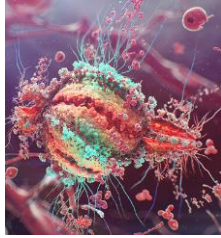


Detection of Anti-HIV Antibodies in Saliva

Kim Wilson
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Scientific Projects Manager
NRL, Melbourne, Australia

Holt M, Lea T, Asselin J, Hellard M,
Prestage G, Wilson D, De Wit J,
Stoové M



“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)



Oral Fluid

- Saliva is a mixture of secretions from
 - the salivary glands
 - and transudate from the capillary bed beneath the buccal mucosa
 - Especially gingival crevicular fluid which flows from the crevice between the gum margin and the tooth.
 - Crevicular fluid has an antibody content similar to that of plasma, with IgG, IgM and IgA
 - But once mixed with salivary gland secretions becomes greatly diluted
 - The IgG concentration of whole saliva is thus ~1000 fold less than that of plasma



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)



WHO/UNAIDS Report 13 Phase 1

Operational Characteristics of Current HIV Assays

- Compared with the GACELISA

Assay	Sensitivity (95% CL)	Specificity % (95% CL)
OraScreen	56.0 (44 – 68)	98.6 95 – 100)
Salivax	79.4 (67 – 89)	96.0 91 – 99)
SMLX	62.7 (51 – 74)	74.8 67 – 82)



WHOUNAIDS Report 13 Phase 1

Review of current literature

- Hodinka et al. Clinical and Vaccine Immunology
<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

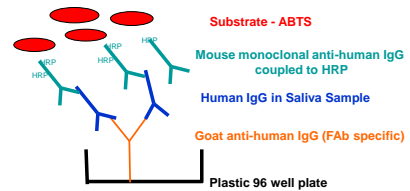


Saliva HIV Confirmatory Testing Algorithm

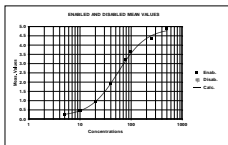
- To ensure that the sample is of adequate quality and contains enough antibody to detect anti-HIV antibodies
 - Total IgG ELISA
- To test for anti-HIV antibodies do a GacElisa
 - Repeat in duplicate if reactive
- Confirm reactive GacElisa result with a saliva HIV Western Blot



Total IgG ELISA



Total IgG Standard Curve



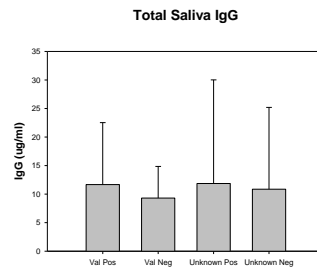
	Conc.	Meas.	CalcCon	Residual
Cal.1	500,000	4.867	959.295	-459.295
Cal.2	250,000	4.361	200.755	-49.245
Cal.3	100,000	3.646	105.700	-5.700
Cal.4	80,000	3.199	80.017	-0.017
Cal.5	40,000	1.854	39.145	0.855
Cal.6	20,000	0.952	20.040	-0.040
Cal.7	10,000	0.466	10.217	-0.217
Cal.8	5,000	0.261	4.971	0.029

Fit type: Sigmoid logistic: $y = b/(a-b)(1+xc)^d$
 Meas. transformation: Linear
 Conc. transformation: Linear
 Parameters: a b c d
 0.157 4.921 0.018 1.573
 Corr. coeff. R2: 0.964

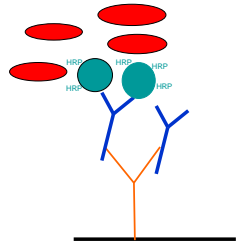
For surveillance purposes 0.1µg/ml IgG was considered adequate



Total Saliva IgG



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

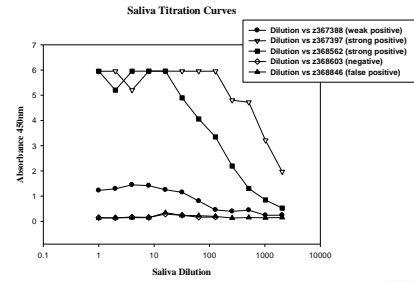
Human IgG in Saliva Sample

Rabbit anti-human IgG

Plastic 96 well plate



Saliva Titration Curves - GACELISA



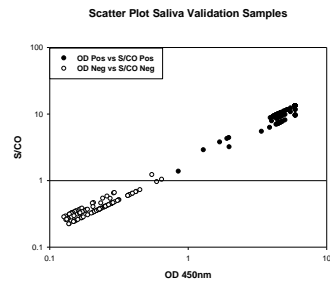
Samples to Validate "in-house" GacElisa

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)

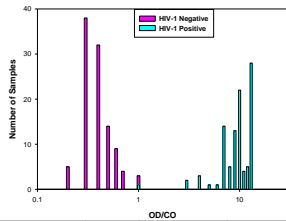


GACELISA on Validation Samples



GACELISA - Validation

HIV-1 Validation Samples

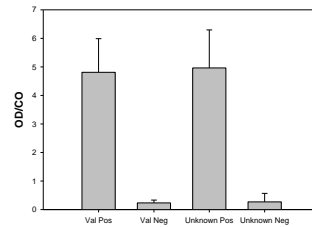


Study	Anti-HIV-1 Status	Number	Mean (OD/CO)	Median (OD/CO)	Range (OD/CO)
Connell	Positive	50	8.6	8.9	1.5 – 11.2
Burnett	Positive	99	9.9	9.75	1.37 – 13.21
Connell	Negative	127	0.41	0.37	0.27 – 0.90
Burnett	Negative	105	0.412	0.364	0.221 – 1.218

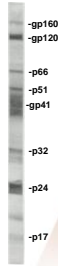
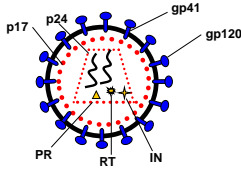
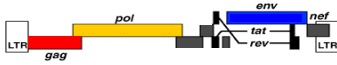


GACELISA

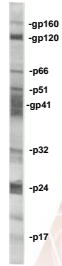
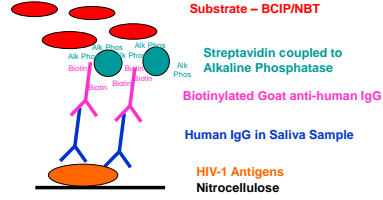
GACELISA



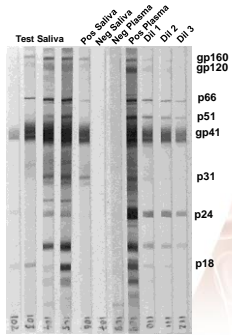
HIV-1 Western Blot



Saliva Western Blot



Saliva HIV Western blot

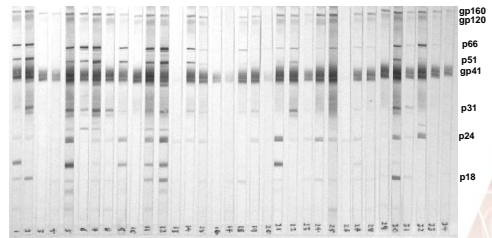


- Genelabs Diagnostic HIV blot (Parry et al.) for saliva
- Presence of gp160 with or without any other band
- For our "in house" Western blot we require the presence of an envelope band (usually gp41) with or without p24



John Parry and Eddie Murphy, Virus Reference Division, Central Public Health Laboratory London, UK

Confirmatory Western Blot on Saliva Samples



"Suck it and See..."

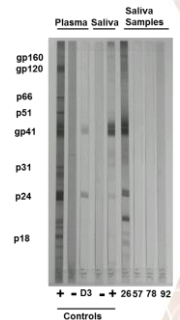


- 745 men recruited
 - 36.9% from bars and clubs
 - 48.9% from sex on premises venues
 - 14.2% from gay men's health clinics
- 100 were positive for anti-HIV antibodies
 - 13.4% 95% CI 11.1 – 16.0
- 20% of the reactive samples were from participants who were previously undiagnosed



"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)

GET IT ON!
BIG
GAY OUT



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.



Saxton et al. BMC Public Health 2012, 12:92
<http://www.biomedcentral.com/1471-2458/12/92>

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



PROUDLY SUPPORTED BY



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

	COUNT participants		Undiagnosed HIV				
	n	n (%)	HIV-positive (by test results)		As % of HIV-negative and untested (self-reported)		
	n (%)	95% CI	n (%)	95% CI	n (%)	95% CI	
Canberra	86	4 (4.7)	1.8-11.4	0 (0.0)	0.0-49.0	0 (0.0)	0.0-4.3
Melbourne	1001	70 (7.0)	5.6-8.7	5 (7.1)	3.1-15.7	5 (0.5)	0.2-1.2
Sydney	948	65 (6.9)	5.4-8.6	7 (10.8)	5.3-20.6	7 (0.8)	0.4-1.6
Perth	310	21 (6.8)	4.5-10.1	4 (19.0)	7.7-40.0	4 (1.4)	0.5-3.5
Brisbane	377	33 (8.8)	6.3-12.0	2 (6.1)	1.7-19.6	2 (0.6)	0.2-2.1
Adelaide	372	20 (5.4)	3.5-8.2	1 (5.0)	0.9-23.6	1 (0.3)	0.1-1.6
TOTAL	3084	213 (6.9)	6.0-7.8	19 (8.8)	5.8-13.5	19 (0.7)	0.4-1.0

- 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

- NRL
 - Jing Jing Cai, Gelinda Nekarine, Susie Braniff, Terri Sahin
- SydPath
 - Philip Cunningham, Craig Leeman and Leon McNally
- CPHL UK
 - John Parry and Eddie Murphy
- Burnet Institute, UNSW, University of Otago and Rhed
 - Mark Stoove, Martin Holt, Peter Saxton, Richard Griffiths, James Rowe etc. etc. etc.
- DiaSorin
 - Helen
- NHMRC



Melbourne, Australia



Thank you for your attention!

