A reproducible and accurate 14-drug microtitre plate containing bedaquiline and delamanid for susceptibility testing of *Mycobacterium tuberculosis*

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Running title: Validation of a 14-drug microtiter plate for TB DST.

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ABSTRACT

Universal access to drug susceptibility testing is key to ending TB. UKMYC5 is a 96-well microtitre plate designed by the *Comprehensive Resistance Prediction for Tuberculosis: an International Consortium* (CRyPTIC) which has the potential to determine, at low cost, the MICs for 14 different anti-TB drugs, including several new and repurposed compounds. It is a dry-format plate and therefore easy to transport and store. UKMYC5 plates were tested by seven laboratories on four continents using a panel of 19 external quality assessment (EQA) strains, including H37Rv. MICs were measured from each plate by two readers using three methods (mirrored-box, microscope and Vizion™ Digital viewing system) at four different timepoints. All EQA strains were whole-genome sequenced and phenotypically characterized by MGIT960, 7H10/7H11 agar and resazurin microtitre assay. The optimum incubation period for a plate is 14 days. The within- and between-laboratory reproducibilities for the best performing methods (mirrored-box and Vizion) were >95% and >92%, respectively. One site was identified as requiring re-training and one drug (*para*-aminosalicylic acid) produced inconsistent results.

MICs measured using the UKMYC5 microtitre plate (i) are reproducible, (ii) compare well with the results of several established methods and (iii) correlate with the presence or absence of genetic mutations that confer resistance. This study provides evidence that this assay can be deployed by TB reference laboratories world-wide as a diagnostic and research tool.

INTRODUCTION

The proportion of tuberculosis (TB) cases that are multi-drug resistant (MDR) is increasing worldwide. Although set against a background of a falling global incidence of TB, the net effect is that the number of MDR-TB cases continues to grow (1). Improving the treatment success rate for MDR-TB requires each patient to receive an individual antimicrobial regimen tailored to maximize efficacy whilst minimizing toxicity; this necessitates being able to measure minimum inhibitory concentrations (MIC) to direct both the choice of drug and dose. Universal access to prompt and comprehensive drug susceptibility testing (DST) is therefore a key component of the WHO's *End TB Strategy* (2, 3). Although molecular approaches have the potential to deliver universal DST methods, they require further development work and any resulting solutions are likely to be expensive.

Liquid and solid media assays that measure MICs for TB exist (4–9), but are time consuming and, often, costly. Microtitre plates offer a way of testing in parallel the effectiveness of a large number of drugs at a range of concentrations on small aliquots taken from a single clinical isolate. Broth microdilution methods, including several using colorimetric indicators, have previously been developed that assess the MICs for a panel of compounds using a single microtitre plate (5, 10, 11). A dry-format, 96-well microtitre plate assay (the Sensititre™ MYCOTBI plate; Thermo Fisher Scientific Inc., USA) containing 12 drugs has been commercially available since 2010 and early validation studies have returned promising results (12–16). No plate-based assays, however, have so far included both new and re-purposed drugs that will be key to the successful treatment of individual MDR-TB cases in the future.

In this paper, we validate a bespoke, dry-form 96-well microtitre plate (UKMYC5) that has been designed by the global CRyPTIC (*Comprehensive Resistance Prediction for Tuberculosis: an International Consortium)* project. Since the UKMYC5 plate contains 14 drugs, including two

new compounds (delamanid and bedaquiline) and several re-purposed drugs (Fig. S1, Table S1), it could form the basis of a new DST protocol for tailoring regimens to treat individual cases of MDR-TB. We shall assess, therefore, both the reproducibility of MIC measurements using this microtiter plate and its accuracy by comparing it to a range of established DST methods.

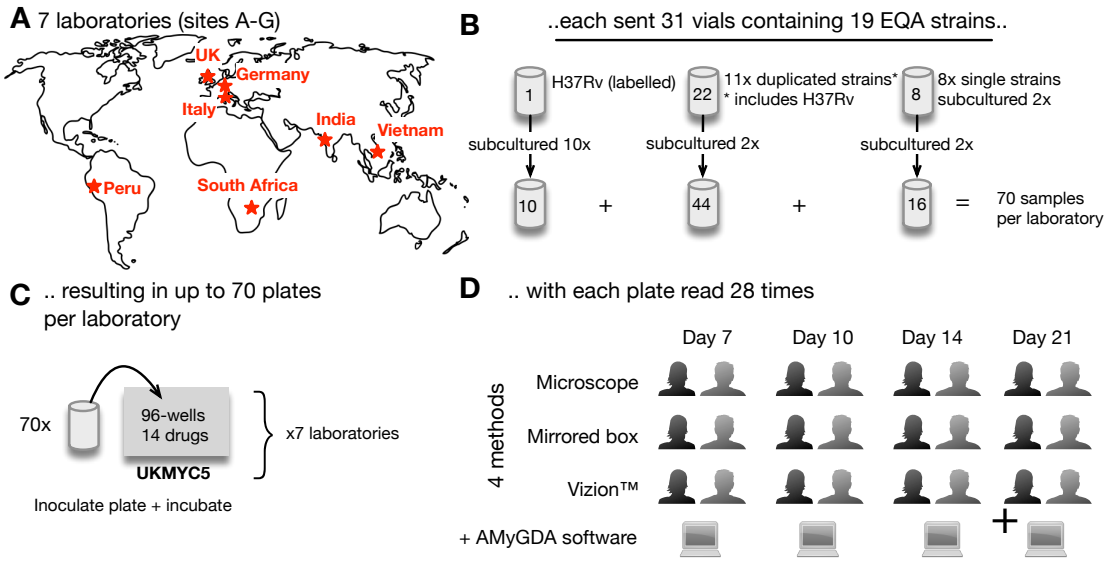

FIG 1. The study design for validating the UKMYC5 plate. (**A**) Seven laboratories (**B**) were each sent 31 vials containing 19 different WHO external quality assessment (EQA) strains. The sample of H37Rv was subcultured ten times, with all other vials sub-cultured in duplicate, making a maximum total of 70 samples. (**C**) Each was inoculated onto a UKMYC5 96-well plate. (**D**) The minimum inhibitory concentrations for each drug were independently read at 7, 10, 14 & 21 days post-inoculation by two laboratory scientists using three methods. Each plate was also photographed and the image analysed using the AMyGDA software (Fig. S2).

RESULTS

Study design

Each of the seven participating laboratories (Fig. 1A) received nineteen external quality assessment strains, the majority of which were blinded. These were sub-cultured either 2 or 10 times (Fig. 1B) and then inoculated onto a UKMYC5 plate (Fig. 1C). Each plate was incubated for 28 days, and two laboratory scientists measured the MICs for all 14 anti-TB drugs at four different timepoints using three different methods (Fig. 1D). In addition, a photograph was taken at each timepoint, and this was retrospectively analysed using some bespoke plate-reading software, AMyGDA (Fig. S2) (17).

The proportion of readable plates

The proportion of readable results (defined in the Materials and Methods) increased with elapsed time since inoculation. For Site F the proportion of readable results was anomalously high (≥94.2%), regardless of reading day (Fig. S3, Table S2), yet the reproducibility within Site F was anomalously low, varying between 77.5%-80.2% depending on the reading day (Fig. S3, Table S2). Site F also had an anomalously low between-site reproducibility of only 72.2%-75.3% (Fig. S3, Table S2). Logistic mixed-effect models confirmed that for Site F between reader overall essential agreement (OEA, within a doubling dilution) and within- and between-site reproducibilities were all significantly lower than the other laboratories ($p<0.001$ for all comparisons; Table S3). Data from Site F was consequently excluded from subsequent analyses. For the remaining six sites, the proportion of readable results was 57.8-66.1% (depending on reading method) after 7 days of incubation, but then increased to 85.7-93.0% after 14 days, reaching ≥95.9% after 21 days (Fig. 2A, Table S4).

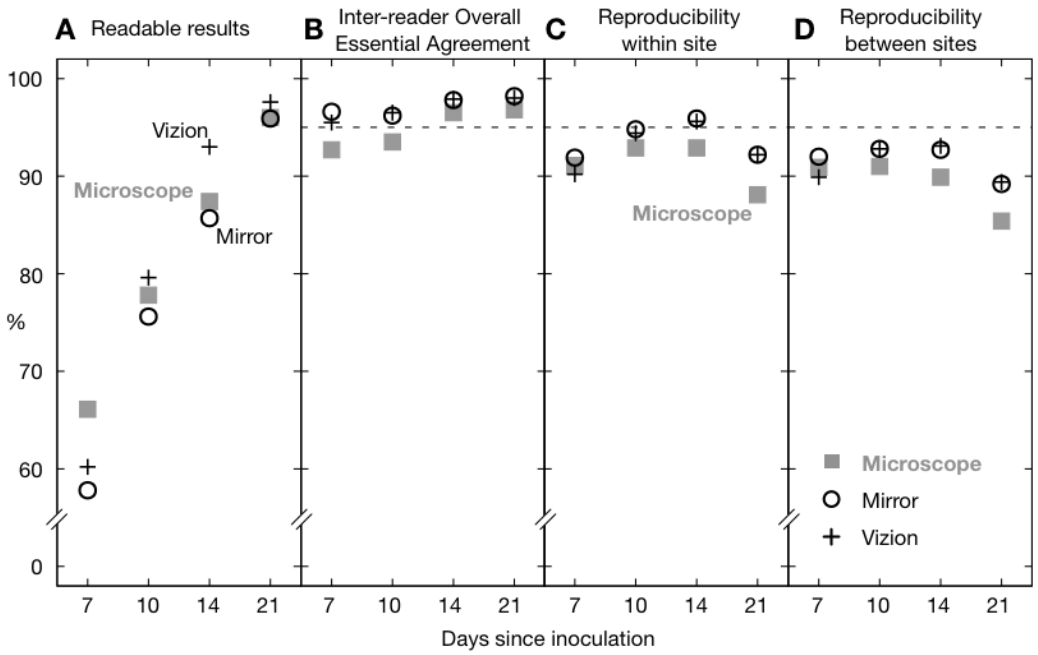

FIG 2. For each reading method, (**A**) the percentage of readable results, (**B**) the Overall Essential Agreement (OEA) between two readers, and the reproducibility (**C**) within and (**D**) between sites (Table S4). Site F is excluded from this analysis. The dashed line indicates 95%, which is the level of reproducibility required for an antimicrobial susceptibility test device to conform to ISO-20776-2.

Overall within and between laboratory reproducibility.

At least 92.7% of all MICs read by two scientists from the same laboratory were in OEA, regardless of reading day or method (Fig. 2B, Table S4). The OEA was ≥96.5% 14 and 21 days after inoculation, regardless of reading method. Reproducibility of MIC readings (measurements within a doubling dilution of the mode) within sites was ≥88.1% (Fig. 2C, Table S4) regardless of reading-day and method. The maximum values were observed after 14 days of incubation, when the mirrored-box and Vizion within-site reproducibilities were 95.9% and 95.6%, respectively. The reproducibility between-sites was slightly lower, as one would expect,

being ≥85.4% regardless of reading-day and method combinations, peaking again after 14 days at 93.1% and 92.7% when using Vizion and mirrored box, respectively (Fig. 2D, Table S4).

Selection of reading day and method.

After 7 days, only between 57.8-66.1% of results were readable, depending on the method used, compared to 85.7-93.0% at day 14, and between 95.9%-97.6% at day 21 (Fig. 2A). If a plate was not readable, this was usually because there was insufficient growth of *M. tuberculosis* in both positive control wells. The corresponding logistic mixed-effects model demonstrated that significantly fewer plates were readable at day 7 and 10 than at day 14, and that significantly more plates were readable at day 21 compared with day 14 ($p<0.001$ for all comparisons, Table S5). The proportion of results readable by inverted-light microscope or mirrored box was significantly lower than for Vizion™ ($p<0.001$ in both instances, Fig. 2A, Table S5).

The OEA between two scientists examining the same plate within a laboratory increased with time (Fig. 2B). For Vizion™ and mirrored box, the OEA was ≥95.5% for all reading-days, while for the inverted-light microscope it increased from 92.7% at day 7 to 96.8% at day 21 (Fig. 2B). The corresponding model showed there was significantly lower OEA between readers when they used the inverted-light microscope compared to the Vizion™, or when they read the plates at day 7 compared to day 14 ($p<0.001$ for both comparisons, Table S5).

Overall, the greatest reproducibility (both within and between laboratories) was observed at day 14 (Fig. 2C & 2D) using either the Vizion™ or the mirrored box: the within-site reproducibilities were 95.6% & 95.9%, respectively, whilst the between-site reproducibilities were slightly lower at 93.1% & 92.7%, respectively. For the inverted-light microscope, the corresponding

reproducibilities were 92.9% and 89.9%. The logistic mixed-effects models confirmed that within- and between-site reproducibility for the inverted-light microscope was overall significantly lower with respect to Vizion™ ($p<0.001$ for all, Table S6). The Sensititre™ Vizion™ Digital MIC Viewing System was therefore selected for all subsequent analyses, although we note that the mirrored box performed similarly.

The individual MICs measured using Vizion™ were then analyzed to establish the optimal reading-day for each drug (Fig. S4, Table S7). *Para*-aminosalicylic acid showed the lowest OEA between readers at each reading day and the lowest reproducibility both within and between sites at days 10, 14 and 21. The results of the corresponding logistic mixed-effects models (Table S8) showed that, overall, readings had a significantly lower probability of being reproducible within- and between-sites at days 7 and 21, than at day 14 ($p=0.001$ and $p=0.004$, respectively for day 7, and $p=0.001$ and $p<0.001$ respectively for day 21). Reproducibility was similar at days 10 and 14 ($p=0.465$ and $p=0.784$, respectively).

Within the same model, reproducibility using Vizion™ was assessed also on an individual drug basis by using rifampicin as a reference standard (Table S8). Although there were statistically significant differences between many of the drugs, the greatest difference was seen for *para*-aminosalicylic acid where the reproducibilities within- and between-sites were significantly lower than rifampicin ($p<0.001$). Although the reproducibilities of the drugs were similar after 10 and 14 days (Table S8), since more results were readable after two weeks, reading day 14 was preferred and was used in subsequent analyses.

We shall use the minimum standards set out in ISO-20776-2 that any new antimicrobial susceptibility test must meet as our success criteria (18) – the reproducibility as defined in the

Materials and Method must be ≥ 95%. After 14 days of incubation, all drugs had within-site reproducibilities ≥ 95% (Fig. S4, Table S7), with the exception of bedaquiline (94.3%), linezolid (93.4%) and *para*-aminosalicylic acid (74.2%). Seven drugs do not meet the more stringent test of requiring between-site reproducibility ≥ 95%, although five of these return between site reproducibilities of ≥ 90%: bedaquiline (87.1%), clofazimine (92.2%), delamanid (94.7%), linezolid (91.6%), moxifloxacin (93.7%), *para*-aminosalicylic acid (67.5%) and rifabutin (92.4%) (Fig. S4, Table S7).

Minimum inhibitory concentrations measured using our chosen combination of Vizion™ and UKMYC5 plate at day 14 are 95.6% reproducible when determined at the same laboratory, but the reproducibility between sites (i.e. same strain, but different plate, reader and laboratory) is 93.1%, which is below the specified threshold of 95%, although comparing between different laboratories is a very stringent test (18).

Results with AMyDGA software

MICs measured from photographs of the UKMYC5 plates using the AMyGDA software (Fig. S2) (17) were then compared with those based on the Vizion™ reading method. The overall OEA between the two methods starts at 87.9% at day 7 and increases with incubation time, reaching 93.8% after 21 days (Fig. 3, Table S9). At day 14, the OEA between readings by AMyGDA and Vizion™ is ≥90% for all drugs, except for moxifloxacin (89.3%) and *para*-aminosalicylic acid (73.8%), and therefore conditionally satisfies ISO-20776-2 (18). Using a logistic mixed-effect model, we found that the overall OEA at days 7, 10 and 21 is not significantly different from the overall OEA at day 14 ($p=0.143$, $p=0.479$ and $p=0.525$, respectively, Table S10).

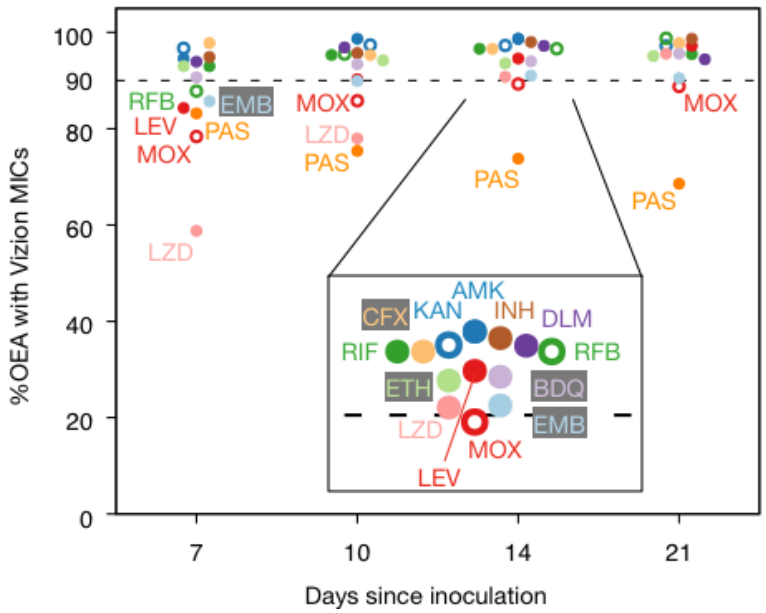

FIG 3. The proportion of Overall Essential Agreement (OEA) between MICs read by Vizion™ and determined by the AMyGDA software (Table S9). The dashed line at 90% indicates the minimum level of essential agreement between a new antimicrobial susceptibility test and an existing method according to ISO-20776-2. Drugs are labelled using the abbreviations defined in Table S1.

MIC distributions for H37Rv and agreement with reference standards.

MICs measured by Vizion™ at day 14 for the H37Rv reference strain were compared with MICs obtained by the agar proportion method (APM) and REMA (16, 19). For the majority of the drugs, the MIC values obtained by the APM were mostly within one doubling dilution of UKMYC5 readings, indicating a good correlation between the two methods (Fig. 4A). Similar results were obtained for the comparison with MICs obtained by REMA. MICs for bedaquiline, isoniazid, clofazimine, rifampicin, levofloxacin, moxifloxacin, amikacin, linezolid, ethambutol and kanamycin were compared to the corresponding mode of MICs measured using frozen-form microtitre plates (16). We shall again use the condition set out in ISO-20776-2: this states that a new antimicrobial susceptibility test must be in essential agreement with a reference

standard in ≥90% of cases (18). After 14 days incubation, the overall essential agreement was ≥ 90% for all drugs (Fig. 4B, Table S11), while by day 21 the OEAs of KAN and EMB had decreased to 65.2% and 83.0%, respectively, and hence were below the threshold (Fig. S5B, Table S11). The overall essential agreement between the two methods was 98.3% at day 14, dropping to 92.4% at day 21 (Table S11), both of which are above the prescribed 90% threshold.

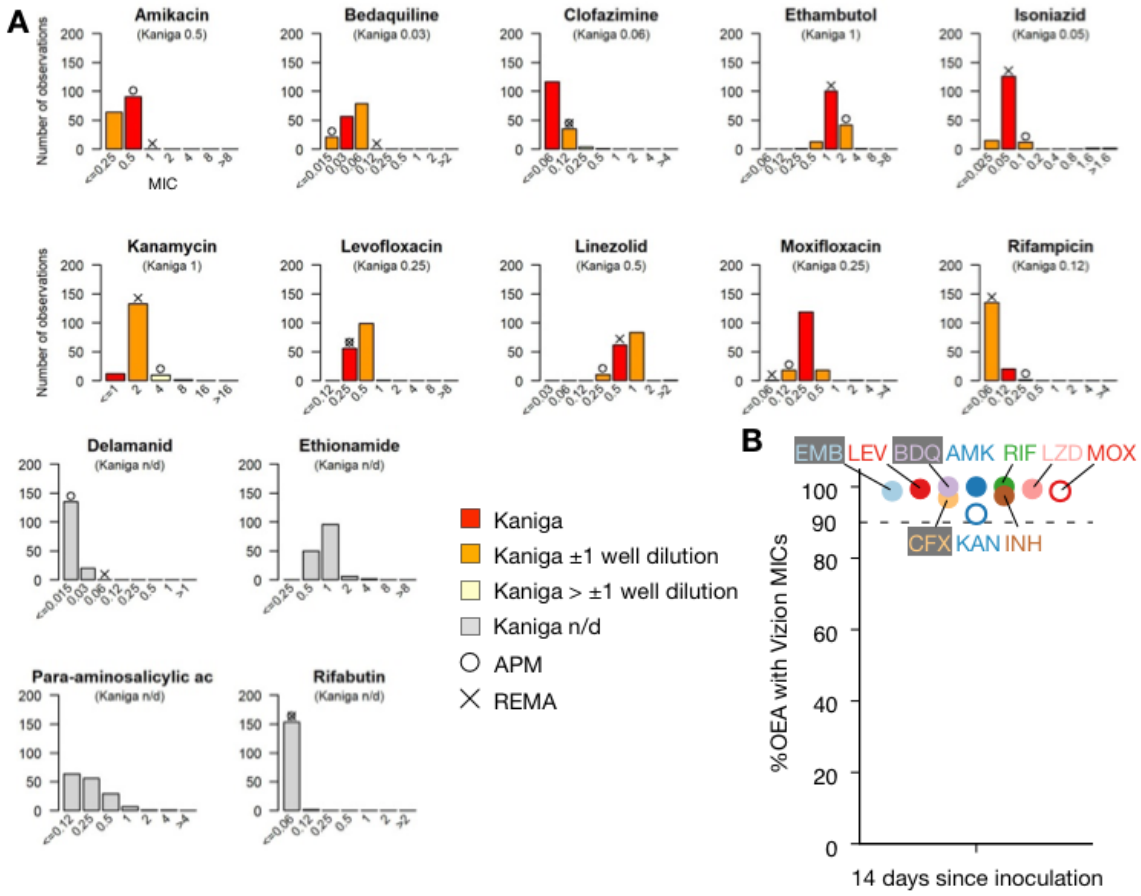

FIG 4. Minimum Inhibitory Concentration (MIC) distributions for H37Rv, as measured at day 14 by Vizion™ and compared with the corresponding mode of frozen-form microtitre assay MICs. (**A**) Red bars correspond to identical frozen-form assay and UKMYC5 values; orange bars correspond to frozen-form microtitre values falling within one dilution from UKMYC5's; yellow bars correspond to frozen-form microtitre values falling beyond one dilution from UKMYC5's. Grey bars indicate that the drug is not present on the frozen plates. (**B**) The Overall Essential Agreement between the MICs as measured by Vizion™ and frozen-form microtitre plates. The dashed line at 90% indicates the minimum threshold for an antimicrobial susceptibility test to be considered accurate by ISO-20776-2. Drugs are labelled using the abbreviations defined in Table S1.

For all strains, the mode of UKMYC5 MICs was calculated by drug and then compared with the categorical results (susceptible or resistant) obtained from MGIT and APM, for drugs with established breakpoints (Fig. 5) (13, 20, 21). Categorical and conditional agreement between UKMYC5 MICs and those obtained from MGIT and the APM were also computed (Table S12). To infer 'sensitivity' or 'resistance' using the UKMYC5 plate, we assumed shared breakpoints with each comparator method (APM or MGIT). Discrepancies are shown in Table S13.

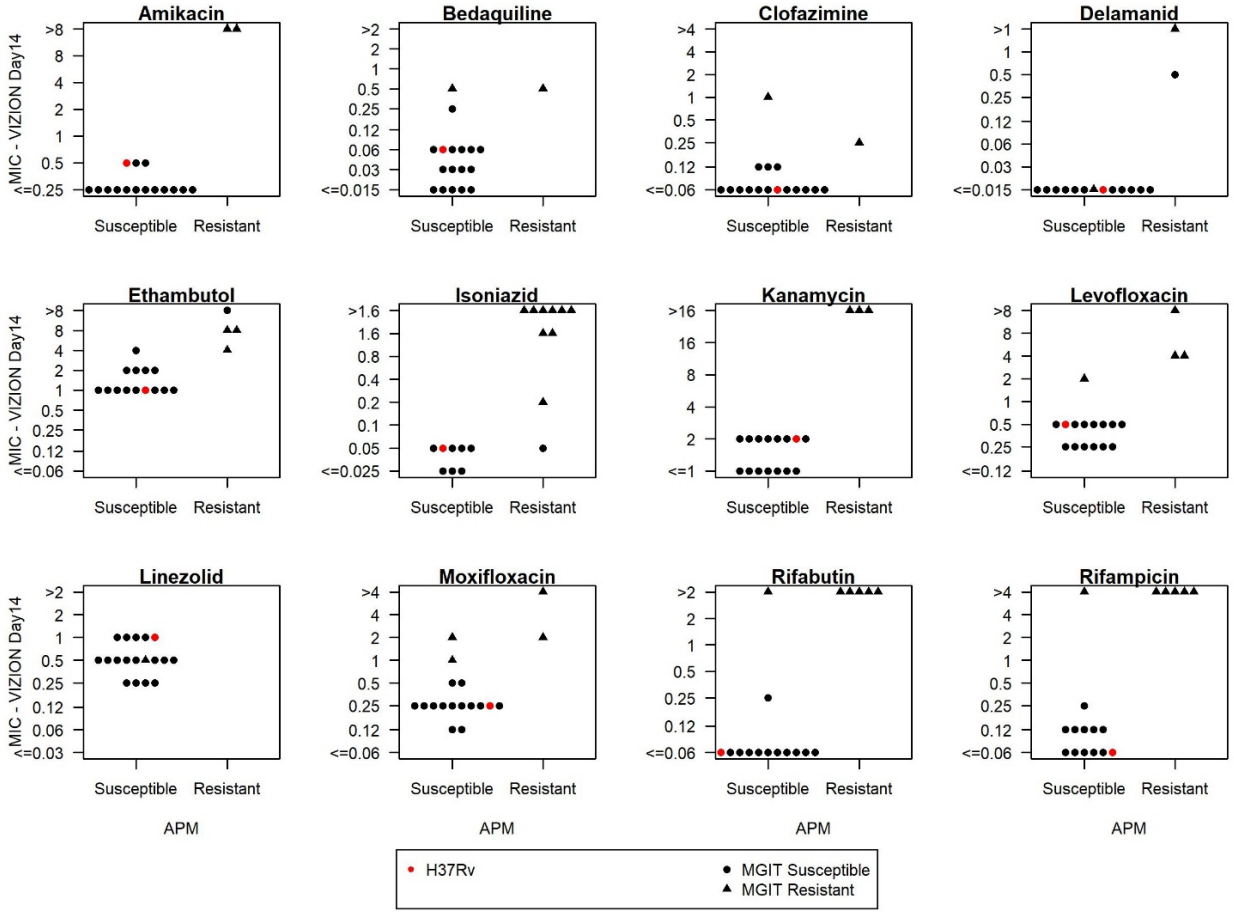

FIG 5. UKMYC5 Vizion™ MICs Day 14 versus APM and MGIT results for all tested strains. The critical concentrations used are reported in Table S12. Filled red circles indicate the mode MIC of H37Rv ATCC 27294.

Comparison between genotype and UKMYC5 results

For all drugs, the presence of mutations in genes in which there is a high confidence that they confer resistance was associated with MICs greater than observed in wild type strains (Fig. 6) (22). Mutations whose role in the drug resistance was not well established in the literature were included in the analysis. Only bedaquiline, ethambutol, linezolid and the fluoroquinolones showed some overlap in MICs between wildtype strains and strains containing mutations in genes associated with resistance. For linezolid, no difference of MICs was observed between wild type and mutant strains, but this was expected since these mutations were shown to be susceptible by MGIT and APM (Table S18), although mutations in the *rrL* gene have been reported to shift MICs (23). As expected, mutations associated with low-level resistance, such as *inhA*_C-15T, were associated with smaller MIC increases than mutations in *katG* that are associated with high-level resistance (Fig. S6) (24). Interestingly, the EQA panel included two isolates that were resistant to delamanid according to the APM. One of these had a mutation in the *ddn* gene, and the other, in *fbiC*. The *ddn* mutation was associated with higher MICs than the *fbiC* mutation (Fig. S6).

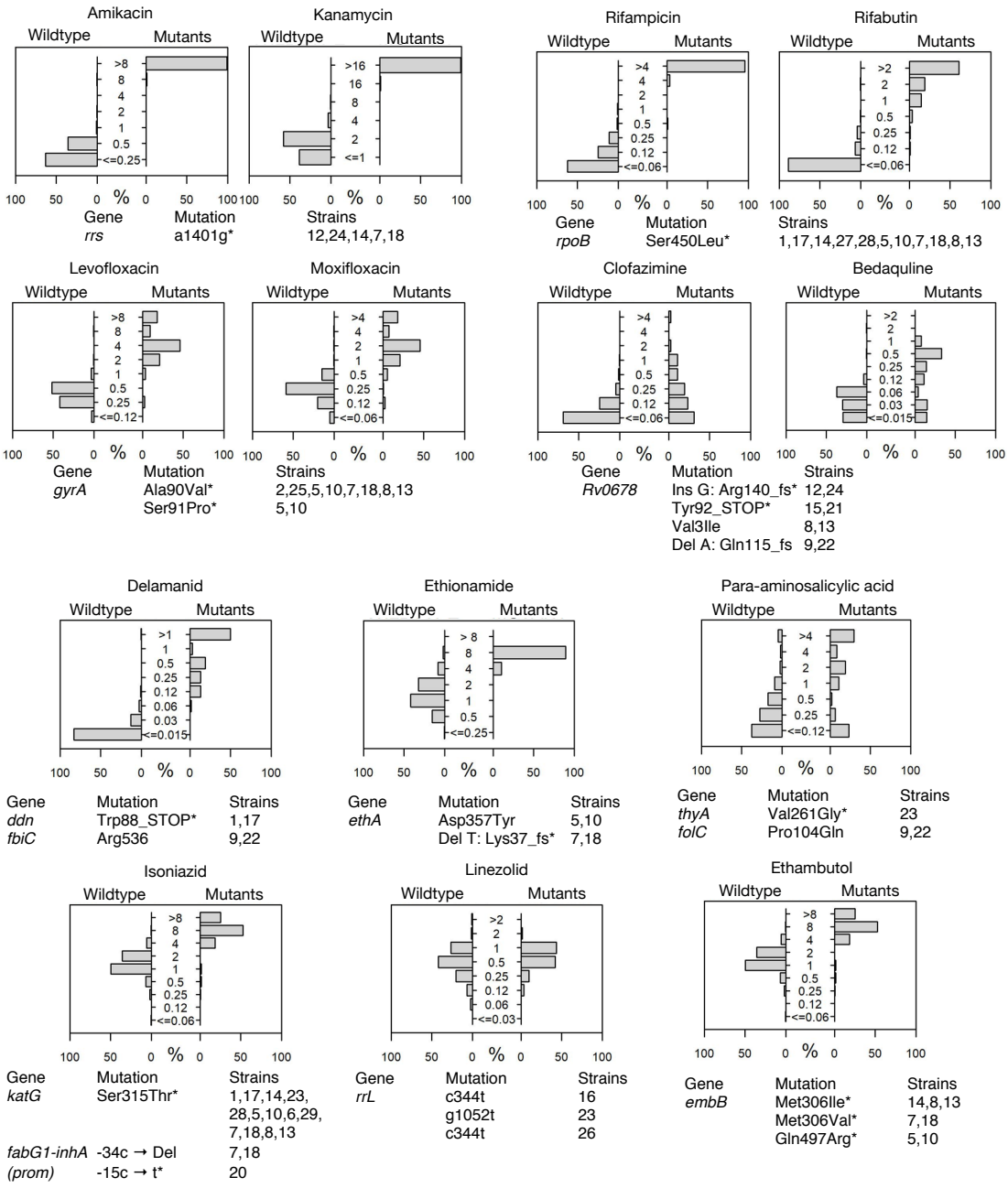

FIG 6. The MIC distributions for each drug, split by whether the resistance genes are wild-type, or contain mutations. Mutations that confer resistance with a high degree of confidence are marked with an asterisk**.** Drugs that share the same resistance genes are paired.

DISCUSSION

Broth-based microdilution methods for TB DST have been proposed in the past that either directly (8) or indirectly (5, 10, 11) measure mycobacterial growth at a range of drug concentrations, potentially enabling the determination of MIC values. In 2011, the WHO examined whether non-commercial microdilution assays would be suitable to screen patients at risk of MDR-TB and conditionally recommended their use (25). Subsequent studies assessed their performance (12–14) and established MIC distributions for the H37Rv reference strain (15, 16), demonstrating a good correlation with reference methods.

This validation study moves the field forward: UKMYC5 is the first microtitre plate assay to incorporate both new (delamanid, bedaquiline) and repurposed drugs (linezolid, clofazimine) in a dry-well format that is convenient to transport and store. In this paper, we have demonstrated that the measurement of MICS within and between participating laboratories is ≥90% reproducible for 11 of the 14 drugs on the plate. Fourteen days after inoculation was shown to be the optimal time to read a plate and the Thermo Fisher Sensititre™ Vizion™ Digital MIC viewing system was selected as the optimal reading method. We note, however, that the mirrored box performed equally well and, since it is cheaper, is likely to be the preferred better solution in low-income countries. The Vizion™, however, also allowed us to photograph each plate, thereby not only providing an audit trail but also enabling the use of the AMyGDA software (17).

By using a well characterised set of nineteen EQA strains we have been able to not only determine the conditional agreement with results from established DST assays that measure MICs but also assess the categorical agreement with MGIT and the APM by assuming the same critical concentrations (CC) applied. All comparisons to existing methods demonstrated

encouraging consistency and accuracy. Furthermore, mutations in genes that are known to confer resistance had a clear impact on MIC values. Taking these results together, we conclude that that the UKMYC5 plate has the potential to be used not only as a research tool but also as a clinical diagnostic assay, although we note that the sample size is not yet sufficient for formal validation by the FDA.

The determination of MIC values for a range of drugs allows therapeutic decisions to be more nuanced since they can be guided by the degree of resistance to a drug and an understanding of the tolerability of drug doses required to overcome it. Unlike CC-based DST methods, where all errors are categorical in nature, MIC errors can be marginal, and thereby may be potentially less disruptive to treatment decisions. In addition to these advantages, the UKMYC5 plate assays 14 drugs at once, at low cost -- a clear advantage over MGIT and the APM. Including both new and re-purposed drugs is a clear advantage over other microtitre plate-based quantitative DST methods.

As different countries have different settings, it would be optimal if one could modify the design of the microtitre plate to reflect what drugs are locally available, or the make-up of locally recommended regimens. It is therefore key that, whilst the ranges of MICs are standardised, the combination of drugs included on the plate can be adjusted according to need. A challenge to regulators is therefore whether the performance of the plate can be accredited for a menu of *individual* drugs from which country-specific plates can be constructed, rather than a fixed plate layout.

Since the increasing global numbers of MDR-TB cases are a major obstacle to TB control, let alone its elimination, there is growing need for a clinical assay that can provide quantitative

data on the second-line, new and repurposed drugs clinicians will have to prescribe with increasing frequency in the future. The UKMYC5 plate has the potential to guide the appropriate treatment of MDR-TB either directly, through implementation in clinical microbiology laboratories. Or indirectly, by producing the data necessary to characterise the effect of individual genomic mutations on drug resistance or by providing detailed and timely surveillance of the prevalence of different strains by country.

Although the MICs measured using the UKMYC5 plate were shown to be reproducible within participating centres (as defined by ISO-20776-2 (18)) the readings from one laboratory were excluded from our analysis due to their relatively poor reproducibility. A retrospective analysis of the plate images recorded by Vizion™ revealed that readers at Site F had frequently mistaken sediment from the inoculum with bacterial growth, thereby allowing us to offer targeted training to the laboratory scientists involved. This process highlights the importance of storing images of all the plates as part of an audit trail. As these images can also be read automatically by the AMyGDA software (17), we are evaluating whether incorporating the AMyGDA software into our standard operating procedure will allow us to detect significant differences in plate interpretation much earlier.

The UKMYC5 plate does have several limitations; these include the need for a pre-culture step, entailing a delay of up to six weeks before the plate is inoculated. This currently prevents the rapid turn-around of results, however, we expect this time could be reduced significantly by further development work to define the optimal inoculum from MGIT culture. Given the notable advantages inherent in this microtitre plate, such further development work should clearly be pursued. The UKMYC5 plate would also benefit from a minor re-design; PAS, which performed poorly, should be excluded and the vacated wells used to expand the range of concentrations

for drugs where measurements were most frequently reported at the extremes of the dilution range.

How the UKMYC5 microtitre plate could be optimally incorporated in a clinical laboratory's workflow remains to be determined, but the numerous advantages it offers suggests its adoption should be pursued with vigour. The Foundation for Innovative and New Diagnostics (FIND) have already expressed interest in pursuing endorsement by the WHO. In parallel, its impact on our understanding of the effect of genomic mutations on the MICs of various drugs is likely to inform the WHO's planned assessment of WGS-based DST in 2018 (1). Whether it be through direct measurement of MICs, or predictions of MIC from genomic data, a new era of quantitative TB DST may be with us.

MATERIALS AND METHODS

Participating laboratories.

Vials containing nineteen external quality assessment (EQA) TB strains were distributed by the WHO Supranational Reference Laboratory at San Raffaele Scientific Institute (SRL), in Milan, Italy, to six other participating laboratories (Fig. 1A). These were located in Germany (Institute of Microbiology and Laboratory Medicine, IML red GmbH, Gauting), UK (Public Health England Regional Centre for Mycobacteriology, Birmingham), India (P.D. Hinduja National Hospital and Medical Research Centre and Foundation for Medical Research, Mumbai), South Africa (Centre for Tuberculosis at the National Institute for Communicable Diseases, Johannesburg), Peru (Mycobacterial Laboratory, Cayetano Heredia University, Lima) and Vietnam (Oxford University Clinical Research Unit in Vietnam, Ho Chi Minh City).

M. tuberculosis strains.

Each participating laboratory received up to 31 culture vials of *Mycobacterium tuberculosis* (Fig. 1B & S7). One contained the H37Rv *Mycobacterium tuberculosis* reference strain ATCC 27294 (GenBank AL123456) (26) and was labelled as such. Eleven EQA strains were duplicated (one of which was also H37Rv) by the SRL prior to sending, along with one representative of eight additional EQA strains, bringing the total to 31 culture vials containing nineteen distinct strains. All vials, except the labelled H37Rv vial, were labelled CRY-1 to CRY-30, and therefore were blinded.

Preparation of replicates.

Ten replicates were derived from the unblinded H37Rv ATCC27294 vial (Fig. 1B), each being sub-cultured on solid media as described below before being subbed onto a UKMYC5 plate. Two replicates were created from each of the remaining thirty vials and, again following sub-

culture, were inoculated onto a UKMYC5 plate. Each participating centre therefore tested up to 70 UKMYC5 plates (Fig. 1C).

UKMYC5 design.

The UKMYC5 plate was designed by the CRyPTIC consortium and manufactured by Thermo Fisher Scientific Inc., UK. Fourteen anti-TB drugs (rifampicin, rifabutin, isoniazid, ethambutol, levofloxacin, moxifloxacin, amikacin, kanamycin, ethionamide, clofazimine, *para*-aminosalicylic acid, linezolid, delamanid and bedaquiline) were included, at 5 to 8 doubling dilutions (Fig. S1, Table S1). Janssen Pharmaceutica and Otsuka Pharmaceutical Co., Ltd provided bedaquiline and delamanid pure substances, respectively. Although pyrazinamide-only plates containing lyophilised substance in different stocks were tested, poor performance due to suboptimal broth pH conditions resulted in pyrazinamide being excluded from UKMYC5.

Inoculation protocol.

Laboratory scientists from all centres received training in plate inoculation and reading at the SRL. The standard operating procedure involved preparing a 0.5 McFarland suspension in saline tween with glass beads (Thermo Fisher, Scientific Inc., USA) from 20-25 day-old colonies (or no later than 14 days after visible growth) grown on Löwenstein-Jensen or 7H11 agar media after initial MGIT culture. Suspensions were diluted 100-fold with the addition of 100 µL of the suspension to 10 mL of enriched 7H9 broth. Aliquots of 100 µL of standard $1.5x10^5$ CFU/mL inoculum (approximate range $5x10^4$ – $5x10^5$) were dispensed into wells by the semi-automated Sensititre™ Autoinoculator (Thermo Fisher, Scientific Inc., USA).

Measurement of MICs.

In each centre, two laboratory scientists independently read each microtitre plate using three different methods (Thermo Fisher Sensititre™ Vizion™ Digital MIC viewing system, a mirrored

box and an inverted-light microscope) at 7, 10, 14 and 21 days post-inoculation (Fig. 1D). MIC results and additional data were recorded locally onto paper and into a shared web-enabled database (Table S14). An image of each plate was captured using the Vizion™ and was stored and subsequently analysed by software, the Automated Mycobacterial Growth Detection Algorithm (AMyGDA) (17). AMyGDA analysis was performed at the University of Oxford using default settings (Fig. S2).

Independent characterisation of panel strains.

Each of the 19 strains used (Fig. 1B) were phenotypically characterised by BACTEC™ MGIT960 (BD Lifesciences, New Jersey, USA), Middlebrook 7H10/7H11 agar dilution method (Table S15) and (with the exception of ethionamide and *para*-aminosalycilic acid) resazurin microtitre assay (REMA, Table S16) for drugs for which the WHO has endorsed critical concentrations (CCs) (21). Middlebrook 7H11 agar was used for bedaquiline and delamanid. All strains were also whole genome sequenced; genomic DNA was extracted from Löwenstein-Jensen cultures using either FastPrep24 for cell lysis and ethanol precipitation or the cetyl-trimethylammonium bromide method as described elsewhere (27). Paired-end libraries of 101 bp were prepared using the Nextera XT DNA Sample Preparation kit (Illumina Inc., San Diego, CA, USA) and sequenced on Illumina HiSeq 2500 instruments (with the exception of one site which used NextSeq 500 instruments). A minimum genome coverage of 30x was required for SNP analysis. Variant calling in genes associated with resistance was performed by the PhyResSE web tool and the bioinformatics pipeline at the SRL (28) and the results are given in Tables S17 & S18. The sequences reported in this paper have been deposited in the Sequence Read Archive of the National Center for Biotechnology Information under study accession numbers SRP068011 & SRP130092.

Statistical analysis.

Both descriptive and modeling analyses were conducted. For the latter logistic mixed-effects models were constructed since the data consist of repeated measurements. A drug on a plate is defined as readable if (i) there is acceptable growth in both the positive control wells, (ii) there is no contamination in the wells for that drug and (iii) the wells of that drug were not evaporated. Let us define two measurements as being in overall essential agreement (OEA) if the two MICs are within one doubling dilution of each other. Furthermore, the reproducibility is defined as the proportion of MICs that are within one doubling dilution of the mode. According to the International Organization for Standardization (ISO-20776-2), a clinical antimicrobial susceptibility test is required to have a reproducibility of ≥ 95 % (18). To assess the reproducibility within a site, the mode MIC for each drug was computed for that site, pooling the results across reading methods, days, replicates and readers. To test the reproducibility between sites, the mode was calculated only for each drug, pooling the results also across sites (besides reading methods, days, replicates and readers). To assess the accuracy of the plate we compared UKMYC5 MICs to MICs obtained by other established DST methods – the OEA is required to be ≥ 90% according to ISO-20776-2 (18). Results were assessed in three ways: (i) the OEA between the UKMYC5 and a reference method was calculated, (ii) the categorical agreement was defined as concordant reporting of either sensitivity or resistance as defined by the critical concentration (CC) of the comparator phenotypic test (if the MIC was lower or equal to the CC the strain was defined as susceptible, otherwise it was defined as resistant). Finally, (iii) the conditional agreement was defined as resistance by the comparator method and a MIC equal to or higher than the CC on the UKMYC5, or susceptibility by the comparator method and a MIC equal to or lower than the CC plus one doubling dilution on the UKMYC5 (11).

ACKNOWLEDGMENTS

We acknowledge the CRyPTIC members for their hard work which made this study possible, and thank the following collaborators for their crucial roles: Marco Rossi and Giovanna Graziano from the SRL Milano for the organisation of the training sessions in Milan; Meera Gurumurthy (The Union) for the training session in Beijing; Hien Ho Van and Tien Nguyen Thanh (Oxford University Clinical Research Unit, Ho Chi Minh City) for the development and maintenance of the CRyPTIC database; Koné Kaniga (Janssen Ltd) and Yongge Liu (Otsuka Pharmaceutical) for making available bedaquiline and delamanid powder, respectively; Thermo Fisher Scientific Inc, for the manufacture of the microtitre plate assays. Finally, we thank Claudio Köser for constructive discussions.

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FUNDING

The research was funded by the National Institute for Health Research (NIHR) Oxford Biomedical Research Centre; the CRyPTIC consortium which is funded by a Wellcome Trust/Newton Fund-MRC Collaborative Award [200205/Z/15/Z] and the Bill & Melinda Gates Foundation Trust [OPP1133541]. T.E.A.P. and D.W.C. are affiliated to the National Institute for Health Research Health Protection Research Unit (NIHR HPRU) in Healthcare Associated Infections and Antimicrobial Resistance at University of Oxford in partnership with Public Health England (PHE). T.M.W. is an NIHR Academic Clinical Lecturer. The views expressed are those of the author(s) and not necessarily those of the NHS, the NIHR, the Department of Health or Public Health England.

CONFLICTS OF INTEREST

None to declare

SUPPLEMENTAL MATERIAL

Supplementary material (Figures S1 to S7 and Tables S1 to S18) for this article may be found online

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