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Evaluation of QuantiFERON TB gold plus among TB household contacts in high incidence settings

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Abstract

Background Accurately identifying and categorizing individuals who are latently infected is critical for developing prevention strategies against tuberculosis (TB) disease. The QuantiFERON-TB Gold Plus (QFT-Plus), a set of two antigen tubes, was used to assess TB household contacts, aiming to induce CD4 + and CD8 + T cell responses.

Methods We examined fifty-six TB household contacts for TB infection using the QFT-Plus and QFT-Gold In-Tube (QFT-GIT) tests. In addition, we evaluated 616 samples from the parent study to determine whether there was any association between the QFT-Plus CD8 + T cell responses and variables that were clinically significant. This was done by analyzing the difference in interferon-gamma (IFNγ) levels between TB2 and TB1 tubes. We utilised a cut-off of 0.6 IU/mL.

Results To assess agreement between tests, a Cohen's kappa of 0.71 was observed across 56 TB contacts. Eight participants reported discordance: four reported positive QFT-Plus and negative QFT-GIT, and four reported negative QFT-Plus and positive QFT-GIT. The QFT-Plus CD8 + T cell responses did not show any significant correlation with the age, sex, history of BCG vaccination, HIV infection, TB risk score and baseline blood draw among adult TB household contacts.

Conclusion The QFT-Plus and QFT-GIT tests significantly agree with one another. No clinically significant variable was observed to be associated with CD8 + T cell responses in QFT-Plus.

Keywords QFT-plus, QFT-GIT, IGRA, Tuberculosis

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Introduction

Tuberculosis (TB) remains a significant public health concern in Uganda, with a high incidence rate of 196 active disease per 100,000 people [1]. There is a high risk of developing active TB disease mostly among immunocompromised latent TB individuals. Currently, screening for TB infection (TBI) is done using tests like the tuberculin skin test (TST) and interferon-γ release assays (IGRAs) [2, 3].

The QuantiFERON Gold In-Tube test (QFT-GIT), the third generation of IGRA, measures the amount of IFN-γ that CD4 + T cells generate in response to *Mtb* long peptides of TB7.7, CFP10, and ESAT-6 [4]. It is limited, nevertheless, in patients receiving immune suppressive medication, pediatric patients, patients recently exposed to active TB and patients with advanced HIV infection. With its cytotoxic and cytokine secreting capabilities, CD8 + T cells have been shown to play a major role in host defense during *Mtb* infection [5, 6]. This is taken into consideration by QFT-Plus, a modified version of GIT. Considering this, antigen TB7.7 was excluded from both antigen tubes and CFP10 and ESAT-6 shorter peptides were added to QFT-Plus's TB2 tube [7].

Research conducted in nations with low or intermediate TB incidence settings has demonstrated a high degree of agreement between the QFT-Plus and QFT-GIT tests. This high agreement was also observed when previous studies included low and high risk participants with *Mtb* infections. The high-risk groups included were: children under five years old, immunocompromised people such as those who had solid organ or hematopoietic stem cell transplants, patients receiving immunotherapy, and HIV-positive people. Most of these studies had a significant agreement of ≥ 93.7% [8–10]. QFT-Plus had higher positive results compared to QFT-GIT among children and immunocompromised individuals as well as diagnose recent infections [11, 12]. Information about QFT-Plus's evaluation in high-incidence TBI settings is limited.

The aim of this study, was to assess if QFT-Plus test would not cause ambiguity in the diagnosis and/or continuing classification of TBI patients using IGRA data for our ongoing longitudinal studies. To evaluate QFT-Plus performance in TB household contacts in a highly burdened TB setting, we compared it to QFT-GIT. Furthermore, to investigate potential associations between QFT-Plus CD8 + T cell responses and clinically relevant variables such as age, history of BCG vaccination, weeks between baseline and blood draw, TB risk score, and HIV infection.

Materials and methods

Study population and design

Participants were recruited as part of a larger (parent) study presenting at the Uganda-Case Western Research University Research Collaboration (UCRC) tuberculosis clinic in Kampala, Uganda. The design of the parent study, participant selection, inclusion and exclusion criteria, and procedures are described in detail by Gutierrez et al. [13].

In summary, participants who had been household contacts (HHCs) of a GeneXpert or culture-positive pulmonary tuberculosis index case were recruited if they were ≥ 15 years. Participants underwent clinical, radiological and microbiological assessments at baseline and then every three months for a total of twelve months. HHCs were defined as people who, during the preceding three months prior to enrollment, resided in the same house as a pulmonary TB index case for at least seven consecutive days. Both TST and Interferon Gamma Release Assay (IGRA) were done to rule out *Mtb* infection. Clinical presentation, positive chest x-rays, positive GeneXpert, or positive sputum culture for Mtb were done to exclude active pulmonary TB disease.

In the current study, participants were included following an IGRA test request for the parent study. For the paired analysis, fifty six participants were enrolled at any of their clinical visits between May 2017 and November 2018. In order to assess whether there are potential associations of QFT-Plus CD8 + T cell response, we included 616 results from the parent study.

The study was approved by Institutional Review Board of Makerere School of Biomedical Sciences and Uganda National Council for Science and Technology. We obtained written informed consent from patients.

QFT-plus and QFT-GIT assays

We used 3mL and 4mL of whole blood containing lithium heparin for QFT-GIT and for QFT-Plus, respectively. Blood was processed in compliance with the guidelines provided by the manufacturer (QuantiFERON-TB Gold In-Tube package insert, document no. 1075115, Cellestis Inc, Aug 2013) and (QuantiFERON-TB Gold Plus (QFT-Plus) package insert, document no. 1095849, Cellestis Inc, Jan 2023) [4, 7]. To summarize, 1mL of heparinized blood was transferred into prelabelled *Mtb* antigen tubes TB for QFT-GIT, TB1 and TB2 for QFT-Plus and Nil and Mitogen tubes. Blood was thoroughly mixed using a roller for at least 5 min, and incubated at 37^0C for 16–20 h. Following incubation, we centrifuged the blood and plasma was harvested and stored at -80^0C. The plasma from QFT-GIT and QFT-Plus tubes was subsequently batch tested for interferon gamma (IFN-γ) using an Enzyme-linked Immunosorbent assay (ELISA) according to the manufacturer's instructions. The optical densities that were generated from the automated VersaMax ELISA processor (Molecular Devices LLC, CA, USA) through a software called SoftMax Pro version 5.2, were then transferred to Qiagen QFT-GIT or

Table 1 Variables associated with CD8 + T cell peptide response (QFT-Plus TB2-TB1 IFNγ) in 616 samples from the larger study

Characteristics	POS: TB2 – TB1 > = 0.6 *n* = 58 assays	NEG: TB2 – TB1 < 0.6 *n* = 558 assays	*P*-value
Weeks from baseline to blood draw, mean, (± SD)	20.8(± 18.5)	22.6(± 18.9)	0.47
Age in years, mean, (± SD)	29.9 (± 10.2)	31.2(± 13.2)	0.35
Male, n (%)	20 (35%)	214 (38%)	0.66
HIV negative, n (%)	51 (88%)	511 (92%)	0.48
BCG scar present, n (%)	47 (82%) *1	409 (74%) *6	0.22
risk score range 4–9	4: 11 (2%)	4: 1 (2%)	0.66
	5: 61 (11%)	5: 7 (12%)	
	6: 174 (32%)	6: 15 (26%)	
	7: 157 (28%)	7: 18 (31%)	
	8: 76 (14%)	8: 5 (9%)	
	9: 79 (14%)	9: 12 (21%)	

BCG: Bacille Calmette-Guerin * missing data

QFT-Plus analysis software to obtain quantitative values. The quantitative values were reported in International units (IU) per milliliter (IU/mL). The NIL tube provided background concentration hence all the responses from both QFT-GIT and QFT-Plus were evaluated by subtracting the NIL response. A cutoff value of 0.35 IU/mL was used. For the QFT-GIT, a response was considered positive when the TB antigen response minus the NIL response was ≥ 0.35 IU/mL and ≥ 25% of the NIL. For the QFT-Plus assay, a positive response was considered when either a single antigen response of TB1 or TB2 tube minus Nil or from both tubes was ≥ 0.35, and TB1 or TB2 minus Nil was ≥ 25% of the Nil value.

Statistical analysis

Both agreements between QFT-Plus and QFT-GIT tests, and between QFT-Plus TB1 and TB2 tubes were done by using Cohen's kappa and the prevalence and bias-adjusted kappa (PABAK). Coefficients were interpreted according to the Landis and Koch definitions [14, 15]. Agreement between quantitative IFN-gamma values (Nil adjusted) was assessed using the Bland-Altman method [16]. To evaluate association of CD8 + T cell response (Table 1) with other clinical variables, a comparison was done using the t-test for quantitative variables and the chi-square test for categorical variables. Mandalakas AM et al.'s 2012 risk score was used in our study ((Ma N & Unit, Clinical and epidemiological characteristics of individuals resistant to *M.tuberculosis* infection in a longitudianl TB household contact study in Kampala, Uganda., 2014) [17]. The risk score included a range of 1–10 variables, but in our data, there were no subjects with scores < 4 or > 9. A *p*-value of < 0.05 was considered statistically significant. R version 3.4.1 (2023) with the Tidyverse package was used for all analyses [18]. For this paper, OpenClinica 3.16, an open-source software, was used for both clinical data collection and management (Waltham, Massachusetts, USA; copyright OpenClinica LLC and associates; www.OpenClinica.com).

Table 2 Demographics of 56 household contacts

Characteristics	Summary Measure
Age in years, mean (SD)	35.8 (12.2)
Sex = Female, n (%)	37 (66%)
TST in mm at baseline, mean (SD)	6.6 (7.1)
TST negative at baseline, n (%)	32 (57%)
HIV positive, n (%)	19 (34%)
CD4 count in cells/μL, (HIV + only), median (IQR)	404 (323.5-606.5)

Abbreviations: TST, tuberculin skin test; HIV, human immunodeficiency virus, IQR, interquartile range, SD, standard dévіation

Table 3 Assessment between QFT-GIT and QFT-plus among 56 contacts

	QFT-GIT POSITIVE	QFT-GIT NEGATIVE
QFT-Plus POSITIVE	**20**	4
QFT-Plus NEGATIVE	4	**28**

Concordant results are in bold; QFT-GIT: QuantiFERON Gold In-Tube test; QFT-Plus: QuantiFERON TB Gold Plus

Results

Demographic characteristics

For the purpose of performing the paired analysis of the QFT-Plus and QFT-GIT tests, 56 Household contacts donated 9mL of blood. Out of these, 37 were females and 34% were HIV positive with a median (interquartile range) absolute CD4 count of 404 (323.5–606.5) cells/μL. Participants had a mean of 35.8 years and at baseline more than half ($n = 32$, 57%) were TST negative and where the mean TST reading was 6.6 mm (Table 2).

Agreement and discordance between QFT-GIT and QFT-plus

As shown in Table 3, the two tests produced concordant results among 48 contacts. Concordance was shown among 20 positive and 28 were negative results for both tests. Using a Cohen's kappa and a prevalence and bias-adjusted kappa (PABAK) of 0.71 (95%, kappa coefficient range 0.52–0.89), the similar results yielded an 86% agreement. We observed discordance in 8 contacts where

Table 4 Eight discordant QFT-GIT and QFT-Plus test results

Participant	Study Visits	HIV status	CD4 Absolute Count	QFT-GIT results Qualitative (TB1 - Nil)	QFT-Plus results Qualitative (TB1-Nil, TB2-Nil)	TST result Qualitative (mm)
1	M0	positive	781	neg (0.22)	pos (0.35[a], 0.27)	neg (4.0)
2	M0	positive	1207	neg (0.10)	pos (0.28, 0.36[a])	neg (0.0)
3	M6	positive	767	neg (-0.03)	pos (-1.43, 4.14)	neg (0.0)
4	M12	negative		neg (0.04)	pos (-0.21, 1.20)	neg (0.0)
5	M0	negative		pos (0.80)	neg (0.0, -0.11)	pos (10.9)
6	M0	negative		pos (0.77)	neg (0.14, 0.11)	pos (12.6)
7	M6	negative		pos (0.48)	neg (-2.91, 0.43*)	pos (10.0)
8	M0	negative		pos (1.30)	neg (0.0, -0.05)	pos (11.8)

QFT quantitative values are nil subtracted IFN-gamma (IU/ml). a: results at the borderline, *: This value was not considered positive because neither quantitative value was greater than 25% of the nil, M: Month

Table 5 Assessment between QFT-Plus TB1 and QFT-Plus TB2 IFNγ results

	QFT-Plus TB2 POSITIVE	QFT-Plus TB2 NEGATIVE
QFT-Plus TB1 POSITIVE	**20**	1
QFT-Plus TB1 NEGATIVE	3	**32**

Concordant results are in bold; QFT-GIT: QuantiFERON Gold In-Tube test

QFT-Plus: QuantiFERON TB Gold Plus

4/8 were QFT-GIT - /QFT-Plus + and 4/8 were QFT-GIT + /QFT-Plus -.

Assessment of discordant test results

Discordance was noted in 8 of the subjects in Table 4. All the three HIV-positive subjects were QFT-GIT negative/QFT-Plus positive. Three of four positive results in QFT-Plus demonstrated positivity in TB2 tube. Half of positive results in QFT-Plus were on borderline. The three HIV + contacts were TST negative. We reran the QFT-Plus test for participant samples with either TB1 or TB2 IFNγ yields at the borderline. TB1-Nil and TB2-Nil for Participant 1's sample were 0.08 and 0.11, respectively, indicating a negative result. The results for the second participant's sample were negative, with TB1-Nil and TB2-Nil values of 0.03 and 0.20, respectively.

Assessment of the QFT-plus TB1, TB2 and QFT-GIT TB tube IFNγ values

Furthermore, when we evaluated the agreement between the three antigen tube IFNγ values, we observed an agreement of 93% (Cohen's kappa of 0.86) in TB1 and TB2 tubes (Table 5). Discordance was noted in 4 contacts where 1 TB1 positive/TB2 negative and 3 were TB2 positive/TB1 negative. We used Bland-Altman plots to evaluate agreement and any biases between IFNγ values from QFT-Plus TB1 and TB2 tubes (Plot A) and QFT-GIT TB and QFT-Plus TB1 tubes (Plot B) in Fig. 1. We observed good agreement between IFNγ values from TB1 and TB2 as well as TB and TB1 tubes. The HIV- and HIV + groups both agreed on this point. In both comparisons, only 2 differences were random. When compared to TB1 and TB2 of QFT-Plus, IFN-γ values in the QFT-GIT TB tube were generally higher.

Examination of the TB2-TB1 CD8 + T cell responses in the QFT-plus test

Furthermore, we examined a total of 616 IGRA results from the parent study to determine whether there was any association of QFT-Plus TB2 CD8 + T cell responses and variables of clinical significance. More than one result per participant was used. The variables included age, sex, BCG vaccination history, HIV status, weeks from baseline to blood draw, and a risk score that included exposure to multiple index cases, duration of exposure, proximity to the index case, and infectivity of the index case. We used differences between TB2 and TB1 IFNγ values to estimate CD8 + T cell responses. We considered a cutoff of ≥ 0.6 IU/mL as a positive and < 0.6 IU/mL as a negative, following Barcellini et al.'s description [12]. We used univariate and multivariate logistic regression to identify variables that were associated with CD8 + T cell responses (Table 1). Positive CD8 + T cell responses were observed in 58 IGRA results. However, there was no significant association of these positive CD8 + T cell responses with any of the variables.

Discussion

The current study's goal was to validate QFT-Plus against QFT-GIT results for ongoing prospective studies, primarily for quality control purposes. We investigated the hypothesis that, in our longitudinal studies, the QFT-GIT and QFT-Plus results could be regarded as identical due to their high concordance. Overall, we observed 86% agreement between QFT-Plus and QFT-GIT tests (kappa = 0.71) on matched blood samples. These results are comparable to the majority of studies that showed agreements ranging from 89.9 to 95% when Mtb infections were detected in areas with low TB incidence settings [8–10, 12]. This analysis revealed no indeterminate

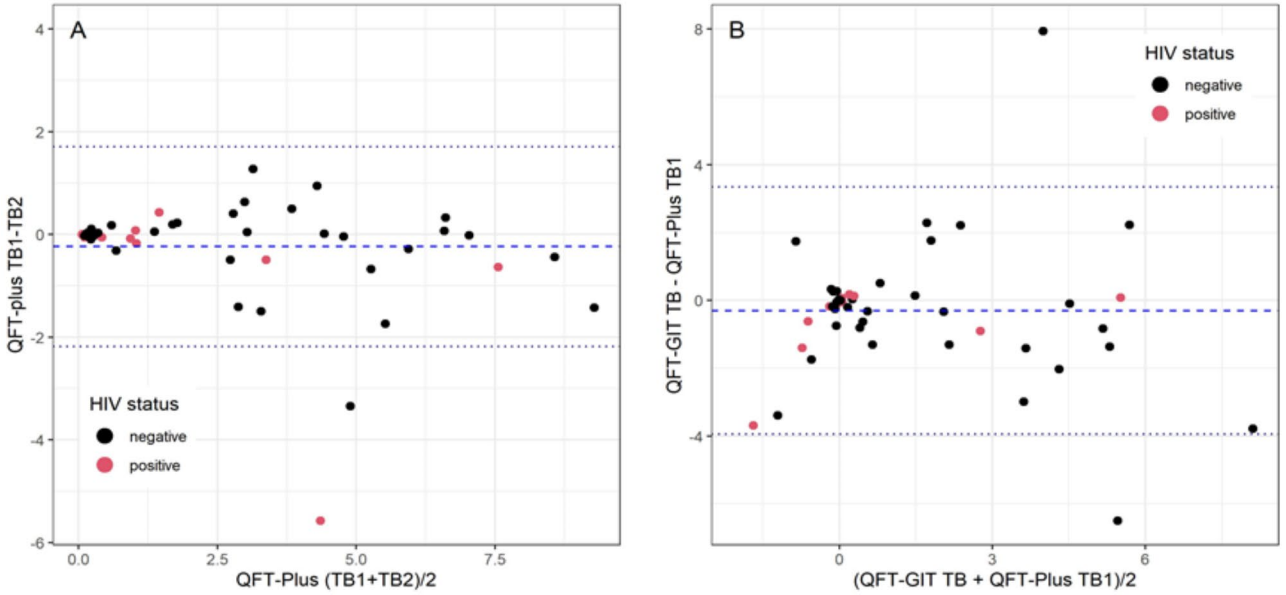

Fig. 1 Comparison between IFN-γ values (IU/mL) in QFT-GIT TB, QFT-Plus TB1 and TB2 tubes. The Bland-Altman plots show agreement between QFT-Plus TB1 and TB2 (**A**) and between QFT GIT TB and QFT-Plus TB1 (**B**). The horizontal dashed lines show the mean difference and dotted lines show the upper and lower 95% confidence intervals for the mean difference

IGRAs. The discordance between the two tests were in both directions. Three of four QFT-GIT-/QFT-Plus+, the positivity was in TB2 and 2 out of 3 the intense positivity was at follow-up visits suggesting that QFT-Plus may be sensitive in detecting new infections or reactivation of *Mtb* in HHC. Furthermore, 3 out of 4 of QFT-GIT-/QFT-Plus + results showed positivity was among HIV + individuals. However, 2 out of the 3 of QFT-GIT-/QFT-Plus + were at borderline. Earlier research has shown that in scenarios were IFNγ concentrations are low such that the final results are near the cut-off, it is associated with reversion rates of up to 50% with QFT-GIT [19, 20]. When these samples with near cut-off QFT-Plus IFNγ values were run again, the results shifted from positive to negative. These two participant samples' IFNγ values were likewise comparable to those of their QFT-GIT counterparts. The fact that two of these QFT-Plus were at the borderline complicates reproducibility, and the sample size of eight contradictory results was too small to make a determination.

When we examined agreement of QFT-Plus TB1 and TB2 in paired blood samples, we observed a perfect agreement of 93% (kappa = 0.86). Notably, the disagreement existed in only four contacts suggesting that sensitivity in TB1 is close to that in TB2 in high TB settings among HHC. Although there is a good similarity in IFNγ releases between QFT-GIT TB and QFT-Plus TB1 tubes, generally, there were higher IFNγ levels from QFT-GIT TB than QFT-Plus TB1 tube. The TB7.7 antigen is only in QFT-GIT TB tube and may be in some cases this extra antigen contributes to higher IFNγ release.

In this study we observed that among QFT-Plus discordant TB1 and TB2 results, sensitivity of TB2 was higher in HIV + group. We examined to find out if the shorter peptides added to the TB2 tube contributes to this sensitivity. We noted no association between HIV status and excess IFNγ value in TB2 tube suggesting that a combined response from CD4 + and CD8 + T cells may be the cause. We could contend that because of the participants' HIV status, which was not sufficiently advanced (323.5–606.5 cell/μL), the CD4 + T cells were able to function more or less like those of a healthy individual. Although, a Zambian study found that QFT-Plus high sensitivity individuals with HIV + and active tuberculosis [21]. Additional research has demonstrated that patients with active tuberculosis have higher rates of TB2 positivity, and it has also demonstrated the linkage between this positivity and the antigenic burden [22]. We did not include those with active TB in this study, and for those with TB infections, we were unable to determine whether the infection was new or had recently cleared up. All of these findings suggest that QFT-Plus TB2 CD8 + T cell response may have a minor role in the diagnosis of TB infections in both HIV-positive and HIV-negative individuals. Sources of discordance could be to a number of reasons subject-specific, technical, or random errors which could confound surveillance TBI data. A study that considered positivity to be from both tubes demonstrated that when a follow-up retest was done on seven TB1and TB2 discordants, only 1 remained positive [9] suggesting that within subject variations are pretty minimal, majority being technical and random errors. A school of

thought suggests increasing the cut-off for positive IGRA in high-incidence settings to 0.70 IU/mL. Additionally, using a borderline zone as an alternative to a cut-off value has been suggested. Research teams should consider quantitative values when rendering decisions [23, 24].

TST positivity is confused by BCG sensitization, which yields false positive results [25]. We discovered that QFT-Plus was not connected to the BCG vaccination through univariate analysis. Four TST results were positive (TST ≥ 10 mm) in our discordant results (QFT-GIT positive and QFT-Plus negative). This high positive in the TST results could be attributed to environmental non-tuberculous mycobacteria as well as BCG sensitization. It will be speculative to claim that the inconsistent positive results in QFT-GIT were false positives because there is no gold standard; instead, it may be because the test tube antigen formulations differed [26].

The study examined the relationship between age, sex, TB risk score, time since exposure, HIV status, BCG vaccination, and differences in TB2 and TB1 IFNγ releases in QFT-Plus. Results showed no correlation between weeks between baseline and blood draws and CD8 + T cell response. The endemic nature of Uganda may have affected immune responses.

There was no discernible relationship between the CD8 + T cell response and the TB risk score. In the larger study, the variables that represented the infectivity of index cases, duration of exposure, exposure to multiple index cases, and proximity of HHC to the index comprised the epidemiological risk score [17]. The high number of HHCs in risk scores 5, 6, and 7 ($n = 61$, 174, and 157, respectively) during this exposure suggested that the index cases were communicative. These risk scores did not correlate with the CD8 + T cell response. This suggests that even in people who have lived with an active TB case for a long time, the response by CD8 + T cells during TB infections is so weak as to be detectable. Positive TB2-TB1 differences were found to be substantially correlated with sleeping close to the index case by Barcellini et al. and may thus serve as a helpful proxy marker of recent exposure [12].

In summary, the study found a high sensitivity of QFT-Plus in immunocompromised individuals but limited by a small number of HIV + subjects. No significant factor was found for QFT-Plus indirect CD8 + response estimate. Further research is needed to assess QFT-Plus TB2 CD8 + T response function.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12879-025-10812-x.

Supplementary Material 1

Supplementary Material 2

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Author contributions
M.N, N.S, K.C and N.A.R provided conception and designed the study. B.T, L.L.M. and N.S. analyzed and interpreted the data. W.H.B and H.M.K. secured the funding and confirmed the study's conclusions. N.S. wrote the first draft of the manuscript. Each author the reviewed the manuscript before approving the finished copy.

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Data availability
Data is provided within the manuscript or supplementary information files.

Declarations

Ethics approval and consent to participate
Publication of these data was approved by the Institutional Ethics Review Board (IRB) of the Makerere University School of Biomedical Sciences and the Uganda National Council for Science and Technology. Every procedure was carried out in compliance with the of Helsinki and all applicable rules and regulations.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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