Influence of pre-analytic conditions on the rate of indeterminate T-SPOT®.*TB* tests

To the Editors:

A prior study has demonstrated that the rate of indeterminate T-SPOT®.*TB* (Oxford Immunotec, Abingdon, UK) test results performed under routine field conditions was low (3.4%) [1]. Indeterminate test results were more common in individuals aged >75 yrs and may be influenced by the transport conditions (cold weather in winter). Since this study was published, two technical improvements in the pre-analytic conditions were introduced. The first is the use of lithium heparinate blood sample collection tubes instead of the CPT™ Cell Preparation Tubes with sodium citrate (Vacutainer®; BD, Franklin Lakes, NJ, USA), as used in the prior study; the other is the use of the T-Cell *Xtend*™ reagent (Oxford Immunotec) in samples >8 h old [2].

The Meditest reference laboratory (Lausanne, Switzerland) receives blood samples from remote places in Switzerland, so that many samples travel overnight or for ⩽36 h before being analysed. All samples that have been collected >8 h earlier are processed with T-Cell *Xtend*™. We therefore performed an updated analysis of the data from the beginning of 2010 to compare the rate of indeterminate tests with those previously reported.

Heparinised samples received by Meditest were analysed using the T-SPOT®.*TB* test according to the manufacturer's instructions [3]. Samples >8 h old had 25 μL T-Cell *Xtend*™ reagent added per mL of blood. The tube was inverted two to three times and incubated at room temperature for an additional 20 min before being processed in the T-SPOT®.*TB* assay.

Between January 1 and March 31, 2010, 445 samples were analysed. 29% were from the city of Lausanne, 44% from the local region (canton of Vaud) and 27% from more remote areas in Switzerland. By default, the samples from local places, which were received the same day, were processed using standard methodology. Among samples received from regional collecting places, 38 out of 194 were received and processed the same day (<8 h) whereas 156 were received and processed the next day. All samples from remote places travelled by postal service overnight, and were processed between 12 and 36 h after blood collection. Samples sent from regional or remote areas that requested overnight transportation were all processed using T-Cell *Xtend*™. One sample was received >3 days after blood collection and was excluded from the analysis, leaving 444 samples for the final interpretation (table 1).

Six samples were from young patients (6–15 yrs; all negative), 34 were from elderly patients (>65 yrs) and all others from adults between 16 and 65 yrs of age. 256 (57%) samples were from males. No test result from very young or very old patients was indeterminate.

The rate of positivity for the samples from Lausanne, Vaud and remote places was 10, 17 and 10%, respectively. Nine tests were borderline (having five, six or seven more spots than the nil control) and one was indeterminate (from a remote location). This subject was tested again 1 month later and was again indeterminate. On both occassions, there were >10 spots in the nil control. The subject was an adult between 16 and 65 yrs of age.

The rate of indeterminate results obtained using the T-SPOT®.*TB* assay, which was already low in a prior study performed under field conditions [1], was reduced to an even lower value (one (0.2%) out of 444). The only indeterminate result was sent from a remote place and remained indeterminate after repeat processing. We therefore assume that this subject had some abnormality in their lymphocyte function. Interestingly, there were no indeterminate results in samples sent from local sources. The decline in indeterminate results in this group compared to the previous study may be due to the exclusive use of lithium heparinate blood tubes instead of Vacutainer® CPT™ tubes. We assume that the survival of the lymphocytes may be altered by the CPT™ tube content (citrate), particularly during transport, with partial separation and resuspension of cells, whereas the lymphocytes are more stable in a plain lithium heparinate tube, where the separation

TABLE 1 Number of positive, negative, indeterminate and borderline test results, by the provenance of the blood samples

Provenance	Samples	Same day (<8 h)	Overnight (>8 h)	Positive	Negative	Indeterminate	Borderline
Lausanne city	128	128	0	14	111	0	3
Vaud canton	194	38	156	34	156	0	4
Remote	122	0	122	12	107	1	2

Data are presented as n. All samples from remote places and the majority of samples from regional sources travelled overnight by train or postal service and were processed by default with T-Cell *Xtend*™.

takes place only once the tubes are in the laboratory. If we consider only the samples sent from regional or remote sources, the rate of indeterminate test result is still very low (one (0.3%) out of 317). The use of T-Cell *Xtend*™ for all samples shipped overnight is likely to have had a beneficial effect on older samples. The cold weather conditions had no influence on the rate of indeterminate results (all samples were sent and processed in winter). The number of tests performed in very young or very old patients being small, we cannot assess the rate of indeterminate in these population groups.

The slightly higher rate of positivity in samples referred from Vaud county (17% *versus* 10% in other groups) can be explained since these samples were mostly from recent immigrants from countries with a high prevalence of tuberculosis, whereas the samples from the city or from remote places were more often taken for contact tracing investigations where the rate of infection may be expected to be lower.

In conclusion, these results demonstrate that the use of lithium heparinate blood collection tubes for all samples and T-Cell *Xtend*™ for samples shipped overnight allows the processing of samples from all over Switzerland while maintaining the rate of indeterminate test results at a very low level in spite of longer travel time (8–36 h). In our experience, the only indeterminate test results are observed in patients with some immune abnormality and do not represent a laboratory failure.

A. Zellweger* and J-P. Zellweger#
*Meditest Laboratory, Lausanne, #Chest physician, Fribourg, Switzerland.

Correspondence: J-P. Zellweger, Chest physician, Rue de Locarno 1, 1700 Fribourg, Switzerland. E-mail: zellwegerjp@swissonline.ch

Statement of Interest: A statement of interest for J-P. Zellweger can be found at www.erj.ersjournals.com/site/misc/statements.xhtml

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DOI: 10.1183/09031936.00179310

Is MDR-TB on the rise in Mozambique? Results of a national drug resistance survey

To the Editors:

Tuberculosis (TB) remains a serious public health problem in many low- and middle-income countries in Africa, Asia and the former Soviet Union [1]. Mozambique is one of them. Despite the fact that in this country detection of patients with TB has drastically increased during the past decade due to improved notification (from nearly 20,000 new TB cases notified in 1998 to nearly 40,000 in 2008), less than half of the cases of TB estimated to emerge annually are currently being detected. As in other southern African countries, the HIV epidemic is fuelling the TB epidemic in Mozambique, with 60% of patients with TB being co-infected with HIV [1]. Drug-resistant TB is thought to be a major problem, although the latest information available on the magnitude of resistance to anti-TB drugs is from 1998, when the first national drug resistance survey (DRS) was conducted. At that time, multidrug-resistant (MDR)-TB (defined as TB resistant to at least isoniazid and rifampicin, the two main first-line drugs in the treatment of TB) was found among 3.5 and 3.3% of new and previously treated TB cases, respectively [2]. Since then, there has been no further investigation of the magnitude of drug resistance in the country. Based on the latest available data, >3,500 MDR-TB cases are estimated to have emerged in Mozambique in 2008 [3]. Detecting and treating patients with MDR-TB is feasible but substantially more complex and costly than treating patients with fully susceptible *Mycobacterium tuberculosis* strains. Knowing the levels of anti-TB drug resistance in the country is essential for any national TB programme (NTP) to evaluate the efficacy of TB control measures and treatment practices in the community, as well as to design effective treatment regimens for all patients with TB [4, 5].

For this reason, after nearly 10 yrs from the first survey, a new DRS on a national representative sample of TB patients has been conducted, with the objectives to investigate levels and patterns of resistance to first- and second-line anti-TB drugs among new and previously treated TB cases, compare the new findings with the existing data, and explore the association between the HIV epidemic and anti-TB drug resistance. The findings will be used by the NTP to develop treatment and management policies for TB and MDR-TB patients.

The survey was designed according to the guidelines of the World Health Organization (WHO) [6], and conducted between February 2007 and May 2008 in 40 diagnostic centres sampled from all centres distributed in all 11 provinces of the country. The sampling was probability proportional to size, based on the number of new sputum smear-positive cases in 2006. Each cluster was required to enrol 30 new sputum smear-positive TB patients. During the enrolment period, all previously treated TB patients presenting to the 40 diagnostic