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Performance Evaluation of H-FABP and Myoglobin/CK-MB/Cardiac Troponin I Combo Rapid Test Cassette (CMA-445) via Clinical Validation

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Abstract: The H-FABP and Myoglobin/CK-MB/Cardiac Troponin I Combo Rapid Test Cassette (Whole Blood/Serum/Plasma) is a chromatographic immunoassay designed for the qualitative detection of human heart-type fatty acid-binding protein (H-FABP), myoglobin (MYO), creatine kinase MB (CK-MB) and cardiac troponin I (cTnI) in whole blood, serum, or plasma, thereby aiding in the diagnosis of acute myocardial infarction (AMI); this study aimed to evaluate both the consistency between the test results of CITEST DIAGNOSTICS INC.'s combo rapid test and the reference device, as well as its clinical applicability, so it conducted tests on representative specimens, analyzed the results using scientific statistical methods, and adopted enzyme-linked immunosorbent assay (ELISA) as the reference method, specifically testing 281 H-FABP specimens, 514 myoglobin specimens, 887 cTnI specimens, and 797 CK-MB specimens, which showed the test had high sensitivity (89.9% for H-FABP, 98.7% for myoglobin, 98.1% for cTnI, 98.2% for CK-MB), specificity (91.0% for H-FABP, 97.2% for myoglobin, 98.5% for cTnI, 99.2% for CK-MB), and accuracy (90.7% for H-FABP, 97.5% for myoglobin, 98.4% for cTnI, 99.1% for CK-MB), with Kappa values ranging from 0.76 to 0.97 (indicating good to excellent consistency with the reference method) and additionally, the test exhibited no cross-reactivity with common interfering substances and remained stable under the recommended storage conditions (2-30 ℃), confirming it as a reliable, rapid, and practical tool for the simultaneous detection of key cardiac biomarkers and facilitating timely AMI assessment.

1. Introduction

Acute myocardial infarction (AMI), often referred to as a heart attack, is a life-threatening condition caused by abrupt occlusion of a coronary artery, leading to myocardial cell necrosis [1]. Prompt diagnosis and intervention are pivotal for reducing mortality and optimizing patient outcomes, as every minute of delayed treatment heightens the risk of irreversible cardiac damage [2].

The diagnosis of AMI generally depends on a combination of clinical manifestations, electrocardiographic signs of myocardial injury and the detection of cardiac biomarkers [3].

Cardiac biomarkers are crucial for the timely identification of AMI. Myoglobin (MYO), a 17.8 kDa heme-protein present in both skeletal and cardiac muscle, is quickly released into the bloodstream 2-4 hours after myocardial injury, peaks at 9-12 hours and returns to normal levels within 24-36 hours, thus serving as an early indicator of AMI[4]. However, its insufficient cardiac specificity - attributed to comparable concentrations in skeletal muscle - restricts its application as an independent marker[5]. Creatine kinase MB (CK-MB), an 87.0 kDa creatine kinase isoenzyme with greater cardiac specificity than MYO, is released 3-8 hours after infarction, reaches a peak at 9-30 hours and normalizes within 48-72 hours [6]; yet, its sensitivity during the early stage of AMI is inferior to that of newer biomarkers [7]. Cardiac troponin I (cTnI), a 22.5 kDa regulatory protein specific to cardiac muscle, is recognized as the gold standard for AMI diagnosis: it is released 4-6 hours after symptom onset and stays elevated for 6-10 days, providing an extended detection window characterized by high specificity and sensitivity[8,9]. Heart-type fatty acid-binding protein (H-FABP), a 15 kDa protein, is released within 2 hours of AMI onset and returns to baseline within 18-24 hours. With a concentration 20-fold higher in cardiac tissue compared to skeletal muscle, it constitutes a valuable early marker for evaluating acute cardiac injury and infarct size [10].

Conventional laboratory-based methods such as enzyme-linked immunosorbent assay (ELISA) offer high accuracy but demand specialized equipment, trained staff and prolonged turnaround times (often several hours), which may delay clinical decision-making[11]. In contrast, rapid chromatographic immunoassays facilitate point-of-care testing with results available within minutes. This study seeks to assess the performance of the H-FABP and Myoglobin/CK-MB/cTnI combo rapid test cassette (for whole blood/serum/plasma) - including its sensitivity, specificity, precision, cross-reactivity and stability - by comparing it with the reference ELISA method, so as to verify its clinical applicability as a rapid and reliable tool for AMI diagnosis.

2. Materials and Methods

2.1 Specimen Collection

A total of 2,479 clinical specimens from symptomatic and asymptomatic individuals were collected, including 281 for H-FABP (69 positive, 212 negative), 514 for myoglobin (78 positive, 436 negative), 887 for cTnI (267 positive, 620 negative) and 797 for CK-MB (114 positive, 683 negative), all confirmed by ELISA.

Specimens were whole blood (venipuncture/fingerstick), serum or plasma. Fingerstick blood was collected post-cleaning, with first drop wiped; venipuncture blood stored at 2-8 ℃ if tested within 2 days. Serum/plasma was separated within 1 hour, stored at 2-8°C (≤2 days) or -20 ℃ (long-term). All equilibrated to room temperature before testing; frozen ones thawed and mixed.

2.2 Test Kits and Procedures

The evaluated test device was the H-FABP and Myoglobin/CK-MB/cTnI Combo Rapid Test Cassette (Citest, CMA-445) , including test cassettes, droppers, buffer and inserts. The reference method was ELISA, using Getein Biotech kits for H-FABP, Myoglobin, cTnI and CK-MB , all stored and used per manufacturers' instructions.

Testing followed the combo kit insert: materials equilibrated to 15-30 ℃; cassettes placed on a level surface. For serum/plasma, venipuncture whole blood or fingerstick blood (50 μL), 2 drops (or 50 μL) of specimen plus 1 drop of buffer were added to the specimen area. Results were read at 10 minutes (invalid after 20 minutes). Positive (C+T lines), negative (only C line) and invalid (no C

line) results were defined accordingly.

3. Results and Discussion

3.1 Performance Characteristics

3.1.1 Sensitivity, Specificity, and Accuracy

The combo rapid test exhibited high sensitivity, specificity, and accuracy for all four biomarkers in comparison with the ELISA reference method. (Tables 1-4).

H-FABP Detection: Among 281 specimens, the combo test yielded 62 true positives, 193 true negatives, 19 false positives and 7 false negatives (Table 1). The relative sensitivity was 89.9% (95% CI: 80.2%~95.8%), specificity was 91.0% (95% CI: 86.4%~94.5%) and accuracy was 90.7% (95% CI: 86.7%~93.9%). The total conformity rate was 90.7%, with a Kappa value of 0.76, indicating good consistency with ELISA.

Table 1: H-FABP Detection Results (Combo Test vs. ELISA).

Method		ELISA		Total Result
H-FABP Rapid Test Cassette (Whole Blood/Serum/Plasma)	Results	Positive	Negative	
	Positive	62	19	81
	Negative	7	193	200
Total Result		69	212	281

Relative sensitivity: 89.9% (95%CI*: 80.2%~95.8%);
Relative specificity: 91.0% (95%CI*: 86.4%~94.5%);
Accuracy: 90.7% (95%CI*: 86.7%~93.9%).
Kappa=0.76

Myoglobin Detection: In 514 specimens, the combo test identified 77 true positives, 424 true negatives, 12 false positives and 1 false negative (Table 2). Relative sensitivity was 98.7% (95% CI: 93.1%~99.9%), specificity was 97.2% (95% CI: 95.2%~98.6%) and accuracy was 97.5% (95% CI: 95.7%~98.6%). The total conformity rate was 97.5%, with a Kappa value of 0.91, indicating excellent consistency.

Table 2: Myoglobin Detection Results (Combo Test vs. ELISA).

Method		ELISA		Total Result
Myoglobin Rapid Test Cassette (Whole Blood/Serum/Plasma)	Results	Positive	Negative	
	Positive	77	12	89
	Negative	1	424	425
Total Result		78	436	514

Relative sensitivity: 98.7% (95%CI*: 93.1%~99.9%);
Relative specificity: 97.2% (95%CI*: 95.2%~98.6%);
Accuracy: 97.5% (95%CI*: 95.7%~98.6%).
Kappa=0.91

cTnI Detection: Among 887 specimens, there were 262 true positives, 611 true negatives, 9 false positives and 5 false negatives (Table 3). Relative sensitivity was 98.1% (95% CI: 93.2%~98.2%), specificity was 98.5% (95% CI: 97.3%~99.3%), and accuracy was 98.4% (95% CI: 96.7%~98.7%). The total conformity rate was 98.4%, with a Kappa value of 0.96, reflecting near-perfect agreement with ELISA.

Table 3: cTnI Detection Results (Combo Test vs. ELISA).

Method		ELISA		Total Result
Cardiac Troponin I Rapid Test Cassette (Whole Blood/Serum/Plasma)	Results	Positive	Negative	
	Positive	262	9	271
	Negative	5	611	616
Total Result		267	620	887

Relative sensitivity: 98.1% (95%CI*: 93.2%~98.2%);
Relative specificity: 98.5% (95%CI*: 97.3%~99.3%);
Accuracy: 98.4% (95%CI*: 96.7%~98.7%)
Kappa=0.96

CK-MB Detection: In 797 specimens, the combo test showed 112 true positives, 678 true negatives, 5 false positives and 2 false negatives (Table 4). Relative sensitivity was 98.2% (95% CI: 98.2%~99.8%), specificity was 99.2% (95% CI: 98.3%~99.8%) and accuracy was 99.1% (95% CI: 98.2%~99.6%). The total conformity rate was 99.1%, with a Kappa value of 0.97, indicating excellent consistency.

Table 4: CK-MB Detection Results (Combo Test vs. ELISA).

Method		ELISA		Total Result
CK-MB Rapid Test Cassette (Whole Blood/Serum/Plasma)	Results	Positive	Negative	
	Positive	112	5	117
	Negative	2	678	680
Total Result		114	683	797

Relative sensitivity: 98.2% (95%CI*: 98.2%~99.8%);
Relative specificity: 99.2% (95%CI*: 98.3%~99.8%);
Accuracy: 99.1% (95%CI*: 98.2%~99.6%)
Kappa=0.97

The high sensitivity and specificity observed for myoglobin, cTnI and CK-MB are particularly notable, as these markers are critical for different stages of AMI. Myoglobin's early release (2-4 hours) and cTnI's prolonged elevation (6-10 days) make their combined detection valuable for both early diagnosis and late confirmation . The lower but still robust sensitivity for H-FABP (89.9%) is acceptable given its role as an early marker, where even moderate sensitivity can aid in ruling out AMI in the acute phase.

3.1.2 Cross-reactivity and Interference

The combo test showed no cross-reactivity with high concentrations of potentially interfering agents, encompassing skeletal troponin I (10,000 ng/mL), troponin T (2,000 ng/mL), cardiac myosin (20,000 ng/mL), CK-MM (1,800 ng/mL), CK-BB (1,200 ng/mL), and a range of infectious disease markers (e.g., HBsAg, HBsAb, HBeAg, HBeAb, HBcAb, syphilis antibodies, anti-HIV, anti-H. pylori, anti-CMV, anti-Rubella, anti-Toxoplasmosis). Besides, common substances like acetaminophen (20 mg/dL), bilirubin (1,000 mg/dL), ascorbic acid (20 mg/dL), cholesterol (800 mg/dL), and hemoglobin (1,000 mg/dL) failed to interfere with the test outcomes at the tested concentrations. This lack of cross-reactivity and interference guarantees the specificity of test results for the target biomarkers, lowering the risk of false positive or negative results caused by confounding factors - a key feature for the accurate diagnosis of acute myocardial infarction (AMI), as misdiagnosis can lead to improper treatment or missed intervention opportunities.

3.1.3 Precision

For intra-assay precision, 15 replicates of each specimen (spanning the detection range) for all four biomarkers showed a correct identification rate of >99%, indicating minimal within-run variability. For inter-assay precision, testing across 3 different lots of the combo test kit yielded a correct identification rate of >99% for all specimens, demonstrating consistent performance between batches. These results confirm that the combo test kit has good reliability, with minimal variation regardless of the run or lot. This is crucial for Point-of-Care Testing (POCT), as test consistency is critical for clinical decision-making.

3.2 Discussion

3.2.1 Performance Characteristics

The test ensures operational safety through strict precautions: users must wear protective clothing (laboratory coats, disposable gloves, eye protection) when handling specimens, avoid eating/drinking/smoking in the testing area and dispose of used tests per local regulations. All specimens are treated as infectious, with adherence to microbiological hazard precautions to prevent contamination.

For effectiveness, it detects H-FABP (≥8ng/ml), Myoglobin (≥50ng/ml), CK-MB (≥5ng/ml) and cTnI (≥0.5ng/ml) with high sensitivity (89.9%-98.7%) and specificity (91.0%-99.2%) vs. ELISA. Kappa values (0.76-0.97) indicate good to excellent consistency. No cross-reactivity with skeletal troponin I, troponin T or infectious markers, and no interference from substances like bilirubin or hemoglobin. An internal control line confirms valid operation, ensuring reliable results.

3.2.2 Limitations

The test is qualitative and cannot determine the quantitative value or the rate of increase of H-FABP, Myoglobin, CK-MB or cardiac Troponin I. It cannot replace quantitative methods for tracking biomarker trends over time. But it compensates with rapid results (readable at 10 minutes), a critical advantage in acute myocardial infarction scenarios where timely decision-making directly impacts patient outcomes. Its high consistency with ELISA (Kappa values 0.76-0.97) further validates its reliability for qualitative screening.

The test fails to detect biomarkers below specific thresholds: H-FABP <8ng/ml, Myoglobin <50ng/ml, CK-MB <5ng/ml and cardiac Troponin I <0.5ng/ml. A negative result does not entirely rule out myocardial infarction in very early stages. But within clinically relevant concentration ranges (above these thresholds), it demonstrates strong sensitivity (89.9%-98.7%) and specificity (91.0%-99.2%), ensuring most cases of myocardial injury are correctly identified.

Whole blood samples with excessively high viscosity or stored for over 2 days may fail to perform properly, necessitating retesting with serum or plasma. In addition, samples with abnormally high titers of heterophile antibodies or rheumatoid factor can impact test outcomes. However, these problems are alleviated through clear procedural guidelines, and the test exhibits no cross-reactivity with common interfering substances (e.g., skeletal Troponin I, cardiac myosin, or infectious disease markers), thus guaranteeing stable performance in most clinical scenarios.

3.2.3 Comparison with Other Diagnostic Methods

Compared to single-marker rapid tests, the combo test reduces the need for multiple assays, saving time and resources. Its performance is comparable to other multi-marker immunoassays but offers the advantage of including H-FABP, an early marker with higher cardiac specificity than

myoglobin.

When compared to laboratory-based methods like ELISA, the combo test sacrifices quantitative capability but gains speed and accessibility—a trade-off that is justified in acute care settings where rapid diagnosis is paramount. The high Kappa values (0.76-0.97) indicate that the qualitative results align well with the reference standard, ensuring reliability.

4. Conclusion

The H-FABP and Myoglobin/CK-MB/Cardiac Troponin I Combo Rapid Test Cassette (Whole Blood/Serum/Plasma) exhibits high sensitivity, specificity and accuracy in the qualitative detection of four key cardiac biomarkers, with excellent concordance with the ELISA reference method (Kappa values: 0.76 - 0.97). Coupled with its precision, absence of cross-reactivity and stability under recommended storage conditions, these attributes underscore its reliability for clinical application.

Notably, the test's rapid turnaround time, versatility across specimen types and user-friendly operation position it as a valuable tool in point-of-care settings, enabling timely diagnosis of acute myocardial infarction (AMI) and facilitating early intervention. While it has inherent limitations - including its qualitative readout and potential for invalid results with suboptimal specimens - its advantages in accessibility and efficiency outweigh these drawbacks, particularly for initial screening and triage.

In conclusion, this combo rapid test emerges as a robust, practical addition to the armamentarium of cardiac diagnostics. When used in conjunction with clinical findings and electrocardiographic results, it facilitates timely and accurate assessment of myocardial infarction, thereby enhancing diagnostic efficacy in clinical practice.

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