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Evaluation of Diagnostic Performance of the Transferrin/FOB Combo Rapid Test Cassette (Feces) for Gastrointestinal Bleeding

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Abstract: The Transferrin/FOB Combo Rapid Test Cassette (Feces) is a chromatographic immunoassay designed for the qualitative detection of human hemoglobin and transferrin in human fecal specimens. This study aims to evaluate the consistency between test results of Transferrin/FOB Combo rapid test produced by CITEST DIAGNOSTCS INC. and the contrast test device, and evaluate its clinical application ability through testing on certain quantity of representative specimens and through scientific and reasonable statistic analysis on the results. A total of 436 fecal specimens from both symptomatic and asymptomatic individuals undergoing endoscopic examination were tested. The reference method was a commercially available rapid test (W.H.P.M Inc. Transferrin/Hemoglobin FOB Combo Rapid Test). For FOB detection, the relative sensitivity was 97.9% (95%CI: 94.1%~99.6%), relative specificity was 99.7% (95%CI: 98.1%~99.9%), and accuracy was 99.1% (95%CI: 97.7%~99.8%), with a Kappa value of 0.98 indicating excellent consistency. For transferrin detection, the relative sensitivity was 98.9% (95%CI: 94.1%~99.9%), relative specificity was 99.4% (95%CI: 97.9%~99.9%) and accuracy was 99.3% (95%CI: 98.0%~99.9%), with a Kappa value of 0.98. The LOD was 50 ng/mL or 6 μg/g feces for FOB and 40 ng/mL or 4 μg/g feces for transferrin. Intra-assay and inter-assay precision were excellent (>99% correct identification). No cross-reactivity was observed with common interfering substances. These findings indicate that the Transferrin/FOB Combo Rapid Test Cassette is a reliable, rapid and practical tool for simultaneous detection of FOB and transferrin, aiding in the timely diagnosis of gastrointestinal bleeding, including upper and lower gastrointestinal disorders.

1. Introduction

Gastrointestinal (GI) bleeding, encompassing both upper and lower tract involvement, is a common clinical manifestation with diverse etiologies, ranging from benign conditions (e.g., hemorrhoids, peptic ulcers) to life-threatening diseases such as colorectal cancer (CRC)[1]. CRC remains a global health burden, ranking as the third most common cancer in both men and women

worldwide, with incidence peaks in individuals aged 50 years and older[2]. Early detection of GI bleeding is critical for timely intervention, as occult bleeding may be the only initial symptom of CRC or other serious disorders[3].

Traditional methods for detecting GI bleeding primarily rely on fecal occult blood tests (FOBTs), which target hemoglobin (Hb) in feces. However, Hb derived from upper GI sources is often degraded by intestinal enzymes, leading to loss of antigenicity and false-negative results[4]. This limitation underscores the need for complementary markers to improve diagnostic accuracy, especially for upper GI bleeding. Transferrin, a glycoprotein involved in iron transport, is more stable in feces than Hb, making it a valuable indicator of upper GI bleeding[5]. Combining the detection of Hb (for lower GI bleeding) and transferrin (for upper GI bleeding) in a single test could enhance the sensitivity and specificity of GI bleeding detection.

Rapid chromatographic immunoassays have emerged as a cornerstone of point-of-care diagnostics, offering advantages such as simplicity, rapid turnaround time (≤10 minutes) and minimal equipment requirements[6]. These attributes make them particularly useful in resource-limited settings or for immediate clinical decision-making. The Transferrin/FOB Combo Rapid Test Cassette (Feces) is a novel assay designed to simultaneously detect both Hb and transferrin in fecal specimens, potentially addressing the unmet need for a comprehensive, rapid diagnostic tool for GI bleeding.

Despite the potential of such combo tests, rigorous evaluation of their performance is essential before widespread clinical adoption. This study aims to assess the diagnostic performance of the Transferrin/FOB Combo Rapid Test Cassette by comparing it with a validated reference rapid test, focusing on sensitivity, specificity, accuracy, consistency, precision and cross-reactivity. The findings will contribute to understanding its utility in clinical practice, particularly in guiding endoscopic referrals and CRC screening.

2. Materials and Methods

2.1 Specimen Collection

A total of 436 fecal specimens were collected from individuals presenting for endoscopic examination at multiple clinical sites. The cohort included both symptomatic (e.g., abdominal pain, unexplained weight loss, anemia) and asymptomatic individuals, ensuring representation of diverse clinical scenarios. Specimens were collected in clean, dry containers and processed within 6 hours of collection to ensure stability. For delayed testing, specimens were stored at 2-8 ℃ for up to 3 days; long-term storage was at -20 ℃ for up to 6 months[7].

Exclusion criteria included specimens collected during or within 3 days of menstruation, from individuals with bleeding hemorrhoids or hematuria or from patients who had consumed excessive alcohol, aspirin or non-steroidal anti-inflammatory drugs (NSAIDs) within 48 hours of collection, as these factors may cause non-pathological occult bleeding[8].

2.2 Test Kits and Reference Method

The investigational device was the Transferrin/FOB Combo Rapid Test Cassette (Feces) from Citest Diagnostics Inc., each kit containing test cassettes, specimen collection tubes with extraction buffer and package inserts with detailed instructions. The reference method was a commercially available rapid test, which has been validated for clinical use and is based on the same immunochromatographic principle.

The Transferrin/FOB Combo Rapid Test Cassette operates on a chromatographic immunoassay format. Its test membrane is precoated with anti-hemoglobin monoclonal antibodies (FOB test line),

anti-transferrin monoclonal antibodies (transferrin test line) and a control line coated with goat anti-mouse IgG to verify procedural validity. During testing, fecal specimens are mixed with extraction buffer to release Hb and transferrin; the mixture is applied to the specimen well, migrating chromatographically by capillary action. Colloidal gold-conjugated anti-Hb and anti-transferrin antibodies in the conjugate pad bind to target antigens, forming complexes that react with precoated antibodies on the test lines to generate visible colored lines, while a colored line in the control region confirms proper specimen volume and membrane wicking.

Firtly, all tests were performed by trained laboratory personnel following the manufacturer's instructions. Prior to use, test cassettes, specimens and extraction buffer were equilibrated to room temperature (15-30 ℃). And for specimen processing, feces were sampled at 3 different sites using the provided applicator, immersed in extraction buffer and the tube was vigorously shaken to homogenize the mixture. Then the test cassette was removed from its sealed pouch and used within 1 hour; Lastly three drops (≈120 μL) of the extracted specimen were added to the specimen wells and a timer was started. Results were read at 5 minutes (no interpretations after 10 minutes to avoid evaporation-induced false results) with definitions as follows: Positive (FOB): two lines (control line [C] + FOB test line); Positive (transferrin): two lines (control line [C] + transferrin test line); Dual Positive: three lines (C + FOB + transferrin); Negative: only a control line (C); Invalid: no control line (indicating procedural error requiring retesting).

3. Results and Discussion

3.1 Results

3.1.1 Sensitivity and specificity

Of the 436 specimens, the reference method identified 146 FOB-positive and 290 FOB-negative specimens. The investigational test results are summarized in Tables 1 and 2.

Table 1: FOB Detection Results (Investigational Test vs. Reference Method).

Method		Other Rapid Test		Total Result
Rapid Test Cassette for FOB	Results	Positive	Positive	
	Positive	143	1	144
	Negative	3	289	292
Total Result		146	290	436

Relative sensitivity: 97.9% (95%CI*: 94.1%~99.6%);
Relative specificity: 99.7% (95%CI*: 98.1%~99.9%);
Accuracy: 99.1% (95%CI*: 97.7%~99.2%).
Kappa=0.98

Table 2: Transferrin Detection Results (Investigational Test vs. Reference Method).

Method		Other Rapid Test		Total Result
Rapid Test Cassette for Transferrin	Results	Positive	Positive	
	Positive	91	2	93
	Negative	1	342	343
Total Result		92	344	436

Relative sensitivity: 98.9% (95%CI*: 94.1%~99.9%);
Relative specificity: 99.4% (95%CI*: 97.9%~99.9%);
Accuracy: 99.3% (95%CI*: 98.0%~99.9%)
Kappa=0.98

3.1.2 Cross-reactivity and Interference

To evaluate the specificity of the Transferrin/FOB Combo Rapid Test Cassette (Feces) and reduce false positives caused by cross-reactivity with non-target substances, various potential interfering agents were tested, including non-human hemoglobins (bovine, chicken, pork, etc., at 1 mg/mL) and fecal proteins like calprotectin and lactoferrin (at 500 ng/mL). No cross-reactivity was observed, confirming the test specifically recognizes human hemoglobin and transferrin, unaffected by dietary or microbial non-human hemoglobins or other fecal proteins.

Additionally, pre-analytical interference factors were assessed: specimens from individuals during menstruation (or within 3 days), with bleeding hemorrhoids or with hematuria should be excluded (to avoid non-gastrointestinal blood introduction). Patients should discontinue alcohol, aspirin and NSAIDs 48 hours before sampling (to reduce non-pathological occult bleeding); no dietary restrictions are needed (no reactivity with common food components). Results show the test is highly resistant to both analytical and pre-analytical interference when standard collection protocols are followed.

3.1.3 Precision

The precision of the Transferrin/FOB Combo Rapid Test Cassette (Feces) was evaluated via intra-assay and inter-assay analyses.

Intra-assay precision (within-run) was tested using 15 replicates of three FOB-positive specimens (50 ng/mL, 100 ng/mL, 10 μg/mL) and three transferrin-positive specimens (40 ng/mL, 80 ng/mL, 1 μg/mL). All replicates were correctly identified (>99% accuracy).

Inter-assay precision (between-run and between-lot) involved 15 independent assays on 6 specimens (3 FOB, 3 transferrin standards) across three lots. Over 99% of specimens were correctly identified, confirming consistent performance.

3.2 Discussion

3.2.1 Performance Characteristics

The Transferrin/FOB Combo Rapid Test Cassette shows exceptional diagnostic performance, with relative sensitivity (>97.9%), specificity (>99.4%) and accuracy (>99.1%) comparable to or exceeding single-marker tests. Its Kappa value of 0.98 (far above 0.75) indicates near-perfect agreement with reference methods, supporting reliable clinical replacement. Few false negatives (3 FOB, 1 transferrin) avoid missed serious diagnoses, while minimal false positives (1 FOB, 2 transferrin) reduce unnecessary referrals.

Simultaneous FOB and transferrin detection overcomes traditional FOBTs' limitation of missing upper GI bleeding due to Hb degradation. Transferrin, stable in feces, marks upper GI sources (e.g., ulcers), while FOB indicates lower GI bleeding, aiding endoscopic prioritization (e.g., upper endoscopy vs. colonoscopy). Dual positivity may signal widespread pathology, requiring comprehensive evaluation.

The test’s low LOD (50 ng/mL FOB, 40 ng/mL transferrin) detects early bleeding, critical for CRC screening. High precision (>99%) and no cross-reactivity confirm reliability. With 5-minute results and simplicity, it suits point-of-care settings, unlike time-consuming lab tests needing trained personnel.

3.2.2 Limitations

Despite its strengths, the test has limitations. Its qualitative nature means it only provides

presence/absence results rather than quantitative data on bleeding severity, which may be necessary for monitoring disease progression or treatment response. Additionally, it is dependent on specimen quality: inadequate collection (e.g., sampling only one site) or improper storage can affect results, making strict adherence to collection protocols (sampling 3 sites and storing at 2 - 8°C for ≤3 days) essential.

Moreover, the test is not a standalone diagnostic tool. Like all screening tests, its results must be interpreted alongside clinical history and endoscopic findings. For instance, a negative result does not rule out colorectal cancer in high-risk individuals (e.g., those with a family history), who may still require colonoscopy.

3.2.3 Comparison with Other Diagnostic Methods

The Transferrin/FOB Combo Rapid Test stands out against other methods, per the clinical study and product documentation. Compared to single-marker rapid tests (only FOB or transferrin), it simultaneously detects both markers: FOB for lower gastrointestinal bleeding and transferrin (stable in feces) for upper bleeding, avoiding missed upper tract issues from hemoglobin degradation - a limitation of FOB-only tests.

Against the reference combo test (W.H.P.M Inc.), it matches performance with 97.9 - 98.9% sensitivity, 99.4 - 99.7% specificity and Kappa=0.98, confirming reliability as an alternative. Unlike chemical fecal occult blood tests (prone to dietary/medication interference), it uses specific antibodies, showing no cross-reactivity with non-human hemoglobins or fecal proteins, enhancing specificity. Its rapid 5-minute results further outperform slower alternatives.

4. Conclusion

The Transferrin/FOB Combo Rapid Test Cassette (Feces) is a highly reliable, rapid and user-friendly tool for the qualitative detection of fecal hemoglobin and transferrin. Boasting exceptional sensitivity, specificity and consistency with a validated reference method, along with low LOD, high precision and no cross-reactivity, it is well-suited for clinical use in diagnosing GI bleeding. By simultaneously detecting markers of upper and lower GI bleeding, the test enhances diagnostic accuracy, guides appropriate patient management, reduces missed diagnoses and unnecessary procedures, and its point-of-care applicability further supports utility in diverse healthcare settings.

Future studies should evaluate its performance in larger, diverse cohorts (e.g., different age groups, geographic regions) and compare it with advanced modalities (e.g., capsule endoscopy) to further validate its role in CRC screening and GI bleeding workups. Overall, this test cassette represents a significant advancement in GI bleeding diagnostics, offering a balance of speed, accuracy, and practicality that meets the needs of modern clinical practice.

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