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Rapid, accurate determination of multidrug resistance in *M. tuberculosis* isolates and sputum using a biochip system

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SUMMARY

OBJECTIVE: To develop and evaluate a rapid biochip system for the determination of multidrug-resistant tuberculosis (MDR-TB) in *Mycobacterium tuberculosis* isolates and clinical sputum samples.

DESIGN: We developed a total solution-based system, including a biochip kit, apparatus for sample preparation, hybridisation, washing and data acquisition, and dedicated software for automated diagnosis. The biochip simultaneously identifies *M. tuberculosis* and detects the most commonly found mutations in the *rpo*B, *kat*G and *inh*A genes. The system was assessed with 330 mycobacterial isolates and 129 sputum samples for rifampicin (RMP), and with 205 isolates and 105 sputum samples for isoniazid (INH), and then compared to DNA sequencing and conventional drug susceptibility testing (DST).

RESULTS: The entire biochip assay took 6 h. The concordance rate between the biochip assay and the DNA sequencing results was 100%. Compared to conventional DST, the concordance rates were 91.8% for isolates and 94.6% for sputum samples for RMP resistance, and 70.2% for isolates and 78.1% for sputum samples for INH resistance.

CONCLUSION: The biochip system provides a simple, rapid, reliable and accurate clinical assay for the parallel detection of *M. tuberculosis* and prevalent MDR-TB in a 6 h procedure, using either culture isolates or sputum samples for diagnosis.

KEY WORDS: *Mycobacterium tuberculosis*; MDR; biochip

MYCOBACTERIUM TUBERCULOSIS is a leading cause of death worldwide and strains resistant to at least rifampicin (RMP) and isoniazid (INH), multidrug-resistant tuberculosis (MDR-TB), are associated with high fatality.[1,2] Rapid identification of resistant strains would facilitate early administration of appropriate treatment and is crucial to reducing the spread of MDR-TB.[3–5] Conventional drug susceptibility testing (DST) is time-consuming and takes several weeks to complete. Automated systems such as the BACTEC/BacTAlert (Becton Dickinson, Sparks, MD, USA) are currently used widely, yet complete resistance testing still takes about 14 days from the time the culture is obtained.[6]

Considerable progress has been made recently in delineating the mechanisms of resistance to RMP and INH.[7,8] The vast majority of RMP resistance is caused by mutations located in the 81 base-pairs (bp) region of the *rpo*B gene. Investigations of strains with high-level INH resistance found that between 50% and 95% of the strains contain mutations in codon 315 of the *kat*G gene, while between 20% and 35% possess mutations in the *inh*A regulatory region. Knowledge of these resistance mutations allows the direct testing of patient specimens by fast molecular detection methods.[9–12]

We report here a biochip-based assay system which includes a biochip, apparatus for sample preparation, chip hybridisation, washing and data acquisition, and dedicated software for automated diagnosis. The biochip is designed to detect the most frequently observed mutations for RMP and INH resistance in the *rpo*B and *kat*G genes and promoter of the *inh*A gene. The total process is semi-automatic, and the automatic software analysis for the diagnosis of drug resistance eliminates some elements of operator error.

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MATERIALS AND METHODS

Culture strains and clinical specimens

Samples comprising 330 *M. tuberculosis* cultures and 129 sputum specimens obtained from the National TB Reference Laboratory, Beijing, China, were analysed. A control panel for sensitivity analysis and 63 standard strains (Table 1) for specificity analysis were obtained from the National Institute for the Control of Pharmaceutical and Biological Products of China. For sputum specimens, 0.5 ml of sputum was firstly liquefied by the addition of an equal volume of 10% NaOH and then incubated for 30 min at 37°C before centrifugation at 10 000 × *g* for 5 min to pellet the bacteria. The supernatant was discarded and the pellet resuspended in 1 ml of 0.9% (w/v) saline and then centrifuged at 10 000 × *g* for 5 min. This supernatant was discarded and the pellet was resuspended in 50 μl of 10 mM Tris-EDTA buffer, then transferred to an extraction tube. The extraction materials and reagents were supplied in the CapitalBio Universal Kit for bacterial DNA extraction (CapitalBio, Beijing, China). The total DNA was isolated by vortexing at maximum speed in an Extractor™ 36 (CapitalBio) for 5 min. The extraction tube was then incubated at 95°C for 5 min, centrifuged briefly and then stored at −20°C until use. For cultured strains, 80 μl of DNA extraction buffer was added to a bacterial DNA extraction tube, and the bacterial colony was collected with a sterile tip and transferred into the tube. The extraction then followed the same procedure as for sputum samples. The mycobacteria samples were heated at 85°C for 30 min to decontaminate before use.

Biochip preparation

Oligonucleotide probes were printed onto OPAldehydeSlide™ aldehyde-activated slides at a concentration of 10 μM in DNA Spotting Solution using a SmartArrayer-48 microarrayer (all CapitalBio) and were covalently immobilised on the slides via an amino group at their 5′ ends[13,14] to create the biochips (Figure). All oligonucleotide probes and primers listed in Table 2 were obtained from Invitrogen (Beijing, China).

Multiplex asymmetric PCR

Multiplex asymmetric polymerase chain reaction (MAPCR) was performed as described previously,[15,16] with some minor modifications. Uracil DNA glycosylase (UNG, 0.02 U/20 μl) and dUTP (400 nM) was used to prevent carry-over contamination during amplification. MAPCR was performed in a Peltier PTC225 thermal cycler (MJ Research, Watertown, MA, USA) in two amplification rounds, with an initial activation step at 37°C for 10 min, then DNA denaturation at 94°C for 10 min, followed by the first round of exponential amplification of 35 cycles of 94°C for 30 s, 60°C for 30 s and 72°C for 40 s; the second round of linear amplification of 10 cycles of 94°C for 30 s and 72°C for 60 s; and a final extension step at 72°C for 5 min. Polymerase chain reaction (PCR) products (2 μl) were analysed by electrophoresis in 2.0% agarose.

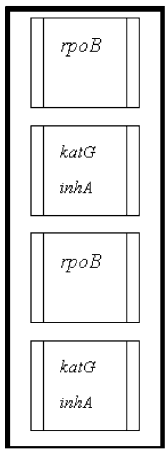
Table 1 Oligonucleotide probes and primers used in this study

Oligonucleotide probe	Sequence (5′–3′)*
QC	NH2-(T)25-GCAAGACAAGTGGAAGTGTG-HEX
EC	NH2-(T)25-GCAACCACCACCGGAGG
NC	NH2-(T)25-CCTCTCTCGGACTAATCGCC
Mycobacterium spp.	NH2-(T)25-ACAAGACATGCATCCCGT
M. tuberculosis	NH2-(T)25-GCGGGCTCATCCCACAC
*rpo*B IC	NH2-(T)25-CGGGCACATCCGGCCG
511 WT(CTG)	NH2-(T)25-GAATTGGCTCAGCTG
CTG 511CCG	NH2-(T)25-ATTGGCTCGGCTGGaT
513 WT(CAA)	NH2-(T)25-CCATGAATTGGCTCA
CAA513AAA	NH2-(T)25-ATGAATTTGCTCAGCT
CAA 513CCA	NH2-(T)25-CCATGAATGGGCTCA
516 WT(GAC)	NH2-(T)25-TTGTTCTGGTCCATGA
GAC516GTC	NH2-(T)25-GTTCTGGACCATGAA
GAC516GGC	NH2-(T)25-GTTCTGGCCCATGAA
GAC516TAC	NH2-(T)25-TTGTTCTGGTACATGA
526 WT(CAC)	NH2-(T)25-GCGCTTGTGGGTCAA
CAC526TAC	NH2-(T)25-GGCGCTTGTAGGTCAAC
CAC526GAC	NH2-(T)25-CGGCGCTTGTCGGTCA
CAC526CGC	NH2-(T)25-TCGGCGCTTGCGGGT
CAC526CTC	NH2-(T)25-gGGCGCTTGAGGGTC
531 WT(TCG)	NH2-(T)25-CCCAGCGCCGACAGTCG
TCG531TGG	NH2-(T)25-AGCGCCCACAGTCGG
TCG531TTG	NH2-(T)25-AGCGCCAACAGTCGG
533 WT(CTG)	NH2-(T)25-GCCCCAGCGCCGACAGTC
CTG533CCG	NH2-(T)25-GGGCCCCGGCGCCGACA
*kat*G IC	NH2-(T)25-GGCCGGCGCCATGGG
*kat*G 315 WT(AGC)	NH2-(T)25-GATGCCGCTGGTGATC
*kat*G AGC315ACC	NH2-(T)25-ATGCCGGTGGTGATC
*kat*G AGC315AAC	NH2-(T)25-GATGCCGTTGGTGAT
*inh*A IC	NH2-(T)25-GGTTTGGCCCCTTCAGTG
*inh*A -15 WT(C)	NH2-(T)25-CTATCGTCTCGCCGCGG
inhA -15(C→T)	NH2-(T)25-AACCTATCATCTCGCCG
BC	DMSO
16S UT primer	TAMRA-TCACTTGCTTCCGTTGAGGTGGCT CAGGACGAACG (66.2/48.0°C, 0.4 μmol/l)
16S SS primer	AGCCGTGAGATTTCACGAACA (50.9°C, 0.2 μmol/l)
*rpo*B UT primer	TAMRA-TCACTTGCTTCCGTTGAGGAGGC GATCACACCGCAGACGT (68.5°C, 0.4 μmol/l)
*rpo*B SS primer	CGAGCCGATCAGACCGATGT (54.5°C, 0.2 μmol/l)
*kat*G UT primer	TAMRA-TCACTTGCTTCCGTTGAGGGGCC ATGAACGACGTCGAAACAGC (68.8°C, 0.4 μmol/l)
*kat*G SS primer	TTCGTCAGCTCCCACTCGTAGCCG (60.5°C, 0.2 μmol/l)
*inh*A promoter UT primer	TAMRA-TCACTTGCTTCCGTTGAGGTGCTG AGTCACACCGACAAAC (66.4°C, 0.4 μmol/l)
*inh*A promoter SS primer	CAGGACTGAACGGGATACGAA (52.8°C, 0.2 μmol/l)

*Underlined letters indicate true point mutations, while lowercase letters indicate the nucleotides introduced to form artificial mismatches. Tm and final concentration of primers are also listed. Tm was calculated using the Primer Premier 5 programme.

QC = surface chemistry control; EC = hybridisation control; NC = negative control; IC = mycobacteria and PCR control; HEX = hexachloro-6-carboxyfluorescein; T_{25} = 25 consecutive thymidines; DMSO = dimethyl sulfoxide; UT primers = universal unrelated sequence-tagged primers; TAMRA = 6-carboxytetramethylrhodamine; SS primer = sequence-specific primers; Tm = melting temperature.

A



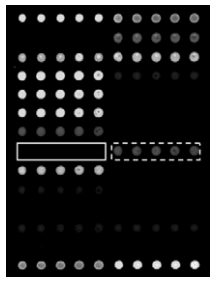
B

	1	2	3	4	5	6	7	8	9	10
1	QC					EC				
2	BC					*rpo*B-IC				
3	*Mycobacterium spp.*					*M. tuberculosis*				
4	511 WT(CTG)					CTG 511CCG				
5	513 WT(CAA)					CAA513AAA				
6	516 WT(GAC)					CAA 513CCA				
7	533 WT(CTG)					CTG 533CCG				
8	531 WT(TCG)					TCG531TTG				
9	526 WT(CAC)					TCG531TGG				
10	CAC526TAC					CAC526GAC				
11	CAC526CTC					CAC526CGC				
12	GAC516GTC					GAC516TAC				
13	GAC516GGC					NC				
14	EC					QC				

C

	1	2	3	4	5	6	7	8	9	10
1	QC					EC				
2	BC					BC				
3	*Mycobacterium spp.*					*M. tuberculosis*				
4	*katG*-IC					*inh*A-IC				
5	*kat*G 315 WT(AGC)					*inh*A -15 WT(C)				
6	*kat*G AGC315ACC					*inh*A -15(C→T)				
7	*kat*G AGC315AAC					NC				
8	EC					QC				

D



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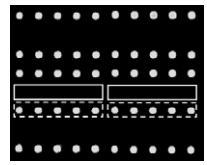

Figure Sketch map of the chip, array layout of the *rpo*B, *kat*G and *inh*A genes, and images of the microarray hybridisation results. **A.** The biochip contains four arrays; each array has five types of control to verify the test procedures. All probes were printed in five replicates. Each chip has two assays; one sub-array is for RMP and the other is for INH. **B.** Two patient samples can be analysed on each chip. For the detection of RMP resistance, six *rpo*B wild-type probes encompass the region of the *rpo*B gene encoding amino acids 509–534 (WILD 511, 513, 516, 526, 531, 533). Thirteen mutation-type probes target the most common mutations for the same region (MUT CTG 511CCG, CAA513AAA, CAA513CCA, GAC516GGC, GAC516 GTC, GAC516TAC, CAC526GAC, CAC526TAC, CAC526 CTC, CAC526CGC, TCG531TTG, TCG531TGG and CTG533CCG). **C.** For the detection of INH resistance, one probe covers the wild-type 315 region of *kat*G and two probes detect for the most common mutations for the same region (*kat*G MUT AGC315 ACC and AGC315AAC), while two probes detect for the *inh*A promoter region (WILD-15 and MUT-15C-T). Representative images of the microarray hybridization results are shown as **D** and **E**. **D.** RMP resistance with the TCG531TTG mutant, the hybridisation signal of probe TCG531TTG (dashed rectangle) being higher than corresponding wild-type probe (solid rectangle). **E.** Indicates INH resistance with the *kat*G AGC315ACC mutant and *inh*A15C→T mutant; the hybridisation signal of probe *kat*G AGC315ACC and *inh*A15C→T (dashed rectangles) were higher than corresponding wild-type probe (solid rectangles). QC = surface chemistry control; EC = hybridisation control; BC = blank control; IC = mycobacteria and PCR control; WT = wild-type; NC = negative control; RMP = rifampicin; INH = isoniazid.

Table 2 The 63 standard strains used for biochip hybridisation specificity analysis

1	*Corynebacterium pseudodiphtheriticum*	33	*Mycobacterium intracellulare*
2	*C. xerosis*	34	*M. avium*
3	*Neisseria subflava*	35	*M. gordonae*
4	*Proteus mirabilis*	36	*M. kansasii*
5	*P. vulgaris*	37	*M. fortuitum*
6	*Citrobacter freundii*	38	*M. scrofulaceum*
7	*Enterobacter cloacae*	39	*M. gilvum*
8	*Enterobacter aerogenes*	40	*M. terrae*
9	*Serratia marcescens*	41	*M. chelonae*
10	*Escherichia coli*	42	*M. abscessus*
11	*Klebsiella pneumoniae*	43	*M. phlei*
12	*Stenotrophomonas maltophilia*	44	*M. nonchromogenicum*
13	*Pseudomonas aeruginosa*	45	*M. marinum*
14	*Acinetobacter calcoaceticus*	46	*M. ulcerans*
15	*Staphylococcus epidermidis*	47	*M. aurum*
16	*S. aureus*	48	*M. szulgai*
17	*Streptococcus salivarius*	49	*M. xenopi*
18	*Streptococcus mutans*	50	*M. smegmatis*
19	*Alcaligenes faecalis*	51	*M. aichiense*
20	*Rhodococcus rhodochrous*	52	*M. asiaticum*
21	*Nocardia asteroides*	53	*M. austroafricanum*
22	*N. otitidiscaviarum*	54	*M. chubuense*
23	*Actinoplanes italicus*	55	*M. diernhoferi*
24	*Micrococcus luteus*	56	*M. duvalii*
25	*Enterococcus faecalis*	57	*M. elephantis*
26	*Enterobacter faecium*	58	*M. gadium*
27	*Streptococcus mitis*	59	*M. gastri*
28	*Streptococcus pyogenes*	60	*M. leprae*
29	*N. brasiliensis*	61	*M. simiae*
30	*Candida albicans*	62	*M. thermoresistibile*
31	*Neisseria gonorrhoeae*	63	*M. triviale*
32	*Fusobacterium nucleatum*		

Biochip hybridisation and data analysis

Chip hybridisation was performed for 2 h at 50°C in a BioMixer II hybridisation oven (CapitalBio). After hybridisation, slides were washed once at room temperature for 3 min in 2 × 0.15 M NaCL plus 0.015 sodium citrate (SSC)—0.2% sodium dodecyl sulfate (SDS), and for an additional 3 min in 0.2 × SSC. Finally, the slides were dried by spinning them at 1000 × *g* for 2 min. Microarrays on the slides were analysed using a confocal LuxScan-10K laser scanner (CapitalBio). Fluorescent intensities were quantified by using dedicated software, the *M. tuberculosis* Drug Resistance Detection Array Test System (CapitalBio). A set of 210 non-*M. tuberculosis* cultures and 586 non-*M. tuberculosis* sputum specimens was used for threshold determination. All the hybridisation signals were processed using the following statistical method. In theory, the 99% non-specific hybridisation signal should be lower than the threshold produced by mean ± 3 SD (standard deviation) for each probe. For sensitivity determination, the biochip was hybridised by using diluted *M. tuberculosis* samples (10^4, 10^3, 10^2, 50, 25, 10 copies). The detection limit was the lowest concentration of *M. tuberculosis*, with a hybridisation signal above the threshold.

DNA sequencing

The sequences of the PCR products of the *rpo*B, *kat*G and *inh*A genes from individual patient samples were determined by standard Sanger DNA sequencing.

Drug susceptibility testing

DST was performed using the proportional and absolute methods in parallel, using indirect DST; Löwenstein-Jensen medium was used for culture. For the proportional method, concentrations of INH and RMP were respectively 0.2 μg/ml and 40 μg/ml. For the absolute method, concentrations of INH and RMP were respectively 1 μg/ml and 10 μg/ml, and 50 μg/ml and 250 μg/ml. The proficiency of DST was evaluated in the 12th round of the DST Quality Assurance Programme (QAP) by the Supranational Reference Laboratory of the Hong Kong Public Health Centre.

RESULTS

Sensitivity, reproducibility and specificity of the biochip system

Detection limits were determined using different concentrations of *M. tuberculosis* suspensions and 129 culture-positive sputum samples. The *M. tuberculosis* suspensions were positive when the template concentration was ⩾25 copies per PCR reaction. All the 129 culture-positive sputum samples, including 41 smear-positive and 88 smear-negative samples, were successfully detected by the biochip system. The reproducibility was evaluated with 10 negative, 16 low-positive (200 copies/PCR reaction) and 16 high-positive (10^5 copies/PCR reaction) samples, which were tested repeatedly (10 times) to evaluate variation. All the negative samples were determined as a negative result, and all low-positive (200 copies/PCR reaction) and high-positive (10^5 copies/PCR reaction) samples obtained positive results. To determine the specificity, 63 types of standard strains (31 types of non-tuberculous mycobacteria and 32 types of respiratory tract-related bacteria) and 361 non-tuberculous clinical sputum samples were tested: none produced PCR amplification products, nor were signals detected on the TB-specific probes.

Genotyping results of the biochip test using culture isolates

RMP resistance was determined by the DST assays and the biochip genotypic assays on a total of 330 culture isolates; the results are summarised in Table 3. In 303 (91.8%) of the 330 isolates, the biochip genotypic assay results were in agreement with the results of the DST. INH resistance was determined in a total of 205 culture isolates (Table 4). The genotypic results were in agreement with the DST results in 144/205 (70.2%) specimens. Concordance between the biochip assay results and DNA sequencing was 100%. Among the RMP-susceptible isolates identified by culture-based DST, it was notable that of the 14 mutations found by the biochip method and by DNA sequencing, two of the mutations were T511C, while 12 were T533C. This result is in agreement with previous studies which showed that mutations in codons 511 and 533 may exhibit low-level resistance or susceptibility to some rifamycins.[17–19] Genotyping analysis also revealed that the MUT 531 (C→T) mutation was the most frequent genotype in *rpo*B (142/257, 55.3%) and that MUT 315 (G→C) mutation was the most frequent genotype in *kat*G (92/ 154, 59.7%).

Table 3 Comparison of the results of DST assays and the biochip genotyping tests for the detection of RMP susceptibility among 330 *M. tuberculosis* isolates and 129 sputum samples

	DST assays			
	Isolates		Sputum	
	RMP Susceptible	RMP Resistant	RMP Susceptible	RMP Resistant
Genotype biochip test				
Wild type	106	13	50	4
Mutant type	14	197	3	72

DST = drug susceptibility testing; RMP = rifampicin.

Table 4 Comparison of the results of DST assays and the biochip genotyping tests for the detection of INH susceptibility among 205 *M. tuberculosis* isolates and 105 sputum samples

	DST assays			
	Isolates		Sputum	
	INH Susceptible	INH Resistant	INH Susceptible	INH Resistant
Genotype biochip test				
Wild type	80	20	45	11
Mutant type	41	64	12	37

DST = drug susceptibility testing; INH = isoniazid.

Genotyping results of the biochip test using sputum samples

RMP resistance was determined using DST and the biochip genotype assay in 129 sputum samples (results given in Table 3). In 122/129 (94.6%), the genotype assay agreed with the DST results. INH resistance was determined for 105 sputum samples (Table 4). The genotypic assay agreed closely with the DST results in 82/105 (78.1%) specimens. The biochip assay and DNA sequencing were 100% concordant. The proficiency of DST testing for RMP and INH is shown in Table 5. Significantly, for control of the spread of resistant genes, the biochip genotypic assay

Table 5 Results of DST proficiency for RMP and INH

	Rate %	
	RMP	INH
Susceptibility detection rate	100	100
Resistance detection rate	100	100
Reproducibility	100	100
Concordance rate	100	100

DST = drug susceptibility testing; RMP = rifampicin; INH = isoniazid.

detected strains that were resistant to both RMP and INH in 15/129 (11.6%) sputum samples; these MDR strains were also confirmed by the DST assays.

DISCUSSION

With the increasing spread of MDR-TB, it is imperative that the time required for the identification of *M. tuberculosis* and DST be reduced. Reducing diagnosis time would allow the early administration of appropriate treatment and prevent the further spread of drug-resistant strains. These requirements can be achieved by the use of rapid molecular detection methods. In the present study, we present a newly designed biochip system specific for detecting genotypic RMP and INH resistance. The instrumentation and microarray components of the platform have also been independently evaluated previously for detection of other infectious entities, such as *Enterobacteriaceae*,[15] staphylococcal isolates[16] and severe acute respiratory syndrome (SARS).[20] The biochip genotype assay is accurate, sensitive and uses a simple semi-automatic protocol which can be completed within 6 h.

This study indicates that the biochip system has a good overall performance. The concordance rates were respectively 91.8% for isolates and 94.6% for sputum for RMP resistance, and 70.2% for isolates and 78.1% for sputum for INH resistance. A lower concordance for the detection of INH resistance was observed in samples because the biochip assay detects only two loci, the *kat*G 315 mutation and a mutation in the *inh*A regulatory region. This finding indicates a deficiency in the current array content. There is a need to continue identifying resistance-related genes and mutations and to add to the array content; as genetic research advances and the mutation type is proven, probes for new gene mutations can be designed and tested for application to the biochip. The agreement between the genotype and the phenotype of samples will increase accordingly with increased biochip content. We recognise that the strains used for this study from the different regions of China still do not represent all genotypes found in the country, and the concordance rate needs to be further validated in a larger multi-regional study due to the geographical variations of prevalent strains.

Some novel molecular diagnostic methods based on PCR and reverse hybridisation procedures, such as the INNO-LiPA Rif TB assay (Innogenetics, Zwijndrecht, Belgium), and the Genotype MTBDR assay (Hain Lifescience GmbH, Nehren, Germany)—both line-probe assays—have recently become available.[3–5,21–24] These line-probe technologies have some limitations, e.g., the number of probes printed on the membrane is low and most of the mutation types encountered clinically are not present on the assay strips. This limitation alone prevents the identification of many resistance-causing mutations and prevents identification of some nonsense mutations from wild type. The assay also requires manual manipulation, as the strips have to be pasted onto an evaluation sheet and compared with the interpretation chart, requiring experienced technical skills for interpretation. It is also difficult to interpret the strip results if the reaction bands are not well-defined.

Our assay is similar in some respects to both the INNO-LiPA Rif TB assay and the Genotype MTBDR assay. Each of the assays analyses the most commonly found mutations in the *rpo*B, *kat*G and *inh*A genes, but the biochip system has several clinically advantageous differences compared to other molecular diagnostic assays. First, the extraction of DNA has been simplified by using a bacterial DNA extraction kit. Second, the biochip contains probes for most of the frequently seen mutation types. This is useful for molecular epidemiological surveys and provides higher confidence in determining the causative mutation. Third, a modified spotting buffer[25] and a newly developed advanced hybridisation system were employed in this study. These measures have both previously demonstrated large improvements in the local signal intensity and global signal uniformity as well as the elimination of the 'doughnut spots' commonly seen on spotted oligonucleotide arrays.[26] These effects are thought to be due to the alteration in the surface tension during slide spotting, resulting in higher reproducibility and more uniform signals. Fourth, a higher sensitivity was achieved by improving the MAPCR process. The sensitivity was 25 copies per PCR reaction and, importantly, most smear-negative TB sputum samples could be successfully tested. Because of the higher sensitivity, even apparently smear-negative sputum, with around 1×10^3 *M. tuberculosis* bacilli in 1 ml sputum, could also be accurately diagnosed. Such higher assay sensitivity is a key issue for the early administration of appropriate treatment of (false) smear-negative pulmonary TB patients. Lastly, the process is semi-automatic, and the interpretation software set to predetermined detection ranges was used to analyse scan data and to generate test reports. This automation of test reporting averts some of the potential diagnostic variabilities due to operator interpretation, and also permits the operator to scrutinise the primary data for confirmation. We believe that the improved sensitivity and uniformity of the signals from this system also contributes to the reliability of the automatic test reporting operations by contributing to the better definition of each potential signal spot. We believe that the platform presented addresses many of the key bottlenecks that have prevented same-day TB analysis and diagnosis.

CONCLUSION

The biochip system provides a rapid (6 h procedure), simple (semi-automatic) and reliable diagnostic tool

for the simultaneous detection of *M. tuberculosis* as well as the most prevalent forms of MDR-TB from culture isolates or sputum samples. The system has the potential for a major impact on the speed of detecting infection and on the clinical management of TB patients.

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RÉSUMÉ

OBJECTIF : Elaborer et évaluer un système rapide « biochip » pour déterminer les germes multirésistants (TB-MDR) dans les isolats de *Mycobacterium tuberculosis* et dans les échantillons cliniques de crachats.

SCHÉMA : Nous avons élaboré un système total basé sur les solutions incluant un kit biochip et un appareil pour la préparation de l'échantillon, l'hybridisation, le lavage et l'acquisition des données ainsi qu'un logiciel voué au

diagnostic automatisé. Le biochip identifie *M. tuberculosis* et détecte simultanément les mutations les plus couramment trouvées dans les gènes *rpo*B, *kat*G et *inh*A. Le système a été évalué sur 330 isolats mycobactériens et 129 échantillons de crachats pour la rifampicine (RMP) et sur 205 isolats et 105 échantillons de crachats pour l'isoniazide (INH) et a été ensuite comparé au séquençage de l'ADN et aux tests conventionnels de sensibilité aux médicaments.

RÉSULTATS : Le test complet biochip a duré 6 h. Le taux de concordance entre le test biochip et les résultats du séquençage de l'ADN est de 100%. Par comparaison avec les tests conventionnels de sensibilité aux médicaments, le taux de concordance est de 91,8% pour les isolats et de 94,6% pour les crachats en ce qui concerne la résistance à la RMP et de 70,2% pour les isolats et de 78,1% pour les crachats en ce qui concerne la résistance à l'INH.

CONCLUSION : Le système biochip est un outil clinique simple, rapide, fiable et précis pour la détection parallèle de la TB-MDR dans une procédure d'une durée de 6 h, que le diagnostic provienne d'isolats de culture ou d'échantillons de crachats.

RESUMEN

OBJETIVO : Elaborar y evaluar un sistema rápido de chips biológicos destinados a detectar la multidrogoresistencia (TB-MDR) a partir de aislados de cultivos de *Mycobacterium tuberculosis* y de muestras de esputo de los pacientes.

MÉTODOS : Se concibió un sistema de análisis en solución que comporta el estuche del microcircuito integrado, el equipo para preparar la muestra, realizar la hibridación y el lavado y la adquisición de los datos y el programa informático destinado al diagnóstico automático. El biochip detecta en forma simultánea la presencia de *M. tuberculosis* y de las mutaciones más frecuentes de los genes *rpo*B, *kat*G e *inh*A. Se evaluó con este sistema la resistencia a rifampicina (RMP) en 330 aislados de micobacterias y 129 muestras de esputo y la resistencia a isoniazida (INH) en 205 aislados y 105 muestras y se compararon luego con los resultados de la secuenciación del ADN y con las pruebas convencionales de sensibilidad a los medicamentos.

RESULTADOS : La duración total del ensayo con el biochip fue de 6 h ; se observó una concordancia del 100% de sus resultados con la secuenciación del ADN. Cuando se comparó el sistema con las pruebas de sensibilidad a RMP, la concordancia fue del 91,8% en los aislados y del 94,6% en las muestras de esputo ; con respecto a la INH, la concordancia fue del 70,2% en los aislados y del 78,1% en las muestras de esputo.

CONCLUSIÓN : El sistema del biochip ofrece un ensayo clínico sencillo, rápido, fiable y preciso para la detección simultánea de *M. tuberculosis* y de la TB-MDR predominante, en un procedimiento de 6 h, a partir de aislados de cultivos o de muestras de esputo.