MEDS Clinical Medicine (2025)
Clausius Scientific Press, Canada

DOI: 10.23977/medsc.2025.060501
ISSN 2616-1907 Vol. 6 Num. 5

Distinctive Integrated Design and Clinical Utility of the Transferrin/FOB and Hb-Hp Combo Rapid Test in Gastrointestinal Bleeding Diagnosis

Zhang Lei[1,*], Yang Feng[2], Zhu Junzhe[3]

[1]*Zhejiang Gongshang University, Hangzhou, Zhejiang, 310018, China*
[2]*Community Health Service Center, Hangzhou, Zhejiang, 310000, China*
[3]*Wenzhou Medical University, Wenzhou, Zhejiang, 310000, China*

Keywords: Transferrin; Fecal Occult Blood (FOB); Hemoglobin-Haptoglobin (Hb-Hp) Complex; Gastrointestinal Bleeding; Rapid Immunoassay; Colorectal Cancer Screening

Abstract: Gastrointestinal bleeding, a critical indicator of various disorders including colorectal cancer, upper gastrointestinal lesions and inflammatory bowel disease, requires timely and accurate detection for effective clinical management. The Transferrin/FOB and Hb-Hp Combo Rapid Test (Feces) is a chromatographic immunoassay designed for the qualitative simultaneous detection of transferrin, fecal occult blood (FOB, based on human hemoglobin) and hemoglobin-haptoglobin (Hb-Hp) complex in fecal specimens. This study aimed to systematically evaluate the clinical performance of this combo test, including its relative sensitivity, specificity, accuracy, precision and cross-reactivity, by comparing it with a commercially available reference rapid test. A total of 730 clinical specimens (240 for transferrin, 300 for FOB and 190 for Hb-Hp) were collected and tested in parallel using both the combo test and the reference test. The results showed that the combo test achieved high relative sensitivity (99.1% for transferrin, 98.3% for FOB, 97.9% for Hb-Hp), specificity (98.5% for transferrin, 99.4% for FOB, 98.6% for Hb-Hp) and accuracy (98.8% for transferrin, 99.0% for FOB, 98.4% for Hb-Hp), with narrow 95% confidence intervals indicating robust reliability. Intra-assay and inter-assay precision studies demonstrated >99% correct identification rates for all three analytes. No cross-reactivity was observed with common interfering substances. These findings confirm that the Transferrin/FOB and Hb-Hp Combo Rapid Test is a reliable, sensitive and specific tool for the detection of gastrointestinal bleeding, suitable for clinical application in screening and diagnostic evaluation of related disorders.

1. Introduction

Gastrointestinal bleeding serves as a critical clinical indicator for a range of disorders, from benign conditions such as hemorrhoids and peptic ulcers to life-threatening malignancies like colorectal cancer (CRC)[1]. CRC, in particular, is a major global health concern, affecting both men and women across all racial and ethnic groups, with a higher incidence in individuals aged 50 years or older[2]. For men, it ranks as the third most common cancer after prostate and lung cancers[3]; for

women, it is the third most common after breast and lung cancers. Fecal occult blood (FOB) has long been recognized as a key marker for gastrointestinal bleeding[4], but traditional FOB tests, which primarily detect human hemoglobin (Hb), have limitations: Hb from upper gastrointestinal sources (e.g., stomach, duodenum) is often degraded by intestinal enzymes, leading to loss of antigenicity and false-negative results[5]. This underscores the need for more stable markers to enhance detection accuracy.

Transferrin and hemoglobin-haptoglobin (Hb-Hp) complex have emerged as complementary markers to address these limitations. Transferrin, an iron-transporting glycoprotein, is more stable in feces than Hb, retaining its antigenicity even after passing through the upper gastrointestinal tract, making it a reliable indicator of upper gastrointestinal bleeding[6]. Meanwhile, the Hb-Hp complex, formed by the binding of Hb to haptoglobin in the bloodstream, exhibits greater stability in the digestive tract compared to free Hb, thereby improving the sensitivity of occult blood detection, including bleeding from the upper gastrointestinal tract[7]. Together, these three markers—transferrin, FOB (Hb) and Hb-Hp—cover both upper and lower gastrointestinal bleeding, providing a more comprehensive evaluation of bleeding etiology.

The Transferrin/FOB and Hb-Hp Combo Rapid Test (Feces) is designed to simultaneously detect these three markers using a rapid chromatographic immunoassay, aiming to overcome the limitations of single-marker tests. This study aims to systematically evaluate the clinical performance of this combo test, including its relative sensitivity, specificity, accuracy, precision and cross-reactivity, by comparing it with a commercially available reference rapid test. The goal is to validate its reliability as a non-invasive tool for the diagnosis of gastrointestinal bleeding, supporting timely clinical decision-making in the screening and management of related disorders.

2. Materials and Methods

2.1 Specimen Collection

A total of 730 fecal specimens were collected, these specimens were categorized based on reference test results as follows: 110 positive and 130 negative for transferrin (total 240); 120 positive and 180 negative for FOB (total 300); and 48 positive and 142 negative for Hb-Hp (total 190).

Specimen collection and handling adhered to strict protocols. Specimens were not collected during or within 3 days of menstruation, nor from individuals with bleeding hemorrhoids or hematuria. Patients were instructed to discontinue alcohol, aspirin and non-steroidal anti-inflammatory drugs (NSAIDs) at least 48 hours prior to collection to avoid interference from drug-induced gastrointestinal irritation. No dietary restrictions were imposed. Approximately 1-2 g of feces was collected in clean, dry containers. Specimens were tested within 6 hours of collection; if delayed, they were stored at 2-8 ℃ for up to 3 days.

2.2 Test Kit and Procedure

The Transferrin/FOB and Hb-Hp Combo Rapid Test (Feces) used in this study was provided by CITEST Diagnostics Inc. This test employs a chromatographic immunochromatographic principle: for transferrin and FOB detection, the membrane is precoated with anti-transferrin and anti-hemoglobin antibodies on respective test lines and colloidal gold particles conjugated with these antibodies react with analytes in the specimen to form visible lines if positive; for Hb-Hp detection, the membrane is precoated with anti-haptoglobin antibodies, while colloidal gold-conjugated anti-hemoglobin antibodies react with Hb-Hp complexes to form a visible line if positive. A control line (C) is included to confirm valid test performance. A commercially available

chromatographic immunoassay for the simultaneous detection of transferrin, FOB and Hb-Hp was used as the reference rapid test comparator, with details masked for commercial confidentiality.

All tests were performed by trained laboratory technicians following the kit's package insert. Firstly, Prior to use, the test kit and specimens were equilibrated to room temperature (15-30℃). Using a specimen collection applicator, approximately 50 mg of feces (equivalent to 1/4 of a pea) was collected from at least 3 different sites in the specimen to ensure representativeness. Secondly, The applicator was inserted into the test cup (containing dilution buffer), the cap was tightened and the cup was shaken vigorously for 10-15 seconds to mix the specimen and buffer, followed by a 2-minute standing period. Then after removing the plastic seal at the base of the test cup, the cup was placed on a level surface and the timer was started immediately after pressing the cup to initiate flow. Results were read at 5 minutes, with readings after 10 minutes considered invalid.

Results were interpreted as follows: Transferrin positivity was indicated by two colored lines in the transferrin/FOB window (control line [C] and transferrin test line [T1]); FOB positivity by two colored lines in the same window (C and FOB test line [T2]); concurrent positivity for both transferrin and FOB by three colored lines in this window (C, T1, and T2); and Hb-Hp positivity by two colored lines in the Hb-Hp window (C and Hb-Hp test line [T]). A negative result was characterized by a single control line (C) with no lines in the test regions. An invalid result was noted when no control line was visible, indicating a procedural error or insufficient specimen volume.

3. Results and Discussion

3.1 Results

3.1.1 Sensitivity Specificity and Accuracy

A total of 240 specimens (110 positive, 130 negative by the reference test) were evaluated for transferrin. The combo test results are summarized in Table 1.

Table 1: Clinical Results for Transferrin Detection

Method		Other Rapid Test		Total Result
Transferrin result of Transferrin/FOB/Hb-Hp Combo Rapid Test (Feces)	Results	Positive	Negative	
	Positive	109	2	111
	Negative	1	128	129
Total Result		110	130	240

Relative sensitivity: 99.1% (95%CI*: 95.6%~99.9%)

Relative specificity: 98.5% (95%CI*: 94.6%~99.8%)

Accuracy: 98.8% (95%CI*: 96.4%~99.7%)

For FOB detection, 300 specimens (120 positive, 180 negative by the reference test) were analyzed. The results are shown in Table 2.

Table 2: Clinical Results for FOB Detection.

Method		Other Rapid Test		Total Result
FOB result of Transferrin/FOB/Hb-Hp Combo Rapid Test (Feces)	Results	Positive	Negative	
	Positive	118	1	119
	Negative	2	179	181
Total Result		120	180	300

Relative sensitivity: 98.3% (95%CI*: 94.1% ~99.8%)

Relative specificity: 99.4% (95%CI*: 96.9%~99.9%)

Accuracy: 99.0% (95%CI*: 97.1% ~99.8%)

A total of 190 specimens (48 positive, 142 negative by the reference test) were tested for Hb-Hp. The results are presented in Table 3.

Table 3: Clinical Results for Hb-Hp Detection.

Method		Other Rapid Test		Total Result
Hb-Hp result of Transferrin/FOB/Hb-Hp Combo Rapid Test (Feces)	Results	Positive	Negative	
	Positive	47	2	49
	Negative	1	140	141
Total Result		48	142	190

Relative sensitivity: 97.9% (95%CI*: 88.9%~99.9%)

Relative specificity: 98.6% (95%CI*: 95.0%~99.8%)

Accuracy: 98.4% (95%CI*: 95.5%~99.7%)

3.1.2 Cross-reactivity and Interference

The cross-reactivity assessment demonstrated that the Transferrin/FOB and Hb-Hp Combo Rapid Test showed no false-positive results when tested against a panel of potential interfering substances, including bovine, chicken, pork, goat, horse, rabbit and turkey hemoglobin (each at 1 mg/mL), calprotectin (500 ng/mL) and lactoferrin (500 ng/mL). This confirms the test's high specificity for human transferrin, hemoglobin and Hb-Hp complex, with no interference from non-human hemoglobins or common fecal inflammatory proteins.

In terms of interference mitigation, the implementation of targeted pre-analytical controls—including excluding specimens from individuals with menstruation (within 3 days), bleeding hemorrhoids or hematuria, and discontinuing alcohol, aspirin and NSAIDs 48 hours prior to collection—effectively minimized the risk of false positives from non-pathological bleeding sources. Additionally, the absence of dietary restrictions required for the test indicates its insensitivity to common food components, further supporting the reliability of results in reflecting genuine gastrointestinal bleeding.

3.1.3 Precision

The precision of the Transferrin/FOB and Hb-Hp Combo Rapid Test was evaluated through both intra-assay and inter-assay studies. For intra-assay precision, 15 replicates of three positive specimens with different concentration levels were tested in a single run for each analyte. Specifically, for transferrin, specimens with concentrations of 40 ng/mL, 80 ng/mL and 1 μg/mL were used; for FOB, specimens with 50 ng/mL, 100 ng/mL, and 10 μg/mL were tested; and for Hb-Hp, specimens with 50 ng/mL, 200 ng/mL and 2 μg/mL were analyzed. In all cases, the correct identification rate of the specimens exceeded 99%.

For inter-assay precision, 15 independent assays were conducted on the same set of 12 specimens, which included low, medium and high concentration levels of transferrin (40 ng/mL, 80 ng/mL, 1 μg/mL), FOB (50 ng/mL, 100 ng/mL, 10 μg/mL) and Hb-Hp (50 ng/mL, 200 ng/mL, 2 μg/mL), as well as a standard sample. Three different lots of the combo test kit were used in these assays, and the correct identification rate of all specimens remained above 99% across all runs and lots. These results confirm the high reproducibility of the test, ensuring consistent performance within a single run and across different runs and kit lots.

3.2 Discussion

3.2.1 Performance Characteristics

The results of this study demonstrate that the Transferrin/FOB and Hb-Hp Combo Rapid Test (Feces) exhibits high sensitivity, specificity and accuracy for the detection of transferrin, FOB and Hb-Hp in fecal specimens. With relative sensitivities exceeding 97.9% and specificities exceeding 98.5% for all three analytes, the combo test aligns with or surpasses the performance of existing single-marker or dual-marker rapid tests.

For transferrin detection, the 99.1% sensitivity and 98.5% specificity indicate robust performance in identifying upper GI bleeding, where transferrin's stability is critical. This is particularly valuable given that traditional Hb-based FOB tests often fail to detect upper GI bleeding due to Hb degradation. The FOB component, with 98.3% sensitivity and 99.4% specificity, maintains strong performance for lower GI bleeding detection, consistent with established FOB assays.

The Hb-Hp component, with 97.9% sensitivity, enhances detection of both upper and lower GI bleeding by leveraging the stability of the Hb-Hp complex. This complements the transferrin and FOB markers, as Hb-Hp is resistant to digestive enzymes, making it a reliable indicator even in cases where free Hb is degraded.

3.2.2 Limitations

The Transferrin/FOB and Hb-Hp Combo Rapid Test (Feces) has several inherent limitations that should be considered in clinical application. First, as a qualitative assay, it only provides a binary result (positive or negative) and cannot quantify the concentration of transferrin, FOB or Hb-Hp complex in fecal specimens, which limits its ability to assess the severity of gastrointestinal bleeding.

Second, the test merely indicates the presence of human transferrin, hemoglobin and Hb-Hp complex, but cannot definitively identify the source of bleeding (e.g., distinguishing colorectal bleeding from upper gastrointestinal bleeding) or differentiate between benign and malignant causes of bleeding. Thus, test results must be interpreted in conjunction with other clinical information, such as patient symptoms, medical history and additional diagnostic tests.

Third, like all fecal-based assays, its performance is dependent on proper specimen collection and handling. Inadequate sampling (e.g., insufficient fecal material, non-representative collection from a single site) or failure to adhere to pre-collection protocols (e.g., not discontinuing NSAIDs) may lead to erroneous results.

Finally, if questionable results are obtained (e.g., inconsistent with clinical findings), further confirmation using alternative diagnostic methods is required to validate the outcome. These limitations highlight the need for cautious integration of the test into clinical decision-making rather than reliance on it as a standalone diagnostic tool.

3.2.3 Comparison with Other Diagnostic Methods

The Transferrin/FOB and Hb-Hp Combo Rapid Test (Feces) differs significantly from other diagnostic methods for gastrointestinal bleeding in its integrated design and clinical utility. Unlike single-marker rapid tests that target only one indicator (e.g., fecal occult blood [FOB] based on hemoglobin or transferrin alone), this combo test simultaneously detects three key markers—transferrin, FOB and hemoglobin-haptoglobin (Hb-Hp) complex—in a single assay. This eliminates the need for multiple separate tests, reducing the number of specimens required and streamlining the diagnostic workflow.

Traditional FOB tests, which focus solely on hemoglobin, often miss upper gastrointestinal bleeding because hemoglobin from the upper tract is degraded by intestinal enzymes, losing antigenicity. In contrast, the inclusion of transferrin (stable in feces and resistant to such degradation) and Hb-Hp complex (more stable in the digestive tract than free hemoglobin) allows the combo test to capture bleeding from both upper and lower gastrointestinal regions, a capability not typically offered by single-marker tests.

Moreover, unlike standalone transferrin or Hb-Hp tests that address only specific segments of the gastrointestinal tract, the combo test provides a comprehensive assessment of bleeding etiology in one step. This breadth of detection, combined with its rapid chromatographic immunoassay format, makes it more efficient for clinical settings where timely and holistic evaluation of gastrointestinal bleeding is critical.

4. Conclusion

The Transferrin/FOB and Hb-Hp Combo Rapid Test (Feces) is a reliable, sensitive and specific tool for the qualitative detection of GI bleeding. Its high performance across transferrin, FOB and Hb-Hp markers, combined with excellent precision and minimal cross-reactivity, makes it suitable for clinical application in screening and diagnostic evaluation of GI disorders. By simultaneously addressing upper and lower GI bleeding detection, the test fills a critical need in point-of-care diagnostics, enabling timely and informed clinical decisions. Future studies with larger, diverse cohorts (e.g., different age groups, geographic regions) are warranted to further validate its performance in real-world settings.

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