**Advancing HIV Diagnostics: Evaluating the Emerging Multisure HIV1/2 Rapid Confirmatory Test as an Alternative to the Traditional Gold Standard Assays**

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**Abstract**

**Background:** HIV remains a significant global health challenge, demanding routine testing for early detection. CLIA screening assay, particularly Architect HIV, followed by immunoblot confirmation assay such as INNO-LIA™, is the standard procedure. However, due to indeterminate results and limitations of immunoblot assays, the CDC recommended utilizing Geenius HIV1/2 assay for confirmatory testing. This study compares two advanced HIV1/2 rapid tests as efficient alternatives for HIV confirmation. **Method:** 224 Architect HIV positive and HIV negative samples were utilized. These included true positives (*n*=38; Architect positive & INNO-LIA™ positive), true negatives (*n*=139; Architect negative & INNO-LIA™ negative), false positives (*n*=20; Architect positive & INNO-LIA™ negative) and INNO-LIA™ indeterminate (*n*=27). Samples were screened with Architect HIV and confirmed by INNO-LIA™ and PCR. All samples were re-tested by Multisure HIV1/2 and Geenius HIV1/2. Assessment performed via performance evaluation metrics. **Results:** Both rapid tests showed 100% sensitivity and specificity compared to INNO-LIA™. For IND cases, Multisure HIV1/2 classified 81.5% as negative, while Geenius HIV1/2 classified 55.6%. Multisure had higher specificity (89.2%) and PPV (89.5%) than Geenius (82.9% and 84.6%) when compared to PCR. **Conclusion:** Multisure HIV1/2 is a reliable potential addition to the CDC algorithm as an alternative to immunoblot assays.

**Keywords:** HIV diagnosis, rapid confirmatory tests, Multisure HIV1/2, Geenius HIV1/2, indeterminate results

**Introduction**

Family retroviridae, genus lentivirus, species *Human immunodeficiency virus* (HIV) remains a major global public health threat and is one of the primary infections transmitted through blood transfusions (1). In 2023, 1.3 million new HIV infections were reported, with 39.9 million people living with the virus and 630,000 deaths attributed to AIDS-related illnesses (2). The rapid spread of HIV underscores the urgent need for heightened prevention strategies that are reliable and accessible diagnostic methods (3, 4). When such a demand is fulfilled, early detection, diagnosis and treatment prior to tragic individual and public health consequences is facilitated (5-7).

Historically, the third generation enzyme-linked immunosorbent assay (ELISA) was utilized as a primary screening method (8). Although ELISA remains valuable, it is less common due to the emergence of more sensitive and specific fourth generation assays like the Architect HIV Ag/Ab combo assay (8-10). More recently, Architect HIV has been adopted as a preferred screening method for its heightened.

HIV testing strategies vary drastically from one region to another depending on societal needs (11). Several testing approaches are present, including: routine testing, integrated testing, community-based testing, etc. (11). In Qatar, where this study was conducted, routine HIV testing is the primary detection strategy (12). In 2014, the U.S. Centers for Disease Control and Prevention (CDC) and the Association of Public Health Laboratories (APHL) updated their recommendations for HIV diagnosis, suggesting that specimens reactive on antigen/antibody (Ag/Ab) combination assays, like the Architect HIV, be followed by a differentiating antibody immunoassay, such as the FDA-approved Geenius HIV1/2 Supplemental Assay (13). This algorithm aims to replace the immuno-blot with more rapid and reliable differentiation, and is widely adopted in global HIV diagnostic guidelines, including in Qatar. The Medical Commission (MC) conducts medical tests for applicants immigrating into the country, including routine HIV screening. This process begins with preliminary screening using the Architect HIV Ag/Ab combo assay, followed by confirmatory testing with the INNO-LIA™ HIV I/II Score, a immunoblot assay. Subsequent testing, such as PCR (Polymerase Chain Reaction) is also performed for further validation (9, 12).

Despite advancements in screening methods, confirmatory testing continues to rely heavily INNO-LIA™, which has shown an inability to classify a significant proportion of samples as definitive positive or negative, resulting in indeterminate (IND) results(14-16). Additionally, INNO-LIA™ demonstrates reduced sensitivity to HIV-2, further complicating its reliability in certain cases (17).

The development of confirmatory rapid diagnostic tests (RDTs), such as Geenius HIV1/2,has transformed HIV diagnostics by offering fast, accurate, and scalable solutions that address many of the challenges associated with INNO-LIA™ and other immunoblots (18). Recent studies have focused on investigating such confirmatory rapid tests as a replacement for both screening and confirmatory assays (19, 20). It has been demonstrated previously that rapid tests can outperform immunoblot in several aspects (19), specifically when it comes to differentiating between HIV-1 and HIV-2 instances (14, 17, 19, 21).

The growing demand for confirmatory RDTs reflects the need for affordable and accessible diagnostic tools, especially in resource-limited settings (22-24). Immunoblot-based assays can cost up to three times more than rapid tests (22), making them impractical for widespread use in low-resource regions (25). Rapid tests provide same-day results and can be deployed in community-based settings, expanding access to diagnostics in limited healthcare infrastructure (26). This accessibility plays a critical role in preventing the spread of HIV by ensuring that undiagnosed individuals are identified and linked to care quickly (26).

This study aims to evaluate and compare the performance of two advanced confirmatory HIV1/2 rapid tests, CDC-recommended Bio-Rad Geenius HIV 1/2 Supplemental Assay and novel MP Diagnostics Multisure HIV 1/2 Confirmatory Test. It seeks to demonstrate their concordance with INNO-LIA™ and the potential of incorporating Multisure HIV1/2 confirmatory rapid test to the CDC HIV diagnostic algorithm. Additionally, this study investigates their ability to resolve the indeterminate (IND) results often seen with INNO-LIA™, illustrating more definitive outcomes. While Geenius HIV1/2 has been the focus of previous studies (17, 27-29), the performance of the Multisure HIV1/2 test remains underexplored in this context, representing a novel contribution in this study to literature (30).

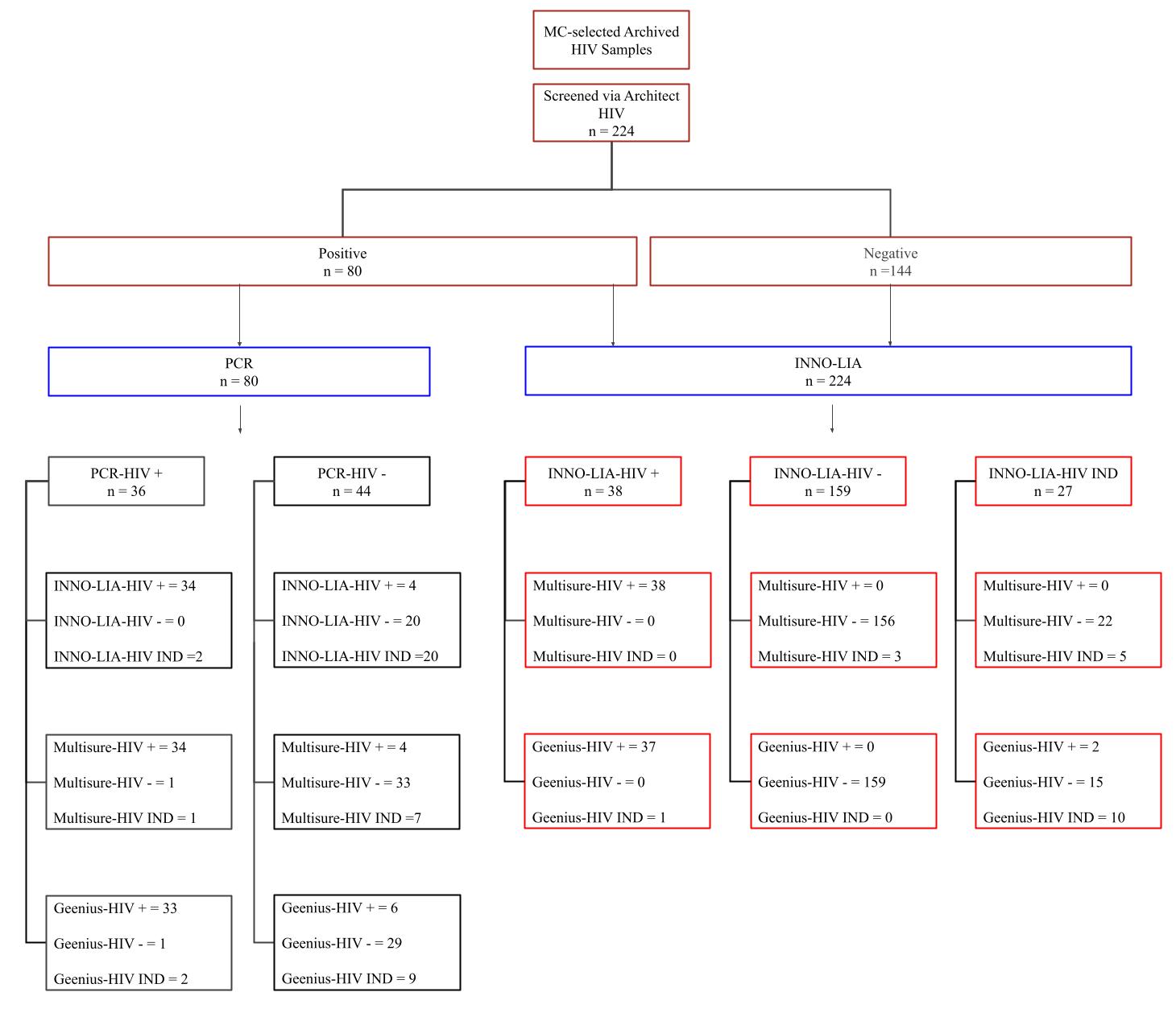
1. **Methodology**
2. **Ethical approval**

Previously collected samples from our previous studies were utilized (9, 12). This study was approved by IRB at Qatar University (QU-IRB 017/2024-E).

1. **Study population and study design:**

The Medical Commission (MC) is an institution that functions under the jurisdiction of the Ministry of Public Health (MOPH). As part of the recruitment procedure requirements in Qatar, the MC performs a number of medical tests, including routine screening for HIV1/2 among applicants immigrating into the country. The MC re-tests samples showing discrepancies (indeterminate) with fresh blood samples four weeks after for confirmation. In this study, archived HIV1/2 samples were provided by the MC under a uniquely generated test-result-dependent coding system. Such a system ensures applicant confidentiality and avoids disclosing their identity. The generated coding system relies on Architect HIV Ag/Ab combo assay screening and INNO-LIA™ results. Cases that are Architect HIV and INNO-LIA™ positive were assigned as PP, Architect HIV positive and INNO-LIA™ negative cases as PN, Architect HIV positive and INNO-LIA™ indeterminate cases as PI and samples that are negative in both as N. There was no patient recruitment nor was there any direct or indirect contact with any of the study’s subjects.

Out of the large dataset provided, samples suggesting agreement as well as potential discrepancies between Architect HIV and INNO-LIA™ were selected for a comprehensive comparison. The sample dataset comprised of true positives (*n* = 38; Architect HIV positive & INNO-LIA™ positive, PP), true negatives (*n* = 139; Architect HIV negative & INNO-LIA™ negative, N), false positives (*n* = 20; Architect HIV positive & INNO-LIA™ negative, PN) and INNO-LIA™ indeterminate (*n* = 27). Indeterminate samples consisted of 22 PI samples (Architect HIV positive & INNO-LIA™ indeterminate) and 5 samples negative by Architect HIV and indeterminate by INNO-LIA™.

In total, 224 samples were screened initially with the Architect HIV Ag/Ab combo assay. Confirmatory testing was performed using INNO-LIA™, which served as the gold standard reference, and PCR testing was conducted for Architect positive samples. All samples were subsequently tested using Multisure HIV1/2 and Geenius HIV1/2, as illustrated in Figure 1.

**Figure 1:** Flowchart outlining MC sample selection algorithm, depicting 224 archived samples screened by Architect re-tested via INNO-LIA™, Geenius HIV1/2, Multisure HIV1/2 and PCR. As part of MC standard procedure, only Architect-HIV positive samples were run by PCR.

1. **Abbott Architect HIV** **Ag/Ab Combo Assay**

The Architect HIV Ag/Ab Combo Assay (Abbott Diagnostics, Abbott Park, Illinois, USA) is a chemiluminescent test that detects both HIV p24 antigen and antibodies to HIV-1 (groups M and O) and HIV-2 in human serum and plasma (EDTA and heparin). It is designed to help diagnose HIV-1/HIV-2 infections, including acute HIV-1 infections. Initially, the sample is mixed with a wash buffer, assay diluent, and microparticles coated with HIV antigens and antibodies. Any HIV-1 p24 antigen and antibodies present in the sample bind to these microparticles (31). After washing, acridinium-labeled conjugates are added to the bound antigens and antibodies. A chemiluminescent reaction occurs, and the emitted light is measured in relative light units (RLU). The amount of HIV antigen and antibodies correlates with the detected RLU. By comparing this signal to a predetermined cutoff value, samples with a signal to cutoff (S/CO) value of 1.00 or higher are deemed reactive for HIV-1 p24 antigen or antibodies, while those with lower values are considered nonreactive.

According to MC laboratory protocols, any reactive sample undergoes retesting. If both tests return non-reactive results, the sample is considered non-reactive. However, if one or both tests are reactive, the sample is confirmed as reactive. All samples initially reactive from the Architect HIV assay were re-tested using Architect HIV analyzer, INNO-LIA™, and PCR using fresh blood.

1. **Fujirebio INNO-LIA™ HIV I/II Score**

The INNO-LIA™ is a Line immuno-assay (Innogenetics, Ghent, Belgium; now: Fujirebio Europe N.V.) used to confirm antibodies against HIV-1 (including group O) and HIV-2 in human serum or plasma as described previously (9, 12, 32). It is able to differentiate between HIV-1 and HIV-2 infections. The INNO-LIA™ uses a nylon strip coated with recombinant proteins and synthetic peptides from HIV-1 and HIV-2 to detect antibodies in human serum or plasma. It comprises of five HIV-1 antigens, including sgp120 and gp41, and two HIV-2 antigens gp36 and sgp105 (32). Control lines for various antibodies are also present. The test operates on the enzyme immunoassay principle: the sample is incubated with the antigen-coated strip, allowing any HIV antibodies present to bind. Then, a goat antihuman IgG labeled with alkaline phosphatase is added to bind to these complexes. A color change occurs when a substrate is added, indicating the amount of HIV antibodies. A darker color means more antibodies are present. If no antibodies are detected, only a low background color develops. All strips tested were examined and interpreted using automated LiRAS for Infectious Diseases software (32), specifically designed for the interpretation of LIA results, all in accordance with the manufacture instruction of the kit.

1. **Bio-Rad Geenius HIV1/2 Supplemental Assay**

The Geenius HIV1/2 Supplemental Assay (Hercules, California, United States) is a lateral flow test that uses a cassette with HIV-1 and HIV-2 recombinant antigens attached to a membrane. As the sample migrates along the test strip, specific anti-HIV antibodies are captured by immobilized recombinant antigens representing HIV-1 and HIV-2. A buffer is added to release conjugated colloidal gold protein A, which binds to the captured antibodies, making the antibody bands visible (33).

1. **MP Diagnostics** **Multisure HIV1/2 Confirmatory Test**

The Multisure HIV1/2 Confirmatory Test (2 Pioneer Place, Singapore) is a rapid qualitative test for detecting and differentiating antibodies of HIV-1 and HIV-2 in human serum or plasma. It features highly purified recombinant antigens (gp120, gp41, and p24 for HIV-1, and gp36 and gp105 for HIV-2) arranged in five test lines on a membrane. As the sample migrates upward from the sample well, antibodies form complexes with the immobilized antigens (34). These complexes are then detected by a goat anti-human IgG gold conjugate carried by a chase buffer, producing a pink-purplish color. An indicator/control line contains pyronin Y dye and protein A, which captures human IgG and binds with gold conjugate. The change of the control line from pink to purplish pink indicates proper sample addition and migration, as well as the presence of gold conjugate. In the analysis, the iPeak 4.3" Lateral Flow Reader was utilized for reading the results (35).

1. **Statistical Analysis**

Collected dataset comprised of categorical data, as a result, descriptive statistical analysis was performed. To successfully assess concordance of Multisure HIV1/2 and Geenius HIV1/2 against standard references, performance evaluation metrics were measured as previously described (36, 37). These metrics included: Sensitivity, Specificity, Positive Predictive Value *(PPV)*, Negative Predictive Value *(NPV)*, Overall Percent Agreement *(OPA)*, Positive Percent Agreement *(PPA)*, Negative Percent Agreement *(NPA)* and agreement coefficient, Cohen’s Kappa. Level of agreement values of 95% confidence interval (CI) are considered as follows: 𝜅 < 0 indicates no agreement, 𝜅= 0.00-0.20 indicates slight agreement, 𝜅= 0.21-0.40 indicates fair agreement, 𝜅= 0.41-0.60 indicates moderate agreement, 𝜅= 0.61-0.80 indicates substantial agreement and 𝜅= 0.81-1.00 indicates almost perfect agreement (38, 39). All statistical analyses were conducted using GraphPad Prism software, version 10.4.1 (San Diego, CA, US).

1. **Results**
2. **Multisure HIV1/2 and Geenius HIV1/2** **demonstrate excellent sensitivity and specificity with no false positives or false negatives compared to INNO-LIA**™ **confirmatory assay**

A total of 224 samples tested with INNO-LIA™ were used as the basis for assessing the performance of Multisure HIV1/2 and Geenius HIV1/2. Out of this sample set, 27 IND samples were excluded from statistical analysis. Multisure HIV1/2 identified 17% (38/224) of samples as true positive and 69.6% (156/224) as true negative. Notably, no false positives or false negatives were observed when comparing Multisure HIV1/2 to INNO-LIA™. Indeterminate results accounted for 3.5% (8/224) of the cases.

Similarly, when assessing Geenius HIV1/2, there were no false positives nor false negatives. It was found that 16.5% (37/224) were confirmed as true positive and 71% (159/224) were confirmed as true negative, while 5% (11/224) of cases presented as IND (Table 1).

Performance evaluation metrics were applied to assess the concordance of Multisure HIV1/2 and Geenius HIV1/2 relative to INNO-LIA™. Multisure HIV1/2 and Geenius HIV1/2 demonstrated 100% specificity and sensitivity in the detection of HIV1/2, indicating that both are able to recognize negative and positive cases accurately. This concordance is further supported via the 100% OPA, PPA, NPA, PPV and NPV, exhibiting the kits’ ability to successfully reflect the state of infection. Similarly, Cohen’s Kappa coefficient (𝜅 = 1.000) confirmed perfect agreement between INNO-LIA™ and both rapid tests (Table 2).

**Table 1:** A comparative summary of Multisure HIV1/2 and Geenius HIV1/2 Assays’ results relative to INNO-LIA™ and PCR.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Test | **Multisure HIV1/2** | | |  | **Geenius HIV1/2** | | |
| **INNO-LIA™** | **+** | - | INDᵟ |  | **+** | - | IND |
| HIV Positive (nᴔ=38) | **38**  (17%) | **0**  (0%) | **0**  (0%) |  | **37**  (16.5%) | **0**  (0%) | **1**  (0.5%) |
| HIV Negative (n=159) | **0**  (0%) | **156** (69.6%) | **3**  (1.3%) |  | **0**  (0%) | **159**  (71%) | **0**  (0%) |
| HIV IND (n=27) | **0**  (0%) | **22**  (9.2%) | **5**  (2.2%) |  | **2**  (0.9%) | **15**  (6.7%) | **10**  (4.5%) |
| **Total** | **224 (100%)** | | |  | **224 (100%)** | | |
| **PCR** | **+** | - | IND |  | **+** | - | IND |
| HIV Positive (n=36) | **34**  (42.5%) | **1**  (1.3%) | **1**  (1.3%) |  | **33**  (41.3%) | **1**  (1.3%) | **2**  (2.5%) |
| HIV Negative (n=44) | **4**  (5%) | **33**  (41.3%) | **7**  (8.8%) |  | **6**  (7.5%) | **29**  (36.3%) | **9**  (11.3%) |
| **Total** | **80 (100%)** | | |  | **80 (100%)** | | |

*ᴔ n: Number of samples*

*ᵟ IND: Indeterminate*

1. **Multisure HIV1/2 outperforms Geenius HIV1/2 against PCR in performance evaluation metrics**

A total of 80 PCR samples were utilized to assess Multisure HIV1/2 and Geenius HIV1/2. 8 and 11 IND samples were excluded during statistical analysis in Multisure HIV1/2 and Geenius HIV1/2, respectively. When evaluating Multisure HIV1/2, it demonstrated 42.5% (34/80) as true positives and 41.3% (33/80) as true negatives. The rapid test recorded a single false negative (1.3%) and 5% (4/80) as false positives. 10.1% (8/80) of the cases were defined as IND (Table 1).

Likewise, when evaluating Geenius HIV1/2, 41.3% (33/80) presented as true positives and 36.3% (29/80) as true negatives. Similar to Multisure HIV1/2, Geenius HIV1/2 also presented a single false negative (1.3%). 7.5% (6/80) of the cases were found to be false positives. Total IND samples made up 13.8% (11/80) (Table 1).

In light of these findings, performance evaluation metrics were considered. Both Multisure HIV1/2 and Geenius HIV1/2 showed high sensitivity at 97.1%. Specificity for Multisure HIV1/2 outperformed that of Geenius HIV1/2, where it was found to be 89.2% and 82.9%, respectively. This was also true regarding PPV, NPV and OPA, where Multisure HIV1/2 recorded 89.5%, 97.1% and 93.1%, respectively. On the other hand, Geenius HIV1/2 recorded 84.6% PPV, 96.7% NPV and 89.9% OPA. PPA for both tests were 97.1% and NPA was observed as 89.2% for Multisure HIV1/2 and 82.9% for Geenius HIV1/2. Moreover, Cohen’s Kappa was considered, where Multisure HIV1/2 was 0.861 and Geenius HIV1/2 was 0.797. Overall, the analysis shows that both rapid tests are able to identify individuals who do not have the disease and those who do to a high caliber. Additionally, it can be concluded from Cohen’s Kappa that Multisure HIV1/2 had almost perfect agreement while Geenius had substantial agreement.

**Table 2:** A comparative concordance assessment of Multisure HIV1/2 and Geenius HIV1/2 against gold standard INNO-LIA™ and PCR

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Reference | Test | **Sensitivity (%)** | **Specificity (%)** | **Positive Predictive value (%)** | **Negative Predictive value (%)** | **Overall Percent Agreement (%)** | **Positive Percent Agreement (%)** | **Negative Percent Agreement (%)** | **Cohen's Kappa Coefficient** |
| (CI:95%) | | | | | | | | | |
| **INNO-LIA™** | **Multisure HIV 1/2** | **100** | **100** | **100** | **100** | **100** | **100** | **100** | **1.000** |
| (90.8 - 100) | (97.7 – 100) | (90.8 - 100) | (97.7% - 100) | (100 - 100) | (100 - 100) | (100 - 100) | (1.000 - 1.000) |
| **Geenius HIV 1/2** | **100** | **100** | **100** | **100** | **100** | **100** | **100** | **1.000** |
| (90.6 - 100) | (97.7 - 100) | (90.6 - 100) | (97.7 - 100) | (100 - 100) | (100 - 100) | (100 - 100) | (1.000 - 1.000) |
| **PCR** | **Multisure HIV 1/2** | **97.1** | **89.2** | **89.5** | **97.1** | **93.1** | **97.1** | **89.2** | **0.861** |
| (85.5 - 99.9) | (75.3 - 95.7) | (75.9 - 95.8) | (85.1 - 99.9) | (85.8 - 97.9) | (86.5 - 99.3) | (74.8 - 97) | (0.744 - 0.978) |
| **Geenius HIV 1/2** | **97.1** | **82.9** | **84.6** | **96.7** | **89.9** | **97.1** | **82.9** | **0.797** |
| (85.1 - 99.9) | (67.3 - 91.9) | (70.3 -92.8) | (83.3 - 99.8) | (81.4 - 97.2) | (86.9 - 99) | (71.8 - 94) | (0.657 - 0.938) |

1. **Discussion**

It was observed in the results of this study that both Multisure HIV1/2 and Geenius HIV1/2 had excellent performance when compared to INNO-LIA™ and a high overall-concordance with PCR. While both rapid tests showed perfect results when compared to INNO-LIA™, having no false positives or false negatives, analysis of Multisure HIV1/2 and Geenius HIV1/2 against PCR showed slight discrepancies.

Due to the presence of a single false negative for both Multisure HIV1/2 and Geenius HIV1/2, identical almost perfect sensitivity and NPV are seen (97.1%). As for false positive results, Multisure HIV1/2 had less instances recorded than Geenius HIV1/2 (2:3), nonetheless, it had an effect on its performance. This is in regard to specificity and PPV, where it scored very good specificity and PPV (89.2% and 89.5%). As for Geenius HIV1/2, good specificity and PPV (82.9% and 84.6%) and very good OPA (89.9%) were observed. Substantial agreement was also seen (𝜅= 0.797). Despite discrepancies, Multisure HIV1/2 surpassed Geenius HIV1/2. This is evident in performance metrics due to a higher incidence of false positives in Geenius HIV1/2 (6/80). However, given the potential for PCR to yield false negative or false positive results, the observed discrepancies may reflect limitations in PCR accuracy rather than errors in the rapid assays. Previous studies have documented instances where PCR produced false negative results in patients during early or late stages of infection, potentially leading to misclassification of samples (40). Reasons for this misclassification include bias in amplification, primer mismatch, high viral load and primer dimer formation (40). This highlights the need for caution when interpreting discordant findings and emphasizes the importance of using multiple diagnostic tools in parallel to ensure accurate HIV detection.

Despite these discrepancies, both Multisure HIV1/2 and Geenius HIV1/2 demonstrated high overall percent agreement (OPA) and exhibited performance consistent with their role as robust alternatives to traditional immunoblot assays. The results suggest that rapid tests could provide reliable diagnostic outcomes, even in cases where confirmatory PCR results appear inconsistent.

Beyond the scope of performance analysis, another noteworthy observation was made in the raw data, which was excluded previously. Discrepancies observed in originally INNO-LIA™ IND samples shed light on valuable insights relating to strengths and limitations of each rapid test in comparison to INNO-LIA™. It is believed that conclusions drawn from IND samples’ analysis are the primary and key contribution of this paper.

For INNO-LIA™ IND cases *(n=27)*, 81.5% (22/27) of samples tested via Multisure HIV1/2 presented as negative whereas only 5/27 samples presented as IND, highlighting its ability to resolve 22/27 of the cases as definitive negative. On the other hand, Geenius HIV1/2 identified 37% of the cases as IND. The remaining 63% mainly consisted of negative samples (15/27) with the exception of 2 positive cases. When compared to PCR and Multisure HIV1/2, these two particular samples were seen as negative. Although it has been demonstrated previously that PCR unveils false positive outcomes in alternate HIV testing, literature also emphasizes the potential errors in PCR, necessitating further testing (41).

Collectively, it is concluded that both Multisure HIV1/2 and Geenius HIV1/2 are able to resolve IND cases present in INNO-LIA™ (9, 12, 17, 19, 42, 43). This has been previously demonstrated, where even rapid HIV tests utilized for screening were able to conclude definitive results when immunoblot assays failed to (42). Specifically, several studies have observed this in Geenius HIV1/2 as well (14, 17, 44). Multisure ---HIV1/2, however, has not been studied in this context previously, and so is a novel finding in this study. It is worth mentioning that Multisure HIV1/2 had higher efficacy in resolving IND cases than Geenius HIV1/2, classifying them predominantly as negative, adding on its reliability.

When analyzing INNO-LIA™ negative samples, three cases were identified where Multisure HIV1/2 classified the samples as IND, while Geenius HIV1/2 categorized them as negative. This highlights an important distinction between the two rapid tests. While Multisure HIV1/2 demonstrated greater efficacy in resolving INNO-LIA™ IND cases, Geenius HIV1/2 exhibited a higher sensitivity in accurately categorizing true negative samples. This suggests that Geenius HIV1/2 may be more reliable in ruling out HIV infection. Conversely, there was a case where both INNO-LIA™ and Multisure HIV1/2 identified a sample as positive, while Geenius HIV1/2 classified it as IND. This suggests that Multisure HIV1/2 may be more effective at accurately recognizing true positive cases compared to Geenius HIV1/2.

The findings of this study highlight the importance of assessing such advanced HIV rapid tests, potentially further enhancing and easing diagnostic procedures. Rapid tests were reliably used as a screening tool, not only in HIV diagnosis, but also in other infections such as Coronavirus (11, 13, 14). Numerous challenges are faced in testing strategies nowadays. Whether it was cost, accessibility, accuracy or reliability, advanced rapid tests are promising tool. This is especially when confirmatory immunoblots sought for reliable and robust diagnosis encounter similar challenges as those of ELISA (13). Limitations include high rate of false positivity, tendency to produce IND results and non-specific reactions (9, 10, 13-16). Non-definitive outcomes are strongly undesirable as they produce uncertainty in the diagnostic process as well as necessitates additional testing, not to mention stress-induced anxiety in patients (15). Additionally, as mentioned previously, one of immunoblots’ limitations was its misclassification of HIV-2 (17). It is seen in a newly conducted study that Geenius HIV1/2 surpasses immunoblots in this regard, where mentioned immunoblot categorized 17% of HIV-2 cases as a dual HIV-1/HIV-2 infection (45). On the other hand, Geenius HIV1/2 only misclassified 9% of the samples. It has been also suggested that Geenius HIV1/2 was able to outperform immunoblots in sensitivity, where Geenius HIV1/2 sensitivity had a range of 91% - 100%, while immunoblot assessed had a sensitivity of 83% (45).

The present study demonstrated the high sensitivity and specificity of the Multisure HIV1/2 and Geenius HIV1/2 compared to the gold standard INNO-LIA™. This finding, coupled with the ability to resolve indeterminate cases, particularly by the Multisure test, underscores their potential as reliable and efficient alternatives to traditional immunoblot assays. The comprehensive dataset, including a diverse range of samples, further strengthens the study’s validity and reliability. The practical implications of these findings are significant, as these rapid tests can be implemented in resource-limited settings, offering same-day results, and expanding access to HIV diagnostics. However, the study presents certain limitations. The relatively small sample size of indeterminate cases limits the generalizability of conclusions drawn from this specific subset. Furthermore, the occurrence of a single false negative result in both tests, although rare, highlights the potential for missed diagnoses, emphasizing the need for continued vigilance and quality control measures. Future studies can focus on investigating discrepancies in the threshold of such kits in determining IND as definitive positive or definitive negative.

While immunoblot -based assays are considered confirmatory assays for the detection of HIV1/2, the rise of advanced rapid tests with comparable results to that of immunoblot assays (46) paves the way for a more readily available, robust, and facilitated method for HIV detection.

1. **Conclusion**

In this study, a comparative evaluation of the performance of two advanced rapid HIV tests, Multisure HIV1/2 and Geenius HIV1/2, was done against the gold standard INNO-LIA™ and PCR, aiming to address limitations associated with immunoblot assays, particularly indeterminate results. Both rapid tests demonstrated excellent sensitivity and specificity, matching INNO-LIA™ with no false positives or negatives. Despite this, Multisure HIV1/2 consistently outperformed the CDC-recommended, FDA-approved Geenius HIV1/2.

Ultimately, integrating a rapid test like Multisure HIV1/2 into CDC HIV diagnostic protocols shows promise in enhancing diagnostic accuracy. It also could increase accessibility and efficiency, especially in under-resourced regions, while overcoming the challenges posed by indeterminate results in confirmatory immunoblots.

However, the study highlights the importance of careful interpretation of results, especially for samples with complex serological profiles. Further research is needed to understand the fate of indeterminate samples across different assays and to refine HIV testing algorithms.

**Conflict of Interest Disclosure:** All kits of the MP Diagnostics Multisure HIV1/2 used in this study were received from MP Biomedicals as in-kind support to Dr. Gheyath. However, it is important to note that MP Biomedicals had no influence or involvement in the study design, data collection, analysis, interpretation, or the decision to publish the results.

**Ethics Approval Statement:** Ethical approval exemption has been granted (QU-IRB 017/2024-E) from Qatar University.

**Data Availability Statement:** All data generated or analyzed during this study are included in this published article [and its supplementary information files].

**Funding:** Gheyath K. Nasrallah, Houssein Ayoub and Laith J. Abu-Raddad would like to acknowledge receiving funding QUCG-CAS-23/24-114 from Qatar University, UREP31-172-3-045 from Qatar Research, Development and Innovation (QRDI), member of Qatar Foundation, and NPRP13S-0128-200185 from Qatar National Research Fund (QNRF). Houssein Ayoub would like to acknowledge receiving fund ARG01-0524-230321 from QRDI. Nadine Younes would like to acknowledge receiving fund GSRA8-L-1-0501-21022 from QNRF. It is important to note that no funding party played any role in the design of study, collection of data, analysis, decision to publish or preparation of manuscript. All statements made in this report are solely the responsibility of the authors.

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