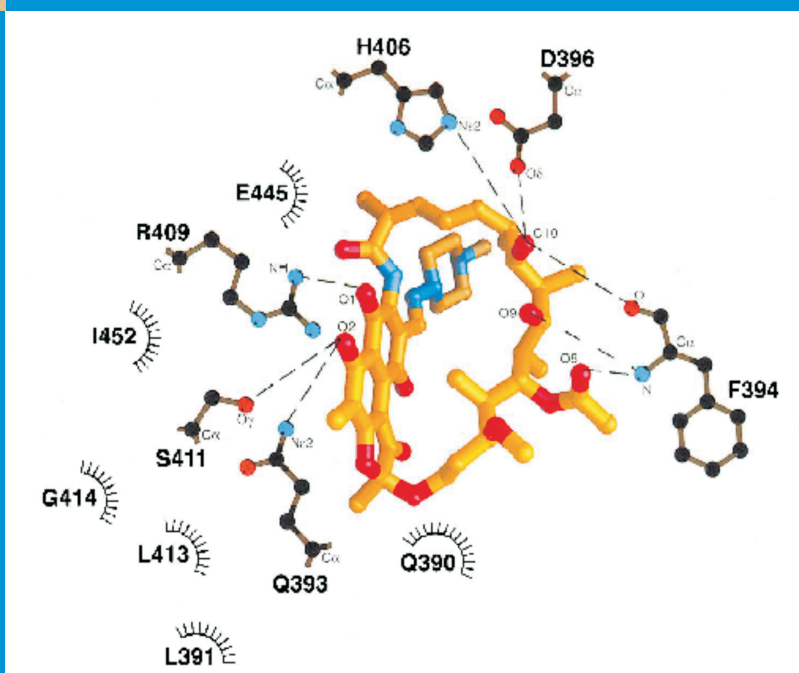




Guidelines for Global Surveillance of Drug Resistance in Leprosy



**World Health
Organization**

Regional Office for South-East Asia

Guidelines for Global Surveillance of Drug Resistance in Leprosy



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Regional Office for South-East Asia

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Foreword

The emergence of drug resistance is a cause for concern and a threat for any infectious disease intervention programme. For leprosy, a chronic disease with social stigma, drug resistance poses a serious impediment especially at the stage where a dramatic decline in prevalence and new case detection has been achieved due to intensive and concerted chemotherapy interventions made by the national programmes and its global partners. There seems to be an extraordinary degree of complacency about drug resistance, in spite of current challenges faced by TB control programmes and the history of dapsone-resistance and its negative effects on the leprosy control strategies. This has resulted in lack of priority and absence of information on current magnitude of drug resistance in leprosy which, of course, is not evidence of an absence of drug resistance. It is assumed that a combination of three drugs, if taken regularly will prevent the emergence of drug resistance. In addition, there is limited information on patient adherence with the unsupervised components of multidrug therapy (MDT). Although the problem of drug resistance is presently not acute, it is important that we collect data more systematically and monitor the trend carefully so that effective measures to combat this problem can be developed. With the recent development of more practical and quick DNA sequencing methods to detect drug resistance, several reports of rifampicin, dapsone and ofloxacin resistance have been published which further highlights the emerging threat.

In order to contain the threat, WHO has planned the following two-pronged strategy:

- (1) To closely monitor trends in occurrence of relapses after treatment with MDT due to drug resistance, particularly to rifampicin, and
- (2) To promote research on developing new drugs for non-rifampicin containing regimens to limit and treat patients who relapse after completing one or more courses of MDT due to resistant strains of *M. leprae* (secondary resistance) and those new patients who are not responding to standard MDT regimen (primary resistance).

The establishment of a network for global surveillance of drug resistance in leprosy is primarily to keep a close vigil on the drug resistance scenario at many vulnerable settings. To accomplish this, WHO has developed a simple guidelines to carry out sentinel surveillance and this initiative is expected to be conducted annually on a routine basis. This initiative will be coordinated by WHO' Global Leprosy Programme with support and collaboration from national programmes and major research institutes around the world. The research institutes have offered to provide free-of-cost testing of samples sent to them from the sentinel sites in several endemic countries. The results will be published annually in the Weekly Epidemiological Record of WHO in agreement with the national programmes.

Dr. V. Pannikar
Team Leader, Global Leprosy Programme
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Rationale 1

The emergence of drug resistance is a concern and a threat for many infectious disease intervention programmes especially when secondary prevention (chemotherapy) is the main component of the control strategy. The fight against leprosy has been a great success largely due to the development of multidrug therapy (MDT) in 1981. Since 1995, as a result of donations to WHO from The Nippon Foundation and Novartis Foundation for Sustainable Development all leprosy patients have had access to MDT free of cost. The effectiveness of MDT in curing leprosy in a short time has brought about a dramatic decrease in the disease burden in all leprosy-endemic countries. The disease prevalence has declined significantly especially in countries where leprosy has been highly endemic for decades and along with the decline in prevalence the annual new case detection has also started to decline in some countries.

Since rifampicin is the backbone of MDT, it is important to monitor the emergence of rifampicin-resistant mutants, as recent reports and publications have indicated instances of rifampicin resistance in several endemic areas. Resistance to dapsone has been reported since the late 1960s but convincing data supporting the existence of clofazimine resistant strains of *M. leprae* have not been reported. To meet the challenge of containing the disease and to sustain the on-going declining trend of leprosy in endemic countries, it is essential to keep a vigil on drug sensitivity patterns in vulnerable settings.

This document describes a drug resistance surveillance programme for leprosy to be carried out by WHO in selected endemic countries which have the necessary clinical, field and laboratory support systems in place to undertake this activity.

2 Objectives

2.1 General objective

The overall aim is to establish a surveillance network using a standardized approach to detect secondary drug resistance, particularly rifampicin resistance among leprosy patients and to monitor its trend.

2.2 Specific objectives

To monitor the trend of rifampicin resistance occurring among multibacillary (MB) leprosy patients who have taken a full course of treatment with standard WHO recommended MDT for MB leprosy and have relapsed.

To monitor the trend of dapson resistance occurring among MB leprosy patients who have taken a full course of treatment with standard WHO recommended MDT for MB leprosy and have relapsed.

To monitor ofloxacin resistance occurring among MB leprosy patients who may or may not have been treated with WHO recommended MDT and have relapsed.

Surveillance methodology 3

Surveillance of drug resistance in leprosy will be carried out based on a sentinel surveillance model with the aim to monitor the trends over a period of time. Certain health facilities will be identified in selected endemic countries as sentinel sites where tissue samples will be collected and transported to the reference laboratories.

As it is based on a sentinel surveillance model, it does not intend to cover routinely all relapse cases diagnosed throughout a country or continent and it is not a crosssectional survey.

4

Broad outline of the sentinel surveillance system

4.1 Anti-leprosy drugs to be investigated

The main aim of the sentinel surveillance system is to detect secondary rifampicin resistance among patients who have relapsed after completing a full course of treatment with MDT for MB leprosy as prescribed by WHO. The inclusion of dapsone resistance would further enhance the preparedness to face rifampicin resistance. Since clofazimine resistance has not been described, its mechanism and molecular methods to detect this, are unknown.

Ofloxacin, apart from being used in combination with rifampicin and minocycline as a single dose treatment for single lesion paucibacillary (PB) leprosy cases has not been used for the treatment of leprosy extensively in national programmes. However, it is well known that this drug is easily available in many leprosy-endemic countries and could be used by private practitioners in treating leprosy. As the mutations for ofloxacin-resistant strains have been reported recently, it was decided to include monitoring of ofloxacin resistance along with rifampicin and dapsone.

Though primary resistance is a possibility, it will not be included in this current (first generation) surveillance activity taking into consideration the large sample size and high cost to undertake such a study. It is possible that after reviewing data and experiences generated by the proposed activities, in the next steps, drug resistance surveillance can be expanded to include testing for primary drug resistance.

4.2 Components

The sentinel surveillance system is composed of two parts:

- (1) The first component is the systematic collection of samples in the field. This involves proper identification of relapse cases, collection of appropriate tissue specimens from these patients and transportation of samples to the respective reference laboratory. In order to monitor trends over a period of time and to ensure that data can be interpreted in a meaningful way it is important that specimens be collected systematically at the sentinel sites.
- (2) The second component is the laboratory part which will be carried out by referral laboratories receiving samples from the field and carrying out tests for rifampicin, dapsone and ofloxacin resistance.

4.3 Selection of endemic countries

The surveillance activity will be carried out in selected endemic countries that are detecting significant numbers of new cases annually. Regional representation will also be taken into account. Surveillance will be an ongoing activity and participating centres will have to take into account the need to maintain the sentinel surveillance work over a period of time. Centres will be identified in countries which are already conducting surveillance activity for drug resistance and have the necessary human resources to carry out the surveillance on a long-term basis.

4.4 Documentation

The results of the surveillance activity will also be utilized for appropriate patient management by providing feed-back information to the health facilities at the peripheral levels where patients included in the surveillance system are currently undergoing treatment. In order to achieve this, standard clinical information forms have been developed to record data that will be sent to the reference laboratories along with the samples. Here too, appropriate international networking is planned (*please see Annexes 1, 2 and 3*).

5 Definitions and procedures

MB relapse cases referred to the selected referral facilities will be examined by an expert (designated experienced physician) to confirm the diagnosis of relapse using strict criteria so as to reduce selection bias.

5.1 Relapse definition

A relapse is defined as the re-occurrence of the disease at any time after the completion of a full course of treatment with WHO recommended MDT. Relapse is diagnosed by the appearance of definite new skin lesions and/or an increase in the bacteriological index (BI) of two or more units at any single site compared to BI taken from the same site at a previous examination. Care should be taken to exclude patients suffering from leprosy reactions.

5.2 Criteria for inclusion of MB relapse cases

A person who was initially classified as an MB case and has taken at least 12 monthly doses of MB MDT as recommended by WHO and who is now showing signs and symptoms of relapse without any evidence of lepra reaction is to be recruited for sentinel surveillance. Taking into consideration the present limitation in PCR sensitivity (due in part to extracting DNA from tissue specimens and to the small volume examined) only MB relapse cases with a BI of +2 and above will be recruited. MB classification is based on having six and more skin lesions or having a positive BI at any single site.

5.3 Patient consent

Necessary patient consent must be obtained as per the ethical guidelines of the country.

5.4 At the referral facility

It is important that all MB relapse cases diagnosed at the sentinel surveillance facility/referral centre having a BI of +2 and more are included in the study. This would minimize selection biases that can affect the trend analysis. Samples are then to be sent to the designated reference laboratory (identified in Annex 3) to carry out polymerase chain reaction (PCR) and direct sequencing for drug resistance. It is expected that each sentinel site will be able to identify and collect the necessary tissue samples from at least 20 MB relapse cases a year.

Information regarding past treatment history and current clinical presentations are to be collected using the Case Report Form as shown in Annex-1. Each MB relapse case will undergo a slit skin smear evaluation using the standard technique as for a routine skin smear for bacteriological index (BI).

From each case, two slit skin smear samples will be collected. Each sample will be taken from a different skin lesion that is most prominent. It has been shown that materials collected from a slit skin smear (tissue scrapings) contain enough bacilli for PCR amplification.

For those MB relapse patients who refuse to participate in the study, basic information should be collected from them without carrying out further tests as required for surveillance. Such patients should be given appropriate MDT treatment as per the national or WHO guidelines.

5.5 Information to be collected

Basic information about the patients will be collected using the Case Report Form shown in Annex 1.

- The first part of the report deals with reporting details such as case identification number, date of report and particulars relating to the institute sending the specimen.

- The second part deals with the demographic details of the case such as age and sex.
- The third part deals with the present clinical presentation of the case along with the tests undertaken. Detailed description of clinical status of the skin and nerve lesions, particularly of any new skin lesions should be recorded. It is important to record the date and BI results of the slit skin smears for each site. The clinical features and smear results will be considered for arriving at the current classification of the relapse case.
- The fourth part covers clinical history which includes date of initial diagnosis and details about clinical presentation at the time of diagnosis, classification, including reasons and date if any, re-classification done subsequently, treatment prescribed with indication of patient's adherence to treatment and results of any tests that were performed. Should the patient treatment card be available the above-mentioned information could be collected from it. Usually, old patient cards may not be available, in which case the information will have to be collected based on the recall of the patient.
- The fifth part deals with the description of the site and BI results of the two skin smear specimens to be sent to the collaborating reference laboratory for DNA sequencing.

Collection of samples and transportation

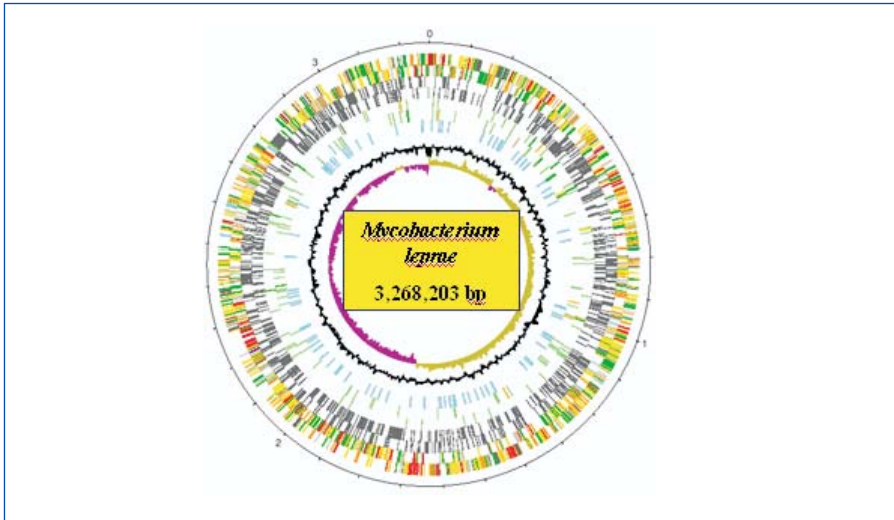
6

Slit skin smear samples are collected in the same manner as taking skin smears for Bacterial Index (BI) examination using a disposable stainless steel blade. Samples are to be collected from two different sites from the most prominent skin lesions or from sites which showed the highest BI in previous examinations. Caution should be taken to prevent cross-contamination. The stainless steel blade containing the tissue scrapings are to be rinsed into a 1.8 ml centrifuge tube (screw type) pre-filled with 1ml of 70% ethanol (molecular biology grade absolute ethanol 70% + sterile de-ionized water from MilliQ or use water for human injection 30% , the mix should be prepared in the laboratory) making sure that the tissue scrapings are washed from the surface of the blade and are suspended in the solution. This will mean that from each relapse case two slit skin smear samples will be collected, each placed in separate tubes.

Samples could be kept at room temperature and sent to laboratories for sequencing later. Bacilli are inactivated by ethanol which means that samples can be sent by routine transport without the need to control the temperature during transportation, or to take additional precautions for biohazard control.

Should DNA extraction not be successful from the skin smear sample, a second slit skin smear or a biopsy may be taken after having discussions with the referral centre and the respective reference laboratory on a case-by-case basis.

Figure 1: *Mycobacterium leprae* genome

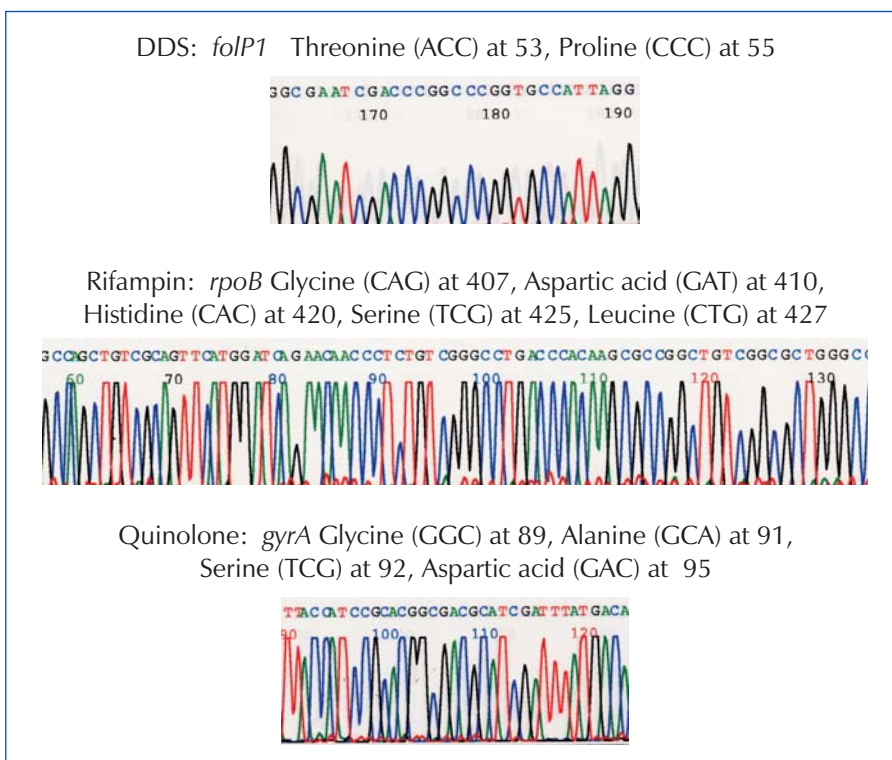


6.1 Molecular basis of rifampicin, dapson and ofloxacin resistance and methods for detection

The recent mapping of the *M. leprae* genome has identified sites at which mutations occur, conferring resistance to dapson, rifampicin and the quinolones. Rifampicin binds the beta-subunit (coded by the *rpoB* gene) of the RNA polymerase and certain mutations in the *rpoB* gene lead to rifampicin resistance in *M. leprae* and *M. tuberculosis*. Missense mutations leading to the substitution of any one of the following positions (positions 407, 410, 416, 420, 425 and 427) or an insertion of amino acids between position 408 and 409 confers rifampicin resistance to *M. leprae*. Missense mutations in the sulphone resistance determining region of the *folP* gene (codes dihydropteroate synthase), resulting in alterations of amino acids at positions 53 and 55, confer dapson resistance to *M. leprae*. Missense mutations in the *gyr A* gene at position 89, 91, 92 and 95 are correlated with ofloxacin resistance.

DNA in skin smear samples from relapsed MB cases will be amplified by PCR. The use of PCR sequencing ensures detection of drug resistance with greater certainty.

Figure 2: Mutations and drug resistance to anti-leprosy drugs.



The first step will be DNA extraction followed by amplification by PCR of the drug resistant determining regions (DRDR) in the *rpoB*, *folP* and *gyrA* genes. Amplification of the target region will be confirmed by agarose electrophoresis. PCR products are purified and followed by sequencing reaction. Big Dye Terminator v1.1 (Applied Biosystems) is adequate for sequencing of short fragment. Samples are applied to sequencer for analysis of nucleotide mutation.

6.2 DNA extraction protocol

- Centrifuge for at least 20 min at max speed
- Remove the supernatant
- Re-suspend the pellet in 500ul PBS (120mM NaCl; 2.0mM KCl; 100mM

Na₂ HPO₄; 6mM KH₂ PO₄, pH7.3) 10mM

- Allow to stand more than 30min at room temperature
- Centrifuge for 20min at max speed ($>5,000 \times g$)
- Suspend in 50 μ l (low amount of tissue) or 100 μ l (high amount of tissue) of Lysis buffer (Proteinase K 1mg/ml 0.05% Tween 20 in 0.1M Tris-HCl, pH.8.5. Reference: de Wit et al. *J. Clin Microbiol* 29: 906-910, 1991)
- Over lay mineral oil to prevent evaporation
- Incubate overnight at 60°C
- Heat 10 min at 97°C
- Transfer the suspension to a DNA low binding tube (DNA LoBind tube, Eppendorf)

6.3 PCR protocol

Amplification will be done using primers described in the literature for detection of mutation in *rpoB*, *gyrA* and *folP1*. If possible, primers described in the Figure 3 will be used. Alternatively, reference centres may use the primers they are currently using with the condition that these primers would have been qualified in a comparison study.

The PCR protocol has been previously approved by the Initiative for Diagnostic and Epidemiological Assays for Leprosy (IDEAL) group.

The PCR mixture contains:

- 12.5 ul PCR master mix (2X) from Applied Biosystem (ABI) containing Hot Start Taq polymerase
- 1.25 ul Forward primer at final concentration 0.5uM
- 1.25 ul Reverse primer at final concentration 0.5uM
- 15.0 ul water to final volume 25ul
- 5 ul Template DNA

Protocol for PCR

folP primers:

OMSfolPF ctt gat cct gac gat gct gt
OMSfolPR cca cca gac aca tcg ttg ac

rpoB primers

OMSrpoBF gtc gag gcg atc acg ccg ca
OMSrpoBR cga caa tga acc gat cag ac

gyrA primers

OMSgyrAF atg gtc tca aac cgg tac at c
OMSgyrAR tac ccg gcg aac cga aat tg

Figure 3: Primers for direct sequencing

Primers for Direct sequencing

	1	gtgagttgg	cgccagtga	ggttattggg	gtttgaacg	tcactgacaa	ttcgttctca
	61	gatggcggac	gttacctga	tcctgacgat	gctgtccagc	acggcctggc	aatggtcgcg
<i>folP1</i>	121	gaaggcgcg	cgattgtcga	cgtcggggc	gaatcgaccc	ggcccgggtg	cattagacc
	181	gatcctcgag	ttgaactctc	tcgtatcgtt	cctgtcgtaa	aagaactgc	agcacagggg
	241	attacagtaa	gtatcgatac	tacgcgcgct	gatgttgac	ggcgggcgct	gcaaagcggc
	301	gcacggatcg	tcaacgatgt	gtctggggg	cgagcagatc	ccgcgatggc	tcctctggtg
	361	gctgaagccg	gtgtgcgtg	ggtgttgatg	cactggcgac	tgatgtcggc	tgaacggccg
	1201	cgggagcgga	tgaccacca	ggacgtcgag	gcgatcacgc	cgcagacgct	gatcaatc
	1261	cgtccggtgg	tcgccgctat	caaggaaftc	ttcggcacca	gccagctgtc	gcagttcatg
	1321	gatcagaaca	accctctgtc	gggcctgacc	cacaagcgcc	ggctgtcggc	gctgggccc
<i>rpoB</i>	1381	ggtggtttgt	cgctgagcg	tgccgggcta	gaggtccgtg	acgtgcaccc	ttcgactac
	1441	ggccgatgt	gccgatcga	gactccggag	ggcccgaaca	taggtctgat	cggttcattg
	1501	tcggtgtacg	cgcggtcaa	ccccttcggg	ttcatcgaaa	caccgtaccg	caaagtgtt
	1561	gacggtgtgg	tcagcgacga	gatcgaatac	ttgaccgctg	acgaggaaga	cccgatgtc
	1	atgactgata	tcacgtgcc	accaggtgac	ggttctatac	agcgggttga	gccggtcgac
	61	attcagcagg	aaatgcagcg	cagctatatt	gattacgcga	tgagtgtgat	tgtggccgg
	121	gcgttgctg	aagtcgcga	tggtctcaaa	ccggtacatc	gtcgggtctt	gtacgcgatg
	181	ttagactccg	gtttccgcc	ggaccgtagc	cacgctaagt	cagcacggtc	agtcgctgag
<i>gyrA</i>	241	acgatgggca	attaccatcc	gcacggcgac	gcacgatatt	atgacacgtt	agtgcgatg
	301	gcgcagccgt	ggctgctcg	gtatcccttg	gttgatgggc	aaggcaattt	cgggtcgccg
	361	ggtaaatgacc	caccggcagc	gatcggttat	tgtgtgtcag	gaaattcctt	ggtgaggttg
	421	ctatttggga	aatcaatacg	aatcggatgat	atcggttactg	gagctcagtt	caattcgagc
	481	aatccgatcg	actgaaggt	tcttgatcgg	catggtaatc	cggttgtagc	cgattattta

The following is the PCR run:

- Denaturation at 95°C for 2 min
- Then 40 cycles of
 - Denaturation at 95°C for 15 sec
 - Annealing at appropriate temp (58°C) for 15 sec
 - Extension at 72 °C for 60 sec
- Final extension at 72 °C for 7 min

Negative (pure water) and positive controls should be processed at the same time. The PCR thermal cycler should be calibrated periodically.

For confirmation of PCR results, 5ul of PCR products will be loaded into an agarose gel electrophoresis. Only PCR products showing one clear single band will be processed for DNA sequencing.

6.4 DNA sequencing protocol

The standard operational procedures are as follows:

- Purify PCR products using QIA quick PCR purification kit as per manufacturer's instructions.
- Estimate the concentration of DNA available for sequencing (OD_{260} or by visual observation of amplicon band on gel in comparison to DNA standards).
- For each sequencing reaction, add the following reagents:
 - 8.0 μ l of 1X Big Dye 1.1 Terminator Ready Reaction Mix
 - Up to 11 μ l of 3-5 ng of DNA in dH₂O
 - 1.0 μ l of 3.2 pmol/ μ l primer
 - Adjust volume to a total of 20.0 μ l.
 - Alternative schemes using Big Dye Terminator 3.1 are optional

- Mix well and spin briefly. Place the tubes in a thermocycler and set the volume to 20 μ l.
- Programme the thermocycler as follows:
 - 1 cycle of 96°C for 30 sec
 - 25 cycles of [96°C for 10 seconds, 50°C for 5 sec, and 60°C for 4 min]
 - Hold cycle at 4°C
- Final purification step and preparation for sequencing will be done according to the sequencer present in each laboratory.

6.5 Reporting results of DNA sequencing

Results of PCR and sequencing will be reported directly to the referral centre/institute that sent the specimen (clinician in charge of the patient) on Form-2 (shown in Annex 2). The result will be either: no amplification of DNA, and for each gene the presence or absence of mutations known to confer drug resistance.

DNA sequences will also be sent to the National Reference Centre on Mycobacteria, Faculte de Medecine Pitie-Salpetriere, Paris, France, which will compile all the sequence data and reference the mutations. The data base will be shared with all national programmes and other laboratories for research purposes.

6.6 Quality control

Quality control of the reference laboratories that are carrying out DNA sequencing for drug resistance will be conducted following standard procedures.

The laboratory that will conduct quality control will be identified in consultation with all partners. This laboratory will send representative DNA samples (no mutation, known mutation) before and during the study.

In addition, quality checks will be made from time to time regarding patient selection, data entry and transportation of specimens by the national leprosy control programme in collaboration with WHO.

All positive and negative slit skin smear specimens from each case are to be archived for quality assurance and further testing. Specimens will be kept by the laboratory for at least ten years.

6.7 Reference laboratories

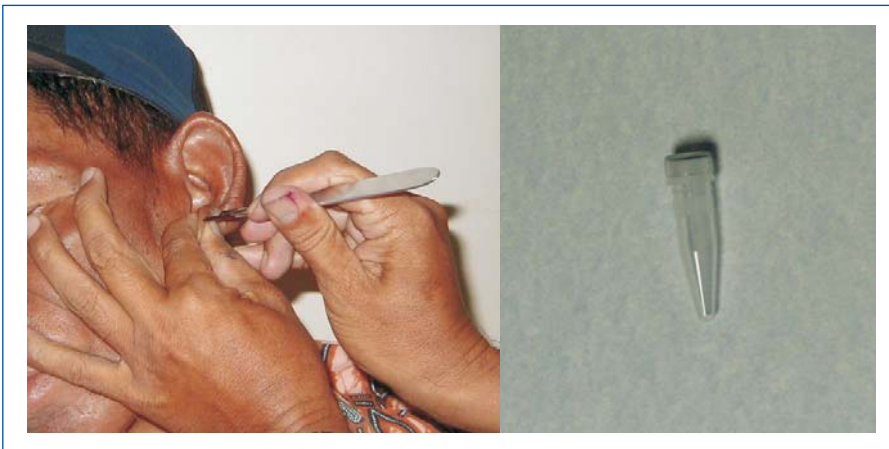
The following reference laboratories (contact details are given in Annex-3) have been identified for conducting the DNA sequencing tests to confirm rifampicin and dapsone resistance:

- (1) Department of Microbiology, Yonsei University College of Medicine, Seoul, South Korea.
- (2) Leprosy Research Centre, National Institute of Infectious Diseases, Tokyo, Japan.
- (3) Central JALMA Institute for Leprosy and other Mycobacterial Diseases. Agra, India.
- (4) National Reference Centre on Mycobacteria, Faculte de Medecine Pitie-Salpetriere, Paris, France.
- (5) Laboratorio de Hanseniase, Instituto Oswaldo Cruz, Rio de Janeiro, Brazil.
- (6) Laboratory Research Branch, National Hansen's Disease Programs, Baton Rouge, USA.
- (7) Global Health Institute, Ecole Polytechnique Federal de Lausanne, Department of Immunology, Lausanne, Switzerland.

Laboratory tests 7

Nucleotide sequencing of the drug resistance determining region in the *rpoB*, *folP1* and *gyrA* genes will be determined using PCR and direct sequencing. Although only a small number of cases have been tested so far, high concordance between mouse footpad technique and DNA sequencing methods have been observed. Isolates with one or more amino acid substitutions in one or more drug-resistance determining regions, those substitutions which have been confirmed to confer rifampicin, dapsone and ofloxacin resistance by the mouse footpad method, will be scored as resistant to the respective drug(s).

Figure 4: Skin smear examination and centrifuge tube for collection of tissue specimens



Management of MB relapse cases included in the surveillance system

All MB relapse cases included in the surveillance study should immediately be put on treatment with standard MB-MDT without waiting for the results from the reference laboratory on the status of drug resistance. If the result comes back as sensitive to rifampicin, MB-MDT treatment is to be continued accordingly. For patients who are reported to be resistant to dapsone only, standard MB-MDT can be safely continued.

In case the reference laboratory reports that the patient is harbouring rifampicin resistant *M. leprae* the following treatment should be given¹:-

- administration of 50 mg of clofazimine, together with 400 mg of ofloxacin and 100 mg of minocycline, daily for six months;
- administration of 50 mg of clofazimine, together with 100 mg of minocycline or 400 mg of ofloxacin daily for at least an additional 18 months.

The above-mentioned treatment regimen is also to be used for patients reported to be harbouring both rifampicin and dapsone resistant *M. leprae*.

1 Reference: WHO Expert Committee on Leprosy. Seventh Report. Geneva, World Health Organization, 1998 (WHO Technical Report Series, No. 874)

Reporting and dissemination of information

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The reference laboratory will be reporting information regarding the susceptibility of *M. leprae* to rifampicin and dapsone to the respective surveillance centres in each country. Each surveillance centre will then take the necessary steps to inform the health facility where the MB relapse patient is currently undergoing treatment and, at the same time, provide the necessary second-line drugs to treat the patient as recommended.

Reference laboratories will also send a copy of the results along with case report forms (submitted to them by various participating surveillance centres from endemic countries) to the WHO Global Leprosy Programme (GLP) for data compilation and analysis, and an annual report of all the samples tested and the results. GLP will publish information on surveillance of drug resistance annually in the *WHO Weekly Epidemiological Record*.

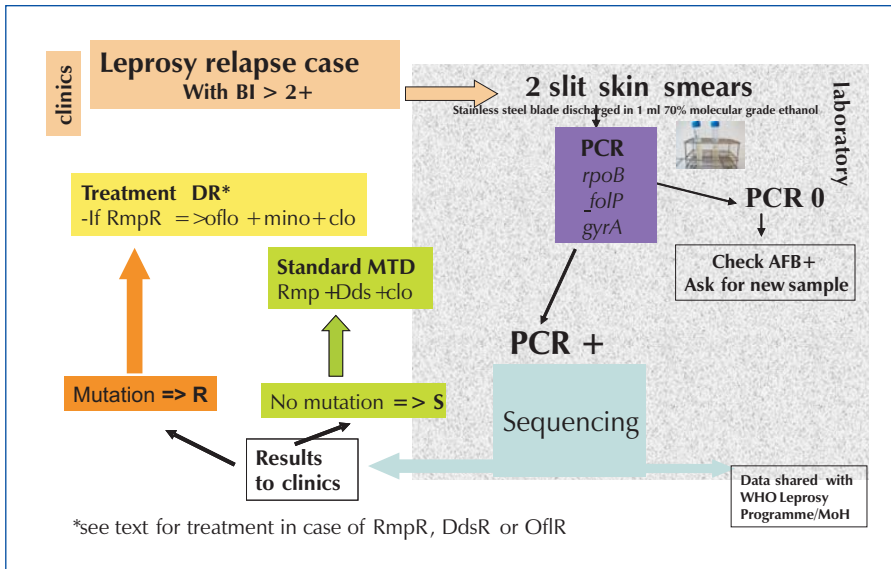
10 Conclusion

The global burden of leprosy in many endemic countries has declined and many leprosy patients are leading normal and productive lives as a result of MDT treatment. It is important that the achievements made with MDT over the past two decades be sustained so that the disease burden is further reduced and leprosy ceases to be a dreaded disease in the community.

To ensure that MDT continues to be effective, all efforts must be made to make sure that patients are able to complete their full course of MDT treatment as prescribed. Rifampicin is the most important component of MDT and it is essential that emergence of resistance is closely monitored. This would greatly improve the effectiveness of the MDT treatment regimen. MDT is the best treatment available for leprosy and it is hoped that it will continue to be effective for many more years. In addition, efforts must continue to search for more effective, safer and shorter combination regimens, using new classes of anti-mycobacterial drugs, to manage patients who harbour *M. leprae* strains resistant to standard MDT drugs or patients who cannot be treated by the standard MDT regimens due to contraindications or other reasons.

Annex 1

Workflow of molecular detection for drug resistance in leprosy



Annex 2

Form 1. Case Report Form

(MB Relapse cases only)

1. Reporting details

1.1 Case identification number: _____ (given by national authorities)

1.2 Name of country _____

1.3 Date of report __/__/____ (dd/mm/yy)

1.4 Name of National Health Authority/Institute sending specimen

Address: _____

Telephone: _____ Fax: _____ Email: _____

2. Demographic details of case

2.1 Year of Birth: _____ 2.2 Sex: Male/female

3. Clinical presentation at time of relapse

3.1 Clinical features _____

3.2 Number of old skin lesions _____

3.3 Number of new skin lesions _____

3.4 Patient consent to participate: Yes/No (If No, skip steps 3.5, 3.6 and 3.7)

Date of Consent: _____

3.5 Skin smear results from specific sites (if any, along with the date of test)

1. Site BI Date of Smear.....

2. Site BI Date of Smear.....

3. Site BI Date of Smear.....

4. Site BI Date of Smear.....

5. Site BI Date of Smear.....

6. Site BI Date of Smear.....

3.6 Biopsy Yes/No

If 'YES' Date: _____ Sites _____

Biopsy Report _____

3.7 Present classification (MB PB and Ridley Jopling if available)

4. Past clinical history

4.1 Date of diagnosis _____ (dd/mm/yy)

4.2 Past clinical history _____

4.3 Classification at diagnosis _____

4.4 MDT taken or not: Yes/No

4.5 If "YES":

a) Date when treatment was first taken ____/____/____ (dd/mm/yy)

b) What type (PB or MB blister packs) _____

c) How many months MDT was taken _____

d) Date when treatment was completed ____/____/____ (dd/mm/yy)

4.6 Skin smear results at diagnosis

1. Site BI Date of Smear

2. Site BI Date of Smear

3. Site BI Date of Smear

4. Site BI Date of Smear

5. Site BI Date of Smear

6. Site BI Date of Smear

4.7 Past change in classification (if any, during the course of treatment) Yes / No

4.8 If yes, date of change in classification ____/____/____ (dd/mm/yy)

5. Skin smears sent for DNA sequencing (please write code numbers on the tube)

Date: (dd/mm/yy)

5.1 No: _____ Site _____ Result _____

5.2 No: _____ Site _____ Result _____

Annex 3

Form 2. Reporting results of DNA sequencing

Name of Central/Reference laboratory _____

Address: _____

Telephone: _____ Fax: _____ Email: _____

Case identification

Case identification number: _____

Name of Institute sending specimen

Address: _____

Telephone: _____ Fax: _____ Email: _____

Date of reception ____/____/____ (dd/mm/yy)

Type of specimen received: Skin sample / DNA extraction / PCR product

Results

- **rpoB gene**
 - negative PCR
 - no mutation (do not report silent mutation)
 - presence of a mutation involved in rifampin resistance

- **folP gene**
 - negative PCR
 - no mutation (do not report silent mutation)
 - presence of a mutation involved in dapsone resistance
- ***gyrA* gene**
 - negative PCR
 - no mutation (do not report silent mutation)
 - presence of a mutation involved in fluoroquinolone resistance

Name of corresponding biologist (and signature):

Date of reporting:

Annex 4

Collaborating Reference Laboratories

- (1) Laboratório de Hanseníase, Instituto Oswaldo Cruz,
Manguinhos Pavilhão Mourisco S. 105,
21045-900 Rio de Janeiro, Brazil
Tel. 55 21 2590 4712; Fax: 55 21 2590 9741
Email: euzenir@uol.co.br, psuffys@ioc.fiocruz.br
- (2) National Reference Center on Mycobacteria,
Department of Bacteriology and Hygiene,
Faculty of Medicine, Pitié-Salpêtrière,
91 Boulevard de l'Hôpital, 75634 Paris Cedex 13, France
Tel: +33 1 40779746, Fax: +33 1 4856 2222
Email : emmanuelle.cambau@hmn.aphp.fr
- (3) Central JALMA Institute for Leprosy and
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Tel. No.: 81-42-391-8211; Fax No.: 81-42-394-9092
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Tel. No.: +822-2228-1819; Fax No.: +822-392-9310
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- (6) National Hansen's Disease Programs,
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Annex 5

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The eventual emergence of drug resistance is a significant cause for concern and threat to many infectious disease control programmes, especially when secondary prevention (chemotherapy) is the main component of the control strategy. For leprosy, a chronic disease accompanied by social stigma, drug resistance poses a serious impediment to its control. This is particularly worrying at the stage where a dramatic decline in prevalence and new case detection has been achieved after to intensive and concerted chemotherapy interventions made by the national programmes and its global partners.

In order to meet the challenges of containing the disease and to sustain the ongoing declining trend in leprosy cases in endemic countries, it is essential to keep a vigil on drug sensitivity patterns in vulnerable settings. WHO has developed a simple guideline that outlines the standard tools and procedures for key components of laboratory and field protocols to be followed for surveillance of drug resistance in leprosy.



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