HIV testing – a laboratory perspective

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HIV testing process
Diagnostic methods of choice
  + Adults
    × Problems in adults
  + Babies
    × Problems in babies
Problems with serology
Problems with PCR
  + Case
HIV testing process

- Step 1
  - Request form

[Image of a laboratory request form with annotations]

- Baby of???
HIV testing process

- Step 1
  - Request form
HIV testing process

- Step 2
  - Taking the sample
    - Label tube first
      - Min 2x personal identifiers, e.g. name + DOB/barcode
    - Make sure it’s the correct patient
    - Correct tube order
    - Mix well
    - Full tube if possible — 2x baby tubes if necessary
Sample problems
HIV testing process

- Step 3
  - Arrival in the lab
    - Sorting
    - Registration
    - Lab barcode label
    - Send to testing lab
HIV testing process

- Step 4
  - Testing in the lab
HIV testing process

× Step 5

+ Results going back to Sr/Dr
Problems along the way

Incorrect details
Too few details
Baby vs mother
Contact details
Clinical info
Wrong test
Unclear request

Wrong patient
Unlabelled tube
Wrong tube type
Incorrectly labelled
Wrong sample in bag
Not mixed: clots
Insufficient

Lost in transit
Leaked
Wrong tests assigned
Sent to the wrong lab
Mislabelled

Wrong test done
Mislabeled
Instrument error
Sample failure
Insufficient
Problem result, e.g. equivocal

Result goes “missing”
Problem result, e.g. equivocal
Result not seen
Result not acted on
Result not understood

Request form  Taking sample  Lab arrival  Testing  Results
Examples

- Incorrect labelling
  + Clinician phones lab to say the baby’s sample is really the mother’s sample
  + Clinician puts unlabelled tubes in pocket – has to remember which tube belongs to which patient later at home

- Instrument breaks down during a run
  + Infant sample used up on a failed test
  + Insufficient for repeating

- Lab error
  + Sample mislabelled with the wrong lab barcode
  + Lab sends HIV PCR sample to FBC bench
  + Laboratory contamination – negative samples test positive
Diagnostic methods of choice
Adults, children >18 months

- Serology – rapid test or ELISA
  + Know which one to request at your local lab

- Positives
  + 2x specimens, different dates
  + False positive results
    - Patient identity wrong – mislabelled
    - Patient / sample factors resulting in non-specific reaction

- When in doubt – ask the lab
  + Clinical picture mismatch
    - Sick patient, candida, TB, wasting – but HIV negative serology?
Babies <18 months

- PCR

- Serology detects antibodies
  - Babies have their mother’s antibodies too

- Polymerase chain reaction
  - Amplifies HIV genome (DNA)

- Difficult to get enough sample
  - 250ul for PCR; 2ml or 750ul for HIV viral load
Problems – serology

- False negatives
  - Window period
  - Wrong sample tested
  - Test not done properly, e.g. rapid read too soon

- False positives
  - Sample splashes
  - Wrong sample tested
  - Test not done properly, e.g. rapid read too late

- Problems with babies
  - Sometimes maternal antibody lasts till 24 months!!
Problems – PCR

- **False negative**
  - Levels of virus too low, e.g. early in infection, ARVs
  - Virus suppressed, e.g. by PMTCT / ARVs
  - Wrong sample, test not done properly
  - Small DBS spots – full circle needed

- **False positive**
  - Maternal cells in birth blood
  - Maternal cells in cord blood ← bad specimen
  - Wrong sample, test not done properly
Case study: HIV PCR problems
Case example

- Baby born to HIV+ mother on ARVs
- Baby given ARVs for PMTCT
- Birth PCR = equivocal
Measurement Details

**Negative**

- CH1: Target
- CH2: QS/IC

<table>
<thead>
<tr>
<th>CH1: Target</th>
<th>Cycle#</th>
</tr>
</thead>
<tbody>
<tr>
<td>-</td>
<td>27.8</td>
</tr>
</tbody>
</table>

**Positive**

- CH1: Target
- CH2: QS/IC

<table>
<thead>
<tr>
<th>CH1: Target</th>
<th>Cycle#</th>
</tr>
</thead>
<tbody>
<tr>
<td>29.3</td>
<td></td>
</tr>
<tr>
<td>Test</td>
<td>Result</td>
</tr>
<tr>
<td>--------------</td>
<td>------------</td>
</tr>
<tr>
<td>HI2QLD96</td>
<td>Detected DBS</td>
</tr>
</tbody>
</table>

**Workflow**

<table>
<thead>
<tr>
<th>Process Steps</th>
<th>Name</th>
<th>System ID</th>
<th>Position</th>
<th>Timestamp</th>
<th>Clip#</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary Pipetting</td>
<td>HI2QLD96</td>
<td>Manual</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td>AmpliPrep Preparation</td>
<td>HI2QLD96</td>
<td>393161 (393161)</td>
<td>0076 - 22</td>
<td>05/27/2016 15:03:20</td>
<td>$SA20A9FF4</td>
</tr>
<tr>
<td>TaqMan Amplification</td>
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<td>391935 (391935)</td>
<td>TCC 076 - 05</td>
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<td>$SA20A9FF4</td>
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<tr>
<td>TaqMan Detection</td>
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<td>391935 (391935)</td>
<td>0076 - 22</td>
<td>05/27/2016 18:15:46</td>
<td>$SA20A9FF4</td>
</tr>
</tbody>
</table>

**Measurement Details**

![Graph showing fluorescence intensity over cycles](image)

- CH1: Target  Cycle#: 39.9
- CH2: QS/IC   Cycle#: 28.1

Equivocal
Case example

- Baby born to HIV+ mother on ARVs
- Baby given ARVs for PMTCT
- Birth PCR = equivocal
- Viral load on that sample = LDL
- Repeat PCR at 3 days = negative
- Repeat PCR at 6 weeks = negative
- Baby stops PMTCT after breastfeeding stops
- 2 months later:
  - Scenario 1 – Baby PCR negative
  - Scenario 2 – Baby PCR positive, viral load 10000 c/ml
Equivocal results

- Scenario 1 – May be false positive
  + Very low levels of maternal cell contamination
  + Faint background signal – non-specific

- Scenario 2 – May be true positive
  + Infected infant with virus suppressing due to
    - Maternal ARVs during pregnancy
    - Infant ARVs from PMTCT
The end