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Point-of-care urine test for assessing adherence to isoniazid treatment for tuberculosis

To the Editor:

Good adherence to treatment for tuberculosis (TB) is essential, both to cure disease and prevent development of drug resistance. Adherence to chemoprophylaxis (preventive therapy) for latent TB infection (LTBI) is particularly poor [1].

Methods for measuring adherence to TB medication include detecting urine colour change due to the presence of rifampicin. However, this effect is short lived, peaking at 2–6 h and is only seen in <50% of patients [2]. Directly observed therapy (DOT) will ensure adherence to antituberculous treatment, but this can be unacceptable and many patients do not tolerate a three times a week regimen. DOT is costly in terms of personnel and is seldom employed in chemoprophylaxis patients [1]. The highly reliable Arkansas method for detecting isoniazid metabolites in urine relies on a laboratory colorimetric assay, involving adding drops of prepared solutions of reagents, including potassium cyanide, to a urine sample [3]. There are obvious risks involved in handling and storing the reagents.

IsoScreen (GFC Diagnostics, Bicester, UK) uses the reagents of the Arkansas Method but in an enclosed plastic testing device (SafeTube; GFC Diagnostics), allowing safe and rapid point-of-care testing in clinics and patients' homes [2]. The urine colour change is apparent within a few seconds but 5 min is allowed to ensure stable colour development. Purple or blue samples are deemed positive, green intermediate and

yellow negative. High sensitivities and specificities have been reported, but without a consistent relationship to the timing of the previous dose of isoniazid or measurement of acetylator status [2, 4].

In this prospective study, we analysed the relationship over time of the colour change seen with IsoScreen over 72 h to determine whether different colours could accurately inform when the last dose was taken, and the influence on this of acetylator phenotype. *N*-acetyltransferase 2 (NAT2) is the enzyme responsible for metabolising isoniazid to acetyl-isoniazid. The *NAT2* gene is highly polymorphic, with over 50 allelic variants identified. Of these variants, single nucleotide polymorphism (SNP) substitutions at positions $G^{191}A$, $T^{341}C$, $G^{590}A$ and $G^{857}A$ result in a significant decrease in the enzyme's acetylation capacity [5]. Individuals with these mutations exhibit a ''slow acetylator'' phenotype, while those with the wild-type allele are known as ''fast acetylators''. A third phenotype, the ''intermediate acetylator'', may also exist, corresponding to the heterozygous genotype [6].

105 patients completing treatment for either active TB or LTBI were recruited from two UK TB clinics (Mayday University Hospital, Croydon, UK and Guy's and St Thomas' Hospitals, London, UK). Urine samples for testing with IsoScreen were provided within 12 h and at 24, 48 and 72 h following ingestion of the final treatment dose containing isoniazid. 83% received this final dose by direct observation. Single urine samples from 38 healthy controls of similar age, sex and ethnic origin were randomly tested using IsoScreen. Blood samples were taken from 46 patients to measure acetylator status by genotyping, using a previously described protocol [7]. Assays were developed for six commonly occurring SNPs in the *NAT2* gene: $C^{282}T$, $T^{341}C$, $C^{481}T$, $G^{590}A$, $A^{803}G$ and $G^{857}A$, and proved to be robust [5]. All patients provided informed written consent.

Binary and ordinal logistic regression analysis, fitted with robust standard error to take into account the multiple measures per patient, was used to analyse IsoScreen reliability at different time-points, and the effect of demographic data, medication, alcohol consumption, smoking habit and acetylator status on IsoScreen responses (Minitab version 16; Minitab, State College, PA, USA). Sensitivity, specificity, and positive and negative predictive values were assessed. Significance was determined as $p \leqslant 0.05$.

Full demographic data are shown in table 1. Renal function, measured in 95 patients, was normal; abnormal liver function at presentation in four patients improved following treatment and one patient was HIV positive. Data on smoking habit, alcohol consumption and additional medication are shown in table 1. Results of urine colour changes at different time-points are also shown in table 1. All healthy controls gave a negative result.

The majority (32 out of 46) were slow acetylators (homozygous mutant), five were fast (homozygous wild-type) and nine were intermediate (heterozygous). The most common allele, found in 19 (29%) of the Asian patients, was the NAT2*5B variant and the most common genotype was NAT2*5B/6A, giving a deduced slow phenotype in 65.5% of this, the largest, group.

There was a highly significant probability of observing a purple result at 12 h, a blue result at 24 h, a green result at 36 h and a yellow result at 72 h (OR 0.84, robust SE 0.01 (95% CI 0.81–0.88); $p=0.0001$). As the concentration of metabolites falls over time, the odds ratio would be expected to be <1. There was very little variation between individuals at 12 and 72 h in particular (table 1). Multivariate analysis showed no associations between urine colour change, acetylator status and other collected data. Sensitivity, specificity, and positive and negative predictive values for a positive test at 12 and 24 h were especially high: 100% at 12 h; and 99%, 100%, 100% and 94%, respectively, at 24 h. For a green colour change at 48 h, these results were 78%, 100%, 100% and 45%, respectively.

This study demonstrates that a simple, rapid, point-of-care test for isoniazid metabolites in urine, the IsoScreen, reliably demonstrates adherence to isoniazid containing regimens, with positive results at 12 and 24 h after ingestion, while a green (intermediate) colour change is likely at 48 h and a yellow (negative) result at 72 h. The operator is not exposed to toxic chemicals, unlike the original Arkansas method, as the reagents are sealed before use and the reactants after use. A recent study of 54 patients also found high sensitivity (93.2%) and specificity (98.7%) of IsoScreen, but the time intervals from ingestion of isoniazid to urine collection were not defined and varied from 30 min to 26 h [8]. Our study assessed colour change over time using defined time-points and also assessed acetylator status.

We found that the detection of isoniazid metabolites by IsoScreen was not affected by patient demographics, smoking, alcohol or additional medication. Carbamazepine can increase and corticosteroids decrease plasma isoniazid concentrations. Although three patients taking these drugs did not differ in their results from others, one taking carbamazepine still had an intermediate result at 72 h (data not shown). None of the other prescribed drugs should have affected isoniazid metabolism. Both prescribed and illicit drugs were previously found not to affect results obtained by the Arkansas method [3]. The presence of

TABLE 1 Patient demographics[#] and urine colour change at different time-points

Patient demographics (n=105)					
Sex	59 males (56%)	46 female (44%)			
Age years	Median (range) 33 (17–74)	Mean±SD 35.7±12.1			
Ethnicity	30 Black African/ Caribbean (29.1%)	53 Indian/Pakistani Asian (50%)	15 Asian other (14.6%)	3 mixed race (2.9%)	4 White (3.9%)
Smoking habit	76 never-smoked (72.4%)	8 ex-smokers/recently stopped (7.6%)	19 current smokers (18.1%)	2 chewing tobacco (1.9%)	
Alcohol consumption	93 non-drinkers (88.6%)	12 drinkers (11.4%)	Mean±SD 8.8±11.5 units per week	Median 4.0 units per week	
Tuberculosis drugs	97 standard chemotherapy (92.4%)	8 chemoprophylaxis (7.6%)			
Other prescribed drugs	3 possible drug effect on isoniazid metabolism (2.9%)	35 unlikely drug effect on isoniazid metabolism (33.3%)	67 none prescribed (63.8%)		
Recreational drug use (n=98)	2 reported (2%)	96 not reported (98%)			

Urine colour change at different time-points after final isoniazid dose					
Time from last dose of isoniazid	**Urine samples tested**	**Purple (positive)**	**Blue (positive)**	**Green (intermediate)**	**Yellow (negative)**
⩽12 h	99	75 (76)	24 (24)	0	0
24 h	105	17 (16)	69 (66)	18 (17)	1 (1)
48 h	105	0	5 (5)	84 (80)	16 (15)
72 h	104	0	0	14 (13)	90 (87)

Data are presented as n or n (%), unless otherwise stated. [#]: rifampicin and vitamin B6 ceased 12 h before first sample.

nicotine metabolites in the urine could, theoretically, interfere with the colour change, due to the similarities in molecular structure to isoniazid. We found no evidence of such interference.

HIV testing is offered to all patients treated for active TB but only one patient was known to be HIV positive. Those on chemoprophylaxis were not routinely tested for HIV infection but no patient has subsequently presented with evidence of infection. Therefore, HIV infection would be unlikely to affect our results.

Although acetylator status could affect the rate at which isoniazid metabolites appear in the urine, we did not find this in 46 patients studied. The presence of acetylator phenotypes varies in different populations [9]. In our study 29 out of 46 patients were Asian and 65.5% of these were slow acetylators. Similarly, 75% of a South Indian population were slow acetylators [10]. Fast acetylation was postulated as an explanation for negative urine results at 24 h in five out of 94 patients after isoniazid ingestion, although acetylator status was not measured [4]. Using logistic regression analysis, we found no effect on Isoscreen results in five fast acetylators, whose individual results did not differ from others. Overall, we found no negative results at 12 h, but one at 24 h. Acetylator status had not been measured in this patient of Indian Subcontinent ethnic origin.

Improving adherence to treatment of both LTBI and active disease is essential for TB control and the prevention of drug resistance. The IsoScreen test for isoniazid metabolites in urine gives a reliable and immediate indication of adherence to isoniazid-containing treatment regimens. Unexpected negative or intermediate results up to 24 h post dose should raise the possibility of nonadherence and stimulate enhanced supervision. In this cohort, patient characteristics, acetylator status, smoking, alcohol or prescription medication were not detrimental to the performance of IsoScreen, making it a cheap and practical alternative to other methods for assessing adherence.

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Adherence to TB treatment regimens containing isoniazid can be reliably assessed using a rapid point-of-care urine test http://ow.ly/rPZxR

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Detecting active pulmonary tuberculosis with a breath test using nanomaterial-based sensors

To the Editor:

Detecting active tuberculosis (TB) remains a major global public health challenge [1]. The tuberculin skin test does not distinguish latent from active TB [2]. The interferon-γ release assays have similar limitations [3]. Acid-fast bacilli staining of sputum has a high false-negative rate (up to 50%) [4]. Nucleic acid amplification tests (NAATs), such as GeneXpert MTB/RIF (Cepheid, Sunnyvale, CA, USA), are accurate but require a good infrastructure and the necessity to obtain a good quality sputum sample, which is often unobtainable in more than a third of HIV-infected persons [5, 6].

Given these unmet needs, we explored the use of a novel, rapid, simple and inexpensive point-of-care test for the diagnosis of TB [7]. The approach is based on the detection of volatile organic compounds (VOCs) that are emitted from infected cells and released in exhaled breath [8, 9]. Using gas chromatography linked with mass spectrometry, researchers have previously reported identification of TB-related VOCs in the exhaled breath, though there has been low accuracy in detection (80–85%) [10]. In this study, we explore