

Test Performance of Expired Malaria Rapid Diagnostic Tests: A Pilot Diagnostic Accuracy Study Conducted in Western Uganda

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We evaluated the diagnostic agreement of expired malaria rapid diagnostic tests (mRDTs) with nonexpired mRDTs in a clinical setting in western Uganda. Expired mRDTs (SD Bioline Ag Pf/Pan and First Response Ag pLDH/HRP2) were administered to each participant in parallel with a nonexpired mRDT serving as the reference. Of 200 participants, 21 (10.5%) tested positive and 179 (89.5%) tested negative for malaria. All tests up to 9 months postexpiration were valid and consistent with reference mRDTs. All mRDTs 10–12 months postexpiration were invalid. These preliminary findings support further research into the use of expired mRDTs in resource-limited settings.

Keywords. malaria; expired RDT; rapid diagnostic testing.

Since 2010, the World Health Organization (WHO) has recommended parasitological confirmation of all suspected malaria cases before treatment [1]. This recommendation has led to an extensive use of malaria rapid diagnostic tests (mRDTs), which currently account for >80% of all diagnostic tests performed in endemic regions. Approximately 3.9 billion mRDTs were delivered between 2010 and 2022, with >82% of these being sold to sub-Saharan countries. The widespread usage of mRDTs is attributed to their several operational advantages over blood smear microscopy including ease of use,

comparatively rapid results (within 15–20 minutes), and minimal training and infrastructure requirements [2].

In Uganda, the Ministry of Health distributes the mRDTs through the National Medical Stores to government health centers and hospitals [3]. Yet challenges regarding their supply, storage, and utilization remain [4]. Interruptions to global and local supply chains, intensified by events such as the recent COVID-19 pandemic, can lead to major inventory imbalances including both stockouts and oversupply (ie, stocks of mRDTs that are nearing expiration) [3]. Excess of mRDTs approaching expiration present few options, with some facilities attempting to donate to other users (eg, community health workers [CHWs]), discard the mRDTs, or use them beyond their manufacturer-established expiration dates, albeit with uncertain reliability.

The expiration date of a diagnostic tool defines the end of shelf-life or timeframe within which the manufacturer can guarantee utmost stability and test performance [5]. Outside this timeframe, degradation of reagents may compromise the validity, thus increasing the likelihood of invalid—or, more concerning—inaccurate results [6]. Despite these potential issues, usage of expired mRDTs for malaria diagnosis is likely to persist in many resource-limited settings.

Currently, there are scant published data evaluating the validity and test performance of expired mRDTs, as no studies have been undertaken to assess the diagnostic reliability and diagnostic agreement of expired mRDTs compared to nonexpired mRDTs. This knowledge gap is particularly relevant in high-transmission settings where there is significant reliance on mRDTs, yet sustained supply is difficult to achieve. To address this gap, our study sought to evaluate the diagnostic performance of 2 brands of expired mRDTs up to 1 year postexpiration. Our larger goal was to generate evidence that may minimize waste.

METHODS

Study Setting

The study was conducted at Bugoye Level III Health Center (BHC) in the Kasese District of western Uganda. The health center maintains general, pediatric, and obstetric wards for inpatient services, along with a busy outpatient department that cared for 60–80 patients per day at the time of the study. Clinical officers, nurses, midwives, and laboratory technicians employed by the Ugandan Ministry of Health staff the health center. The climate in Bugoye permits year-round malaria transmission marked by semiannual transmission peaks typically following the end of the wet seasons, traditionally occurring March–May and September–November. *Plasmodium*

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Table 1. Test Brands and Sourcing

Test	Time Postexpiration	Source			
		Mbarara Regional Referral Hospital	Local Healthcare Centers	Joint Medical Stores ^a	CHWs
SD Bioline Ag Pf/Pan	1–3 mo	200	0	0	0
	4–6 mo	40	0	0	0
	7–9 mo	0	0	0	0
	10–12 mo	0	0	0	50
First Response Ag pLDH/HRP2 combo	1–3 mo	0	0	0	0
	4–6 mo	0	160	0	0
	7–9 mo	0	0	200	0
	10–12 mo	0	0	0	0

Brands of malaria rapid diagnostic tests differed across the spectrum of expiration.

Abbreviation: CHWs, community health workers.

^aA private, nonprofit organization that distributes medicine and health supplies in Uganda to private health facilities and research groups.

falciparum is responsible for the majority (>95%) of the infections, although *Plasmodium malariae* and *Plasmodium ovale* are isolated and coinfections with *P. falciparum*. The most recent malaria indicator survey undertaken in the region found a parasitemia prevalence of 7.3% in 2018–2019 [7].

Study Design

We conducted a diagnostic agreement study incorporating testing of expired mRDTs in parallel with routine testing using nonexpired, clinic-provided mRDTs. Expired mRDTs were compared with nonexpired mRDTs, as opposed to microscopy and polymerase chain reaction, to reflect the real-world diagnostic trade-offs in resource-limited settings where routine diagnosis relies on nonexpired mRDTs. The mRDTs used were SD Bioline Malaria Ag Pf/Pan (HRP2/pLDH; Abbott) (hereafter “SD Bioline”), and First Response Malaria Ag pLDH/HRP2 combo (Premier Medical) (hereafter “First Response”). Both tests contain a control and 2 antigen bands (ie, histidine-rich protein 2 [HRP2] and *Plasmodium* lactate dehydrogenase [pLDH]).

Eligibility was limited to individuals aged 2–18 years undergoing testing for malaria at BHC with a parent or caregiver who could provide written informed consent. Each participant received up to 5 mRDTs performed in parallel, including 3–4 expired mRDTs and 1 nonexpired mRDT. The expired mRDTs were grouped by expiration date up to 12 months past the manufacturer’s expiration date. Expired mRDTs were donated from local health facilities and commercial vendors (Table 1). The typical shelf-life for both mRDT brands used, SD Bioline and First Response, is on average 24 months [8, 9].

We were unable to obtain the required 200 mRDTs in the 10- to 12-month expiration window, resulting in 150 children receiving only 3 expired mRDTs. mRDTs 10–12 months postexpiration were given to the first 50 participants. Testing procedures, including finger- or heel-stick phlebotomy and testing, were conducted by trained laboratory staff.

Specifically, sample collection by finger-prick was performed to collect approximately 50–100 µL of capillary blood, which was then divided across the mRDTs. mRDTs were performed and interpreted in accordance with the manufacturers’ instructions. Treatment decisions were based on the result of the nonexpired mRDT, which was provided to the attendant clinician as is the current standard of care. Demographic information (eg, age, sex) and mRDT results were recorded on case report forms.

Data Analysis

Validity of the test was defined as the presence of the control band after 15 minutes. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were determined treating the nonexpired mRDT result as reference value. Accuracy was calculated as the number of people classified correctly for each expiration date category [(true positives + true negatives) / (true positives + false positives + false negatives + true negatives)]. Clopper–Pearson 95% confidence intervals (CIs) were calculated for each performance statistic.

Ethical Approvals

The study protocol and the informed consent documents were reviewed and approved by the Mbarara University of Science and Technology Research Ethics Committee, and the Uganda National Council of Science and Technology. Prior to a participant’s participation in the study, the written informed consent form(s) were signed by the participant’s legally authorized representative (parent or guardian) and by the person who conducted the informed consent discussion.

RESULTS

From September to December 2024, 200 participants underwent testing with 21 (10.5%) testing positive and 179 (89.5%) testing negative on the nonexpired mRDT. Over half (121 [60.5%]) of participants were female with 20 (20.0%) of

Table 2. Characteristics of Expired Malaria Rapid Diagnostic Tests

Result	Test	No. of Expired mRDTs			
		1–3 mo postexpiration	4–6 mo postexpiration	7–9 mo postexpiration	10–12 mo postexpiration
Positive	SD Bioline	21	14	0	NA
	First Response	0	7	21	NA
Negative	SD Bioline	179	27	0	NA
	First Response	0	152	179	NA
Invalid	SD Bioline	NA	NA	NA	50
	First Response	NA	NA	NA	0

Positive and negative refer to the results of the expired mRDTs.

Abbreviations: mRDT, malaria rapid diagnostic test; NA, not applicable (no test with that combination of characteristics).

participants under the age of 5. Of the expired mRDTs, all those between 1 and 9 months postexpiration showed a control band and were assessed as valid. All tests 10–12 months postexpiration were invalid (Table 2).

Overall, 21 participants had positive mRDT results, while 179 were negative according to the nonexpired mRDT. All valid, expired mRDT results, regardless of brand and expiration date, were in agreement with the results of the nonexpired mRDTs. Defining the nonexpired mRDT as the reference standard, estimated sensitivity and PPV were 100% (95% CI, 83.9%–100%), while specificity and NPV were 100% (95% CI, 98.0%–100%). Therefore, test accuracy was 100% (95% CI, 98.5%–100%).

DISCUSSION

Our findings suggest that mRDTs can provide valid and accurate results up to 9 months postexpiration for the brands of mRDTs used. The degree of agreement between the nonexpired and expired tests was surprising as we expected results to include disagreements between the 2 tests, especially when using 2 different brands. Reassuringly, however, it appears that so long as the test is valid (ie, presence of control band), the risk of an inaccurate result, which could lead to misdiagnosis, is quite low when using either the SD Bioline Malaria Ag Pf/Pan or First Response Malaria Ag pLDH/HRP2 combo mRDT.

Expiration dates are determined by manufacturers based on stability testing [10]. Expiration dates typically reflect a conservative threshold beyond which performance is no longer guaranteed [11]. While guidelines from the WHO and regulatory agencies emphasize strict adherence to these expiration dates, there is limited publicly available evidence assessing the actual diagnostic reliability of expired mRDTs in clinical settings and there has been little research to date comparing mRDT performance pre- and postexpiration. This study provides promising data that warrant further investigation. Yet our findings should not be interpreted as advocating for the routine use of expired tests. Instead, they offer preliminary data that may inform future research and policy discussions, particularly in emergency

or resource-constrained situations where expired tests might otherwise be discarded without consideration of residual utility. Such policies could include that health facilities' laboratory staff conduct periodic evaluation of expired mRDTs if stock-outs are anticipated.

Invalid test results prompt retesting, which could lead to multiple sticks for 1 patient or lead to empirical treatment. This method could risk overtreatment with antimalarials. Notably, the 50 mRDTs with expiration dates 10–12 months prior to use were all provided by local CHWs, who carry out integrated community case management in their respective villages. These mRDTs were stored in homes, where they may have been more exposed to heat, moisture, and sunlight compared to those provided by health facilities and vendors. This suggests that storage conditions may be an equally, if not more important, factor than expiration date. Because we had so few mRDTs in this category, all of which were provided by the CHWs, we cannot test this hypothesis. The fact that all of these kits appeared invalid does demonstrate that the control band is working as intended. However, the inability to document exact storage conditions limits our understanding of the specific conditions associated with assay degradation. These issues could be mitigated by training facility staff and CHWs on storage requirements and repeat testing in case of invalid results.

Most recent literature on mRDT stability continues to focus on environmental and operational stress rather than on the performance of expired unused kits. Evaluations of highly sensitive mRDTs indicate that these newer tests often have more limited temperature-stability and shelf-life claims compared to conventional mRDTs [12]. Quality assurance reviews also highlight how storage conditions, manufacturing variability, and supply chain temperature control remain central to maintaining mRDT reliability [13]. Other studies demonstrated that used mRDT cassettes stored under typical health facility conditions retain >95% of their results over a month [14]. While interesting and relevant, these studies do not address the issue of test validity after labeled expiration, highlighting the lack of research on this topic. Moreover, operational evaluations of

pLDH-based mRDTs have documented decreased heat stability at high temperatures (eg, >35°C–45°C), which could compromise diagnostic performance during storage [15]. As few studies explicitly test unused mRDTs past their expiration, our study fills a critical gap by directly assessing whether expired mRDTs maintain diagnostic accuracy of nonexpired mRDTs.

This study does have limitations. First, due to the low test positivity for malaria on the nonexpired mRDT, we are unable to draw strong conclusions about performance of expired mRDTs in this population. Due to the pilot nature of the study, we limited enrollment to children at 1 health facility. Therefore, results may not be generalizable to other transmission settings or populations.

Further, we include just 2 types of mRDTs, which were not distributed evenly across the postexpiration period. Therefore, we were unable to disentangle the effects of different expiration windows and test brands, which may confound results. Due to differences in test performance and characteristics, these results are not generalizable to other brands of mRDTs. This is particularly true for the 10- to 12-month postexpiration period where only 50 tests, all the same brand, were used and all returned invalid results.

Last, these data are far cleaner than expected, leading us to recommend caution against overinterpreting the results. While we are confident in the validity of our findings, they are limited in depth and scope, and larger studies will be needed before any recommendation can be made around the use of expired mRDTs; current clinical guidelines should be adhered to regarding expiration dates for medical testing.

In conclusion, the use of expired mRDTs during times of stockouts is one potential strategy to fill gaps in access to diagnostic testing, particularly in resource-limited settings. While our findings suggest strong alignment between expired and nonexpired tests, we do not recommend their use for clinical decision-making without further validation given the limitations in sample size, test variety, and generalizability. However, this study provides an important first step in exploring whether expired mRDTs could have a role in nonclinical or emergency contexts, and further investigation is warranted.

Notes

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Data availability. Deidentified individual data that support the results will be made available provided the investigator who

proposes to use the data has approval from an institutional review board, independent ethics committee, or research ethics board, as applicable, and executes a data use/sharing agreement with the University of North Carolina (UNC). The principal investigator should submit a request to the UNC Industry Contracting team (OSPContracting@unc.edu) to initiate the data use/sharing agreement.

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Potential conflicts of interest. The authors: No reported conflicts of interest.

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