Comparison of HIV Oral Fluid and Plasma Testing During Early Infection in a Longitudinal Nigerian Cohort

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The view expressed in this presentation are those of the authors and do not necessarily represent those of the Centers for Disease Control and Prevention.
Background

- Oral fluid (OF) testing more acceptable to some individuals and testing programs
  - Non-invasive specimen collection
  - No biohazard waste
- OraSure device approved for OF collection
- Specimens collected with OraSure tested with Avioq EIA (previously Vironostika) and OraSure WB
Oral Fluid

- Oral mucosal transudates (OMT) collected by OraSure
  - Fluid from capillaries beneath buccal mucosa and at the base of the crevice between teeth and gums
  - Secretory IgA and IgG and IgM that originate from plasma
  - Higher levels of IgG than whole saliva, but less than plasma
  - Estimated to be >1000 fold less IgG than blood in some individuals
Oral Fluid Reported Sensitivity and Specificity

- **OraSure + Vironostika EIA Sensitivity**
  - Range reported – **99.2-99.9%**
  - References:

- **OraSure + Vironostika EIA Specificity**
  - Range reported - **99.2-100.0%**
  - References:
    - Tamashiro and Constantine Bull *WHO* 1994
Avioq HIV-1 Microelisa System

“PERFORMANCE CHARACTERISTICS OF THE ASSAY FOR THE ORAL FLUID SPECIMENS COLLECTED WITH THE ORASURE® COLLECTION DEVICE

Non-Inferiority Study

A small non–inferiority study was performed between Avioq HIV-1 Microelisa System and the licensed comparator Oral Fluid Vironostika® HIV-1 Microelisa System. The study indicated that the Avioq HIV-1Microelisa System was not inferior to that of the licensed comparator Oral Fluid Vironostika® HIV-1 Microelisa System.”

Limitations of the procedure with oral fluid compared with blood specimens

- False negative results occur more frequently maybe due to lower antibody levels in oral fluid
- False positive results also occur more frequently as a result of nonspecific cross-reacting antibodies, and not from an HIV-1 infection

Source -Avioq package insert
Oral Fluid WB

- OraSure Blots
  - Limited published data
  - Serum and oral fluid comparisons
    - Less intense bands
    - Positive rate is lower with oral fluid but in most cases blots are indeterminate and not negative
  - References
    - Gallo et al. JAMA 1997
Objective

The objective of this study was to compare oral fluid and plasma testing in a cohort of HIV seroconverters from Nigeria

- Concordance between plasma and oral fluid?
  - Avioq
  - WB
Methods

- Specimens collected in the Recruiting Acute Case of HIV (REACH) study conducted between May 2003 and March 2010 in Nigeria.

- Testing algorithm for detection of acute HIV infection:
  - Initial screen
    - 2 rapid HIV tests (Determine HIV, Abbott and Unigold HIV, Trinity), if the results showed discordance, Stat-Pak HIV (Chembio) was used as a tie-breaker.
  - Second screen for negative individuals
    - 4 weeks after initial screen
    - Second screen positive individuals were enrolled into the study.

- Sixteen potential acute infections (age range: 18-40 years) were identified from 28,655 persons screened.
  - 14 had multiple longitudinal paired oral fluid/plasma samples
  - 2 had only 1 time-point of paired oral fluid/plasma samples.
Methods

• Longitudinal follow up, blood (EDTA) and OF (OraSure) collected at 7-10 days, 3, 5, 7, 9 weeks, and then 3, 4, 6, 8, 10, 12, 15, 21, 24 months
• All specimens were stored at -80°C (>3 yrs)
• Paired plasma and oral fluid (n= 152 pairs) were tested using Avioq HIV-1 Microelisa System (Avioq, Inc).
• Oral fluid specimens reactive by Avioq were tested by OraSure HIV-1 WB
• Plasma WB (Genetic Systems HIV-1 Western blot) was performed on the corresponding plasma of the first 2 Avioq-OF positive time points
## Characteristics of Sample Population

<table>
<thead>
<tr>
<th>Subtype</th>
<th>Sex</th>
<th>Pregnancy Status</th>
<th>Risk group</th>
</tr>
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<tr>
<td>SC12</td>
<td>CRF02_AG</td>
<td>F</td>
<td>Y</td>
</tr>
<tr>
<td>SC24</td>
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<td>N</td>
</tr>
<tr>
<td>SC61</td>
<td>CRF02_AG</td>
<td>F</td>
<td>Y</td>
</tr>
<tr>
<td>SC11</td>
<td>CRF02_AG</td>
<td>M</td>
<td>NA</td>
</tr>
<tr>
<td>SC28</td>
<td>CRF02_AG</td>
<td>M</td>
<td>NA</td>
</tr>
<tr>
<td>SC16</td>
<td>CRF02_AG/G</td>
<td>F</td>
<td>N</td>
</tr>
<tr>
<td>SC13</td>
<td>G</td>
<td>F</td>
<td>N</td>
</tr>
<tr>
<td>SC17</td>
<td>G</td>
<td>F</td>
<td>Y</td>
</tr>
<tr>
<td>SC20</td>
<td>G</td>
<td>F</td>
<td>N</td>
</tr>
<tr>
<td>SC21</td>
<td>G</td>
<td>M</td>
<td>NA</td>
</tr>
<tr>
<td>SC26</td>
<td>G</td>
<td>M</td>
<td>NA</td>
</tr>
<tr>
<td>SC62</td>
<td>G</td>
<td>M</td>
<td>NA</td>
</tr>
<tr>
<td>SC27</td>
<td>URF</td>
<td>M</td>
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</tr>
<tr>
<td>SC19*</td>
<td>Not done</td>
<td>F</td>
<td>Y</td>
</tr>
<tr>
<td>SC25*</td>
<td>Not done</td>
<td>F</td>
<td>Y</td>
</tr>
</tbody>
</table>

CSW- commercial sex workers, DC- serodiscordant couples, STI-sexually transmitted infection, NO-no identified risk

*only 1 follow-up time point

*Man Charurat et al, J Infectious Disease, 2012:205
Delayed Antibody response with Oral Fluid Compared to Plasma

Gender

- Less variability of delay among men
- SC19 and SC25 with only 1 time-point are not included in the table. The results of the oral fluid and plasma were concordant
- SC17 remained negative by oral fluid up to 20 months, but had intermittent positive and negative results with plasma testing

Female

Male

Pregnant
**SC17** remained negative by oral fluid up to 20 months, but had intermittent positive and negative results with plasma testing.

- **Delay was not associated with HIV subtype**
- **SC17** remained negative by oral fluid up to 20 months, but had intermittent positive and negative results with plasma testing.
## Delayed Antibody response with Oral Fluid Compared to Plasma Viral Load

<table>
<thead>
<tr>
<th>Specimen</th>
<th>RNA copies/ml at first plasma WB positive</th>
<th>Days to first Avioq OF positive</th>
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<tbody>
<tr>
<td>SC26</td>
<td>400</td>
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<td>SC20</td>
<td>16,827</td>
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<td>SC16</td>
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<td>SC62</td>
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<td>294,068</td>
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<td>2982</td>
<td>55</td>
</tr>
<tr>
<td>SC61</td>
<td>38,023</td>
<td>154</td>
</tr>
<tr>
<td>SC29</td>
<td>151,579</td>
<td>174</td>
</tr>
<tr>
<td>SC17</td>
<td>38,850</td>
<td>&gt;200</td>
</tr>
</tbody>
</table>

- No obvious association between viral load and delay in reactivity with oral fluid

*Man Charurat et al, J Infectious Disease, 2012:205*
Hypothesis tested

- Wilcoxon Signed Rank Test
- Hypothesis Test $\mu_{\text{delay}} = 0$
  - $n = 14 \ p < 0.0039$
  - $n = 13(\text{exclude sc17}) \ p < 0.0078$
  - SC17 pregnant female
  - remained negative by oral fluid up to 20 months
  - had intermittent positive and negative serologic results in plasma
  - Detectable viral load
  - Therapy initiated
Western Blot Comparison

• Oral fluid and plasma had concordant final interpretation

• Generally fewer and less intense bands present on oral fluid WBs

<table>
<thead>
<tr>
<th></th>
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<th>gp120</th>
<th>p65</th>
<th>p55</th>
<th>p51</th>
<th>gp41</th>
<th>p40</th>
<th>p31</th>
<th>p24</th>
<th>p18</th>
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<tbody>
<tr>
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<td></td>
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<td></td>
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</tr>
<tr>
<td>42152 OF OF</td>
<td>+</td>
<td>+/-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>NA</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>42152 P</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+/-</td>
<td>+/-</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>++</td>
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</tr>
</tbody>
</table>
Summary

- Avioq results- 14 individuals with longitudinal specimens:
  - 9 (64.3%) showed discordant results during the early course of infection
    - 8 individuals showed a significant delay in antibody response with OF
      - Range: 5 to 174 days (median 44 days, mean 41 days)
    - One pregnant female remained negative by oral fluid up to 20 months,
  - Final interpretations for plasma and OF WB were concordant
  - No apparent association between delayed OF antibody detection and viral RNA copies or subtype
  - Delay was observed in both males and females with males having less variability in period of delay
    - No apparent difference in pregnant and non-pregnant females
Conclusions

- The long-term storage of the oral fluid specimens at -80°C may be a potential limitation of our study.
- Decreased sensitivity of oral fluid testing with Avioq HIV-1 compared to blood-based testing with specimens obtained early after HIV infection.
- Delay in detection was statistically significant.
Conclusions

- Programs that utilize oral fluid testing in populations with increased risk of acute/early HIV infections should be aware of the potential for delayed detection of antibodies
  - Alternative testing methods would likely improve detection of acute/early infections

- Additional studies are needed to further evaluate antibody detection in OF during early HIV infection
  - Different populations (MSM, IDU)
  - Individuals infected with other viral subtypes (B and C)
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