National Leprosy Eradication Programme

National Guidelines for Surveillance of Antimicrobial Resistance in Leprosy

January 2023

सत्यमेव जयते

Central Leprosy Division
Directorate General of Health Services
Ministry of Health & Family Welfare
Government of India

NATIONAL LEPROSY ERADICATION PROGRAMME

National Guidelines for Surveillance of Antimicrobial Resistance in Leprosy

2023

Central Leprosy Division
Directorate General of Health Services
Ministry of Health & Family Welfare,
Government of India

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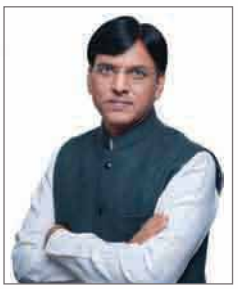

MESSAGE

Anti-Microbial Resistance (AMR) is an urgent global public health challenge that makes it more difficult to treat infections leading ultimately to death. Addressing the drivers and the impact of AMR poses significant threat for the country and hence requires a cross-cutting strategy with extensive work both at inter-departmental and inter-divisional levels and at regional and country level. It actively calls for a rapid standardization of guidelines regarding antibiotic use, pharmaco-vigilance etc.

Leprosy has been endemic in India since time immemorial. It is curable with a combination of drugs known as Multi Drug Therapy (MDT) that has led to a substantial reduction in the disease burden of leprosy globally since 1983. National Leprosy Eradication Programme (NLEP) supplies MDT throughout the year, across the country, free of cost. However, relapse cases of leprosy remain a concern for the country.

Ministry of Health and Family Welfare has framed this tangible document to set up a country-wide surveillance network for AMR management in leprosy to understand the trends and patterns of AMR to the drugs given as MDT, as well as implementing the surveillance model as part of routine NLEP component.

I am affirmative that this document will serve as a pivotal guide to establish a nationwide robust surveillance system for AMR in leprosy. I wish the programme to be successful to win this crucial battle for our country and congratulate all the stakeholders on this significant milestone.

(Dr. Mansukh Mandaviya)

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MESSAGE

Anti-microbial resistance (AMR) poses a significant global threat of extensive capacities. It is estimated that drug-resistant infections contribute to nearly 5 million deaths each year. Although antimicrobial resistance is a natural phenomenon, the main drivers of both its development and spread are 'man-made'. Irrational use of antimicrobials in humans, unregulated over the counter sale of antibiotics and lack of guidance and awareness on antibiotic use are the major drivers of AMR.

With the introduction of Multi Drug Therapy (MDT) and the implementation of the National Leprosy Eradication Programme (NLEP) as a centrally sponsored scheme, there has been a significant acceleration of leprosy elimination as a public health problem in the country. MDT is supplied free of cost in India. Meanwhile, a relatively high number of relapse cases continues to be registered. Central Leprosy Division has developed an evidence based guidelines to set up a systematic surveillance system for AMR management in leprosy to keep a vigil on drug sensitivity patterns in different settings.

Our Prime Minister Shri Narendra Modi ji has said that as a country, we have to leave no stone unturned to not just reach the last mile but also to work together to eliminate the social stigma attached with this disease and I am hopeful that this document will benefit in strengthening the laboratory capacity for detection of leprosy cases and drug resistance thus improving the quality of patient care and bringing down the AMR burden in the country.

I, acknowledge and congratulate the team of leprosy experts, physicians and clinical microbiologists who contributed to the compilation of this document.

(Dr. Bharati Pravin Pawar)

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Message

India is one of the endemic countries for Leprosy. India contributes more than 50% global annual new cases of leprosy. Ministry of Health & Family Welfare, Govt. of India, with the National Leprosy Eradication Program (NLEP), has been continuously re-focusing its strategies towards eradication of this disease. NLEP's continuous efforts so far have witnessed success of various strategies introduced in the past, as a result, the movement is now shifting from elimination towards eradication.

With reducing number of new leprosy cases, there are challenges which require focused approach and proactive action to detect new cases and prevent transmission. Simultaneously, program needs introduction of tools & techniques to counter the upcoming challenges.

India now envisions for further strengthening of healthcare system with provision of improved detection & surveillance mechanism and establishing technical architecture for surveillance of Anti-microbial resistance (AMR) and management of cases with antimicrobial resistance. As such AMR guidelines have been developed to address issues of AMR to antibiotics used in Multi-Drug Therapy regime for treatment of leprosy.

AMR surveillance guidelines for leprosy will help the States and UTs to develop and establish a structure for AMR surveillance and management of resistance cases. They will guide and help in framing out strategies for establishing a better case detection, management and referral linkage structure for AMR surveillance.

Place : New Delhi
Date : 27th January 2023

(**Rajesh Bhushan**)

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MESSAGE

Antimicrobial Resistance (AMR) has emerged as a public health concern which is recognized as high priority area by the Government of India. Recent trends clearly illustrate the growing political commitment at the highest levels to have a cogent response in place that can provide the necessary gravitas for nation-wide surveillance for containment of AMR.

With the introduction of Multi Drug Therapy (MDT) in 1982 and implementation of the National Leprosy Eradication Programme (NLEP) since 1983 as a centrally sponsored scheme, there has been an accelerated reduction in prevalence of incidence of leprosy as a public health problem. NLEP ensures uninterrupted supply of Multi Drug therapy (MDT) to all patients of leprosy, free of cost. But despite decrease in leprosy prevalence in the country, relapse cases were seen that burdened the health system.

India had previously instituted surveillance of the emergence of drug resistance in disease causing microbes in the context of vertical programmes, like the Revised National Tuberculosis Control Programme, the National Vector Borne Disease Control Programme, to name a few. This document will build a country-wide surveillance network for AMR management in leprosy to understand the drug sensitivity trends of MDT and its management.

The fight against AMR requires everyone's commitment. I compliment the Central Leprosy Division for developing guiding document under NLEP for the country's fight against Antimicrobial Resistance. I am hopeful that this document will strengthen the laboratory detection of leprosy cases with drug resistance thus contributing in improving the outcomes.

(S Gopalakrishnan)

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MESSAGE

The National Leprosy Eradication Program (NLEP) has been launched by Government of India with aim of control & eradication of leprosy disease. The key strategies adopted under the program include promotive interventions with focus on generating awareness, promoting diagnosis and providing management for disease and its complications.

As a result of strategies initiatives adopted in past, the number of annual new cases have reduced from 1,35,485 in 2015 to 75,394 cases in 2021 and disability among new cases has reduced from 3.9% (2016) to 2.4% (2019) as per WHO Report 2022. Now, with this declining trend, there is a need to incorporate strategies and initiatives which will help in intensification of efforts, towards providing an upgraded sustainable, platform to expand its reach and coverage up to last level.

NLEP with the National Strategic Plan & Roadmap-2030 has focused on introduction of newer interventions. Antimicrobial Resistance (AMR) Surveillance being one such initiative. As, leprosy cases decrease, drug resistance poses a challenge to disease eradication. This surveillance guideline will provide guidance on setting a surveillance mechanism for resistance cases and will clearly define management protocols.

States have successfully demonstrated their dedication towards achieving various targets under NLEP and have shown successful results in case finding activities and rolling out prophylactic measures in leprosy. We need to replicate such efforts in rolling out Antimicrobial resistance surveillance for leprosy. I hope this guideline will help all stakeholders in implementing and operationalizing AMR surveillance in leprosy.

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Message

The roll-out of MDT in the late 1980s has been a major factor in bringing down dramatically the burden of leprosy until the year 2005, after which a plateau was observed in the number of cases on treatment, and a much slower reduction in notification. As rifampicin is the backbone of the MDT regimen, it is important to monitor the emergence of rifampicin-resistant strains, as recent reports and publications have indicated the existence of rifampicin resistance in several endemic areas. Prior to the introduction of MDT, patients were treated with dapsone monotherapy for several years. Resistance to dapsone has been reported since the early 1960s and the development of drug resistance was recognized as an obstacle to case management and control for leprosy. Moreover, to date, there have been no structured data available on resistance.

To overcome the obstacle and establishing the effective surveillance system, Anti-Microbial Resistance Surveillance guideline for leprosy may be helpful in keeping the system in place and guiding all the officials under National Leprosy eradication programme to work effectively and hassle free to achieve the programmatic goals.

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दिनांक / Dated.....

Message

Anti-Microbial Resistance (AMR) is one of the key public health concern in recent time. AMR Surveillance in leprosy plays crucial role under NLEP.

The leprosy new case detection showed a gradual decline from 1,35,485 new cases in 2016 to 1,14,451 in 2019. In 2020, the COVID-19 pandemic affected leprosy programmes resulting in a significant drop in case detection: only 65,147 new cases were reported during the year 2020-21. Relapses are defined as a patient who has completed a full course of treatment and returns with signs and symptoms of leprosy that are not deemed to be due to a reaction. In 2019, 896 relapses were reported while this number was 498 in 2020.

National guidelines are developed through a series of consultations between 2017 and 2022. AMR surveillance was included as one area of work under the National Leprosy Eradication Programme with objectives as to establish a nationwide robust surveillance system for AMR in leprosy, to estimate the burden and monitor trends of AMR in leprosy among new cases and relapses and to provide evidence-based inputs for programme intervention. The AMR surveillance plan includes identifying apex laboratories to which all states and union territories are linked.

I take this opportunity to congratulate CLD, stakeholders, experts, contributors, State Leprosy Officers, partners who have contributed in developing National Guidelines for Surveillance of Antimicrobial Resistance in Leprosy.

Anil Kumar

(Dr Anil Kumar)

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January 24, 2023

MESSAGE

Anti-Microbial Resistance (AMR) is emerging as a major public health challenge towards achievement of leprosy eradication programme goals, which is recognized as high priority area by the Government of India. The resistance towards certain Multi Drug Therapy (MDT) drug regime for Leprosy is the key factors of concern in Anti-Microbial Resistance. The 'National Health Policy' (2017), addresses Anti-Microbial Resistance as one of the key issues and prioritizes development of guidelines regarding sensible use of antibiotic in Leprosy.

In view of above, Central Leprosy Division, Dte.GHS, MoHFW has prepared guidelines for surveillance of the Anti-Microbial Resistance that may be used right from the PHC / base functional unit level to the tertiary care level. This guideline will be a reference tool for all the medicos / non-medicos under NLEP in establishing Anti-Microbial system for Leprosy related matters in their area / zone.

Central Leprosy Division is complimented for developing Anti-Microbial Surveillance guidelines in leprosy, which will be of immense help to the NLEP officials in establishing the system. I am thankful to all the experts who have contributed in preparation of the guidelines.

(Rajiv Manjhi)

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Message

The introduction of Multi Drug Therapy (MDT) in the year 1983 has marked the recognition of Anti-Microbial Resistance (AMR) Surveillance also as a key area of public health for Leprosy and MDT leads to the gradual decline of leprosy burden to a great extent. With the program efforts, ultimately, India achieved the leprosy elimination in 2005 at the national level. However, India contributes about 52% of new leprosy cases detected globally.

According to the World Health Organization (WHO) data from 19 countries, it was observed that out of the relapse patients of leprosy, 5.1% were tested positive for Rifampicin, while 2% tested positive among new cases. Resistance to Dapsone was observed in greater proportions both in relapses and new cases. From 2016 onwards, data on AMR surveillance was collected through annual leprosy statistics which further reported increased Dapsone resistant cases. Where, multi drug resistance was found in 4.2% of the retreatment cases, resistance among new patients were comparatively lower for all the three drugs.

As per Global Leprosy Strategy 2021–2030, AMR is identified as one of the major challenges and its monitoring is a key component under the strategy's first pillar. In line with strategy, Central Leprosy Division (CLD) has developed the National Guidelines for Surveillance of Antimicrobial Resistance in Leprosy under the guidance of the Technical Advisory Group of NLEP.

This guideline will help all the States/UTs to implement the AMR Surveillance. I am confident that this activity will be rolled out effectively to reach our goal of zero transmission of leprosy by 2030.

(Dr. Sudarsan Mandal)

ACRONYMS

AMR	Antimicrobial Resistance
BI	Bacillary Index
CLD	Central Leprosy Division
CLTRI	Central Leprosy Teaching & Research Institute, Chengalpattu, Tamil Nadu
DDS	Diaminodiphenyl Sulfone
DLO	District Leprosy Officer
DNA	Deoxyribo-Nucleic Acid
DNT	District Nucleus Team
DRDR	Drug Resistance-Determining Region
GLP	Global Leprosy Programme
GOI	Government of India
HF	Health Facility
IEC	Information Education and Communication
ILEP	International Federation of Anti-Leprosy Associations
LEPRA-BPHRC	LEPRA-Blue Peter Public Health and Research Centre, Hyderabad, Telangana
LT	Laboratory Technician
MB	Multi Bacillary
MFP	Mouse Foot Pad
MDT	Multi- Drug Therapy
MO	Medical Officer
MI	Morphological Index
NCDC	National Centre For Disease Control
NGO	Non- Governmental Organization
NJIL&OMD	National JALMA Institute for Leprosy and Other Mycobacterial Diseases, Agra
NLEP	National Leprosy Eradication Programme
NMA	Non-Medical Assistant
NMS	Non-Medical Supervisor
OFL	Ofloxacin
PB	Pauci Bacillary
PBS	Phosphate Buffered Saline
PCR	Polymerase Chain Reaction
RMP	Rifampicin
RLTRI	Regional Leprosy Training and Research Institute
SIH-R &LC	Schiefflin Institute of Health Research & Leprosy Centre, Karigiri, Tamilnadu
SLO	State Leprosy Officer
TLMTI	The Leprosy Mission Trust India
WHO	World Health Organization

1.BACKGROUND

Leprosy is one of the oldest diseases of mankind, it is chronic infectious disease, if not treated timely, may lead to deformity and disabilities. It is caused by Mycobacterium leprae. The disease is curable and the fight against leprosy has been a great success, largely due to the development of Multi-Drug Therapy (MDT) in 1981. Early detection and prompt treatment by MDT remains the cornerstone of leprosy control. MDT is a combination of.bactericidal and bacteriostatic antibiotics which together act as a robust regimen for treatment of leprosy. India introduced MDT in the yera 1982 and Post-exposure Prophylaxis (PEP) with single dose Rifampicin in 2019.

Emergence of Antimicrobial Resistance is a growing global concern. The emergence of drug resistance is a concern and a threat for many infectious disease intervention programmes, especially those that have secondary prevention (chemotherapy) as the main component of their control strategy. This is reflected in the World Health Assembly's resolution on Anti-Microbial Resistance (AMR) in 2015 (WHA 68/20) backed with a global action plan. National Health Policy of India (2017) had also highlighted the need for immediate action to tackle the problem of AMR. Global reports indicate the emergence of 'Mycobacterium leprae' strains showing resistance to Rifampicin both in relapse and new cases. The Global Leprosy Strategy (2016-2020) included Anti-Microbial Resistance (AMR) as one of the key areas of intervention.

Antibiotics used in Multi-Drug Therapy are Rifampicin, Clofazimine and Dapsone. Rifampicin is the backbone. Therefore, it is important to monitor the emergence of Rifampicin resistance. Recent reports and publications have indicated the existence of rifampicin resistance in several endemic areas. Data from 19 countries on AMR surveillance between 2009 and 2015 indicated that 5.1% of the tests on relapse patients tested were positive for rifampicin while 2% were positive in new cases. Prior to the introduction of MDT, patients were treated with dapsone monotherapy for several years. Resistance to dapsone has been reported since the early 1960s. Clofazimine resistance is still rare but this antimicrobial cannot be given alone. In case of resistance to rifampicin, fluoroquinolones become the preferred category of second-line drugs. Unfortunately, quinolone-resistant strains of Mycobacterium leprae have also been reported in several countries, probably due to the extensive use of quinolones for treating several types of infections.

While resistance to leprosy drugs appears relatively low, this should not be taken for granted and all efforts must be made to prevent amplification.

The Global Leprosy Strategy 2021-2030 includes a paradigm shift, with a focus on moving towards interruption of transmission and elimination of disease. To meet the challenge of containing the disease and being able to respond to the increase in circulation of drug-resistant strains, it is essential to assess drug-sensitivity patterns and monitor resistance among both new and retreatment cases. Today, India needs to strengthen AMR surveillance and develop systems for roll out of mechanisms for testing and treating resistant cases for achieving the aim of interruption in transmission of leprosy.

Antimicrobial resistance (AMR) is one of the key areas of intervention of the National Strategic Plan (NSP) and Roadmap 2023–2027, under its Pillar I "Strengthen leadership, commitment, and partnerships". All states and union territories that detect leprosy are shall strengthen the existing and develope new system as required to ensure testing for AMR in a laboratory.

The National Guidelines for Surveillance of Antimicrobial Resistance in Leprosy aims to guide the states and union territories on how to test for drug resistance in leprosy. It highlights the clinical, field, and laboratory support systems that need to be organized to undertake this activity.

The National Guidelines for Surveillance of Antimicrobial Resistance in Leprosy

This guideline aims to promote the use of a two-fold standardized approach:

1. To detect primary and secondary drug resistance to antileprosy drugs, namely, rifampicin, dapsone, and ofloxacin.
2. To standardize reports of AMR testing done and outcomes of treatment given to patient. Data on drug resistance in leprosy will allow national and subnational monitoring of trends of drug resistance over time among new and retreatment cases of leprosy. It would also help to identify risk factors for drug resistance (age, sex, area of residence, patient's category/type).

The target audience for these guidelines comprises state program managers, clinicians, medical staff at health facilities and experts at referral facilities, AMR laboratories etc. Roles and responsiblities of stakeholders is given in Annexure - I

2. ANTIMICROBIAL RESISTANCE IN LEPROSY

India accounts for 54 percent (March 2022) of the global new case burden. The new case detection showed a gradual decline from 135,485 new cases in 2016 to 114,451 in 2019. In 2020, the COVID-19 pandemic affected leprosy programme (with restriction to active case detection and reduced access to health services) resulting in a significant dip in case detection: only 65,147 new cases were reported during the year. Relapse is defined as per the WHO guidance, as a patient who has completed a full course of treatment and returns with signs and symptoms of leprosy that are not deemed to be due to a lepra reaction. In 2019, 896 relapse cases were reported. This number was 498 in 2020.

Relapse is an indirect measure of the efficacy of the treatment regimen besides treatment adherence. MDT was introduced in 1983 and for the initial three decades, relapses were mainly attributed to sensitive organisms and could be effectively treated with standard MDT. Relapses continue to occur in pateints that have completed a full course of treatment with MDT. Additionally, it is also important to monitor resistance among new cases (a proxy for primary resistance) to assess the circulation of resistant strains. AMR surveillance protocols shall include all cases of relapses and about 10 percentage of new MB cases. Data on AMR were only available from 2010 to 2015 (Table 1).

Early reports from India's AMR surveillance network published in Weekly Epidemiological Record (June 2011) indicated the absence of resistance in the small specimen of 25 relapse cases tested for DNA sequencing by PCR. However, in the period 2011-2015, reports revealed M. leprae strains resistant to both Rifampicin, and Dapsone and also to second-line drugs such as quinolones (Table 1a). The resistant strains were observed across both relapses as well as new cases.

Table 1a: Leprosy case notification and AMR data, India, 2011-2020.

Indicator	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020
New Cases	127295	134752	126913	125785	127334	135485	126164	120334	114451	65164
Other Retreatments	5945	5831	5359	5199	5311	5893	5040	5158	5332	3376
Relapse After MB	557	595	664	587	459	536	457	436	455	464
Relapse After PB									50	34
New Cases Tested	311									
Rifampicin Resistant	3.5 %									
Dapsone Resistant	2.3 %									
Ofloxocin Resistant	2.3 %									
Retreatments Tested	355									
Rifampicin Resistant	8.2 %									
Dapsone Resistant	4.8%									
Ofloxocin Resistant	2.3 %									

Table 1b: Summary of AMR cases reported by different labs 2018 -2020

Sl No	Institute	No. of Cases Tested (2018-2020)	Dapsone Resistance	Rifampicin Resistance	Clofazimine Resistance	Ofloxacin Resistance	Fluroqunilones Resistance
1	CLTRI	30	3.3%	0.0	0.0	0.0	0.0
2	Blue Peter, PH&RC	35	0.0	0.0	0.0	0.0	0.0
3	JALMA Institute	0	0.0	0.0	0.0	0.0	0.0
4	Schieffelin Institute	74	5.4%	2.7%	0.0	0.0	0.0
5	Stanley Brown Lab	1893	3.1%	4.2%	0.0	0.7%	0.8%
	Total	2032	3.1 %	4.0 %	0.0 %	0.6 %	0.7 %

In Central Leprosy Training & Research Institute, Chengalpattu, Tamil Nadu, from 2015-2017, a total of 9 resistance cases among new cases and 6 among relapse cases were identified.

Table 2 shows the details of mutations detected by year, centre and type of patient during the period 2011-2015. It was observed that 732 specimens from 365 'New' (fresh untreated) cases and 367 'Relapse' cases were investigated. Resistance by molecular testing to Dapsone was detected in 7/365 (1.92 percent) among new MB cases and in 14/367(3.83 percent) in relapse (MB) cases, to Rifampicin was detected in 11/365 (3.01 percent) in new cases and in 21/367(3.27 percent) in relapse MB cases and to Ofloxacin was detected in 7/365 (1.92 percent) in new cases and 6/367(1.63 percent) in relapse cases.

However, this data mostly pertains to few patients tested at few tertiary care facilities. In order to understand the factual information on drug resistance, it is pertinent that a systematic country wide surveillance of drug resistance must be undertaken.

Table 2: Type of mutation detected, India, 2011-2015

Year	Reference Laboratory	New Cases				Relapse Cases			
		Isolates	RMP 'R'	DDS 'R'	OFL 'R'	Isolates	RMP 'R'	DDS 'R'	OFL 'R'
2011	Number	45	1		1	53	NA	NA	NA
	Mutation		Transition 2171 CGA→CAA 723 Arg→Gln		Transition 273 GCA→GTA 723 Ala→Val				
2012	Number	109	1		3	44	NA	NA	NA
	Mutation		Deletion 2007 GAA→GAC 667 Glu→Asp		Transition 272 GCA→GTA 91 Ala→Val				
2013	Number	116	2	1	2	114	NA	NA	
	Mutation		Transition 2171 CGA→CAA 723 Arg→Gln	Transition 158 ACC→ATC 52 Thr→Ile	Transition 297 CGC→CGT 99 No Change Amino Acid				Transition 297 CGC→CGT 99 No Change Amino Acid
2014	Number	5	1			41	1	1	
	Mutation		Transition 1376 TCG→TTG 458 Ser→Leu				Transition 1343 GGC→GAC 447 Gly→Asp	Transition 158 ACC→ATC 52 Thr→Ile	Transition 297 CGC→CGT 99 No Change Amino Acid
2015	Number	36				46	1	1	
	Mutation						Transition 1343 GGC→GAC 447 Gly→Asp	Transition 158 ACC→ATC 52 Thr→Ile	
2015	Number	1	NA	NA	NA	4	NA	NA	NA
	Mutation								
2016	Number	5	NA	NA	NA	5	NA	1	
	Mutation						ACC→AGA Thr→Arg		
2011-2014	Number	48	NA	NA	NA	60	NA	2	1
	Mutation							55 Pro→Arg (CCC→CGC)	89 Pro→Arg (GGC→TGC)

National AMR guidelines were developed through a series of consultations between 2017 and 2020. AMR surveillance has the following objectives:

• To establish a nationwide robust surveillance system for AMR in leprosy;

• To estimate the burden and monitor trends of AMR in leprosy among new cases and relapses.

• To provide evidence-based inputs for programme intervention.

The AMR surveillance plan included identifying six apex laboratories to which all states and union territories were linked (Table 3).

Roll-out of AMR surveillance was planned in three phases: preparatory, appraisal and implementation phases. Health facilities were divided into three levels

Level 1: patients are diagnosed and treated:

Level 2: laboratory technicians available for slit skin smear examinations and collection of specimens for AMR

Level 3: facilities for skin biopsy are available.

All relapses and 20% of new MB patients will be referred to Level 2 for slit skin smear. samples with bacterio logical index, BI ≥ 2+ shall be sent to Apex Lab for AMR testing.

For samples showing negative on PCR testing at Apex Lab concerned patients shall be referred to Level 3 for skin biopsy. Skin biopsy specimen shall be sent to Apex Lab for PCR and resistance testing.

Table 3: National and Regional Laboratories and allotted states and UTs

	Laboratory	Designation NRL, RRL	Allotted States and UTs	
			Numbers	Names
1	Central Leprosy Training and Research Institute (CLTRI), Chengalpattu, Tamil Nadu	NRL & RRL	4	Kerala, Puducherry, Andaman & Nicobar, Lakshadweep
2	National JALMA Institute for Leprosy and other Mycobacterial Diseases (NJIL&OMD), Agra, UP	NRL & RRL	9	Uttar Pradesh, Bihar, West Bengal, Assam, Nagaland, Tripura, Meghalaya, Gujarat, Rajasthan
3	Schieffelin Institute of Health Research, Karigiri, Tamil Nadu	RRL	3	Tamil Nadu, Karnataka, Goa
4	LEPRA-Blue Peter Public Health and Research Centre, Telangana	RRL	5	Andhra Pradesh, Odisha, Telangana, Maharashtra, Madhya Pradesh
5	Stanley Browne Lab, Leprosy Hospital, TLM, Shahdara, Delhi	RRL	8	Delhi, Haryana, Uttarakhand , Chandigarh, Punjab, Jammu & Kashmir, Leh Ladakh, Himachal Pradesh
6	Regional Leprosy Training and Research Institute (RLTRI) Raipur, Chhattisgarh	RRL	7	Chhattisgarh, Arunachal Pradesh, Manipur, Mizoram, Sikkim, Jharkhand, Dadra & Nagar Haveli and Daman & Diu

The plan further included:

- Training of Medical Officer (In-charge) on referral; and laboratory technicians; about the case identification, sample collection, smear microscopy and packaging of specimen for transport to Apex Lab
- Procurement of laboratory reagents, equipment and logistics for transport of specimens;
- Arrangements for delivery of specimens;
- Logistics for recording and reporting;
- Appraisal of preparedness of the levels 1, 2 and 3 facilities by experts;
- Roll-out of surveillance; and
- Gradual expansion to more districts.

An appraisal was carried out reviewing the availaible data of AMR testing by Apex Lab . On the basis of the number of new MB patients and relapse cases of 2019 it was planned that approx. 6718 tests for AMR shall be conducted per year (505 relapses and 10% of 62119 new MB cases i.e. approx. 6213) . Details given in Table4. For testing 6213 samples of new MB cases for AMR testing slit skin smear samples shall be collected from twice the number that is 12,426. Only samples with BI ≥ 2+ shall be sent for AMR testing.

Table 4: Planned number of test for AMR testing, by Apex Laboratories, as per 2019 data

	Number Of New MB Cases samples	Number Of Relapse Cases	Estimated Number Of Specimens
LEPRA-BPHRC, Hyderabad	2354	65	2419
Central Leprosy Teaching And Research Institute, Chengalpattu	51	30	81
JALMA, Agra	2319	168	2487
Regional Leprosy Training And Research Institute, Raipur	800	50	850
Schieffeiln Institute For Health Research And Leprosy Centre, Karigiri	390	170	560
Stanley Brown, New Delhi	298	22	320
Total	**6212**	**505**	**6717**

India has setup a strong surveillance network for Leprosy. However, more needs to be done to expand it.

3. RATIONALE

The Global Leprosy Strategy 2021-2030 includes a paradigm shift, with a focus on moving towards interruption of transmission and elimination of disease. To meet the challenge of containing the disease and being able to respond to the increase in circulation of drug-resistant strains, it is essential to assess drug-sensitivity patterns and monitor resistance among both new and retreatment cases. Today, India needs to strengthen AMR surveillance and develop systems for roll out of mechanisms for testing and treating resistant cases for achieving the aim of interruption in transmission of leprosy. The AMR test results emphasize the need for establishing as well as expanding the surveillance network not only in terms of increasing laboratory networks, and increasing the number of specimens. AMR Surveillance model should be made part of routine programme. Since AMR is a core area of intervention under the umbrella of the Global Leprosy Strategy 2021-2030, it is necessary to develop a surveillance system to keep a vigil on drug sensitivity patterns in different settings. These guidelines describe the framework for setting up surveillance for drug resistance against anti-leprosy drugs in India.

Objectives of the surveillance system:

- To establish a nationwide robust surveillance system for AMR in Leprosy.
- To estimate burden and monitor trends of AMR in Leprosy among both new and relapse cases including patients seeking treatment in government health facilities, medical colleges, private practitioners and private dermatologists.
- To provide evidence-based inputs for programme intervention.
- To establish National Reference Lab and Regional Reference Lab network across the country.

4. DEFINITIONS AND PROCEDURES

New case (of leprosy): a patient diagnosed with leprosy that has never been treated for the disease.

PB case: a case of leprosy with 1–5 anesthetic skin lesions, upto 1 nerve involvement and without demonstrated presence of bacilli in a skin smear.

MB case: a case of leprosy with >5 anesthetic skin lesions; or with more than 1 nerve involvement (pure neuritis or any number of skin lesions and neuritis); or with demonstrated presence of bacilli M. leprae in a slit-skin smear/biopsy, irrespective of the number of skin lesions.

Retreatment case (of leprosy): A patient diagnosed with leprosy who has already received treatment for the leprosy with MDT drugs in the past.

Relapse: 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 lesion and / or an increase in the Bacillary Index (BI) of two or more units on Ridley scale, at any single site. Care should be taken to exclude patients suffering from leprosy reactions. Type 1 reaction is a differential diagnosis of Relapse. Investigation for relapse should take place when reaction subsides after steroid therapy.

Information regarding past treatment history and current clinical presentations are to be collected using the

EPIDEMIOLOGICAL INVESTIGATION FORM FOR DRUG RESISTANT CASES IN LEPROSY (Annexure-II). Each selected case will undergo a slit skin smear examination using the standard technique as for an ordinary skin smear for bacteriological index (BI).

Types of specimens

- Slit skin scraping (from four sites and blade is for PCR)
- Skin biopsy (if required)

5.BROAD OUTLINE OF THE SURVEILLANCE PROGRAMME

The surveillance system is composed of three parts:

I. Systematic collection of samples

II. Testing of samples at the Regional/Apex Laboratories

III. Collection of additional epidemiological information

i) The first component is the systematic collection of specimens in the field. This involves listing of all relapse and MB diagnosed cases in the identified health facilities. The listed cases will be contacted by District Nucleus Team (DLO, MO and NMAs) for filling of patient cum laboratory form. Keeping convenience and access of the cases, filling of the patient cum laboratory form will be planned in decentralized manner. Decentralized plan for specimen collection and filling of investigation form can be based on the availability of facility for slit skin smear examination in that particular district and area. Mobilization of cases needs to be ensured by local health workers before the planning of tissue specimen collection. Further, handing over packed specimens will be done on daily basis to the District Nucleus Team (DNT) and DNT will ensure that specimen with investigation form is shipped to the respective reference laboratory within a week. It is important that specimens are collected systematically at the identified sites designated and mapped for this purpose.

(ii) The second component is the laboratory part which will be carried out by identified referral laboratories receiving specimens from the field, and carry out PCR for RLEP gene and if positive then test for Rifampicin, Dapsone and Ofloxacin resistance. Specimens that are found positive will be subjected to Sanger (gene) sequencing.

(iii) Collection of additional epidemiological information will be carried out based on the lab result for resistance. Additional epidemiological information of drug resistance cases will be collected in field, collated and computerized for data analysis at all levels including district.

The AMR surveillance shall include testing of specimen from 10 percent of new MB cases and all relapse cases. Only samples from new MB cases with BI ≥ 2+ shall be sent for AMR testing. SSS samples shall be collected from 20 percent of new MB cases as it assumed that only 50 percent of the samples shall have BI ≥ 2+.

The AMR results will be utilized for appropriate patient management by providing appropriate feedback information to the health facilities at the peripheral levels where patients included in the surveillance system are currently undergoing treatment.

6. PREPARATORY ACTIVITIES

Identification Apex and Regional Reference Laboratories

Regional Reference Labs will be identified in states which are already conducting surveillance activity for drug resistance and currently have the necessary human resources to carry out the surveillance on a long-term basis. Each Regional Reference Lab will be allotted specified state areas from where it will receive tissue specimens routinely. Surveillance activity will be an ongoing activity and participating laboratories will take into account the need to maintain the surveillance work over a period of time.

Categorizing Health Facilities:

Health facilities are to be divided into three levels:

Level 1: patients are diagnosed and treated:

Level 2: laboratory technicians available for slit skin smear examinations and collection of specimens for AMR

Level 3: facilities for skin biopsy are available. Designated Level 3 health facilities will be either government, private or NGO facilities. These facilities should also be able to collect and transport sample to the identified reference laboratories. (Table 4)

Table 5: Role of various levels of Health Facilities

Category	Role Of Health Facility	Centers Included
Sub District Health Facility Level-I	Diagnosis And Treatment	Selected Block PHC/CHC
Sub District Health Facility Level-II	Level I plus SSS Collection and Microscopic Examination	
District Health Facility, Medical Colleges Level-III	SSS Collection and Microscopic Examination, Collection of Skin Biopsy Specimen	District Hospital/ Medical College/ NGO

In a district, the prior mapping of health facilities / laboratories for diagnosis, collection of slit skins, smear preparation, microscopic examination, obtaining biopsy specimen, transportation of samples, performing PCR and gene sequencing for assessment of resistance may be undertaken and enlisted as per the format below.

Table 6: Format for Mapping of Health Facilities and Labs

Activity	Facility-1	Facility-2	Facility-3
Diagnosis (as MB/Relapse Case)			
SSS specimen collection & reporting			
Skin Biopsy			
PCR			
Gene sequencing			

Designated health facilities will be either government, private or NGO facilities. These facilities should also be able to carry out tissue sampling and transport to the identified reference laboratories. Essential prerequisite to be considered for designating health facilities as level 2/3 are:

a) Human Resources

- Qualified Microbiologist (Preferably MD Microbiology)
- Senior technical officer (Preferably MSc Microbiology, Biotechnology with PhD preferably in Leprosy field)
- Skilled Lab technician (preferably BSc. MLT)
- Storekeeper/ Trained staff for laboratory work flow and logistics management
- Lab Assistant/ Attendant. (MTS)
- Non-medical assistant/ field supervisor (PMW/MPW/CHO)

b) Logistics

- Microscopy Facility
- Arrangements for procurement of laboratory reagents, equipment and other replenishables;
- Mechanisms to ensure a continuous availability of reagents/kits, electric power supply etc
- Arrangements for regular shipment of samples for testing, recording, and reporting of related information with clear guidance for officials / staff at all levels

c) Other Activities

- Plans for Roll-out of surveillance in a phase wise manner. Selected states will start the pilot work, followed by all remaining states in phases.
- Planning for Supervision to ensure that standard protocols are followed by all the facilities, including appraisal of preparedness of the levels 1, 2 and 3 health facilities by experts;
- Planning and organizing Training of Medical Officer (In-charge) on referral; and laboratory technicians;
- Plan and arrangements for Quality Control
- Preparation of Communication Plan and its implementation

7. COLLECTION OF SPECIMEN AND TRANSPORTATION

Selection of patient for AMR surveillance

1. AMR testing must be done for all relapse cases in any part of the country.
2. 20 percent of new MB cases should be tested for M.leprae. This is for surveillance purposes.
3. Only tested samples of new MB patients found to have bacterial index (BI) ≥ 2+ (more than or equal to 2+) from at least 1 site should be sent to AMR Apex Lab for AMR testing.
4. PB patients are usually negative for M.leprae and hence not to be included for AMR testing

Table 7: Selection of patient for AMR Surveillance

INCLUSION CRITERIA	
Resistance among patients never treated before	Resistance among patients who have been treated before
New case of leprosy: A patient diagnosed with leprosy that has never been treated for the disease	**Retreatment case of leprosy:** A patient diagnosed with leprosy who has already received treatment for the disease in the past
10 percent of total new MB cases (with BI ≥ 2+) will be included for AMR to detect primary resistance	All retreatment leprosy cases i.e. relapse, retreatment after loss to follow-up and treatment failure cases must be included for AMR to detect secondary resistance
Note: To have a positive and successful testing for drug resistance, only MB cases confirmed to be smear positive with a BI≥2 at any one site would be tested, as these have a higher chance of a positive PCR	

Patient Information to be collected:

Basic information about the patients is to be collected using the form as shown in Annexure-II.

- Part 1 includes reporting details such as case identification number, date of report and particulars relating to the health facility sending the specimen.
- Part 2 records the demographic details of the case such as age and sex.
- Part 3 records the present clinical presentation of the case and test/s undertaken.
- Part 4 covers the past clinical history and includes the clinical presentation at the time of previous diagnosis, classification, treatment undertaken and the results of the tests that were performed on the patient.

Specimen for microscopy and PCR:

After due counselling of the patient (if below 18 years, then parents'/ guardian's to be counselled), slit skin smear (SSS) would be done with aseptic technique and following infection prevention practices and guidelines:

- Slit-skin smear specimens from four different sites which include two ear lobes and two active sites. Two ear lobes (will be coded as E1 and E2 respectively), two active sites of the patches (will be coded as P1 and P2 respectively).
- The slides will be stained and examined for BI/ morphological index (MI).
- Blades will be washed in container containing 70 percent ethyl alcohol, kept for few minutes and after the scrap material Is dissolved in the alcohol blade will be removed with sterile forceps, container will then be sealed with parafilm, labeled, and sent for PCR. Figure 1 illustrates skin smear examination.

Figure 1: Skin smear examination and centrifuge tube with 1 ml of 70 percent ethanol for collection of tissue specimens

Slit Skin Smear | Centrifuge/Eppendorf tube

Skin Biopsy:

Skin biopsy will be carried out for cases where the skin scrapping specimens were negative for *M. leprae* but PCR indicated strong suspicion of AMR.

- Biopsy sample must be collected after due counseling of the patient.
- Use of punch of 4-6 mm is appropriate. Biopsy would be done with aseptic technique and following infection prevention practices and guidelines.
- The site showing highest skin activity will be selected for skin biopsy.
- Biopsy material will be put in 1.8 ml screw capped disposable conical tubes containing 70 percent ethyl alcohol (Figure 2)
- Specimens could be kept at room temperature and sent to assigned testing laboratory for PCR and sequencing within 7 days of collection.
- Bacilli are inactivated by ethanol, specimens can be sent by normal routine transport without the need to control the temperature during transportation.
- Figure 3 summarizes process for sample collection and storage in a flow chart.

Figure 2: Skin punch biopsy sampling and tube for transportation

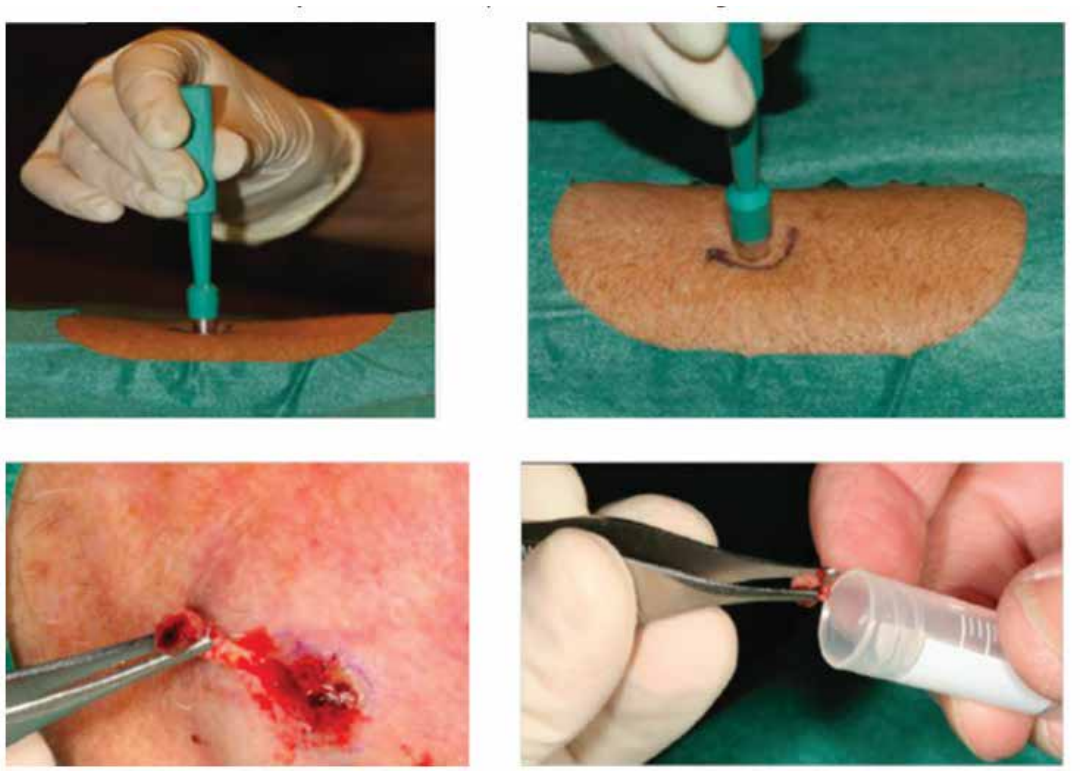
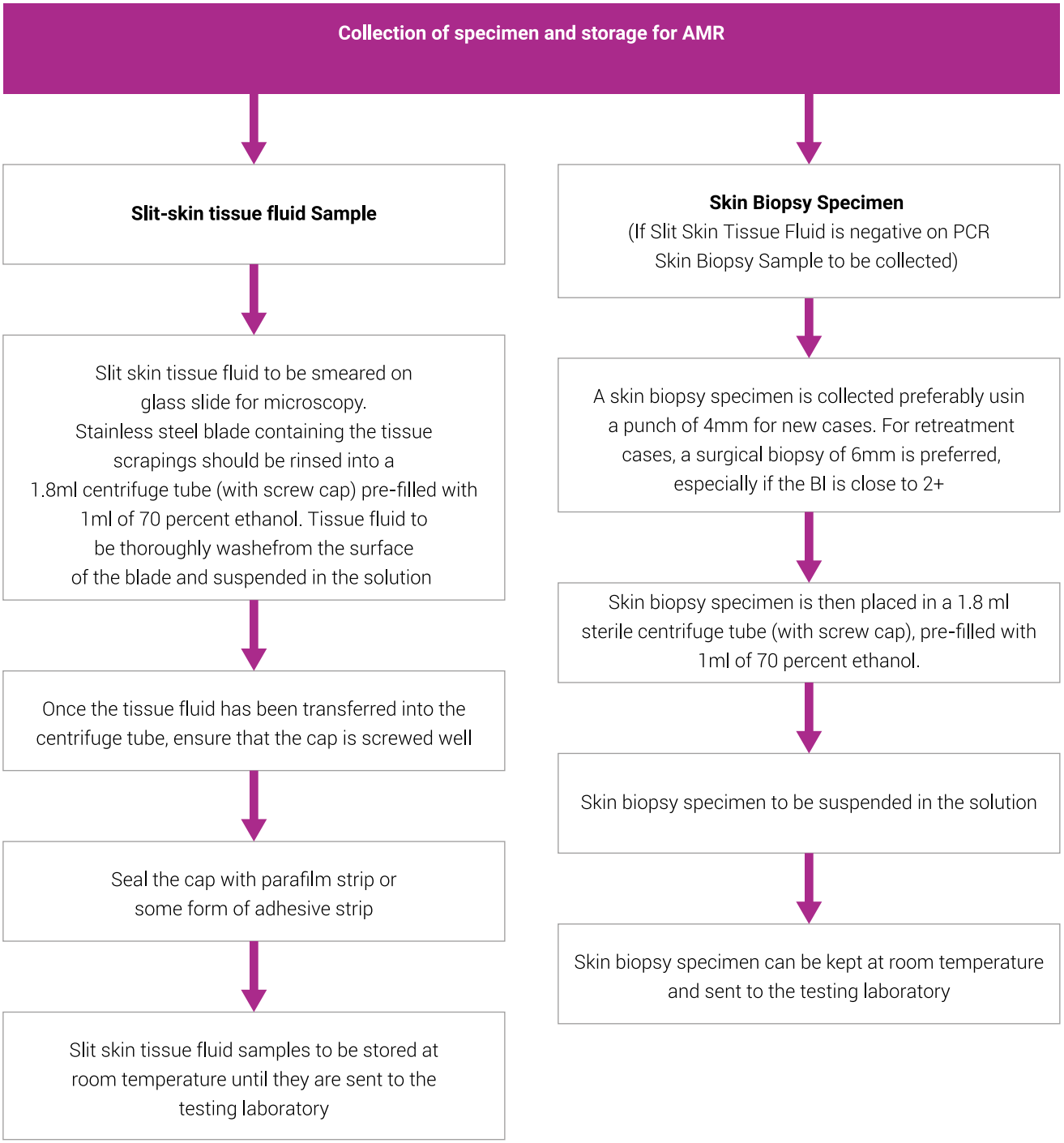


Photo Courtsey: Beatrice Flageul

Figure 3: Process of Specimen collection and storage for AMR



Footnote: Capacity of sample collection of health facilities needs to be enhanced in taking skin biopsies.

Standard Protocol for Specimen packaging:

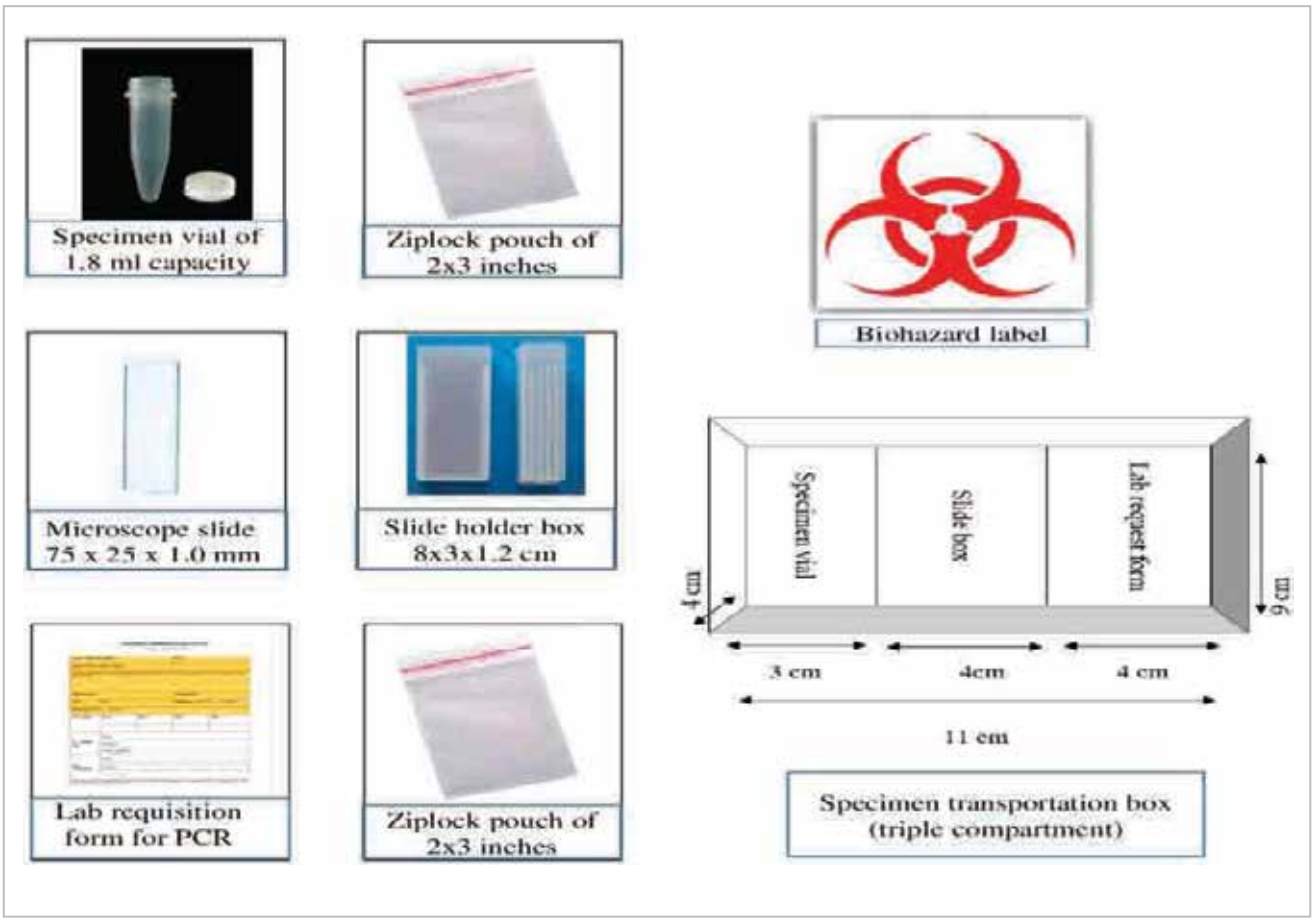
Specimens thus collected can be kept at room temperature, less than 25 º Celsius for a maximum of 5 days, untill further processing or transportation to the laboratory.

• After capping the Eppendorf tube, seal with Para-film and put in Cryo-box vertically. (Figure 6)

• Label the specimen with details of surveillance site from where specimen collected. (Patient unique ID, geographical location, health facility, site of collection etc.)

All the containers must be coded/labeled and sent to Regional/national reference labs for PCR after packaging.

Figure 4: Packaging the samples



Transportation

• Transportation can be done when specimens from 5 patients (20 specimens) are collected or once in a week whichever is earlier

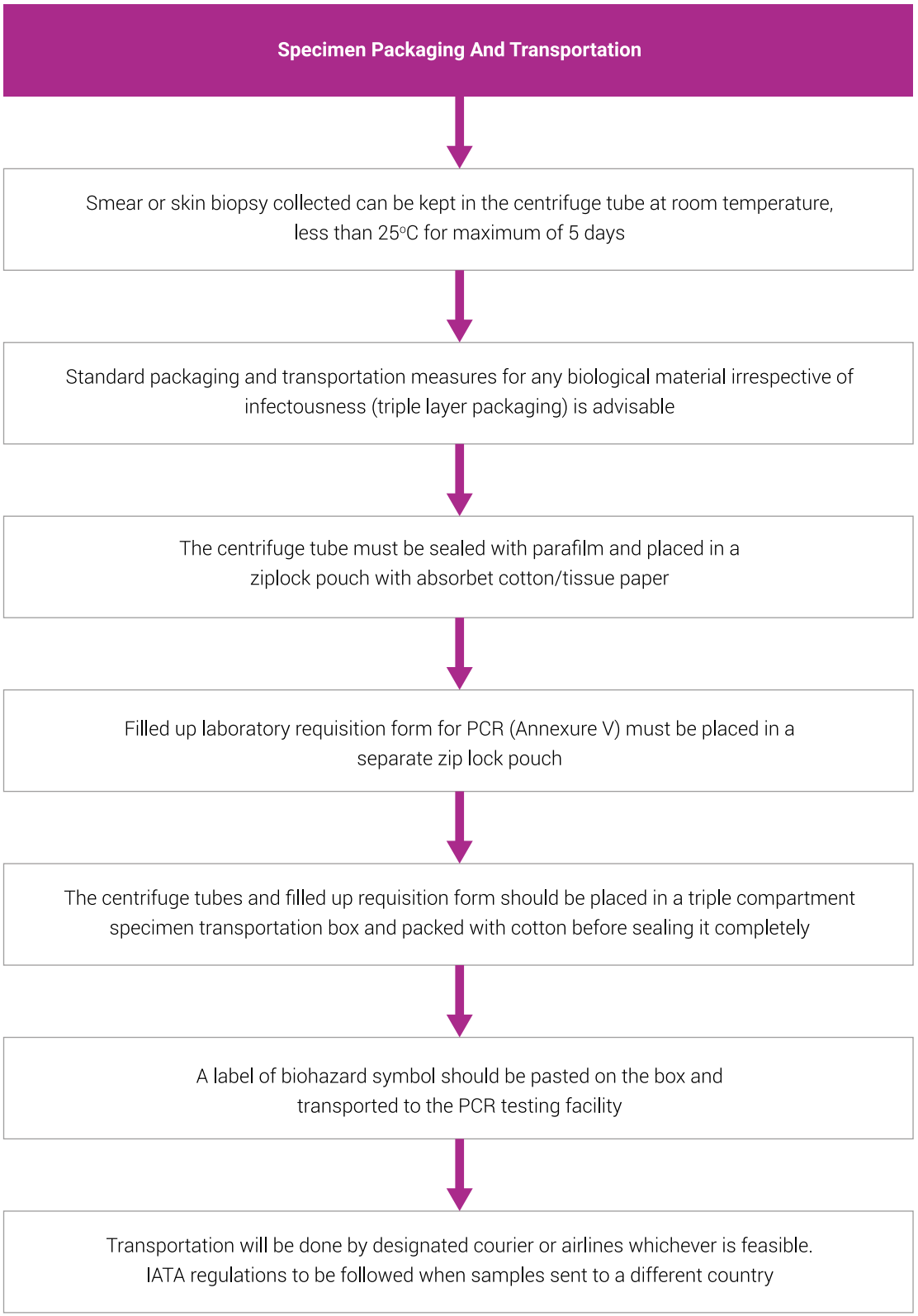
• Samples will be transported to the designated laboratory by courier or airlines as per transportation protocols for state / nation.

• Clinical reporting form will be sent with the samples being transported to the designated laboratory.

• Specimen transportation will be confirmed (Online/Offline) by both receiver and sender.

• Specimen packaging and testing process has been summarized in a flow chart (Figure 5). The logistic requirement details with cost of test and recommendatory equipment list is given in Annexure II.

Figure 5: Packaging and transportation to the designated testing facility



8.LABORATORY TESTS

Once the specimen (SSS) is received in the laboratory, DNA extraction will be done using either manual or kit-based method (following manufacturer's instructions).

Manual method of DNA extraction for skin slit smear samples:

Lysis buffer method (de Wit et al, J Clin Microbiol. 1991;29:906–10)

- Centrifuge the specimen for 10–20 minutes at max speed (should be more than 15000 x g).
- Discard the supernatant. Resuspend the precipitate in phosphate buffered saline (PBS).
- Allow to stand for more than 30 minutes before suspending in PBS.
- Centrifuge for 10–20 minutes at max speed.
- Resuspend the sediment in 50 µL of lysis buffer (a mixture of 1 volume each of proteinase K 1 mg/mL in 100 mM Tris-HCl pH 8.5 and 0.5 percent Tween 20 are diluted 5 times with 100 mM Tris-HCl, pH. 8.5 or distilled water).
- Overlay mineral oil to prevent evaporation of the buffer.
- Incubate overnight at 60 °C
- Heat for 10 min at 97 °C to inactivate proteinase K.
- Transfer to a DNA low-binding tube such as Eppendorf product DNA LoBind.

Kit Method:

Standard testing kits will be used following the manufacturer's instructions.

PCR for detection of M.leprae and drug resistance determining regions:

The drug resistant determining region (DRDR) in the rpoB, gyrA and folP gene is amplified by standard PCR condition. The primers which will be used are as below:

- Amplification maybe carried out in a Thermal cycler.
- For PCR Condition folP1 - The initial denaturation to be done at 95°C for 10 min; 95°C for 1 min , 58°C for 1 min and 72°C for 1 min for 40 cycles; extension at 72°C for 10 min.
- Conditions for amplification of rpoB and gyrA – The initial denaturation to be done at 95°C for 10 min; 95°C for 1 min ,60°C for 1 min and 72°C for 1 min for 40 cycles; final extension at 72°C for 10 min

Table 8: Primers for PCR of DRDR of rpoB, gyrA and folP

Dapsone	*folP*F : CTTGATCCTGACGATGCGGT
	*folP*R : CCACCAGACACATCGTTGAC
Rifampicin	*rpoB* F : GTCGAGGCGATCACGCCGCA
	rpoB R : CGACATGAACCGATCAGAC
Ofloxacin	*gyrA* F : ATGGTCTCAAACCGGTACATC
	gyrA R : TACCCGGCGAACCGAAATTG

Amplification of target region is to be confirmed by agarose gel electrophoresis. Nucleotide sequencing of the drug resistance determining region in the rpoB, gyrA and folP genes will be determined using PCR and sequencer.

Molecular basis of Rifampicin, Dapsone and Ofloxacin resistance and methods for detection.

The mapping of the Mycobacterium leprae genome has identified sites at which mutations occur, conferring resistance to Dapsone, 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 Mycobacterium leprae.

- Missense mutations leading to the substitution at any one of at least six amino acids (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 (Dapsone) resistance determining region of the folP gene (codes dihydropteroate synthase), resulting in alterations of amino acids at positions 53 and 55, confer Dapsone resistance to M. leprae.
- Missense mutations in the quinolone (Ofloxacin) resistance determining region of the gyrA gene, resulting in alterations of amino acids at positions 89 and 91, confer quinolone resistance to M. leprae.

DNA sequencing protocol:

PCR products are purified by commercially available kit followed by sequencing reaction. Big Dye Terminator v1.1 (Applied Biosystems) is considered adequate for sequencing of short fragment. Specimens are applied to sequencer for analysis of nucleotide mutation. The standard operational procedures are as follows:

1. Purify PCR products using QIA quick PCR purification kit as per manufacturer's instructions. Optional (some workers simply dilute the PCR product prior to sequencing).
2. Estimate the concentration of DNA available for sequencing (at OD260 or by visual observation of amplicon band on gel in comparison to DNA standards)
3. For each sequencing reaction, add the following reagents:
   -8.0 microlitre of 1X Big Dye 1.1 Terminator Ready Reaction Mix
   -Up to 11/ul of 3-5 ng of DNA in dH2O
   -1.0/ul of 3.2 pmol/ul primer
   -Adjust volume to a total of 20.0/ul.
   -Alternative schemes using Big Dye Terminator 3.1 are optional.
4. Mix well and spin briefly. Place the tubes in a thermocycler and set the volume to 20µl.
5. 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

Purify the cycle sequencing reaction products with Performa DTR Ultra 96-well plate kit/cartridge Edge Bio Systems 41453 to remove primers, nucleotides, etc. Elute the products in a final volume of 20/ul Tris-EDTA, pH 8.0. Dry up products and dissolve in 25/ul of Hi-Di Formamide (Applied Biosystems) followed by heating for 2 minutes at 95°C and quick chilling in ice.

6. Apply the specimen for capillary electrophoresis using ABI 3130 instrument with 36cm capillaries and POP7 acrylamide matrix for a running time of 2780 sec (Adjust to suitable running time for other types of sequencing instruments).

Note: The above steps are suggestive, chemistry and steps may change. Reference Labs may be required to amplify the target genes and after confirmation may need to ship the amplicons in frozen conditions to a designated laboratory identified for automatic sequencing.

Isolates with already known amino acid substitution in codon positions in one or more drug resistance determining regions will be recorded for confirmed resistance. Changes in new codon position will have to be confirmed to confer Rifampicin, Dapsone and Ofloxacin drug resistance by the reference mouse footpad method laboratory (CLTRI, Chengalpattu, Tamil Nadu/ NJIL&OMD, Agra/ Schiefflin Institute of Health Research, Karigiri, Tamil Nadu).

Antimicrobial agents to which molecular evidence of resistance will be investigated:

Specimens will be tested for presence of genes and mutations known to be associated with resistance to Rifampicin, Dapsone and Ofloxacin through PCR and genomic sequencing. Nucleotide sequencing of the drug resistance determining region in the rpoB, folP and gyrA genes will be determined using PCR and sequencer. However, for quality control, the fresh specimen from 10 percent of the patients showing amplification of M. leprae gene can be obtained for MFP (Mouse Foot Pad) inoculation and further confirmation obtained.

Quality Control

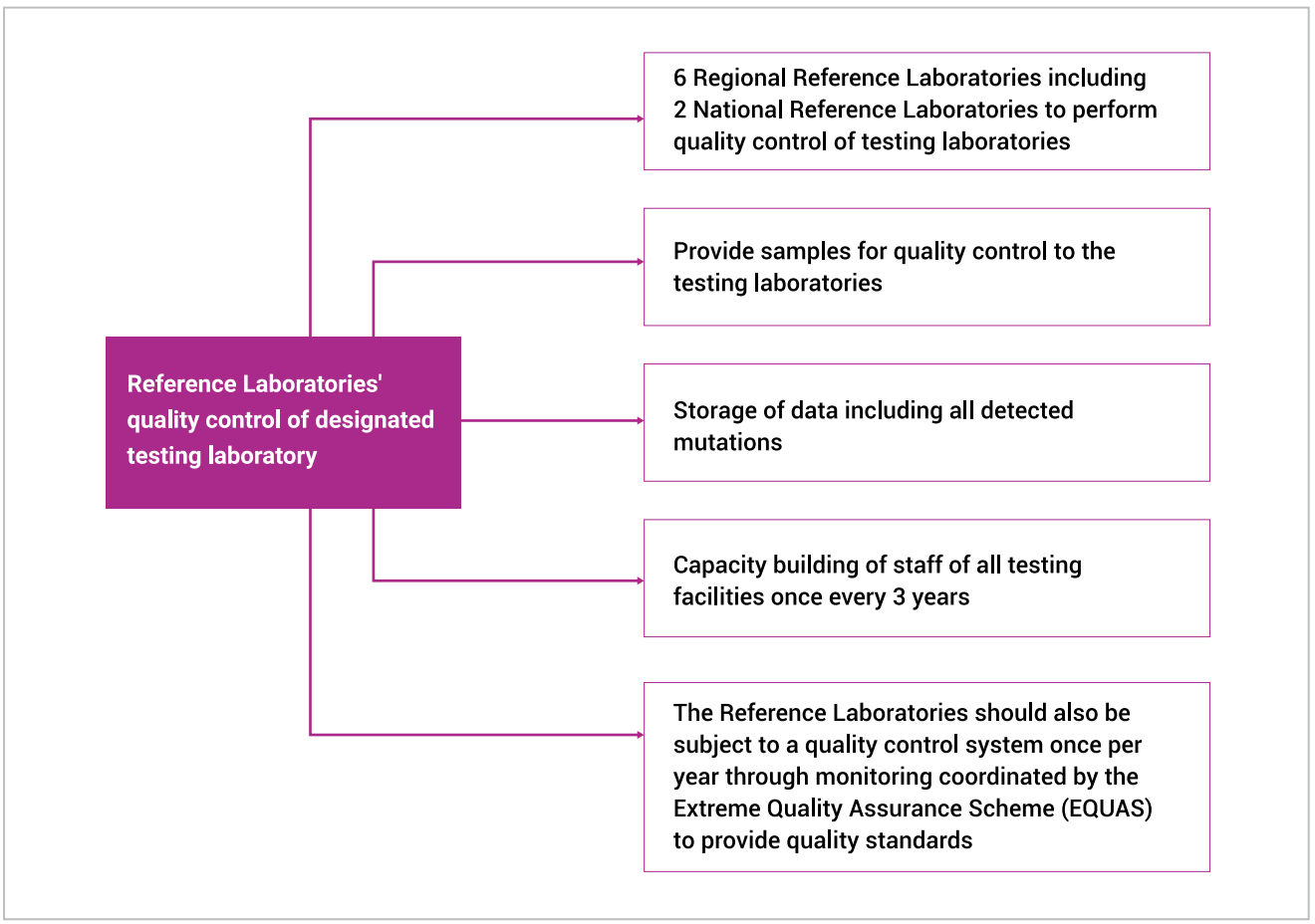
Quality control of the reference laboratories that are carrying out DNA sequencing for rifampicin resistance will be conducted following standard procedures. All positive and negative slit skin smear or punch biopsy specimens from each case are to be frozen and stored for quality assurance.

CLTRI, Chengalpattu, Tamil Nadu, and ICMR- NJIL & OMD, Agra will be working as National Reference Laboratory (NRL). They will conduct quality control in consultation with all identified laboratories by a defined procedure to be informed to all concerned laboratories. In addition, quality checks will be made from time to time regarding patient selection, data entry and transportation of specimens by the National Leprosy Eradication Programme. Table 6 sums up the quality control mechanisms.

Table 9: Quality control mechanism

Activity	Facility Performing the activity	Frequency	Assessor
Patient selection, Patient records, SSS Collection, Packaging and transportation Skin biopsy collection, packaging and transportation	Block PHC/CHC/SSS Microscopic center	Monthly	State Leprosy Officer (SLO), District Leprosy Officer (DLO)
SSS Collection and SSS microscopy	District Hospital/Medical College, etc.	**All SSS slides with BI ≥ 2+** received at PCR lab will be evaluated before scraping the specimen for PCR. **All SSS slides with BI <2+** stored at SSS microscopy centre will be evaluated quarterly	Team from respective PCR reference lab

Figure 6: Reference laboratories' quality control of designated testing facilities



9. REFERENCE LABORATORIES

Seven regional, reference laboratories (RRL) have been identified for conducting the amplification of target genes followed by hybridization or DNA sequencing at a particular laboratory/ lab to confirm Rifampicin, Dapsone and Ofloxacin resistance. Each laboratory has been allotted a few states from the region in which it is located. States will collect tissue specimens send them for testing to the designated regional laboratory. Of these regional laboratories, two laboratories CLTRI and NJIL&OMD have an addition function of serving as National Reference Laboratories (NRL). Refer to Table 3 for give allocation of states to the NRL & RRLs.

10. REPORTING PROTOCOLS AND ANALYZING RESULTS

Once the reference laboratory receives the specimens, the following steps will be undertaken:

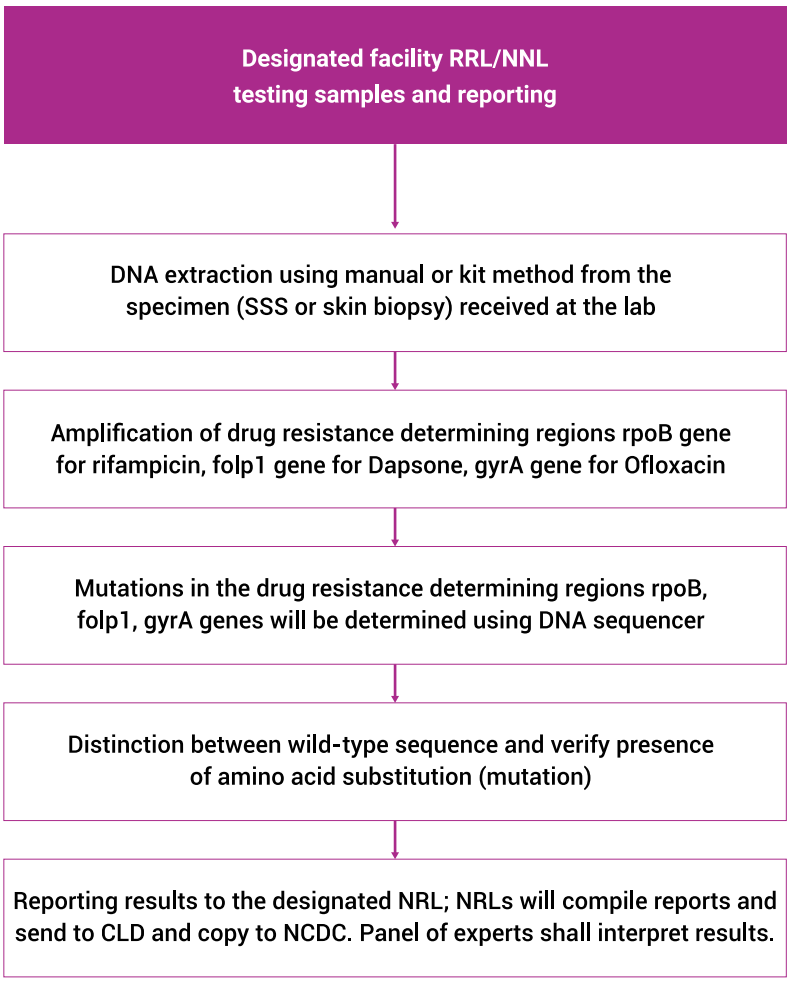
- Extraction of Mycobacterium leprae DNA present in skin specimens using manual or kit methods.
- Amplification of drug resistance-determining regions from of rpoB, folP1 and gyrA genes
- Sequencing of the amplified PCR products preferably with sequencers if available. Each laboratory applies its own approved method.
- Distinction between wild-type sequence and presence of mutation
- Reporting of results will be done as per the details listed in the Annexure V.

-After the laboratory tests have been performed, lab will report the result to designated NRL.

-NRLs shall share compiled report with Central Leprosy Division (CLD) with copy to NCDC.

-Results to be interpreted by an expert panel electronically and communicated to SLO and concerned doctor electronically and receipt as well as follow up action to be ensured.

Figure 7: Testing and Reporting

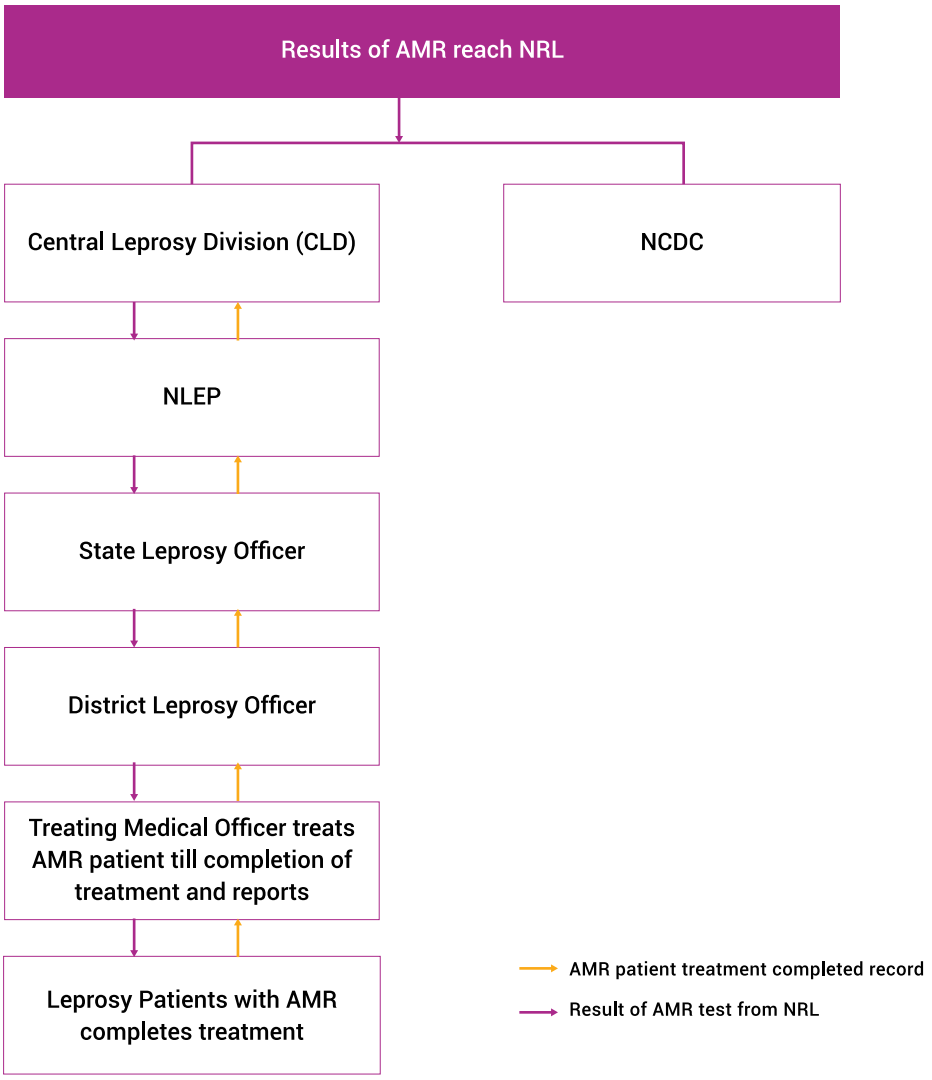


11. SYSTEMATIC RECORDING OF INFORMATION BY THE PROGRAMME

The results of the testing will be utilized by the referring facility for appropriate patient management and by the NLEP for appropriate monitoring. The SLO and DLO will be informed, they will in turn inform the treating medical officer who will treat and monitor the AMR, follow-up and report completion of treatment, when it occurs. NRL will send the result to CLD and NCDC. The CLD records results in the National Leprosy AMR Register, maintaining

data confidentiality. Standard clinical information forms have been developed to record data that will be sent to the reference laboratories and back to the health centres without revealing identity of the patient at various points. At the end of treatment of cases detected with resistance, a copy of the patient treatment card must be sent to the CLD for recording the treatment outcomes.

Figure 8: Flow of information for action and recording AMR results



12. MONITORING AND SUPERVISION

CLD, NRL and RRL will monitor the trends of resistance among new and relapse cases along with monitoring the proportion of patients tested. After the detection of resistance cases, the further detailed information regarding epidemiological data of the resistance cases will be collected as Annexure II. Need for screening for all contacts of those confirmed to have resistant leprosy. If any contacts confirmed to have leprosy, then resistance testing must be done for primary resistance.

It is suggested to duly compile the drug resistance register to be able to analyse data among different categories and monitor treatment outcomes. This would help to identify risk groups for resistance by age, sex, place of residence and/or socio-economic factors, and category of patient (for instance, retreatment after loss to follow up), as well as risk factors for treatment failure.

Annual report of the Leprosy AMR Surveillance shall be published and shared with all stakeholders. NLEP will ensure that each level of activity is adequately supervised to ensure that standard protocols are followed by all the facilities involved. In addition to supervising the clinical aspects from identification of patients to testing and reporting of samples, supervision will also include monitoring logistics, flow of commodities and supplies, timeliness observed, checking transport arrangements, accurate recording, and reporting. Standard supervision checklist will be developed to aid supervisors at all levels.

13. LOGISTICS

Smooth functioning of the Surveillance programme will be contingent on ensuring logistics. These will include – ensuring working equipment for microscopy and sample collection at level 1 and designated facilities, arrangements for procurement of laboratory reagents, equipment and other replenishables, logistics for transport of specimens; and logistics for recording and reporting. Procurement, transport arrangements and facilities for digital communication will be done as per existing protocols for the Ministry of Health and its line departments. However, it will be important to monitor and supervise the logistics to ensure smooth operations at all levels. A few details are mentioned below:

- The laboratories will need sample collection kit including Eppendorf tube/ Screw cap tube, the tube prefilled with 1ml of 70 percent ethanol of molecular biology grade in distilled water and Lab requisition form with patient clinical & contact details.

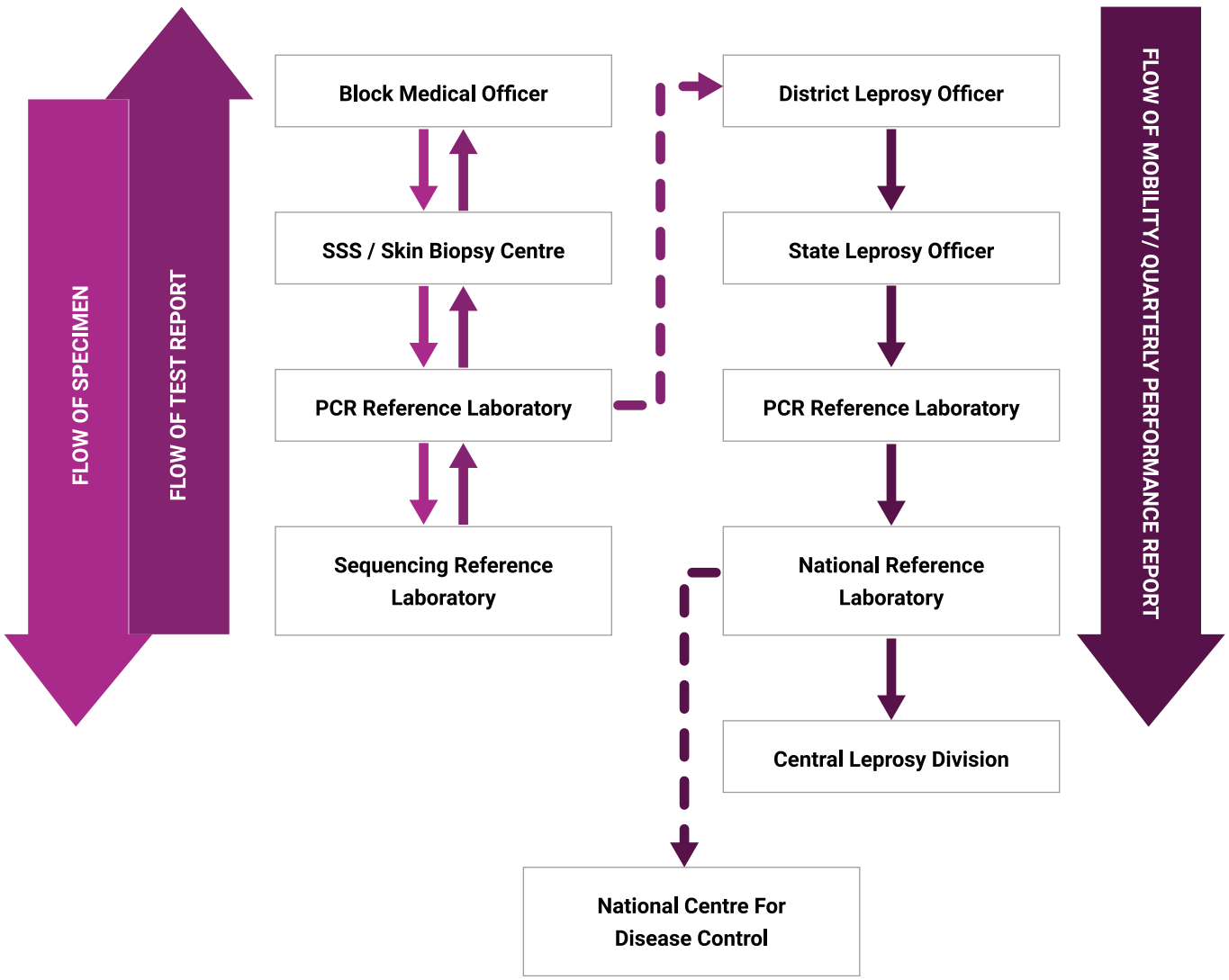
- Procurement of Clofazimine, Ofloxacin and Minocycline, Clarithromycin and Moxifloxacin by the District as per treatment regimen. (As per Annexure 8)

The details of logistics are placed in Annexure III-IV.

14. REPORTING AND DISSEMINATION OF INFORMATION

Further, State-wise and region-wise compiled report will be sent to CLD by CLTRI, Chengalpattu. Central Leprosy Division will send the report to the concerned state by marking a copy to concerned State Leprosy Officer. SLO will provide this feedback to the concerned district and finally district nucleus team (DNT) will in turn facilitate to finally give this feedback to the patient. Electronic communication to be used for timely transmission and follow up action. The National Reference Laboratory will submit a report of all the specimens tested to the Central Leprosy Division, GOI, New Delhi. Further, the draft format for investigation details of all confirmed resistance cases will be done as per the format Epidemiological investigation form kept as Annexure II.

Figure 9: Reporting Mechanism



15. MANAGEMENT OF RELAPSE CASES

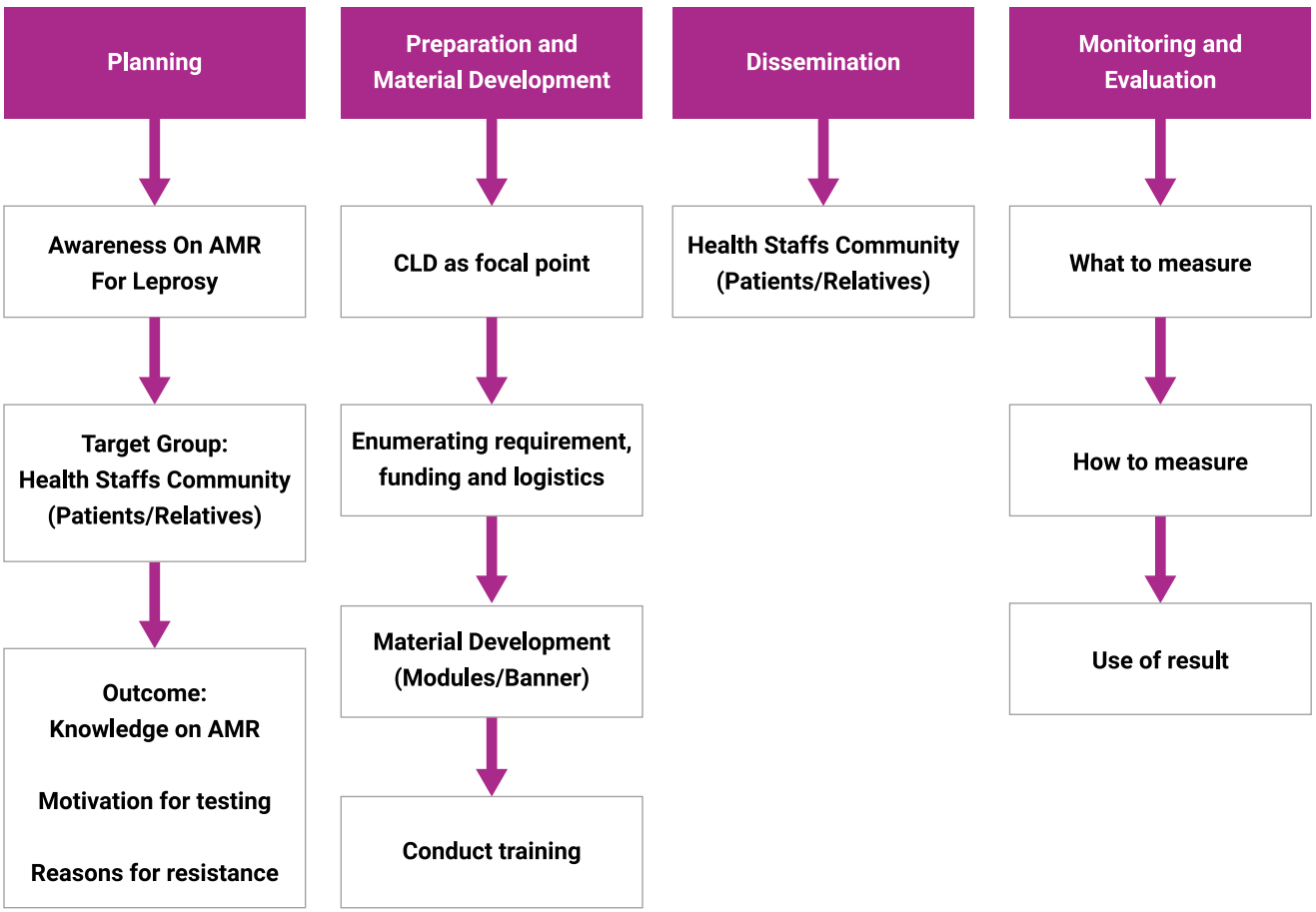
All relapse cases included in the surveillance should immediately be put on treatment with standard MB-MDT without waiting for the status of drug resistance. After the results about resistance become available, these patients are to be treated as per guidelines issued by NLEP.

16. INFORMATION EDUCATION & COMMUNICATION

Central Leprosy Division will be the focal point for administrative and technical support of IEC development and dissemination.

Objective of the IEC activities will be to create awareness among the health care workers on AMR with special reference to Leprosy. The prototype of IEC material will be developed in CLD and disseminated at sub-national level for its implementation. Further active involvement of Panchayati Raj Institutions, ASHA, Village Health Sanitation and Nutrition Committee and School Management Committee will be ensured for extensive dissemination of awareness.

Figure 10: IEC strengthening for AMR



17.TRAINING

Training will be an inbuilt part of the AMR surveillance system. Objective of the training will be to train the participants in the following manner:

- Orientation of AMR surveillance
- Identification of patients for AMR surveillance
- Procedure of slit skin smear technique
- Procedure for collection of biopsy 30
- Procedure for PCR
- Epidemiological investigation
- Planning and implementation of the activities with reporting and feedback mechanism

The details of training including the participants, training venue and number of trainings are depicted in table 9.

Table 10: Capacity building of health personnel at all levels

	Facility	Personnel involved	Capacity to be built for these activities
1	Level 1 Sample collection health facility	• Clinicians/ Medical Officers • Lab Technicians • Health Workers	1. Diagnosis of leprosy 2. Selection of patient for AMR as per inclusion criteria 3. Collection of Slit skin tissue fluid 4. Collection of Skin biopsy 5. Packaging and transportation of specimen
2	Level 2 Designated testing facility	• Molecular Biologist	1. DNA extraction 2. PCR and sequencing for folp1, rpoB and gyrA gene mutations 3. Reporting of results 4. Sending specimens to the reference laboratory for quality control
3	All levels	• Clinicians/ Medical Officers • Programme managers	1. Epidemiological investigation 2. Planning and implementation of the activities with reporting and feedback mechanism

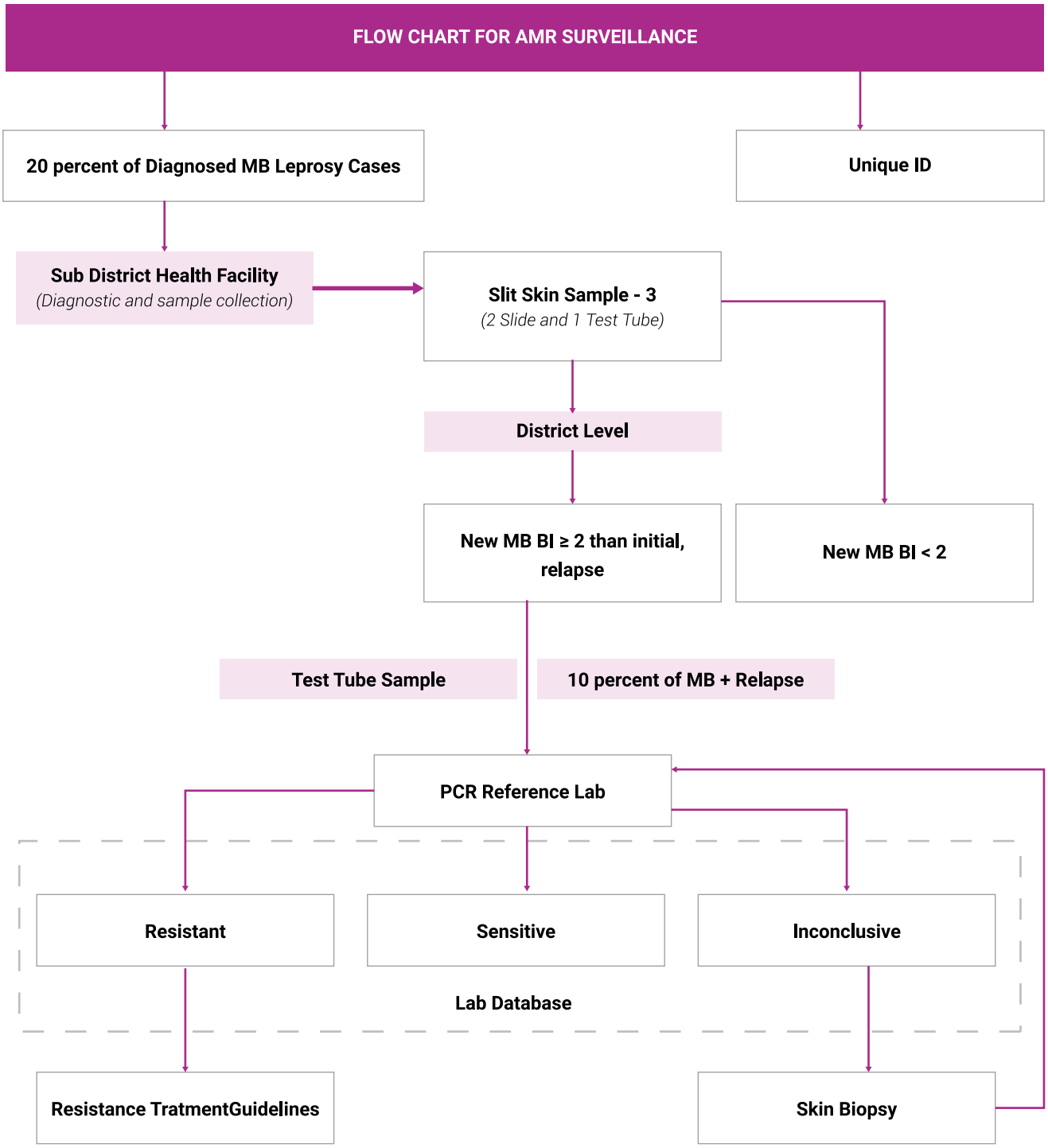
Table 11: Details of trainings for AMR Surveillance

	Trainer	Training venue	Trainee	No. of Trainings	Content
1	NRL team	NRL Institute	PCR Ref. Lab Incharge & teams	1-2 batches	PCR & AMR
2	PCR Reference Lab team	PCR Reference Lab	SLOs & teams	1-2 batches	AMR planning, evaluation & implementation
3	State leprosy Team	State Leprosy Office	DLOs & teams, skin specialists	5-6 batches	AMR planning, evaluation & implementation
4	District leprosy team	District Leprosy Office	MOs & LTs	2-3 batches	Diagnosis & AMR SSS & AMR

18. SUMMARY

The programme for surveillance of antimicrobial resistance in Leprosy has been designed to make it a routine activity, in which all new and other cases will undergo SSS and BI ≥ 2+ will go through PCR and DNA sequencing. Data thus collected will be analysed quarterly and feedback will be given to all participating units by NRL. NRL will share data with CLD where national data will be analysed, feedback will be given to NRLs. Data will be used for all administrative and technical planning, PIP preparations, budget sanctions and planning of existing and new activities. Annual report will be prepared by CLD and will be shared with AMR division of NCDC and all other stakeholders.

Fig. 11: Flow of patient/specimen for AMR Surveillance



ANNEXURES

ANNEXURE I

ROLES & RESPONSIBILITIES OF STAKEHOLDERS

Person responsible	Activity
In-Charge MO & team	• Diagnosis and filling of lab requisition form. • Mobilization of cases to SSS microscopy facility. • Supervision of SSS specimen packaging and transportation PCR reference lab through DLO. • Management of resistance cases with second line drugs in consultation with DLO follow up healthy contacts. • A copy of NLEP patient card of all cases to be sent to DLO.
Lab technician Under supervision of In- Charge MO	• SSS collection, microscopy and reporting In-Charge MO. • Packaging & transport of SSS specimen for AMR to PCR reference Lab.
Dermatologist/ trained Medical Officer	• Skin biopsy collection, packaging and transport PCR reference Lab through DLO.
DLO	• Mapping of Level I, II and III health facilities in each District and submission to SLO. • Setting up of SSS labs. • Training of MOs on diagnosis and treatment of leprosy including AMR surveillance. • Training of LTs on SSS collection, microscopic examination, specimen packaging and transport to PCR reference laboratories. • Establish accessible transportation mechanism for specimen transport. • Maintenance of AMR register at District. • Submission of MPR to SLO. • Provision of second line drugs for resistance cases to In-Charge MO. • Epidemiological investigation of resistance cases. • Submission of a copy of epidemiological investigation form for resistant cases and NLEP patient card for all cases to SLO.
In-Charge MO & team	• Consolidation of mapped facilities (Level I, II, III to be involved in AMR) of all Districts of State. • Submission of consolidated MPR to CLD. • Submission of a copy of epidemiological investigation form for resistant. cases and NLEP patient card for all cases to PCR Reference Lab.
I/C PCR lab	• PCR testing. • Packaging and transportation of PCR products to sequencing laboratories. • Analysis of sequenced data for mutation. • Submission of Drug resistance report to In-Charge MO and DLO. • Submission of consolidated regional quarterly report to NRL. • Appropriate storage of tested samples. • Submission of stored samples to NRL for Quality Control.
I/C Sequence lab	• Sequencing of PCR products and communication of sequenced data to PCR reference lab. • Storage of tested samples for Quality Control.

Person responsible	Activity
I/C NRL	• Consolidation and Submission of nation-wide quarterly report to CLD with a copy to NCDC. • Co-ordination of QC activity for PCR among PCR reference labs. • Monitor the trend of resistance among new and relapse cases and monitor the proportion of patients tested. • Monitor the treatment outcome of resistance cases in terms of clinical improvement. • Co-ordinate half yearly review meeting of stake holders.

ANNEXURE II

EPIDEMIOLOGICAL INVESTIGATION FORM FOR DRUG RESISTANT CASES IN LEPROSY

1. Health Facility details:

Nikusth ID:	**NLEP Registration No:**
State:	**District:**
Block:	**PHC:**

2. Socio- Demographic details:

Name of the patient: …… **Age:** …… (Completed years)

Sex: Male [ ] Female [ ] Transgender

Complete residential address with nearest landmark:

……

……

Contact number: (1) ……..……(2) ……..……

Residence: Urban [ ] Rural [ ]

Religion: Hindu [ ] Christian [ ] Muslim [ ] Others [ ]

Category: General [ ] OBC [ ] SC [ ] ST [ ]

Education: Illiterate [ ] 1 – 5th [ ] 6 – 8th [ ] 9 – 10th [ ] 11 – 12th [ ] Degree [ ] Professional [ ]

Occupation: Unemployed/ Not working [ ] Unskilled Worker [ ] Semiskilled/Skilled Worker

[ ] Clerical [ ] Farmer [ ] Business or petty shop [ ] Professional [ ]Others ……..

Monthly income: Rs. ……

Total number of family members: ……

Marital Status: Never Married [ ] Currently Married [ ] Divorced/Separated [ ] Widow(er) [ ]

Type of house: Kutcha [ ] Semi-pakka [ ] Pakka [ ]

Overcrowding: Yes [ ] No [ ]

Sanitation and Hygiene: Good [ ] Poor [ ]

Away from home due to education/occupation during a year's time: Yes [ ] No [ ]

If yes, frequency …… Duration (Months)

3. Clinical details:

Duration of Disease ……Months / years

Disease Classification: PB [ ] MB [ ]

Type of Case: New [ ] Others - Relapse [ ] Reentered for treatment completion[ ] Referral [ ] Reclassification [ ]

Duration of delay in diagnosis:……Months

Disability status: Nil [ ] Grade I [ ] Grade II [ ]

History of Lepra Reactions: Yes [1] No [2], If Yes - Type 1 / Type II

4.Previous treatment history:

Treatment with MDT: Complete [ ] Incomplete [ ] (Specific drug duration details)……

Treatment for lepra reactions: No [ ] Yes [ ] If yes - Duration…… (Weeks)

History of treatment for TB: Yes [ ] No [ ]

History of taking Ofloxacin or other quinolones (Name) for more than one month: Yes [ ] No [ ] Duration in months if yes……

History of Chemoprophylaxis with single dose Rifampicin: Yes [ ] No [ ]

History of taking Rifampicin for any other conditions: Yes [ ] No [ ]

5.Contact history and others

History of contact with leprosy case: No [ ] Yes - Family [ ] Neighbourhood [ ] Social [ ] Don't Know [ ]

Workplace conditions: Good [ ] Poor [ ]

6.Health care utilization:

Health facilities visited: Govt [ ] Private [ ] Others [ ]

Nearest health facility: Govt [ ] Private [ ]

Preference for health facility: Govt [ ] Private [ ]

7. Evaluation of treatment and outcome of drug resistance:

Type of resistance detected: Rifampicin alone [ ] Dapsone alone [ ] Ofloxacin alone [ ]

Rifampicin + Dapsone + Ofloxacin [ ]

Rifampicin + Dapsone [ ]

Rifampicin + Ofloxacin [ ] Dapsone + Ofloxacin [ ]

Second line treatment regimen initiated (Adult dose):

- Option A: 400 mg of ofloxacin + 100 mg of minocycline + 50 mg of clofazimine, daily for 6 months followed by 400 mg of ofloxacin + 50 mg of clofazimine for 18 months daily
- Option B: 400 mg of ofloxacin + 100 mg of minocycline + 50 mg of clofazimine, daily for 6 months followed by 100 mg of minocycline + 50 mg of clofazimine daily for 18 months
- Other treatment (Specify): ……

Treatment outcome: Treatment completed [ ] Died [ ] Lost to follow up [ ]

Transferred out [ ] Unsatisfactory response to treatment [ ]

Defaulter [ ]

ANNEXURE III

1.CHECKLIST FOR PREPAREDNESS OF SUB DISTRICT/DISTRICT LEVEL HEALTH FACILITY (SSS CENTRE)

S.No.	Requirements	Remarks	
		Yes	**No**
1	**Infrastructure**		
	Waiting hall		
	SSS collection room		
	Staining area with sink facility, Microscope and electricity,		
	Biomedical waste bins		
2	**Personnel**		
	Lab technician		
3	**Training**		
	Lab. technician whether trained in SSS collection, microscopic examination and specimen packaging for transport to PCR reference lab		
4	**Consumables for SSS microscopy**		
	1 percent Carbol fuchsin		
	1 percent Acid alcohol		
	1 percent Methylene blue		
	Spirit lamp		
	Tincture benzoin		
	Scalpel		
	Surgical blade (No.15)		
	Match box		
	New glass slides (75mm X 25mm X 1.0 mm)		
	Diamond pencil		
	Sterile cotton		

S.No.	Requirements	Remarks	
		Yes	**No**
5	**Spicemen Packaging and Transportation**		
	Specimen collection vial		
	Parafilm		
	Ziplock pouch		
	Slide holder box		
	Laboratory report form for SSS and Laboratory requisition form for PCR		
	Triple compartment specimen transport box		
	Biohazard label		
	Accessible transport services		

ANNEXURE IV

2.CHECKLIST FOR PREPAREDNESS OF TERTIARY LEVEL (MEDICAL COLLEGES) HEALTH FACILITY (SSS AND SKIN BIOPSY CENTRE)

S.No.	Requirements	Remarks	
		Yes	**No**
1	**Infrastructure**		
	Waiting hall		
	Skin biopsy collection room		
	Biomedical waste bins		
2	**Personnel**		
	Dermatologist / Trained Medical Officer		
	Staff nurse		
3	**Consumables for collection of skin biopsy**		
	Tincture benzoin		
	Biopsy punch (4-6mm)		
	Scalpel		
	Surgical blade (No.15)		
	Sterile gauze		
	Suture materials		
	Sterile cotton		
4	**Specimen packaging and transportation**		
	Specimen collection vial		
	Parafilm		
	Ziplock pouch		
	Lab requisition form for PCR		
	Triple compartment specimen transport box		
	Accessible transport services		

ANNEXURE V

(A) LABORATORY REQUISITION FORM FOR SSS/BIOPSY

(To be filled by In-Charge MO, Level I Facility)

Name of Diagnosis facility:		**Date:**
Name and address of SSS / biopsy facility (to where this request form and patient has to be sent):		
Patient name:		**ID:**
Age:	**Sex:**	**Diagnosis:** New
To collect specimen:	☐ SSS ☐ Biopsy	**Previous SSS result:**
In-Charge Medical Officer	**Name:**	
	Signature:	
	Email id:	
	Contact number:	

Please fill the form completely and send the patient along with the lab requisition form to the nearest SSS/Biopsy facility.

(B) LABORATORY REPORT FORM FOR SSS

(To be filled by LT, Level II facility)

Name of SSS facility:			**Date:**	
Name and address of Diagnosis facility (to where this report form has to be sent):				
Patient name:			**ID:**	
Age:	**Sex:**		**Diagnosis:** New MB Relapse	
Site	**Site 1**	**Site 2**	**Site 3**	**Site 4**
Result:				
Lab. Technician	**Name:**			
	Signature:			
	Contact number:			

Please fill the form completely and send to the In-Charge MO.
Result should be reported with BI if positive (refer below for BI grading) or negative.

Bacteriological Index	Criteria
1+	1-10 bacilli on an average in 100 oil immersion fields
2+	1-10 bacilli on an average in 10 oil immersion fields
3+	1-10 bacilli on an average in each oil immersion field
4+	10-100 bacilli on an average in each oil immersion field
5+	100-1000 bacilli on an average in each oil immersion field
6+	> 1000 bacilli on an average in each oil immersion field / innumerable bacilli / globi

(C) LABORATORY REQUISITION FORM FOR PCR

(To be filled by LT, Level II facility)

Name of Health facility:			**Date:**	
Name of SSS / Skin Biopsy Facility:				
Name and address of PCR Reference Laboratory (to where this request form, specimen vial and SSS slide has to be sent)**:**				
Patient name:			**Nikusth ID:**	
Age:	**Sex:**		**Diagnosis:** New	
Specimen:	☐ SSS	☐ Biopsy	**Previous SSS result:**	
Site	**Site 1**	**Site 2**	**Site 3**	**Site 4**
Result:				
In -Charge MO and DLO (Add row)	**Name:**			
	Email id:			
	Contact number:			
Lab. Technician	**Name:**			
	Signature:			
	Contact number:			

Please fill the form completely and send this form along with specimen vial containing SSS scraping/biopsy and SSS slide through designated courier.

(D) LABORATORY REPORT FORM FOR PCR

(To be filled by In-Charge, PCR Reference Lab.)

Name of PCR reference lab:			**Date:**
Name of Diagnosis facility (to where this report form has to be sent):			**Name of the DLO:**
Patient name:			**ID:**
Age:	**Sex:**		**Diagnosis:** New
Specimen:	☐ SSS	☐ Biopsy	
PCR result	**Rifampicin**	**Dapsone**	**Ofloxacin**
Amplified (Yes/No)			
Resistant (R) / Sensitive (S)			
Details of mutation:			
Collection of biopsy specimen:	☐ Required	☐ Not Required	
In-charge, PCR Reference Lab	**Name:**		
	Signature:		
	Contact number:		

Please fill the form completely and send to the In-Charge MO and DLO through Email/online reporting system.

ANNEXURE VI

CONTACT DETAILS OF PCR REFERENCE LABORATORIES (NODAL OFFICER) UPDATE

1.Central Leprosy Teaching and Research Institute

Allen Road, Tirumani, Chengalpattu, Tamil Nadu- 603001 Tel. No.: (044)27426274, 27426275, 27426065

E-Mail: cltriadmn@gmail.com, dircltri.tnchn@nic.in,

2.National JALMA Institute for Leprosy and Other Mycobacterial Diseases

Dr. M. Miyazaki Marg, Taj Ganj, Agra – 282001

Tel No.: +91-562-2331751; Fax No.: +91-562-2331755

E-Mail: jalma@sancharnet.in

3.Stanley Brown Laboratory

Shahdara Leprosy Hospital, The Leprosy Mission, Nandnagri, Delhi – 110093 Tel. No.: 011 – 22581451, 22110788

E-Mail: tlmshahdra@tlmindia.org

4.Blue Peter Public Health and Research Centre

Near TEC Building, Cherlapally, Hyderabad, Telangana- 501301 Tel. No.: 040 27264547

E-Mail: info@leprahealthinaction.in

5.Schieffelin Institute of Health Research and Leprosy Centre

Karigiri, Vellore, Tamil Nadu -632106

Tel. No.: 0416 – 2274221, 2274222, 2274223

E-Mail: publicrelation@karigiri.org, directorate @karigiri.org

6.Regional Leprosy Training and Research Institute

Dhamtari Road, Rishabh Nagar, Pawan Vihar Colony, Lalpur, Raipur, Chattisgarh -492001 Tel. No.: 07712412792

E-Mail: rltri.cg@gov.in

ANNEXURE VII

(A) AMR SURVEILLANCE MONTHLY PERFORMANCE REPORT FORM

(To be prepared by In-Charge MO at Diagnosis facility and submitted to DLO)

Block PHC/CHC:	**Reporting Month:**
District:	**Year:**

1	Number of new cases diagnosed in the reporting month			**PB**	**MB**	**Total**
			Adult			
			Child			
			Total			
2	Number of relapse cases detected in the reporting month		**Adult**			
			Child			
			Total			
3	Number of SSS performed in the reporting month		**New MB**	**Relapse**		**Total**
4	Number of patients with positive SSS in the reporting month		**BI <2 +**	**BI≥ 2+**		**Total**
5	Number of SSS specimens sent for PCR in the reporting month		**New MB**	**Relapse**		**Total**
6	Number of patients referred to skin biopsy facility in the reporting month		**New MB**	**Relapse**		**Total**
7	PCR results of SSS/biopsy samples obtained in the reporting month		**No. Sensitive**	**No. Resistant**		**Inconclusive**
		Rifampicin				
		Dapsone				
		Ofloxacin				

Enclose a copy of NLEP patient card

Remarks if any:

Signature of the
In-Charge Medical Officer

(B) AMR SURVEILLANCE MONTHLY PERFORMANCE REPORT FORM

(To be prepared by DLO and submitted to SLO)

District:	**Reporting Month:**
State:	**Year:**

1	Number of diagnosis facility involved in AMR surveillance in the district				
2	Number of new cases diagnosed in the reporting month		**PB**	**MB**	**Total**
		Adult			
		Child			
		Total			
3	Number of relapse cases detected in the reporting month	**Adult**			
		Child			
		Total			
4	Number of SSS performed in the reporting month	**New MB**	**Relapse**		**Total**
5	Number of patients with positive SSS in the reporting month	**BI <2+**	**BI≥ 2+**		**Total**
6	Number of SSS specimens sent for PCR in the reporting month	**New MB**	**Relapse**		**Total**
7	Number of patients for whom biopsy was taken and sent for PCR in the reporting month	**New MB**	**Relapse**		**Total**
8	PCR results of SSS/biopsy samples obtained in the reporting month	**No. Sensitive**	**No. Resistant**		**Inconclusive**
	Rifampicin				
	Dapsone				
	Ofloxacin				
9	Epidemiological investigation of drug resistant cases	**Performed**		**Not Performed**	

Enclose a copy of NLEP patient card for all cases and a copy of epidemiological investigation form for resistant cases along with this MPR.

Remarks if any: *Signature of the District Leprosy Officer*

(C) AMR SURVEILLANCE MONTHLY PERFORMANCE REPORT FORM

(To be prepared by SLO and submitted to In-Charge, PCR Reference Lab.)

State:	**Reporting Month & Year:**

1	Number of districts involved in AMR surveillance in the State					
2	Number of new cases diagnosed in the reporting month		**PB**	**MB**	**Total**	
		Adult				
		Child				
		Total				
3	Number of relapse cases detected in the reporting month	**Adult**				
		Child				
		Total				
4	Number of SSS performed in the reporting month	**New MB**	**Relapse**		**Total**	
5	Number of patients with positive SSS in the reporting month	**BI <2 +**	**BI≥ 2+**		**Total**	
6	Number of SSS specimens sent for PCR in the reporting month	**New MB**	**Relapse**		**Total**	
7	Number of biopsy specimens sent for PCR in the reporting month	**New MB**	**Relapse**		**Total**	
8	PCR results of SSS/biopsy samples obtained in the reporting month	**No. Sensitive**	**No. Resistant**		**Inconclusive**	
	Rifampicin					
	Dapsone					
	Ofloxacin					
9	Epidemiological investigation form of drug resistant cases	**Received**		**Not Received**		

Enclose a copy of NLEP patient card for all cases and a copy of epidemiological investigation form for resistant cases along with this MPR

Remarks if any:

Signature of the State Leprosy Officer

(D) AMR SURVEILLANCE MONTHLY PERFORMANCE REPORT FORM
PCR REFERENCE LAB

(To be prepared by In-Charge, PCR Reference Lab and submitted to NRL)

Name of the PCR Reference Lab:	**Reporting Year & Quarter: I/ II/ III/ IV**

1	Number of States involved in AMR surveillance under the PCR Reference Lab					
2	Number of specimens received for PCR in the reporting quarter	**New MB**	**Relapse**	**Total**		
3	Number of specimens received for PCR in the reporting quarter (State wise with type of specimen)	**State**	**No. of SSS**	**No. of Biopsy**		
	Total					
4	Number of SSS specimens positive for PCR in the reporting quarter					
5	Number of biopsy specimens positive for PCR in the reporting quarter					
6	Number of PCR products sent for sequencing in the reporting quarter	**Rifampicin**	**Dapsone**	**Ofloxacin**	**Total**	
7	Number of sequencing results obtained in the reporting quarter	**Non-specific result**	**Specific result**		**Total**	
			Sensitive	Resistant		
8	Number of drug resistant cases detected in the reporting quarter	**New MB cases**	**Relapse cases**		**Total**	
	Rifampicin					
	Dapsone					
	Ofloxacin					
9	Epidemiological investigation forms of drug resistant cases	**Received**		**Not Received**		

(E) DETAILS OF MUTATION OF DRUG RESISTANT CASES

(To be prepared by In-Charge, PCR Reference Lab and submitted to NRL)

Name of the PCR Reference Lab:Reporting Year & Quarter: I/ II/ III/ IV

Total No. of drug resistance detected in the reporting quarter	**Among New MB cases**			**Among Relapse cases**		
	RMP 'R'	**DDS 'R'**	**OFL 'R'**	**RMP 'R'**	**DDS 'R'**	**OFL 'R'**
Describe the mutation in terms of: 1.Transitiont/deletion/silent 2.Change in nucleotide with position 3.Change in amino acid with position						
No. of unusual mutations detected in the reporting quarter						
Unusual mutation cases sent for MFP inoculation (Yes/No)						

Enclose the details of mutation (in the given format), a copy of epidemiological investigation form for resistant cases and a copy of NLEP patient card for all cases along with the QPR

Remarks if any:

Signature of the In-Charge, PCR Reference Laboratory

(F) AMR SURVEILLANCE MONTHLY PERFORMANCE REPORT FORM (ANNUALLY)

(To be prepared by In-Charge, NRL (CLTRI) and submitted to CLD with a copy to NCDC)

Reporting quarter: I/ II/ III/ IV	**Year:**

State	New MB cases	Relapse cases	No. of specimens tested by PCR		No. of cases sensitive to all drugs		No. of resistant cases (any drug)		No. resistant to Rifampicin		No. resistant to dapsone		No. resistant to ofloxacin	
			New	Relapse	New	Relapse	New	Relapse	New	Relapse	New	Relapse	New	Relapse

Enclose the details of mutation (in the given format), a copy of epidemiological investigation form for resistant cases and a copy of NLEP patient card for all cases along with the QPR

(G) CONSOLIDATED REPORT – NRL

Reporting details	Total	percent
Number of new MB cases tested during the quarter		
Number of drug resistance detected among new cases during the quarter		
Resistant to Rifampicin		
Resistant to Dapsone		
Resistant to Ofloxacin		

Reporting details	Total	percent
Number of relapse cases tested during the quarter		
Number of drug resistance detected among relapse cases during the quarter		
Resistant to Rifampicin		
Resistant to Dapsone		
Resistant to Ofloxacin		

Enclose a copy of NLEP patient card for all cases and a copy of epidemiological investigation form for resistant cases, a copy of details of mutation for resistant cases along with this QPR

Remarks if any:

Signature of the In-Charge, NRL

ANNEXURE VIII

GUIDELINES FOR TREATMENT OF DRUG RESISTANT CASES

After collection of specimens from patients (either SSS or biopsy), treatment should be initiated immediately without waiting for the PCR results on drug resistance. On the availability of PCR and sequencing results, the following should be considered:

- Sensitive to all three drugs: Routine MB-MDT treatment to be continued.

- Resistant to Dapsone/ Quinolone only: Routine MB-MDT treatment to be continued and to follow up regularly for possible relapse.

- Resistant to Rifampicin: the following treatment regimen should be prescribed

  -Option A: 400 mg of Ofloxacin + 100 mg of minocycline + 50 mg of clofazimine, daily for 6 months followed by 400 mg of ofloxacin + 50 mg of clofazimine for 18 months daily

  (OR)

  -Option B: 400 mg of ofloxacin + 100 mg of minocycline + 50 mg of clofazimine, daily for 6 months followed by 100 mg of minocycline + 50 mg of clofazimine daily for 18 months

ANNEXURE IX

STANDARD OPERATIVE PROTOCOL FOR SPECIMEN COLLECTION

Selection of the specimen (4 specimens from each patient)

Two ear lobes (will be coded as E1 and E2 respectively)
Two active sites of the patches (will be coded as P1 and P2 respectively)

Slit skin scraping specimen: specimens will be collected in the same manner as taking skin smears for Bacterial Index (BI) examination using a disposable stainless steel surgical blade such as No. 15 (4 Specimens are to be collected).

Specimen collection-

- Slit skin scraping specimens are to be collected as per prescribed protocol with the help of stainless steel BP blade number 15 size.
- Put the blade (with the skin scrapings) in the Eppendorf containing 70 percent ethanol. Skin scrapping from all the four sites to be pooled into single Eppendorf tube and keep this for 2 hours and then remove the blade with the help of sterile forceps (should be done at Molecular Lab only).
- Label the Eppendorf tube.

Specimen for PCR- All specimens with BI 2+ shall be sent for PCR. The skin scrapping to be collected for SSS and further the remaining specimen on the scalpel to be washed in container containing 70 percent ethyl alcohol. The skin scrapings from all the sites can be mixed in the same container which is to be sent for PCR.
Caution should be taken to prevent cross-infection and proper disposal of used blades, test tubes and biopsy materials will be done. The blade along with tissue scrapings are to be soaked in tube containing 1ml of 70 percent ethanol of molecular biology grade in distilled water and transported as such with blade in the tube to Molecular biology laboratory. Tube should be properly labelled indicating patient register number, site of collection and date.

Skin biopsy: A skin specimen is collected using a disposable punch biopsy needle. The biopsy specimen is then placed in a 1.8 mL centrifuge sterile tube (with screw cap), pre-filled with 1 mL of 70 percent ethanol (molecular biology grade absolute ethanol at 70 percent v/v + sterile de-ionized water .

Specimens can be kept at ambient room temperature (upto 25 degree centigrade) until they are sent to the laboratory, possibly in batches, depending on the cost of transportation and on the number of specimens per month. Bacilli are rapidly inactivated, which means that specimens can be sent by routine transport without the need to control the temperature during transportation or take additional precautions for biohazard control.

Standard Protocol for Specimen packaging

Specimens thus collected can be kept at room temperature, less than 25 degrees Celsius for maximum of 5 days, till further processing or transportation to the laboratory. After capping the Eppendorf tube, seal with Para-film and put in Cryo-box vertically. Label properly and in concordance with surveillance site from where specimen collected.

The mapping of the Mycobacterium leprae genome has identified sites at which mutations occur, conferring resistance to Dapsone, 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 Mycobacterium leprae.

- Missense mutations leading to the substitution at any one of at least six amino acids (positions 407, 410, 416, 420, 425, 427, 441, 451 and 456) or an insertion of amino acids between position 408 and 409 confers Rifampicin resistance to Mycobacterium leprae.
- Missense mutations in the sulphone (Dapsone) resistance determining region of the folP gene (codes

dihydropteroate synthase), resulting in alterations of amino acids at positions 53 and 55, confer Dapsone resistance to Mycobacterium leprae.

- Missense mutations in the quinolone (Ofloxacin) resistance determining region of the gyrA gene, resulting in alterations of amino acids at positions 89 and 91, confer quinolone resistance to M.leprae.

DNA sequencing protocol

The methods for isolation of DNA are considered the most appropriate, either by a freeze-boiling (simple process) or by a physiochemical method using lysozyme. The drug resistant determining region (DRDR) in the rpoB and folP gene is amplified by standard PCR condition. Amplification of target region is to be confirmed by agarose electrophoresis. PCR products are purified by commercially available kit such as Minielute Gel extraction kit (Quiagen) followed by sequencing reaction. Big Dye Terminator v1.1 (Applied Biosystems) is considered adequate for sequencing of short fragment. Specimens are applied to sequencer for analysis of nucleotide mutation. The standard operational procedures are as follows: -

(i) Purify PCR products using QIA quick PCR purification kit as per manufacturer's instructions. Optional (some workers simply dilute the PCR product prior to sequencing).

(ii) Estimate the concentration of DNA available for sequencing (OD260 or by visual observation of amplicon band on gel in comparison to DNA standards)

(iii) For each sequencing reaction, add the following reagents:

- 8.0 microlitre of 1X Big Dye 1.1 Terminator Ready Reaction Mix
- Up to 11/ul of 3-5 ng of DNA in dH2O
- 1.0/ul of 3.2 pmol/ul primer
- Adjust volume to a total of 20.0/ul.
- Alternative schemes using Big Dye Terminator 3.1 are optional.

(iv) Mix well and spin briefly. Place the tubes in a thermocycler and set the volume to 20/ul.

(v) 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

(vi) Purify the cycle sequencing reaction products with Performa DTR Ultra 96-well plate kit/cartridge Edge Bio Systems 41453 to remove primers, nucleotides, etc. Elute the products in a final volume is 20/ul Tris-EDTA, pH 8.0. Dry up products and dissolve in 25/ul of Hi-Di Formamide (Applied Biosystems) followed by heating for 2 minutes at 95°C and quick chilling in ice.

(vii) Apply the specimen for capillary electrophoresis using ABI 3130 instrument with 36cm capillaries and POP7 acrylamide matrix for a running time of 2780 sec (Adjust to suitable running time for other types of sequencing instruments).

Note: the above steps are suggestive, chemistry and steps may change. Reference Labs may be required to amplify the target genes and after confirmation may need to ship the amplicons in frozen conditions to a designated laboratory identified for automatic sequencing, this will be decided and instructions communicated. (refer to annexures).

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