frontiers in Parasitology

TYPE Original Research
PUBLISHED 10 November 2026
DOI 10.3389/fpara.2026.1974692

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RECEIVED 21 August 2026
REVISED 08 September 2026
ACCEPTED 16 September 2026
PUBLISHED 10 November 2026

CITATION
de Jong D, Carrell C, Maganga JK, Shigella PS, Mhango L, Hoekstra PT, van Dam GJ, Mullins B, Changalucha JM, Downs JA, Warrick JW and Corstjens PLAM (2026) Flow-S enables field-based detection of low-intensity *Schistosoma* infections for monitoring and evaluation of control programs. *Front. Parasitol.* 5:1974692. doi: 10.3389/fpara.2026.1974692

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Flow-S enables field-based detection of low-intensity *Schistosoma* infections for monitoring and evaluation of control programs

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Introduction: Schistosomiasis remains a major public health challenge, particularly in sub-Saharan Africa. Detecting low-intensity infections is difficult because conventional parasitological methods lack sufficient sensitivity. Circulating anodic antigen (CAA), a genus-specific biomarker released by *Schistosoma* worms, enables detection of active *Schistosoma* infections. However, existing CAA assays require laboratory-based sample processing. We evaluated Flow-S, a field-deployable device for high-sensitivity quantitative CAA detection directly from large-volume, untreated urine samples. Flow-S integrates CAA concentration and quantitative upconverting reporter particle lateral flow (UCP-LF) detection with a portable standalone battery-powered reader for field-use.

Methods: A prospective study was conducted by local healthcare staff in a remote *Schistosoma haematobium*-endemic low-to-moderate infection setting. Fresh urine samples (n = 158) from women of reproductive age were analyzed using Flow-S. Paired urine, serum, and stool samples were examined by urine filtration microscopy, Kato-Katz (to exclude *S. mansoni*), and laboratory-based UCP-LF CAA reference assays (serum SCAA20 and urine UCAA*hT*417). A composite reference standard (CRS) classified individuals as infected when eggs or either reference CAA assay was positive. Flow-S performance was evaluated against the CRS and further assessed using latent class analysis (LCA).

Results: Complete datasets were available for 115 samples. Flow-S achieved an area under the receiver operating characteristic (ROC) curve of 0.92 and detected all egg-positive infections. Using a positivity threshold of 1.10 pg/mL, Flow-S achieved 80.8% sensitivity and 96.6% specificity, meeting the World Health Organization ideal target product profile requirements for monitoring and evaluation of schistosomiasis control programs. Latent class analysis provided complementary model-based evidence consistent with the high discriminatory performance of Flow-S observed using the composite reference standard.

Conclusions: Flow-S enables quantitative, field-deployable detection of *S. haematobium* infections directly from untreated large-volume urine without laboratory pre-treatment. Its high analytical sensitivity and diagnostic performance support its potential for detecting low-intensity infections. At an operational threshold of 1.10 pg/mL, Flow-S met the WHO TPP ideal targets for monitoring and evaluation and showed potential for cost-effective surveillance strategies, including pooled-sample screening for schistosomiasis control and elimination programs.

KEYWORDS

circulating anodic antigen (CAA), composite reference, Flow-S, latent class analysis (LCA), lateral flow (LF), point-of-care (POC), *Schistosoma haematobium* (*S. haematobium*), upconverting reporter particles (UCP)

1 Introduction

Infection from the parasitic flatworm *Schistosoma* affects humans as well as animals and causes urinary or intestinal schistosomiasis. The most common species involved in human infection are *S. mansoni*, *S. haematobium*, *S. japonicum* and *S. mekongi* with the first two mainly in sub-Saharan Africa and the latter two mainly in South East Asia (McManus et al., 2018; Hu et al., 2024; Rivera et al., 2024). Adult worm pairs migrate to and reside in blood vessels of bowel and bladder, where they produce eggs that are shed via stool or urine into the environment. Transmission of infection requires an intermediate host, species-specific freshwater snails, which are infected by miracidia that are released when excreted *Schistosoma* eggs hatch. This is followed by asexual reproduction in the snail. Large numbers of cercariae are released in freshwater by individual snails which can then infect humans who contact infested water (Colley, 2014).

School-aged children are often a major source in the transmission cycle due to frequent recreational water contact, lack of hygiene, and the development of partial immunity, resulting in lower egg excretion in adults (Mutapi et al., 2008; Mbanefo et al., 2014; WHO, 2021a). Moreover, occupational water-contact activities in agriculture, fishing, or car washing can also lead to infection. Schistosomiasis often arises in women as a consequence of infection when performing domestic tasks (collecting water, washing clothes) and, when it involves the female genital tract, may increase the risk for HIV infection (Hotez et al., 2019; Sturt et al., 2020; WHO, 2023). Therefore, multi-pronged interventions providing safe water, adequate sanitation and hygiene, as well as education to decrease risk of infection are likely the most effective approaches to reduce transmission (Wang et al., 2009; Colley et al., 2020).

Morbidity in schistosomiasis greatly exceeds mortality (Li et al., 2025). It is estimated that globally over 200,000 deaths per year are attributable to direct effects of schistosomiasis, most commonly due to hepatosplenic schistosomiasis resulting in liver fibrosis, urinary tract obstruction and renal failure, and bladder cancer (LoVerde, 2024). Worldwide, over 250 million people are infected and about 800 million are at risk, over 90% of whom live in Africa (McManus et al., 2018; WHO, 2023). The drug used to treat schistosomiasis, praziquantel (PZQ), is effective against adult schistosomes, but its activity against juvenile worms is limited and reinfection after treatment is common (Lamberton et al., 2017; Xiao et al., 2018). Vaccines are not yet available but progressing faster since the development of a safe controlled human *Schistosoma* infection protocol (Langenberg et al., 2020).

The Neglected Tropical Disease (NTD) road map, published in 2021 (WHO, 2021b), set the elimination of schistosomiasis as a public health problem as its main goal. Elimination was defined as <1% prevalence of heavy intensity infections in all endemic countries by 2030, as measured using standard parasitological methods (egg count by Kato-Katz or urine filtration) (Wiegand et al., 2022). Besides better focused mass drug administration, implementation of WASH (water, sanitation and hygiene) and snail control measures, WHO also recognized the need for new diagnostic methods to determine accurate infection prevalence as parasitological methods lack sensitivity (Clements et al., 2018; WHO, 2021a; Mwinzi et al., 2025). A current available and well recognized rapid point-of-care (POC) test demonstrating active infection is the POC-CCA test, which detects a schistosome-derived circulating cathodic antigen (CCA) in a urine sample of the host. The test is particularly useful for *S. mansoni* infections and a good alternative for more labor-intensive Kato-Katz stool analysis, but it lacks sensitivity for other species (Ashton et al., 2011; Hoekstra et al., 2024; Vaillant et al., 2024).

Circulating anodic antigen (CAA) is a distinct schistosome-excreted carbohydrate that is genus-specific and can therefore be used to detect infections across all species (Corstjens et al., 2020; Fasogbon et al., 2023). A CAA-specific lateral-flow (LF) based diagnostic test has demonstrated very high analytical sensitivity and is suitable for detecting very low-intensity infections, including infections that may be missed by conventional parasitological methods (Knopp et al., 2015; Langenberg et al., 2020). Sensitivity of the test is due to high affinity of the antibody used to capture and detect CAA (a repetitive di-saccharide), in combination with a background free high-sensitivity label, up-converting reporter particles (UCP) (Corstjens et al., 2014). The current UCP-LF CAA test is applicable to blood (serum and plasma) and urine samples, but is still a laboratory-based test as it requires pretreatment and extraction of the sample with trichloroacetic acid (TCA) (Krijger et al., 1994; Corstjens et al., 2020). Further, for ultimate sensitivity (single worm detection) an ultrafiltration concentration step is required. Flow-S is a novel field-deployable, single-use disposable device

developed for the detection of active *Schistosoma* infections. The device enables concentration of *Schistosoma* genus-specific circulating anodic antigen (CAA) from large-volume urine samples (5–18 mL) without the need for additional laboratory equipment, thereby increasing its sensitivity for detecting low worm burdens. In addition, Flow-S incorporates an integrated quantitative lateral flow (LF) test strip for rapid CAA detection using the highly sensitive UCP label. The complete device is compatible with a battery-operated portable reader that provides automated output, thereby eliminating potential operator bias in test result interpretation. The development of field-deployable and quantitative diagnostics is consistent with the broader movement toward portable point-of-care platforms for schistosomiasis surveillance and control (Rivera et al., 2024).

In a previous study, we evaluated the accuracy and acceptability of Flow-S using frozen urine samples in a laboratory setting (de Jong et al., 2024). The device was tested under laboratory conditions following a field-deployable protocol, demonstrating its potential as a sensitive and user-friendly diagnostic tool for detecting active *Schistosoma* infections. In the current study, we evaluated an improved version of the Flow-S device in a real-world field setting, where testing was conducted by local staff using freshly collected large-volume urine samples (18 mL).

2 Materials and methods

2.1 Test cohort, clinical samples and study design

Ethical approval for field collection and testing of samples was obtained from the National Institute for Medical Research in Dar es Salaam (NIMR/HQ/R8.a/Vol. IX/3625) and Weill Cornell Medicine in New York (20-10022745). Prospective testing with Flow-S was conducted over a 5-month period from January to May 2024. The study was conducted in an *S. haematobium* endemic region of Tanzania as part of an ongoing cohort study on female genital schistosomiasis (FGS) in women of reproductive age (Maseke et al., 2022; Lamberti et al., 2024). The cohort comprised women with predominantly low- to moderate-intensity *Schistosoma* infections, as well as women without *Schistosoma* infection. Women provided written informed consent and underwent collection of blood, urine, and stool samples for assessment of *Schistosoma* infection status. Stool samples were processed using the Kato-Katz method with five slides prepared per sample for detection of *S. mansoni* eggs (Katz et al., 1972; Berhe et al., 2004). Participants with *S. mansoni* infection were excluded from the cohort, which focuses on genital mucosal manifestations of *S. haematobium* infection. Participants were enrolled at different stages of the cohort: some were screened for infection for the first time, while others had previously received praziquantel treatment during earlier visits following detection of circulating anodic antigen (CAA) positivity or urine egg positivity. Fresh urine samples were tested immediately in the field. A 10 mL aliquot was filtered and examined microscopically for *S. haematobium* eggs (WHO, 1991), while 18 mL was analyzed using the Flow-S device (de Jong et al., 2024), equipped with a wick to improve flow rate and prevent clogging. The remaining urine (up to 10 mL) was transported to the laboratory and stored at −80 °C for later analysis using the UCP-LF CAA urine assay [UCAA*hT*417 (Corstjens et al., 2020)]. Venous blood was collected into serum tubes, and serum was separated upon return to the NIMR laboratory and stored at −20 °C for later analysis using the UCP-LF CAA serum assay [SCAA20 (Corstjens et al., 2020)].

2.2 Flow-S description, stability and field application

Flow-S comprises integrated 5 mm width LF strips with the UCP reporter-conjugate dried into a conjugate release pad (Figure 1A); further details have been described previously (de Jong et al., 2024). A video showing how the device is run is provided as a separate Supplementary Material (Supplementary Video 1). A single batch of Flow-S devices was produced for field and stability testing and stored individually in desiccated pouches (Figure 1B). To test field performance and confirm device stability, calibration curves (triplicates) using artificial urine (Pickering Laboratories, CA, USA; #1700-0602) spiked with known concentrations of CAA were run at three time points during the study: after receipt at NIMR, halfway (3rd month) and at the end (5th month) of the study (Figure 2).

In the field, 18 mL fresh urine was added to a 50 mL tube containing 2 mL buffer to ensure pH-neutral antibody-antigen binding conditions. The mixture was applied to the Flow-S urine reservoir initiating passive capillary flow and CAA concentration on the CAA-specific capture pad (Figure 1C, zoomed-in part). After 60 min the concentrator pad was flipped (Figure 1C) to elute bound CAA and initiate LF with 225 µL IgG elution buffer (Pierce^{TM}; Thermo Fisher #21004). LF was sustained for at least 25 minutes, after which strips were analyzed using a portable, battery-operated UCP-Quant reader (version LR3; DIALUNOX GmbH, Stockach, Germany). The reader was custom adapted for UCP detection and modified to fit the Flow-S device (Figure 1C). Laser power was optimized and fixed for scanning wet LF strips. LF strips were removed from the Flow-S devices and stored for reanalysis in the laboratory (dry scans) to compare with field measurements (wet scans) and explore the alternative when immediate field-based LF strip reading is not possible.

2.3 UCP-LF CAA laboratory testing

Serum samples (stored at −20 °C) were analyzed using the laboratory-based UCP-LF SCAA20 assay, with a positivity threshold of 30 pg/mL (Corstjens et al., 2020). The 'Serum CAA 20' (SCAA20) assay analyzes the equivalent of 20 µL serum; this assumes equal volume of pellet and clear supernatant after TCA extraction. At 100% capture efficiency the 30 pg/mL from a 20 µL sample would translate to 0.6 pg CAA captured on the Test line of the 4 mm width LF strip. Briefly, 50 µl of serum was extracted with 50 µl 4% (w/v) trichloroacetic acid (TCA), vortexed, and centrifuged at 12,000 rpm. From the clear supernatant, 20 µL was added to 50 µL high-salt lateral flow (HSLF: 200 mM Tris pH 8, 270 mM

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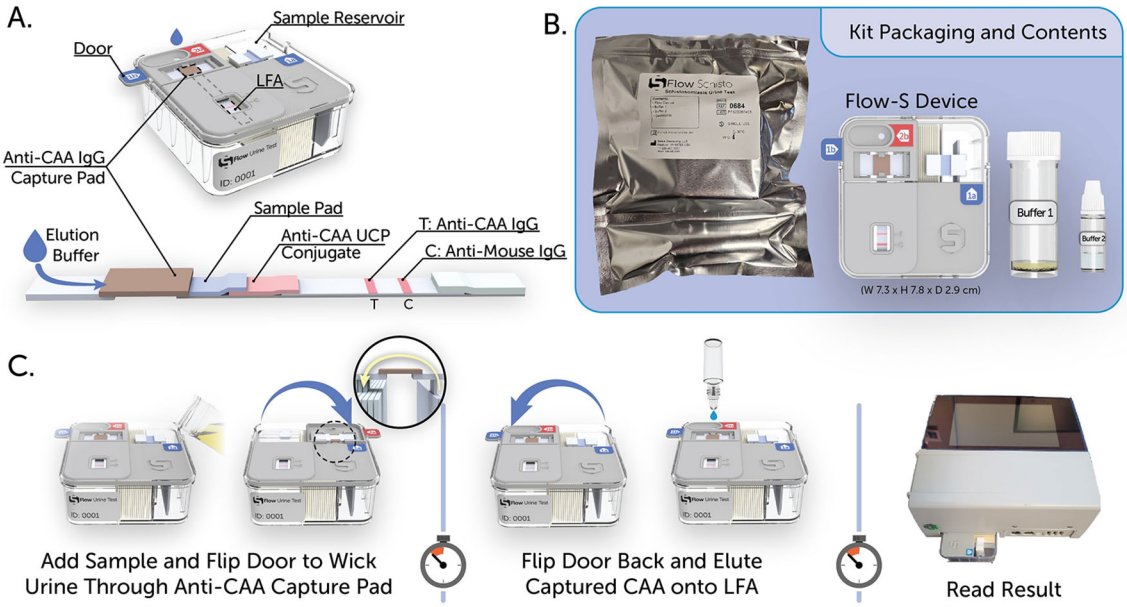


FIGURE 1
Flow-S and assay workflow. **(A)** Flow-S cartridge showing the integrated components and fluid flow. **(B)** Flow-S kit packaging and components. **(C)** Step-by-step assay workflow, including addition of urine, CAA concentration, elution and lateral-flow detection, followed by quantitative reading using a portable battery-operated reader.

NaCl, 0.5% Tween-20 (v/v), 1% BSA (w/v)) buffer and mixed with 50 µL HSLF buffer containing 100 ng UCP particles ($NaYF_4$:Yb^{3+}, Er^{3+}) covalently coupled to anti-CAA antibody (147-3G4) as previously described (Corstjens et al., 2008). A regular 4 mm width UCP-LF CAA strip was inserted to initiate lateral flow. LF strips were manufactured by Maxim Biomedical Inc. (Rockville, MD, USA) according to protocols identical to our in-house procedure. LF strips were analyzed using a dedicated UPCON benchtop

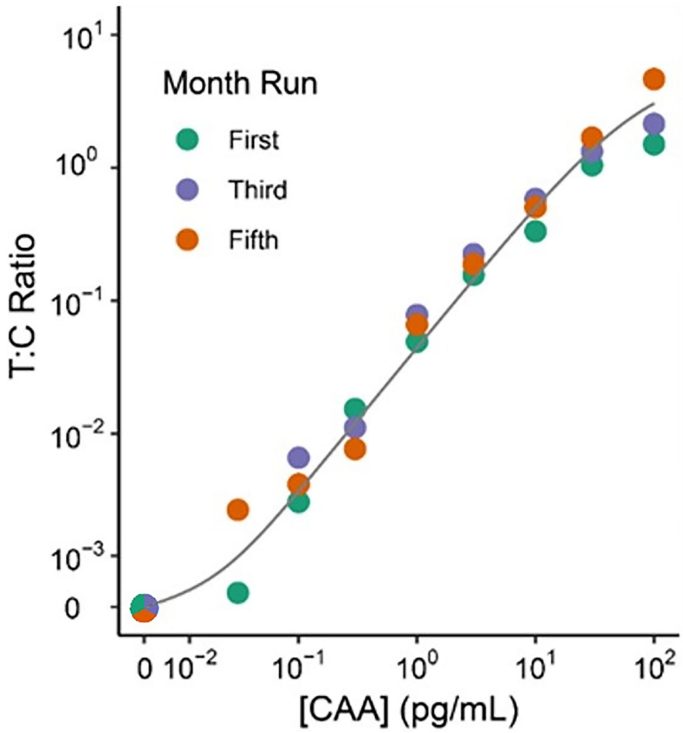


FIGURE 2
Stability and detection limit of Flow-S in the field. Repeated standard curves were performed to monitor device stability over the 5-month study period. Each point represents the mean ratio of three independent runs. CAA concentrations of 0 pg/mL overlapped across all three time points and were below the lowest concentration measured at 0.03 pg/mL in months 1 and 5. The 0.03 pg/mL concentration was not included in the standard curve at month 3. T, Test line; C, Control line.

reader (Labrox Oy, Turku, Finland). As a reference standard for the quantitative determination of CAA, a TCA-soluble fraction of *Schistosoma mansoni* adult worm antigen (AWA-TCA) was used (Deelder et al., 1980). CAA standard curves were prepared by spiking AWA-TCA into negative human serum and were run in parallel to validate the cut-off and quantify CAA concentrations (Polman et al., 2000).

Urine samples (stored at −80 °C) were tested with the UCP-LF UCAA*hT*417 assay, with a 2 pg/mL positivity threshold (Corstjens et al., 2020). The 'Urine CAA high TCA 417' (UCAA*hT*417) assay analyzes the equivalent of 417 µL urine. Briefly, 500 µL urine was mixed with 100 µL 12% (w/v) TCA to obtain a final 2% TCA concentration, vortexed, and centrifuged (5 min, 13,000 rpm). From the clear supernatant, 500 µL was concentrated to 20 µL using 10 kDa Amicon Ultra-0.5 centrifugal filters (Merck Millipore). The concentrate was then analyzed using the same procedure as described above for SCAA20. CAA standard curves were prepared using negative human urine instead of serum.

2.4 Processing of LF strip reader scan data

To read the UCP reporter signal at the Test (T) and Control (C) lines of the LF strips, a portable reader for field work and a laboratory-based benchtop reader for batch processing were available. The portable reader (UCP-Quant LR3) is an ESEQuant reader version LR3 (DIALUNOX GmbH, Stockach, Germany), custom adapted for the UCP reporter technology and in-house modified to allow scanning of the LF strip while still integrated in the Flow-S device. The benchtop lab-based reader is a dedicated UCP plate reader (UPCON; Labrox Oy, Turku, Finland) which can read a plate with up to 20 LF strips at a time. The LF strip readers scan the nitrocellulose membrane containing the T and C capture lines using 980 nm infrared (IR) light to excite the UCP label, generating 540 nm emission via up-conversion. Signals, peak areas in relative units, at the T and C lines are measured, and Ratio values (R) are calculated by dividing the T signal by the C signal. CAA concentrations in clinical samples are determined using standard curves generated from R values obtained with standard dilution series of CAA in the appropriate biological matrix. T signals and corresponding R values increase proportionally with CAA concentration. A (minimum) flow time of 25 minutes was used in all Flow-S experiments to guarantee a stable Ratio value. UCP-LF strips can be stored as a permanent record and rescanned any time. Dry strips will generate higher signals and may need adaptation of the IR laser to a lower power to prevent signal saturation (Sedlmeier et al., 2016). A unified algorithm implemented in R was used to standardize data processing. The script applies rolling window operations (using the roll apply function from the zoo package (Zeileis and Grothendieck, 2005) for background correction, peak detection, and signal integration across all reader outputs (Iliopoulou, 2026).

2.5 Analysis – composite reference standard

In the composite reference standard (CRS) approach, a sample was classified as positive (CRS-P) if eggs were detected or if either the serum or urine sample was positive for CAA based on predefined assay thresholds. Samples that were negative in all three reference tests were classified as negative (CRS-N). Consequently, the specificity of each reference assay relative to the CRS was 100% by definition. Only samples from participants with complete data for all reference tests were included in the analysis. Sensitivity and specificity were determined at the field-determined threshold, the threshold corresponding to the maximum Youden index, and thresholds corresponding to the WHO TPP minimum and ideal specificity targets, using receiver operating characteristic (ROC) curve analysis.

2.6 Analysis – latent class model

Latent class analysis (LCA) has previously been used to evaluate schistosomiasis diagnostics in settings where no single diagnostic test can be regarded as a perfect reference standard (Koukounari et al., 2021; Mesquita et al., 2022). In the current study, we used a Bayesian hierarchical latent-variable framework, hereafter referred to as the hierarchical LCA, to combine binary and numerical measurements as described in Supplementary Material 2 and Supplementary Table S1. LCA Models #1 and #2 classified CAA measurements as positive or negative based on the positivity thresholds of the reference tests and therefore did not retain the quantitative information in the CAA measurements. Model #3 retained the numerical CAA measurements and was selected for the primary LCA analysis. It incorporated numerical CAA measurements, including values below positivity thresholds, together with binary egg results to estimate latent infection and evaluate Flow-S performance. Because Flow-S measurements were included directly in the latent-variable model, the resulting LCA-based classification is not independent of Flow-S and was therefore considered complementary to, rather than an independent validation of, the CRS-based analysis. LCA models were encoded in R (Team RC, 2025) using the *nimble* package (de Valpine et al., 2017; Team ND, 2026), with the mathematical functions and code described in detail in Supplementary Material 2, "Latent Class Modeling."

3 Results

3.1 Stability and analytical performance of Flow-S

Flow-S device stability was evaluated at three time-points over the 5-month study period to assess long-term performance. Triplicate standard curves spiked in artificial urine were measured upon in-country arrival (after ambient-temperature air- and ground-transport) and again at months 3 and 5 (Figure 2). Performance remained stable under ambient storage conditions, with a demonstrated field detection limit of 0.10 pg/mL at all three time points.

3.2 Analysis against the composite reference standard

Flow-S field results were compared with a CRS based on the results of egg microscopy, serum SCAA20, and urine UCAA*hT*417.

The Standards for Reporting Diagnostic Accuracy Studies (STARD) diagram (Figure 3) provides an overview of Flow-S testing and sample inclusion in the CRS analysis. A total of 158 urine samples were tested in the field using Flow-S. No devices showed visual failure; however, results from four devices were excluded because of missing Control lines, and 14 field scans were unavailable because the results were not stored. Of the remaining 140 samples, 25 were excluded because of incomplete reference test results (Supplementary Material 1; Supplementary Table S2), leaving 115 sample sets for analysis (Supplementary Material 1; Supplementary Table S1). These comprised seven samples positive by all three reference tests, nine positive by both SCAA20 and UCAA*hT*417, four positive by SCAA20 only, six positive by UCAA*hT*417 only, and 89 negative by all three reference tests. Thus, the CRS classified 26 samples as CRS-P and 89 as CRS-N.

Flow-S detected all seven egg-positive samples. Against the CRS, Flow-S showed a sensitivity of 88.5% and specificity of 86.5% at the field-determined positivity threshold of 0.10 pg/mL CAA. ROC analysis demonstrated high discriminatory performance, with an area under the curve (AUC) of 0.92 (Table 1; Supplementary Material 1; Supplementary Table S1). The WHO Target Product Profile (TPP) for tools supporting monitoring and evaluation specifies minimum clinical sensitivity and specificity of >60% and >95% and ideal targets of >75% and >96.5%, respectively (WHO, 2021a). At 0.10 pg/mL, Flow-S met the ideal sensitivity target but did not meet the minimum specificity target. Increasing the Flow-S CAA positivity threshold improved specificity while maintaining sensitivity above the WHO TPP ideal target. At a threshold of 0.27 pg/mL, corresponding to the maximum Youden index, sensitivity remained 88.5% and specificity increased to

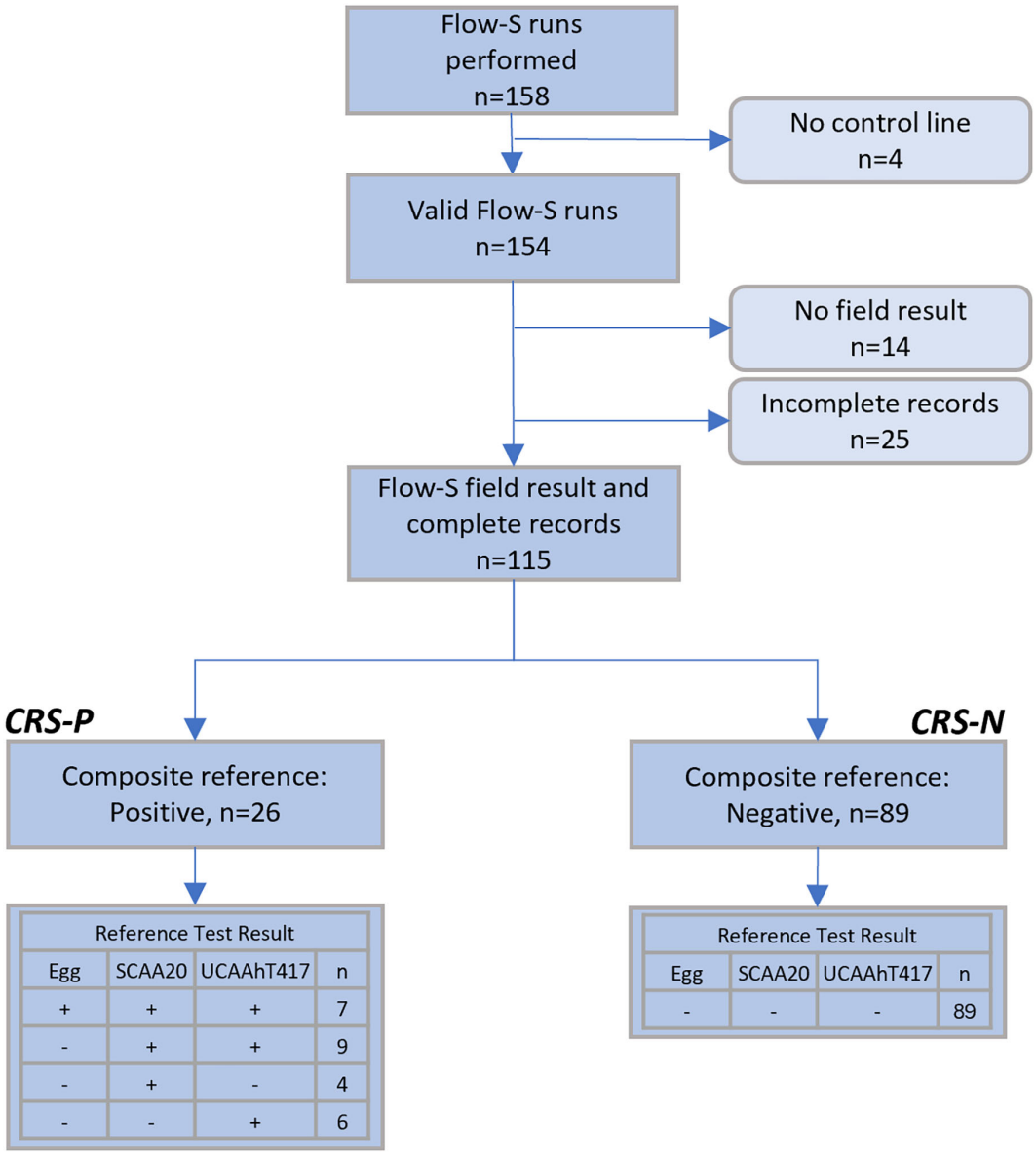


FIGURE 3
STARD diagram. Sample inclusion and classification based on the composite reference standard.

TABLE 1 Diagnostic performance of Flow-S at different CAA positivity thresholds.

Test (cut-off)	Threshold rationale	TP	FN	TN	FP	Sn (%)	Sp (%)	AUC
Flow-S ≥ 0.10 pg/mL CAA	Field-determined	23	3	77	12	88.5	86.5	0.92
Flow-S ≥ 0.27 pg/mL CAA	Highest Youden index	23	3	83	6	88.5	93.3	0.92
Flow-S ≥ 0.81 pg/mL CAA	Achieving WHO TPP minimum	21	5	85	4	80.8	95.5	0.92
Flow-S ≥ 1.10 pg/mL CAA	Achieving WHO TPP ideal	21	5	86	3	80.8	96.6	0.92
Egg microscopy ≥ 1 egg	Parasitological reference	7	19	89	0	26.9	100.0	0.64
SCAA20 ≥ 30 pg/mL	Predefined antigen threshold	20	6	89	0	76.9	100.0	0.95
UCAA*hT*417 ≥2 pg/mL	Predefined antigen threshold	22	4	89	0	84.6	100.0	0.96

Clinical parameters calculated for Flow-S, egg microscopy, SCAA20, and UCAA*hT*417 for 115 clinical samples (26 reference-positive and 89 reference-negative) against CRS. .

93.3%. At thresholds of 0.81 and 1.10 pg/mL, sensitivity was 80.8%, while specificity increased to 95.5% and 96.6%, respectively. The 0.81 pg/mL threshold met the WHO TPP minimum specificity requirement, whereas the 1.10 pg/mL threshold met the ideal specificity requirement; both also met the ideal sensitivity requirement. At these two thresholds, five CRS-P samples were classified as false negatives (FN, Supplementary Material 1; Supplementary Table S1) by Flow-S. All five were egg-negative but positive by UCAA*hT*417, with one also positive by SCAA20. On the other hand, four CRS-N samples were positive by Flow-S at 0.81 pg/mL and three at 1.10 pg/mL. When the field-determined positivity threshold of 0.10 pg/mL was used, 12 CRS-N samples were classified as positive by Flow-S.

The individual reference tests showed lower sensitivity than Flow-S. Egg microscopy detected 7 of the 26 CRS-P samples (sensitivity, 26.9%), whereas SCAA20 and UCAA*hT*417 detected 20 (76.9%) and 22 (84.6%), respectively. All three reference tests had 100% specificity against the CRS because CRS positivity was defined by a positive result in at least one of the reference tests. The two CAA-based reference assays did not identify identical sets of positive samples: four samples were positive by SCAA20 only and six by UCAA*hT*417 only. This difference is consistent with previously reported variation in the relationship between serum and urine CAA concentrations among individuals (de Dood et al., 2018; Sousa et al., 2019; Hoekstra et al., 2024).

3.3 Comparison of CRS and latent class analysis results

Flow-S results were evaluated using both the CRS and LCA approaches. The ROC curves showed similar discriminatory performance, with an AUC of 0.923 when sample classification was based on the CRS and 0.947 when based on the LCA model (Figure 4; Supplementary Figure S3). The similar AUCs indicate broadly consistent discriminatory patterns between the CRS- and LCA-based classifications; however, because Flow-S measurements were incorporated directly into the LCA model, the LCA-derived classification is not an independent reference standard. The differences between the CRS- and LCA-based classifications, particularly for samples near the positivity thresholds of the reference assays, reflects the different ways in which the two approaches handle uncertainty. The CRS classifies a sample as positive when at least one reference test exceeds its predefined threshold, whereas the LCA incorporates the numerical CAA measurements and estimates the probability of latent infection. Samples with CAA concentrations below the positivity thresholds of the reference assays may

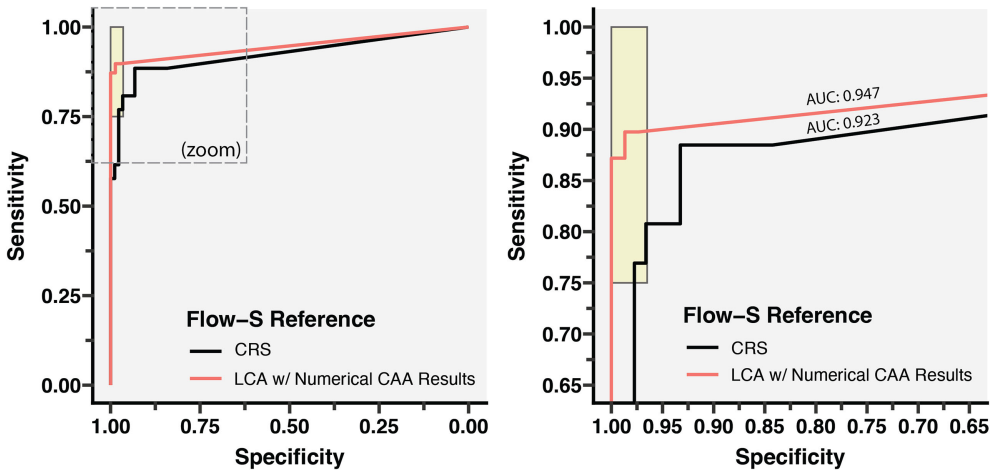


FIGURE 4
ROC analysis. (Left) ROC curves of Flow-S against CRS and LCA Model #3. (Right) Magnified view of the upper-left region of the ROC curves, showing the WHO TPP ideal targets (yellow rectangle).

receive a higher probability of positivity in the LCA than in the CRS. This general issue has been recognized in latent class analyses of schistosomiasis diagnostics, particularly when conventional reference tests have limited sensitivity at low infection intensities (Clements et al., 2018; Koukounari et al., 2021; Mesquita et al., 2022). Table 2 summarizes the samples classified differently by the two approaches. These findings illustrate the potential limitations of using fixed reference-test thresholds when evaluating a highly sensitive quantitative assay such as Flow-S.

3.4 Correlation between UCP-LF wet scan vs laboratory-based dry scan

In the current study, UCP-LF strips were scanned in the field immediately after testing (wet scan), transported to the laboratory, and re-scanned the following day after drying (dry scan). Wet- and dry-scan results correlated well, with an R^2 of 0.96 for samples within the standard-curve range (≤100 pg/mL CAA; 112 of 115 samples). Dry UCP-LF strips produced higher emission intensity, which could result in signal saturation and deviations in Ratio values. This effect can be reduced by lowering laser power. Overall, differences in Ratio values and corresponding CAA concentrations were minor for samples within the standard-curve range (Figure 5).

4 Discussion

Circulating anodic antigen (CAA) is a highly specific biomarker of active *Schistosoma* infection and can be detected in both blood and urine (de Dood et al., 2018; Corstjens et al., 2020). Laboratory-based UCP-LF CAA assays provide high analytical sensitivity but require relatively large sample volumes, trichloroacetic acid (TCA) extraction, and ultrafiltration-based concentration steps (Krijger et al., 1994; Corstjens et al., 2020). TCA pretreatment has historically been used as a standard procedure for CAA and CCA detection in serum and urine, but can add substantial sample-processing requirements (Krijger et al., 1994). Flow-S replaces these laboratory-intensive procedures with passive filtration and antibody-based antigen concentration, enabling POC application. Combined with the high sensitivity of the UCP reporter technology, Flow-S enables detection of CAA concentrations in the sub-pg/mL range (de Jong et al., 2024). The ability to process large urine volumes (18 mL) using a simple workflow that does not require separate laboratory equipment supports deployment in remote and resource-limited settings. In addition, quantitative LF detection with automated readout reduces operator-dependent interpretation and variability. These characteristics address several of the operational requirements identified by WHO for field-based schistosomiasis monitoring and evaluation, including portability, minimal infrastructure, and limited training requirements (WHO, 2021a). In this study, Flow-S was successfully applied in an endemic *S. haematobium* setting, including a cohort with predominantly medium- to low-intensity infections and expected low CAA concentrations in serum and urine. This is particularly relevant because diagnostic sensitivity of conventional parasitological methods decreases at low infection intensities (Knopp et al., 2015; Clements et al., 2018).

In this prospective field evaluation, Flow-S demonstrated high diagnostic performance for detection of low-intensity *S. haematobium* infection. All egg-positive samples were detected, while Flow-S also identified most of the additional infections detected by the laboratory-based CAA reference assays. Against the CRS, Flow-S achieved 80.8% sensitivity and 96.6% specificity, meeting the WHO TPP ideal targets for monitoring and evaluation

TABLE 2 Data of samples classified differently by CRS and LCA.

ID	Reference test results			Flow-S CAA (wet) [pg/mL]	CRS - egg | UCAA | SCAA	LCA w/all numerical CAA (model #3) - % likelihood of positivity
	Egg count	SCAA20 CAA (30 pg/mL thresh)	UCAA*hT*417 CAA (2 pg/mL thresh)			
76	0	18.9	0.03	0	Neg	89.7%
75	0	8.13	0.22	0.09	Neg	65.5%
55	0	6.72	0.01	0.15	Neg	65.9%
71	0	4.72	0.02	0.16	Neg	62.7%
15	0	8.38	0.15	0.17	Neg	77.7%
7	0	0	0.41	0.22	Neg	79%
116	0	21.15	0.36	0.26	Neg	100%
100	0	3.02	0.11	0.48	Neg	94.8%
25	0	21.82	0	0.54	Neg	100%
8	0	14.37	1.03	1.07	Neg	100%
94	0	5	0.99	1.14	Neg	100%
4	0	4.18	0.51	2.14	Neg	100%
70	0	2.37	0	3.74	Neg	100%

Green cell background indicates negative classification or values below assay threshold while Red indicates positive status or values above assay threshold. For the purposes of this table, data was sorted by Flow-S measurement, and highest Youden index was used for color-coding Flow-S results.

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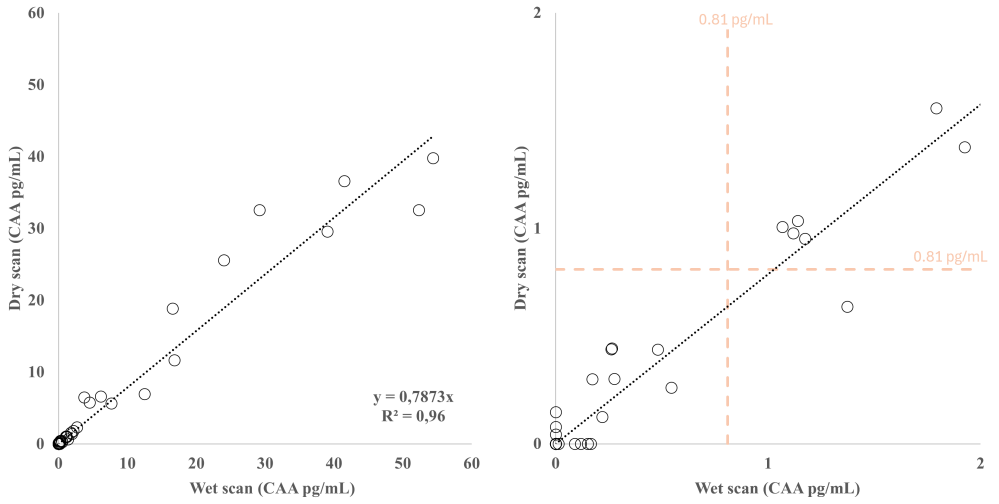


FIGURE 5
Comparison of UCP-LF wet and dry test-strip scans. (Left) Correlation between CAA concentrations measured by Flow-S in the field (wet scan) and after drying and rescanning of test strips in the laboratory (dry scan). (Right) Zoomed-in view of the correlation at low CAA concentrations. The dotted lines represent the linear regression between wet and dry scan results. Dashed lines indicate the 0.81 pg/mL CAA threshold.

using an operational threshold for positivity of 1.10 pg/mL. The maximum Youden index was observed at 0.27 pg/mL, where sensitivity was 88.5% and specificity was 93.3%. The operational threshold can be based on intended use-case linked to the targeted sensitivity or specificity as defined in appropriate TPPs. A lower threshold may be preferable when maximizing detection of low-intensity infections is the primary objective, whereas the higher threshold of 1.10 pg/mL provides the specificity required by the WHO TPP while retaining sensitivity above the ideal target.

The field detection limit of 0.10 pg/mL CAA demonstrates the high analytical sensitivity achievable with Flow-S. However, the clinical significance of measurements at this concentration cannot be established independently because the reference assay formats used are less sensitive than Flow-S. CRS was based on pre-set positivity thresholds and excludes infection in samples with CAA concentrations below those thresholds. The apparent superiority of Flow-S to detect CAA concentrations requires confirmation in future studies. Longitudinal studies focusing on early detection of infection could provide proof in case more sensitive reference assays are not available.

The LCA provided complementary, model-based evidence consistent with the high discriminatory performance of Flow-S observed using the CRS. The ROC analyses showed similar discriminatory performance, with AUCs of 0.947 for the LCA-based classification and 0.923 for the CRS-based classification. Because Flow-S measurements were incorporated directly into the LCA model, the LCA classification is not independent of Flow-S and should not be interpreted as an independent validation of its diagnostic performance. Rather, the LCA provides a model-based assessment that retains the numerical CAA measurements and accounts for uncertainty in the latent infection status. The slightly higher AUC obtained with LCA may therefore reflect the use of continuous CAA measurements rather than reducing the reference assays to binary positive or negative classifications. The CRS defines positivity when at least one reference test exceeds its positivity threshold, whereas the LCA incorporates the quantitative CAA measurements and uncertainty around these thresholds. Consequently, samples with CAA concentrations below a reference-test positivity threshold can still receive a substantial probability of positivity. This may explain why some Flow-S-positive/CRS-negative samples were classified as having a relatively high probability of infection by LCA. These findings provide additional support for the possibility that some Flow-S-positive/CRS-negative results represent detection of CAA below the positivity thresholds of the reference assays rather than false-positive Flow-S results. The CRS-based analysis therefore remains the primary assessment of clinical performance.

The contribution of egg microscopy to reference classification was limited in this cohort. Only seven of the 26 CRS-positive samples were egg-positive, and these samples were also positive by both CAA-based reference assays and Flow-S. Consequently, inclusion or exclusion of egg results had little effect on the overall LCA classification. This confirms the known limitations of parasitological methods in low-intensity infections and supports the use of CAA-based approaches for detecting infections (Knopp et al., 2015; Koukounari et al., 2021; Mesquita et al., 2022; Fok et al., 2024). LCA is useful in this context because it allows evaluation of diagnostic performance when no single reference test can be considered a perfect standard and when a test with potentially better analytical sensitivity is being evaluated (Pepe and Janes, 2007; van Smeden et al., 2014; Clements et al., 2018; Fok et al., 2024).

LCA also provided insight into the relationship between urine and serum CAA concentrations. The estimated urine-to-serum relationship for UCAA*hT*417 corresponded to approximately an 8.1-fold lower CAA concentration in urine than in serum (95% CI, 4.9–13.0), broadly consistent with the approximately 10-fold difference previously described for serum and urine CAA measurements (de Dood et al., 2018; de Jong et al., 2024; Hoekstra et al., 2024). The corresponding estimate for Flow-S was a 13.2-fold lower concentration in urine than in serum (95% CI, 8.1–20.4). These

differences are likely to reflect variation in antigen clearance and excretion between individuals. The estimated ratios also help explain why low-level infections may be detected by one CAA assay but not another. In particular, the SCAA20 threshold of 30 pg/mL and the UCAA*hT*417 threshold of 2 pg/mL represent a 15-fold difference in operational concentration thresholds, which is broadly consistent with the estimated serum-to-urine relationships. Flow-S measurements were generally lower than those obtained with UCAA*hT*417, despite its ability to detect low concentrations of CAA. The reason for this difference may relate to differences in assay workflows, including TCA extraction used for UCAA*hT*417, antibody-binding efficiency, or antigen retrieval yield during the sequential concentration and elution steps of Flow-S.

An important practical consideration is the use of fresh urine. Flow-S is designed to process urine immediately after collection, avoiding potential changes in stored urine that could interfere with passive filtration. Frozen, refrigerated, or otherwise stored urine may form precipitates that could bind CAA or interfere with filtration (de Jong et al., 2024). Immediate testing therefore remains preferable for obtaining a result at the point of sampling. Wet-versus-dry scan comparison showed that UCP-LF strips can subsequently be dried and rescanned, with strong agreement between field and laboratory measurements (R^2 = 0.96) for samples within the standard-curve range. This provides operational flexibility: urine processing can be performed in the field, while strip analysis can, when necessary, be completed later using a laboratory reader. The UCP label also provides a stable record of the measurement, allowing strips to be retained for subsequent analysis.

This study has several limitations. First, the reference assays used in the CRS had lower analytical sensitivity than Flow-S, limiting the ability to conclusively classify samples that were positive by Flow-S but negative by the reference assays. In particular, the CRS was based on predefined positivity thresholds and therefore may classify samples with CAA concentrations below these thresholds as uninfected. Second, the identifiability of the hierarchical latent-variable model warrants consideration. Although the MCMC simulations showed good convergence (R-hat ≤1.01), convergence alone does not establish that all model parameters are independently identifiable. The model therefore relies on its specified parameterization and priors, and the absolute scale of the latent disease-burden variable is defined by fixing one β parameter. In Model #3, serum CAA was used as this reference scale, while the β parameters for urine tests describe relative scaling. The hierarchical model also makes assumptions about the underlying distribution of disease burden. We modeled a latent disease-burden variable on a log scale, reflecting the observed distribution of CAA concentrations and the reported association between CAA and infection burden. This latent variable was defined as proportional to CAA concentration and should therefore be interpreted as a relative disease-burden scale rather than a direct estimate of worm burden. Although this formulation provides a useful framework for integrating quantitative CAA measurements, the influence of the assumed burden distribution should be considered when interpreting the model-based results. The LCA therefore provides complementary model-based evidence rather than an independent validation of Flow-S performance, with the CRS-based analysis remaining the primary assessment of clinical performance. Third, the evaluation was conducted in a single endemic setting and included a modest sample size, which provided an initial evaluation of performance against the WHO TPP targets. Finally, the study focused on *S. haematobium*, and Flow-S performance may differ for other *Schistosoma* species. Limited available evidence suggests that circulating worm antigen levels may be higher in infections with other major human schistosome species, such as *S. mansoni* (De Jonge et al., 1989; Hoekstra et al., 2024), but further evaluation is needed.

Future studies should include how urine volume affects analytical and clinical performance; the modular design of Flow-S allows adaptation of sample volume. Lower volume could reduce time to result and facilitate POC applications, whereas larger volume could further increase analytical sensitivity and potentially support pooled-sample strategies in low-endemicity settings. Pooled urine has previously been investigated as a potentially cost-saving approach for population-level assessment of *S. haematobium* infection, although sensitivity depends on pool size and infection intensity (Degarege et al., 2015). Such approaches are useful for surveillance and stratification of populations for targeted mass drug administration and for monitoring program impact. The choice of sample volume and detection format will ultimately depend on the required balance between sensitivity, time to result, and cost.

In conclusion: Flow-S enables quantitative detection of low-intensity *S. haematobium* infections directly from large-volume fresh urine without laboratory-based sample pretreatment. In this field evaluation, Flow-S achieved 80.8% sensitivity and 96.6% specificity at a 1.10 pg/mL CAA threshold, meeting the WHO TPP ideal targets for monitoring and evaluation. Latent class analysis provided complementary model-based evidence consistent with the high discriminatory performance observed against the composite reference standard, while not constituting an independent validation of Flow-S. Further evaluation across transmission settings and *Schistosoma* species is warranted to demonstrate the full potential of Flow-S.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

Ethics statement

The studies involving human participants were reviewed and approved by the National Institute for Medical Research in Dar es Salaam (NIMR/HQ/R8.a/Vol. IX/3625) and Weill Cornell Medicine in New York (20-10022745). Written informed consent was obtained from all participants. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

DJ: Conceptualization, Data curation, Formal analysis, Writing – original draft, Writing – review & editing. CC: Conceptualization, Data curation, Formal analysis, Writing – original draft, Writing – review & editing. JM: Data curation, Writing – review & editing, Writing – original draft. PS: Data curation, Writing – review & editing. LM: Writing – review & editing, Data curation. PH: Writing – review & editing. GD: Writing – review & editing. BM: Conceptualization, Funding acquisition, Project administration, Writing – review & editing. JC: Writing – review & editing. JD: Conceptualization, Resources, Writing – review & editing. JW: Formal analysis, Software, Writing – original draft, Writing – review & editing, Conceptualization. PC: Conceptualization, Resources, Writing – original draft, Writing – review & editing, Formal analysis.

Funding

The author(s) declared that financial support was received for this work and/or its publication. Research reported in this publication was supported by the National Institute of Allergy And Infectious Diseases of the National Institutes of Health under Award Number R43AI172578.

Conflict of interest

CC, BM, and JW are employees of Salus Discovery, which developed the Flow-S device.

The remaining author(s) declared that this work was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Generative AI statement

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpara.2026.1974692/full#supplementary-material

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