Development and evaluation of an antigen targeting lateral flow test for Crimean-Congo Haemorrhagic Fever

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Summary

Background Crimean-Congo Haemorrhagic Fever (CCHF) is a viral haemorrhagic fever with a case fatality rate of 5–25% that has been prioritised for research and development by the World Health Organisation. There are no CCHF rapid diagnostic tests (RDTs) commercially available. We describe the development and evaluation of an antigen-targeting lateral flow immunoassay RDT for CCHF.

Methods Prospective clinical samples were collected and tested between July and October 2023 in Türkiye. Retrospective stored samples were obtained from the Central Public Health Laboratory, Baghdad, Iraq. The sensitivity and specificity of the CCHF RDT was compared to reverse transcription quantitative polymerase chain reaction assays.

Findings On prospective clinical samples in Türkiye, the sensitivity and specificity of the CCHF RDT was 90.4% [95% CI 81.5–95.3%] (n = 73) and 96.2% [95% CI 87.0–99.3%] (n = 52), respectively with a sensitivity of 92.9% [95% CI 84.3–96.9%] (n = 70) in samples with a cycle threshold (Ct) ≤30. On retrospective stored samples in Iraq, sensitivity and specificity of the RDT was 71.7% [95% CI 59.2–81.5%] (n = 60) and 92.5% [95% CI 80.1–97.8%] (n = 40), respectively with a sensitivity of 82.2% [95% CI 68.7–90.7%] (n = 45) in samples of Ct ≤30.

Interpretation The CCHF RDT was an effective rapid diagnostic test in this preliminary clinical evaluation, showing this RDT has the potential diagnostic capability for use at the point-of-care. Definitive evaluation is now required to ensure the RDT meets the regulatory requirements for commercialisation.

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Keywords: Crimean-Congo Haemorrhagic Fever; CCHF; Rapid diagnostic test; RDT; Lateral flow test

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Research in context

Evidence before this study

Crimean-Congo Haemorrhagic Fever (CCHF) is the most widespread tick-borne viral disease globally. The causative agent, Crimean-Congo Haemorrhagic Fever Virus (CCHFV), can cause severe and often fatal haemorrhagic illness, and is endemic in many countries in Africa, the Middle East, the Balkans, Europe and Asia. The World Health Organisation (WHO) has identified CCHF as a priority disease for research and development as it poses a significant public health risk due to its epidemic potential and the insufficient countermeasures in place.

Current diagnostic capabilities remain primarily polymerase chain reaction (PCR) in reference laboratories which are often unsuitable for rapid diagnosis due to the need for high-containment facilities, trained staff and specialised equipment. Timely diagnosis is critical for early triage of the patients, clinical management, surveillance and outbreak containment. WHO has identified development of near patient CCHF diagnostic tests as a strategic goal of its research and development roadmap.

There is currently no antigen targeting lateral flow test available for CCHF. We searched Pubmed, Scopus and Google Scholar using search terms "Crimean Congo Haemorrhagic Fever Virus", "CCHF", "rapid diagnostic test", "lateral flow test", "RDT" and "LFT". From this search we were unable to find any evidence of an antigen targeting CCHF RDT available or that had been evaluated. This newly developed CCHF antigen rapid diagnostic test (RDT) uses monoclonal antibodies generated against recombinant nucleoprotein of the Matin strain (Asia I clade). Monoclonal antibodies were screened and triaged for their binding potential to recombinant CCHF nucleoprotein and optimal monoclonal antibodies were transferred to a lateral flow platform. The prototype RDT was then evaluated on stored, inactivated CCHF serum samples collected from Türkiye between 2015 and 2016 and provided a sensitivity of 82.2% and a specificity of 98.0% compared to RT-qPCR.

Added value of this study

In this study, the prototype RDT was prospectively evaluated across six regional hospital facilities between July and October 2023 in Türkiye and in retrospective, stored samples in the Central Public Health Laboratory in Baghdad, Iraq. The RDT demonstrated high sensitivity (90.4%) and specificity (96.2%) in Türkiye and high specificity (92.5%) with slightly lower sensitivity (71.7%) on stored samples in Iraq. In both countries the RDT met the minimal specificity requirement set out by the World Health Organisation of ≥90%. For prospectively collected samples in Türkiye, the RDT met the requirement of ≥80% sensitivity (90.4%). Increasing positivity of RDT results correlated with decreasing RT-qPCR cycle threshold values in both countries. For cycle threshold values of less than 30, the RDT exceeded the sensitivity requirement in both countries (92.9% and 82.2% in Türkiye and Iraq, respectively). These results demonstrate the feasibility of an antigen targeting CCHF RDT and the usability of a CCHF RDT in primary health facilities.

Implications of all the available evidence

This study provides the first data on a rapid lateral flow immunoassay targeting the CCHF virus. This research is critical as no antigen-targeting rapid lateral flow tests are currently available for this major emerging infectious disease threat. This study demonstrates the capability of a rapid lateral flow immunoassay to provide critical early detection of CCHF. We aim to take this test forward through the complete product development pipeline, to pursue our goal of high-quality accessible diagnostic testing for all settings.

Introduction

Crimean-Congo Haemorrhagic Fever (CCHF) is the most widespread tick-borne viral disease globally with a case-fatality rate of 5–25%.[1] The World Health Organisation (WHO) has identified CCHF as a priority disease for research and development as it poses a significant public health risk due to its epidemic potential and the insufficient countermeasures in place.[2] Diagnostic capabilities are lacking at the point-of-care and require high containment laboratories and skilled laboratory scientists to carry out diagnostic testing.

Crimean-Congo Haemorrhagic Fever Virus (CCHFV) is transmitted to humans through ixodid tick bite (largely Hyalomma spp.) or by direct contact with blood or tissue from an infected animal or human.[3] The disease is asymptomatic in animals but can develop into a potentially fatal illness in humans. It is estimated that >80% of CCHF infections are subclinical and therefore infection rate is difficult to determine.[4,5] However, in a recent systematic review of seroprevalence in the European region, estimated seroprevalence was between approximately 0 AND 15%,[6] with seroprevalence in an endemic region of Türkiye as high as 19.6%.[7] The incubation period is typically 3–7 days with sudden onset of non-specific febrile illness including malaise, headache, joint pain, back pain and vomiting which can progress to severe haemorrhagic syndrome.[8] There is currently no approved vaccine available and treatments for CCHF remain limited and are primarily supportive.[9]

Timely diagnosis is critical in CCHF cases to initiate appropriate therapy and infection, prevention and control measures. Lack of CCHF diagnostics has also severely limited surveillance activities and continues to be a barrier to ongoing therapeutic and vaccine development pathways. Due to the non-specific early clinical presentation of CCHF, diagnostic confirmation is of

vital importance both to rule in and rule out CCHF in endemic settings. The current routine diagnosis for CCHF is based on viral RNA identification through reverse transcription polymerase chain reaction (RT-PCR) and identification of anti-CCHF virus IgM/IgG antibodies through enzyme-linked immunosorbent assay (ELISA) and Immunofluorescence assay (IFA).

At present, CCHF is endemic to countries in Africa, Asia, the Middle East and Europe with increasing frequency of sporadic cases in non-endemic areas being reported over the last few years through nosocomial and community transmission.[10–12] CCHF is primarily found in rural settings[13,14] that can lack the infrastructure or facilities necessary for diagnosis, relying on reference laboratories often found only in major cities. This delay in diagnosis can limit access to effective treatment both for patients with CCHF, and also for those suspected cases who need CCHF to be excluded.[15] Delay in diagnosis also increases the risk of human-to-human and nosocomial transmission that is well reported in CCHF.[16] There is an urgent need for sensitive and specific rapid point-of-care diagnostic tests to be available in rural and low-infrastructure settings. A strategic goal of the WHO Roadmap for Research and Product Development against CCHF is the development and introduction of near-patient and/or point-of-care tests.[17] Rapid diagnostic tests (RDTs) are an ideal screening test for rapid triage in low-infrastructure settings and have been proven successful for other high-risk diseases such as SARS-CoV-2 and Dengue.[18,19] However, not all high-risk disease RDTs have been successful. Both Ebola and Lassa fever RDTs have struggled to achieve acceptable sensitivity levels when evaluated prospectively.[20,21] There is currently no commercially available, regulated rapid diagnostic test targeting CCHF.

Here, we report on the development of an antigen targeting lateral flow immunoassay rapid diagnostic test. This test has been evaluated prospectively on serum samples from patients suspected of CCHF virus infection in regional hospital sites across Türkiye between July and October 2023 and retrospectively on stored CCHF serum samples at the Central Public Health Laboratory (CPHL) in Baghdad, Iraq, comparing RDT result to reverse transcriptase quantitative polymerase chain reaction (RT-qPCR).

Methods

Study design and participants

The CCHF antigen RDT (CCHF RDT) was evaluated on serum samples from patients suspected of having CCHF in Türkiye and Iraq. In Türkiye, prospective clinical samples were collected between July and October 2023 from patients who presented with suspected CCHF infection at six sites (Sivas Cumhuriyet University Hospital, Kastamonu State Hospital, Amasya State Hospital, Bolu Izzet Baysal Hospital, Samsun Ondokuz Mayis University Hospital and Tokat State Hospital). At the sites, venous whole blood was centrifuged for serum separation for Real-Time Reverse Transcription Quantitative Polymerase Chain Reaction (RT-qPCR), with excess serum used to carry out RDT testing per protocol provided by the Liverpool School of Tropical Medicine (LSTM). RDT testing was carried out at the site of sample collection before the sample was sent to the Ministry of Health CCHF reference laboratory in Ankara for diagnostic RT-qPCR testing.

In Iraq, retrospective clinical samples were obtained from a biobank of CCHF patients serum samples stored at the Central Public Health Laboratory (CPHL) in Baghdad. Blood samples were collected from suspected patients, serum was separated by centrifuging and then transported to CPHL, Baghdad in a cooled, triple packed system. A total of 100 suspected specimens were selected for RDT testing and included 40 CCHFV RT-qPCR negative samples and 60 CCHFV RT-qPCR positive samples. Positive samples were selected to span a wide range of cycle threshold (Ct) values from 14 to 39. Samples selected were collected between May and August 2023 from patients in 18 different Iraqi provinces. Samples were frozen in storage at –80 °C before being defrosted and used to carry out RDT testing per protocol provided by the Liverpool School of Tropical Medicine.

Research and Ethics approval was obtained from LSTM research and Ethics committee (IRB00001960). In Türkiye, Ondokus Mayis University Research and Ethics committee (OMUKAEK-2023/118) approved the prospective RDT evaluation with all participants providing written informed consent. In Iraq evaluation of the RDT against stored anonymised diagnostic samples was approved by Central Public Health Laboratory, Baghdad in accordance with ethical approval of the Ministry of Health, Iraq.

Procedures

CCHF lateral flow test development

The CCHF nucleoprotein antigen lateral flow test has been developed using affinity purified monoclonal mouse antibodies specific for the Nucleoprotein (NP) of CCHFV Matin strain (Accession AF527810.1). Nucleoprotein antigen was recombinantly expressed in HEK293 cells using the FreeStyle™MAZ293 Expression System (Life Technologies, USA) and purified using fast protein liquid chromatography. Recombinant CCHF nucleoprotein (NP) was used to develop 47 nucleoprotein specific mouse monoclonal antibodies (mAbs) which were screened against the recombinant Matin strain nucleoprotein and a commercial CCHF nucleoprotein of the IbAr10200 strain (Nigeria 1996, Accession NP_950237) (Native Antigen Company, UK).

Using an IBIS MX96 surface plasmon resonance imager, anti-CCHF NP monoclonal antibodies were screened for their binding affinity and kinetic to CCHF

NP. Optimal monoclonal antibodies were used to produce a lateral flow immunochromatographic assay. Six versions of the prototype lateral flow assay were taken to the Ministry of Health CCHF reference laboratory in Ankara, Türkiye in July 2022 to test on a small number of prospective RT-qPCR confirmed positive (n = 32) and negative (n = 31) samples for proof-of-concept evaluation. These results informed decisions on optimisation of the two optimal performing prototypes. The two optimised prototypes were subsequently evaluated on inactivated RT-qPCR confirmed CCHF serum samples collected between 2015/2016 from Türkiye and available at LSTM before the prospective clinical evaluation in Türkiye and retrospective evaluation in Iraq, reported in this study.

RDT testing procedure

RDT testing was carried out by trained healthcare workers or laboratory staff in Türkiye depending on the site, and laboratory staff in Iraq. Appropriate biosafety precautions were taken when handling samples in accordance with local and national guidelines. All staff were given an identical protocol and training by a member of the research team was provided. Briefly, 10 µl of serum sample was added to 70 µl sample running buffer in a 2 ml screw cap tube and incubated at ambient temperature (18–30 °C) for 5 min. Eighty microlitres of sample and sample running buffer were added to the sample well of the lateral flow device and incubated at ambient temperature for 20 min (no longer than 30 min) for full signal development. Results were scored using the visual score card (Fig. 1a). In Türkiye, RDTs were carried out prior to obtaining RT-qPCR results. In Iraq RT-qPCR results were used to determine samples selected for RDT evaluation.

CCHFV RT-PCR testing procedure

In Türkiye, serum samples were sent to the Ministry of Health CCHF reference laboratory in Ankara and were handled in a category 2+ laboratory in accordance national biosafety guidelines. The Bio-Speedy CCHFV RT-qPCR detection kit (Bioeksen, Istanbul, Türkiye) was used as a reference standard for protocol testing of suspected CCHF patient samples. The Bio-Speedy CCHFV RT-qPCR detection kit targets the N gene of the CCHFV virus. Viral RNA isolation was performed using EZ1 Virus Mini Kit v2.0 and the EZ1 advanced robotic system (Qiagen, Germany), according to manufacturer's instructions with a finale elute volume of 60 µl. RT-qPCR was performed on the Rotor-Gene 5-plex Real-Time PCR system, (Qiagen, Germany) following the manufacturer's instructions for use (IFU). Briefly, the protocol follows a reverse transcription holding step at 52 °C for 5 min, denaturation holding phase at 95 °C for 5 min, followed by 40 cycles of amplification at 95 °C and 55 °C, respectively. A volume of 5 µl of extracted RNA was used per reaction for a final volume of 20 µl.

In Iraq, stored serum samples at the CPHL were handled in a category 2 laboratory in accordance with national biosafety guidelines. Viral RNA was isolated from the samples using the QIAamp viral RNA mini kit (QIAGEN, Germany), according to manufacturer's instructions. Prior to CCHF testing using real-time reverse transcriptase polymerase chain reaction (RT-qPCR), the viral RNA was kept at –80 °C. RT-qPCR testing was performed using the RealStar® CCHFV RT-PCR Kit 1.0 detection kit (Altona Diagnostics GmbH, Germany) on the QuantStudio™ 5 PCR thermocycler (Thermofisher, US) following the manufacturer instructions for use (IFU). The RealStar® CCHFV RT-PCR Kit 1.0 detection kit targets the S segment of the CCHFV virus. Briefly, the protocol follows a reverse transcription holding step at 50 °C for 10 min, denaturation holding phase at 95 °C for 2 min, then 45 cycles of amplification at 95 °C, 55 °C, and 72 °C, respectively. A volume of 10 µl of extracted RNA was used per reaction for a final volume of 25 µl.

Statistical analysis

Data analysis was performed using GraphPad Prism version 10.0 (GraphPad Software, USA). The sensitivity and specificity of the RDT were assessed using 2 × 2 contingency tables. Fisher's exact test was applied for contingency analysis, and the Mann–Whitney U test was utilised to compare differences between groups. All statistical tests were two-tailed, with a significance level set at a P-value of <0.05 and 95% confidence intervals.

Role of the funding source

RDT design and evaluations were funded by the NIHR Health Protection Research Unit in Emerging Zoonotic Infections, The Pandemic Institute, the Medical Research Council and LSTM. Data was analysed and interpreted at LSTM. The corresponding author had full access to all data in the study. The funders had no role in study design, data collection, analysis or report writing.

Results

Development and evaluation of NP-CCHF RDT prototypes in Türkiye

Prior to the clinical evaluations taken place in this study, six prototype versions of the NP-CCHF RDT were taken to the Ministry of Health CCHF reference laboratory in Türkiye for evaluation (Figure S1, Table S1). Two prototypes showed the highest overall results; Prototype 1 had a sensitivity of 75.0% [95% Cl 57.9–86.8%] and a specificity of 90.6% [95% CI 75.8–96.8%] and prototype 2 had a sensitivity of 71.9% [95% CI 54.6–84.4%] and a specificity of 96.8% [95% CI 83.8–99.8%] (Figure S1). These prototypes were optimised by varying the nanoparticle, conjugation pad and

Fig. 1: (a) Visual score card to produce a quantitative score for a positive RDT result. (b) A positive result scoring a 7 on the visual score card (left) and a negative result scoring a 0 on the visual score card (right).

nitrocellulose membrane to increase sensitivity then tested on inactive RT-qPCR CCHF serum samples. The optimised prototypes resulted in a sensitivity for prototype 1 of 82.2% [95% 72.0–89.3%, n = 73] and a specificity of 98.0% [95% CI 89.7–99.9%, n = 51] and for prototype 2, a sensitivity of 78.9% [95% CI 68.0–86.8%, n = 71] and a specificity of 100% [95% CI 93.0–100%, n = 51] (Figure S3). Prototype 1 NP-CCHF RDT was taken forward as the final NP-CCHF RDT prototype for the prospective evaluation in Türkiye and retrospective evaluation in Iraq.

Prospective evaluation of final NP-CCHF RDT prototype in Türkiye

A total of 105 prospective consecutive samples and 20 stored RT-qPCR CCHF negative samples were included in the study. Samples were obtained from patients attending Sivas Cumhuriyet University Hospital (n = 120, including 20 stored negative samples), Samsun Ondokuz Mayis University Hospital (n = 1), Amasya State Hospital (n = 2) and Kastamonu State Hospital (n = 2). Thirty-seven (30.1%) patients were female and 86 were male (69.9%) (Self-reported, two provided no data). The median age was 52 (37–65 interquartile range). Seventy three of the 125 samples were CCHF RT-qPCR positive (58.4%) (Fig. 2) and 52 were negative (41.6%) (no indeterminate results).

The clinical sensitivity of the final NP-CCHF RDT prototype in prospective clinical samples from Türkiye was 90.4%, [95% CI 81.5–95.3%]. The clinical specificity of the final NP-CCHF RDT prototype was 96.2%, [95% CI 87.0–99.3%] (Fig. 2). Sixty-six of the 73 RT-qPCR positive samples (90.4%) were RDT positive, seven were RDT negative (9.6%). The RDT had a 100% test completion rate with no invalid results. Of the 52 RT-qPCR confirmed negative samples, two were RDT positive (3.8%) and 50 were RDT negative (96.2%). The sensitivity of the RDT was inversely correlated to RT-qPCR Cycle threshold (Ct) value (Table 1). Ct ≤30 in positive RT-qPCR samples had an RDT sensitivity of 92.9% [95% CI 84.3–96.9%], Ct ≤25 had an RDT sensitivity of 93.3% [95% CI 84.1–97.4%] and Ct ≤20 had an RDT sensitivity of 100% [95% CI 91.2–100%].

Country	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Türkiye	66	2	7	50	0·904 [0·815, 0·953]	0·962 [0·870, 0·993]
Iraq	43	3	17	37	0·717 [0·592, 0·815]	0·925 [0·801, 0·978]

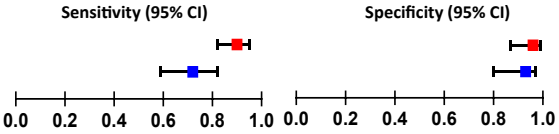

Fig. 2: Forest plot showing the diagnostic accuracy (sensitivity and specificity, 95% CI) of the CCHF RDT in prospective samples in Türkiye and retrospective stored samples in Iraq. TP, True Positive; FP, False Positive; FN, False Negative; TN, True Negative.

Retrospective evaluation of final NP-CCHF RDT prototype in Iraq

In retrospective stored samples from Iraq, 100 patient samples were included. Thirty-Eight (38.4%) were female and 61 (61.6%) were male (self-reported, one provided no data). The median age was 35 (24–48 interquartile range). Sixty (60%) were CCHF RT-qPCR positive and 40 (40%) were RT-qPCR negative (Fig. 2). The clinical sensitivity of the final NP-CCHF RDT prototype was 71.7% [95% CI 59.2–81.5%] and specificity was 92.5% [95% CI 80.1–97.8%] (Fig. 2). Forty-three (71.7%) RT-qPCR positive samples were also RDT positive, while 17 (28.3%) were RDT negative. Of the 40 RT-qPCR confirmed negative samples, 37 were also RDT negative (92.5%) while 3 were RDT positive (7.5%). In this sample set, the RDT also had a 100% test completion rate with no invalid results. Sensitivity in this sample set varied between Ct range (Table 1). Ct ≤30 in positive RT-qPCR confirmed samples had an RDT sensitivity of 82.2% [95% CI 68.7–90.7%], Ct ≤25 had an RDT sensitivity of 95.8% [95% CI 79.8–99.8%], Ct ≤ 20 had an RDT sensitivity of 90.9% [95% CI 62.3–99.5%].

Final NP-CCHF RDT comparative analysis

There was a weak correlation between RDT visual score and Ct value for both the Bio-Speedy CCHFV RT-qPCR detection kit (Bioesken, Istanbul, Türkiye) in Türkiye ($R^2 = 0.22$, $P \leq 0.0001$) and the RealStar® CCHFV RT-PCR Kit 1.0 detection kit (Altona Diagnostics GmbH, Germany) in Iraq ($R^2 = 0.39$, $P \leq 0.0001$).

Comparison between RDT visual score results of positive and negative RT-qPCR samples showed a significant difference in both Türkiye ($P \leq 0.0001$) and Iraq ($P \leq 0.0001$) between positive and negative RDT results (Fig. 3a). There was also a significant difference ($P = 0.002$) in RDT visual score between positive RT-qPCR confirmed Turkish and Iraqi samples (Fig. 3a). The median RDT visual score in Türkiye was 4 whereas the median RDT visual score in Iraq was a score of 1. There was no significant difference in RDT visual score and negative RT-qPCR between the Turkish or Iraqi sample set, both scoring a median of 0 RDT visual score ($P = 0.65$). When RT-qPCR positive results were grouped by Ct value (Fig. 3b), Ct values of <26.00 resulted in an identical RDT visual median score[5] between the two sites (although Turkish samples result in a larger interquartile range), as did the Ct values >31.00 and the negative RT-qPCR samples. The Ct values that range between 26.00 and 30.99 differed in median RDT visual score between the Turkish sample (2.5) and Iraqi sample set[1] ($P = 0.03$).

Category	Value for country	
Sensitivity by Ct (95% CI), N	Türkiye	Iraq
≤20	100% (91.2–100%), 40	90.9%, (62.3–99.5%), 11
≤25	93.3% (84.1–97.4%), 60	95.8%, (79.8–99.8%), 24
≤30	92.9% (84.3–96.9%), 70	82.2%, (68.7–90.7%), 45
≤40[a]	90.4%, (81.5–95.3%), 73	71.7%, (59.2–81.5%), 60
Specificity (95% CI), N	96.2%, (87.0–99.3%), 52	92.5%, (80.1–97.8%), 40

CI, Confidence Interval; Ct, Cycle Threshold; RT-qPCR, Real-time quantitative polymerase chain reaction. [a]Cut off for the Bio-Speedy CCHFV RT-qPCR detection kit (Bioesken, Istanbul, Türkiye) used for the RT-qPCR in Türkiye is Ct 34.

Table 1: **NP-CCHF RDT results based on CCHF RT-qPCR Ct results in Türkiye and Iraq.**

Discussion

Rapid diagnostic tests, specifically lateral flow immunoassays are cost-effective, easily deployable, require minimal specimen processing and provide results within 10–30 min. They have been used successfully in the COVID-19 pandemic to alleviate some of the demands of laboratory testing throughout the world.[22] Despite this urgent need, there are no licenced RDTs that are in routine use for endemic or outbreak prone viral haemorrhagic fevers. CCHF was deemed by the WHO in the 2018 R&D blueprint to be a high priority pathogen in need of urgent development of point-of-care diagnostic tests. The WHO subsequently produced a draft target product profile and a call out for a point of care diagnostic test with a minimum sensitivity of 80% and a specificity of 90%.[23] Here, we report results of an antigen targeting CCHF RDT in two CCHF endemic countries recording the highest cases of CCHF each year,[13,24,25] including the first prospective clinical evaluation across multiple sites in Türkiye.

In the clinical evaluations carried out in this paper, the CCHF prototype lateral flow test was 90.4% (95% CI 81.5–95.3%, n = 73) sensitive and 96.2% (95% CI 87.0–99.3%, n = 52) specific in prospectively enrolled patients in Türkiye. On retrospective stored samples from the CPHL in Baghdad, Iraq the sensitivity was 71.7% (95% CI 59.2–81.5%, n = 60), and specificity was 92.5% (95% CI 80.1–97.8%, n = 40). Prospective clinical

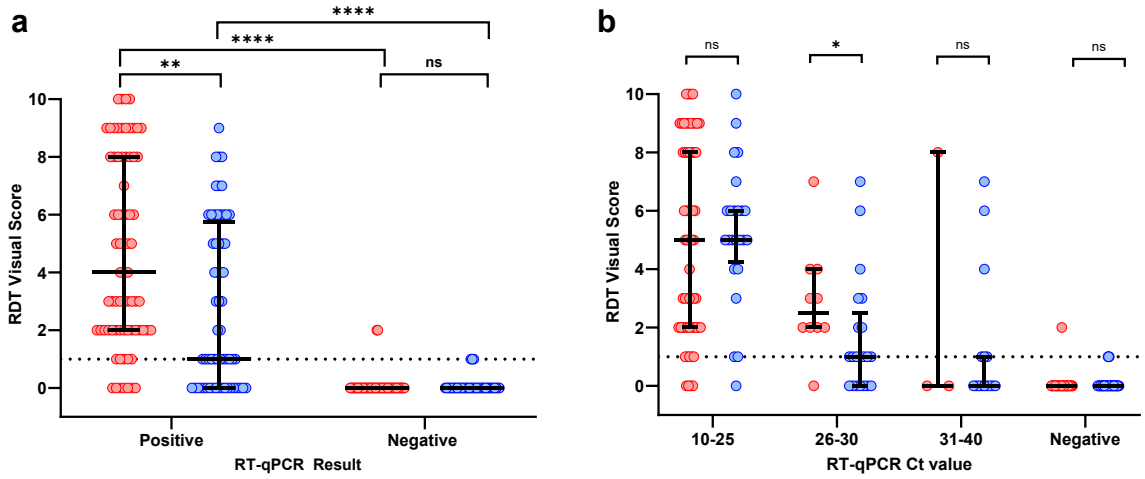

Fig. 3: Correlations of Real-time quantitative polymerase chain reaction (RT-qPCR) results with RDT results in Türkiye and Iraq. (a) Correlation of RT-qPCR result to RDT visual score of Türkiye (Red) and Iraq (Blue) positive (P = 0.002) and negative samples (P ≤ 0.0001). Median and interquartile range shown. (b) Correlation between RT-qPCR Ct ranges and RDT visual score (Türkiye in Red, Iraq in Blue). Ct range 26–30 (P = 0.03). Median and interquartile ranges shown.

serum samples collected from hospital sites across Türkiye between July and October 2023 met the performance criteria determined by the draft target product profile outlined by the WHO (>80% sensitivity and >90% specificity).[23] Although, the sensitivity of the stored samples in the CPHL, Baghdad missed out on the performance criteria, in samples of Ct values ≤30 the RDT performed well within the criteria with a sensitivity of 82.2% (95% CI 68.7–90.7%, n = 45).

Although the performance of the test was below the WHO requirement for sensitivity in samples tested in Iraq, the samples were from a historic stored collection, chosen specifically to cover a range of Ct values to assess the final CCHF RDT prototype in a country not previously evaluated for limit of detection data. As a result, Iraq had a higher proportion of samples with Ct value 30–40 compared to Türkiye (25.0% of samples >Ct30 in Iraq compared to 4.3% of samples in Türkiye). The prospective clinical evaluation across Turkish regional hospitals is closer to an accurate representation of sensitivity and specificity of the RDT compared to patient diagnostic RT-qPCR results. For viral haemorrhagic fevers such as CCHF, it is critical to detect patients with the highest viral load, as these patients present the highest risk of progression to severe disease and death as well as an increase in the risk of nosocomial transmission.[26] The RDT works especially well in Ct <20, with the test detecting 100% of cases (40/40) in the prospective evaluation in Türkiye. The evaluation and approval of RDTs is also a key enabler for future antiviral trials that are planned in Türkiye and Iraq.

No significant correlation between RDT visual score and RT-qPCR Ct value may be due to stability variation of CCHF NP and CCHF RNA in serum or varying quantity released into blood during patients' clinical evaluation period. The prospective clinical evaluation presented in this paper was relatively small (n = 125) and the retrospective sample evaluation may have had other influencing factors (sample storage, freeze/thaw cycles, sample condition), therefore further investigation on a larger prospective sample set is required.

Although the median RDT visual score between the Turkish and Iraqi sample set differs (4–1, respectively), this is likely due to the variation in average Ct value between the two sample sets (average Ct value in Türkiye, 20.67, average Ct value in Iraq, 27.00). Iraqi samples were specifically chosen to cover a wide range of Ct values with the prospective Turkish samples a truer representation of likely Ct values within an exposed population. When categorised into Ct ranges, there was no statistically significant difference in RDT visual score between the two sites in Ct values <26.00 and >30.99. However, there was a difference in sample sets between Ct 26.00 and 30.99 (P = 0.03). The small sample size within the Ct ranges (10 samples in the Turkish sample set with Ct values between 26.00–30.99 and 21 samples in the Iraqi sample set) indicate a larger sample size will be needed to discover if there is a significant difference in RDT score between these geographical regions.

There are a number of limitations within this study, there is currently no gold standard reference test for diagnosis of CCHF virus infection.[23] As a result, two different RT-qPCR kits were used, the Bio-Speedy CCHFV RT-qPCR detection kit (Bioesken, Istanbul, Türkiye) in Türkiye and the RealStar® CCHFV RT-PCR Kit 1.0 detection kit (Altona Diagnostics, Germany) in Iraq. This may have affected the sensitivity and

specificity of the RDT in the two settings, given that strain variation can affect the performance of RT-PCR assays.[27] Additionally, the stored, retrospective sample collection from Iraq was also a limitation. However, as the RDT had yet to be evaluated in any setting, access to these data sets was necessary and valuable for the development of this diagnostic test.

The next stage of this development pathway, based on these key data, has been the refinement of the prototype RDT in collaboration with Global Access Diagnostics (GADx). Further funding has been secured from the UK Medical Research Council Developmental Funding Pathway Scheme to continue to a larger scale, multi-national evaluation across all regions affected by CCHF, including Africa, to demonstrate pan-linage detection of this test including obtaining sequence data from confirmed cases. All collaborating sites will carry out conformational PCR using the same RT-PCR kit to ensure comparative analysis can take place. In addition to this, the next steps in the development of the RDT will be to evaluate this prototype test on pathogens that present with similar symptoms and occupy the same geographical regions as the CCHF virus to evaluate cross-reactivity of this test.

Samples tested on this prototype to obtain initial clinical sensitivity and specificity were serum. Initial development of this test accounted for whole blood samples in addition to serum samples. Therefore, further development and access to whole blood clinical samples will enable the optimisation of this lateral flow assay to provide true point-of-care diagnostic testing.

This study demonstrates the capability of a rapid lateral flow test to provide critical early detection of CCHF. We aim to take this test forward through the complete product development pipeline, to pursue our goal of high-quality accessible diagnostic testing for all settings.

Contributors

Conceptualisation: CRT, TEF, EA. Data curation: CRT, AD, MJ, YC, RIK. Formal analysis: CRT, YC, AICA. Funding acquisition: EA, TEF, AICA. Investigation: CRT, IB, PB, AD, MJ, HH, FGA, BAU, YC, HCB, ANP, TNT, MB, SAB, ET, YO, FAY, MA, RIK, HAS, ZAM, AAH, NAA, AICA, IMA, ZAM, IRA, RAA, SGM. Methodology: CRT, TEF, EA, AD, AICA. Project administration: CRT, IB, YC, SGM, GK, TEF, EA, AICA. Resources: AD, MJ, PB, YC, SGM, RIK, GK. Software: CRT. Supervision; TEF, EA, GK, YC, AICA. Validation: TEF, EA. Visualisation: CRT. Writing, original draft: CRT. Writing, reviewing and editing: All Authors. TEF and EA accessed and verified the underlying data. All authors have read and approved the final version of the manuscript.

Data sharing statement

The raw data for this evaluation is available from the corresponding author upon reasonable request.

Declaration of interests

LSTM intends to out-license the antibodies to facilitate the future commercialisation of the rapid diagnostic test (RDT). Additionally, we are evaluating the potential for filing a patent application for the antibodies.

EA collects salary from the Liverpool School of Tropical Medicine and Global Access Diagnostics.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.ebiom.2024.105460.

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