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# Fingerstick test quantifying humoral and cellular biomarkers indicative for *M. leprae* infection

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ABSTRACT

*Objectives:* New user-friendly diagnostic tests for detection of individuals infected by *Mycobacterium leprae* (*M. leprae)*, the causative pathogen of leprosy, can help guide therapeutic and prophylactic treatment, thus positively contributing to clinical outcome and reduction of transmission. To facilitate point-of-care testing without the presence of phlebotomists, the use of fingerstick blood (FSB) rather than whole blood-derived serum is preferred. This study is a first proof-of-principle validating that previously described rapid serum tests detecting antibodies and cytokines can also be used with FSB.

*Methods:* Quantitative detection of previously identified biomarkers for leprosy and *M. leprae* infection, anti-*M. leprae* PGL-I IgM antibodies (αPGL-I), IP-10 and CRP, was performed with lateral flow (LF) strips utilizing luminescent up-converting reporter particles (UCP) and a portable reader generating unbiased read-outs. Precise amounts of FSB samples were collected using disposable heparinized capillaries. Biomarker levels in paired FSB and serum samples were determined using UCP-LF test strips for leprosy patients and controls in Bangladesh, Brazil, South-Africa and the Netherlands.

*Results:* Correlations between serum and FSB from the same individuals for αPGL-I, CRP and IP-10 were highly significant ($p < .0001$) even after FSB samples had been frozen. The αPGL-I FSB test was able to correctly identify all multibacillary leprosy patients presenting a good quantitative correlation with the bacterial index.

*Conclusions:* Reader-assisted, quantitative UCP-LF tests for the detection of humoral and cellular biomarkers for *M. leprae* infection, are compatible with FSB. This allows near-patient testing for *M. leprae* infection and immunomonitoring of treatment without highly trained staff. On site availability of test-result concedes immediate initiation of appropriate counselling and treatment. Alternatively, the UCP-LF format allows frozen storage of FSB samples compatible with deferred testing in central laboratories.

## 1. Introduction

Leprosy is an infectious disease caused by *Mycobacterium leprae* (*M. leprae*) that is still a significant health threat in low and middle income countries where it exist in pockets of high endemicity [1,2]. Diagnosis of leprosy in endemic areas is based on the presence of one of three cardinal signs: skin patch with loss of sensation, enlarged peripheral nerves and detection of the causative pathogen *Mycobacterium leprae* (*M. leprae*). The so-called elimination of leprosy as public health problem (prevalence < 1 per 10,000 population) in many previously endemic countries has led to integration of leprosy control into the general healthcare and a decrease in the number of clinical leprosy experts. Consequently, late- and misdiagnosis, as evidenced by the considerable number of leprosy-associated grade 2 disability when diagnosed, has

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become a critical issue [3]. Moreover, the unabated rate of new case detection indicates that transmission of *M. leprae* is persistent and that current measures for prevention and multidrug therapy (MDT) are insufficient [1].

Contact with leprosy patients, particularly multibacillary (MB) patients who have the highest bacillary load (BI) [4], is a risk factor to develop leprosy or contract infection [5,6]. Although the vast majority of *M. leprae* infected individuals do not develop leprosy, highly infected individuals without clinical symptoms may form a reservoir of infection [7]. Immuno-prophylaxis by vaccination or prophylactic drug treatment with single dose rifampicin (SDR) has been demonstrated to be a successful and safe method to prevent disease in contacts of newly diagnosed leprosy patients with a protective effect of 56% reached in the first two years [8–12]. Thus, although not actually proven to reduce infection and transmission, it is plausible that decreased incidence is caused at least in part by reduced transmission as supported as well by modelling studies [13]. Hence, detection and treatment of these *M. leprae* infected individuals without clinical symptoms, is considered essential to interrupt transmission and can help prevent leprosy [13].

Considering this, it is clearly recognized [14], that accurate, affordable tests are needed to be applied in leprosy control programs, in particular aiming at non-expert settings to detect asymptomatic *M. leprae* infection or leprosy closer to where patients first seek care.

To identify individuals spreading leprosy bacilli for prophylactic treatment, *M. leprae* infection needs to be measured objectively. Levels of antibodies directed against the *M. leprae*-specific Phenolic Glycolipid I (PGL-I) closely correlate with bacterial load and higher risk of developing leprosy [15–17]. Thus, it would be rationale to focus on highly seropositive individuals in current post-exposure prophylactic trials [10,12], especially if a stronger antibiotic regimen is applied [18]. Additionally, since MDT treatment of MB leprosy patients leads to reduction of bacillary load [19,20], detection of *M. leprae* specific antibodies to monitor treatment efficacy, represents an additional application for POC (point of care) tests.

Besides determination of the levels of antibodies we recently showed that additional assessments of cytokines in blood such as IP-10 (interferon-γ induced protein 10), contributed to identification of *M. leprae* infection [21,22]. This indicates the value of combining detection of both cellular-and humoral biomarkers in new diagnostic tests.

Leprosy endemic areas are often short of sophisticated laboratories stressing the need for low complexity diagnostic tests. Using the luminescent upconverting reporter particle (UCP) technology combined with low-cost immune-chromatography (i.e. lateral flow), we have developed and field-evaluated quantitative lateral-flow assays (LFAs) suitable for detection of cytokines and anti-*M. leprae* PGL-I IgM Ab (αPGL-I) in serum [21,23–25] as an alternative for the more elaborate and time consuming laboratory-based enzyme linked immunosorbent assay (ELISA). For detection of the presence of antibodies against *M. leprae*, rapid test relying on visual detection of immunogold particles have been used [15,26–29]. Using UCP-LFA it is also possible to quantify the amount of any type of biomarker present in biological samples [30–33], which will allow assessment of differences in biomarker levels in time as well as adjustable cut-off values to meet sensitivity and specificity requirements for areas with variable leprosy endemicity [21,34].

The user-friendly UCP-LFAs do not require sophisticated analytical laboratory equipment. An inexpensive, lightweight portable reader provides a full instrument-assisted analyses and thereby avoids operator bias. Previously developed UCP-LFAs were applied for the analysis of venous blood and thus required the presence of certified medical staff. A less invasive approach, not requiring the presence of a phlebotomist, is the collection of capillary blood using a finger prick. This method is widely used e.g. by diabetics to check glucose levels [35] and is more suitable for large scale, POC screening efforts for leprosy.

In this study we have investigated whether levels of targeted antibodies and cellular biomarkers could be detected in fingerstick blood (FSB) using previously in-house developed UCP-LFA serum assays [24,25] for αPGL-I and the cytokine IP-10. In addition, we included a newly developed UCP-LFA for the acute phase protein CRP (C-reactive protein) [36] which has been described as a sensitive biomarker for active tuberculosis [37–39] as well as for Crohn's disease [40] (a disease which shares susceptibility genes with leprosy). The correlation between FSB and serum was examined in leprosy patients and contacts from Bangladesh, Brazil, the Netherlands and South Africa.

## 2. Materials and methods

### 2.1. Study participants

HIV-negative, treated and untreated leprosy patients and controls were recruited on a voluntary basis between August 2016 and May 2018 at the Dept. Dermatology, Erasmus MC, University Medical Center, Rotterdam, The Netherlands; The Dr. Marcello Candia Reference Unit in Sanitary Dermatology of the State of Pará, Marituba, Brazil; The Leprosy Mission International Bangladesh (TLMIB) in Nilphamari, Bangladesh and the Dept. Dermatology; Groote Schuur Hospital, Cape Town, South Africa (Table 1 and Supplementary Table S1). In Bangladesh leprosy was diagnosed based on clinical, and bacteriological observations. At the other 3 institutes leprosy histology of biopsies was additionally applied for classification according to Ridley and Jopling [41]. Bacterial index (BI) was determined routinely for MB leprosy patients in Bangladesh and Brazil by bacilloscopic analyses of intradermal smears from the two ear lobes, both elbows and knees, and from skin and/or nerve biopsy samples [17].

### 2.2. Ethics

This study was performed according to the Helsinki Declaration. Ethical approval of the study was obtained from all local ethical boards in The Netherlands (MEC-2012-589), Bangladesh (BMRC/NREC/2010–2013/1534), Brazil (Ethical Appreciation Certificate N° 26,765,414.0.0000.0018) and South Africa (HREC REF: 202/2017). Participants were informed about the study-objectives, the samples and their right to refuse to take part or withdraw from the study without consequences for their treatment. Written informed consent was obtained before enrolment. All patients received treatment according to national guidelines.

**Table 1**
Study participants.

| Test group[a] | Origin[b] | n[c] |
|---|---|---|
| MB or BL/LL | Bangladesh | 27 |
| PB or TT/BT | Bangladesh | 15 |
| HC | Bangladesh | 27 |
| EC | Bangladesh | 12 |
| MB or BL/LL | Brazil | 8 |
| PB or TT/BT or I | Brazil | 4 |
| HC | Brazil | 4 |
| ODD | Brazil | 5 |
| MB or BL/LL[d] | South Africa | 4 |
| HC | South Africa | 1 |
| MB or BL/LL | Netherlands | 3 |
| PB or TT/BT | Netherlands | 6 |
| ODD | Netherlands | 1 |
| | | 117 |

[a] Confirmed clinical status of the patients. BL: borderline lepromatous leprosy; BT: borderline tuberculoid leprosy; EC: endemic control; HC: household contact; ENL: erythema nodosum leprosum; I:indeterminate leprosy; LL: lepromatous leprosy; ODD: other dermatological diseases; MB: multibacillary leprosy; PB: paucibacillary leprosy; TT: tuberculoid leprosy.
[b] Country of origin of patients and controls.
[c] Number of samples tested in this group.
[d] One MB patient was diagnosed with ENL (purple dot in Fig. 1).

### 2.3. Synthetic PGL-I

The disaccharide epitope (3,6-di-*O*-methyl-β-D-glucopyranosyl(1 → 4)2,3-di-*O*-methylrhamnopyranoside) of *M. leprae* specific native PGL-I glycolipid was synthesized and coupled to human serum albumin (synthetic PGL-I; designated ND-O-HSA, approximately 40 disaccharides per molecule) [42]. It was obtained through the Biodefense and Emerging Infections Research Resources Repository (http://www.beiresources.org/TBVTRMResearchMaterials/tabid/1431/Default.aspx).

### 2.4. UCP Conjugates

Lateral flow assays were developed and performed using luminescent up-converting reporter particles (UCP) allowing quantitative detection of the targeted biomarker [43–45]. Sodium yttrium fluoride upconverting nano materials (200 nm, $NaYF_4$:$Yb^{3+}$,$Er^{3+}$) functionalized with polyacrylic acid were obtained from Intelligent Material Solutions Inc. (Princeton, New Jersey, USA). UCP conjugates were prepared with goat anti-human IgM (I0759, Sigma-Aldrich, St. Louis, Missouri, USA), mouse-anti-IP-10 (BC-50; Diaclone Research, Besancon, France) or mouse-anti-CRP (CRP135; Labned.com, Amstelveen, Netherlands) at a concentration of 50 μg antibody per mg UCP according to the method described previously [25].

### 2.5. Lateral Flow (LF) strips

LF strips were assembled by mounting 10 mm glass fiber sample/conjugate pad (Ahlstrom 8964), 25 mm laminated nitrocellulose membrane (Sartorius UniSart CN95) and 20 mm absorbent pad (Whatman Cellulose 470) on a plastic backing. Sample pad and absorbent pad each overlap 2.5 mm with the nitrocellulose, respectively at the beginning and the end. All LF strip components were obtained via Kenosha (Amstelveen, the Netherlands). Using a CAMAG ATS-4 (BCON Instruments B.V., Sint-Annaland, the Netherlands) the nitrocellulose was provided with an assay-specific test (T) line and an upstream Flow Control (FC) line. Ready to use LF strips were stored at ambient temperature in plastic containers with silica dry pad.

For αPGL-I strips the test (T) line comprised of synthetic PGL-I (ND-O-HSA, see above) and the flow-control (FC) line of rabbit anti-goat IgG (RαG; G4018, Sigma-Aldrich) at a concentration of 100 and 50 ng per 4 mm width, respectively. For IP-10 and CRP LF strips the T line comprised mouse-anti-IP-10 mAb (Clone BC-55; Diaclone) or mouse-anti-CRP mAb (Clone C5; LabNed.com, Amstelveen, the Netherlands) respectively, at a concentration of 200 ng per 4 mm width. The FC line comprised goat-anti-mouse IgG antibody (M8642; Sigma-Aldrich). The corresponding UCP reporter conjugate was applied to the sample/conjugate-release pad at a density of 400 ng per 4 mm in a buffer containing 5% (*w/v*) sucrose, 50 mM Tris pH 8.0, 0.6 mM Boraat pH 8, 135 mM NaCl, 0.5% (*w/v*) BSA, and 0.25% Tween-20. The pads were dried 1 h at 37 °C.

### 2.6. UCP-LFAs

FSB was collected using disposable 20 μl Minivette® collection tubes (Heparin coated; Sarstedt). The fingerstick sample was directly mixed with 980 μl (for αPGL-I and CRP) and for IP-10 with 80 μl high salt finger stick buffer supplemented with 1% (*v*/v) Triton X-100 (HSFS; 100 mM Tris pH 8, 270 mM NaCl, 1% (w/v) BSA). The diluted fingerstick sample (50 μl) was immediately flowed on LF strips. Remaining material was frozen at −20 °C.

Serum samples from venous blood were analysed using 50 μl of a 1:50 (αPGL-I), 1:500 (CRP) or 1:5 (IP-10) dilution of serum in HSFS. LF strips were analysed with a portable LF strip reader adapted for the UCP label (ESEQuant *LFR* reader, 980 nm excitation and 550 nm emission; QIAGEN Lake Constance GmbH, Stockach Germany). Results are displayed as the ratio value between Test and Flow-Control signal based on relative fluorescence units (RFUs) measured at the respective lines. For αPGL-I UCP-LFA the Ratio (R) threshold was set at 0.2 according to our previous studies [23].

### 2.7. Statistical analysis

Graphpad Prism version 7.00 for Windows (GraphPad Software, San Diego CA, USA) was used to calculate $R^2$ values for correlations and perform Mann-Whitney *U* tests).

## 3. Results

### 3.1. αPGL-I UCP-LFA - quantification in FSB and correlation with serum

The correlation between αPGL-I levels measured in paired fingerstick blood (FSB) and serum was tested with UCP-LFAs in patients with leprosy, their household contacts, patients with other dermatological diseases (ODD) and healthy controls in cohorts in Bangladesh, Brazil, South-Africa and the Netherlands (Table 1 and Supplementary Table S1). FSB samples were collected using disposable heparinized capillaries that allow safe dispensing of precise volumes of whole blood in a mild lysis buffer. Of 117 locally tested individuals (67 patients; 32 household contacts; 12 endemic controls; 6 ODD) from areas with different levels of leprosy endemicity, 36 patients (Bangladesh: n = 25, Brazil: n = 6, Netherlands: n = 2, South Africa: n = 3) and 7 household contacts (Bangladesh) tested (borderline) positive with the αPGL-I UCP-LFA for FSB in line with their seropositivity levels. Ratio values, the read-out of the UCP-LFA, obtained with FSB and serum demonstrated similar levels of αPGL-I in FSB and serum ($p < .0001$, $R^2 = 0.92$; Fig. 1).

The FSB αPGL-I UCP-LFA was analysed on samples collected from six patients with other dermatological diseases (ODD) such as psoriasis and bullous pemphigoid (Supplementary Table S1). These ODD patients were negative in the FSB αPGL-I UCP-LFA (Ratio values were zero; data not shown). Clinical and histopathological workup of the patient presenting with a leonine facies suggestive of either lepromatous leprosy (LL) or post kala azar derma leishmaniasis (PKDL) diagnosed this patient with scleromyxedema.

### 3.2. αPGL-I UCP-LFA – sensitivity with FSB and correlation with BI in MB patients

As part of the routine diagnostic procedure for multibacillary (MB) patients in Bangladesh and Brazil, the bacterial index (BI) was determined for 33 patients using the invasive technique to obtain slit skin smears (SSS). Eighteen MB patients were BI positive varying form BI 1+ to 6+. BI values correlated well with αPGL-I UCP-LFA ratio values for FSB performed in the field: all BI positive patients were positive in the FSB test whereas six of them without detectable acid fast bacilli in SSS were also positive using this test (Table 2). This indicates that FSB analysis for αPGL-I not only is more patient-friendly but likely more sensitive as well to determine infection.

### 3.3. IP-10 and CRP UCP-LFAs – quantification in FSB and correlation with serum

In previous studies, additional assessment of serum proteins such as IP-10 [21,46] and CRP [36,37] contributed significantly to the identification of mycobacterial infection and detection of leprosy reactions [24]. Therefore, besides UCP-LFAs for the humoral marker αPGL-I, UCP-LFAs for these cellular serum biomarkers were also applied to the FSB sample set of leprosy patients from Bangladesh and the Netherlands (n = 56). Of note is that most leprosy patients were already receiving antibiotic treatment or finished treatment at the time that FSB was collected. As a control cohort 29 Dutch healthy individuals were tested

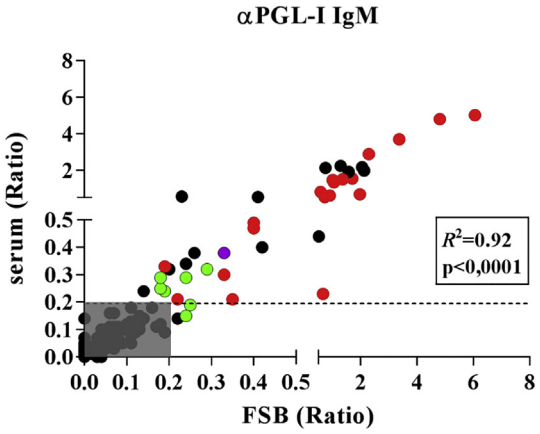


**Fig. 1.** Correlation between αPGL-I IgM levels in FSB and serum.
Levels of αPGL-I (n = 117) determined by UCP-LFA in FSB and serum from HIV-negative, treated and untreated leprosy patients and controls at clinics in Bangladesh, Brazil, the Netherlands and South Africa. Results are shown as Ratio value (R), being relative fluorescence units (RFUs) measured at Test line divided by the signal measured at the Flow-Control line. $R^2$ is the square of the Pearson correlation coefficient. Dotted line ($R = 0.2$) indicates the threshold for positivity applied to serum [23] and FSB assays. Grey area indicates αPGL-I UCP-LFA negative samples (Ratio values below threshold). αPGL-I positive individuals are indicated as follows: MB: red dots; PB: black dots; MB/ENL: purple dots; HHC green dots; none of the EC was seropositive. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

**Table 2**
Correlation of BI with αPGL-I UCP-LFA in FSB from leprosy patients.

| Origin[a] | αPGL-I[b] | BI[c] | Origin[a] | αPGL-I[b] | BI[c] |
|---|---|---|---|---|---|
| Brazil | 9.2 | 6 | Brazil | 0.1 | 0 |
| Bdesh | 2.3 | 6 | Bdesh | 0.1 | 0 |
| Bdesh | 4.8 | 6 | Bdesh | 0.1 | 0 |
| Bdesh | 1.6 | 6 | Bdesh | 0.3 | 0 |
| Bdesh | 1.4 | 6 | Bdesh | 0.2 | 0 |
| Bdesh | 3.4 | 5 | Bdesh | 0.7 | 0 |
| Bdesh | 0.9 | 5 | Bdesh | 0.1 | 0 |
| Bdesh | 6.1 | 5 | Bdesh | 0.6 | 0 |
| Bdesh | 2.0 | 5 | Bdesh | 0.1 | 0 |
| Brazil | 6.6 | 4.5 | Bdesh | 0.0 | 0 |
| Bdesh | 0.4 | 4 | Bdesh | 0.1 | 0 |
| Brazil | 3.6 | 3.5 | Bdesh | 0.0 | 0 |
| Brazil | 1.0 | 3.3 | Bdesh | 0.1 | 0 |
| Brazil | 0.3 | 3 | Bdesh | 0.4 | 0 |
| Bdesh | 1.1 | 3 | Bdesh | 0.2 | 0 |
| Bdesh | 1.0 | 2 | | | |
| Brazil | 0.4 | 1.3 | | | |
| Bdesh | 0.4 | 1 | | | |

[a] Country of origin of patients.
[b] αPGL-I UCP-LFA test results shown as Ratio value. A test is considered positive for αPGL-I if the Ratio value > 0.2.
[c] BI (bacterial index; scale from 0 to 6) is determined by the acid fast bacilli (AFB) staining of the slit-skin smear (SSS) reported as BI WHO (www.who.int/lep/microbiology/en).

(nonendemic controls; NEC). Both CRP and IP-10 could be detected well in FSB showing significantly higher levels in leprosy patients compared to controls and with a highly significant ($p < .0001$) correlation between serum and FSB-values (Fig. 2). Quantitatively, the correlation between FSB and serum was good for CRP ($R^2 = 0.71$; Fig. 2C) and reasonable for IP-10 ($R^2 = 0.61$; Fig.2D). The latter is in line with the reported dependency of cytokine levels on the blood collection method: e.g. for TNFα it was reported that higher levels were detected in venous blood compared to capillary blood, whereas the reverse was found for IL-10 [47]. Possible effects of the anticoagulants [48] were not investigated.

In addition, the in vivo IP-10 concentration is relevantly lower (10 to 100 fold) than generally found for CRP [37,49]. IP-10 FSB assays were therefore performed with 10-fold higher sample load than assays for CRP and αPGL-I. This implies a potentially larger influence of the biological matrix leading to slightly different values in FSB but still with a highly significant correlation with UCP-LFA values obtained with the paired serum samples.

### 3.4. Comparison of fresh FSB vs frozen FSB

The reporter technology applied in the UCP-LFA format is not hampered by lysis of the erythrocytes (the red colour of heme groups of hemoglobin) upon dilution of the FSB in assay buffer [43]. This allows convenient field sampling by collecting FSB and later analysis in a central laboratory. Stability of the biomarkers in lysed FSB samples, stored at −20 °C for 2–6 months in UCP-LFA buffer, was analysed with αPGL-I UCP-LFA and CRP UCP-LFA and compared to results obtained immediately after FSB sampling (fresh). Since the 1:5 diluted FSB samples were completely used for analysis of fresh samples, IP-10 frozen samples could not be tested. For the markers assessed in this study, fresh and frozen FSB samples correlated significantly ($p < 0,0001$; Fig. 3 with $R^2$ of 0.85 and 0.74, respectively for the αPGL-I and CRP LFA). Moreover, all αPGL-I positive samples remained positive after storage at −20 °C using the previously determined cut-off of 0.2 or higher for Ratio (R) values [23].

## 4. Discussion

Although leprosy is one of the oldest recorded debilitating diseases it has remained a health problem in several countries, predominantly in poor-resourced regions, and imposes a significant social and financial burden on society in these economically underprivileged areas. New tools to break the ongoing transmission are required, in particular low-complexity diagnostic tests for detection of *M. leprae* infection and early diagnosis of leprosy could definitely be game-changers [24,50–52].

Characteristic for leprosy is its unique disease spectrum [41], in susceptible individuals, with on one hand paucibacillary disease (tuberculoid leprosy) accompanied with enhanced serum levels of pro-inflammatory cytokines and chemokines (IFN-γ, IP-10) and on the other hand multibacillary disease (lepromatous leprosy) with high *M. leprae*-specific antibody titres and increased levels of regulatory cytokines like IL-10 [41,53,54].

In our research on test development for mycobacterial diseases we have previously described the use of UCP-LFA to detect antibodies for *M. leprae*-specific PGL-I (αPGL-I) as well as cytokines such as IP-10, CCL4, IL-10 and IFN-γ in serum, plasma, stimulated whole blood [25,55,56] or pleural fluid [50]. It was demonstrated at various field sites [25,51] that UCP-LFA allowed quantitative detection of these biomarkers also indicating the possibility of biomarker monitoring e.g. to assess drug-efficiency during treatment [24]. The combined detection of humoral αPGL-I and cellular immune-markers (IP-10, CCL4 and IL-10) in *M. leprae* antigen stimulated whole blood allowed significant distinction between *M. leprae* infected and non-infected individuals [21]. Moreover, the inclusion of cellular markers increased the sensitivity of the assay for leprosy by 39% compared to the UCP-LFA based on antibodies alone.

In the current study we investigated and successfully implemented the use of FSB in the UCP-LFA rather than analysing serum samples. FSB is minimally invasive (more patient-friendly) and a trained phlebotomist is not required. Additionally to permit full POC use, the applied $NaYF_4$ UCP were integrated in the sample/conjugate-release omitting the in previous studies applied pre-flow sample-reporter incubation step [55]. Hence the here described UCP-LFAs can be performed by general staff in modest field- or mobile clinics and even in patients' homes. In fact FSB can be collected anywhere, and upon dilution in

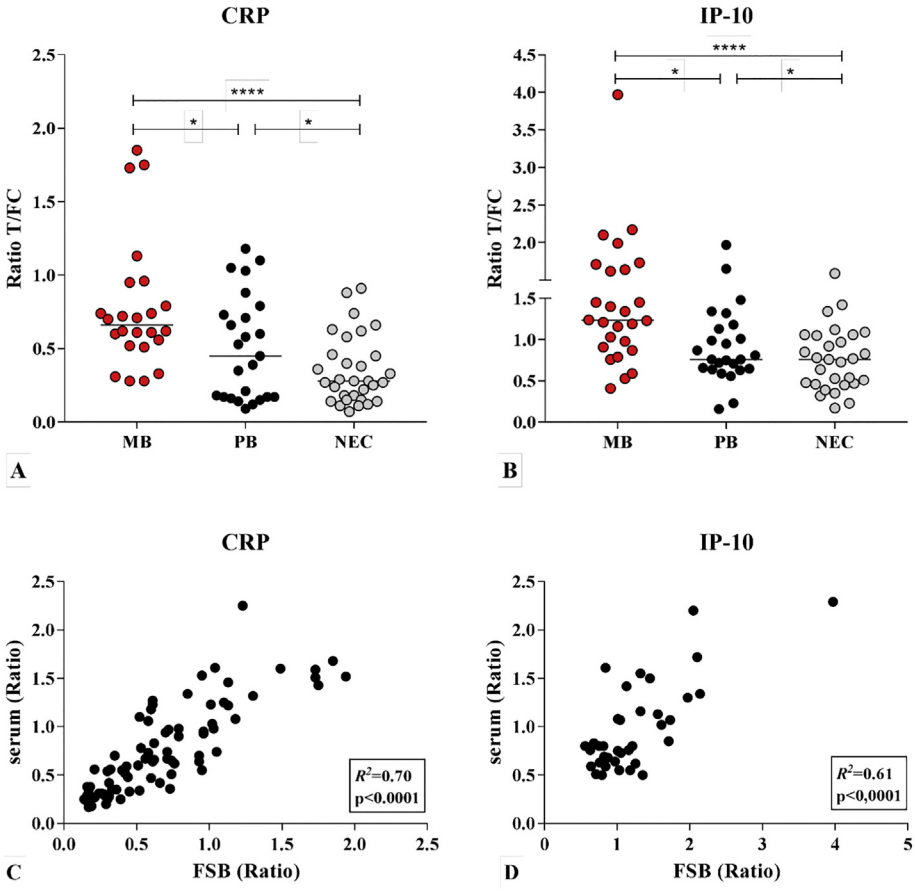


**Fig. 2.** CRP and IP-10 levels in FSB and serum. Levels of CRP (A) and IP-10 (B) were determined in FSB of leprosy patients at clinics in Bangladesh and the Netherlands (n = 56; MB: red dots; PB: black dots) and Dutch healthy individuals (n = 29; NEC). Correlations between serum and FSB are shown for CRP (C) and IP-10 (D). Results are shown as Ratio value, being relative fluorescence units (RFUs) measured at Test line divided by the signal measured at the Flow-Control line. For IP-10 only FSB samples Ratio > 0,5 were considered relevant for correlations according to the quality control of the applied batch of LF strips. $R^2$ is the square of the Pearson correlation coefficient. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

assay buffer (for lysis of the blood cells) stored for later analysis with the UCP-LFAs at a different location making the technology accessible to everyone. The FSB-based UCP-LFA presented in the current study, as with serum, allows quantitative detection of the biomarkers previously shown to be associated with *M. leprae* infection [21,23,25]. Like the analysis with serum, it holds promise for detection of different phenotypes of clinical leprosy when multiplexing humoral and cellular immune biomarkers. After testing, LF strips can be stored indefinitely for reanalysis or as a permanent record [43], the UCP label of this autofluorescence-free reporter technology does not fade [43].

Potential applications of the *M. leprae*-specific FSB-based UCP-LF immunodiagnostic tests described in this study are: i) the identification of *M. leprae* infection and bacterial load (by way of αPGL-I, CRP and IP-10 levels [21,34]) to guide who needs post-exposure prophylactic treatment [57]; ii) the assistance in classification of leprosy in field situations, mostly by αPGL-I levels but since IP-10 levels are increased in MB leprosy [21] measurements of IP-10 in FSB allow for increased sensitivity of the test for detection of leprosy, particularly in populations with low seropositivity for αPGL-I such as Bangladesh; iii) monitoring treatment efficacy [24] by reduction of αPGL-I; and iv) early detection of type 1 leprosy reactions by detecting IP-10 increases [24,58,59]. Moreover, UCP-LFAs may also be applied for differential diagnosis, particularly in endemic areas where other diagnostic aids such as histopathology may not be available. A clinical diagnosis which includes lepromatous leprosy (such as in the patient with leonine facies) with a negative UCP-LF should prompt further investigations. Lastly, the possibility to use frozen FSB samples can be very useful within evaluation studies including samples from different field sites. In large population screening efforts this will facilitate more convenient assessment of biomarkers in e.g. post exposure prophylactic (PEP) and vaccine trials as highly trained staff is not needed for the FSB sample collection.

*In conclusion:* Previously developed UCP-LFAs for detection of humoral (αPGL-I) and cellular markers (IP-10 and CRP) in serum were successfully applied to FSB. The less invasive and user-friendly UCP-LF FSB approach can be utilised to improve early diagnosis of leprosy. Implementation of specific POC diagnostic tests in general healthcare settings has the potential to help reduce disability and eventually social stigma and discrimination, leading to a significant improvement of the quality of life of leprosy patients, reduce medical costs associated with leprosy care and as such contribute to sustainability of healthcare.

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## Author contributions

Designed test device and research: AG, PC.
Enrolled patients, performed and registered clinical diagnosis: CH, CS, LV, MB, JS, SD,
Performed research: AG, AH, ET, MB.
Analysed the data: AG, AH, PC.
Wrote the paper: AG, PC.
Critically reviewed and agreed with the manuscript: all authors.

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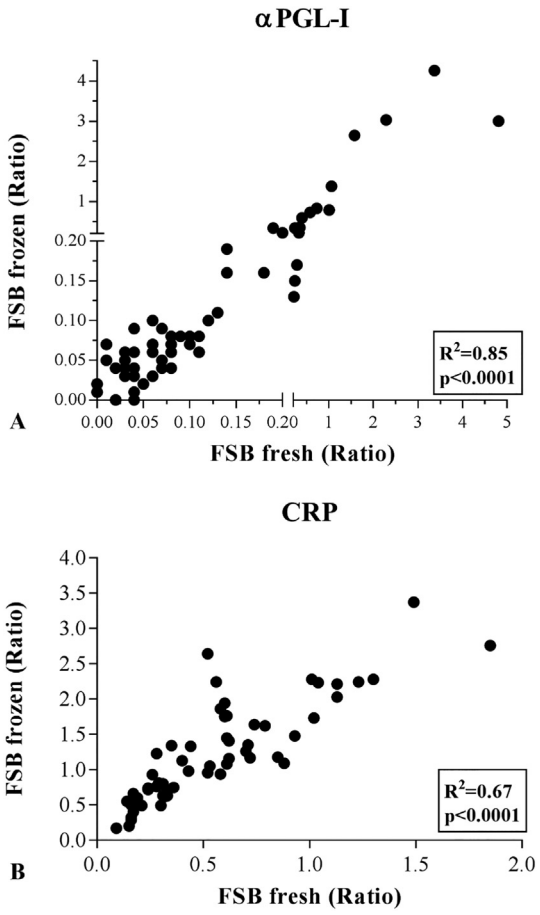


**Fig. 3.** Correlation between biomarker levels in fresh and frozen FSB samples. Levels of αPGL-I (A; n = 81) and CRP (B; n = 61) were determined by UCP-LFA in fresh and frozen FSB at the field clinic in Bangladesh. Equal sample dilution was used for the αPGL-I and CRP comparisons of fresh vs. frozen. Test results are shown as the Ratio value, being relative fluorescence units (RFUs) measured at Test line divided by the signal measured at the Flow-Control line. $R^2$ is the square of the Pearson correlation coefficient.

## Competing financial interests

The authors declare to have no financial/commercial conflicts of interests.

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