Correspondence: Simona M. Cristescu, Dept of Molecular and Laser Physics, Institute for Molecules and Materials, Radboud University, Heyendaalseweg 135, 6525 AJ, Nijmegen, The Netherlands. Email: s.cristescu@science.ru.nl

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Pouched rats as detectors of tuberculosis: comparison to concentrated smear microscopy

To the Editor:

In 2014, 1.5 million people died of tuberculosis (TB), a disease that can be cured in nearly every case if detected in time. Rapid and accurate detection of TB is a crucial component of the World Health Organization's 2016–2035 End TB Strategy [1]. Pouched rats, *Cricetomys ansorgei* (previously called *Cricetomys gambianus* [2]), are able to detect *Mycobacterium tuberculosis* by sniffing sputum samples [3]. Since 2007, they have been used for second-line screening of sputum samples previously evaluated by Ziehl–Neelsen microscopy (ZN) at clinics in Tanzania. Use of the rats increases new case detections by ~40% [3].

In 2013, a TB-screening programme using rats opened in Maputo (Mozambique). Here, as in Tanzania, presumptive TB patients provide two samples, which the rats evaluate by sniffing holes above pots containing the heat-inactivated sputum. The rats are taught to pause above samples deemed TB positive by ZN. Samples deemed TB negative by ZN but TB positive by rats are evaluated by concentrated smear light-emitting diode fluorescence microscopy (LED-FM) [4, 5]. Presumptive TB patients with at least one LED-FM-positive sputum sample are assumed to have active TB and constitute additional case detections. Information about the disease status of such patients is conveyed to clinics so that they can be treated. Although sputum concentration and the use of LED-FM (rather than ZN) result in higher sensitivity, TB clinics in Mozambique and other financially impoverished countries are rarely able to implement these methods due to resource constraints [5, 6].

Cricetomys sniff samples quickly and have minimal housing and maintenance requirements, allowing diagnostic centres to evaluate each sample with multiple animals [7, 8]. By altering the number of rats evaluating samples and the group performance criterion used to determine whether a given sample is rat positive, the number of ZN-negative samples deemed rat positive and rat negative can vary substantially [9]. Previous studies have established the accuracy of the rats relative to culture [9, 10]. However, the practical value of the rats resides in their ability to reduce the number of samples that need to be evaluated by LED-FM, thereby reducing the requirement for laboratory staff and equipment in resource-limited settings. Because LED-FM is used to determine the final status of samples, the theoretical maximum number of additional case detections by rats is the number that would occur if they evaluated every ZN-negative sample as TB positive. But in this case the rats would be of no practical value. If, in contrast, the rats evaluated every LED-FM-positive sample as TB positive while evaluating no LED-FM-negative sample as TB positive, their predictive and practical value would be maximised.

To assess the rats' predictive and practical value, we determined the number of ZN-negative patients who could be detected as TB positive by LED-FM and the number of such patients who could be detected by a group of four rats in a sample of 447 presumptive TB patients from Maputo. Comparing these values provides an index of the rats' predictive value, specifically their sensitivity with ZN-negative but LED-FM-positive individuals. We also examined the total number of sputum samples provided by presumptive TB patients relative to the number of samples evaluated as TB positive by the rats. The larger the difference in these measurements, the fewer samples needed to be sent for confirmation with LED-FM, hence the greater the practical value of the rats.

A power analysis was carried out as recommended for diagnostic test studies [11], which resulted in a minimum sample size of 307 individuals to establish 95% confidence intervals for sensitivity with a maximal distance of 0.10 from the estimated value, assuming a TB prevalence of 20% for individuals visiting TB clinics in Maputo and rat sensitivity of 80%. Sputum samples were obtained from presumptive TB patients who had visited any of the eight collaborating TB clinics in September 2014. Any individual who provided two sputum samples on two consecutive visits to a clinic was eligible for inclusion in the study. Ethical approval was obtained from the National Bioethics Committee (Ministry of Health, Maputo, Mozambique) and, because there was no direct contact with patients, the need for informed consent was waived.

This study was conducted as part of routine second-line screening operations in which samples identified as ZN-positive at clinics are included in rat evaluation sessions, which enables handlers to reward correct indications of positive samples. Samples graded at clinics as 3+ (high concentration of bacilli) were not evaluated by the rats because training with high-concentration samples impairs the rats' detection of low-concentration samples [12].

The evaluation procedures are detailed elsewhere [3]. In brief, a rat holding its nose in the hole above a sample for >3 s was treated as an indication. Indications over ZN-positive samples were reinforced with a "click" and food. Indications over ZN-negative samples were recorded as indications but had no programmed consequence. Following rat evaluation, every sample was evaluated with LED-FM. Data were entered into a database and analysed using MedCalc Software (Ostend, Belgium).

Our findings are presented in tables 1 and 2. Of the 447 presumptive TB patients, 38 (8.5%) were ZN-positive. If an indication by one of the four rats was used to define a sample as rat positive, then 36 (95%) out of these 38 patients were identified by the rats. When all samples were analysed *via* LED-FM, 35 additional cases were revealed; 21 of these patients were identified by at least one rat yielding a patient-wise sensitivity relative to LED-FM (with ZN-positive individuals excluded) of 60% (95% CI 42–76%). With ZN-positive individuals included, sensitivity relative to LED-FM was 78% (95% CI 67–87%). If samples

TABLE 1 Summary of rats' performance: status of presumptive tuberculosis patients

	ZN⁺ individuals[#]	Additional case findings[¶]	LED-FM⁻ individuals	Total
Subjects	38	35	374	447
≥ 1 rat	36 (95%)	21 (60%)	175 (47%)	235 (53%)
≥ 2 rats	34 (89%)	17 (49%)	103 (28%)	157 (35%)
≥ 3 rats	34 (89%)	12 (34%)	65 (17%)	114 (26%)
4 rats	29 (76%)	8 (23%)	35 (9%)	76 (17%)

ZN: Ziehl-Neelsen microscopy; LED-FM: light-emitting diode fluorescence microscopy. Bold indicates key measures. [#]: all ZN⁺ individuals confirmed as positive with LED-FM; [¶]: LED-FM⁺ but ZN⁻ individuals, percentages indicate rats' sensitivity relative to LED-FM.

TABLE 2 Summary of rats' performance: status of individual sputum samples[#]

	From ZN ⁺ individuals		From additional case findings [¶]		From LED-FM ⁻ individuals	From ZN ⁻ individuals ⁺
	LED-FM ⁺	LED-FM ⁻	LED-FM ⁺	LED-FM ⁻		
Subjects	74	2	48	22	748	818
≥ 1 rat	67 (91%)	0 (0%)	28 (58%)	7 (32%)	242 (32%)	277 (34%)
≥ 2 rats	61 (82%)	0 (0%)	25 (52%)	3 (14%)	136 (18%)	164 (20%)
≥ 3 rats	57 (77%)	0 (0%)	19 (40%)	3 (14%)	79 (11%)	101 (12%)
4 rats	43 (58%)	0 (0%)	12 (25%)	3 (14%)	39 (5%)	54 (7%)

ZN: Ziehl-Neelsen microscopy; LED-FM: light-emitting diode fluorescence microscopy. Bold indicates key measures. [#]: two sputum samples were collected from each individual; [¶]: samples from LED-FM⁺ but ZN⁻ individuals; ⁺: percentages indicate how many samples were indicated by rats and would be evaluated by LED-FM.

indicated by one or more rats were treated as rat positive, 34% of the samples from ZN-negative individuals were indicated and would require evaluation by LED-FM in routine operations. Progressively increasing the number of rat indications required to define a sample as rat positive systematically reduced sensitivity, hence additional case findings, but increased specificity, thereby reducing the number of samples from ZN-negative individuals that would need LED-FM confirmation.

Previous studies have suggested that results are optimised with a one-rat indication criterion when employing a group of four rats [9, 10]. The present results are consistent with those findings. Had ordinary operational procedures been used in the present study, and a one-rat indication criterion been applied, 34% of the sputum samples would have required LED-FM confirmation and examining those samples would have revealed 60% of the total TB patients that LED-FM could detect. Although 40% of LED-FM-positive individuals were not indicated by the rats, it is likely that some of these positives were false-positives because of the presence of non-TB mycobacteria [10].

Using rats to pre-screen sputum samples in centralised, second-line screening operations appears to be an effective means of increasing the rate of TB detection in circumstances where resource constraints make it impossible to re-evaluate samples from all, or most, smear-negative presumptive TB patients with concentrated smear LED-FM or an alternative technique that is equally, or more, accurate. The development of better triage tools for systematic screening of high-risk individuals, such as miners and occupants of refugee camps [13, 14], is considered high-priority among key stakeholders [15]. High throughput and low variable costs make the TB-detection rats a promising triage tool for systematic screening. Therefore, their accuracy compared to Xpert MTB/RIF (Cepheid Inc., Sunnyvale, CA, USA) and their cost-effectiveness are now being assessed to further clarify the potential of this technology.

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Pouched rats find 60% of TB patients that are missed by clinics but identifiable with concentrated smear microscopy <http://ow.ly/jnUr300i2I7>

Timothy L. Edwards^{1,2}, Emilio Valverde^{2,3}, Christiaan Mulder^{2,4}, Christophe Cox² and Alan Poling^{2,5}

¹School of Psychology, University of Waikato, Hamilton, New Zealand. ²Anti-Persoonsmijnen Ontmijnende Product Ontwikkeling (APOPO), Morogoro, Africa. ³Medical School, Vanderbilt University, Nashville, TN, USA. ⁴Amsterdam Institute for Global Health and Development, Amsterdam, The Netherlands. ⁵Dept of Psychology, Western Michigan University, Kalamazoo, MI, USA.

Correspondence: Timothy L. Edwards, School of Psychology, University of Waikato, Private Bag 3105, Hamilton 3240, New Zealand. E-mail: edwards@waikato.ac.nz

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Is bedaquiline as effective as fluoroquinolones in the treatment of multidrug-resistant tuberculosis?

To the Editor:

Bedaquiline (Bdq) is approved for the treatment of multidrug-resistant (MDR) tuberculosis (TB). In a phase IIb trial, Bdq allowed a significant reduction in time to culture conversion and improved outcome in MDR-TB patients [1, 2]. Preliminary reports of Bdq compassionate use have shown promising results [3–5]. However, in an early bactericidal activity (EBA) study, the association of moxifloxacin (Mfx) with PA-824 and pyrazinamide showed better activity than Bdq-based associations [6]. In addition, resistance to fluoroquinolones (Fq) has been associated with poorer outcome in MDR-TB before Bdq use [7]. These data reinforce the pivotal role of Fq. Comparing Bdq to Fq in interventional studies is challenging. Indeed, the paucity of drugs available for MDR-TB treatment, and the need for combination therapy, often impose the need to use all available drugs.

In an interim analysis of a Bdq-treated MDR-TB cohort, we showed that culture conversion reached 96% with 6-month Bdq-containing treatment regimens [8]. Following these encouraging results, we sought to compare the microbiological efficacy of Bdq- and Fq-containing regimens.

A retrospective study comparing microbiological outcome in Bdq-treated and Fq-treated patients was designed using the 2006–2014 cohort of MDR-TB patients hospitalised at Bligny's Sanatorium (Briis-sous-Forges), a French referral TB centre. The first group included patients treated for ≥ 30 days with Bdq, who either had not received any Fq or had received Fq but harboured *Mycobacterium tuberculosis* isolates with high-level phenotypical Fq resistance. The second group comprised patients treated for ≥ 30 days with any Fq but without Bdq, with isolates susceptible to ofloxacin (Ofx) and Mfx. All patients received any second-line injectable drug and linezolid for ≥ 30 days, and had positive sputum cultures at treatment start.