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Performance Evaluation of CARBA-5 Rapid Test (Culture) OCAR-902 via Clinical Validation

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Abstract: This study performed comprehensive clinical performance verification of CARBA-5 Rapid Test (Culture, REF OCAR-902, Hangzhou AllTest Biotech Co., Ltd.), an in vitro diagnostic rapid chromatographic immunoassay for the qualitative detection of carbapenemase enzymes [KPC (K), OXA-48-like (O), IMP (I), VIM (V), NDM (N)] in bacterial colonies. A leading commercial CARBA-5 rapid test was adopted as the reference standard to evaluate diagnostic performance, with a total of 200 clinical culture specimens consisting of 50 carbapenemase-positive isolates and 150 carbapenemase-negative isolates collected from symptomatic and asymptomatic hospitalized patients. The investigational test achieved a relative sensitivity of 96.0% (95% CI: 86.3%–99.5%), relative specificity of 99.3% (95% CI: 96.3%–>99.9%), and overall diagnostic accuracy of 98.5% (95% CI: 95.7%–99.7%). Limit of detection (LoD) testing using purified recombinant carbapenemase proteins demonstrated ultra-low analytical thresholds: KPC 0.3 ng/mL, OXA-48 0.5 ng/mL, VIM 0.6 ng/mL, IMP 4.6 ng/mL, NDM 1.0 ng/mL. Intra-assay and inter-assay precision assessments confirmed >99% consistent correct identification for negative and carbapenemase-positive specimen panels across three independent production batches. The assay exhibits straightforward operation, stable storage tolerance (2-30℃ without freezing), and interpretable results within 10 minutes after sample loading, with readout validity limited within 20 minutes post-test initiation. This evaluation demonstrates that OCAR-902 CARBA-5 Rapid Test (Culture) delivers reliable, reproducible, and user-friendly point-of-laboratory detection of dominant carbapenemase variants, enabling rapid laboratory identification of carbapenemase-producing Enterobacterales and Pseudomonas aeruginosa to support timely hospital infection control and targeted antimicrobial stewardship.

1. Introduction

Carbapenem β-lactam antibiotics serve as the last-resort first-line agents for invasive infections caused by Enterobacterales and Pseudomonas aeruginosa, exerting broad-spectrum bactericidal effects against multidrug-resistant Gram-negative pathogens [1]. Nevertheless, decades of clinical overuse and inappropriate empirical administration have led to the global emergence and spread of carbapenemase-producing organisms (CPOs). These organisms produce hydrolytic β-lactamases

that inactivate all carbapenem antibiotics [2]. Given their high mortality rates, limited therapeutic options and frequent nosocomial outbreaks, carbapenem-resistant Enterobacterales (CRE) have been designated as critical priority pathogens by the World Health Organization for urgent global surveillance [3].

Before the prevalence of carbapenemases, clinical β-lactam resistance was mainly driven by extended-spectrum β-lactamases [1]. Since the early 2000s, five predominant carbapenemase families (KPC, IMP, VIM, NDM, and OXA-48-like) have become endemic worldwide with distinct geographical distributions [4,5]. CPOs predominantly colonize hospitalized patients with indwelling devices, prolonged antibiotic exposure or long-term ICU stays, and represent the major cause of severe nosocomial bloodstream, urinary tract, respiratory and intra-abdominal infections [6]. Rapid and accurate carbapenemase detection is essential for implementing isolation measures, halting hospital transmission and guiding targeted anti-CPO treatment [7].

Traditional detection methods have inherent limitations. Phenotypic assays including mCIM and Carba NP test require overnight incubation with a 12-24 hour turnaround time and professional operational skills [8]. Molecular diagnostics such as real-time PCR and whole-genome sequencing offer high sensitivity but rely on costly equipment, specialized laboratories and trained personnel, restricting their routine use in resource-limited facilities [9]. Conventional multiplex PCR may produce false-positive results and fail to verify gene expression, while mass spectrometry demands high bacterial inoculum and expensive instruments [10].

Multiplex lateral flow immunochromatographic assays have emerged as superior rapid detection platforms, which directly detect expressed carbapenemase protein antigens from bacterial isolates without nucleic acid extraction or amplification. Featuring simple pretreatment, minimal operation, 10-15 minute visual results and good compatibility with routine culture workflows, they eliminate the reliance on high-end devices [11]. This study performed clinical validation of the novel CARBA-5 Rapid Test developed by Hangzhou AllTest Biotech Co.,Ltd, evaluating its limit of detection, intra- and inter-assay precision and clinical performance against commercial reference assays, so as to confirm its clinical value for routine CPO screening in microbiology laboratories.

2. Experimental Procedures

2.1 Clinical Specimen Source and Preparation

A total of 200 archived clinical bacterial culture isolates were enrolled for clinical performance evaluation, comprising 50 carbapenemase-positive isolates and 150 carbapenemase-negative isolates previously confirmed by a leading commercial CARBA-5 lateral flow assay as reference standard. Specimens were recovered from clinical cultures of symptomatic and asymptomatic hospitalized patients, covering major target pathogens including Klebsiella pneumoniae, Klebsiella oxytoca, Klebsiella aerogenes, Escherichia coli, Enterobacter cloacae complex, Serratia marcescens, and Pseudomonas aeruginosa.

All bacterial isolates were propagated following standardized microbiological culture protocols: Enterobacterales and Pseudomonas aeruginosa isolates were cultured on 5% sheep blood agar or MacConkey agar and incubated in ambient air for 16-24 hours, while Escherichia coli and KES complex isolates were cultured on CRE selective agar with incubation for 18–24 hours. Prior to sample pretreatment and testing, all test reagents, cultured isolates, buffers, and test cassettes were equilibrated to room temperature (15-30℃) for at least 30 minutes. All specimen handling procedures strictly complied with national biosafety regulations governing the transport and disposal of pathogenic microorganisms, and all solid agar colonies were freshly harvested to ensure optimal antigen yield.

2.2 Test Kits and Procedures

The investigational device adopted in this study was the CARBA-5 Rapid Test (Culture, Cat.No.: OCAR-902, Hangzhou AllTest Biotech Co., Ltd.), which was composed of supporting test cassettes and matched assay buffer. A commercially available mainstream multiplex CARBA-5 lateral flow assay, capable of synchronous detection of KPC, OXA-48, VIM, IMP, and NDM carbapenemase subtypes, was applied as the gold-standard reference assay for all 200 enrolled clinical bacterial isolates. To ensure experimental consistency, parallel testing with the OCAR-902 investigational kit and the commercial reference assay was conducted for every specimen under identical ambient temperature conditions ranging from 15 ℃ to 30 ℃.

All testing operations were performed strictly according to the manufacturer's guidelines. Firstly, the test cassette was removed from the sealed pouch and used within 60 minutes on a clean and flat bench. Secondly, specimen pretreatment was conducted separately for liquid and solid cultures. For liquid bacterial cultures, the bacterial suspension was mixed with the assay buffer, vortexed thoroughly, and incubated statically for 10 minutes at room temperature. For solid agar colonies, pure bacterial colonies were collected and fully suspended in the buffer, followed by identical 10-minute static incubation. After incubation, the processed sample mixture was loaded into the test cassette. Results were read exactly 10 minutes after sample loading, while readings beyond 20 minutes were considered invalid. In addition, opened assay buffer should be used within six months to ensure stable detection performance.

Standardized and simplified criteria were applied for result interpretation. A positive result was confirmed by the presence of a valid control line plus any visible test band for KPC, OXA-48-like, VIM, IMP, or NDM. A negative result was defined as only a clear control line with no visible testlines. Tests without an effective control line were invalid and required retesting. For analytical performance evaluation, the limit of detection (LoD) of each carbapenemase subtype was determined using serially diluted recombinant proteins with 20 replicate tests. Assay precision included intra-assay and inter-assay reproducibility, which was assessed via repeated tests using identical specimen panels and different kit batches.

3. Performance Analysis

3.1 Clinical Diagnostic Performance Metrics

3.1.1 Cross-tabulation of Test versus Reference Assay Results

Table 1 summarizes concordance and discordance results between OCAR-902 CARBA-5 Rapid Test (Culture) and the commercial reference CARBA-5 assay across all 200 clinical isolates.

Table 1: Comparative results of OCAR-902 versus commercial reference CARBA-5 assay

Method		**Commercial test**		**Total Results**
CARBA-5 Rapid Test (Culture)	Results	Positive	Negative	
	Positive	48	1	49
	Negative	2	149	151
Total Results		50	150	200

Relative Sensitivity: 96.0% (95% CI: 86.3%-99.5%)

Relative Specificity: 99.3% (95% CI: 96.3%->99.9%)

Overall Diagnostic Accuracy: 98.5% (95% CI: 95.7%-99.7%)

The test showed reliable diagnostic performance with high sensitivity and specificity, confirming its validity for routine CPO screening. Only two false-negative and one false-positive result

occurred in 200 specimens. The false negatives came from isolates with barely detectable carbapenemase near the assay's LoD, while the false-positive weak OXA-48 band was caused by minor bacterial protein interference from mixed cultures. Its high sensitivity avoids missed CPO cases, and excellent specificity prevents unnecessary patient isolation to cut infection control costs.

3.1.2 Precision

Precision was evaluated through intra-assay and inter-assay studies.

Intra-assay reproducibility was evaluated via three parallel replicate tests on negative and single carbapenemase-positive specimen panels, achieving a classification accuracy of over 99% across all replicates. No inconsistent line profiles, missing control lines, or ambiguous faint lines were observed in the parallel tests, which verified the reliable repeatability of the immunochromatographic membrane and conjugated antibody particle formulation within a single test batch.

Inter-assay reproducibility was assessed through three independent testing runs using three different production lots of OCAR-902 test cassettes. All standardized specimen panels yielded more than 99% consistent and accurate detection results. No lot-to-lot variations including decreased band intensity, altered limit of detection, or elevated invalid test rates were identified. These findings confirmed stable manufacturing consistency and satisfactory shelf stability of antibody-coated test strips under standard storage conditions (2-30℃, sealed packaging without freezing).

3.2 Discussion

3.2.1 Overall Diagnostic Performance Advantages of OCAR-902

The OCAR-902 CARBA-5 Rapid Test (Culture) achieved 96.0% relative sensitivity and 99.3% relative specificity against a validated commercial reference assay, with an overall diagnostic accuracy of 98.5% across 200 clinical culture isolates, matching or exceeding performance benchmarks reported for comparable multiplex carbapenemase lateral flow assays in peer-reviewed clinical evaluations. The high clinical sensitivity is clinically critical for hospital infection control workflows: missed CPO carriers create unrecognized transmission reservoirs within wards, driving outbreaks of carbapenem-resistant Gram-negative infections with elevated mortality risk among immunocompromised, ICU and surgical patients. The near-perfect specificity minimizes false-positive results that would trigger unnecessary patient contact isolation, increased nursing workload, and inappropriate administration of expensive last-resort anti-CPO antibiotics.

Ultra-low analytical LoD values for KPC, OXA-48, VIM and NDM enable detection of weakly carbapenemase-expressing isolates that produce borderline minimum inhibitory concentrations (MICs) to carbapenem antibiotics, which are frequently missed by phenotypic screening assays such as disk diffusion and single-substrate Carba NP tests. The precision study results confirm consistent test performance within single testing runs and across separate manufacturing batches, eliminating lot-to-lot variability concerns that complicate routine laboratory quality control. The assay's broad storage temperature window (2-30℃) removes cold-chain transportation requirements for regional laboratories and satellite clinic microbiology stations without refrigeration infrastructure, expanding real-world deployment flexibility.

Operationally, OCAR-902 delivers actionable results within 10 minutes of sample loading, a dramatic reduction in turnaround time relative to overnight phenotypic carbapenemase confirmation methods and multi-hour molecular PCR workflows. The protocol accommodates both solid agar colony suspensions and liquid bacterial cultures, integrating seamlessly into standard clinical

microbiology workflows without additional pre-processing steps like nucleic acid extraction or centrifugation enrichment. Visual band readouts require no specialized equipment or digital interpretation software, allowing accurate result classification by entry-level laboratory technicians after minimal training.

3.2.2 Assay Limitations

As a qualitative immunochromatographic protein detection platform, OCAR-902 carries inherent diagnostic limitations clearly outlined in the product insert. First, the test only confirms presence or absence of target carbapenemase antigens and does not provide quantitative measurement of enzyme concentration; test line intensity does not linearly correlate with bacterial load or carbapenemase expression levels, prohibiting semi-quantitative susceptibility prediction. Second, negative test results does not definitively rule out carbapenemase production in isolates with extremely low enzyme expression below the LoD threshold, as illustrated by the two false-negative isolates recovered in this clinical cohort. Third, the assay exclusively targets five major carbapenemase families (KPC, OXA-48-like, IMP, VIM, NDM) and does not detect rare carbapenemase variants including SME, IMI, NMC or other emerging metallo-β-lactamase subtypes, requiring supplementary molecular sequencing for epidemiological surveillance of uncommon resistance mechanisms.

Environmental and procedural variables may negatively impact test reliability: excessive ambient humidity, temperatures outside the 15-30℃ testing range, expired buffer solution, incomplete colony suspension, or insufficient incubation of sample-buffer mixture for 10 minutes can produce faint lines or invalid control lines. All test results must be interpreted alongside full clinical context, patient antimicrobial exposure history, carbapenem susceptibility MIC data, and hospital infection control surveillance data; equivocal or discordant results require confirmatory testing via molecular PCR or phenotypic mCIM assays per clinical laboratory standard operating procedures.

3.2.3 Comparative Analysis with Competing Diagnostic Modalities

Relative to phenotypic carbapenemase screening assays (Carba NP, mCIM), OCAR-902 drastically reduces testing turnaround time from 12-24 hours to 10 minutes, enabling same-shift identification of CPO isolates and immediate activation of infection control isolation protocols. Phenotypic assays rely on carbapenem hydrolysis color change and frequently fail to detect low-level carbapenemase producers, whereas OCAR-902’s antibody-antigen recognition directly targets enzyme protein molecules with superior analytical sensitivity.

Compared to molecular PCR platforms (Xpert Carba-R, multiplex carbapenemase PCR), OCAR-902 eliminates high capital equipment costs, certified molecular laboratory space requirements, and technical expertise for nucleic acid amplification and melt curve analysis. Unlike PCR, lateral flow antigen detection only identifies transcriptionally active carbapenemase protein production, avoiding false-positive signals from silent, non-expressed carbapenemase genes carried on mobile plasmids. However, molecular assays retain superior analytical sensitivity for direct clinical specimens (rectal swabs, blood cultures) without prior bacterial culture enrichment, making PCR the preferred modality for pre-culture surveillance screening, while OCAR-902 is optimized for post-culture isolate confirmation in routine microbiology labs.

Other commercial CARBA-5 lateral flow assays share similar multiplex detection capacity but often exhibit higher IMP LoD thresholds or narrower storage temperature ranges. OCAR-902’s broad 2-30℃ storage window and simplified colony harvesting protocol deliver logistical advantages for decentralized laboratory networks in resource-limited healthcare settings globally.

4. Conclusion

Validated against a commercial reference assay using 200 clinical Gram-negative bacterial isolates, the OCAR-902 CARBA-5 Rapid Test achieved robust diagnostic performance, with a relative sensitivity of 96.0% (95% CI: 86.3%–99.5%), specificity of 99.3% (95% CI: 96.3%–>99.9%), and overall accuracy of 98.5% (95% CI: 95.7%–99.7%). Ultra-low limits of detection support reliable identification of weakly expressed KPC, OXA-48, VIM and NDM carbapenemases, while intra-assay and inter-assay tests confirmed over 99% consistent classification across replicates and production batches. The assay allows flexible storage at 2-30 ℃ without freezing, adapts to both solid agar and liquid bacterial cultures, and provides visual qualitative results within 10 minutes, requiring no specialized laboratory equipment.

This multiplex immunochromatographic cassette offers a reliable, cost-effective and user-friendly approach for routine post-culture detection of the five major carbapenemase families in Enterobacterales and Pseudomonas aeruginosa. Incorporation of OCAR-902 into laboratory workflows accelerates CPO reporting, enables timely isolation strategies to reduce nosocomial transmission, and informs targeted anti-carbapenemase treatment. Combined with antimicrobial susceptibility testing, clinical epidemiological data and supplementary confirmatory assays for ambiguous results, OCAR-902 facilitates effective antimicrobial resistance surveillance and carbapenem-resistant infection management, helping alleviate the global public health threat posed by carbapenemase-producing Gram-negative pathogens.

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