Validation of PCR for the diagnosis of Leprosy in Kiribati

Jacqueline Gardner
Steve Chambers
Emma Trowbridge
Trevor Anderson

Canterbury
District Health Board
Te Poari Hauora o Waitaha
Christchurch, New Zealand
Kiribati
- Island nation equatorial pacific
- Population of 116,000
- Rising sea levels likely due to climate change have put the islands in danger
- Endemic leprosy
Leprosy

• Quite possibly the oldest infectious disease known to man
• Molecular evidence has been found in 100,000 year old skeletal remains
• The first bacterium ever identified
  • Norwegian physician Dr Hansen in 1893
Clinical features are those of unsightly skin lesions and damage to peripheral nerves which can be highly deforming and destructive.

Not highly infectious unless living at close quarters.

Thought to be spread through nasal secretions and open skin lesions.

Incubation period is long on average 5 years but can be up to 20 years.

- This feature makes contact testing a problem.

90% of some populations have natural immunity.
Leprosy

• *mycobacterium leprae*
• An acid fast bacillus best shown by modified Ziehl-Neelsen stain
• A wimpy pathogen that can survive only a few hours outside the host

Mycobacterium leprae in the skin from a patient diagnosed with leprosy. The bacteria stain red. *Courtesy of Wellcome Collection.*
WHO Eradication of Leprosy

• 1991 WHO aimed to eliminate Leprosy worldwide by 2000
• Prevalence rate of below 1/10000 population
• Free multi drug therapy MDT
Number of Leprosy Cases

1997

2015
Pacific Leprosy Foundation

• 2015 Pilot
  • Raise community awareness
  • Train health care workers
  • School screening
  • Contact tracing from the previous 5 years

• 2016
  • 3 day skin camps with local and NZ leprosy and skin specialists
  • Set up real time PCR for the detection of the *mycobacterium leprae*
2016

- Suitable cases were selected to get the test up and running
- Punch biopsies of skin were cut in half and fixed in 70% ethanol with one half submitted for histology and one half for PCR
- PCR was successfully set up
- The lepromatous and some tuberculoid cases yielded positive results with reads ranging from 12 to 32
- Ethanol was suitable for the PCR but the histology was affected and suboptimum
Validation

• 2017 and 2018
• 40 clinical cases of leprosy selected
  • Lepromatous: multibacillary
  • Tuberculoid: paucibacillary
  • Indeterminate: early cases
Specimen collection

• Histology and modified ZN stains: Punch biopsy of lesional skin fixed in 10% buffered formalin
• Real time PCR: Punch biopsy of lesional skin fixed in 70% ethanol
• 36/40 cases had positive PCR

• 19/40 MZN positive
Results

<table>
<thead>
<tr>
<th>Category</th>
<th>Total</th>
<th>PCR +ve</th>
<th>ZN +ve</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lepromatous</td>
<td>13</td>
<td>13</td>
<td>13</td>
</tr>
<tr>
<td>Tuberculoid</td>
<td>20</td>
<td>18</td>
<td>2</td>
</tr>
<tr>
<td>Indeterminate</td>
<td>7</td>
<td>5</td>
<td>0</td>
</tr>
</tbody>
</table>

Legend:
- **Total**
- **PCR +ve**
- **ZN +ve**
<table>
<thead>
<tr>
<th>Number of reads</th>
<th>Lepromatous</th>
<th>Tuberculoid</th>
<th>Indeterminate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>15-24</td>
<td>24-42</td>
<td>32-35</td>
</tr>
</tbody>
</table>
Negative controls

• 10 cases of other skin conditions were PCR negative
PCR

• Sensitive
• Specific
• Quick
• Easy to interpret
• No need to involve an expensive pathologist in a sometimes futile search for acid fast bacilli!
Modified ZN stain as compared to PCR

• Less sensitive than PCR
• Less specific
  • Stains mycobacteria other than *mycobacterium leprae*
• In cases other than lepromatous leprosy, it is dependent on skill of pathologist!!
Application of PCR

• To confirm the diagnosis in endemic areas
  • Especially tuberculoid and indeterminate cases
  • PCR lab in a suitcase in the clinic

• To test for the Rifampicin resistance gene
  • Relevant for contact testing

• Reference laboratory test
  • Canterbury Health is now reference lab for NZ

• Anthropologic interest
  • Where did the leprosy on Kiribati come from?
  • Whole genome sequencing
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