Public Health England

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Comparison of pooled RNA and 4th Gen Ag/Ab testing to identify acute HIV Infection

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Background

- The high viral load associated with early HIV infection and the sensitivity of HIV RNA detection assay means that specimens can be diluted many fold and HIV RNA still be detected
- This allows for the potential cost effective screening of anti-HIV negative specimen to identify acute infection
- Recent introduction of 4th Generation HIV antigen/antibody assays bring US in line with European standards
- British HIV Association Guidelines recommend that patients be screened with a 4th generation HIV Ag/Ab combination assay
- Use of pooled RNA screening is not recommended due to limited advantages in terms of earlier detection over 4th generation assays

OBJECTIVE – In high risk HIV populations does screening of pooled anti-HIV negative specimens for HIV RNA identify more acute infections than testing specimens by individual 4th generation assays?

Case for RNA testing

- First marker to appear – Allows infection to be determined earlier than using antibody or antigen assays
- Pooled RNA testing allows cost effective screening with the ability to identify acute infections
- High level viraemia close to infection minimises the possibility of missing infection through pooling specimens

Case against RNA testing

- Specimens with low viral load may be missed in pooled testing
- It may take many days to obtain the required number of specimens to constitute the pool
- Individual RNA testing is too expensive to be performed on a routine basis
- The reduction in the window period through RNA testing may be negated by slow turnaround times

Study Design

- Samples were selected from residual specimens archived from an Unlinked anonymous survey into HIV prevalence in UK
- The survey monitors HIV Prevalence in 15 STI Clinics in England and Wales using specimens collected for syphilis serology
- Specimens were selected from four centres in London over the period 2005-2008 which had a population demographic known to be at higher risk of acquisition of HIV
- Three of the clinics are in central London area and the other on the eastern outskirts of the city
- 3570 specimens from men who have sex with men (MSM) from 2 STI Clinics
- 1837 specimens from heterosexual men of black African ethnicity 2 clinics
- Specimens were collected from 4 age ranges (20-24, 25-34, 35-44, >45)

Testing Details

- All specimens had been found to be anti-HIV negative by a 3rd generation anti-HIV assay (Murex GE94/95)
- Specimens aliquoted to create mini-pools of 8 specimens each
- An aliquot from up to 6 mini pools was then mixed to create master pools containing a maximum of 48 specimens
- The master pools were then tested for presence of HIV specific RNA (see assay details)
- Reactive pools were broken down to reveal the individual reactive specimen
- RNA positive but 3rd generation Negative specimens were tested with a 4th generation Ag/Ab assay (Murex 7G79-11)

Assay Details

In-house RT-PCR

- Adaptation of an in-house real time quantitative taqman probe based assay developed by Paul Grant at University College Hospital, London
- Assay targets both the *ltr* and *pol* regions of HIV-1; multiplexed with an internal control in the form of BMV RNA
- The assay functions as both a sensitive qualitative and quantitative assay with a range from 100-10,000,000 copies per ml (cpml)

Murex - GE94/95

- 3rd generation assay – detects HIV-1/HIV-2 antibody only

Murex - 7G79-11

- 4th generation combined assay – detects HIV p24 Antigen and HIV antibody

Results

- Of the 5407 anti-HIV negative specimens six were found to be HIV RNA positive (Table 1)
- In 3rd generation antibody only testing the specimens were clearly negative with a range of activity of 0.041 -0.508 OD/CO
- On testing with the fourth generation HIV antigen/antibody combination assay all six were found to be positive (OD/CO ranges 4.66 – 19.64)
- All of the six specimens came from men who have sex with men and five of the six came from one centre
- All the men were aged between 25-44 years Two of the men had an acute STI at the time of sampling (Table 2)

Table 1 – Distribution of Anti-HIV Negative specimens tested for evidence of acute HIV infection

STI Clinic	HIV Antibody Negative Number Tested	Number PCR Positive	Number 4th generation Antigen/Antibody EIA Positive
Centre 1 Outer London Predominately Black African	547	0	0
Centre 2 Inner London Predominately MSM	234	1	1
Centre 3 Inner London Predominately Black African	1290	0	0
Centre 4 Inner London Predominately MSM	3336	5	5
TOTALS	5407	6	6

Table 2 – Demographic description and assay results for anti-HIV negative specimens shown to be acutely HIV infected.

	Year of Collection	PCR Result	Abbott Murex HIV 4th Gen AG/AB Combination (OD/CO)	ABBOTT MUREX HIV 3rd gen Ab only (OD/CO)	Risk Group	Age Group	Acute STI diagnosed at visit
G0704482	2007	pos	4.66	0.293	MSM	25-34 yrs	No
G0707767	2007	pos	19.924	0.372	MSM	35-44 yrs	No
G0706653	2007	pos	19.1	0.295	MSM	35-44 yrs	Yes
G0715760	2007	pos	8.579	0.241	MSM	25-34 yrs	No
G0515075	2005	pos	19.639	0.508	MSM	25-34 yrs	No
G0801548	2007	pos	13.207	0.456	MSM	25-34 yrs	Yes

Conclusion

- This study confirms expected benefits of 4th generation HIV Ag/Ab assays over the 3rd generation for identifying these acute infections
- The recommendation by BHIVA that HIV screening should be performed using 4th Generation assays is supported by our data
- Those with acute infection are at their most infectious and likely to pass on infection thus early recognition of infection is crucial
- In this study, use of individual 4th generation assays negated the need to perform pooled RNA testing of anti-HIV negative specimens
- These results raise concerns for situations where 3rd generation assays are still used as screening test for HIV
- The recent CDC recommendations to use 4th generation bring the US in line with many other countries
- Testing algorithms between the US and Europe are now likely to converge as similar technologies are available

Implications for home / Near patient HIV testing

- This study shows 4th generation antigen/antibody or RNA testing is required to identify acute HIV infections
- However, most home testing kits detect HIV antibody alone
- Those accessing Home HIV tests may do so due to recent high risk activity
- Therefore, home testing may provide false reassurance of HIV status
- In Near patient testing scenarios, sample should be taken from individuals with anti-HIV negative result for Antigen or RNA detection to rule out acute infection
- Patients with reactive NPT results should be referred for HIV confirmation and care

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