researched area [6]. To date, our validation of the Leicester Cough Monitor is the most extensive for cough monitoring systems. We are aware of fully automated cough detection systems where the same level of validation is not available [7]. We encourage our colleagues to publish similar validation data so that independent researchers are able to compare the merits of the available system.

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STATEMENT OF INTEREST
None declared.

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Local immunodiagnosis of pulmonary TB: ELISPOT or flow cytometry, PPD or ESAT-6?

To the Editors:

Immunodiagnosis is a significant addition to the diagnostic armamentarium in tuberculosis (TB) infection and disease. For some time it has been known that cells secreting type-1 cytokines, such as interferon (IFN)-γ and tumour necrosis factor-α, selectively accumulate in the lung in higher frequencies than in the blood [1], as would be expected in a disease dominated by pulmonary pathology.

In a recent issue of the *European Respiratory Journal*, JAFARI *et al*. [2] detailed the TB-specific response in bronchoalveolar lavage (BAL) and blood from 12 patients with culture-positive but smear negative disease. JAFARI *et al*. [2] used a combination of flow cytometry to determine the phenotype of the CD4 and CD8 lymphocytes concentrating on memory and activation markers, and enzyme-linked immunospot (ELISPOT), to measure the response to the TB-specific proteins early secretory antigenic target (ESAT)-6 and culture filtrate protein (CFP)-10. This study is similar in design and findings to another paper by JAFARI *et al*. [3].

One of the disadvantages of ELISPOT when compared with flow cytometry is that it cannot identify the phenotype of the cells secreting the measured cytokines (usually IFN-γ). Hence in the study by JAFARI *et al*. [2] it is through association, rather than direct measurement, that the TB-specific lymphocytes are characterised as predominantly memory phenotype, as evidenced by CD45RO expression. Indeed the percentage of CD45RO+ CD4+ lymphocytes in TB (50%) and the control group (35%) appeared to be surprisingly low. True memory CD4+ lymphocytes, determined by the absence of both CD45RA and CD27 expression, were 93% in BAL in 46 controls and patients with a variety of different pathologies, compared with 23% in blood samples [4].

In the study by JAFARI *et al*. [2], the region of difference-1 antigens, ESAT-6 and CFP-10, were used as the stimulatory antigens. JAFARI *et al*. [2] noted that the TB-specific responses to these peptides in BAL were ~10 times those in peripheral blood. However, ESAT-6 and CFP-10 only encompass a small part of the TB genome and larger proteins, such as purified protein derivative (PPD), have been shown to generate much larger responses. For example, in a previous study [5] the median CD4 T cell IFN-γ response to PPD in BAL was 24% but only 0.07% in blood, a 300-fold difference. More importantly, ESAT-6 responses in BAL in a large cohort of patients were negative in 19% of cases with culture confirmed TB [6].

Most enthusiasts for using region of difference-1 antigens in TB immunodiagnosis point out the difficulties of using PPD on blood samples when patients have received the bacilli Calmette–Guerin (BCG) vaccination. However, BCG vaccination has no influence on lung antigen-specific responses when PPD is used

as the stimulatory antigen [5, 6]. This is probably due to lymphocytes that are primed in dendritic cells in the skin recirculating to local lymph nodes and the spleen, but not the lung.

Therefore, we suggest that flow cytometry provides more relevant information regarding lung lymphocyte responses and that purified protein derivative has several advantages over RD-1 antigens in this context.

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STATEMENT OF INTEREST

None declared.

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From the authors:

We would like to thank S. Barry and colleagues for their comments on our recent article describing the local immunodiagnosis of active smear negative tuberculosis (TB) by enzyme-linked immunospot (ELISPOT) [1]. In contrast to a previous article [2], we demonstrated that relating numbers of spot forming cells by ELISPOT to lymphocytes, rather than to all mononuclear cells, improves the analysis of local recruitment of antigen specific cells to the compartment of infection for the detection of smear-negative pulmonary TB (pTB). Although the ELISPOT is unable to simultaneously assign the quantity and phenotype of a cell, it is known from the use of flow-cytometry that responding cells represent CD4+ T-lymphocytes [3, 4]. Based on this knowledge, the combination of ELISPOT results with lymphocyte counts seems a reasonable strategy to increase diagnostic accuracy, and may be of particular interest for resource-poor settings where ELISPOT assays are easier to perform than flow-cytometric approaches.

When analysing mycobacterial-specific immune responses, purified-protein derivate (PPD) naturally generates more intense immune responses in mononuclear cells from the human lung than region of difference-1 (RD) restricted antigens. In a previous publication [1] PPD, early-secretory antigenic target (ESAT)-6 and culture-filtrate protein (CFP)-10 specific immune responses were compared in blood and bronchoalveolar lavage (BAL) by ELISPOT of patients with smear-negative pTB and in patients with nontuberculous pulmonary diseases. Approximately twice the number of PPD specific interferon (IFN)-γ releasing BAL mononuclear cells (BALMCs) than RD-1 antigen-specific BALMCs were reported in patients with pTB [2]. In contrast to the BALMC responses to RD-1-restricted proteins, PPD-specific BALMC responses were less specific as they did not discriminate well between patients with active pTB and non-TB control patients. In the non-TB control group, BALMCs from seven (28%) of a total 25 patients who were latently infected with *Mycobacterium tuberculosis*, yielded positive results by ELISPOT when stimulated with PPD [2]. While bacille Calmette–Guérin vaccination does not seem to influence PPD-specific immune responses in the human lung (which might be related to its limited protection against TB disease), BAL cells are preferentially enriched for PPD-specific lymphocytes in individuals with latent *M. tuberculosis* infection [5]. Recently, it was also demonstrated that the frequency of PPD-reactive interferon-γ producing CD4+ T-cells in the sputum of patients with active TB did not differ from individuals with presumptive latent TB infection [6].

While we constantly observe PPD-specific responses of BALMCs in ELISPOT of patients with a past history of pTB, these BALMC responses to RD-1-restricted antigens are usually absent in these patients if they do not have reactivation of TB.

A higher sensitivity of an assay that analyses PPD-specific immune responses *versus* RD-1-restricted immune responses in BALMCs as observed by BREEN *et al.* [7] might, therefore, result in a lower test specificity and could result in a higher number of indeterminate test results. Interim results of an ongoing multicentre study of the TB-NET, comparing the performance of the BAL ELISPOT with RD-1-restricted antigens and *Mycobacterium tuberculosis*-specific nucleic acid amplification technique, still demonstrate a sensitivity of the BAL ELISPOT with ESAT-6 and CFP-10 for the detection of smear-negative pTB of >90% (data not shown) [8].

In a small case series of five individuals with infections due to nontuberculous mycobacteria (NTM) at our institution, BALMCs of four patients had positive PPD-specific ELISPOT responses. However, BALMC responses to ESAT-6 and CFP-10 were negative in four individuals in this group. The one individual with an NTM infection and a positive BALMC immune response to the RD-1-selected antigens had an infection with *M. kansasii*, which is known to encode RD-1 proteins.

While the frequency of region of difference-1-specific T-cells for the detection of smear negative tuberculosis in the sputum