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MOLECULAR LINE PROBE ASSAY IN THE DETECTION OF ANTIBIOTIC RESISTANCE IN LEPROSY PATIENTS

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DISCLOSURE

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EXECUTIVE SUMMARY

Background

Leprosy or Hansen's disease is a chronic infectious disease caused by Mycobacterium leprae (*M. leprae*) which affects the skin and peripheral nerves, causing permanent morbidity and disability as well as social stigmatization and discrimination against patients and their families in many societies. Early diagnosis and treatment with multidrug therapy (MDT) are crucial in eliminating this disease as a public health issue. Since its introduction by the World Health Organisation (WHO) in 1995, MDT has had significant impact in reducing the global burden of the disease. Malaysia has reached a stage of elimination in 1994, but up to today, new cases are still being detected. Resistance to these drugs has been reported in some parts of the world, usually in association with relapse after an insufficient therapy.

Drug resistance in leprosy patients can be attributable to chromosomal mutations in *M. leprae* genes encoding the targeted drug and as a result of errors in DNA replication. Detection of these mutations and errors has been a challenge since *M. leprae* cannot be cultivated in vitro. The Mouse Footpad (MFP) Inoculation test which was developed 50 years ago has been the 'gold standard' for leprosy drug susceptibility testing. However, this test has been described as labour-intensive and time-consuming and is available only in a number of highly specialised laboratories. This highlighted the urgency for rapid screening methods to identify mutations associated with resistance to antileprosy drugs. Molecular Line Probe Assay (MLPA) is a novel technology which permits simultaneous detection of *M. leprae* and its resistance to Rifampicin, Dapsone and Ofloxacin. It is claimed to be of low operational cost, easier to manage with the capability of producing results in less than seven days.

This technology review was conducted following a request from the Head of Tuberculosis and Leprosy Sector, Disease Control Division, Ministry of Health Malaysia.

Objective / aim

The objective of this technology review was to evaluate the evidence on effectiveness, safety and cost-effectiveness of Molecular Line Probe Assay (MLPA), compared to Mouse Footpad Inoculation test (MFP), in detecting antibiotic resistance among leprosy patients.

Results and conclusions

A total of 67 titles were identified through the Ovid interface and PubMed. After applying the inclusion and exclusion criteria, only one feasibility study was finally selected.

The feasibility study was conducted by Cambau et al. (2012) to evaluate the performance of DNA probe assay by comparing its results on 120 *M. leprae* strains, previously studied for resistance against the reference drug using Mouse Footpad Inoculation (MFP) test and for mutations in the gene regions by polymerase chain reaction (PCR) sequencing. The test sensitivity for detecting *M. leprae* was 100%. It was also found to be 100% concordant with DNA sequencing and the MFP assay for DRDRs encoding most of the major mutations in rpoB, folP1 and gyrA. For the susceptible strains, concordance was 98.3% when two strains showing a mutation at the codon 447 that does not induce Rifampicin resistance in *M. leprae.*

There was no retrievable evidence that evaluate the safety and cost-effectiveness of MLPA, in detecting antibiotic resistance among Leprosy patients. A MLPA test unit is less costly compared to a single MFP test unit (priced at RM 147.08 and RM 710.58, respectively). The MLPA test is also less burdensome and is capable of producing more rapid results than MFP.

In conclusion, there was limited fair level of retrievable evidence that evaluated the effectiveness of MLPA compared to MFP test, in detecting antibiotic resistance among leprosy patients. The test in the retrieved evidence revealed good performance in detecting mutations in three specific genes responsible for resistance towards three antibiotics; Dapsone, Rifampicin and Ofloxacin but not to Clofazimine, which is one of the drug used as first-line MDT in Malaysia.

Methods

Electronic databases were searched through the Ovid interface; MEDLINE(R) In-Process and Other Non-Indexed Citations and Ovid MEDLINE (R) 1946 to present, EBM Reviews – Cochrane Central Registered of Controlled Trials – July 2015, EBM Reviews – Database of Abstracts of Review of Effects – 2nd Quarter 2015, EBM Reviews – Cochrane Database of Systematic Reviews – 2005 to July 2015, EBM Reviews – Health Technology Assessment – 3rd Quarter 2015, EBM Reviews - NHS Economic Evaluation Database – 2nd Quarter 2015. Other databases used include PubMed and Embase. Search was also conducted through some official websites such as World Health Organisation (WHO) and INAHTA. General database such as Google was used to search for additional web-based materials and information. Additional articles retrieved from reviewing the bibliographies of retrieved articles. The search was limited to articles on human only. **Appendix 1** showed the detailed search strategies. The last search was conducted on 25 August 2015.

MOLECULAR LINE PROBE ASSAY IN THE DETECTION OF ANTIBIOTIC RESISTANCE IN LEPROSY PATIENTS

1.0 BACKGROUND

Leprosy or Hansen's disease is a chronic infectious disease caused by *Mycobacterium leprae* (*M. leprae*) which affects the skin and peripheral nerves.[1] It is classified into paucibacillary or multibacillary leprosy based on the number of skin patches.[1] The disease gradually spreads over the entire body, causing permanent morbidity and disability as well as social stigmatization and discrimination against patients and their families in many societies.[1] Early diagnosis and treatment with multidrug therapy (MDT) are therefore crucial in eliminating this disease as a public health issue.[2] Since its introduction by the World Health Organisation (WHO) in 1995, MDT has had significant impact in reducing the global burden of the disease and had cured around 16 million patients.[1] In the year 2000, global elimination of leprosy was achieved (i.e. a prevalence rate of leprosy less than 1 case per 10 000 persons).[3] Malaysia has reached a stage of elimination in 1994, but up to today, new cases are still being detected.[4]

The first - line MDT that is being used in Malaysia, as recommended by WHO, consists of a combination of Rifampicin, Dapsone and Clofazimine in a 6-month and 12-month regimens for paucibacillary and multibacillary (MB) cases, respectively.[5] Resistance to these drugs has been reported in some parts of the world, usually in association with relapse after an insufficient therapy.[6, 7] It can be attributable to chromosomal mutations in *M. leprae* genes encoding the targeted drug and as a result of errors in DNA replication.[8] These mutational processes can be acquired during the initial infection from an infection source containing drug-resistant leprosy (primary drug resistance) or from inadequate treatment (secondary drug resistance). The Global Sentinel Surveillance for Drug Resistance in Leprosy program which was established in nine countries and coordinated by WHO to monitor global leprosy drug resistance in relapse cases, reported nine cases of resistance to Dapsone and one case tested positive for resistance to Rifampicin in 2010.[9]

Since MDT is currently the only effective means to control leprosy, it is therefore important that we keep a vigil on drug resistance trends. Leprosy presents a challenge for detecting drug resistance because *M. leprae* cannot be cultivated in vitro. It is an obligate intracellular parasite that grows very slowly in estimated 12 to14 days in the mouse footpad and nine-band armadillo.[2] The mouse footpad (MFP) inoculation test which was developed 50 years ago has been the 'gold standard' for leprosy drug susceptibility testing.[8] This in-vivo method requires the recovery of a sufficient number of viable organisms from a patient to inoculate the footpads of 20 to 40 antibiotic-treated mice (depending on the number of drugs to be tested). These mice are then sacrificed after a defined period of time (usually six months or longer) and the numbers of bacilli in the footpads of treated mice and untreated mice are compared.[8] The test is labour-intensive and time-consuming and is available only in a number of highly specialized laboratories. As a result, it is not being widely used in poor, leprosy endemic countries.

In Malaysia, the National Public Health Laboratory also utilised the MFP method for antibiotic sensitivity testing of *M. leprae*.[4] This method usually takes around 13 to 15 months to determine the susceptibility of *M. leprae* isolates and delivery of the biopsy samples from treatment facilities especially in Sabah and Sarawak to the National Public Health Laboratory often incurred high cost and cumbersome. During this time, patients may be inappropriately treated, the drug resistant strains may continue to spread, and amplification of resistance may occur. This highlighted the urgency for rapid screening methods to identify mutations associated with resistance to antileprosy drugs.

Over the past two decades, the availability of genomic sequence of *M. leprae* and an improved understanding of the genetic basis of drug resistance in mycobacteria has led to the development of molecular assays for detection of drug-resistant mutations.[7] In WHO Guidelines for Global Surveillance of Drug Resistance in Leprosy, the use of polymerase chain reaction (PCR) sequencing is recommended for detection of drug resistance in leprosy patients.[10] Other non DNA sequencing-based methods for detecting mutation in *M.leprae* include DNA array technology using reverse hybridisation method, real-time PCR high resolution melt assay and Molecular Line Probe Assay (MLPA).[11-13] The latter is a novel technology which permits simultaneous detection of *M. leprae* and its resistance to Rifampicin, Dapsone and Ofloxacin.[14] It is claimed to be of low operational cost, easier to manage with the capability of producing results in less than seven days.[13, 14] Furthermore, the test is done in-vitro which translated to no animal testing.

This technology review was conducted following a request from the Head of Tuberculosis and Leprosy Sector, Disease Control Division, Ministry of Health Malaysia to review the performance of MLPA in view of using it as an alternative to MFP in detecting resistance to antileprosy drugs.

2.0 OBJECTIVE / AIM

The objective of this technology review was to evaluate the evidence on effectiveness, safety and cost-effectiveness of MLPA, compared to MFP test, in detecting antibiotic resistance among leprosy patients.

3.0 TECHNICAL FEATURES

Molecular Line Probe Assay (MLPA) technology is claimed to be able to detect *M. leprae* as well as resistance towards three antileprosy drugs; Dapsone, Rifampicin and Ofloxacin.[14] The molecular basis of this lies in the identification of missense mutations in the drug resistance-determining region (DRDR) in the genes responsible for resistance; *rpoB* for Rifampicin, *folP* for Dapsone, *gyrA* for quinolones.[10]

There are several steps in MLPA which include DNA extraction from *M. leprae* isolates or directly from clinical specimens, polymerase chain reaction (PCR) amplification of the DRDR using biotinylated primers and hybridization of labelled PCR products with specific probes immobilized on a strip. The hybrids are then visualised as coloured bands on the strip at the site of probe binding, enabling detection of the presence of *M. leprae* complex, as well as the presence of wild-type and mutation probes for resistance. Mutations are

detected by the lack of binding to wild-type probes, but positive binding to the mutation probes.[13, 14]

Figure 1: Molecular Line Probe Assay

4.0 METHODS

4.1. Searching

Electronic databases were searched through the Ovid interface; MEDLINE(R) In-Process and Other Non-Indexed Citations and Ovid MEDLINE (R) 1946 to present, EBM Reviews – Cochrane Central Registered of Controlled Trials – July 2015, EBM Reviews – Database of Abstracts of Review of Effects – 2nd Quarter 2015, EBM Reviews – Cochrane Database of Systematic Reviews – 2005 to July 2015, EBM Reviews – Health Technology Assessment – 3rd Quarter 2015, EBM Reviews - NHS Economic Evaluation Database – 2nd Quarter 2015. Other databases used include PubMed and Embase. Search was also conducted through some official websites such as World Health Organisation (WHO) and INAHTA. General database such as Google was used to search for additional web-based materials and information. Additional articles retrieved from reviewing the bibliographies of retrieved articles. The search was limited to articles on human only. **Appendix 1** showed the detailed search strategies. The last search was conducted on 25 August 2015.

4.2. Selection

A reviewer screened the titles and abstracts against the inclusion and exclusion criteria and then evaluated the selected full-text articles for final article selection.

The inclusion and exclusion criteria were:

Inclusion criteria

Problem Population	Leprosy patient
Intervention	Molecular line probe assay (MLPA)
Comparator	Mouse footpad inoculation test (MFP)
Outcomes	Detection of resistance towards antileprosy drugs
Study design	Systematic reviews (SRs), randomised controlled trials (RCTs), cohort study, diagnostic test accuracy study, cross-sectional study, qualitative study, case control study
	English, full text articles

Exclusion criteria

Study design	Anecdotal, animal studies
	Non-English

Relevant articles were critically appraised using Critical Appraisal Skills Programme (CASP) and evidence graded according to NHS Centre for Reviews and Dissemination (CRD) University of York, Report Number 4 (**Appendix 2**). Data were extracted and summarised in the evidence table (**Appendix 3)**.

5.0 RESULTS AND DISCUSSION

A total of 67 titles were identified through the Ovid interface and PubMed. After applying the inclusion and exclusion criteria, only one feasibility study was finally selected. No economic analysis was retrieved.

5.1 Effectiveness

A feasibility study was conducted by Cambau et al. (2012) to evaluate the performance of GenoType LepraeDR®.[13,Level 3] Hundred and twelve skin biopsies containing *M. leprae* were sent for leprosy diagnosis to the National Reference Center for Mycobacteria in France from 1989 to 2010 and were all smear-positive for acid fast bacilli (AFB). Around half of the collection were selected consecutively for MFP and the molecular studies.

The test includes DNA probes for the wild-type sequence of regions of *rpoB*, *gyrA* and *folP* genes and probes for the prevalent mutations involved in acquired resistance to Rifampicin, Dapsone and Ofloxacin. The performances of the test were evaluated by comparing its results on 120 *M. leprae* strains, previously studied for resistance by the reference drug using MFP and for mutations in the gene regions by PCR-sequencing. Mutations are detected by the lack of binding to wild-type probes, but positive binding to the mutation probes.

The DNA probe test with *M. leprae* identification band was positive with an intensity equal or higher than that obtained with the universal positive control, demonstrating the presence of *M. leprae* DNA. Thus, the overall sensitivity of GenoType LepraeDR for detecting *M. leprae* was 100%. The test result was found to be 100% concordant with DNA sequencing and the MFP assay for DRDRs encoding most of the major mutations in *rpoB*, *folP1* and *gyrA*. Among 120 *M. leprae* strains previously analysed for resistance by MFP, 16 were resistant to Rifampicin, 22 resistant to Dapsone and four resistant to Ofloxacin. For the susceptible strains, concordance was 98.3% when two strains showing a mutation at the codon 447 that does not induce Rifampicin resistance in *M. leprae* .[13, Level 3]

The test's detection of resistance towards antibiotics Dapsone, Rifampicin and Ofloxacin is limited on gene *rpoB*, *folP* and *gyrA*, respectively. [13, Level 3] The test is also limited by its inability to detect resistance to Clofazimine, which is one of the drug recommended by WHO and used as first-line MDT in Malaysia.

5.2 Safety

There was no retrievable evidence that evaluate the adverse event of MLPA, in detecting antibiotic resistance among leprosy patients. However, it is claimed that this product has CE mark.[14]

5.3 Cost-effectiveness

There was no retrievable evidence that evaluate the cost-effectiveness of MLPA in detecting antibiotic resistance among leprosy patients.

A single MLPA test unit is priced at RM 147.08, compared to a MFP test unit at RM 710.58 (Communication letter from TB/Leprosy Sector, Ministry of Health Malaysia).

Limitation

Our review has several limitations. The selection of the studies and appraisal was done by one reviewer. Although there was no restriction in language during the search, only English full text articles were included in the report.

6.0 CONCLUSION

In conclusion, there was limited fair level of retrievable evidence that evaluate the effectiveness of MLPA compared to MFP test, in detecting antibiotic resistance among leprosy patients. The diagnostic test in the retrieved evidence revealed good performance of MLPA in detecting mutations in three specific genes responsible for resistance towards three antibiotics; Dapsone, Rifampicin and Ofloxacin but not to Clofazimine, which is the drug used as first-line MDT in Malaysia. There was lack of evidence on safety and cost-effectiveness of MLPA in detecting resistance towards antileprosy drugs. A MLPA test unit is less costly compared to a single MFP test unit (priced at RM 147.08 and RM 710.58, respectively). The MLPA test is also less burdensome and is capable of producing more rapid results than MFP.

7.0 REFERENCES

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12. Li W, Matsuoka M, Kai M et al. Real-time PCR and high-resolution melt analysis for rapid detection of Mycobacterium leprae drug resistance mutations and strain types. Journal of Clinical Microbiology. 2012;50(3):742-753.
13. Cambau E, Chauffour-Nevejans A, Tejmar-Kolar L et al. Detection of antibiotic resistance in leprosy using GenoType LepraeDR, a novel ready-to-use molecular test. PLoS Neglected Tropical Diseases. 2012;6 (7)(e1739).
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8.0 APPENDIX

8.1 Appendix 1: LITERATURE SEARCH STRATEGY

Ovid MEDLINE® In-Process & Other Non-indexed Citations and Ovid MEDLINE® 1948 to present

1. Leprosy/
2. Lepros*.tw.
3. Leprosy, Borderline/ or Leprosy, Paucibacillary/ or Leprosy/ or Leprosy, Multibacillary/ or Leprosy, Lepromatous/ or Leprosy, Tuberculoid/
4. Leprosy, borderline/
5. (Lepros* adj1 (dimorphous or borderline)).tw.
6. Leprosy, Lepromatous/
7. (Lepros* adj1 (lepromatous or cutaneous or nodular)).tw.
8. Leprosy, Multibacillary/
9. ((Lepros* or lepromatous) adj1 (midborderline or multibacillary or borderline)).tw.
10. Leprosy, Paucibacillary/
11. ((Borderline or undeterminate or paucibacillary) adj1 tuberculoid*).mp. or lepros*.tw. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier]
12. Leprosy, Tuberculoid/
13. ((Neural or tuberculoid or macular) adj1 lepros*).tw.
14. (Disease adj1 hansen*).tw.
15. 1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9 or 10 or 11 or 12 or 13 or 14
16. Mycobacterium leprae/
17. Microbial Sensitivity Tests/
17. Drug Resistance, Microbial/
18. Mouse footpad inoculation test.mp.
19. mouse footpad inoculation test.tw.
18. Microbial Sensitivity Tests/
19. (minimum adj2 inhibitory concentration*).tw.
20. (test* adj2 microbial sensitivity).tw.
21. ((microbial or bacterial or antibiogram bacterial) adj2 drug sensitivity test*).tw.
22. Drug sensitivity assay.tw.
23. (Sensitivity test* adj2 bacterial).tw.
24. Antibiogram*.tw.
25. ((Antibacterial or antimicrobial) adj2 susceptibility breakpoint).tw.
26. (Concentration* adj2 minimum inhibitory).tw.
27. Molecular line probe assay.tw.
28. DNA strip test.tw.
29. reverse hybridization.tw.
30. 16 or 17 or 18 or 19 or 20 or 21 or 22 or 23 or 24 or 25 or 26 or 27 or 28 or 29
31. 15 and 30
32. limit 31 to (english language and humans)

OTHER DATABASES	
EBM Reviews – Cochrane Central Registered of Controlled Trials	Same MeSH, keywords, limits used as per MEDLINE search
EBM Reviews – Database of Abstracts of Review of Effects	
EBM Reviews – Cochrane database of systematic reviews	
EBM Reviews – Health Technology Assessment	
NHS economic evaluation database	
Embase	

PubMed

((("leprosy"[MeSH Terms] OR "leprosy"[All Fields]) OR (((((indeterminate[All Fields] OR paucibacillary[All Fields]) OR borderline[All Fields]) OR (("leprosy, tuberculoid"[MeSH Terms] OR ("leprosy"[All Fields] AND "tuberculoid"[All Fields]) OR "tuberculoid leprosy"[All Fields] OR ("neural"[All Fields] AND "leprosy"[All Fields]) OR "neural leprosy"[All Fields]))) OR (("leprosy, tuberculoid"[MeSH Terms] OR ("leprosy"[All Fields] AND "tuberculoid"[All Fields]) OR "tuberculoid leprosy"[All Fields] OR ("tuberculoid"[All Fields] AND "leprosy"[All Fields])) OR (("leprosy, tuberculoid"[MeSH Terms] OR ("leprosy"[All Fields] AND "tuberculoid"[All Fields]) OR "tuberculoid leprosy"[All Fields] OR ("macular"[All Fields] AND "leprosy"[All Fields]) OR "macular leprosy"[All Fields]) AND ((((((((((((((molecular[All Fields] "line"[All Fields]) AND ("Probe"[Journal] OR "probe"[All Fields] OR "Probe (Lond)"[Journal] OR "probe"[All Fields] OR "Probe (Adelaide)"[Journal] OR "probe"[All Fields]) AND ("analysis"[Subheading] OR "analysis"[All Fields] OR "assay"[All Fields] OR "biological assay"[MeSH Terms] OR ("biological"[All Fields] AND "assay"[All Fields]) OR "biological assay"[All Fields])) OR (minimum[All Fields] AND inhibitory[All Fields])) OR (microbial[All Fields] AND ("sensitivity and specificity"[MeSH Terms] OR ("sensitivity"[All Fields] AND "specificity"[All Fields]) OR "sensitivity and specificity"[All Fields] OR "sensitivity"[All Fields]))) OR (bacterial[All Fields] AND drug[All Fields] AND ("sensitivity and specificity"[MeSH Terms] OR ("sensitivity"[All Fields] AND "specificity"[All Fields]) OR "sensitivity and specificity"[All Fields] OR "sensitivity"[All Fields]))) OR (microbial[All Fields] AND drug[All Fields] AND ("sensitivity and specificity"[MeSH Terms] OR ("sensitivity"[All Fields] AND "specificity"[All Fields]) OR "sensitivity and specificity"[All Fields] OR "sensitivity"[All Fields]))) OR (("microbial sensitivity tests"[MeSH Terms] OR ("microbial"[All Fields] AND "sensitivity"[All Fields] AND "tests"[All Fields]) OR "microbial sensitivity tests"[All Fields] OR "antibiogram"[All Fields]) AND bacterial[All Fields] AND drug[All Fields] AND ("sensitivity and specificity"[MeSH Terms] OR ("sensitivity"[All Fields] AND "specificity"[All Fields]) OR "sensitivity and specificity"[All Fields] OR "sensitivity"[All Fields]))) OR (drug[All Fields] AND ("sensitivity and specificity"[MeSH Terms] OR ("sensitivity"[All Fields] AND "specificity"[All Fields]) OR "sensitivity and specificity"[All Fields] OR "sensitivity"[All Fields]))) OR (("anti-bacterial agents"[Pharmacological Action] OR "anti-bacterial agents"[MeSH Terms] OR ("anti-bacterial"[All Fields] AND "agents"[All Fields]) OR "anti-bacterial agents"[All Fields] OR "antibacterial"[All Fields]) AND ("disease susceptibility"[MeSH Terms] OR ("disease"[All Fields] AND "susceptibility"[All Fields]) OR "disease susceptibility"[All Fields] OR "susceptibility"[All Fields]))) OR (("anti-infective agents"[Pharmacological Action] OR "anti-infective agents"[MeSH Terms] OR ("anti-infective"[All Fields] AND "agents"[All Fields]) OR "anti-infective agents"[All

Fields] OR "antimicrobial"[All Fields]) AND ("disease susceptibility"[MeSH Terms] OR ("disease"[All Fields] AND "susceptibility"[All Fields]) OR "disease susceptibility"[All Fields] OR "susceptibility"[All Fields]) AND breakpoints[All Fields] AND .tw[All Fields])) OR (concentration[All Fields] OR breakpoint[All Fields]) OR ("anti-bacterial agents"[Pharmacological Action] OR "anti-bacterial agents"[MeSH Terms] OR ("anti-bacterial"[All Fields] AND "agents"[All Fields]) OR "anti-bacterial agents"[All Fields] OR "antibacterial"[All Fields])) OR ("disease susceptibility"[MeSH Terms] OR ("disease"[All Fields] AND "susceptibility"[All Fields]) OR "disease susceptibility"[All Fields] OR "susceptibility"[All Fields])) OR ("anti-bacterial agents"[Pharmacological Action] OR "anti-bacterial agents"[MeSH Terms] OR ("anti-bacterial"[All Fields] AND "agents"[All Fields]) OR "anti-bacterial agents"[All Fields] OR "antibiotic"[All Fields])) OR ("anti-infective agents"[Pharmacological Action] OR "anti-infective agents"[MeSH Terms] OR ("anti-infective"[All Fields] AND "agents"[All Fields]) OR "anti-infective agents"[All Fields] OR "antimicrobial"[All Fields])) OR (molecular[All Fields] AND ("long interspersed nucleotide elements"[MeSH Terms] OR ("long"[All Fields] AND "interspersed"[All Fields] AND "nucleotide"[All Fields] AND "elements"[All Fields]) OR "long interspersed nucleotide elements"[All Fields] OR "line"[All Fields]) AND ("Probe"[Journal] OR "probe"[All Fields] OR "Probe (Lond)"[Journal] OR "probe"[All Fields] OR "Probe (Adelaide)"[Journal] OR "probe"[All Fields]))) OR ((("mice"[MeSH Terms] OR "mice"[All Fields] OR "mouse"[All Fields]) AND footpad[All Fields] AND ("vaccination"[MeSH Terms] OR "vaccination"[All Fields] OR "inoculation"[All Fields]) AND (("mice"[MeSH Terms] OR "mice"[All Fields] OR "mouse"[All Fields]) AND footpad[All Fields] AND "inoculation"[All Fields])))) AND "humans"[MeSH Terms] AND English[lang])

8.2 Appendix 2

HIERARCHY OF EVIDENCE FOR TEST ACCURACY STUDIES

Level	Description	
1.	A blind comparison with reference standard among an appropriate sample of consecutive patients	
2.	Any one of the following	Narrow population spectrum
3.	Any two of the following	Differential use of reference standard
4.	Any three or more of the following	Reference standard not blind Case control study
5.	Expert opinion with no explicit critical appraisal, based on physiology, bench research or first principles.	

SOURCE: NHS Centre for Reviews and Dissemination (CRD) University of York, Report Number 4 (2nd Edition)

8.3 APPENDIX 3: Evidence Table

Question: Is Molecular Line Probe Assay effective in detecting antibiotic resistance in leprosy patients?

Bibliographic citation	Study Type/Methods	LE	Number of Patients & Patient Characteristic	Intervention	Comparison	Length of Follow Up (If Applicable)	Outcome Measures/Effect Size	General Comments
Cambau E, Chauffour-Nevejans A, Tejmar-Kolar L et al. Detection of antibiotic resistance in leprosy using GenoType LepraeDR, a novel ready-to-use molecular test. PLoS Neglected Tropical Diseases. 2012;6 (7)(e1739)	Feasibility study Objective: to compare the effects of DNA strip test in detecting antibiotic resistance in leprosy patient. It includes DNA probes for the wild-type sequence of regions of *rpoB, gyrA and folP* genes and probes for the prevalent mutations involved in acquired resistance to Rifampicin, fluoroquinolones and Dapsone, respectively. The performances of the GenoType LepraeDR test were evaluated by comparing its results on 120 *M. leprae* strains, previously studied for resistance by the reference drug in MFP and for mutations by PCR-sequencing.	3	• 112 skin biopsies containing M. leprae sent for diagnosis to the National Reference Center for Mycobacteria in France from 1989 to 2010 • 54% of these biopsies were selected consecutively for MFP and the molecular studies. • 120 *M. leprae* strains tested	Molecular line probe assay			• Overall sensitivity of GenoType LepraeDR for detecting *M. leprae* was 100%. • The results of the test were 100% concordant with those of PCR sequencing and the mouse footpad test for the resistant strains: 16 strains resistant to Rifampicin, 22 to Dapsone and 4 to Ofloxacin with mutations in rpoB, folP1 and gyrA, respectively. • Concordance was 98.3% for the susceptible strains, two strains showing a mutation at the codon 447 that in fact was not conferring resistance as shown by MFP method.	