

SUMMARY OF SAFETY AND EFFECTIVENESS

I. GENERAL INFORMATION

Device Generic Name: *Mycobacterium tuberculosis*, Cell mediated immune response, enzyme-linked immunospot test

Device Trade Name: T-SPOT[®].TB Test

Applicant's Name & Address: Oxford Immunotec Ltd.
2 Mount Royal Avenue
Marlborough, MA 01752

PMA Number: P070006

Date of Panel Recommendation: None

Date of FDA Notice of Approval: July 30, 2008

II. INDICATIONS FOR USE

T-SPOT[®].TB is an in vitro diagnostic test for the detection of effector T cells that respond to stimulation by *Mycobacterium tuberculosis* antigens ESAT-6 and CFP-10 by capturing interferon gamma (IFN- γ) in the vicinity of the T cell in human whole blood collected in sodium citrate or sodium or lithium heparin. It is intended for use as an aid in the diagnosis of *M. tuberculosis* infection.

T-SPOT.TB is an indirect test for *M. tuberculosis* infection (including disease) and is intended for use in conjunction with risk assessment, radiography and other medical and diagnostic evaluations.

III. CONTRAINDICATIONS

There are no known contraindications for the T-SPOT.TB test.

IV. WARNINGS AND PRECAUTIONS

The warnings and precautions are contained in the T-SPOT.TB Package Insert.

V. DEVICE DESCRIPTION

A. Principle of Test

The immune response to infection with *Mycobacterium tuberculosis* is mediated predominantly through T cell activation. As part of this response, T cells are

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sensitized to *M. tuberculosis* antigens and the activated effector T cells, both CD4+ and CD8+, produce the cytokine interferon gamma (IFN- γ) when stimulated by these antigens¹⁻². T-SPOT.TB uses the enzyme-linked immunospot (ELISpot) methodology to enumerate *M. tuberculosis*-sensitized T cells by capturing interferon-gamma (IFN- γ) in the immediate vicinity of the T cell from which it was secreted³.

Peripheral blood mononuclear cells (PBMCs) are separated from a whole blood sample, washed and then counted before being added into the assay.

Isolated PBMCs (white blood cells) are placed into microtiter wells where they are exposed to a phytohemagglutinin (PHA) control (a mitogenic stimulator indicating cell functionality), nil control, and two separate panels of *M. tuberculosis* specific antigens. The PBMCs are incubated with the antigens to allow stimulation of any sensitized T cells present.

Secreted cytokine is captured by specific antibodies on the surface of the membrane, which forms the base of the well, and the cells and other unwanted materials are removed by washing. A second antibody, conjugated to alkaline phosphatase and directed to a different epitope on the cytokine molecule, is added and binds to the cytokine captured on the membrane surface. Any unbound conjugate is removed by washing. A soluble substrate is added to each well; this is cleaved by bound enzyme to form a (dark blue) spot of insoluble precipitate at the site of the reaction.

Evaluating the number of spots obtained provides a measurement of the abundance of *M. tuberculosis* sensitive effector T cells in the peripheral blood.

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B. Specimen Collection & Handling

Individual laboratories should validate their procedures for collection and separation of PBMCs to obtain sufficient numbers of PBMCs.

- Blood samples are collected into either sodium citrate or sodium heparin Vacutainer® CPT tubes (Becton Dickinson) and then the PBMCs separated in the tube using the manufacturer's instructions.
- Blood samples are collected into lithium heparin blood collection tubes with PBMCs being subsequently separated using standard separation techniques such as Ficoll-Paque®. **Note EDTA tubes are not recommended.**
- A donor's cells can be pooled, if necessary to obtain sufficient cells, from multiple tubes of blood which were collected and processed concurrently.

Typically, for an immunocompetent patient, sufficient PBMCs to run the assay can be obtained from venous blood samples, using the above methods, according to the following guidelines:

- Adults and children 10 years old and over: one 8mL or two 4mL tubes (CPT) or two lithium heparin 6mL tubes
- Children 2-9 years old: one 4mL tube (both methods)
- Children up to 2 years old: one 2mL pediatric tube (both methods)

Blood samples should be processed within 8 hours.

C. Test Procedure

Peripheral blood mononuclear cells (PBMCs) are separated from a whole blood sample, washed and then counted before being added into the assay's four wells, phytohemagglutinin (PHA) control; nil control; and two separate panels of *M. tuberculosis* specific antigens, ESAT-6 and CFP10. The viable PBMCs are incubated with the antigens to allow stimulation of any sensitized T cells present. Secreted cytokine is captured by specific antibodies on the surface of the membrane, which forms the base of the well. A second antibody is added and binds to the cytokine captured on the membrane surface. A soluble substrate is added to each well; this is cleaved by bound enzyme to form a (dark blue) spot of insoluble precipitate at the site of the reaction. These dark blue spots are counted and depending on how many of them are present, the sample is recorded as positive, borderline/equivocal, or negative for exposure to *Mycobacterium tuberculosis*.

D. Quality Control of Test

The Nil Control should have few or no spots and the Positive Control should have 20 or more spots.

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High numbers of spots in the Nil Control, and high background staining in one or more wells may occur, which makes counting of spots difficult. These results are usually due to operator technique, such as suboptimal plate washing, medium contamination or inappropriate specimen handling and PBMC separation methods. A Nil Control spot count in excess of 10 spots is 'Invalid'.

The cell functionality Positive Control spot count should be ≥ 20 or show saturation (too many spots to count). A small proportion of patients may have T cells which show only a limited response to PHA⁴. If the Positive Control spot count is < 20 spots, it is 'Invalid', unless either Panel A or Panel B are 'Positive' or 'Borderline (equivocal)' as described in the Results Interpretation and Assay Criteria (see below), in which case the result is valid.

Invalid results should be reported as "Invalid" and the user should collect a further sample and re-test.

E. Interpretation of Results

NOTE: Diagnosing or excluding tuberculosis disease, and assessing the probability of LTBI, requires a combination of epidemiological, historical, medical and diagnostic findings that should be taken into account when interpreting T-SPOT TB. Refer to the most recent CDC guidance (<http://www.cdc.gov/nchstp/tb>) for detailed recommendations about diagnosing TB infection (including disease) and selecting persons for testing.

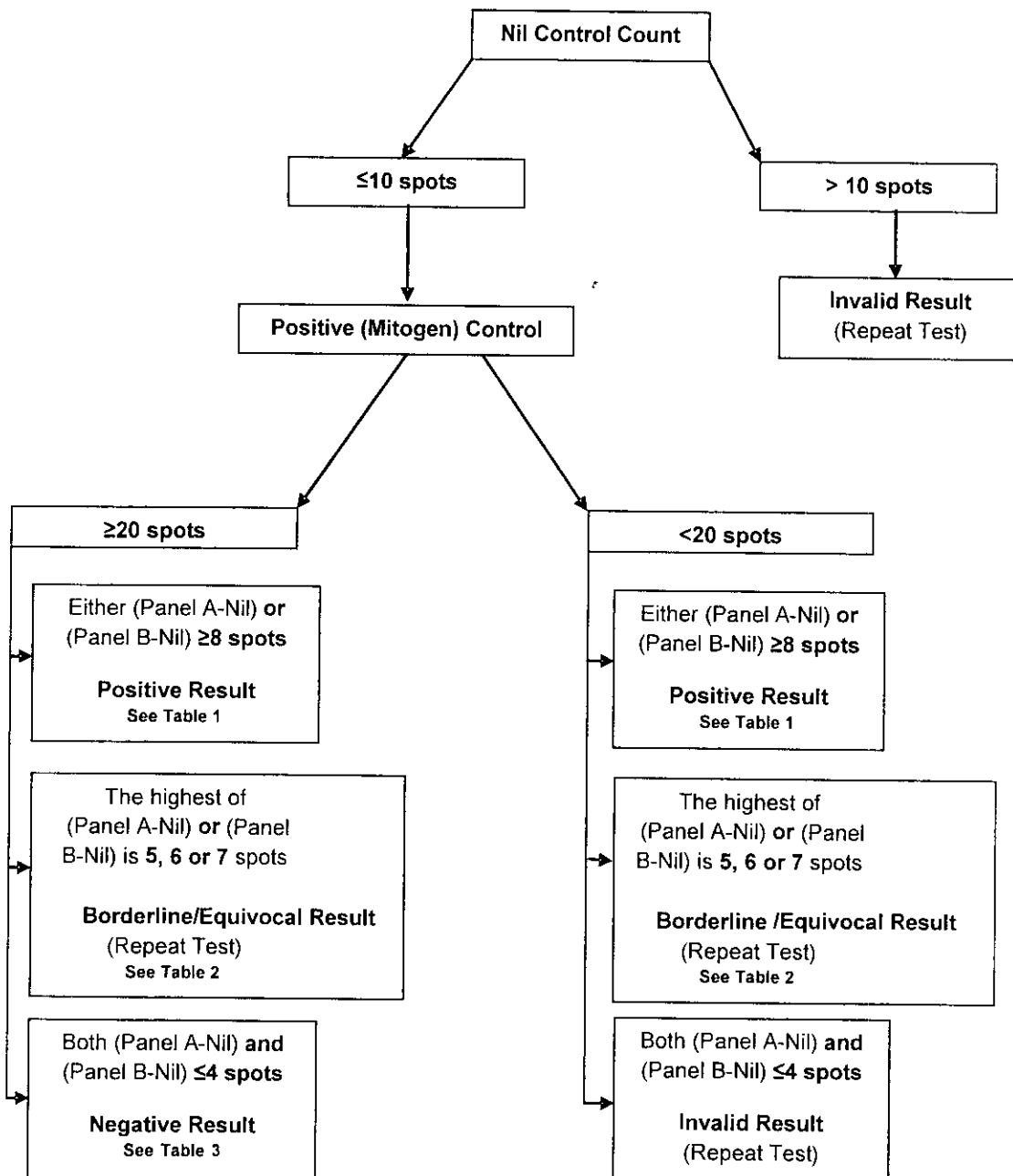
Results for T-SPOT.TB are interpreted by subtracting the spot count in the Nil control well from the spot count in each of the Panels, according to the following algorithm:

- The test result is Positive if (Panel A-Nil) and/or (Panel B-Nil) ≥ 8 spots.
- The test result is Negative if both (Panel A-Nil) and (Panel B-Nil) ≤ 4 spots. This includes values less than zero.
- Results where the highest of the Panel A or Panel B spot count is such that the (Panel minus Nil) spot count is 5,6 or 7 spots is Borderline or Equivocal and retesting by collecting another patient specimen is recommended.

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The interpretation algorithm, including the quality control criteria is shown in Figure 1 below.

Figure 1 Algorithm Flow Diagram.



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Table 1: Positive Interpretation: Either (Panel A-Nil) or (Panel B-Nil) ≥ 8 spots

Nil Control Well Count	Either Panel A or Panel B has the following number of spots*	Result Interpretation
0	≥ 8	Positive
1	≥ 9	Positive
2	≥ 10	Positive
3	≥ 11	Positive
4	≥ 12	Positive
5	≥ 13	Positive
6	≥ 14	Positive
7	≥ 15	Positive
8	≥ 16	Positive
9	≥ 17	Positive
10	≥ 18	Positive
>10 spots	n/a	Invalid**

***Note:** The highest Panel-Nil spot count is used to determine the test outcome.

Table 2: Borderline (equivocal) Interpretation: The highest of (Panel A-Nil) or (Panel B-Nil) is 5, 6 or 7 spots

Nil Control Well Count	The highest of Panel A or Panel B has the following number of spots	Result Interpretation
0	5, 6, or 7	Borderline (equivocal)*
1	6, 7, or 8	Borderline (equivocal)*
2	7, 8, or 9	Borderline (equivocal)*
3	8, 9, or 10	Borderline (equivocal)*
4	9, 10, or 11	Borderline (equivocal)*
5	10, 11, or 12	Borderline (equivocal)*
6	11, 12, or 13	Borderline (equivocal)*
7	12, 13, or 14	Borderline

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		(equivocal)*
8	13, 14, or 15	Borderline (equivocal)*
9	14, 15, or 16	Borderline (equivocal)*
10	15, 16, or 17	Borderline (equivocal)*
>10 spots	n/a	Invalid**

Table 3: Negative Interpretation: Both (Panel A-Nil) and (Panel B-Nil) ≤4 spots

Nil Control Well Count	Both Panel A and Panel B has the following number of spots	Result Interpretation*
0	≤4	Negative
1	≤5	Negative
2	≤6	Negative
3	≤7	Negative
4	≤8	Negative
5	≤9	Negative
6	≤10	Negative
7	≤11	Negative
8	≤12	Negative
9	≤13	Negative
10	≤14	Negative
>10 spots	n/a	Invalid**

* Results where the highest of the Panel A or Panel B spot count is such that the (Panel minus Nil) spot count is 5,6, or 7 spots is Borderline (Equivocal) and the test should be repeated by collecting another patient specimen.

** In the case of Invalid results, the test result should be reported as “Invalid”, and a new sample should be collected and re-tested.

VI. ALTERNATIVE PRACTICES AND PROCEDURES

Laboratory methods for the diagnosis of active pulmonary disease, such as *M. tuberculosis*, include chest x-ray, sputum (phlegm that is coughed up from deep in the lungs) smear microscopy – including Acid Fast Bacilli (AFB) detection, bacteriological and mycobacteriological culture and PCR.

The Tuberculin Skin Test (TST) is a biologic device used for the determination of latent and active *M. Tuberculosis*. Another legally marketed cell mediated immune response

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assay is the QuantiFERON-TB Gold (QFT-G), a whole blood test, which is based on ELISA methodology.

VII. MARKETING HISTORY

The T.SPOT.TB test has not been marketed in the United States.

Starting in 2004, T-SPOT.TB has been marketed in the following countries as a CE-marked IVD:

Australia	Latvia
Bulgaria	Mexico
China	Netherlands
Denmark	Portugal
Finland	Saudi Arabia
France	Singapore
Georgia	South Africa
Germany	South Korea
Greece	Spain
Hong Kong	Switzerland
Ireland	Taiwan
Italy	Turkey
Japan	UAE
Kuwait	UK

T-SPOT.TB has also been marketed in Canada since November 2005 and in Korea since November 2006.

T-SPOT.TB has not been withdrawn from any market for any reason related to the safety and effectiveness of the device.

VIII. POTENTIAL ADVERSE EFFECTS OF THE DEVICE ON HEALTH

An erroneous result can lead to an adverse effect for the individual. A false negative test result could lead to an individual developing active TB disease, adversely affecting their health, and possibly facilitating the spread of *M. tuberculosis* to other individuals in their community. Tuberculosis is contagious and can be transferred from one person to another via air particles expelled from an infectious individual while coughing. A false positive response could lead to an individual being administered unnecessary prophylaxis for tuberculosis infection.

The only direct adverse effects on patient health are those associated with collecting the specimen for testing. Taking blood samples by venipuncture may result in a slight risk of bleeding, hematoma, and infection. Pain and redness at the site of injection occur and some people become dizzy and/or faint when blood is drawn.

IX. SUMMARY OF PRECLINICAL STUDIES

A. Cut-Off Studies:

The objective of the study was to determine the optimal Cut-off point. The cut off value used to determine a positive (positive) or negative (negative) result was established using Receiver Operating Characteristic (ROC) curve analysis⁵ and chosen to maximize observed sensitivity and specificity. A cut-off value was established for sensitivity by testing subjects with culture-confirmed TB (n=87), and for specificity by testing subjects with no known risk for TB exposure (n=93). A cut-off value of ≥ 6 spots maximized assay sensitivity and specificity (Figure 2).

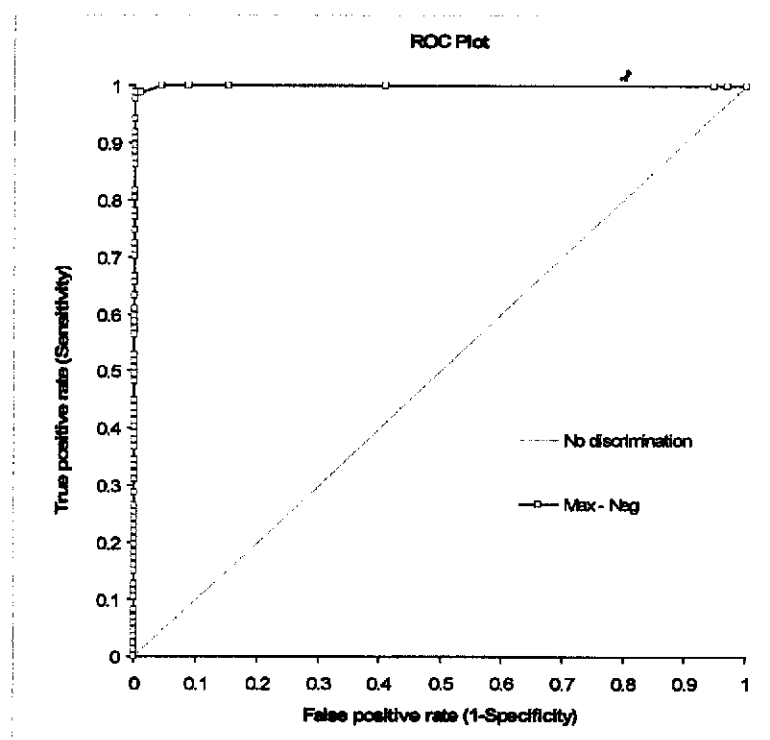


Figure 2: ROC curve analysis for T-SPOT.TB

B. Specificity and Interfering Substances:

Interference from heterophilic antibodies or intrinsic IFN- γ in the blood sample is avoided by the separation and washing of the PBMC fraction from whole blood. This removes background amounts of IFN- γ , other potentially interfering plasma moieties, hemoglobin and any heterophilic antibodies.

Cytokines expected to be produced by leucocytes, including IL-2, IL-4, IL-5, IL-6, IL-10, IL-12, TNF α , IFN- α , and IFN- β were examined for cross-reactivity with the antibody pair used in the T-SPOT.TB assay. Results demonstrated that

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the antibody pair used in the T-SPOT.TB assay did not show any evidence of cross-reactivity with other cytokines.

C. Cross Reactivity:

T-SPOT.TB did not cross react with the *M. avium* patients tested; however, it is known that other NTMs do contain DNA sequences homologous to ESAT-6 and CFP10. Therefore, individuals who are infected with *M. kansasii*, *M. xenopii*, *M. szulgai*, *M. gordonae* or *M. marinum* may also show an IFN-gamma signal in the test in response to the T-SPOT antigens. Results are shown in Table 4 below. There were no Borderline (equivocal) results.

Species of NTM identified	Number	# T-SPOT.TB Positive	# TST Positive
<i>M. avium</i>	12	0	Not done
<i>M. xenopii</i> *	1	1	1
<i>M. kansasii</i>	1	1	1
<i>M. gordonae</i>	4	4	4

Table 4 T-SPOT.TB and TST results amongst 18 patients with confirmed Non-Tuberculosis Mycobacterial (NTM) infection. 1 invalid T-SPOT.TB result was excluded from the results. There were no Borderline (equivocal) results

** Note that this study participant was known to have had contact to an infectious source case and was strongly suspected of also having LTBI. However, no confirmatory testing for the presence of Mycobacterium tuberculosis was performed throughout this limited study.*

D. Analytical Sensitivity of T-SPOT.TB

The minimum detectable unit of response of T-SPOT.TB is one *M. tuberculosis* specific T cell, which corresponds to a lower limit of detection of 1 reactive T cell in 250,000 PBMCs.

E. Reproducibility:

Intra-assay variability was analyzed by comparing the T-SPOT.TB assay run on the same plate by the same operator. Experiments were carried out by three operators on nine plates which resulted in a range of % CVs representative of the inherent variation in the test. The range that was obtained for the high spot counts ($210.40 \pm$

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11.59) was between 2.21% - 7.7% CV (mean % CV = 4.43), mid-range spot counts (71.17 ± 8.47) gave a range of 6.57% - 16.49% CV (mean % CV = 11.0%), whereas spot counts close to the cut-off (mean spot count = 5.71 ± 1.25) gave a mean % CV = 21.97%.

Inter-assay precision data were collected, where three kit lots were used by three different operators to run the same three samples on six occasions. The coefficient of variation measured across the three samples, three operators and three lots was 3.68% for samples giving a mean spot count of 210.40. For spot counts close to the T-SPOT.TB cut-off, the inter-assay variation was 24.95%. For moderate spot levels, the mean %CV was 13.86%. The results for the %CV were consistent for each of the batches tested.

Inter-operator reproducibility was assessed using three operators and one plate each from three kit batches. The variation observed between operators was 3.64%-5.76% CV.

Two separate inter-laboratory experiments were run where in each case two tubes of blood were taken from the same patient and processed in parallel at the two sites. Results are shown in Table 5.

	Site 2		
Site 1	Positive	Borderline (equivocal)	Negative
Positive	13 (31.7%)	0	0
Borderline (equivocal)	0	0	1 (2.4%)
Negative	0	1 (2.4%)	26 (63.4%)

Table 5 Results from inter-laboratory experiments.

F. Storage and Shipping, Time and Temperature Studies:

1. Shelf life:

Stability studies (2-8°C) demonstrated no loss of performance of the T-SPOT.TB Test for up to 1 year post-manufacture. A shelf life of 52 weeks is established for the T-SPOT.TB Test.

2. Shipping:

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Shipping studies were conducted by shipping T-SPOT.TB kits to various locations. Kits were subjected to temperature variation between 2°C and 29°C.

G Animal Studies

No studies with T-SPOT.TB have been conducted in animals

X. SUMMARY OF CLINICAL STUDIES

The pivotal clinical study was designed to establish the clinical performance of the T-SPOT.TB test in both culture confirmed active TB disease and in potentially latent TB infection in populations stratified by risk of exposure to *M. tuberculosis*. A total of 2355 subjects were enrolled in the pivotal study; using 11 study sites as detailed in Table 5. Of the 2355 enrolled subjects, 492 samples did not meet the inclusion criteria, leaving 1863 subjects available for analysis.

The 492 excluded samples were for the following reasons: 14 no informed consent/possible coercion, 11 excluded by site, 13 duplicate enrollments, 59 no blood sample collected, 115 insufficient sample collected to run T-SPOT.TB assay, 66 laboratory deviations in performing the T-SPOT.TB test, 18 no risk group assigned (incomplete data) and 196 without a TST result (121 recorded as no TST administered or no result provided, 67 with no record of the TST or missing medical records, 6 with a TST result from greater than a year prior to the study and no current result, 1 non-return for the reading of the TST result, 1 TST given post enrollment).

The study population as a whole was intended to include subjects from all major risk groups indicated for screening for TB infection according to guidelines from the CDC². The performance of T-SPOT.TB was assessed in populations where the TST may likely give false-positive results (e.g. patients exposed to non-tuberculous mycobacteria and those who have previously received the BCG vaccination)⁶. In addition, the performance of T-SPOT.TB was assessed in populations where the TST may likely give false-negative results (e.g., patients of very young or old age and patients with various types of immunosuppression) and who may be at elevated risk of progression of latent TB infection to active TB disease⁷⁻¹¹. All TST results were scored using 5, 10 or 15mm cutoffs according to CDC/ATS guidance¹.

Subjects from the pivotal studies were classified into five main groups for analysis. The allocation of all patients from the pivotal studies is detailed in Figure 5.

- Group 1 – Active TB (n=105)

The sensitivity of the T-SPOT.TB test was estimated from subjects where it was known that active, culture confirmed TB infection was present. Only subjects with positive culture confirmed results were included in this group. It was not a requirement for this group to have a TST result. Sixty-nine subjects were from Brazil, thirty-six remaining subjects were from three Texas sites. In addition, results from studies in Germany (n=34), Italy (n=22) and the UK (n=28) were included into this Group for sensitivity calculations only. Thus, the sensitivity calculations were based on a combined group of 189 subjects, of which 6 T-

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SPOT.*TB* results were invalid, leaving 183 samples for analysis. Results are presented in Table 8.

- Group 2 – Low Risk Controls (n=311)

The specificity of the T-SPOT.*TB* test was estimated from subjects that were presumed to have a lower probability of TB infection. “Low risk” subjects were selected on the basis of the absence of clinical, epidemiological and diagnostic risk factors for TB infection.

Although common risk factors for TB infection were controlled, a complete epidemiological background on each participant could not be obtained and, as such, this group may have contained subjects who had a genuine TB or NTM infection detectable by T-SPOT.*TB*. This group was used to estimate the specificity of T-SPOT.*TB*.

Factors used to determine a low risk of TB infection, based on patient self report, was the absence of any of the following factors:

- More than 3 months spent living in a TB endemic country (TB prevalence > 40/100,000)
- Occupational history of work in a high risk setting, e.g., TB laboratory, health care worker
- Time spent in a high risk environment, e.g., jail, nursing home or homeless shelter
- Known contact with TB Index case
- IV Drug use
- Heavy alcohol use
- Known Non-tuberculous (NTM) infection
- HIV infection or other immunosuppressive conditions
- History of having had TB or taken TB medication
- Radiological or microbiological test results consistent with TB infection
- History of positive TST result

Of the 311 results, 5 had invalid T-SPOT.*TB* results leaving 306 results for analysis. Results are presented in Table 9.

- Group 3 – LTBI Suspects (n=1403)

This group, the largest in the study, included candidates for routine screening for LTBI infection according to the prevailing CDC guidelines for screening of risk groups. This group contained a broad mix of subjects screened for TB infection at varying degrees of risk of exposure (for example: recent contacts of known source cases, prison inmates) and risk of progression (for example: those with HIV infection, young children, the elderly, those with immunosuppressive conditions). This group was used to demonstrate the positivity of T-SPOT.*TB* relative to the TST in these populations.

Of the 1403 results, 55 had invalid T-SPOT.*TB* results leaving 1348 results for analysis. Results are presented in Table 11.

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- Group 4 – NTM (n=19)

This group contained a small group of subjects with known Non-Tuberculosis Mycobacterial (NTM) infections. [Note that these are also sometimes called Mycobacteria Other Than Tuberculosis (MOTT) or Atypical mycobacteria]. The group includes subjects with recently diagnosed (within the previous 12 months) NTM infection, or those who were diagnosed more than 12 months previously but listed by the enrolling physician as having an active ongoing infection. TST was not a requirement, but was sometimes included in their normal clinical care. This group was used to demonstrate the estimated cross reactivity of T-SPOT.TB in NTM infection. Of the 19 results, 1 was invalid by T-SPOT.TB, leaving 18 results available for analysis. Results are presented in Table 12.

- Group 5 – Unconfirmed Active TB (n=25)

Among those subjects recruited with active TB; 25 subjects were diagnosed clinically without culture-confirmation. TST was not consistently performed. Of the 25 results, 1 was invalid by T-SPOT.TB leaving 24 results for analysis. Results are presented on page 27.

A summary table of the number of subjects (after exclusions) by site comprising each group in the clinical study is shown in Table 6.

Table 6 – Summary of subjects comprising each clinical study group by site

Study site locations and primary enrolment populations in pivotal study	Group 1, Sensitivity calculations	Group 2, Specificity calculations	Group 3, LTBI suspects	Group 4, NTM infection	Group 5, Unconfirmed Active TB	*Total number tested (including invalids.)
Prison inmates, TX	7	0	462	5	3	477
Active TB patients Brazil	69	0	0	0	0	69
TB contacts New York	0	3	183	2	0	188
Children attending TB clinic, TX	12	13	184	1	18	228
HIV patients with suspected TB, TX	17	0	17	1	3	38
End Stage Renal Disease patients, Canada	0	0	195	0	1	196
Patients attending HIV clinic, GA	0	0	227	0	0	227
Naval Recruits screened on	0	294	52	0	0	346

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recruitment to Military, <i>IL</i>						
Patients infected with NTMs, <i>NH</i>	0	1	2	10	0	13
Rheumatoid Arthritis patients on anti-TNF α therapy, <i>Canada</i>	0	0	34	0	0	34
Rheumatoid Arthritis patients on immunosuppressive therapies, <i>MA</i>	0	0	47	0	0	47
Total	105	311	1403	19	25	1863

1. Patient Demographics

Demographic and key epidemiologic information for all subjects in the US pivotal study by group is shown in Table 7.

Gender	Group 1	Group 2	Group 3	Group 4	Group 5	All Groups
Males	62	260	917	11	13	1263
Females	43	50	483	8	12	596
Gender unknown/not recorded	0	1	3	0	0	4
Place of birth						
US born	20	299	687	18	15	1039
Foreign born	16	10	488	1	10	525
No data	69	2	228	0	0	299
BCG vaccination history						
BCG vaccinated	11	1	290	1	8	311
Not BCG vaccinated	22	212	783	17	15	1049
Unknown/not recorded	72	98	330	1	2	503
Ethnic group						
White	4	213	391	10	3	621
Black	8	40	532	6	1	587
Hispanic	91	34	335	2	16	478
Middle East / Indian	0	2	35	0	1	38
Asian	2	7	87	0	2	98
Native American / Alaskan	0	5	3	1	0	9

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Hawaiian / Pacific Islander	0	3	6	0	0	9
Other	0	5	11	0	2	18
Ethnicity unknown/not recorded	0	2	3	0	0	5
Age group						
0 to 2 years	2	0	37	1	1	41
>2 to 5 years	1	2	41	0	6	50
6 to 17 years	6	9	78	0	11	104
Age not recorded	0	0	1	0	0	1
Age Range	0.4-69 years	3-65 years	0.08-93 years	1.83-77 years	2-52 years	0.08 - 93 years
0 to 17 years (children)	9	11	156	1	18	195
18 to 64 years (adult)	94	299	1117	14	7	1531
65+ years (elderly)	2	1	129	4	0	136
TOTAL	105	311	1403	19	25	1863

Table 7 - Summary of overall US pivotal study subject demographics by group

XI. PANEL MEETING RECOMMENDATION AND FDA'S POST-PANEL ACTION

Pursuant to Section 515(c)(2) of the Act as amended by the Safe Medical Devices Act of 1990, this PMA was not the subject of an FDA Microbiology Devices Advisory Panel meeting because the information in the PMA substantially duplicated information previously reviewed by this Panel.

XII. CONCLUSIONS DRAWN FROM PRE-CLINICAL AND CLINICAL STUDIES

A. Pre-clinical studies

Pre-clinical studies were conducted to establish the performance characteristics of T-SPOT.TB. The high level of specificity is facilitated by the separation of the T cells from any contaminating IFN- γ in the whole blood sample.

The reproducibility of the test has been shown to provide reproducible results for the test at different levels of spot counts. A Borderline (equivocal) region has been established to account for the variation observed at counts close to the cut off, i.e., from 5 – 7 spots. This represents the area of overlap between results obtained for culture confirmed positive samples and low risk TB negative samples.

Stability studies have provided data to establish expiration dating for this device at 52 weeks when stored at 2-8 degrees centigrade.

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B. Clinical Studies

1. Sensitivity Results (US Pivotal Study Group 1 and European Study Subjects)

Source of culture-confirmed samples		T-SPOT.TB					Total	Sensitivity
		Positive ≥8 spots	Borderline (equivocal)			Negative ≤4 spots		
			7 spots	6 spots	5 spots			
US		31	3	0	0	2	36	94.4% (34/36)
BR		60	1	1	1	4	67	92.5% (62/67)
EU	DE	31	1	0	0	1	33	97.0% (32/33)
	IT	19	1	0	0	0	20	100.0% (20/20)
	UK	25	1	1	0	0	27	100.0% (27/27)
TOTAL		166	7	2	1	7	183	95.6% (175/183) [91.6-98.1%]

Table 8 – Sensitivity Results in US pivotal study Group 1 patients and European study subjects

* Out of the 189 total samples, 6 results were invalid: 1 subject with high background, 4 subjects with high Nil Control responses, 1 subject with low positive (mitogen) control response. Invalid results were excluded from calculations, leaving 183 samples for analysis.

Using the cutoff of ≥6 spots, the estimated sensitivity of T-SPOT.TB was 95.6% (175/183) [95%CI 91.6%-98.1%]

If all the Borderline (equivocal) results are considered all positive or all negative, the sensitivity of T-SPOT.TB was either 96.2% (176/183) or 90.7% (166/183), respectively.

2. Specificity Results (Group 2)

US low risk samples	T-SPOT.TB				Negative ≤4 spots	Total	Specificity
	Positive ≥8 spots	Borderline (equivocal)					
		7 spots	6 spots	5 spots			
TOTAL	3	1	5	7	290	306	97.1% (297/306) [94.5-98.7%]

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Table 9 –Specificity Results

*Out of the 311 total samples, 5 results were invalid: 1 subject with high background, 1 subject with high Nil Control responses, 3 subjects with low positive (mitogen) control responses. Invalid results were excluded from calculations, leaving 306 samples for analysis.

Using the cutoff of ≥ 6 spots, the estimated specificity of T-SPOT.TB was 97.1% (297/306) [95%CI 94.5%-98.6%]

If all the Borderline (equivocal) results are considered all positive or all negative, the specificity of T-SPOT.TB was either 94.8% (290/306) or 99.0% (303/306), respectively

3. Results in Subjects in ATS/CDC Risk Groups (Group 3)

There were 1403 subjects recruited in Group 3 who had a total of 2713 ATS/CDC risk factors. Over 50% of these subjects were immunocompromised (for a variety of reasons including HIV, drug-induced immunosuppression, malignancy, end stage renal disease). A substantial number of BCG vaccinated and foreign-born individuals were included, which is consistent with the epidemiology of TB in the US¹³. A wide age range of subjects were also included to incorporate groups at elevated risk of progression to TB disease, including infants and the elderly.

Table 10 shows a breakdown of the numbers of Group 3 subjects, recruited within each ATS/CDC Risk Group and other key epidemiologic information. The table shows the numbers of patients within Group 3 who had each risk factor. Patients may have had more than one risk factor concurrently and are therefore counted more than once in different rows of this table.

ATS/CDC Risk Group	Number of subjects in Group 3 with identified risk factor	% of subjects in Group 3 with identified risk factor
HIV-positive persons	328	23.4%
Recent contacts of TB patients	229	16.3%
Patients with fibrotic changes on chest radiograph consistent with prior TB	26	1.9%
Patients with organ transplants and other immunosuppressed patients (receiving the equivalent of ≥ 15 mg/d of prednisone for 1 mo or more) ⁱ	122	8.7%
Recent immigrants (i.e., within the last 5 yr) from high prevalence countries	41	2.9%
Injection drug users	97	6.9%
Residents and employees of the following high-risk congregate	613	43.7%

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settings: prisons and jails, nursing homes and other long-term facilities for the elderly, hospitals and other health care facilities, residential facilities for patients with acquired immunodeficiency syndrome (AIDS), and homeless shelters		
Mycobacteriology laboratory personnel	5	0.4%
Silicosis	0	0.0%
Diabetes mellitus	108	7.7%
Chronic renal failure (End-stage renal disease)	195	13.9%
Hematologic disorders	5	0.4%
Other specific malignancies ⁱⁱ	23	1.6%
Gastrectomy, and jejunioileal bypass	4	0.3%
Children younger than 4 yr of age or infants, children, and adolescents exposed to adults at high-risk	93	6.6%
Other Groups of Interest		
Previous TB diagnosis	110	7.8%
Pregnant women	8	0.6%
Asthmatics	95	6.8%
Smokers	426	30.4 %
Heavy alcohol users	68	4.8%
Patients with Rheumatoid Arthritis	85	6.1%
Patients with Hepatitis	51	3.6%
Born in a high TB prevalence country ⁱⁱⁱ	232	16.5%
History of prior TB infection	110	7.8%
Immunocompromised ^{iv}	717	51.5%

Table 10 Summary of subjects in Group 3 falling into ATS/CDC risk groups. Note that some subjects may have had multiple coincident conditions and are therefore counted more than once in this table.

i. Subjects taking the following drugs were included in this cohort (provided dosage was high enough): anti-TNF-alpha, steroids, transplant recipients, adalimumab, azathioprine, ciclosporin, etanercept, infliximab, leflunomide, methotrexate, mycophenolate mofetil, prednisone, sulfasalazine, tacrolimus

ii. Defined as any participant undergoing cancer chemotherapy

iii. Defined as a country with a prevalence >40/100,000

iv Immunocompromised includes: all subjects with leukaemia, taking immunosuppressive drug therapy (see footnote i above), transplant, GI bypass, RA, cancer chemotherapy, hepatitis, silicosis, HIV or AIDS, end-stage renal disease

With the exception of silicosis patients, all groups indicated for screening according to prevailing CDC guidelines where represented in Group 3 as shown in Figure 4. T-SPOT.TB has, however, been shown to give valid results in silicosis patients¹⁴. The publication by Leung *et al.*

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found that “*T-SPOT.TB is likely to perform better than tuberculin test in the screening of latent tuberculosis infection among silicotic patients*”¹⁴.

Results comparing the T-SPOT.TB test with the TST are shown in Table 11 below. The TST cut-off utilized for Group 3 was selected based on CDC recommendations for classification of tuberculin reaction.

Group 3		T-SPOT.TB					
LTBI suspects		Positive ≥8 spots	Borderline (equivocal)			Negative ≤4 spots	Total
			7 spots	6 spots	5 spots		
TST	Positive	166	6	4	9	118	303
	Negative	116	23	13	25	868	1045
	TOTAL	282	29	17	34	986	1348

Table 11 Summary of results for Group 3.55 Invalid assays excluded.

55 subjects were invalid, 9 had high background, 22 had high Nil Control responses, and 24 had low positive (mitogen) control responses. The invalid results were excluded from calculations.

Using a cutoff of ≥6 spots, the T-SPOT.TB results were positive in 24.3% (328/1348) and the TST was positive in 22.8% (303/1348) of Group 3 subjects. If all Borderline (equivocal) results are considered either positive or negative, the percentage of T-SPOT.TB positive results was either 26.9% (362/1348) or 20.9% (282/1348), respectively.

The data from Group 3 were also assessed to determine agreement of results between T-SPOT.TB and TST. Based on the cut-off of ≥6 spots, results are as follows:

- Overall Agreement = 79.3% (1069/1348) [95%CI 77.0-81.4%]
- Positive Agreement = 58.1% (176/303) [95%CI 52.3-63.7%]
- Negative Agreement = 85.5% (893/1045) [95%CI 83.2-87.5%]

If all the Borderline (equivocal) results were considered positive:

- Overall Agreement = 78.1% (1053/1348) [95%CI 75.8-80.3%]
- Positive Agreement = 61.1% (185/303) [95%CI 55.3-66.6%]
- Negative Agreement = 83.1% (868/1045) [95%CI 80.7-85.3%]

If all the Borderline (equivocal) results were considered negative:

- Overall Agreement = 81.2% (1095/1348) [95%CI 79.0-83.3%]
- Positive Agreement = 54.8% (166/303) [95%CI 49.0-60.5%]
- Negative Agreement = 88.9% (929/1045) [95%CI 86.8-90.7%]

An exploratory multiple logistic regression was performed to investigate association of test results and selected risk factors (gender, age, ethnicity, BCG vaccinated,

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immunocompromised, born in a TB endemic country, contact to infectious source case and history of prior TB infection status) and was conducted separately for the T-SPOT.TB and TST. The analyses were based on the 963 subjects in Group 3 with complete data for all of the variables included in the model. Therefore, 385 patients with incomplete data were not included. A positive T-SPOT.TB test was based on the prespecified cutoff of ≥ 6 spots. Invalid T-SPOT.TB results were not included. The same dataset was used for both T-SPOT.TB and TST. After controlling for the other variables in the model, positive results for both T-SPOT.TB and TST were significantly associated with history of prior TB infection. A positive result for T-SPOT.TB was significantly associated with contact to an infectious source and birth in a TB endemic country. However, a positive result for TST was not associated with those variables. A positive TST was associated with BCG vaccination; while, no association was observed between T-SPOT.TB results and BCG vaccination. A negative TST was associated with being immunocompromised; while, no association was observed between T-SPOT.TB result and immunocompromised status. A more positive TST results were observed among children (5-17 yrs) than among adults (18 -64 yrs); while, T-SPOT.TB results were not impacted by age.

4. Results in NTM infected (Group 4)

The TST is known to cross-react amongst those with Non-Tuberculosis Mycobacterial infections^{6,12}, the most common of which is *M. avium*. Due to the use of antigens ESAT-6 and CFP10 that are not present in *M. avium*; T-SPOT.TB is not expected to cross-react in patients infected with this NTM. Twelve subjects were identified with *M. avium* infection; none were positive with T-SPOT.TB. Based on this limited sample size of 12 subjects, no cross-reactivity of T-SPOT.TB with *M. avium* was observed. T-SPOT.TB results for subjects infected with *M. avium* and other Non-Tuberculosis Mycobacteria are shown in Table 12 below.

Species of NTM identified	Number	# T-SPOT.TB Positive	# TST Positive
<i>M. avium</i>	12	0	Not done
<i>M. xenopii</i> *	1	1	1
<i>M. kansasii</i>	1	1	1
<i>M. goodii</i>	4	4	4

Table 12 T-SPOT.TB and TST results amongst 18 patients with confirmed Non-Tuberculosis Mycobacterial (NTM) infection. One invalid T-SPOT.TB result was excluded from the results. There were no Borderline (equivocal) results.

* Note that this study participant was known to have had contact to an infectious source case and was strongly suspected of also having LTBI.

5. Results in Clinically Diagnosed TB Patients (Group 5)

Of the 25 patients in Group 5, one was invalid by T-SPOT.TB (low positive control), leaving 24 samples for analysis. Using the cutoff of ≥ 6 spots, 79.2% (19/24) were positive by T-SPOT.TB. If the one Borderline (equivocal) result (7

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spots) was considered negative, the positive rate by T-SPOT.*TB* would be 75.0% (18/24); if the one Borderline (equivocal) result was considered positive, the positive rate by T-SPOT.*TB* would be 79.2% (19/24).

C. Risk / Benefit Analysis

As with all *in vitro* diagnostic tests there are some related safety issues with its use. Taking blood samples by venipuncture may result in a slight risk of bleeding, hematoma, and infection. Pain and redness at the site of injection occur and some people become dizzy and/or faint when blood is drawn. In addition, any unscreened biological material must be handled with caution and local Health and Safety regulations must be followed. Warnings and precautions are included in the labeling.

Results from the T-SPOT.*TB* test may be reported within 1 day from the taking of the blood sample unless the test was an invalid or borderline (equivocal) result. It does not depend on a patient returning to have their results assessed.

D. Proposed Restrictions or Training Requirements:

T-SPOT.*TB* is designed to be used in a laboratory setting with operators trained in Good Laboratory practice (GLP). Users should be familiar with the package insert and should be trained in the conduct of the assay.

This test should be conducted in at least a BioSafety Level II environment. Procedural guides and other technical assistance documents are available by contacting Oxford Immunotec.

XIII. CDRH DECISION

FDA issued an approval order on July 30, 2008.

The applicant's manufacturing facility was inspected on 11/14/07 (Abington, UK) and found to be in compliance with the Quality Systems Regulations (21 CFR 820).

XIV. APPROVAL SPECIFICATIONS

Directions for use: See device labeling.

Hazards to Health from Use of the Device: See Indications, Contraindications, Warnings, Precautions, and Adverse Events in the device labeling.

Post-approval Requirements and Restrictions: See approval order.

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