ClearTrachoma: Evaluation of a Novel Molecular Rapid Diagnostic Device for the Detection of Chlamydia Trachomatis in Trachoma-Endemic Areas

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Abstract

**Background:** The clinical signs of active trachoma are often present in the absence of ocular *Chlamydia trachomatis* infection, particularly following mass drug administration. Treatment decisions following impact surveys and in post-control surveillance for communities are currently based on the prevalence of clinical signs, which may result in further unnecessary distribution of mass antibiotic treatment and the increased spread of macrolide resistance alleles in ‘off-target’ bacterial species. We therefore developed a simple, fast, low cost diagnostic assay (DjinniChip) for diagnosis of ocular *C. trachomatis* for use by trachoma control programmes.

**Methods:** The study was conducted in London, Germany and Tanzania. For clinical testing in Tanzania, specimens from a sample of 350 children between the ages of 7 to 15 years, which were part of a longitudinal cohort that began in February 2012 were selected. Two ocular swabs were taken from the right eye. The second swab was collected dry, kept cool in the field and archived at -80°C before sample lysis for DjinniChip detection and parallel nucleic acid purification and detection/quantification by qPCR assay.

**Results:** Djinnichip was able to reliably detect >10 copies of *C. trachomatis* per test and correctly identified 7/10 Quality Control for Molecular Diagnostics *C. trachomatis* panel samples, failing to detect 3 positive samples with genome equivalent amounts £10 copies. Djinnichip performed well across a range of typical trachoma field conditions and when used by lay personnel using a series of mock samples. In the laboratory in Tanzania, using clinical samples the sensitivity and specificity of DjinniChip for *C. trachomatis* was 66% (95% CI 51–78) and 94.8 (95% CI 91–97%) with an overall accuracy of 90.1 (95% CI 86.4 – 93).

**Conclusions:** DjinniChip performance is extremely promising, particularly its ability to detect low concentrations of *C. trachomatis* and its usability in field conditions. The DjinniChip requires further development to reduce inhibition and advance toward a closed system. DjinniChip results did not vary between local laboratory results and typical trachoma field settings, illustrating its potential for use in low-resource areas to prevent unnecessary rounds of MDA and to monitor for *C. trachomatis* recrudescence.

**Background**

Globally, approximately 40 million people, mostly children, are thought to have active trachoma at any given time[1]. The World Health Organization (WHO) recommends mass community-based antibiotic treatment (MDA) coupled with facial hygiene and environmental improvements in districts where the prevalence of the clinical sign of trachomatous inflammation – follicular is $\geq 10\%$. However, a critical issue for control programmes is the poor correlation between disease signs and infection particularly after MDA; the long-term persistence of clinical signs leads to uncertainty about the impact of programmes [2].
Currently there is no testing for infection within national programmes. There is an urgent need for low-cost, simple to use tests for *Chlamydia trachomatis* to guide district-level treatment decisions, particularly around whether MDA can be discontinued, and to support the WHO-led process to validate elimination in formerly endemic regions. In order to be suitably sensitive and specific, the diagnostic test should detect *C. trachomatis* nucleic acid, require minimal sample preparation and testing should be performed at a district-level laboratory [3, 4]. Several nucleic acid amplification (NAAT) tests are available which have excellent sensitivity and specificity (>95%) for chlamydial infections, including the Cepheid Xpert CT/NG test (result in 90 minutes) and the Atlas Genetics io® CT/NG test (result in 30 minutes) which require minimal sample preparation [5, 6]. Both however require specialised proprietary instruments and a mains power supply, are cost prohibitive and the throughput is too low for programmatic use (>US$10/test).

A novel isothermal NAAT diagnostic device for *C. trachomatis* detection was developed; “DjinniChip”, intended to be utilised by trachoma surveillance and control programmes. The test was designed to be performed primarily at the district level laboratory by lay personnel and requires only a portable heat block, able to be powered by 12V car battery, with a total test time of approximately 50 minutes. The approximate cost per test after scale-up is <US$2, however the test is not yet available commercially and its performance has not been formally evaluated. The aim of this study was to perform a preliminary evaluation of the performance of the DjinniChip from archived ocular clinical samples in a trachoma-endemic region in-country, initially in the controlled environment of a research laboratory. The secondary aim was to determine the DjinniChip’s resilience and usability when used in typical conditions and facilities where trachoma is endemic and by lay personnel.

**Methods**

**Study design, participants and sample size**

A retrospective study was conducted using archived conjunctival swab samples collected from children in trachoma-endemic communities in northern Tanzania to assess the performance of the DjinniChip. In February 2012, we recruited a cohort of children aged 6–10 years from three adjacent trachoma endemic villages in Kilimanjaro and Arusha regions, Northern Tanzania. All children, between the ages 3 and 11 years, who were normally resident in one of the three villages, were eligible for inclusion (n = 666) [7]. 616 children were enrolled and the cohort was followed-up every 3 months for 4 years, for a total of 17 time points. Two conjunctival swab samples were collected consecutively from the right eye at each time point. Timepoint 14 (August 2015) was selected for this evaluation and 428 children were seen, 9 months after the last mass community treatment with antibiotic as detailed by the SAFE strategy. Conjunctival swabs were collected as previously described using sterile polyester-tipped swabs [7, 8]. The first swab was collected into 250µl RNA later and was used elsewhere [7–9], whilst the second swab was collected dry and stored at -80 °C until used in this study. 0.5% of swabs collected were ‘air’ control swabs in order to test for field and laboratory contamination. These samples were collected by passing a swab 10 cm from a participant’s everted eye, these were labelled and processed identically to participant samples.
The study sample size was determined by the number of Djinnichips manufactured and shipped to KCRI-BL for the study (n = 600) and the number of participants seen at timepoint 14 (n = 428). Based on the observed infection prevalence 9 months after MDA treatment round 1 (timepoint 7) and 2 (time point 11) the expected prevalence of ocular infection was estimated at 8%. The precision of the prevalence estimate based on this sample size at the 95% confidence level is 5%. Alternatively, assuming the index test (Djinnichip) achieves sensitivity and specificity of 95%, a sample size of 428 would achieve an odds of diagnosing a true (qPCR) positive of 62% (95% CI 51–72%) and an odds of 1% (95% CI 0–2%) of a false negative.

The WHO endorsed SAFE strategy (surgery for trichiasis [in-turned eyelashes], antibiotics, facial cleanliness, and environmental improvement) was implemented in the study villages as approved by the Tanzanian Ministry of Health (MoH). Education about environmental improvements and facial hygiene was provided by the field team, free trichiasis surgery was offered, and all members of the three villages (including study participants) were offered azithromycin for trachoma control immediately following timepoints 3, 7 and 11 specimen collection in August of the years 2012, 2013, and 2014.

**DNA extraction**

Dry clinical samples were thawed and 400µ l of lysis-amplification buffer (20 mM Tris-HCl, 10 mM (NH₄)₂SO₄, 150 mM KCl, 2 mM MgSO₄, 0.1% Tween® 20, 1% Triton X, pH 8.8) was added to the tube. Samples were vortexed for 30 seconds, pulse centrifuged, and incubated for 10 minutes at 95 °C for sample lysis. Lysates were then pulse centrifuged and incubated at room temperature for 10 minutes. For the positive control (PC), 46 µl of lysis-amplification buffer was spiked with 4 µl *C. trachomatis* DNA (10,000 omcB copies/µl) derived from *C. trachomatis* L2/434 which was originally cultured and prepared at LSHTM. For the negative control (NC), 50 µl of lysis-amplification buffer was used. One hundred microliters of sample lysate was used for DNA extraction and *C. trachomatis* detection by quantitative PCR (qPCR) and 25µ l was used for *C. trachomatis* detection by DjinniChip. Remaining sample lysate was stored at -20°C.

**qPCR detection of C. trachomatis**

One hundred microliters of sample lysate was mixed 1:1 with 100µ l of sterile PBS and DNA was extracted using the QIAamp DNA mini kit (Qiagen Ltd, Manchester, UK) following the manufacturer's instructions. DNA was eluted into 60µ l of buffer AE and stored at 4°C prior to qPCR testing. *C. trachomatis* was detected using a reference qPCR assay, which has previously been evaluated against droplet digital PCR and Artus (Qiagen Ltd, Manchester, UK) *C. trachomatis* diagnostic assays for ocular samples [10]. The triplex qPCR assay detects chlamydial chromosomal (omcB) and plasmid (pORF2) targets and a human endogenous control gene (RPP30). Samples were tested in duplicate, with each 20µ l reaction containing 1X TaqMan Multiplex Master Mix (Thermo Fisher Scientific, Inchinnan, UK), 300 nM of all primers and probes and 4 µl DNA. Samples were considered *C. trachomatis* positive if RPP30 in combination with pORF2 and/or omcB targets amplified in < 40 cycles in either or both replicates. qPCR data were processed in STATA v15 and target concentrations were calculated by
extrapolating from a standard curve. Clinical information from participants at timepoint 14 was available to the assessors of the reference qPCR test but the result of the index test was masked until the laboratory study was completed.

Droplet digital PCR (ddPCR) detection of C. trachomatis

ddPCR was used to detect and quantify *C. trachomatis* infectious load in 12 archived conjunctival clinical samples and in Quality Control for Molecular Diagnostics (QCMD, Glasgow, UK) panel samples for the preliminary evaluation of DjinniChips. Dry, frozen conjunctival swabs from 4 *C. trachomatis*-negative and 8 *C. trachomatis*-positive individuals, previously determined by an in-house 16S rRNA qPCR [11], were incubated for 10 minutes at room temperature in 400 µl elution buffer (1x PBS or 1% Triton X-100 + 10 mM Tris-HCl [pH7]). 200 µl of eluate was frozen for DjinniChip evaluation. DNA was extracted from the remaining 200 µl using the Blood and Serum DNA Isolation Kit (BioChain Institute Inc, Newark, CA, USA). For QCMD samples, DNA was extracted using the QIAamp Mini DNA Extraction (Qiagen Ltd, Manchester, UK). *C. trachomatis* plasmid and genome were quantified as previously described [12].

Preparation of DjinniChips

DjinniChip is an integrated diagnostic platform which amplifies DNA from the chromosomal *porB* gene of *C. trachomatis* using loop-mediated isothermal amplification (LAMP). The chips were produced by injection-moulding of cyclic olefin copolymer in Kunststoff-Zentrum in Leipzig (Germany). The reagents for loop-mediated isothermal amplification (LAMP) were freeze-dried in the channel of the chip. Each portion of reaction mix contained 300 nmol MgSO$_4$, 16 U glycerol-free Bst 3.0 polymerase (New England Biolabs (Frankfurt am Main, Germany), 25 mmol dA, dG, dC, 22.5 mmol dU, 0.5 mmol dT (Jena Bioscience, Jena, Germany), 10 pmol of primer B3 and F3, 80 pmol primer BIP, 60 pmol unlabelled primer FIP, 20 pmol 5’-biotin-labelled FIP, 40 pmol 5’-FAM-labelled primer LB and 40 pmol 5’-biotin-labelled primer LF (Eurofins Genomics, Ebersberg, Germany), 0.25 mg/ml (w/v) BSA and 6% trehalose (Sigma Aldrich, Taufkirchen, Germany). The primer sequences are provided in Table 1. The freeze-dried reagents were overlayed with a 4.3 × 35 mm PES filter membrane (Merck Millipore, Darmstadt, Germany), 0.22 µm pore size. A lateral flow test strip DetectLine Basic (Amodia, Braunschweig, Germany) was placed in the chip, and the bottom surface of the chip was sealed with Masterclear® real-time PCR Film (Eppendorf, Hamburg, Germany). DjinniChips were manufactured < 6 weeks prior to testing and were stored in vacuum-sealed sachets at 4 °C until use. The quality of each batch of the chips was verified by testing a positive (0.001 ng *C. trachomatis* DNA) and a negative control on random chips from the batch.
### Table 1
LAMP primers for the detection of *C. trachomatis* by DjinniChip.

| Primer | Sequence 5'-3' |
|--------|----------------|
| F3     | CTCTGCTTCTGCGGAGAT |
| B3     | CAGCGAACGATCAGGAAG |
| FIP    | AGGAGAAGGACCAACCCCAGCTCAGGTA AAAAGAT GTCCCT |
| BIP    | TTCCGACAACAAAAACGCAAGGAAAAAGCTACAG CTACAC |
| LF     | CTGGTGTACAGAGGTAACGAC |
| LB     | CGATCTCGTGA ACTGTA ATCTCAAT |

**DjinniChip detection of *C. trachomatis***

Twenty-five microliters of sample lysate was mixed with 25µ l lysis-amplification buffer and the entire 50µ l was added to the DjinniChip. Sample extract was driven by capillary effect into the reaction channel, dissolving the lyophilized reagents. The inlets of the chip were sealed with Air-O-Seal membrane (4titude, Berlin, Germany). Following incubation on the flat bed of a heat block for 35 min at 65 °C for the LAMP reaction, the reaction was stopped by placing the DjinniChip on a flat ice block (cooling battery) for 2–5 minutes. The amplified product was then manually pumped towards the integrated lateral flow strip (LFS) by injecting 180 µl chromatographic buffer (Amodia, Braunschweig, Germany) into the sample inlet. Biotin- and fluorescein (FAM)-labelled amplicons allowed LFS detection after 10 minutes incubation at room temperature, by use of gold nanoparticles attached to anti-fluorescein-antibodies (Fig. 1). A positive control signal (C) and signal at the test zone (T) corresponded to *C. trachomatis* in the patient sample. Results were recorded electronically and photographic records were taken of each test result.

Samples were analysed using a second DjinniChip if a) the negative no-template control (NC) was weakly or strongly positive for the analysed batch of samples, or b) if the test band was only weakly positive (Figure S1). In either of these cases positive samples were re-tested, with additional positive and negative controls. The outcome of the second test was used as the final result: if the NC was negative but the sample was weakly positive again, the sample was determined positive. Clinical information and the reference test results were masked to the assessors of the Djinnichip results. The study was conducted and reported according to the STARD guidelines for diagnostic studies (Additional file 1. Table S1).[13]

**Mock samples**

In order to determine the resilience and usability of the DjinniChip, 100 mock samples were tested in field conditions. Mock samples consisted of a 50 µl suspension of *C. trachomatis* A2497 elementary bodies at 4 different concentrations (below) in PBS which were added to sterile polyester-tipped swabs and stored dry in 1.5 ml tubes at -80 °C for a minimum of one week. *C. trachomatis* A2497 was grown in HEp-2 cells...
and purified as previously described and quantified by ddPCR [14]. Four field sites were selected; two humid and warm and two hot, dry and dusty. Results were compared to replicate mock samples tested on DjinniChips at Kilimanjaro Clinical Research Institute-Biotechnology Laboratory (KCRI-BL). At each of the five sites, 20 mock samples ranging in \( C.\ trachomatis \) concentration were tested (quadruplicates of negative control, 160 copies/swab, 800 copies/swab, 3200 copies/swab, 16000 copies/swab); 10 duplicates by an experienced lab technician and 10 duplicates by a field nurse who received basic training. A 12V car battery powered heat block (Thermo Fisher Scientific, Inchinnan, UK) was used to heat samples at field sites and DjinniChip testing was performed in the open boot of a Land Rover. Samples were transported on ice in a cool box and reactions were stopped on the surface of flat ice blocks. Results (including photograph), temperature, humidity and time to process 10 tests was recorded. Remaining sample extract was frozen at \(-20^\circ\text{C}\) prior to DNA extraction (Qiagen Ltd, Manchester, UK) and qPCR testing for \( C.\ trachomatis \).

**Results**

**Preliminary evaluation**

DjinniChip performance was initially tested in a European research and development laboratory (Fraunhofer IZI, Leipzig) using anonymised conjunctival dry swab samples that had been collected and stored in an identical manner from a previous study [15] to that described for time point 14 in parallel with a QCMD panel. Of the 12 clinical swabs tested, the DjinniChip correctly diagnosed all samples as \( C.\ trachomatis \) positive or negative; 8/8 positives and 4/4 negatives (Table 2; Additional file 1 Figure S2). The concentration of the sample with the lowest chlamydial load, correctly identified as positive by the DjinniChip, was 36 genome copies per reaction (corresponding to 573 copies per swab; quantified by ddPCR). The QCMD panel consisted of the extracted DNA of urogenital \( C.\ trachomatis \) serovars from swabs and urine. Of the 10 QCMD samples tested, 2/2 negatives and 5/8 positives were correctly diagnosed by the DjinniChip (Table 3; Additional file 1. Figure S3). The 3 false negatives were the 3 lowest concentration samples, each at 10 target copies per reaction.
Table 2
Evaluation of DjinniChip in eluted ocular swabs

| C. trachomatis genome copies per swab | C. trachomatis genome copies per reaction on chip (25 µl of sample) | DjinniChip test result |
|--------------------------------------|---------------------------------------------------------------|------------------------|
| 0                                    | 0                                                             | negative               |
| 0                                    | 0                                                             | negative               |
| 0                                    | 0                                                             | negative               |
| 0                                    | 0                                                             | negative               |
| 573                                  | 36                                                            | positive               |
| 1579                                 | 99                                                            | positive               |
| 1794                                 | 113                                                           | positive               |
| 6739                                 | 421                                                           | positive               |
| 13270                                | 830                                                           | positive               |
| 14989                                | 936                                                           | positive               |
| 185611                               | 11601                                                         | positive               |
| 627486                                | 39218                                                         | positive               |
Table 3
Evaluation of DjinniChip in QCMD (Quality Control for Molecular Diagnostics) DNA sample panel.

| Sample Code     | Sample content                                | copies/µl of extracted DNA | Results of DjinniChip test, 10 µl of sample | copies/reaction on chip |
|-----------------|-----------------------------------------------|----------------------------|--------------------------------------------|-------------------------|
| CTDNA17S-01     | C. trachomatis (LGV†)                         | 3                          | Positive                                   | 30                      |
| CTDNA17S-02     | C. trachomatis (LGV)                          | 1                          | Negative‡                                  | 10                      |
| CTDNA17S-03     | C. trachomatis (LGV)                          | 11                         | Positive                                   | 110                     |
| CTDNA17S-04     | C. trachomatis (LGV)                          | 6                          | Positive                                   | 60                      |
| CTDNA17S-05     | C. trachomatis (Genovar F)                    | 5                          | Positive                                   | 50                      |
| CTDNA17S-06     | C. trachomatis (LGV)                          | 1                          | Negative‡                                  | 10                      |
| CTDNA17S-07     | C. trachomatis (LGV) + N. gonorrhoeae (St 49226) | 1                          | Negative‡                                  | 10                      |
| CTDNA17S-08     | Negative                                      | 0                          | Negative                                   | 0                       |
| CTDNA17S-09     | C. trachomatis (Swedish strain)               | 433                        | Positive                                   | 4330                    |
| CTDNA17S-10     | Negative                                      | 0                          | Negative                                   | 0                       |

† Lymphogranuloma venereum
‡ Samples with low copy number identified on DjinniChip as negative

**Evaluation with clinical samples**

The demographic and clinical characteristics of the 350 participants retained in the Djinnichip analysis are shown in Table 4. In the field, 430 dry swab specimens were collected (428 clinical samples and 2 ‘air
controls’ as detailed in methods). Of these, 352 dry swab samples were eluted and tested in parallel using the DjinniChip and the reference test (qPCR) at the KCRI-BL research laboratory, Moshi, Tanzania (Table 5. Additional file 1. Table S2). Seventy-eight consecutive dry swab samples (CD101-178) were excluded due to a laboratory error in specimen processing. There were 18 DjinniChip false negatives relative to qPCR diagnosis, 9 of these had high chlamydial load by qPCR, with over 100 copies per µl of extracted DNA (Fig. 2). The performance characteristics of sensitivity, specificity, PPV, NPV and accuracy for all 352 specimens (350 clinical samples and 2 air controls) are shown in Table 5. The Djinnichip identified fewer specimens from those with the clinical signs of trachoma (21 v 30) and tended to identify more specimens from individuals with normal healthy conjunctivae without any clinical signs of trachoma (31 v 23).
Table 4
Demographic details and the distribution of the severity of clinical signs of trachoma in the study and evaluation sample population

|                  | Reference test | DjinniChip test |
|------------------|----------------|-----------------|
|                  | All (Time point 14) | Retained in test evaluation | Ct negative | Ct positive | Ct negative | Ct positive | Ct negative | Ct positive |
| N                | 428 | 350 | 297 | 53 | 298 | 52 |
| Median age (range) | 10 (7–15) | 10 (7–15) | 10 (7–14) | 9 (7–14) | 10 (7–14) | 9 (7–14) |
| Sex: female (%)  | 194 (45.3) | 161 (46) | 152 (52.9) | 32 (60) | 157 (52.7) | 32 (61.5) |
| F-score          | 353 (82.5%) | 283 (80.9%) | 266 (89.6%) | 17 (32.1%) | 257 (86.2%) | 26 (50%) |
| P-score          | 391 (91.3%) | 317 (90.6%) | 284 (95.6%) | 33 (62.2%) | 277 (93.0%) | 40 (76.9%) |
| C-score          | 397 (92.8%) | 328 (93.7%) | 275 (92.9%) | 52 (98.1%) | 278 (93.6%) | 49 (94.2%) |
| FPC-scores: Clinical signs were graded using the 1981 WHO Detailed Trachoma Grading System (FPC). This divides the clinical signs into several four-point (0–3) increasing severity scales: follicles (F), papillary inflammation (P), and conjunctival scarring (C). This system corresponds to the WHO Simplified Trachoma Grading System in the following way: Trachomatous inflammation-Follicular (TF) is equivalent to F2/F3 and Trachomatous inflammation-Intense (TI) is equivalent to P3.
### Table 5
Diagnostic performance of the DjinniChip on archived clinical samples

|                      | qPCR negative | qPCR positive | Total |
|----------------------|---------------|---------------|-------|
| DjinniChip negative  | 282*          | 18            | 300   |
| DjinniChip positive  | 17            | 35            | 52    |
| Total                | 299           | 53            | 352   |

**Djinnichip v non-commercial qPCR**

|                      | Value (%) | 95% CI         |
|----------------------|-----------|----------------|
| Sensitivity          | 66        | 51.7–78.5      |
| Specificity          | 94.3      | 91.1–96.7      |
| PPV                  | 67.3      | 55.5–77.3      |
| NPV                  | 94        | 91.5–95.8      |
| Accuracy             | 90.1      | 86.4–93        |

*includes 2 air controls which tested negative by both qPCR and Djinnichip

### Field evaluation

In order to test the performance and usability of the DjinniChip in field conditions, a series of mock swabs spiked with *C. trachomatis* were tested at four field sites in northern Tanzania. Replicate mock swabs were also tested in the laboratory at KCRI-BL, Tanzania for comparison.

In the laboratory, 8/8 *C. trachomatis*-positive mock swabs, ranging in concentration from 160 to 16,000 copies per swab (equivalent to 10 to 1000 copies per DjinniChip reaction), were correctly diagnosed as positive by the DjinniChip and by qPCR (Additional file 1. Table S2.). Duplicate negative control swabs were correctly diagnosed as negative by both methods.

In the field, 62/64 *C. trachomatis*-positive mock samples were correctly diagnosed as positive by the DjinniChip (Additional file 1. Table S3.) There were two false negatives, both with concentrations of 50 copies per DjinniChip reaction; one tested by an experienced technician and one by a field nurse. In contrast, 10/16 negative control samples tested in the field were incorrectly diagnosed as *C. trachomatis* positive by the DjinniChip. Six false positives were tested by a field nurse and four by an experienced technician. qPCR testing confirmed that all 16 controls were *C. trachomatis* negative, suggesting that cross contamination had occurred in the field after the sample lysis step.

### Discussion
We developed and evaluated a LAMP-based first generation DjinniChip assay using ocular swabs collected in the course of a longitudinal trachoma cohort study in Moshi, Tanzania. DjinniChips enabled fast, simple processing and molecular diagnosis of *C. trachomatis* infection in resource limited environments typical of trachoma endemic regions. The results demonstrated high specificity of the test (94.3%) but modest sensitivity (66%), which requires improvement before the DjinniChip can be considered for use in trachoma control programmes.

Sensitivity was affected by both false-positive results (17/52 (32.6%)) strongly impacting PPV, and false-negative results (18/300 (6%)) impacting NPV. The lower sensitivity of Djinnichip also resulted in a lower frequency of detection of infection in those with clinical signs of active trachoma but a higher frequency of infection in those with no clinical signs of disease. The results overall are more typical of an untreated trachoma endemic population where current infection is detected in around 60% of active trachoma cases [16]. Given the age range of the cohort sampled at timepoint 14 (7–14 yrs) the prevalence of infection (14.9%) and clinical signs of active disease (10.8%) are higher than expected after 3 rounds of MDA and would suggest that TF and infection in the indicator group (TF_1−9 yrs) is likely higher [17]. As result the national trachoma control program delivered a fourth round of community treatment with azithromycin in September 2015 to one of the 3 villages where the TF_1−9 prevalence was >10% after conducting a treatment impact survey.

The majority of the false-negative results were due to inhibition of the reaction, which could be overcome by dilution of the sample lysate. The assay design currently uses 25 µl of sample lysate (combined with 25 µl buffer); this large amount of sample lysate may negatively affect the performance of the test due to the presence of excess proteins (such as DNAses and proteases that are incompletely inactivated by heating), blood, inflammatory exudate, and dust. In 10 of 18 false-negative samples the inhibition was overcome by repeating the test with a 1:10 dilution of sample lysate to diluent. This is a well described characteristic of NAATs which is overcome in the same manner with some commercial tests such as Roche CT/NG Amplicor [18]. Alternatively, stabilizing additives such as increased concentrations of albumin or sheared carrier DNA can be added to the diluent. Further sample dilution and the use of additives will be optimised in the next iteration of the DjinniChip.

The extreme sensitivity of LAMP to contaminating DNA most likely explains the false positivity rate [19–21]. DNA ‘contamination’ may occur either during DjinniChip manufacture or more likely during use. Standard precautions to avoid DNA contamination during manufacture and assembly were taken. The false-positive results observed were predominantly found in cases where the test result was ambiguous, evidenced by a faint band on the lateral flow strip. With the exception of one sample (with a high copy number), true positive samples were characterized by clear distinct bands, even when only a low copy number was present. DNA contamination during testing most likely occurred during sample lysis and loading, or post-amplification when the seal was removed to enable the amplified reaction mix to be pumped towards the lateral flow test strip. DNA contamination is frequently caused by aerosol and in the molecular laboratory this can be more easily controlled with aerosol barrier tips and the spatial/physical
separation of the procedures. This is more difficult to control in a general laboratory, local health facility and under differing environmental conditions in the field where a closed system would offer a solution.

Although the susceptibility to sample inhibition and cross-contamination negatively affected the sensitivity of the test, other characteristics of the DjinniChip favour its use for rapid diagnostics in resource-limited settings. Due to the simplicity of the test, the DjinniChip can be used in the absence of laboratory infrastructure. The isothermal DNA amplification was performed on a simple and cheap 12V heating block powered from a car battery; its use requires minimal operator training and needs no software. All reagents are pre-stored on the chip and it requires storage at only 2-8 °C. Furthermore, sample preparation is relatively simple and the entire test can be completed in 50 minutes, with assessment of test result by visual inspection. Other published isothermal assays for *C. trachomatis* are based on conventional laboratory processing and thus far have not been translated into suitable formats for use in low resource settings [22–24]. Although there are some rapid and point-of-care tests available for *C. trachomatis* detection, there is currently no integrated molecular test developed for trachoma that is characterized by the same level of technical simplicity as the DjinniChip. Shin and colleagues developed a mobile magnetofluidic platform for the extraction and amplification of *C. trachomatis* DNA (primarily designed for sexually transmitted infections (STI)), however their device is reliant on a specialised instrument [25]. Most recently SelfDiagnostics Deutschland GmbH (Leipzig, Germany) released a fully integrated device for the detection of *C. trachomatis* and *N. gonorrehea* in urine (STD Multitest); the device is self-contained and suitable for home-use, however its format (internal complexity and construction) results in high production costs making it sub-optimal for neglected tropical disease control programmes [26].

The DjinniChip maintained stable performance over the entire duration of the study (> 4 weeks including shipping with storage at 4 °C). A preliminary accelerated ageing study performed by Fraunhofer IZI (data not shown) found that DjinniChip performance remained unaffected for at least 10 weeks when stored at ambient temperature (~ 23 °C). Field testing in Tanzania revealed that DjinniChip detection was not impeded by temperature ranges between 25–36 °C with humidity between 19–50%, even in two field sites with very dusty and windy conditions. The main factor affecting DjinniChip performance in the field was the high number of false positives, probably due to cross-contamination. In the field it was not possible to segregate each stage of sample preparation, DNA amplification and detection, as all processes were conducted in the open boot of a Land Rover, and over half of No Template Control samples were positive. Work towards the next iteration of DjinniChip therefore needs to focus on its translation into a fully closed integrated format with no manual handling of liquids in order to improve test specificity. DjinniChip sensitivity will also be improved by further investigation of sample matrix impact on the assay to prevent inhibition.

The lower sensitivity of Djinnichip

**Conclusions**
The DjinniChip rapid molecular diagnostic test for C. trachomatis has promising potential, however it requires further development to improve its suitability for use by trachoma control programmes. Its strengths are the simple operation, fast time-to-result, instrument-free detection, usability in the field with limited infrastructure and only a 12V car battery, and its stability in challenging environmental conditions. The estimated approximate cost per test of < US$2 after scale-up makes it a suitable candidate for diagnostic programmes in the future. The current limitations of the DjinniChip are its susceptibility to DNA contamination due to the manual sample- and detection buffer-handling steps and sporadic inhibition of the reaction by sample matrix, both of which can be eliminated in the next iteration of the device. We were also limited by our reduced sample size due to sample loss and higher than expected prevalence of ocular infection. For the Djinnichip to be useful for trachoma control programmatic decisions post MDA only a modest improvement in sensitivity may be required to maintain sensitivity of the test at lower infection prevalence associated with elimination targets otherwise the large and therefore expensive sample size required may be required. Conversely the higher than expected infection prevalence with the reduced sample size may have inflated the index test specificity. The Djinnichip test detected just over half of qPCR test positives correctly and performed well in typical environmental conditions of trachoma. The test mostly likely currently has sufficiently high specificity since it would be used to make treatment decisions at the community level.

The DjinniChip was designed for use by non-specialists in district level laboratories or health centres in trachoma endemic areas for the surveillance of C. trachomatis. Following improvement of the limitations described above, the DjinniChip holds promising potential to prevent unnecessary rounds of MDA and to rapidly detect recrudescence of infection in the community.

**Abbreviations**

omcB
outer membrane complex B ; ORF:open reading frame; LAMP:loop-mediated isothermal amplification; LFS:lateral flow strip; MDA:mass drug administration; NAAT:nucleic acid amplification test; NPV:negative predictive value; PPV; positive predictive value; QCMD:quality control for molecular diagnostics; qPCR:quantitative polymerase chain reaction; RPP30:Ribonuclease P/MRP Subunit P30; SAFE:Surgery for trichiasis [in-turned eyelashes], Antibiotics, Facial cleanliness, and Environmental improvement; WHO:World Health Organisation.

**Declarations**

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**Ethics approval and consent to participate**
Ethical approval and consent for sample testing was granted by the ethics committees of London School of Hygiene & Tropical Medicine, Medical Research Council Gambia at LSHTM, Kilimanjaro Christian Medical Centre and Tanzanian National Institute of Medical Research. The study adhered to the tenets of the Declaration of Helsinki. Study participants were aged between 4 and 11 years at baseline and 7 and 15 the time of timepoint 14 sample collection. Clinical signs and swab specimens were collected following written informed consent from a parent or guardian.

**Consent for publication**

Not applicable

**Availability of data and material**

Data cannot be shared publicly without a request for a data transfer agreement from the Tanzania national ethics committee. Individual requests for transfer of data can be directed to National Institute for Medical Research in Tanzania (contact via ethics@nimr.or. tz) for researchers who meet the criteria for access to confidential data.

**Competing interests**

The authors have declared that no competing interests exist.

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**Authors’ contributions**

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Formal analysis: TRD, NS

Funding acquisition: TRD, NS, MJH, MJB, DK.

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Figures
Schematic principle of the DjinniChip. a: Components and processes. The DjinniChip contains lyophilized reagents (1) and a lateral flow test strip (2). A liquid sample (3) is loaded onto the chip through its inlet. The liquid sample reconstitutes the reagents (4). C. trachomatis DNA is amplified at 65°C. The reaction mix is pumped gently with a syringe to the lateral flow test (5). The appearance of the Control band (C) indicates the correct function of the lateral flow test. The appearance of the Test band (T) indicates the presence of C. trachomatis in the sample. b: Principle of the detection of LAMP product on LFS. c: Detection on DjinniChips.
Figure 2

Concentration of C. trachomatis positive clinical samples by reference qPCR in target copies per microlitre of extracted DNA, showing those diagnosed as positive (blue circles) and negative (red circles) by the DjinniChip.

Supplementary Files

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