Rapid detection of resistance in *Mycobacterium tuberculosis*: a review discussing molecular approaches

D. García de Viedma

Servicio de Microbiología clínica y enfermedades infecciosas, Hospital general universitario Gregorio Marañón, C/Dr Esquerdo 46, 28007 Madrid, Spain

The last few years have seen the development of several molecular designs to search for mutations encoding resistance to antituberculous drugs in *Mycobacterium tuberculosis*. Most of these are highly efficient for RIF-r detection and are well adapted to search for the most relevant INH-R mutations. In this review, these new molecular approaches are explained and are presented according to the molecular strategies on which they are based. In this sense, techniques based on DNA-sequencing, electrophoresis and hybridization are reviewed and the newer designs based on real-time PCR and microarrays are also included. Molecular methods are sure to transform standard approaches to the issue of resistance in the mycobacteriology laboratory. This will allow laboratories to speed up the performance of resistance assays and provide access to essential information for highly refined detection, follow-up and management of antibiotic resistance in *M. tuberculosis*.

Keywords *Mycobacterium tuberculosis*, resistance, molecular methods

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INTRODUCTION

It is estimated that 3.2% of all new cases of tuberculosis worldwide are caused by multidrug-resistant (MDR) strains [1] and, in some regions, the incidence for MDR-tuberculosis (TB) is alarming (14% in Estonia, 11% in Henan Province, China). Apart from the obvious clinical, therapeutical and epidemiological importance of these figures, MDR–TB also has an economic impact, as the treatment for this 3.2% is more expensive than the estimated cost for all the remaining cases.

Knowledge of the susceptibility pattern of the *Mycobacterium tuberculosis* (MTB) strain involved in each case is essential to avoid inefficient therapy and to optimise the antimicrobial efficacy of the combined-drug regimen from the very beginning. It enables us to increase the possibility of cure and to curb the potential spread of high-risk strains in the community. Therefore, physicians request susceptibility patterns as early as possible, and the Centres for Disease Control recommends reporting resistance patterns within 28 days of receiving the specimen in the laboratory [2]. In the last few years, liquid-culture strategies have made it possible to accelerate the culture of mycobacteria and the availibility of an antibiogram. Nevertheless, as phenotypic susceptibility assays require strains that have been subcultured in the presence of a set of antituberculous agents, the possibilities for accelerating the antibiogram are severely limited.

To obtain more rapid results, recourse must be made to genotypic assays [3,4]. These involve performing the analysis directly on the DNA with the result that only the nucleic acid needs to be extracted without having to subculture the strain. The time necessary to determine the susceptibility pattern is therefore reduced and the possibility remains (still restricted to certain techniques) of obtaining the antibiogram directly from the bacilli in the clinical sample.

Recently, some molecular assays have been tailored to obtain susceptibility patterns in MTB thanks to a better understanding of the genetic

Corresponding author and reprint requests: D. García de Viedma, Servicio de Microbiología clínica y enfermedades infecciosas, Hospital general universitario Gregorio Marañón, C/Dr Esquerdo 46, 28007 Madrid, Spain
E-mail: dgviedma@microb.net

basis of resistance to antituberculous agents. Molecular methods for detecting resistance are based on assigning the presence/absence of certain mutations in specific positions/genetic regions which are known to confer resistance. The detection of resistance to anti-TB drugs by molecular approaches is determined by the degree of understanding of the genetic basis of the resistance for each of the drugs [5–7]. This varies somewhat according to the different antibiotics used in the combined anti-TB regimen, with more information available for Rifampin (RIF) and Isonazid (INH), the two drugs most efficacious against MTB.

GENETIC BASIS OF RESISTANCE IN MTB

Most RIF-resistant strains have a mutation in an 81-bp region (named core *rpoB*, or RIF-resistance-determining region RRDR) which is part of the *rpoB* gene encoding the beta subunit of the DNA-dependent RNA polymerase. Because synonymous changes are very uncommon in this region, the detection of any nucleotidic substitution within this genetic region is a marker for resistance. Some codons in this *rpoB* core region are more frequently associated with resistance-encoding mutations, especially 531, 526 and 516, and some of them have been reported to be associated with high-levels of resistance [8,9] or with resistance against other rifamycins [10].

For INH the situation is more complex. Resistance-mutations have been reported to map in at least four different genetic loci: *katG*, encoding catalase-peroxidase; *inhA* involved in fatty acid elongation; *ahpC* encoding alkyl hydroperoxide reductase, and *oxyR* involved in the regulation of oxidative stress. Despite this complexity, one codon, *katG*315, is most frequently associated with resistance to INH (often at high levels), and therefore, the detection of resistance in many cases is simplified. Additionally, resistant strains with mutations in codon 315 have been reported to be efficiently transmitted and to conserve intact their virulence [11,12].

In the case of ethambutol, resistance mutations have been found in the *emb*CAB operon which codes for different arabinosyl transferases that have a role in lipoarabinomannan and arabinogalactan synthesis. Resistance against the other first-line anti-TB drug, streptomycin, is caused by mutations in *rrs*, which codes for 16SRNA and *rps*L, which in turn codes for the ribosomal protein 12S.

Despite the fact that these are the genetic loci most frequently found to encode resistance-mutations in MTB, resistant strains with no mutations in any of these regions have also been isolated. Hence, the ubiquituous nature of resistance in MTB and the need for additional efforts to complete the genetic sequences involved in the development of resistance.

MOLECULAR METHODS FOR DETECTING RESISTANCE IN MTB

Molecular techniques search for resistance mutations either directly or indirectly. The different techniques that are applied for the genotypic analysis of resistance in MTB can be grouped into three categories: electrophoresis, sequencing and hybridization-based assays. The sequencing and hybridization-based assays directly detect mutations and can define the precise mutations involved, whereas electrophoretic assays provide an indirect method of detecting the existence of mutations without having the ability to define the exact nucleotidic substitution involved.

Electrophoresis-based techniques

The different electrophoresis-based approaches search for mutations after analyzing the electrophoretic mobility of DNA fragments which include the genetic regions involved in resistance. Mobility shifts in these DNA fragments, when compared with the mobility of wild type/reference sequences, indicate the presence of mutations. In order to facilitate the detection of mobility shifts, these methods analyse DNA structural variants (single-strand, heteroduplexes) and select special electrophoretic conditions (pH gradients, denaturing gels).

PCR–SSCP (single-strand-conformation-polymorphisms)

This method is based on the conformational distortion that a nucleotide substitution can cause in a single-strand DNA fragment. This conformational change leads to an electrophoretic mobility different to that of the wild-type single-strand fragment. The procedure involves amplification by PCR of a DNA fragment including the region of interest, denaturation of this fragment, and running it in a polyacrylamide gel together with the denatured

wild-type reference sample. Mobility shifts in the clinical sample would indicate the presence of a mutation in the region analyzed.

The advantages of this method are that it is inexpensive, easy and quick. It does not require complex devices and has a high specificity, and a mobility shift is a good indicator of the existence of a nucleotidic substitution. It has frequently been used to search for resistance mutations against RIF and INH [13–15], with higher sensitivity for RIF-r than INH-r mutations. Trials to adapt this technique to other drugs with more genetically complex mechanisms of resistance such as PIR or EMB have failed [16,17].

Some authors obtained positive results by using SSCP directly with clinical samples, especially to rule out the presence of mutations in susceptible cases [15]. Not all nucleotidic changes are well detected by SSCP, depending on the substitution and the relative position of the affected codon within the amplified fragment. In certain cases interpretation is difficult and ambiguous and only under extreme electrophoretic conditions do some mutant strains lead to SSCPs [18]. Some of these limitations could be eliminated by using long performance gels, highly sensitive staining techniques or fluorescent labeling of the products and by processing them in an automatic sequencer [19], thus increasing the sensitivity of the assay.

Heteroduplex analysis

Another method of detecting mutations based on electrophoretic band-shifts is the detection of heteroduplexes in polyacrylamide gels. Again, the technique depends on the PCR amplification of the region of interest in the clinical isolate. This amplicon from the clinical isolate is denatured and then mixed with an equivalent amount of a denatured amplicon from a reference wild-type strain or, in other designs, from a universal heteroduplex generator [20]. The mixture of amplicons is allowed to cool slowly to reconstitute double-strand DNA-renatured molecules. If the clinical strain has no mutations in the region analyzed, the homology between the single strands of the clinical and reference amplicons would be complete, with the result that the renatured duplexes would be identical to the clinical/reference original DNA homoduplexes. On the contrary, if a mutation exists in the clinical amplicon, heteroduplexes could be obtained by the renaturation of one strand from the clinical mutant amplicon and another from the reference strain. These heteroduplexes have an electrophoretic mobility different than the original amplicons.

Some authors improve the sensitivity of the detection of heteroduplexes by performing special electrophoresis techniques such as DG-DGGE (double-gradient denaturing gradient gel electrophoresis) which uses a double gradient (temperature and polyacrylamide) [21]. A modification of the heteroduplex assay is the RNA/RNA mismatch assay, in which the detection of heteroduplexes is performed on RNA hybrids detected after treatment with RNAse, which cleaves the heteroduplex where there is a mismatch [22].

PCR–DNA sequencing

PCR–DNA sequencing is the most direct way of defining resistance genotypically and is the standard of molecular assignment of resistance. It requires no more than the amplification of a region which includes the codons involved in the resistance, and these amplicons are sequenced to assign to the presence/absence of a specific mutation. Its limitation is that it is expensive and requires expertise. Although automatic sequencers are increasingly available in health-care institutions, they are not readily available in all microbiology laboratories. Nevertheless, DNA sequencing is the technique of choice according to several studies on the genotypic detection of resistant TB strains. It has been applied to isolates from a wide variety of geographic origins, in the search for mutations in multiple loci of: MDR strains [23], RIF-r in *rpo*B [8,9,24–26], RIF-r outside the RRDR core [27], INH in *ndh* [28], and *kat*G [29], and EMB-r [17,30,31].

Hybridization-based techniques

Hybridization-based techniques comprise a heterogeneous group. Some of them are performed on PCR assays which include specific DNA probes, and others are conventional hybridization assays in solid or liquid formats. All of them are based on the hybridization of two strands of DNA, one from the clinical strain and another from the consensus probe/oligonucleotide. The hybridization of the two strands is highly stable when it involves fully complementary sequences; if a mismatch occurs in some nucleotidic position, owing to genetic differences between the clinical strain and the consensus sequence, the binding of the two

strands is thermodynamically impaired. In order to appreciate more clearly the peculiarities of these techniques, all PCR-based assays using hybridization probes have been grouped under the heading 'Real-time PCR', and in this section only the immobilized hybridization formats are reviewed.

Hybridization on strips

One adapted hybridization design for the detection of resistance to rifampicin involves the use of commercial membrane strips in which a set of ten oligonucleotide probes is immobilized (INNO-LipA; Innogenetics, Gent Belgium). Five of these probes are homologous with five subregions which together constitute the *rpo*B core region, four other probes are designed to be homologous with frequent Rif-R mutations, and another probe is an amplification control for the MTB complex. The method follows a reverse-hybridization design in which the labeled probe is constituted by the amplicon of the rpoB core region obtained from the clinical sample by PCR. The label (biotin) is incorporated in the amplicon during the PCR. The labeled amplicon is hybridized with the immobilized probes under stringent conditions. The hybridized complexes are detected by incubation with peroxidase-steptavidine and subsequent addition of a chromogen. If any of the five probes which are homologous with wild-type sequences fail to hybridize, a mutation is suspected for the region covered by that probe. Furthermore, if any of the probes that are homologous with a mutation give a positive hybridization signal, it is possible to specify the mutation responsible for the resistance.

The technique is simple and needs no expertise in molecular biology, but it does require a pre-PCR step to amplify and label the clinical amplicon, and a subsequent hybridization step, which requires a specific device. Its application to clinical isolates shows a good correlation with DNA sequencing (only 2–4% of discrepancies with DNA sequencing/phenotypic assays) [32–34], as well as a good performance directly on clinical samples [35]. Its sensitivity increases when using a nested-PCR approach [36]. With regard to the ability to assign the specific resistance mutation, a number of cases have been reported in which certain mutants, frequently insertions, could not be detected [34,37,38].

Hybridization in microplates

A PCR–ELISA format has also been developed for the detection of RIF-r strains [39]. In this assay, five independent oligonucleotides, homologous to the corresponding wild-type sequences, which cover the core rpoB, were designed as capture probes, and immobilized to the wells of a microtiter plate. The *rpo*B region from the clinical isolate is amplified and labeled with digoxygenin and hybridized to the immobilized probes. After developing, positive signals correspond to amplicons lacking a mutation in the region covered by the probe and negative signals indicate the presence of a mutation in that region. This format was tested successfully with a collection of clinical strains and also directly on clinical samples.

Hybridization on arrays

One of the most recent developments in mutation detection, together with real-time PCR, is the use of oligonucleotide arrays or biochips. Different strategies have been followed to adapt these formats to the analysis of RIF-resistance mutations in MTB. The assay is based on the hybridization of DNA from the clinical sample to a set of oligonucleotides, each one corresponding to a different variant of *rpo*B mutation, immobilized either in polyacrylamide gel pads (MAGIChip) [40] or in miniaturized glass substrates (GeneChip, Affymetrix Inc., Santa Cruz, CA) [41]. The *rpo*B region from the analysis strain is amplified and fluorescent-labeled by PCR, and hybridized to the microarray oligonucleotides. Fluorescence is higher for the immobilized oligonuleotides that were homologous with the amplicon and the presence of a mismatch would destabilize the duplex leading to a reduction in fluorescence. The relative fluorescence intensity between the different positions in the microarray will define the pattern of mutations in the clinical strain. This format allows interrogation for the existence of 30 different *rpo*B variants in the polyacrylamide array [40] in 24 h, and 51 different variants in the GeneChip version [41] in 4 h.

Real-time PCR

The use of fluorescence labels and the inclusion of optical devices or CCD cameras in thermocyclers have recently made it possible to observe an amplification reaction directly while it is occurring. The so called 'real-time PCR' involves real-time monitoring of a DNA amplification reaction by measuring the fluorescence signal accumulated by amplification products. Different real-time PCR instruments are available, some based on

conventional peltier-based thermocyclers and others on air-heating and air-cooling thermocylers which, in combination with capillary tubes, also allow a rapid-cycle PCR.

Different reaction formats have been developed to monitor a PCR reaction in real time by the use of fluorescent dyes. If the purpose is to detect specific mutations in the amplified region, then the fluorescent dye must be bound to DNA probes designed to be complementary to the template DNA under analysis. Different formats of labeled probes can be adapted to the detection of mutations. Among the most frequently used are TaqMan, molecular beacons, and FRET (fluorescence-resonance-energy-transfer) probes. These probes are included in the PCR reaction together with the other reagents and are designed to be homologous with the DNA region in which a mutation is to be searched for.

TaqMan probes

TaqMan probes are fluorescence-labeled at the 5′-end and include a fluorescence quencher at the 3′-end (Figure 1). The probe anneals to the amplified target in each of the annealing steps of the PCR. While it is bound to its homologous sequence, the real-time PCR instrument does not measure fluorescence, because the quencher absorbs the energy of the dye. In the elongation step of the PCR, the 5′-3′exonuclease activity of the Taq polymerase releases the fluorescent dye which enhances the emission of fluorescence owing to the separation of the quencher from the dye (Figure 1). This fluorescence is measured by the real-time PCR instrument. Of note is the fact that fluorescence is only emitted if the probe is correctly anchored to its homologous site to allow the Taq polymerase to release the dye. This is the basis for the detection of mutations using TaqMan probes; if a mismatch (mutation) impairs the binding of the probe, the Taq polymerase would not meet the probe and the fluorescense would remain quenched. Therefore, the absence of fluorescence indicates the presence of a mutation in the genetic region covered by the probe (Figure 1).

One study has used this kind of probe to detect resistance mutations for RIF and INH in MTB in cultured strains and clinical samples [42] obtaining a 100% specificity and a sensitivity of 97% and 40%, for cultured strains or clinical samples, respectively.

A requirement of real-time PCR assays using Taq probes is the addition to the PCR reaction of internal amplification controls, with their respective complementary probes, to guarantee that the absence of fluorescence is due to the existence of a mutation and not to deficiencies in the PCR.

Beacons

Molecular beacons have a stem-loop structure when in solution (Figure 2). The loop consists of the sequence which is homologous with the target DNA. A fluorescent dye and a quencher are in the

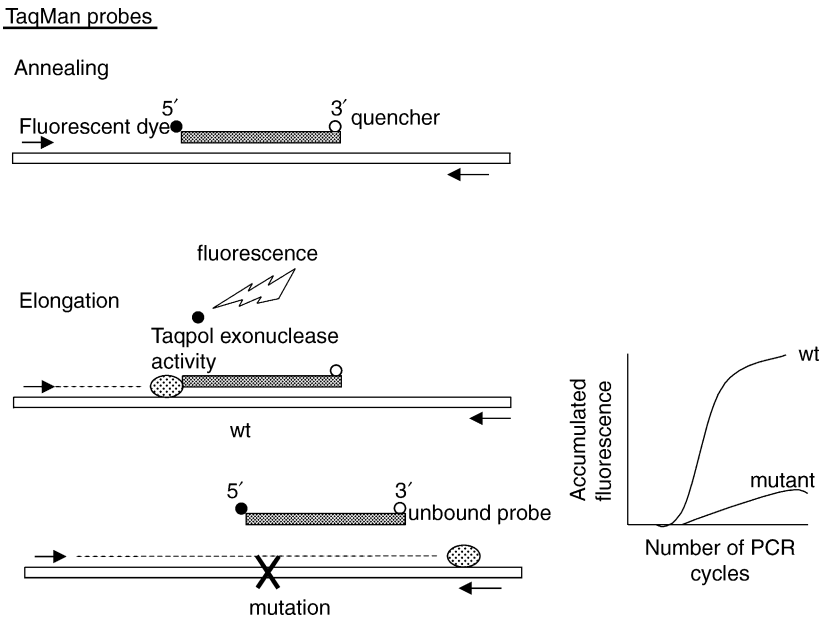

Figure 1 Schema of the mechanism of action of TaqMan probes. In each of the annealing steps, the probe binds to its complementary region in the amplicon. The quencher limits the fluorescence emission. In the elongation step, the Taq polymerase meets the bound probe and its 5′-3′exonuclease activity releases the fluorophore from the probe, which can then emit fluorescence. If a mutation exists in the region covered by the probe, it is not efficiently bound to the template DNA and thus the fluorophore cannot be released by the Taq polymerase. The presence of a mutation is detected by a reduction in fluorescence measured by the real-time PCR instrument.

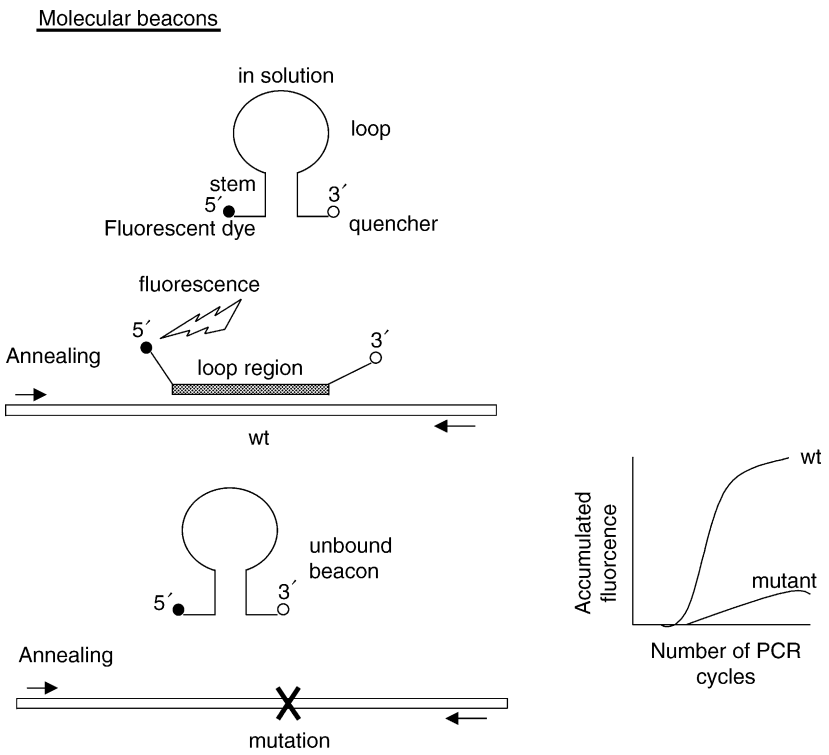

Figure 2 Schema of the mechanism of action of molecular beacons showing the stem-loop conformation of a beacon when in solution, with the fluorescence sequestered by the quencher. In each annealing step, the beacon binds to the template DNA and acquires a linear conformation which increases the distance between the quencher and the dye thus allowing fluorescence emission. If a mutation exists in the region covered by the beacon, binding of the beacon to DNA is not stable and the beacon remains in solution without emitting fluorescence. The presence of a mutation is detected by a reduction in fluorescence measured by the real-time PCR instrument.

5 and 3′ends of the probe and lie in close proximity when the probe is in its hairpin conformation. The stem-loop structure is disrupted when the beacon binds to its homologous sequence in the target DNA and the quencher is located far from the dye which enhances the fluorescence (Figure 2). This works in the same way as TaqMan probes. If a mutation maps in the region covered by the probe, its binding to the target is impaired and the beacon remains in its stem-loop conformation, without emitting fluorescence. Internal amplification controls must be added to the PCR to determine whether the reduction in fluorescence is really due to mutations, or alternatively, to amplification inhibitions. Beacons were first adapted for the detection of RIF-r in MTB [43] by including independent probes in different reaction tubes. Recently, a version of these beacons, called wavelength-shifting molecular beacons, allows each probe to be labeled with different dyes which permit the simultaneous analysis of the whole *rpo*B region in a single reaction tube by following the reaction through independent channels in the real-time instrument [44].

FRET probes

FRET probes are paired probes which are designed to anneal to the DNA template in a head-to-tail orientation with each probe close to the next (Figure 3). One probe (anchor) is fluorescence-labeled at its 3′end and the adjacent probe (sensor) is labeled at 5′ with a dye which can be excited by the fluorescence of the anchor. The sensor probe is designed to overlap with the mutation which is to be searched for. When the FRET probes are correctly bound to their homologous target at the end of the annealing step of the PCR, a process of energy transfer enables emission of the fluorescein to excite the dye in the adjacent probe. The fluorescence emission from the sensor probe is measured by the real-time instrument (Figure 3). This energy transfer only occurs if the distance between the probes is 1–5 nucleotides, which is only the case if the two probes are specifically bound to their homologous target.

In this format, the mutation analysis is different than that explained for TaqMan or beacon probes, and this analysis is performed in a melting step at the end of the PCR. This melting step involves a slow increase in temperature (0.05–0.2 °C/ s) to release the sensor probe from its target and thus monitor the melting of the probes by measuring the progressive reduction in fluorescence emission. The analysis software represents the melting of the probe as a melting peak with a melting temperature value (Tm). This melting temperature

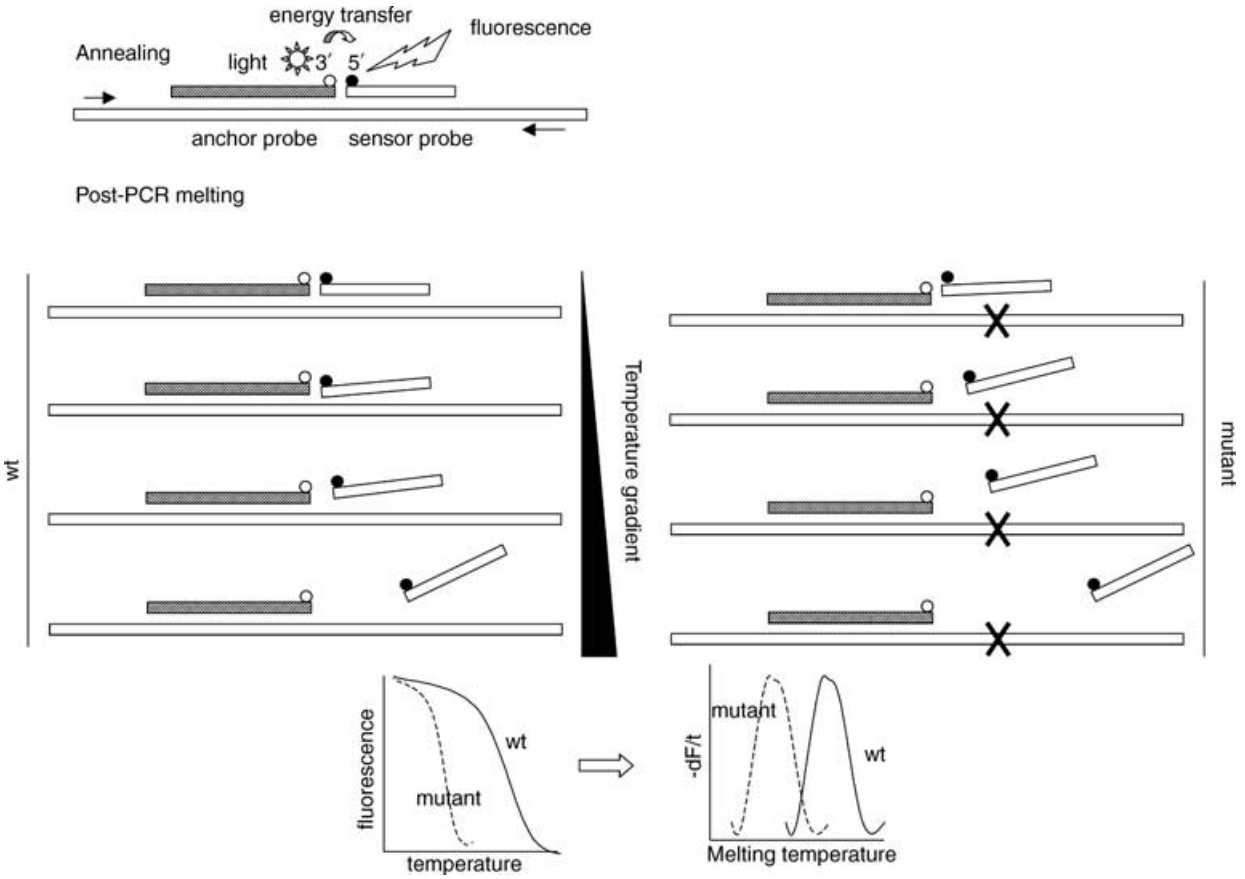

Figure 3 Schema of the mechanism of action of FRET probes. Upper part: in the annealing step the two FRET probes (anchor and sensor) bind to the template DNA. The head-to-tail positioning of the probes allows the two dyes to be close to each other. By a process of energy transfer, excitation of the anchor dye stimulates the sensor dye, which in turn emits fluorescence. Lower part: the behavior of the sensor probe in the post-PCR melting step is shown for a wild-type and mutant sequence. The sensor probe is melted at lower temperature when a mutated sequence is found owing to a thermodynamically impaired binding of this probe. The presence of a mutation is detected by a deviation in the melting temperature of the probe.

is constant for a pair of probes when bound to the *wt* sequence. If a mutation maps in the region covered by the probe, the mismatch between the probes and the template leads to a decrease in the Tm of the probes (Figure 3). Therefore, deviations in the Tm of the probes with respect to the reference wild-type value correspond to the presence of a mutation in the region covered by the probe.

Different studies have adapted FRET probes in different ways to the analysis of RIF and INH resistance. One of the approaches searches for specific mutations in rpoB core, katG [45] and inhA [46] by using multiple FRET probes specialized in specific mutations. This involves performing multiple independent reactions for the whole analysis. The other approach [47] offers a simplified model for detecting, in a single reaction tube, all the mutations related to RIF-r and the most prevalent for INH-r with only three pairs of FRET probes and two independent measurement channels. This is achieved by a design of FRET probes with both paired probes acting as mutation sensors, different from the conventional anchor-sensor design. This design succeeded in detecting 14 different mutations affecting nine codons in the same reaction. This could serve as a reference for adapting real-time PCR to the detection of multiple mutations using simple and less expensive designs which are suitable for different geographic settings.

Unlike TaqMan or beacons, FRET probes do not require amplification controls in the reaction, because the detection of mutations is not based

on the presence or absence of a fluorescent signal but rather, on deviations in the Tm values of a positive amplification signal.

Biprobes

Biprobes are single probes which are fluorescently labeled at the 5′end. In real-time PCR, biprobes are included in the reaction mix together with SybrGreen, a dye that emits fluorescence when it binds to double-strand DNA. Thus, at the end of each annealing step of the PCR, the SybrGreen excites the probe fluorophore by energy transfer, emitting fluorescence which can be measured by the real-time PCR instrument. The analysis of mutations involves monitoring of melting as was explained for FRET probes. Deviations in the Tm of the biprobe indicate the presence of mutations. In one report, this kind of probe correctly assigned all mutations in 46 rif-R strains by using three independent biprobes which involve the performance of three independent reactions [48].

Other PCR-based techniques

Multiplex allele-specific PCR

Some authors have developed PCR assays designed to allow the direct discrimination of certain nucleotidic substitutions at specific codons (Figure 4). These designs are multiplex-PCRs which include outer primers in the reaction to generate the amplicon for analysis, and inner primers (of opposite reading directions), which are designed to alternatively bind one or the other, depending on the substitution found at a certain codon. The codon in the analysis is placed asymmetrically in the amplicon, causing the length of the PCR fragments to vary, according to the direction of the inner primer which binds to the codon in analysis.

Multiplex allele-specific PCRs have been successfully assayed to assign resistance to EMB associated with embB codon 306 [49] and to INH in codon 315 [50] and have shown the ability of these designs to work directly on the clinical sample.

Multiplex allele-specific PCR has also been adpated to be performed on biochips. In this version [40,51], a set of primers which are homologous for different *rpo*B variants is immobilized in the array (MAGIChip) and all the reagents required for the PCR are added onto the chip. Amplification (fluorescence-labeled) only occurs in those positions in which the primer is complementary to the clinical amplicon. The fluorescence intensity of matched or mismatched primers provides information on the genetic content of the amplified DNA from the clinical sample.

FINAL CONSIDERATIONS

Several molecular designs to search for mutations encoding the resistance to antituberculous drugs

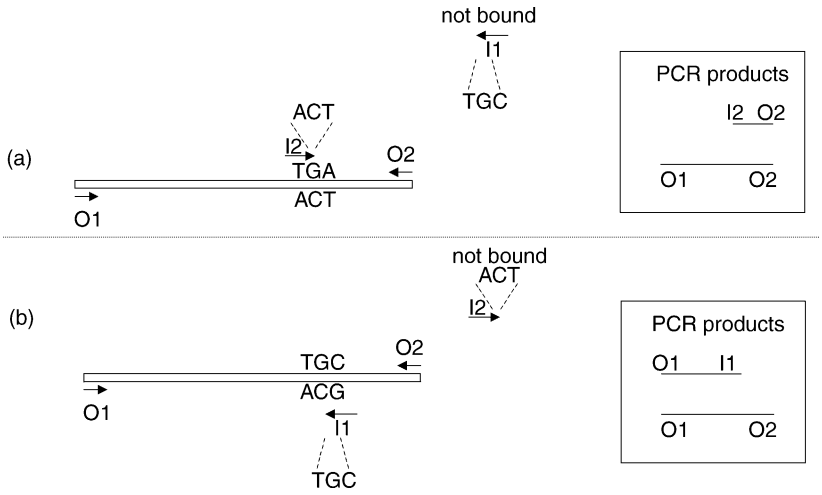

Figure 4 Schema of an multiplex allele-specific polymerase chain reaction (PCR). Outer primers (O1 and O2) and inner primers (I1 and I2), which are all added to the PCR-reagent mix, are indicated. Outer primers always bind to the template generating a long amplicon. Inner primers are designed so that their 3′ends are complementary to different nucleotides in the codon under analysis. Depending on the sequence in the codon, I1 or I2 would alternatively bind to the DNA leading to amplicons of different lengths. Depending on the amplicons obtained, the nucleotidic sequences in the codon analyzed can be specified.

in MTB have been developed in the last few years. Most of these are highly efficient for RIF-R detection and are well adapted to search for the most relevant INH-R mutations. Nevertheless, additional efforts should be made to increase the molecular approaches that could efficiently analyze drug resistances.

These new methods are sure to transform the standard approaches to the issue of resistance in the microbiology laboratory. Speed is a key question in the detection of resistant MTB strains and, in this sense, molecular assays are clearly unrivalled. Their main disadvantages, the need for expertise and their high costs, are becoming less important; the role of molecular biologists is progressively more important in clinical microbiology laboratories and the cost of some 'in-house' molecular designs could be equivalent to, or even less expensive than, the costs of phenotypic antibiograms if the new liquid-culture media are used. However, cost, speed or professional expertise are not the only factors to consider. The molecular analysis of resistance enables us to obtain additional information and essential data that are not available if only phenotypic antibiograms are obtained. Genotypic analysis not only detects the resistant strains, but in many cases it also specifies the mutation involved in the resistance, which helps to identify strains with high levels of resistance or a broad spectrum of resistance. In addition, the assignment of specific mutations could be an epidemiological marker for resistant strains [8]. Furthermore, these molecular methods can detect hetero-resistance by discriminating different subpopulations in the clinical sample and therefore help to detect the emergence of resistance and to follow its developement in certain cases [52]. Genotyping of resistance makes it possible to know which mutations do not diminish the fitness of the strain and thus do not affect the virulence or the ability of the strain to spread [12].

In conclusion, molecular approaches speed up the performance of resistance assays and provide access to essential information for a highly refined detection, follow-up and management of antibiotic resistance in TB.

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