line in the Test Line area. A colored line is a positive result even if the Test Line appears lighter or darker than the Control Line.
- Some specimens may produce a positive Test Line before 5 minutes. To confirm a negative test result, wait a full 5 minutes after adding the Run Buffer before interpreting the results. To avoid misinterpretation of the test, do not interpret the results after 5 minutes.
- If external controls are used, neither the intensity nor the shade of the Test Line produced by the external positive control should be used as a reference for the appearance of a positive test result.
- Discard used Test Cards and any remaining test components in accordance with internal procedures for the disposal ofclinical waste. All specimens and used assay materials should be treated as if they are potentially infectious.
- If the absence of a Control Line occurs repeatedly or for technical assistance, please contact Technical Service, Enterix Inc. in the US: 1-800-531-3681, or your local distributor.

QUALITY CONTROL

Controls Built into InSure® ONE™
This device contains built-in procedural control features which consist of a Control Line and a negative background control area on the Test Strip. The presence of the Control Line indicates that an adequate amount of Run Buffer was used and migrated properly through the Test Card and the Test Strip.
The Control Line contains immobilized conjugate-specific antibody. The presence of a Control Line (pink) indicates that the conjugate was properly hydrated, flowed through the Test and Control Line areas, the Control Line antibodies were immunoreactive and the conjugate is intact. The test is invalid if the Control Line does not appear.
The negative background control area is the region just below the Control Line on the Test Strip. A white to light pink background color in the negative background control area indicates that the reagents and conjugate-sample complex, if formed, flowed properly. If distinct areas of color (dark pink) remain in the window below the Control Line, the test is invalid. The built-in procedural controls should be observed for each patient test performed in order to monitor test validity. Patient test results should not be reported when the built-in controls indicate an invalid test.

External Quality Control
Good laboratory practice recommends the use of external controls to assure the functionality of reagents and proper performance of the test procedures. Operators should always follow the appropriate federal, national, state and local guidelines concerning the use of external controls.

LIMITATIONS OF THE PROCEDURE

InSure® ONE™ is a valuable aid to the physician in early detection of lower gastrointestinal disorders that bleed. However colorectal lesions, including some polyps and colorectal cancers, may bleed intermittently, or not at all. Additionally, blood may not be uniformly distributed in or on the stool and a test result may be negative even when blood or lower gastrointestinal disease is present.
Because blood degrades as it passes through the gastrointestinal tract, possibly losing its immunoreactivity, InSure® ONE™ may be less reactive than guaiac-based FOBTs for detecting upper gastrointestinal bleeding.[2-9, 11-15]
As with any fecal blood test, results obtained with InSure® ONE™ should not be considered conclusive evidence of the presence or absence of gastrointestinal bleeding or pathology. InSure® ONE™ is not intended to replace other diagnostic procedures such as colonoscopy, sigmoidoscopy or double contrast barium x-ray. InSure® ONE™ is not for use in testing urine, gastric specimens or other body fluids.

EXPECTED RESULTS

Positivity rates with immunochemical fecal blood tests may be expected to vary with each patient population depending on the test used, age, ethnicity, predisposition to colorectal disease, and other factors that may be associated with lower gastrointestinal lesions that bleed.[2-7, 10-15]

PERFORMANCE CHARACTERISTICS

Analytical Performance Studies
ANALYTICAL SENSITIVITY
Lower Sensitivity Limit
In vitro studies demonstrated that by following the recommended procedures for sample collection and storage, InSure® ONE™ reliably detected 50 µg Hb/g feces. Test sensitivity is expressed as the lowest Hb concentration in µg Hb/g feces, resulting in at least 95% positive readings.
Studies with hemoglobin (Hb) variants HbS (homozygous) and HbE (heterozygous) indicated that InSure® ONE™ was similarly as sensitive to these forms of hemoglobin as to normal hemoglobin. Other hemoglobinopathies were not tested.
Prozone Effect
In vitro studies demonstrated that InSure® ONE™ reliably detected up to 21 mL of added blood per 100 g of feces (30 mg hemoglobin per g of stool). At this level the blood is generally visible.
Cross-Reactivity
InSure® ONE™ was examined *in vitro* by adding samples of meat extract (myoglobin and hemoglobin) from beef, chicken, fish, horse, pig, rabbit, deer, sheep and kangaroo to the Test Card to determine whether meat extracts cross-react with the test. The samples were added with and without diluted human blood and the cards dried overnight. InSure® ONE™ gave negative test results when tested with all of the extracts, but was positive in all cases when human blood was present. In contrast, the meat extracts, when added to a guaiac FOBT (Hemoccult® SENSA®), consistently gave positive results.
Interference/Effect of Dietary Substances
InSure® ONE™ does not require the patient to follow any special dietary restrictions. Aqueous extracts of raw broccoli, cantaloupe, cauliflower, horseradish, red radish and turnip were added to the Test Card to determine if vegetable extracts cross react with the test. Test cards were also prepared using a 20 mg/mL solution of horseradish peroxidase. The extracts were added with and without diluted human blood and dried overnight. InSure® ONE™ gave negative test results when tested with all of the extracts, but was positive in all cases when human blood was present. Thus, no interference was evident with these substances. In contrast, the same substances when added to a guaiac FOBT (Hemoccult® SENSA®), consistently gave positive results. An iron supplement (ferrous sulfate) and vitamin C did not interfere with the InSure® ONE™ performance.

Interference by Toilet Water Additives and Contaminants
No evidence was found that any of the toilet bowl deodorizers/fresheners or cleaners studied caused false positive test results. The effect of the products on the sensitivity of the test varied. Some decreased the sensitivity of the test by over four-fold, while others appeared to have no effect. The effects that were noted did not appear to correlate with the noted formulation of the deodorizer/freshener. Based on these studies it is concluded that the labeling for InSure® ONE™ should be adhered to and that these products should be removed from the toilet bowl prior to collecting samples with InSure® ONE™ .
A study to assess the effect of residual blood, left in the toilet bowl by an earlier user, showed that provided the InSure® ONE™ instructions for flushing before use were followed, there was no effect on the performance of the test.
A 1:1 dilution of urine appears to increase the sensitivity of the test.
Variations in ion concentrations in the toilet bowl water affected the test. This test should not be used if the toilet contains ocean water (high salt content) or rusty (brown) water.

Assay Cut-off Study
Fecal test samples were prepared by spiking stool samples with human blood of known hemoglobin concentration, to obtain the following fecal hemoglobin concentrations: 0 µg Hb/g stool, 5 µg Hb/g stool, 10 µg Hb/g stool, 20 µg Hb/g stool, 30 µg Hb/g stool, 40 µg Hb/g stool, 50 µg Hb/g stool, 60 µg Hb/g stool and 80 µg Hb/g stool. The pre-measured mass quantities of stool spiked with different levels of hemoglobin were placed in a pre-measured volume of water to simulate toilet water in which stool will be deposited. Following deposition of the stool, water samples were taken at 2 minutes with InSure® sample collection brushes and applied to Test Cards using the InSure® FIT™ and the InSure® ONE™ sampling procedures. Testing was performed side-by-side with the predicate by comparing the test results of the device with that of the predicate. Test Cards were prepared on Day 0, stored at room temperature (20–25°C) and developed on Day 5 to simulate the time span between the patient collecting the fecal water sample on the Test Card and transporting via mail of the sampled Test Card to the lab. The cut-off was determined to be 50 µg hemoglobin/g stool.

***In Vitro* Limits of Detection and Sample Stability**
Fresh whole fecal specimens were divided into approximately equal aliquots and spiked with whole human blood to concentrations of 0, 10, 30, 50, 100, 200, 500, 1000 and 1500 µg Hb/g feces. InSure® ONE™ Test Cards were prepared by the standard collection method. All Test Cards were stored at room temperature 15 - 25º C (59 - 77º F) and tested at 1, 7, 10 and 14 days post preparation. All Test Cards were read by two experienced, independent readers in a blinded fashion.
Test sensitivity is expressed as the lowest Hb concentration in µg Hb/g feces, resulting in at least 95% positive readings.

InSure® ONE™Sensitivity
Day 14 = 50 µg Hb/g feces

Inter-reader Variation and Test Performance
A single stool sample was spiked with whole blood to give hemoglobin concentrations of:
•Negative = 0 µg Hb/g feces
•Low positive = 10 µg Hb/g feces
•Medium positive = 30 µg Hb/g feces

Thirty Test Cards for each aliquot were tested 24 hours post preparation. All Test Cards were read by three, independent readers in a blinded fashion. The table below shows the number of Test Cards read correctly by each reader.

	InSure® FIT™		
Reader	A	B	C
Unspiked (n = 30)	30	29	30
Low Positive (n = 30)	30	30	29
Medium Positive (n = 30)	30	30	30
Disagreements (%)	2.2 (2/90)		
Accuracy (%)	99.3 (2/270)		

Lower Gastrointestinal Analytical Specificity
Acetylsalicylic acid and other Non-Steroidal Anti-inflammatory Drugs (NSAIDS) are known to cause upper gastrointestinal bleeding. To determine the effect of upper gastrointestinal bleeding on InSure® ONE™, two healthy subjects ingested 20 mL of autologous blood immediately after the blood was obtained. Subjects commenced collecting samples with InSure® ONE™, one day prior to blood ingestion and continued to collect samples from each bowel motion thereafter, until six post-ingestion samples had been collected. None of the samples returned by the subjects tested positive by InSure® ONE™. As aspirin and other Non- Steroidal Anti-inflammatory Drugs (NSAIDS) should not cause 20 mL of blood loss, these medications are unlikely to interfere with the InSure® ONE™ test.

CLINICAL PERFORMANCE-METHOD COMPARISON

Clinical Sensitivity
The performance of InSure® ONE™ was assessed in a mixed population of 859 subjects with elevated risk and with normal subjects requesting a colonoscopy. No dietary restrictions were required. Patients who had an incomplete colonoscopy or self- reported rectal bleeding at the time fecal samples were taken were excluded. Statistical analysis of the test results based on clinical positive percent agreement (PPA) and clinical negative percent agreement (NPA) showed that the InSure® ONE™ test results have acceptable overall agreement with InSure® FIT™ test results. See Summary of Agreements Table.

Clinical Specificity
The specificity of InSure® ONE™ for normal subjects with a negative colonoscopy in an elevated risk population of 859 individuals was 89.5%-93.2% (85.4%-95.6%, 95% CI). The specificity of InSure® FIT™ in the same study was 89.8% (88.8%-92.8%, 95% CI)

Non-Neoplastic Finding
In this study, the *non-neoplastic* findings, based on the histopathology reports from the pathologist included:

- Angiodysplasia
- Diverticular Disease
- Hemorrhoids
- Hyperplastic Polyps (1 or 2)
- Inflammatory Bowel Disease
- Inflammatory Polyp
- Leiomyoma
- Proctitis
- Melanosis
- Rectal Varices
- Fibroepithelial Polyp
- Uncharacterized Polyp

Summary of Agreements between InSure® FIT™ and InSure® ONE™

Category	InSure® FIT™	InSure® ONE™ Pos	InSure® ONE™ Neg	InSure® ONE™ Total	Overall Percent Agreement 95% CI[1]	Positive Percent Agreement 95% CI[1]	Negative Percent Agreement 95% CI[1]
Overall	**Pos**	79	51	130	88.7%	63.2%	93.1%
	Neg	46	683	729	86.4% to 90.6%	54.5 to 71.1%	91.0% to 94.7%
	Total	125	734	859			
Cancer	**Pos**	10	0	10	100.0%	100.0%	100.0%
	Neg	0	2	2	75.8% to 100%	72.2% to 100%	34.2 to 100%
	Total	10	2	12			
Advanced Adenoma	**Pos**	12	7	19	80.6%	63.2%	87.5%
	Neg	6	42	48	69.6%-88.3%	41.0%-80.9%	75.3%-94.1%
	Total	18	49	67			
Negative, Non-neoplastic Findings	**Pos**	31	22	53	89.2%	58.5%	94.2%
	Neg	19	309	328	85.7% to 92.0%	45.1% to 70.7%	91.1% to 96.3%
	Total	50	331	381			
Negative, no findings on colonoscopy	**Pos**	**17**	**13**	30	90.8%	56.7%	94.7%
	Neg	**14**	**250**	264	87.0% to 93.6%	39.2% to 72.6%	91.3% to 96.8%
	Total	**31**	**263**	294			

[1]Wilson Score