Exdia Immunochemical Fecal Occult Blood (iFOB)

One Step Immunoassay
for Immunochemical Fecal Occult Blood(iFOB)
For in vitro Diagnostic Use

One–step qualitative time resolved fluorescence immuno-chromatography assay for the detection of Fecal occult blood in human feces

Manufactured by Precision Biosensor Inc.

1. INTENDED USE

Exdia iFOB is a fluorescence immuno-chromatography assay for qualitative detection of fecal occult blood in human feces using Exdia analyzer. The cassette is suitable for use in laboratories and physician's offices as an aid in the diagnosis of lower gastrointestinal disorders such as any suspected colorectal cancer and gastrointestinal bleeding from any source.

2. SUMMARY AND EXPLANATION OF THE TEST

Occult gastrointestinal bleeding is defined as bleeding, which is unknown to the person. It can be caused by several different reasons such as ulcers, hemorrhoids, polyps, colitis, diverticulitis, cancer and fissures. Since these disease conditions may not produce visible symptoms in their early stages, the FOB test results may act as an early warning signal. Occult bleeding increases gradually with growing size of polyps and advancing stage of CRC and FOB tests can potentially detect both CRC and its preliminary stages.

Exdia iFOB is designed to detect fecal occult blood based on Time Resolved Fluorescence(TRF) immuno-chromatography technology, which improved specificity and sensitivity for the detection of lower gastrointestinal disorders, including colorectal cancers and adenomas. Exdia iFOB is qualitative assay but it can be interpreted quantitatively using fluorescence-based Exdia analyzer with enhanced quality while eliminating potential for any visual bias by the observer**.**

3. PRINCIPLE

The Exdia iFOB test is an immuno-chromatography assay for the qualitative determination of hemoglobin concentration in human fecal samples. On the nitrocellulose membrane of test strip, streptavidin is sprayed to capture the biotinylated anti human hemoglobin antibody at test line and Goat anti-chicken IgY is sprayed to capture europium conjugated IgY at control line. The dye pad and biotin pad containing dried form of anti-human hemoglobin antibody and IgY conjugated with europium particles are placed at the end of membrane.

First, sample is collected in a tube with extraction buffer. The fecal sample in the extraction buffer should be vigorously mixed and then 80uL of the mixed sample solution is dispensed into the sample well of the test cassette. When a sample is applied into the sample well, the human hemoglobin present in the feces sample bind to the indicator antibodies coupled with europium particles and biotinylated capture anti hemoglobin antibody. The immune complexes migrate on the nitrocellulose membrane to the test lines, and they bind to streptavidin immobilized on the test line. Chicken IgY coupled with europium particles passed through the test line is captured by goat anti-chicken IgY antibody in the control line. Control line on the membrane provides an internal quality control of the test cassette. To measure the concentration of human hemoglobin, the tested cassette should be read by Exdia analyzer. The fluorescence intensity of the test line is converted to the concentration of human hemoglobin in the feces specimen by the predetermined equation. The converted result is displayed by the Exdia analyzer in a qualitative (positive or negative) manner based on an analytical cut-off of 100 ng/mL (human hemoglobin in human fecal sample mixed with extraction buffer).

4. REAGENT

The Exdia iFOB test contains all the reagents necessary for the detection of hemoglobin in human feces. The cassette contains a membrane strip coated with streptavidin on the test line and dye pad infused with biotinylated monoclonal anti- human hemoglobin antibody and europium particles coupled with anti-human hemoglobin specific antibody. A stabilizer containing 0.05% sodium azide and BSA protein are deposited on the dye pad in dried form.

The extraction buffer contains 0.05% BSA, 0.02% sodium azide to extract the hemoglobin antigen from collected feces specimens.

5. MATERIALS

Provided

- 20 Test cassette sealed in individul pouch with desiccant
- 20 Sample collection tubes with extraction buffer (2mL/tube)
- 20 Storage and transport pouches for collection tubes.
- 1 QR card for calibration (lot-specific)
- 1 Instructions for Use

Required but not provided

- Positive and negative quality control materials
- Timer
- Exdia analyzer

6. STORAGE AND STABILITY

The test cassette should be stored at 2℃ ~30 ℃ in the original sealed pouch for the duration of shelf life.

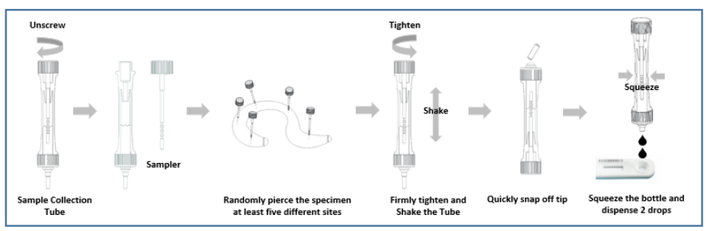
7. PRECAUTIONS

- For *in-vitro* diagnostic and professional use only.
- Handle all specimens as potentially infectious. Proper handling and disposal methods should be established.
- To avoid cross contamination, use a fresh transfer cassette for each clinical sample tested.
- Do not use test cassette if the pouch is damaged or improperly sealed.
- Do not use test cassette beyond expiration date.

8. SPECIMEN COLLECTION

The specimen used in this assay is human fecal samples.

- Collect a random sample of feces in a clean dry container or receptacle.
- Unscrew and remove the collection tube sampler stick. Be careful not to spill or spatter solution from container.
- Collect sample using the sampler. Take sample from various surfaces of the feces specimen at least at five (5) different sites.
- Re-insert the sampler into the tube and screw the cap tightly. Be careful not to break the tip of the Sample Collection Tube.
- Submit sample collection tube to the laboratory immediately. If not, keep it stored under refrigerated condition.



9. TEST PROCEDURE AND PROTOCOL

- Collect specimen according to instructions in **"Specimen Collection"**.
- Test cassette and sample should be brought to room temperature (20℃~30 ℃) prior to testing.
- Remove the test cassette from the sealed pouch immediately before use. Label the cassette with patient or control identification.
- Shake vigorously the collected sample tube for 15-20 second to make sure the sample is well mixed with buffer. After snapping off the tip, squeeze the tube to apply the contents (2 drops) of sample into the sample well.
- The tested cassette should be analyzed by the Exdia analyzer followed by the instruction manual. Read the results at 5 minutes in Exdia analyzer.

10. INTERPRETATION OF RESULTS

Exdia iFOB test results can be interpreted as quantitative and qualitative method by Exdia TRF analyzer. For quantitative results, results are given as the concentration of hemoglobin(Hb) in feces solution (ng/mL) with the measuring range of 25 ng/mL ~ 1,000 ng/mL. If the test result is over or below the measuring range, it will be displayed as 1,000 ng/mL> or <25 ng/mL respectively.

The qualitative results are displayed as Negative or Positive based on the measuring value of hemoglobin(Hb) at cutoff concentration of 100 ng/mL.

11. LIMITATIONS

Results are not conclusive evidence of the presence or absence of gastrointestinal bleeding caused by cancer or pathology.

Immunochemical FOB testing has been shown to be valuable in preliminary screening, particularly of asymptomatic populations, or as an aid to diagnosis. The test is not intended to totally replace other diagnostic procedures such as colonoscopy, flexible sigmoidoscopy, or other imaging studies such as double contrast barium enema or CT colonography.

Because gastrointestinal lesions may bleed intermittently and blood in feces is not distributed uniformly, a negative test result does not assure absence of lesions.

A test result may be negative even when disease is present, because bowel lesions, including some colorectal cancers and significant polyps, may not bleed at all or may bleed intermittently, or the blood may not be uniformly distributed in a fecal specimen and therefore missed during sampling.

Results may be positive for samples from patients without significant bowel pathology. Usually, the reasons for such false positive results are obscure, but in some cases, certain medications may cause gastrointestinal irritation resulting in occult bleeding.

12. CLINICAL CUT OFF AND REFERENCE RANGE

The cutoff values of the Exdia iFOB test were determined by comparison to OC-SENSOR DIANA (Eiken Chemical Co.) Reference range of iFOB of OC-SENSOR DIANA (Eiken Chemical Co.) is 100ng/mL and correlate with Exdia iFOB. The specimens containing human hemoglobin at the concentration of equal or above established cutoff level will give positive result from the Exdia iFOB test.

13. QUALITY CONTROL

The presence of fluorescence band in the Control area of the window acts as an internal control to ensure an adequate volume of sample has been added. In the absence of this Control band, the associated test result is invalid and must be retested. Good laboratory practice recommends quality control to ensure proper test performance. Quality control materials are available from commercial sources, and should be tested by following same procedures as running the patient sample tests. Good laboratory practice suggests that external controls should be tested with every new lot or in case of questionable test result in using the reagent cassette. If the quality control procedures in your laboratory require more frequent use of controls to verify the test results, follow your laboratory-specific procedures. The recommended requirement for testing the Exdia IQC provided with the instrument is for regular time period. When the test result is questionable for any reason, contact the customer support.

14. PERFORMANCE CHARACTERISTICS

14.1 Precision study

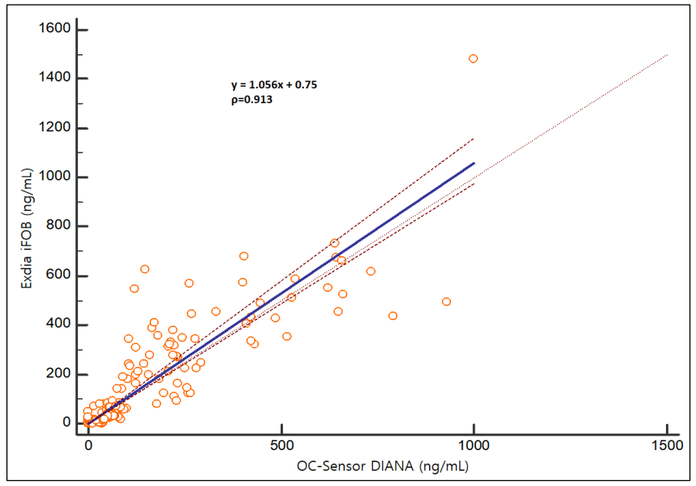
A precision study was performed using controls at 3 different levels in 5 replicates per run at each concentration level for 5 days. Within run and total imprecision of test cassettes were determined according to CLSI guideline EP5-A.

Analyte	Mean (pg/mL)	Within Run	Total Run
		CV (%)	CV (%)
Hemoglobin	75	3.2	4.0
	125	4.9	5.3
	250	8.2	7.3

14.2 Method Comparison Study

Method comparison study was performed for Exdia iFOB in conjunction with Exdia analyzer versus OC-SENSOR DIANA (Eiken Chemical Co.). Fecal samples were collected from 217 peoples whose FOB concentrations distribute in analytical measuring ranges of 25 ~ 1000ng/mL. The comparison result was regressed using Passing-Bablok model and correlation was analyzed using Spearman's ranked correlation. The results showed slope of 1.056 and Spearman's correlation coefficient of ρ=0.913 (95% CI, 0.888 ~ 0.933). The results showed good correlation for iFOB assay between two methods, Exdia iFOB test and OC-SENSOR DIANA (Eiken Chemical Co.).

Analyte	Sample number	Range of detection (ng/mL)	Intercept	Slope	Correlation coefficient
iFOB	217	25 – 1000	0.75	1.056	0.913



14.3 Clinical Accuracy

The clinical accuracy of Exdia iFOB test was evaluated in comparison with OC-SENSOR DIANA predicate device. Total 227 samples were collected and compared to predicative device with 100ng/mL cutoff concentration by blind test. The results are summarized in the following tables.

Cutoff 100 ng/mL			
	OC-SENSOR DIANA (+)	OC-SENSOR DIANA (-)	Total
Exdia iFOB (+)	71	3	74
Exdia iFOB (-)	3	150	153
Total	74	153	227

Analytical sensitivity	96%	95% CI (88.6-99.2%)
Analytical specificity	98%	95% CI (94.4-99.6%)
Agreement	97.4%	

14.4 Cross reactivity and Interference substance effect

The Exdia iFOB test is specific to human hemoglobin. The following substances, when spiked in both positive and negative specimens, did not cross react with the test results and no cross reactivity was found.

Cross reactivity

Cross reacting substance	Concentration
Bovine Hb	1mg/mL
Porcine Hb	0.125mg/mL

Potentially interfering substances were spiked into extraction buffer containing negative and low positive hemoglobin, 125ng/mL. The substances at the following level did not interfere with the performance of the Exdia iFOB .

Interference

Ascorbic acid	1mg/mL
Chloride	0.5mg/mL
Cucumber	Aqueous extract
Cabbage	Aqueous extract
Lettuce	Aqueous extract
Sesame	Aqueous extract
Chili	Aqueous extract

15. REFERENCES

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