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Clinical Performance Assessment of a Dual-Target Rapid Immunoassay: The Newlink One Step HIV 1.2 and HIV p24 Combo Test (Ref: L11-HIBG02C4) in a Multicenter Evaluation

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Abstract: This study evaluated the clinical performance of the Newlink One Step HIV 1.2 and HIV p24 Combo Test (Whole Blood/Serum/Plasma, REF: L11-HIBG02C4), a rapid, fourth-generation immunochromatographic assay designed for the simultaneous, qualitative detection of antibodies to HIV-1/2 and the HIV-1 p24 antigen. Using commercial enzyme-linked immunosorbent assay/chemiluminescent immunoassay (ELISA/CLIA) as the reference standard, the test was evaluated across a substantial cohort of clinical specimens. For HIV-1/2 antibody detection, 2023 specimens (406 positive, 1617 negative) were tested, yielding a relative sensitivity of 99.8% (95% CI: 98.6%-100%), a relative specificity of 99.7% (95% CI: 99.3%-99.9%), and an overall accuracy of 99.7% (95% CI: 99.4%-99.9%), with a Kappa statistic of 0.99 indicating almost perfect agreement. For HIV-1 p24 antigen detection, 1081 specimens (90 positive, 991 negative) were tested, yielding a relative sensitivity of 82.2% (95% CI: 72.7%-89.5%), a relative specificity of 98.8% (95% CI: 97.9%-99.4%), and an overall accuracy of 97.4% (95% CI: 96.3%-98.3%), with a Kappa statistic of 0.83 indicating substantial agreement. Subgroup analyses by gender and age showed consistent performance. The test demonstrated excellent precision (>99% correct identification in within-run and between-run studies) and no cross-reactivity with a panel of common interfering substances and infectious agents. In conclusion, the One Step HIV 1.2 and HIV p24 Combo Test is a highly accurate, reliable, and rapid point-of-care diagnostic tool, particularly for HIV-1/2 antibody detection, while also providing valuable early detection capability for HIV-1 infection through p24 antigen detection, making it suitable for diverse clinical and screening settings.

1. Introduction

Human Immunodeficiency Virus (HIV) remains a critical global public health challenge, with an

estimated 39 million individuals living with HIV by the end of 2022 [1]. Timely and accurate diagnosis is fundamental to initiating effective treatment, preventing transmission, and reducing HIV-associated morbidity and mortality [2]. Fourth-generation assays, which enable simultaneous detection of HIV-1/2 antibodies and HIV-1 p24 antigen, represent a major diagnostic advance by significantly shortening the window period to as early as 2–3 weeks post-exposure compared to antibody-only tests [3,4].

The standard laboratory diagnostic approach typically includes immunoassays such as enzyme-linked immunosorbent assay or chemiluminescent immunoassay (ELISA/CLIA), followed by confirmatory testing with Western blot or nucleic acid tests (NATs) [5]. While highly accurate, these methods require sophisticated laboratory infrastructure and trained personnel, often resulting in prolonged turnaround times that limit their feasibility in resource-limited settings, emergency departments, or community-based screening programs [6]. In contrast, rapid diagnostic tests (RDTs), particularly those based on immunochromatography, can deliver results within 10–30 minutes and are increasingly used for point-of-care testing to expand access to timely diagnosis [7]. Nevertheless, rigorous validation against established reference methods remains essential to ensure the reliability of such RDTs, especially fourth-generation combo assays.

The Newlink One Step HIV 1.2 and HIV p24 Combo Test (Whole Blood/Serum/Plasma) is a single-use, rapid chromatographic immunoassay designed for the qualitative detection of HIV- type 1 antibody, HIV type 2 antibody, and HIV type 1 p24 antigen from whole blood, serum, or plasma. This study aims to comprehensively evaluate its clinical performance, including sensitivity, specificity, accuracy, precision and analytical specificity, using ELISA/CLIA as the reference standard, thereby assessing its suitability as a reliable screening tool in various healthcare environments.

2. Experimental Procedures

2.1 Study Design and Specimen Description

This clinical evaluation was conducted as a multicenter study across three distinct clinical sites: the Dermatology Hospital of Guangxi Zhuang Autonomous Region (China), Bethzatha Advanced Medical Laboratory (Ethiopia), and Wuhan Jinyintan Hospital (China). Specimens were obtained from a population of symptomatic and asymptomatic individuals and were evaluated in a parallel-testing manner, whereby each specimen was tested simultaneously with the investigational One Step HIV 1.2 and HIV p24 Combo Test and the reference standard method.

Two separate analytical panels were constituted based on the target analyte. For the evaluation of HIV-1/2 antibody detection, a panel of 2023 specimens was used, consisting of 406 specimens previously confirmed as positive and 1617 specimens confirmed as negative by the reference ELISA/CLIA methods. For the evaluation of HIV-1 p24 antigen detection, a panel of 1081 specimens was used, comprising 90 positive and 991 negative specimens as confirmed by the same reference methods. Specimen handling strictly adhered to the test's intended use and manufacturer's instructions, utilizing human whole blood (from venipuncture or fingerstick), serum, or plasma. Key handling steps included prompt separation of serum/plasma to avoid hemolysis, storage of venipuncture whole blood at 2-8 ℃ if tested within 2 days, immediate testing of fingerstick whole blood, and thorough mixing of thawed frozen specimens prior to analysis.

2.2 Test Kits and Procedures

The investigational device used was the One Step HIV 1.2 and HIV p24 Combo Test (Whole Blood/Serum/Plasma; REF: L11-HIBG02C4, from Newlink Biotech Co., Ltd). As the reference

standard, commercially available ELISA and CLIA assays were employed, with all procedures strictly adhering to the manufacturers' recommended protocols.

The procedure for the test was performed strictly according to the manufacturer's instructions. First, all test components and specimens were equilibrated to room temperature (15–30 ℃). Next, the test cassette was removed from its sealed pouch. Depending on the specimen type, the appropriate volume was applied: for serum/plasma, one drop (≈25 µL) was added to the specimen well (S), followed by one drop (≈40 µL) of assay buffer; for venipuncture whole blood, two drops (≈50 µL) were applied, followed by two drops (≈80 µL) of buffer; for fingerstick whole blood, approximately 50 µL was transferred using a capillary tube, followed by two drops (≈80 µL) of buffer. The timer was started immediately after buffer addition, and results were read at 10 minutes; readings after 20 minutes were not considered valid.

Interpretation followed these criteria: a positive result was indicated by the presence of both a colored control line (C) and a colored test line (T); a negative result was defined by the appearance of only the control line; if no control line appeared, the test was considered invalid and repeated with a new cassette. All procedures were carried out by trained personnel in accordance with standard biosafety practices.

3. Performance Analysis

3.1 Analysis of Performance Characteristics

3.1.1 Key Diagnostic Metrics

The performance of the One Step HIV 1.2 and HIV p24 Combo Test compared to the reference methods is summarized in the following contingency Tables 1 and 2.

Table 1: Clinical Performance for HIV type 1 and type 2 Antibody Detection vs. ELISA/CLIA

Method		ELISA/CLIA		Total Results
HIV 1.2 Test(Whole Blood /Serum/Plasma)	Results	Positive	Negative	
	Positive	405	5	410
	Negative	1	1612	1613
Total Results		406	1617	2023

Relative Sensitivity (Positive Percent Agreement): 99.8% (405/406; 95% CI: 98.6%-100%)
Relative Specificity (Negative Percent Agreement): 99.7% (1612/1617; 95% CI: 99.3%-99.9%)
Overall Accuracy: 99.7% ((405+1612)/2023; 95% CI: 99.4%-99.9%)
Kappa Statistic : 0.99

Table 2: Clinical Performance for HIV-1 p24 Antigen Detection vs. ELISA/CLIA

Method		ELISA/CLIA		Total Results
HIV p24 Antigen Test (Whole Blood/Serum/Plasma)	Results	Positive	Negative	
	Positive	74	12	86
	Negative	16	979	995
Total Results		90	991	1081

Sensitivity: 82.2% (74/90; 95% CI: 72.7%-89.5%)
Relative Specificity: 98.8% (979/991; 95% CI: 97.9%-99.4%)
Overall Accuracy: 97.4% ((74+979)/1081; 95% CI: 96.3%-98.3%)
Kappa Statistic : 0.83

Subgroup analyses were performed based on gender (Male/Female) and age (<15, 15-64, ≥65 years). For HIV-1/2 antibody detection, sensitivity remained high across all subgroups

(99.7%-100%), and specificity ranged from 99.6% to >99.9%. For p24 antigen detection, sensitivity varied between subgroups (77.4%-88.2%), while specificity remained consistently high (96.6%-99.2%).

3.1.2 Precision

Intra-Assay: Testing of 15 replicates each of four specimens (negative, low positive, medium positive, and high positive) yielded >99% correct identification for all samples.

Inter-Assay: Fifteen independent assays performed on the same four specimen panels using three different manufacturing lots over 10 days also demonstrated >99% correct identification, confirming excellent reproducibility and manufacturing consistency.

3.1.3 Cross-reactivity and Interfering Substances

Cross-reactivity: The test showed no cross-reactivity when tested with specimens positive for a broad panel of potentially interfering agents, including HAMA, HBsAg, HBsAb, HBeAg, HBcAb, HBeAb, anti-HCV, anti-Syphilis, Rheumatoid Factor (RF), anti-MONO, anti-H. Pylori, anti-Rubella (IgG/IgM), anti-CMV (IgG/IgM), and anti-Toxoplasma (IgG/IgM).

Interfering Substances: The addition of potentially interfering substances at high concentrations-Acetaminophen (20 mg/dL), Acetylsalicylic Acid (20 mg/dL), Ascorbic Acid (2 g/dL), Creatinine (200 mg/dL), Bilirubin (1 g/dL), and Gentisic Acid (20 mg/dL)-to HIV-negative and positive specimens did not produce any false-positive or false-negative results.

3.2 Discussion

3.2.1 Performance Characteristics

The One Step HIV 1.2 and HIV p24 Combo Test demonstrated outstanding performance for the detection of HIV-1/2 antibodies, with sensitivity and specificity exceeding 99.5%. This performance is on par with, and in some metrics superior to, other marketed fourth-generation rapid tests.[8, 9] The near-perfect Kappa value (0.99) indicates almost flawless agreement with the laboratory reference standard, supporting its high reliability for antibody screening.

The performance for HIV-1 p24 antigen detection, with a sensitivity of 82.2% and specificity of 98.8%, is consistent with the known performance characteristics of immunochromatographic p24 antigen tests, which generally have lower sensitivity compared to laboratory-based ELISA or NAT for p24.[10] This sensitivity is sufficient to provide a valuable advantage in detecting early, acute HIV-1 infection during the pre-seroconversion "window period," a critical time for transmission risk. The test's ability to use multiple specimen types, including simple fingerstick whole blood, greatly enhances its utility in diverse point-of-care and outreach settings where venous blood draw or centrifugation may not be feasible.

3.2.2 Limitations

As a qualitative screening assay, this test is not quantitative and is not intended as a standalone confirmatory test. Any reactive result must be followed by confirmatory testing as per national HIV testing algorithms. The sensitivity for p24 antigen, while adequate for early detection, is not as high as laboratory-based antigen tests or NAT, meaning some early infections may be missed. Adherence to the specified procedure, particularly specimen volume, buffer addition, and strict 10-minute reading window, which is critical for accurate results. The test's performance in pediatric populations or with novel HIV variants requires further study.

3.2.3 Comparison with Other Diagnostic Methods

Compared to the reference ELISA/CLIA, this rapid test offers the crucial advantages of speed (10 minutes vs. hours) and minimal infrastructure, making it ideal for decentralized testing. While NAT remains the most sensitive method for early infection (detecting viral RNA), its cost and complexity limit widespread use for screening. This fourth-generation rapid test effectively bridges the gap, offering earlier detection than third-generation antibody-only RDTs while maintaining operational simplicity, positioning it as a powerful tool for expanding HIV testing coverage and identifying acute infections.

4. Conclusion

The Newlink One Step HIV 1.2 and HIV p24 Combo Test (Whole Blood/Serum/Plasma, REF: L11-HIBG02C4) is a clinically validated, high-performance, fourth-generation rapid diagnostic test. It demonstrates excellent sensitivity and specificity for HIV-1/2 antibody detection and provides valuable, additional detection capability for HIV-1 p24 antigen to identify early infections. Its excellent precision, lack of cross-reactivity, and flexibility with multiple specimen types, including fingerstick blood, make it a robust, reliable, and practical tool for point-of-care HIV screening in a wide range of clinical and public health settings. Its use can facilitate earlier diagnosis, prompt linkage to care and prevention services, and contribute meaningfully to global HIV control efforts.

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