

Title

Dried plasma spots in the diagnosis of TB: IP-10 release assay on filter paper

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Running head

IGRAs on filter paper

Abstract

Background

Interferon- γ release assays (IGRA) are probably the most accurate tests for detection of latent *M.tuberculosis* infection, but IGRAs are labour intensive and transport of samples over longer distances is difficult. IP-10 is expressed at 100-fold higher levels than IFN- γ , and IP-10 release assays have comparable performance to IGRA. The aim of this study was to explore the diagnostic potential of a novel IP-10 release assay based on dried plasma spots (DPS).

Methods

IP-10 and IFN- γ was determined in plasma and in DPS by ELISA. Diagnostic algorithms for IP-10 plasma and DPS tests were developed on a training cohort comprising 60 TB patients and 59 controls. The diagnostic accuracy was assessed in a validation cohort comprising 78 TB patients and 98 healthy controls. Plasma was measured in Spain and DPS samples were sent to Denmark with conventional postal service for analysis.

Results

IP-10 was readily detectable in both plasma and DPS, and correlation was excellent ($r^2=0.95$). QuantiFERON Gold-In-Tube (QFT-TB) and IP-10 in DPS and plasma rendered comparable sensitivity (78;82;84%), specificity (100;97;97%) and indeterminate rates ($p>0.55$).

Conclusions

The DPS based IP-10 test has comparable diagnostic accuracy as the QFT-TB and samples can be sent with conventional mail over long distances for analysis without affecting the results.

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Key words:

Dried blood spots, Interferon Gamma release assay, IP-10 release assay, Latent tuberculosis infection, monokine-amplified interferon gamma release assay, resource restraint setting

Introduction

IFN- γ release assays (IGRAs) are likely the most accurate diagnostic tests for latent tuberculosis (LTBI)[1]. There are two IGRAs on the market, the T-SPOT.TB (Oxford Immunotec, UK) and the QuantiFERON-TB Gold In-Tube (QFT-TB, Cellestis, Australia). Both tests utilize the ability of sensitized T cells to release IFN- γ when stimulated with peptides from *M.tuberculosis* specific antigens *in-vitro*. While the first method measures the frequency of reactive lymphocytes in the peripheral blood mononuclear cell (PBMC) fraction, the latter measures the concentration of released IFN- γ in plasma of whole blood [2,3]. The IGRAs address two of the major limitations of the Tuberculin skin test: low specificity in BCG vaccinated subjects and logistic challenges with tests having to be read 2-3 days after application[4].

There are major obstacles with implementation of the IGRA, especially in low resource settings[2]: The T-SPOT.TB requires access to skilled technicians to separate cells and set up the assay for incubation. The time window between blood sampling and laboratory analysis is short and requires that the blood be transported to the lab at ambient temperature. For the QFT-TB, incubation is done directly in the blood collection tubes, and following a centrifugation step, samples can be transported refrigerated or frozen. Transport of these samples requires a cold chain and safety precautions must be considered, as the plasma is potentially infectious. Simpler, safer and more flexible solutions are needed in both high and low resource regions.

We have challenged these obstacles using the biomarker IP-10. IP-10 is a chemokine expressed in concert with IFN- γ in the IGRA[5]. The diagnostic accuracy of the IP-10 release assay has been assessed in 26 clinical studies and it established that the IP-10 release assay performs *at par* with an IGRA based on IFN- γ (reviewed in [5–7]). In

contrast to the T cell derived IFN- γ , IP-10 is mainly secreted from the antigen presenting cells. IP-10 expression is stimulated by multiple signals downstream of the T cell signals, mainly IFN- γ and TNF- α but also IL-2, type II IFNs, IL-27, IL-17/IL-23 and IL-1 β [6,8–10] supporting pathogen specific adaptive immune responses by a positive feedback loop [11]. In-vitro, IP-10 is released in 100 fold higher levels than IFN- γ [12], which allows for alternative methods of storing and transportation of samples.

Blood or plasma blotted onto filter paper has been shown to be a safe, robust and convenient method for collection, transport and storage of small volumes of sample for analysis of e.g. biomarkers[13]. In preparation for this study we have developed and validated an ELISA assay for IP-10 detection for both plasma samples and dried blood and plasma spots (DBS and DPS, respectively) from unstimulated, QFT-TB, and QFT-CMV stimulated whole blood[12].

The aim of this study was to investigate if an IP-10 release assay using whole blood stimulated with antigens specific for *M.tuberculosis* stored as DPS could be used to diagnose *M.tuberculosis* infection. We applied the method to two sets of patients with confirmed TB and healthy controls. We defined cut off and established a diagnostic algorithm based on the first set of patients and controls; subsequently we evaluated the test performance in the other set of cases and controls.

Methods

Study participants: For establishing the cut off and diagnostic algorithm we included a training set comprising 60 microbiologically confirmed patients with active TB included at the Copenhagen University Hospital, Hvidovre, Denmark and 59 healthy

unexposed Danish controls (table 1). The controls were included as part of a larger contact investigation at a high school in Copenhagen[14]. This investigation demonstrated very limited exposure to non-classmates, and we included the controls among non-classmates without travel, family or contact exposure. Evaluation of test performance was conducted in the validation set comprising 78 microbiologically confirmed TB patients from Barcelona, Spain and 107 healthy secondary school students from Valencia, Spain. The controls were included as part of the routine screening of Valencia secondary school students and the majority of the students (61%) were recent immigrants or children of recent immigrants. Students were consecutively included and those who reported known TB exposure, previous TB treatment or lymphadenitis of unknown aetiology were excluded (n=9 of 107 screened). The patients used for validation thus comprised 78 patients and 98 healthy controls.

Blood stimulation and QFT-TB testing: All participants had a QFT-TB test done. IFN- γ levels were determined according to the QFT-TB package insert and excess plasma was stored at -80°C for IP-10 measurements and spotting on filter paper.

Filter paper preparation and storage: QFT-TB stimulated plasma supernatant (25 μ L) was spotted in duplicates on filter paper (903 Protein Saver™ cards, Whatman), dried at ambient temperature for 4 hours and stored in plastic bags with desiccant. Training set samples were stored on filter paper at +5°C for 10 days before analysis, validation set samples were stored 2-4 days at +5C before transport with normal two-day postal service at ambient temperature from Spain to Denmark for analysis. Analysis was done two days after arrival.

IP-10 ELISA (plasma and DPS): We used an in-house ELISA for IP-10 detection[12]. In brief, we developed rat and murine hybridoma cell lines producing monoclonal antibodies (mAbs) specific for IP-10. mAbs were combined in an sandwich ELISA which was optimized for the range of IP-10 responses in QFT-TB samples in both plasma and DPS.

For the assay, plasma samples were diluted x33 in assay buffer with HRP-conjugated detection mAb (corresponding to 3 μ L plasma per 100 μ L ELISA well). For DPSs, 2 circular discs of 5.5mm were punched from the centre of the DPS using a standard office paper-puncher (Impega, UK), DPS discs were placed in the ELISA plate well and 100 μ L assay buffer was added. Diluted plasma samples and DPS discs were analysed using the same protocol i.e. incubation for 2 hours at room temperature and wash x3. HRP-substrate (TMB One, Trichem) was added, plates were revealed for 30 minutes, enzyme reaction was stopped and absorbance was read at 450nm, subtracted readings at 630nm. Concentrations were calculated using a standard curve generated for each ELISA plate in duplicates and fitted with linear regression of the expected concentrations against the mean optical density. IP-10 in plasma was measured in duplicate and the mean was used for further analysis. IP-10 in DPS samples was determined with single measurements. The linear range of the ELISA is 2.5-600pg/ml, a full description of the performance characteristics of the ELISA is available in[12]. Plasma levels of IP-10 are presented after multiplication with 33 to compensate for dilution, DPS levels are presented as pg detected per two 5.5mm discs (pg/2 DPS discs). All samples were analysed blinded from the clinical information.

Logistics

All ELISA reagents were prepared in Copenhagen, Denmark. Plasma samples were measured in Barcelona, Spain (validation set) and Copenhagen (training set). DPS were exclusively measured in Copenhagen.

Ethical

The study was approved by the ethical committee of Copenhagen (KF-01-278477), and by the Ethical Committee of the Hospital Universitari Germans Trias i Pujol, Barcelona.

Statistics

Biomarker levels were compared using non-parametric paired and non-paired methods (Wilcoxon signed rank test, Mann-Whitney U test, Spearman). Cut offs were set using ROC curve analysis (at high sensitivity with minimal loss of specificity) and test concordance was compared using McNemars test and kappa-statistics. The sensitivity and specificity were calculated after omission of indeterminate responders. A p-value <0.05 was considered significant. Analyses were done using SAS 9.2 and GraphPad Prism 5.0.

Results

Comparison of IP-10 in plasma and DPS and defining diagnostic test cut offs using training set samples

We compared IP-10 DPS and plasma samples in the training cohort comprising 60 patients with active TB and 59 healthy controls. Compared to controls the TB patients were older and 62% were born in countries outside Western Europe (clinical data available in table 1). The nil levels and antigen induced IP-10 and IFN- γ responses were significantly higher in TB patients compared to controls, and lower for mitogen responses (table 2). There was an excellent correlation between plasma and DPS IP-10 ($r^2=0.95$, figure 1) while the correlation between IFN- γ and IP-10 was only moderate ($r^2<0.48$, data not shown).

We compared the diagnostic potential of the three assays using ROC curve analysis and found comparable AUCs: 0.95, 0.94 and 0.89, for IP-10 plasma, IP-10 DPS and QFT-TB, respectively (figure 2) with no significant difference between the assays. ROC analysis suggested the following cut off values for an assay with high specificity: IP-10 plasma, 2.3 ng/ml (sensitivity 87%, specificity 100%) and IP-10 DPS, 105 pg/2 discs (sensitivity 87%, specificity 100%). For comparison the optimal cut off for QFT-TB on these data was 0.21 IU/ml (sensitivity 83%, specificity 100%). Vis-à-vis the option in the QFT-TB test algorithm, we defined a cut off for an indeterminate test. We chose the cut off values for an indeterminate test at the highest mitogen response value that did not result in indeterminate responders among the controls: 1.5ng/ml for IP-10 in plasma and 71pg/2 discs for IP-10 in DPS. For analysis of IFN- γ results, the QFT-TB algorithm was used as recommended by the manufacturer.

Validation of the diagnostic performance of the DPS based test using clinical samples

We validated the diagnostic potential of the DPS and plasma IP-10 test algorithms in a new cohort of 78 patients with culture confirmed active TB and 98 healthy controls. Patients were older than the controls ($p < 0.0001$), and more were male ($p < 0.0001$). Eighteen patients had a co-morbid condition (table 1). All but two were TST tested of which 19% were TST positive (cut off 10mm for non-vaccinated and 15mm for BCG-vaccinated) and 61% were BCG vaccinated (table 1).

The correlation between plasma levels of IP-10 and filter paper levels of IP-10 was excellent ($r^2 = 0.93$, $p < 0.0001$), (data not shown). Both methods for IP-10 as well as plasma IFN- γ gave ROC curves with similar AUCs of 0.89-0.91 (data not shown).

Test accuracy and concordance

We applied the IP-10 cut off values for positive and indeterminate test results to the validation set and found that the three tests rendered comparable sensitivity (78-84%), specificity (97-100%) and indeterminate rate (<1% in controls and 5,10,8% in TB patients, $p > 0.55$), see table 4. For comparison we also applied the cohort specific optimal cut off for QFT-TB at 0.21 IU/ml, the sensitivity was 82% (61/74) and the specificity was 97% (95/98); 0 of controls and 4 (5%) of TB patients had indeterminate results. Agreement between DPS and plasma IP-10 tests was very high ($k = 0.91$; 95% CI: 0.88-0.99), and higher than the agreement between the QFT-TB test and IP-10 in plasma ($k = 0.83$; 95% CI: 0.75-91). Combining plasma IP-10 results with QFT-TB test results (i.e. a positive result in either or both tests is considered positive;

indeterminate if one test is indeterminate and the other is not positive) increased the sensitivity significantly to 89% ($p=0.032$) without a compromise in specificity.

Discussion

We investigated the potential of combining the user-friendly filter paper method with an IP-10 release assay with the purpose of diagnosing infection with *M.tuberculosis*. We demonstrated that it is possible to dry plasma from QFT-TB tubes on filter paper, transport samples across Europe at ambient temperature in a letter, and extract the diagnostic information without either loss in signal strength or diagnostic accuracy.

IP-10 is an emerging alternative biomarker to IFN- γ for cell mediated immune response assays such as the IGRA[5,6,15]. The findings in this paper support a series of recent papers which have demonstrated that IP-10 release assays have comparable diagnostic accuracy the QFT-TB in TB patients vs controls[6,16,17]. Other studies have suggested that IP-10 has superior diagnostic performance in HIV infected independent of low CD4 count[18–22]; in patients with auto-immune diseases[23]; in young children[24–27] and in patients suspected of active TB who were diagnosed with other bacterial or viral infections[16]. A consistent finding in all studies, is a slight discordance between IP-10 and IFN- γ responders, which opens the possibility of combining the markers for increased sensitivity, seemingly without affecting specificity[16,17].

The concept of spotting blood onto filter paper for diagnostic purposes was introduced by Dr. Guthrie in 1963 as a tool to screen for metabolic diseases in large populations of neonates[13]. The dried blood or plasma matrix stabilizes many analytes, and allows for determination of both phenotype (e.g. biomarkers) and

genotype (e.g. polymorphisms) from a single specimen [13,28]. Transporting samples dried on filter paper is considered safe[13] and the Whatman 903 filter paper is approved by the FDA (Class II medical device). Neonatal screening using DBS is standard of care worldwide [29] and the filter paper technology is also used for HCV/HIV viral load monitoring and HIV screening in many countries including South Africa[28]

We have developed the filter paper based method for IP-10 detection in stimulated and unstimulated whole blood using a simple ELISA based assay. For optimal detection of IP-10 in antigen-stimulated samples we diluted plasma extensively x33 times (3 μ L plasma per ELISA plate well). The signal intensity generated from this low volume, corresponds well to IP-10 signals generated from 2 DPS or DBS discs, and from the 50 μ L plasma sample used for IFN- γ detection in the QFT ELISA. We recently reviewed 22 clinical studies on IP-10 release assays for the diagnosis of infection with *M.tuberculosis*. We found that IP-10 had been detected 5 very different IP-10 assays and at sample dilutions from x2-x20 [6]. This heterogeneity limits comparison between assays and calls for consensus in the field. The easy sample transport enabled by the filter paper method is ideal for international collaborations and larger multicentre studies, and should lead to better quality and comparable studies on IP-10 release assays.

Skogstrand et al pioneered the measurement of cytokines and chemokines in dried blood spots using a Luminex based multiplex approach, and have validated the clinical use in a series of studies of inflammatory conditions from neonates using

heel blood samples [30,31]. Recently a small proof of concept study assessed the multiplex assay using DBS samples from QFT-TB stimulated blood [32]. This study demonstrated a significant difference in IFN- γ responses between QFT-TB positive patients and QFT-TB negative, but the majority of samples – also from TB patients – fell below the lower limit of quantification of the assay [33]. We have also attempted to extract IFN- γ signals from DPS samples using the QFT ELISA, but also failed to detect reliable responses[12]. The likely explanation for these results is that the low volume of plasma available in the filter paper samples contains too little IFN- γ for reliable determination. IP-10 is expressed in 100-fold higher levels and is therefore better suited for this method[12,34].

Limitations

Our long-term aim is to develop a simple test for latent TB, but as there is no true gold standard for latent TB infection we used patients with active TB as proxy of *M.tuberculosis* infection. This approach provides a solid gold standard for infection, but patients with active TB disease may have compromised IFN- γ and IP-10 responses to mitogens, and very likely also to antigens which could lead to inaccurate cut off values for positive test results if used for e.g. latent TB or in patients with immuno-suppression e.g. HIV infected[16,35,36]. Nevertheless described in detail by Zhou et al[37], evaluation of new diagnostic tests (or diagnostic algorithms) should be performed in several steps; first on selected populations in order to ensure assessment of sensitivity and specificity under (for the test) optimal conditions before the test can be applied in a clinical setting, e.g. a prospective study evaluating the predictive values of the test. The “gold standard

problem” in assessing new tests for latent TB is inherent to the field [38], and all tests for LTBI - including the QFT-TB has been assessed using a similar case-controls design[39]

Our sample size in the training set only comprised 119 patients and controls. This relative small cohort will result in an imprecise assessment of cut off values. But, as we found comparable diagnostic accuracy between the training and the validation set - despite controls from very different settings - we suspect this risk to be limited.

Furthermore the age difference between patients and controls both within and across the training and validation cohorts was significant. These differences might have influenced the findings, but we believe the impact to be minor because our youngest controls were teenagers (and not <5 year olds which is the only age group with documented impaired performance of IGRA) and because IP-10 appears less influenced by young age compared to IFN- γ [24,27]. Nevertheless, further studies are needed and these should be designed to allow for adjustments of the cut off value for a positive test result. In contrast to other studies in similar groups of patients and controls we observed a higher number of patients with indeterminate IP-10 responses compared to QFT-TB [16,17], this most likely reflects a relatively lower threshold for indeterminate test in our study. Previous studies on IP-10 have used arbitrary values to mimic the rates seen for QFT-TB (reviewed in [6]) and the method used to set the QFT indeterminate cut off is to our knowledge not published [39,40]. In this study we set the cut off for indeterminate just below the lowest IP-10 mitogen response observed in the cohort of healthy controls, this to optimize sensitivity (by deeming patients with false negative responses indeterminate) without compromising the cost effectiveness (deeming true negative controls as

indeterminate). Other higher values of the cut off rendered indeterminate rates at- par with the QFT-TB (data not shown). Interestingly, the lower threshold for indeterminate IP-10 test result, did not affect the improvements in accuracy seen when combining IP-10 and QFT-TB [16–18,41,42]. Further studies are needed to evaluate and potentially adjust the cut off, and assessing the risk of developing active TB given a false negative/indeterminate test result.

The findings of this study calls for confirmation in other populations e.g. latent versus active TB, and an assessment of the performance of the method in HIV infected and children, as well as prospective evaluations of the positive and negative predictive value of IGRAs based on IFN- γ and IP-10 are urgently needed.

Perspectives

The filter paper method for plasma sample storage and transport enables a simplification and centralisation of the IGRA diagnostic concept. IP-10 is detectable in DBS samples in comparable levels as in DPS samples, wherefore stimulated samples can be prepared without the centrifugation step[12]. With a simpler test format the work load and requirements from the local labs will make IP-10 release assays easier and cheaper to set up e.g. for low volume users, and peripheral or resource restraint settings. Importantly, the easy sample transport allows for better quality control of the analysis, e.g. the possibility for employing a reference laboratory function. Fully automated high-throughput systems for filter paper punching and sample analyses are standard for neonatal screening, and can be adapted for IP-10 measurement [29]. An automated analysis significantly reduces

the risks of human error and assay variability linked to performing manual ELISA and ELISPOT assays, and increases the analytical precision and diagnostic accuracy.

Conclusion

This study describes and validate a method for simplifying sample handling in an IP-10 release assay for the diagnosis of *M.tuberculosis* infection. We show that IP-10 is stable in plasma dried on filter paper, and can be sent with normal mail over long distances for analysis – apparently without affecting the results. Also, the study adds further to the evidence that IP-10 release assays are a valid alternative to IGRAs, and that such IP-10 release assays have operational advantages using the filter paper approach.

Acknowledgements

The authors would like to thank Ove Andersen, MD, PhD for support and for providing the framework; and TBNET for promoting a Trans-European research collaboration without which this work would not have happened.

Funding

This work was supported by the Danish National Advanced Technology Foundation, The Capital Region of Copenhagen, The Danish Lung Association, The Danish Ministry of Science and Innovation, The Clinical Research Centre at Hvidovre Hospital, and The Lundbeck-Foundation (Denmark). Jose Domínguez is a researcher funded from the Miguel Servet programme of the Instituto de Salud Carlos III (Spain).

Figure legends

Figure 1. Comparison of IP-10 detected in DPS and plasma

DPS and plasma samples from nil, antigen and mitogen samples from the training cohort comprising 60 patients with active TB and 59 healthy controls was compared. There was an excellent correlation between plasma and DPS IP-10 ($r^2=0.95$, Spearman)

Figure 1

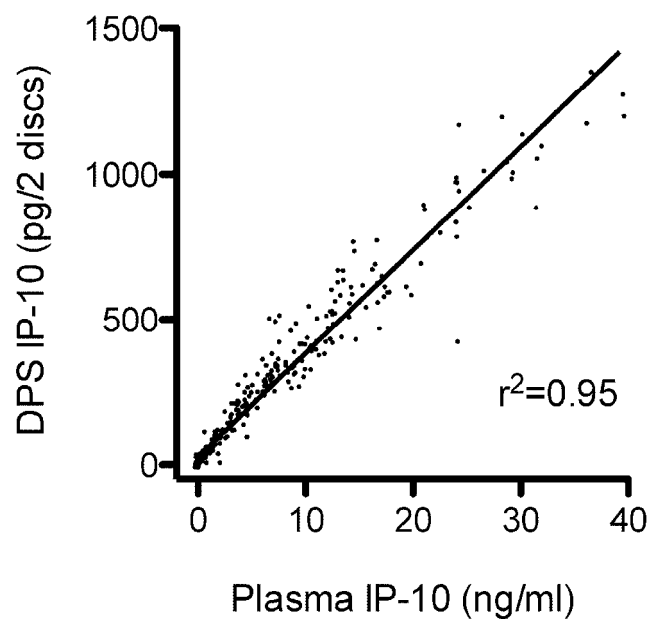
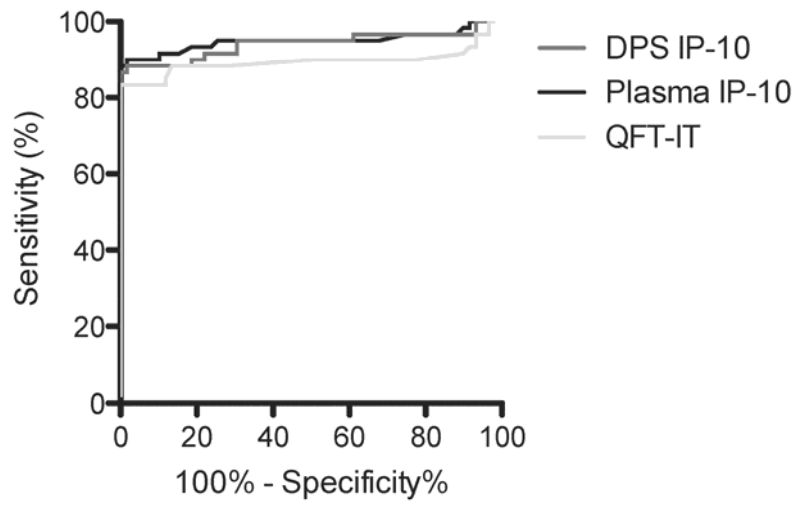


Figure 2. ROC curve analysis

Antigen specific release of IFN- γ and IP-10 was compared in training set samples. IP-10 was detected in DPS and plasma samples, IFN- γ in plasma using the QFT ELISA. AUCs were comparable 0.95, 0.94 and 0.89, for IP-10 plasma, IP-10 DPS and QFT-TB.

Figure 2



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Table 1 – Characteristics of subjects providing training and validation set data. Values are n (%) if not stated as other.

		Training set		Validation set	
		TB	Control	TB	Control
n		60	59	78	98
Age	median (IQR)	40 (31-52)	17 (15-18)	35 (26-45)	14 (13-15)
Male sex		40 (67)	55 (50)	56 (72)	49 (50)
Place of birth	Western Europe	23 (38)	59 (100)	29 (37)	38 (39)
	Africa	19 (32)	0 (0)	15 (19)	1 (1)
	South America	2 (3)	0 (0)	15 (19)	50 (51)
	Eastern Europe	9 (15)	0 (0)	3 (4)	5 (5)
	Asia	6 (10)	0 (0)	11 (14)	4 (4)
	Unknown	1 (2)	0 (0)	5 (6)	0 (0)
BCG status	Yes	0 (0)	0 (0)	10 (13)	59 (60)
	No	0 (0)	59 (100)	28 (36)	38 (39)
	Unknown	60 (100)	0 (0)	40 (51)	1 (1)
Diagnosis	pTB	34 (71)	0 (0)	72 (92)	0 (0)
	EpTB	26 (29)	0 (0)	6 (8)	0 (0)
Comorbidity	None	53 (87)	59 (100)	60 (77)	98 (100)
	Diabetes Mellitus	0 (0)	0 (0)	5 (6)	0 (0)
	HCV	0 (0)	0 (0)	5 (6)	0 (0)
	HIV	7 (13)	0 (0)	3 (4)	0 (0)
	HIV+HCV	0 (0)	0 (0)	2 (3)	0 (0)
	HIV+HBV	0 (0)	0 (0)	1 (1)	0 (0)
	Renal transplant + steroids	0 (0)	0 (0)	1 (1)	0 (0)
	TST (positive/n tested (%))	0/0 (0)	0/53 (0)	16/28 (57)	18/96 (19)
	TST (median mm, IQR)	0.0 (0-0)	0.0 (0-0)	15 (10-18)	9 (6-12)
TST not done /unavailable	60 (100)	6 (10)	50 (64)	2 (2)	
Tests	Culture or NAAT +ve /n tested (%)	60/60 (100)	n.d.	78/78 (100)	n.d.
	Microscopy (+/-/n.d.)	29 (57) / 25(42) / 6 (10)	n.d.	59(76) / 18(23) / 1(1)	n.d.

Table 2 – Training set: Median concentrations of IP-10 in plasma and dried plasma spots (DPS) and IFN-g in plasma

		Training set		p-value
		Controls	TB patients	
IFN-γ Plasma (IU/ml)				
	Nil	0.05 (0.04-0.08)	0.15 (0.07-0.36)	<.0001
	Antigen-Nil	0.01 (0.00-0.02)	2.68 (0.42-6.42)	<.0001
	Mitogen-Nil	8.61 (7.98-9.94)	6.39 (0.76-9.9)	<.0001
IP-10 Plasma (ng/ml)				
	Nil	0.0 (0.0-0.2)	0.6 (0.3-1.4)	<.0001
	Antigen-Nil	0.1 (0.0-0.3)	8.1 (3.6-15.8)	<.0001
	Mitogen-Nil	9.3 (6.7-14.5)	6.3 (2.7-11.6)	0.0024
IP-10 DPS (pg/2 discs)				
	Nil	6 (2-17)	38 (20-69)	<.0001
	Antigen-Nil	5 (0.0-11)	313 (171-567)	<.0001
	Mitogen-Nil	401 (287-729)	287 (113-478)	0.0112

Table 3 – Validation set: Median concentrations of IP-10 in plasma and dried plasma spots (DPS) and IFN- γ in plasma

		Validation set		
		Controls	TB patients	p-value
IFN-γ Plasma (IU/ml)	Nil	0.00 (0.00-0.06)	0.17 (0.05-0.29)	<.0001
	Antigen-Nil	0.00 (0.00-0.05)	1.74 (0.30-4.86)	<.0001
	Mitogen-Nil	10.00 (9.89-10.00)	3.71 (1.13-9.71)	<.0001
IP-10 Plasma (ng/ml)	Nil	0.2 (0.0-0.3)	0.8 (0.4-1.5)	<.0001
	Antigen-Nil	0.2 (0.0-0.4)	8.3 (2.8-17.3)	<.0001
	Mitogen-Nil	13.8 (9.5-17.3)	7.5 (3.6-12.3)	<.0001
IP-10 DPS (pg/2 discs)	Nil	8 (5-19)	35 (16-77)	<.0001
	Antigen-Nil	5 (0.0-17)	355 (137-512)	<.0001
	Mitogen-Nil	467 (388-589)	331 (231-587)	0.0004

Table 4 – Test results, n (%)

		IP-10 plasma	IP-10 DPS	QFT-IT	IP-10 plasma + QFT-IT
TB patients	Positive	61 (78)	58 (74)	58 (74)	64 (82)
	Negative	11 (14)	12 (15)	16 (21)	8 (11)
	Indeterminate	6 (8)	8 (10)	4 (5)	6 (8)
Controls	Positive	2 (2)	2 (2)	0 (0)	2 (2)
	Negative	95 (97)	95 (97)	98 (100)	95 (97)
	Indeterminate	1 (1)	1 (1)	0 (0)	1 (1)