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Article

Improving Quality Assurance in Recent HIV-1 Infection Testing in Uganda: Insights from Proficiency Testing During 2020–2022

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Abstract

Background: To inform epidemic control, Rapid Test for Recent Infection (RTRI) assays, such as the Asanté HIV-1 Rapid Recency Assay (ARRA), have been developed to detect potential signals of increased HIV acquisition. However, ensuring the accuracy of these tests remains a challenge in resource-limited settings. While ARRA has been implemented for surveillance, there is a lack of documented experiences and lessons learned regarding quality assurance through Proficiency Testing (PT). This study examined Uganda's recent HIV infection PT program from 2020 through 2022 to highlight challenges and successes of implementation in resource-limited settings. Methods: We analyzed proficiency testing (PT) implementation for HIV recency testing in Uganda from 2020–2022. The study included biannual PT cycles (Cycle 1: Jan–Jun, Cycle 2: Jul–Dec) across 676 facilities in 133 districts. We assessed performance using pass rates (percentage of testers correctly identifying all samples in a PT panel) and response rates (proportion of testers submitting results within the stipulated timeframe out of the total number expected to participate). To evaluate sustainability, we longitudinally tracked a fixed cohort of the first 175 testers enrolled at the project's inception, representing diverse facility levels and cadres. **Results:** Analysis of six proficiency testing cycles from 2020-2022 revealed two key trends: a significant expansion in program participation and a concurrent longitudinal decline in performance among a consistent cohort. Overall, participation grew from 175 testers in Cycle 1, 2020, to 568 testers by Cycle 2, 2022. Among all participating facilities in each cycle, pass rates fluctuated, ranging from a high of 87.3% (Cycle 2, 2020) to a low of 54.9% (Cycle 2, 2021). A longitudinal analysis of the initial 175-testing-site cohort, however, revealed a significant inverse relationship between participation and performance. Non-response within this cohort increased drastically from 0% to 81.7% by early 2022 (p-value for trend <0.001). Among the diminishing subset of sites that continued to submit results, the pass rate showed a statistically significant declining trend, from 85.1% to 76.6% over the study period (p-value for trend = 0.012). **Conclusion:** This study identified three critical challenges: a steep rise in non-response, declining pass rates, and persistent performance gaps at lower-level health facilities. To address these gaps, we recommend individual tracking with digital feedback and targeted mentorship to re-engage staff and sustain competency at lower-level facilities.

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Introduction

The identification of recently acquired HIV infections can enhance epidemic control by highlighting potential signals of increased HIV acquisition. This has prompted the development of assays like rapid tests for recent infection (RTRIs), such as the Asanté Rapid Recency Assay (ARRA). These assays are used to establish recent infection surveillance, which informs public health interventions by detecting and characterizing recent infections among newly diagnosed cases [1]. Such surveillance, integrated into routine HIV testing services (HTS), paves the way for more targeted HIV prevention and control strategies. However, the utility of this data depends entirely on its accuracy and reliability, especially in countries like Uganda where new surveillance technologies are central to shaping epidemic response [2].

To ensure high-quality testing outcomes, External Quality Assessment (EQA) serves as a critical component of HIV diagnostic programs. Among various EQA methods, proficiency testing (PT) is widely used to assess laboratory performance by comparing test results with those from an external reference laboratory [3]. PT programs involve distributing blinded samples to testing sites, where they are analyzed, and results are sent back to the provider for evaluation and interlaboratory comparison [4]. This process helps laboratories monitor performance, identify errors, implement corrective actions, and enhance result accuracy. Recognizing its importance, the World Health Organization (WHO) recommends PT as a key component of comprehensive quality assurance (QA) programs to improve confidence in HIV testing programs [5].

In Uganda, the dried tube specimen (DTS)-based HIV PT program has been implemented to ensure accuracy and reliability across health facilities. Every three months, PT panels are distributed through the National Hub Transport system to trained HIV testing personnel, including non-laboratory professionals. Upon receipt, testers reconstitute and analyze the DTS panels following the national HIV testing algorithm, which includes rapid antibody assays [6]. Results are then reported to the Uganda Virus Research Institute (UVRI) for evaluation, and testers who fail to achieve 100% accuracy receive follow-up support from the National Reference Laboratory (NRL) technical team.

Uganda has expanded its PT framework under the existing DTS HIV PT program to include newer tests such as ARRA. Since 2019, this RTRI has been integrated into Uganda's HIV testing program for surveillance purposes [7]. This integration necessitated the establishment of an EQA framework to ensure the quality of recent HIV infection testing at implementing facilities.

Health facilities in Uganda operate within a defined tiered system, ranging from Health Centre Level II (HC II) to National Referral Hospitals [8]. This structure creates inherent variations in service capacity, staffing, and resources across facility levels, which can critically influence the implementation and quality of health programs such as PT.

The dynamic landscape of HIV testing in resource-limited settings, such as Uganda, necessitates adaptable and scalable PT programs. Successful QA programs should be tailored to the local infrastructure, with an emphasis on training, data management, and continuous monitoring [9].

This study explored PT trends in Uganda's recent HIV infection surveillance program involving RTRI testing at 676 facilities from 2020 to 2022. We focused on PT implementation and pass rates over time (2020 to 2022), and trends among the first 175 testers. This study also aimed to bridge the gap between the implementation process and the documentation of experiences and lessons learned related to the implementation of PT, specifically focusing on the context of recent HIV infection surveillance implementation in Uganda. This investigation serves to inform strategies for improving training programs and enhancing overall testing quality in recent HIV infection surveillance.

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Methods

Study Design and Setting

This study analyzed the implementation of a national proficiency testing (PT) program for recent HIV-1 testing in Uganda from 2020 to 2022. The program was scaled across all ten subregions of the country, involving health facilities at all levels, from Health Center II to National Referral Hospitals. The program started in March 2020 with 104 accredited facilities and 175 trained testers and was scaled to 676 facilities and 1349 testers by December 2022.–

Study Participants

We conducted two primary, complementary analyses:

(a) Program-wide analysis: We assessed the overall scale-up and performance across all 676 facilities and 1349 testing personnel that participated in the PT program between 2020 and 2022. A total of 2,442 PT panels were administered to these facilities over the six biannual cycles, forming the dataset for this analysis. For this analysis, a pass was defined as the correct identification of all five blinded samples within a PT panel (100% accuracy).

(b) Longitudinal cohort analysis: To track sustainability and individual tester competency, we followed a fixed cohort of the first 175 testers trained at the project's inception in October 2019. Participants were included based on active involvement and documented training completion. The cohort represented diverse cadres and facility levels: Health Center III (47.4%), Health Center IV (21.1%), General Hospital (10.3%), Regional Referral Hospital (6.3%), Health Center II (8.6%), Private Clinic (4.6%), and Special Clinic (1.7%). For this cohort, we defined attrition as the proportion of testers who failed to submit PT results in a given cycle. Tester competence was assessed using the same pass criterion (100% panel accuracy). This measure, alongside pass rates, was used to assess both workforce continuity and tester performance during the study period.

Procedures

Preparation of PT Panels

Samples were obtained from the Uganda Blood Bank. All samples underwent initial HIV screening tests to determine their HIV status according to the Ugandan national testing algorithm in effect at the time of the study. This algorithm utilized rapid tests (Determine HIV-1/2, HIV 1/2 STAT-PAK® Assay, SD BIOLINE HIV). To align with blood bank safety protocols, which require highly sensitive multiplex assays for mass screening, all samples were also tested with a fourth-generation ELISA (Enzyme-Linked Immunosorbent Assay), Genscreen ULTRA HIV Ag-Ab. This test was specifically used due to its ability to detect both HIV antibodies (Ab) and the p24 antigen (Ag), enabling earlier detection of acute infection compared to rapid tests or third-generation ELISAs. Samples that tested positive in initial screenings were subjected to confirmatory testing with another fourth-generation ELISA, the Murex HIV Ag/Ab combination. Finally, samples confirmed positive were tested to determine recency of infection using Asanté™ HIV-1 Rapid Recency Assay.

The National HIV Reference Laboratory (NRL) at UVRI prepared five blinded recency PT panels as dried tube specimens (DTS). The DTS preparation followed the standardized method described by Parekh et al. [10]. Briefly, well-characterized human plasma was mixed with a green dye to a final concentration of 0.1%. A volume of 20 µL of this mixture was aliquoted into 2 mL cryovials. The vials were left uncapped to dry overnight at ambient temperature. Once desiccated, the vials were capped and stored at 4°C, providing a stable and cost-effective material suitable for safe distribution at room temperature to testing sites. For testing, the DTS pellets were rehydrated with 200 µL of PBS-Tween buffer, mixed by tapping, and incubated overnight at room temperature. The following day, the contents were mixed again and used for HIV rapid testing according to the national algorithm.

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Characterization criteria for panel inclusion of specimens with known HIV serology status and recent or long-term was based on: (1) Only samples with strong 3+ bands on the confirmatory Murex HIV Ag/Ab were selected to ensure the accuracy of the panel, (2) Samples that yielded consistent results across multiple tests (e.g., 2/3 testers recording the same result for each sample).

The PT Materials' Packaging and Transportation

Each panel consisted of three characterized quality control (QC) samples (Long-term, Recent, and Negative) and five blinded PT samples (PT1-PT5). Panels were packaged with two vials of DTS dilution buffer, one 0.3 mL disposable Pasteur pipette, DTS rehydrating instructions, and a duplicate PT results form. These PT panels were distributed twice yearly (June and December)to all participating facilities via the Central Public Health Laboratories (CPHL) National Sample Transport Network [11]. A sample of the PT form can be found in *Appendix A* for reference (Figure A1).

PT Testing Procedure Using ARRA Kits

Upon receipt, testing personnel verified sample integrity by confirming the panel was complete and visually inspecting each vial for an intact green pellet, with no signs of damage or moisture. The DTS were reconstituted by adding 7 drops (approximately 200 µL) of the provided dilution buffer to each specimen tube, using the 0.3 mL disposable Pasteur pipette. The tubes were capped, tapped a few times to mix, and incubated at room temperature overnight. The complete dissolution of the pellet was a functional verification of sample integrity. The next day, the contents were tapped again to mix and tested using the ARRA kits according to the manufacturer's instructions and a standardized Asante RTRI job aid (*See Appendix B*).

Assay validity was confirmed by a built-in procedural control line. Its appearance indicated proper test function, while its absence rendered the test invalid, requiring a repeat, regardless of any other lines present on the strip. Testers were required to first run the internal QC samples. Testing and interpretation of the five blinded PT panel only proceeded after correct QC results were obtained, thereby verifying both test kit functionality and tester competency. Results were recorded on the result form, with one copy returned to UVRI within one week and a carbon copy filed at the facility.

PT Marking Scheme and Interpreting Scores

Test results were entered into databases, reviewed, and analyzed. The correct results for the three QC samples (Long-term, Recent, Negative) were known to program implementers and served as a prerequisite for a valid testing session. The five blinded PT samples were then evaluated. Each panel specimen identified correctly received 20 points, for a maximum of 100 points. Performance was evaluated based on the correct identification of all five PT samples. The results were categorized into several outcomes:

Pass: A pass was awarded when the tester correctly identified all five blinded samples (100% accuracy) and had correctly interpreted the three QC samples prior to testing.

Fail: A fail was assigned when the tester correctly interpreted the three QC samples (confirming kit performance and personal competency) but incorrectly identified one or more of the five blinded samples (less than 100% PT score).

Un-graded: An un-graded result was assigned in cases where the response form was submitted but lacked essential information, no final interpretation of the visual results was provided, expired kits were used, or one or more of the three internal QC samples were incorrectly identified, as this indicated a failure in the pre-testing validation process and rendered the subsequent blinded results uninterpretable.

To ensure results were not lost in transport, the program used a two-part tracking system: UVRI received distribution lists from hub coordinators to confirm delivery, and follow-up calls were made to testers who did not submit results.

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Data Analysis

All analyses were performed using the R programming language (v4.5.1). Given the observational and descriptive nature of this analysis, results are presented using descriptive statistics, specifically frequencies and proportions for categorical variables. The statistical significance of trends in binary outcomes - specifically, the pass rates and non-response rates over the six sequential testing cycles - was assessed using the Cochran-Armitage test for trend. A p-value of < 0.05 was considered statistically significant.

Results

Program-Wide Assessment

Between 2020 and 2022, a total of 2,442 proficiency testing (PT) panels were administered across 676 facilities over six biannual cycles. The volume of testing increased over time. The proportion of all panels administered in each specific cycle is shown in Table 1 (e.g., Cycle 1 2020 accounted for 7.2% [175/2,442] of all panels). The highest participation occurred in Cycle 2 of 2021 (n=581 panels, 23.8% of all panels) and Cycle 2 of 2022 (n=568 panels, 23.3% of all panels), as shown in Table 1.

Overall performance declined significantly after an initial peak. Pass rates were high in 2020, rising from 85.1% in Cycle 1 to 87.3% in Cycle 2, but fell sharply in 2021 and failed to recover to initial levels, reaching 73.9% by the end of 2022 (Table 1). In contrast, the rate of un-graded results remained low and stable throughout the study period.

Performance varied by facility level and geographical region. The percentage of testers achieving a 100% correct score (pass rate) was higher at specialized clinics and Regional Referral Hospitals (80.4% and 80.3%, respectively) than at Health Center IIIs (64.7%) and Health Center IIs (62.1%). At the regional level, pass rates ranged from a high of 85.9% in the north-eastern region to a low of 58.1% in the mid-northern region. The distribution of PT panels and overall performance by facility level and region is shown in Table 1.

Table 1. Distribution of overall proficiency testing performance of recency testers by cycle, facility level, and region.

	Pass	Fail	Un-graded
Year			
Cycle 1 2020 (*n=175)/7.2%*	149 (85.1%)	24 (13.7%)	2 (1.1%)
Cycle 2 2020 (*n=267)/10.9%*	233 (87.3%)	30 (11.2%)	4 (1.5%)
Cycle 1 2021 (*n=445)/18.2%*	295 (66.3%)	139 (31.2%)	11 (2.5%)
Cycle 2 2021 (*n=581)/23.8%*	319 (54.9%)	250 (43.0%)	12 (2.1%)
Cycle 1 2022 (*n=406)/16.6%*	245 (60.3%)	155 (38.2%)	6 (1.5%)
Cycle 2 2022 (*n=568)/23.3%*	420 (73.9%)	137 (24.1%)	11 (1.9%)
Facility Level			
HC II	82 (62.1%)	46 (34.8%)	4 (3.0%)
HC III	794 (64.7%)	407 (33.2%)	26 (2.1%)
HC IV	372 (71.8%)	135 (26.1%)	11 (2.1%)
Special Clinics*	41 (80.4%)	10 (19.6%)	
General Hospital	344 (70.1%)	101 (29.4%)	2 (0.6%)
RRH**	53 (80.3%)	13 (19.7%)	
Private Clinic	75 (76.5%)	21 (21.2%)	2 (2.0%)
Others	3 (50.0%)	2 (33.3%)	1 (16.7%)
Subregion			
Central 1	449 (76.0%)	134 (22.7%)	8 (1.4%)
Central 2	156 (63.7%)	85 (34.7%)	4 (1.6%)

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East Central	70 (60.9%)	41 (35.7%)	4 (3.5%)
Kampala	81 (75.7%)	24 (22.4%)	2 (1.9%)
Mid-Eastern	126 (65.3%)	56 (29.0%)	11 (5.7%)
Mid-Northern	118 (58.1%)	83 (40.9%)	2 (1.0%)
Mid-Western	302 (66.4%)	150 (33.0%)	3 (0.7%)
North-East	58 (65.9%)	26 (29.5%)	4 (4.5%)
South-Western	232 (66.5%)	109 (31.2%)	8 (2.3%)
West Nile	69 (71.9%)	27 (28.1%)	

** Regional Referral Hospital *Health facilities with specialized services provided, e.g., TASO (The Aids Support Organization) Sites offer HIV-related services only. **Note:** The percentage for each cycle indicates its proportion of the 2,442 total PT panels administered during the study period (2020-2022).

Longitudinal Assessment

We conducted a longitudinal analysis of a fixed cohort of 175 testers trained at the project's inception (Table 2). The data revealed two critical, statistically significant trends: a sharp increase in non-response rates and a decline in pass rates among the testers who continued to participate (Table 2).

The non-response rate - the proportion of the cohort failing to submit results - rose sharply from 0% at baseline to a peak of 81.7% (143/175) in Cycle 1 of 2022 (p-value <0.001). Concurrently, among the subset of testers who did submit results, the pass rate demonstrated a statistically significant declining trend, from 85.1% in Cycle 1, 2020, to 76.6% in Cycle 2, 2022 (p-value = 0.012) (Table 2).

Table 2. Recency Proficiency Testing Performance for the Cohort of Trained Testers.

	Pass	**Fail**	**Un-graded**	**Non-Response**
Year				
Cycle 1 2020 (*n=175*)	149 (85.1%)	24 (13.7%)	2 (1.1%)	-
Cycle 2 2020 (*n=92*)	82 (89.1%)	10 (10.9%)	-	83 (47.4%)
Cycle 1 2021 (*n=65*)	48 (73.8%)	16 (24.6%)	1 (1.5%)	110 (62.9%)
Cycle 2 2021 (*n=53*)	34 (64.2%)	17 (32.1%)	2 (3.8%)	112 (69.7%)
Cycle 1 2022 (*n=32*)	24 (75.0%)	8 (25.0%)	-	143 (81.7%)
Cycle 2 2022 (*n=47*)	36 (76.6%)	10 (21.3%)	1 (2.1%)	128 (73.1%)
p-value for trend (non-response)				**<0.001**
p-value for trend (Pass Rate)				**0.012**

p-values for trend were calculated using the Cochran-Armitage test. The n-value for each cycle represents the number of testers who submitted results. Non-response is calculated from the fixed cohort of 175 testers.

Discussion

The most critical finding of this study is the statistically significant decline in pass rates among a fixed cohort of recency testers from 2020 to 2022. This trend from an initial high of 85.1% to a final rate of 76.6%, with a low of 64.2%, signals a substantial erosion of tester competency that threatens the quality of Uganda's recent infection surveillance data. While the parallel and dramatic rise in non-response rates is a serious programmatic concern, the declining proficiency among those who do participate is the core quality assurance challenge revealed by this analysis.

Our findings align with established global patterns of quality deterioration in scaled-up HIV testing programs. Similar to Woldesenbet et al. [12] in South Africa and Nkrumah et al. [4] in Ghana, our study documents declining proficiency testing performance over time, a trend often linked to the transition from intensive centralized training to variable facility-level practices. More specifically, our results reflect documented systemic challenges within Uganda's own HIV testing landscape. According to Obeagu and Obeagu [13], laboratory infrastructural deficits, especially in rural areas, compromise testing performance and QA participation by facilities, while facility-level reports cite stock-outs, inventory issues, training, and workforce instability [14]. Furthermore, Gray et al.'s [15]

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study on rapid diagnostic test (RDT) accuracy in Uganda notes that interpretation errors, particularly with weak reactive bands, and deviations from testing algorithms are persistent risks, especially in decentralized settings with less supervision. These shared challenges—human resources, supply chains, supervision, and data systems—create a foundation upon which the unique complexities of recency testing (such as its use for surveillance and its multi-band interpretation) are layered. However, our longitudinal cohort analysis adds a crucial dimension to this understanding by quantifying a twofold sustainability challenge: massive workforce disengagement (81.7% non-response) concurrent with declining competence among those who remain. This indicates that for a complex, surveillance-focused test like the Asanté RTRI—which requires interpretation of three distinct bands (see assay procedure) and more intensive training to achieve competency—programs must combat both attrition and skill erosion simultaneously, a dual burden that may be more acute than for conventional HIV testing.

This decline can be understood as a transition from an intensive initial implementation phase to a challenging sustainability phase. The high pass rates in 2020 are likely attributable to the rigorous, structured training, certification, and heightened oversight from all stakeholders that characterized the program's launch.

As scaling occurred, several factors undermined this early success:

First, staff instability fundamentally compromised program continuity. We documented that among the initial 2019 cohort of 175 testers, only 27% (n=47) remained engaged by the end of 2022. In this study, staff turnover was not captured as a discrete variable. Instead, it was inferred indirectly from the steep rise in non-response within the fixed cohort, supplemented by supervisory reports and informal feedback from facilities. A key limitation is the absence of a formal system for individual-level tracking of tester deployment; we could not distinguish whether testers left government service or were transferred to non-participating facilities. This attrition critically undermined pass rates, as departing experienced staff were often replaced by new personnel who relied on informal on-the-job training rather than the original standardized curriculum.

Second, systemic barriers impeded consistent participation. Initially, addressing PT panels to facilities rather than individuals reduced accountability and contributed to low participation. In direct response, we shifted to sending panels directly to named testers, an intervention that improved accountability and response rates. Another significant limitation was the absence of an electronic tracking system for PT panel delivery and result return, making it difficult to distinguish between non-delivery and non-participation. Other documented barriers, such as delays in the hub transport system [16], commodity stockouts, and slow feedback loops, further hampered consistent participation and performance.

Third, persistent facility-level disparities highlight the need for targeted support. Lower-level facilities (HC IIs: 62.1%; HC IIIs: 64.7%) consistently underperformed higher-level facilities such as General Hospitals (70.1%), Health Center IVs (71.8%), Regional Referral Hospitals (80.3%), and specialized clinics (80.4%). This disparity likely reflects the conjunction of two challenges at these frontline facilities: their greater reliance on non-laboratory personnel, for whom a complex 3-band recency test presents a greater interpretive challenge, and potentially higher workloads or staffing shortages. This suggests solutions must address both training adequacy and staffing sufficiency at these sites.

Recommendations for HIV Recency PT Program Improvement

Based on the key findings of high attrition and declining competency, we recommend the following targeted interventions to strengthen the national HIV recency proficiency testing program:

Mitigate the impact of workforce attrition through formal tracking and accountability. Implement an electronic system to track individual tester deployment, transfers, and PT participation. Such a system would allow the program to identify staff movement early, prompt replacement training, and integrate recency PT performance metrics into national dashboards to hold districts and facilities accountable for maintaining a stable, tested workforce.

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Sustain competency with structured support for PT testers. Establish a program of structured, periodic mentorship and supportive supervision, prioritizing lower-level health centers (HCIIs and HCIIIs) and individual testers who fail PT panels. This should supplement the initial training to combat the erosion of skills over time.

Overcome systemic barriers with logistical reinforcements. Implement digital solutions for PT panel tracking, result submission, and feedback dissemination to create a more responsive and accountable system. Ensure PT panels are consistently addressed to named individuals rather than facilities.

Limitations

Several limitations should inform the interpretation of our findings. First, the longitudinal cohort was restricted to testers with documented pre-2020 training, which may have introduced selection bias by excluding later trainees whose performance patterns could differ. Second, the program lacked an electronic system for tracking individual tester movements and PT panel delivery, making it difficult to distinguish whether attrition reflected testers leaving government service, transfers to non-participating facilities, or logistical failures rather than true non-participation.

Third, we lacked quantitative data on key confounding variables such as individual tester experience and workload, specific supervision frequency, and exposure to refresher training, all of which may influence proficiency. External operational factors known to affect performance—including supply chain disruptions for test kits, staffing shortages, high clinical workloads, and disruptions from the COVID-19 pandemic—were not quantitatively assessed and could explain some outcome variability. The potential effect of environmental conditions on DTS stability during transport was also not measured. Finally, because the ARRA test interpretation is visual, the potential for inter-rater variability exists; however, no formal inter-rater reliability assessments were implemented to quantify this bias. It should also be noted that, while the need for supportive supervision is a key recommendation from our findings, it was not a structured component of the implemented program during the study period, and our results cannot speak to its effectiveness in this context.

Conclusion

Despite these limitations, our study provides critical evidence that the quality of HIV recency testing in Uganda is dynamic and vulnerable to erosion. The significant decline in pass rates among a fixed cohort of testers is not merely a fluctuation but a clear indicator that initial training alone is insufficient. The concurrent rise in non-response rates further reveals systemic disengagement. The core conclusion is that maintaining quality requires a shift from a one-time training model to a continuous, adaptive quality assurance system. This system must be resilient to real-world challenges like staff turnover and supply chain issues and must prioritize targeted support for lower-level facilities and non-laboratory personnel. Ultimately, the sustainability of recent infection surveillance depends on embedding accountability through individual feedback, fostering continuous learning via digital platforms and mentorship, and integrating PT performance into national oversight dashboards to ensure that quality remains a permanent focus.

Authors' Contribution: S.O. and A.M contributed to conceptualization, data extraction, formal analysis, methodology, and writing—original draft. E.M. contributed to formal analysis, methodology, and writing—review and editing. F.J, P.N, and T.A contributed to conceptualization and writing—review and editing. D.O contributed to data extraction and writing—review and editing. R.K, L.B, M.N and A.M contributed to supervision and writing—review and editing.

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Data Availability and Materials: Available upon request from the corresponding author.

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Competing Interests: All authors report no conflict of interest.

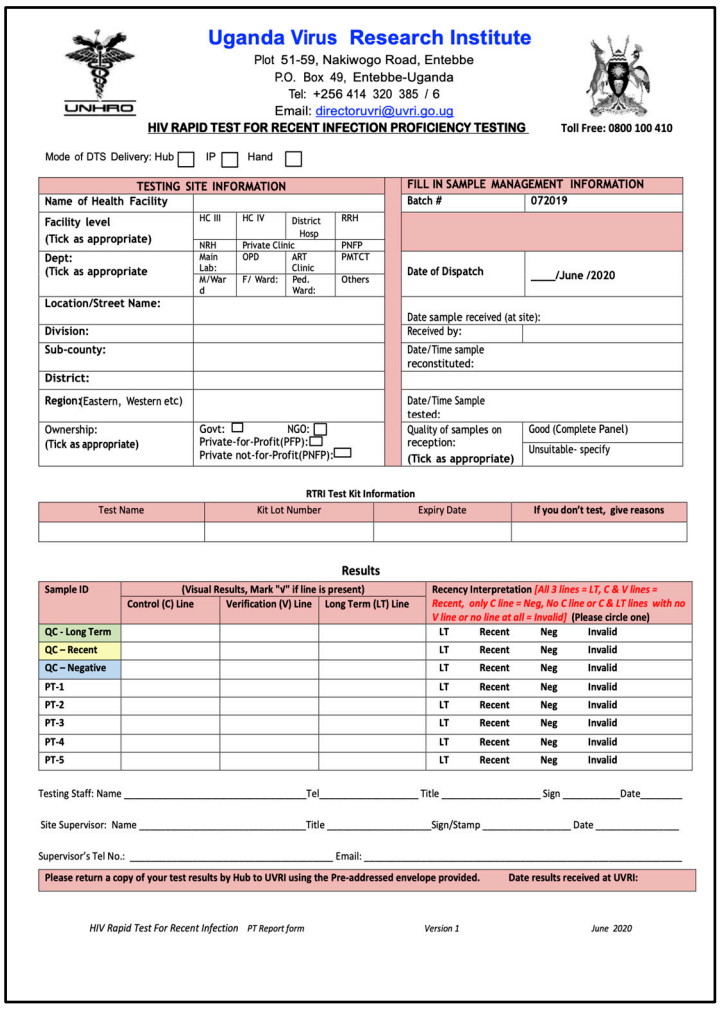
Ethical Considerations: This activity was reviewed and approved by the Uganda Virus Research Institute Research and Ethics Committee (UVRI REC; #GC/127/715), the Uganda National Council for Science and Technology (UNCST; #HS2171ES) and the U.S. Centers for Disease Control and Prevention (CDC), who determined this activity was not research, and it was conducted consistent with applicable federal law and CDC policy 45 C.F.R. part 46.102(l)(2), 21 C.F.R. part 56; 42 U.S.C. §241(d); 5 U.S.C. §552a; 44 U.S.C. §3501 et seq. All procedures involving human data were conducted in accordance with the principles of the Declaration of Helsinki.

Human Ethics and Consent to Participate declarations: Consent to participate was not applicable.

Clinical Trial Number: Not applicable.

Consent for Publication: Not applicable.

Appendix A: Proficiency Testing form Sample



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HIV RAPID TEST FOR RECENT INFECTION PROFICIENCY TESTING

Mode of DTS Delivery: Hub ☐ IP ☐ Hand ☐

TESTING SITE INFORMATION				
Name of Health Facility				
Facility level (Tick as appropriate)	HC III	HC IV	District Hosp	RRH
	NRH	Private Clinic		PNFP
Dept: (Tick as appropriate	Main Lab:	OPD	ART Clinic	PMTCT
	M/War d	F/ Ward:	Ped. Ward:	Others
Location/Street Name:				
Division:				
Sub-county:				
District:				
Region:(Eastern, Western etc)				
Ownership: (Tick as appropriate)	Govt: ☐ NGO: ☐ Private-for-Profit(PFP): ☐ Private not-for-Profit(PNFP): ☐			

FILL IN SAMPLE MANAGEMENT INFORMATION	
Batch #	072019
Date of Dispatch	____/June /2020
Date sample received (at site):	
Received by:	
Date/Time sample reconstituted:	
Date/Time Sample tested:	
Quality of samples on reception: (Tick as appropriate)	Good (Complete Panel)
	Unsuitable- specify

RTRI Test Kit Information

Test Name	Kit Lot Number	Expiry Date	If you don't test, give reasons

Results

Sample ID	(Visual Results, Mark "V" if line is present) Control (C) Line	Verification (V) Line	Long Term (LT) Line	Recency Interpretation *[All 3 lines = LT, C & V lines = Recent, only C line = Neg, No C line or C & LT lines with no V line or no line at all = Invalid]* (Please circle one)
QC - Long Term				LT Recent Neg Invalid
QC – Recent				LT Recent Neg Invalid
QC – Negative				LT Recent Neg Invalid
PT-1				LT Recent Neg Invalid
PT-2				LT Recent Neg Invalid
PT-3				LT Recent Neg Invalid
PT-4				LT Recent Neg Invalid
PT-5				LT Recent Neg Invalid

Testing Staff: Name _____ Tel_____ Title _____ Sign _____Date_____

Site Supervisor: Name _____ Title _____ Sign/Stamp _____ Date _____

Supervisor's Tel No.: _____ Email: _____

Please return a copy of your test results by Hub to UVRI using the Pre-addressed envelope provided. Date results received at UVRI:

HIV Rapid Test For Recent Infection PT Report form Version 1 June 2020

Figure A1. HIV Rapid Test for Recent Infection Proficiency Testing Report Form.

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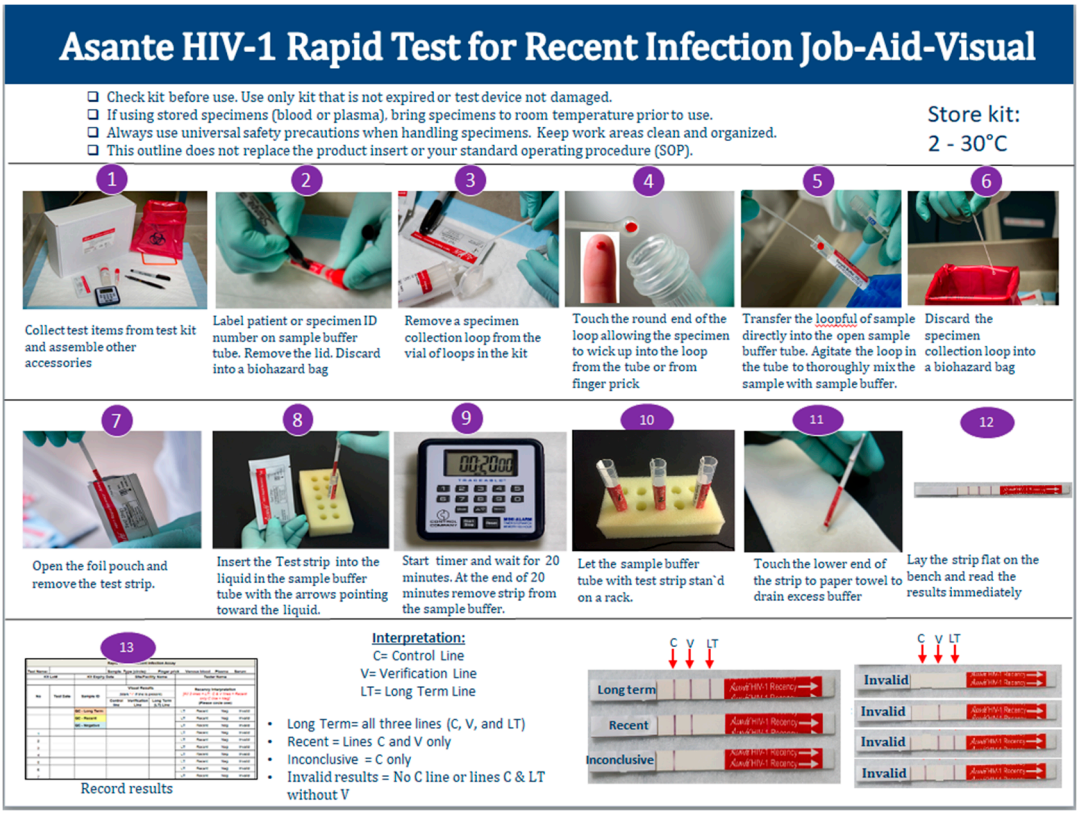
Figure A1 shows the Uganda Virus Research Institute's proficiency testing form for recent HIV infection testing. This quality assurance tool records comprehensive details - including site, sample, and test kit, results, and tester information - to evaluate facility performance and tester competence. The form's purpose is to ensure the accuracy and reliability of recency results and to identify areas needing improvement.

Appendix B

Assay Procedure

The ARRA was performed according to the manufacturer's instructions [17]. Briefly, approximately 5µL of specimen was collected by dipping the provided loop into the plasma. This loopful of specimen was then transferred to a sample buffer tube containing 0.5 mL of buffer and mixed with agitation. The test was initiated by placing the test strip into the buffer tube, ensuring the arrows pointed downward, and a timer was set for 20 minutes. After incubation at room temperature, the strip was removed, the bottom was tapped on a paper towel to drain excess buffer, and it was laid flat with arrows pointing to the right for immediate interpretation. Assay validity was confirmed by a built-in procedural control line. Its appearance indicated proper test function, while its absence rendered the test invalid, requiring a repeat, regardless of any other lines present on the strip. Furthermore, before testing the blinded PT samples, testers were required to first correctly analyze the characterized internal quality control (QC) samples included in each panel. These QCs - Long-term, Recent, and Negative - served a dual purpose: to monitor the performance of the test kits and reagents and to verify tester competency prior to proceeding with the blinded evaluation.

Asante RTRI Job Aid



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