National Institute for Communicable Diseases

(i) Capabilities for Reference Laboratory function.

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Multiplex real-time RT-PCR Testing Algorithm

ALL Specimens (SARI /ILI) → 1-step Multiplex realtime RT-PCR (FastTrack Diagnostics) →

- Influenza A
- Influenza B
- RSV

Subtyping RSV-A / RSV-B (in-house) →

- Sequencing
- RSV isolation
2017 RSV season (Pneumonia surveillance)

<table>
<thead>
<tr>
<th>Hospital (Province)</th>
<th>RSV Positive</th>
<th>Total samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Edendale (KZ)</td>
<td>35</td>
<td>509</td>
</tr>
<tr>
<td>Helen Joseph-Rahima Moosa (GP)</td>
<td>123</td>
<td>844</td>
</tr>
<tr>
<td>Klerksdorp-Tshepong (NW)</td>
<td>43</td>
<td>484</td>
</tr>
<tr>
<td>Mapulaneng-Matikwana (MP)</td>
<td>34</td>
<td>216</td>
</tr>
<tr>
<td>Mitchell’s Plain (WC)</td>
<td>88</td>
<td>322</td>
</tr>
<tr>
<td>Red Cross (WC)</td>
<td>332</td>
<td>984</td>
</tr>
<tr>
<td><strong>Total:</strong></td>
<td><strong>655</strong></td>
<td><strong>3359</strong></td>
</tr>
</tbody>
</table>
**RSV serotypes**

- RSV subtyping assay
- 1-step multiplex rtRT-PCR
- SOP approved- Dec 2017

![Graph showing RSV serotypes for 2016 and 2017 with N=769 and N=826]
RSV genotypes

NICD sequencing core facility

- Sanger sequencing
  - Conventional nested PCR
    - 1\textsuperscript{st} rnd A/B
    - 2\textsuperscript{nd} rnd type specific primers for A and B

- Next generation sequencing
  - 3 Illumina MiSeq’s

- 2018 focus to establish NGS sequencing protocols
RSV isolation

- Established isolation and preservation of isolates
- Share viruses through MTA for research on RSV

Challenges
- Temperature sensitivity of RSV- freeze-thaw
Proficiency testing

- For Real-time RT-PCR assays
  - QCMD programme
    - Influenza, RSV and other respiratory viruses
    - MERS-CoV
  - WHO-EQAP- Flu
  - CDC-EQAP- Flu
  - CDC-EQAP-RSV for WHO pilot

- Virus isolation
  - UKNEQAS