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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DOI: 10.1183/09031936.00043908

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 derivative (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

may be too low [9, 10], assaying the *ex vivo* reactivity of bronchoalveolar lavage T-cells towards *Mycobacterium tuberculosis*-specific antigens by flow cytometry may further improve the diagnostic accuracy of on-site immune based assays for the diagnosis of smear-negative tuberculosis.

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STATEMENT OF INTEREST

A statement of interest for C. Lange can be found at www.erj.ersjournals.com/misc/statements.shtml

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DOI: 10.1183/09031936.00059308

A green humidifier chamber

To the Editors:

A patient presented with a continuous positive airway pressure (CPAP) humidifier chamber that was coated in a green residue (fig. 1). This has never been seen before in our clinic, which has managed >6,500 patients on CPAP. He had been washing the chamber in soapy water, then rinsing it in tap water. Analysis of the patient's water supply found 0.7 mg·L⁻¹ of copper, which is considered to be below acceptable limits set by the 2004 Australian Drinking Water Guidelines for health and aesthetic considerations. Chemical testing of the residue using scanning electron microscopy with energy dispersive X-ray analysis and Fourier transform infrared spectroscopic analysis found that it was predominantly calcium stearate, with trace amounts of copper, calcium, aluminium, silicon and oxygen. Calcium stearate is an insoluble calcium salt of stearic and palmitic acids. It is formed when soap is mixed with "hard" water containing calcium ions. Over time, a build up of calcium stearate had caused the green residue within the chamber.

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STATEMENT OF INTEREST

None declared.

FIGURE 1. A continuous positive airway pressure humidifier chamber coated with a green residue due to a build up of calcium stearate.

DOI: 10.1183/09031936.00046208