Detection of Anti-HIV Antibodies in Saliva

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“Suck it and See...”

In 2008 NRL were approached by the Burnet Institute to set up a confirmatory testing algorithm for antibodies to HIV in saliva samples

-Instigated by the Burnet Institute 2008
-Funding by the Dept. Human Services
-Estimating HIV prevalence and unrecognised HIV infection among men who have sex with men in Victoria (Melbourne)
-Participants were recruited through
-3 gay bars/clubs
-4 gay sex-on-premises venues
-2 gay men’s health clinics
-Were asked to self-complete a behavioural questionnaire and provide an oral fluid specimen for HIV testing.
-Participation was voluntary.

Saliva Collection

Step 1: Collect sample
Place the pad between the cheek and gum for 2-5 minutes.

Saliva Collection

Step 2: Insert the device into the buffer
Snap off the wand. Replace the cap and send to the lab via regular mail
Stable for 21 days at room temperature (in preservative)

Operational Characteristics of Current HIV Assays

WHO/UNAIDS Report 13, 2002
- Oral fluid (saliva) specimens
- matched saliva and serum specimens
- 75 positive specimens
- 147 negative specimens

Assays
- OraScreen HIV rapid test (Beacon Diagnostics)
- Salivax HIV (ImmunoScience Inc)
- SMLX Technologies Diagnostics Test (SMLX Technologies

Reference assay
- Wellcozyme HIV 1 + 2 GACELISA (Murex Biotech Ltd)
Operational Characteristics of Current HIV Assays

- Compared with the GACELISA

<table>
<thead>
<tr>
<th>Assay</th>
<th>Sensitivity (95% CL)</th>
<th>Specificity % (95% CL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>OraScreen</td>
<td>56.0 (44 – 66)</td>
<td>98.6 (95 – 100)</td>
</tr>
<tr>
<td>Salivax</td>
<td>79.4 (67 – 89)</td>
<td>96.0 (91 – 99)</td>
</tr>
<tr>
<td>SMLX</td>
<td>62.7 (51 – 74)</td>
<td>74.8 (67 – 82)</td>
</tr>
</tbody>
</table>

Review of current literature

- Hodinka et al. Clinical and Vaccine Immunology
  [http://cvi.asm.org/cgi/content/full/5/4/419/T1](http://cvi.asm.org/cgi/content/full/5/4/419/T1)
- GACELISA superior sensitivity and specificity
- Philip Cunningham (Craig and Leon)
  SydPath, St. Vincent’s Hospital, Sydney, Australia
- John Parry and Eddie Murphy
  Virus Reference Division, Central Public Health Laboratory London, UK

Saliva HIV Confirmatory Testing Algorithm

- To ensure that the sample is of adequate quality and contains enough antibody to detect anti-HIV antibodies
  - Total IgG ELISA
- To test for anti-HIV antibodies do a GacElisa
- Repeat in duplicate if reactive
- Confirm reactive GacElisa result with a saliva HIV Western Blot

Total IgG ELISA

- Substrate - ABTS
- Mouse monoclonal anti-human IgG coupled to HRP
- Human IgG in Saliva Sample
- Goat anti-human IgG (FAB specific)

Total IgG Standard Curve

<table>
<thead>
<tr>
<th>Conc.</th>
<th>Meas.</th>
<th>CalcCon</th>
<th>Residual</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cal_1</td>
<td>500.000</td>
<td>4.867</td>
<td>959.295</td>
</tr>
<tr>
<td>Cal_2</td>
<td>250.000</td>
<td>4.361</td>
<td>200.755</td>
</tr>
<tr>
<td>Cal_3</td>
<td>100.000</td>
<td>3.646</td>
<td>105.700</td>
</tr>
<tr>
<td>Cal_4</td>
<td>80.000</td>
<td>3.199</td>
<td>80.017</td>
</tr>
<tr>
<td>Cal_5</td>
<td>40.000</td>
<td>1.894</td>
<td>39.145</td>
</tr>
<tr>
<td>Cal_6</td>
<td>20.000</td>
<td>0.952</td>
<td>20.040</td>
</tr>
<tr>
<td>Cal_7</td>
<td>10.000</td>
<td>0.466</td>
<td>10.217</td>
</tr>
<tr>
<td>Cal_8</td>
<td>5.000</td>
<td>0.261</td>
<td>4.971</td>
</tr>
</tbody>
</table>

Fit type: Sigmoid logistic: y = b+(a-b)/(1+xc)^d

- Meas. transformation: Linear
- Conc. transformation: Linear

Parameters: a = 0.157, b = 4.921, c = 0.018, d = 1.573

Corr. coeff. R²: 0.964

For surveillance purposes 0.1µg/ml IgG was considered adequate

Total Saliva IgG

<table>
<thead>
<tr>
<th>IgG (µg/ml)</th>
<th>Val Pos</th>
<th>Val Neg</th>
<th>Unknown Pos</th>
<th>Unknown Neg</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>5</td>
<td>10</td>
<td>15</td>
<td>20</td>
</tr>
<tr>
<td>25</td>
<td>30</td>
<td>35</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
GACELISA

Substrate - TMB
HIV Antigen coupled to HRP
Synthetic peptides and recombinant proteins derived from the envelope proteins of HIV-1 and HIV-2 and HIV core protein
Human IgG in Saliva Sample
Rabbit anti-human IgG

GACELISA

Saliva Titration Curves - GACELISA

Saliva Titration Curves

Samples to Validate “in-house” GacElisa

VALIDATION Saliva Samples

100 Positive controls (A1 – A100)
105 Negative controls
38 from PMC (B1 – B31, B39, B64 – B69)
15 from Centre Clinic (C1, C3 – C11, C13, C26 – C29)
52 from MSHC (D1 – D52)

GACELISA - Validation

GACELISA on Validation Samples

GACELISA

GACELISA

<table>
<thead>
<tr>
<th>Study</th>
<th>Anti-HIV-1 Status</th>
<th>Number</th>
<th>Mean (OD/CO)</th>
<th>Median (OD/CO)</th>
<th>Range (OD/CO)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Connell</td>
<td>Positive</td>
<td>50</td>
<td>8.6</td>
<td>8.9</td>
<td>1.5 – 11.2</td>
</tr>
<tr>
<td>Connell</td>
<td>Negative</td>
<td>127</td>
<td>0.41</td>
<td>0.37</td>
<td>0.27 – 0.90</td>
</tr>
<tr>
<td>Burnet</td>
<td>Positive</td>
<td>99</td>
<td>9.9</td>
<td>9.75</td>
<td>1.37 – 13.21</td>
</tr>
<tr>
<td>Burnet</td>
<td>Negative</td>
<td>105</td>
<td>0.412</td>
<td>0.364</td>
<td>0.221 – 1.218</td>
</tr>
</tbody>
</table>
"Suck it and See…"

- 745 men recruited
  - 36.9% from bars and clubs
  - 48.9% from sex on premises venues
  - 14.2% from gay men’s health clinics
- 100 were positive for anti-HIV antibodies
  - 13.4% 95% CI 11.1 – 16.0
- 20% of the reactive samples were from participants who were previously undiagnosed

"SHANTUSI!"

- In 2010 a second study “SHANTUSI” was conducted by RhED (the sex worker advocate and representative group) investigating HIV in the unregulated sex industry.
  - Total tested n=126
  - Total positive n=1
- Repeatedly reactive on GACELISA
- Positive by Western blot
- (2 samples repeatedly reactive – low S/CO – negative by Western blot)
New Zealand 2011
- Department of Preventive and Social Medicine, University of Otago (Auckland-based), New Zealand
- Total number tested n=1073
  - 837 Big Gay Out (49 Positive)
  - 236 Sex on Site Venues and Bars (18 Positive)

New Zealand Results
- Determine the actual prevalence of HIV and the proportion undiagnosed in a community sample of MSM in Auckland, New Zealand.
- The study was embedded in an established behavioural surveillance programme.
- MSM attending a gay community fair day, gay bars and sex-on-site venues during 1 week in February 2011 who agreed to complete a questionnaire were invited to provide an anonymous oral fluid specimen for analysis of HIV antibodies.
- From the 1304 eligible respondents (acceptance rate 48.5%), 1049 provided a matched specimen (provision rate 80.4%).
- HIV prevalence was 6.5% (95% CI: 5.1-8.1).
- One fifth (20.9%) of HIV infected men were undiagnosed; 1.3% of the total sample.

NHMRC Saliva Study - COUNT
- Run in conjunction with the National Survey
- Option for anonymous or confidential results returned
- ACT, VIC, NSW, WA, SA and QLD
- Increase access to testing
- Determine HIV prevalence
- Determine the number of undiagnosed HIV cases

NHMRC Saliva Study Results - Canberra
- Total number n=87
- Number Positive n=4

NHMRC Saliva Study - Melbourne
- Total number n=1022
  - Confidential n=724
    - Positives n=17
  - Anonymous n=298
    - Positives n=54

NHMRC saliva Study - Sydney
- Total number n=982
  - Confidential n=706
    - Positives n=21
  - Anonymous n=276
    - Positives n=47
NHMRC Saliva Study - Perth

- Total number n=313
- Confidential n=223
- Positives n=5
- Anonymous n=90
- Positives n=16

NHMRC Saliva Study - Brisbane

- Total number n=313
- Confidential n=223
- Positives n=5
- Anonymous n=90
- Positives n=16

NHMRC Saliva Study - Adelaide

- Total number n=379
- Confidential n=279
- Positives n=7
- Anonymous n=100
- Positives n=13

Final COUNT Results

<table>
<thead>
<tr>
<th>COUNT</th>
<th>HIV-positive (by test results)</th>
<th>As % of HIV-positive (by test results)</th>
<th>As % of HIV-positive and undiagnosed (self-reported)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Canberra</td>
<td>86 (5.7) (0.8)</td>
<td>6.3 (0.7)</td>
<td>5.9 (0.8)</td>
</tr>
<tr>
<td>Melbourne</td>
<td>1001 (70.3)</td>
<td>70.3 (6.7)</td>
<td>6.5 (4.0)</td>
</tr>
<tr>
<td>Sydney</td>
<td>375 (21.8)</td>
<td>21.8 (4.6)</td>
<td>4.5 (3.1)</td>
</tr>
<tr>
<td>Perth</td>
<td>310 (20.4)</td>
<td>20.4 (4.5)</td>
<td>3.5 (2.5)</td>
</tr>
<tr>
<td>Adelaide</td>
<td>372 (20.4)</td>
<td>20.4 (4.5)</td>
<td>3.5 (2.5)</td>
</tr>
<tr>
<td>TOTAL</td>
<td>3094 (213)</td>
<td>213 (6.9)</td>
<td>6.0 (6.0)</td>
</tr>
</tbody>
</table>

One potential false negative
- Initially reactive on GACELISA just above the cut-off (1.036)
- On repeat testing just below the cut-off (0.922)

Questionnaire
- Identified as HIV positive
- On treatment
- Anonymous participant could not be followed up

Acknowledgements

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- CPHL UK
- John Parry and Eddie Murphy
- Burnet Institute, UNSW, University of Otago and Rhed
  - Mark Stove, Martin Holt, Peter Saxton, Richard Griffiths, James Rowe etc. etc.
- DiaSorin
- Helen
- NHMRC

Melbourne, Australia

Thank you for your attention!