Validity of oral mucosal transudate specimens for HIV testing using enzyme-linked immunosorbent assay in children in Chirrimimani district, Zimbabwe

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Objective. To assess the validity of oral mucosal transudate (OMT) specimens for HIV testing in children using enzyme-linked immunosorbent assay (ELISA).

Methods. A cross-sectional descriptive study was conducted as part of a community-based behavioural and HIV sero-status survey of adults and children in the Chimanimani district of Zimbabwe. Dried blood spot (DBS) and OMT samples were collected from children aged between 2 and 14 years, inclusive. Both samples were tested for HIV using the Vironostika Uniform II plus O kits. The main study outcomes were the sensitivity and specificity of OMT specimens, with DBS as the gold-standard specimen.

Results. Paired DBS and OMT specimens were available from 1274 (94.4%) of the 1350 children enrolled. Using the DBS, HIV prevalence was 3.2%. Overall sensitivity of OMT was 48.8% (95% confidence interval (CI) 33.3 - 64.5), and specificity was 98.5% (95% CI 97.7 - 99.1).

Conclusion. The overall sensitivity of OMT specimens for HIV testing in children using ELISA was low. Stratifying the analysis by sector showed that OMT samples are good specimens for HIV testing. It is important to note that factors such as the low HIV prevalence in our study population, quality of the OMT, diet and oral hygiene could have influenced the results.
sensitivity and specificity in HIV diagnosis using the Vironostika Uniform II commercial HIV-1/2 plus O kit.

**Methods**

This study was part of a 2004 cross-sectional behavioural risk and HIV sero-status survey conducted in Chimanimani district, Zimbabwe. Children between the ages of 2 and 14 years, inclusive, were enrolled. A structured questionnaire was administered by research assistants to parents/guardians of the 2 - 11-year-old children and directly to children aged 12 - 14 years.

**Specimen collection and testing**

DBS specimens were collected on Whatman No.3 filter paper, following instructions from Whatman International. The blood spots were air-dried for at least 15 minutes away from direct sunlight and placed in appropriately labelled paper envelopes. OMT specimens were collected using the OraSure HIV-1 Oral Specimen Collection Device, following the manufacturer’s instructions (Organon Technika, Netherlands). Before transportation to the laboratory, the DBS specimens were stored in a cool, dry place and the OMT specimens at room temperature. OMT specimens have been shown to be stable at temperatures below 37°C for at least 21 days. In the laboratory, all specimens were stored in a cool, dry place and the OMT specimens at room temperature. OMT specimens have been shown to be stable at temperatures below 37°C for at least 21 days. In the laboratory, all specimens were stored at -20°C.

All OMT specimens were tested within 6 weeks of collection. The Vironostika Uniform II plus O (BioMérieux, Netherlands) commercial ELISA kit, which is licensed for use with the OraSure collection device, was used to test for the presence of HIV antibodies. A similar Vironostika Uniform II kit routinely used with blood products was used to assay the DBS following the manufacturer’s instructions.

**Quality control**

Quality and quantity of specimens were checked in the field and in the laboratory. All specimens were transferred to the laboratory within 14 days of collection. Quality checks included verification of labelling, quantity of each specimen, and checking for contamination of specimens.

Sufficiency of the DBS was determined by the size of the spot and whether the filter paper was soaked. A white filter-paper punch was used to assess intra-run and inter-run variation. In-house controls were also used, and Levy-Jennings charts were constructed (results not shown). Any analytical run (ELISA plate) that violated the standards was rejected, regardless of results obtained for other control materials. The controls were also sent to an external laboratory as part of a national programme of the Zimbabwe National Quality Assurance Programme.

**Ethical considerations**

The study was approved by the Medical Research Council of Zimbabwe and by local and traditional leaders in Chimanimani. Participation in the study and collection of both the DBS and OMT specimens was voluntary. Written informed consent was sought from the children's parents/guardians and assent from the children. HIV testing was anonymous, although bar codes were used in order to link the questionnaire, DBS and OMT specimens. Parents/guardians who wanted to know the results of the tests were referred to the nearest district voluntary counselling and testing centre.

**Data analysis**

Data were entered using EPI Info version 6 and analysed using both SPSS 8.0 and STATA 7.0. Descriptive statistics were presented as medians (with lower and upper quartiles) for interval data and frequencies for categorical data. The chi-square test for association was used to check for associations between categorical variables. Evaluation of sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and test efficiency (TE) was done to determine the validity of the OMT specimen in HIV testing with DBS as the gold standard. TE refers to the overall ability of a test to correctly identify all positives and negatives, i.e. total effectiveness of an assay.

A detailed description of the methodology is provided in a report by Gomo et al.

**Results**

**Characteristics of the study population**

A total of 1 819 children were approached; 1 350 (74.2%) agreed to participate and provided DBS specimens. Of the 1 350 DBS specimens collected, 1 290 (95.6%) were considered sufficient for HIV testing. OMT specimens were collected from 1 335 (73.4%) of the 1 819 children who were approached. Of these, 1 334 (99.9%) were considered sufficient for HIV testing. A total of 1 274 children provided specimens that were adequate as both DBS and OMT samples, and the remainder of the analysis presented refers only to these children (Fig. 1).

The median age of the 1 274 children who had paired DBS and OMT specimens was 10 years (lower quartile 5, upper quartile 13), and 46.6% were aged 12 - 14 years. There was an equal distribution of males and females (Table 1), and 49.4% of children were from the communal areas and 24.6% from the resettlement areas.

**HIV prevalence**

Using the DBS as the gold standard test, HIV prevalence was 3.2% overall. Prevalence was highest in the 6 - 8-year age group, but the difference according to age group was not statistically significant ($p=0.168$). The prevalence was twice as high in females as in males (4.2 v. 2.2%; $p=0.040$).

![Fig. 1. Flow chart showing recruited participants.](image-url)
These specimens are difficult to collect from children, especially specimens for HIV diagnosis in Zimbabwe and the rest of the world. Serum, plasma or dried blood spots are the most commonly used Discussion

PPV to 89.0%. 86.5), and specificity increased to 99.7% (95% CI 99.2 - 99.9) and excluding the two wards gave a sensitivity of 70.4% (95% CI 49.8 - 95%). Further analysis was higher than the overall for all the sectors except the urban sector predictive value 98.3%.

Overall sensitivity of OMT was therefore low (48.8%), while specificity was high (98.5%) (Table III). The test efficiency was 96.8% (95% confidence interval (CI) 95.8 - 97.8). At a prevalence of 3.2%, the positive predictive value (PPV) was 52.4% and the negative predictive value 98.3%.

Sensitivity was low (less than 70%) in most of the sectors, but it was higher than the overall for all the sectors except the urban sector (take note of the large CIs). Two wards (one rural, one urban) had much higher discordant results than other wards. Further analysis excluding the two wards gave a sensitivity of 70.4% (95% CI 49.8 - 86.5), and specificity increased to 99.7% (95% CI 99.2 - 99.9) and PPV to 89.0%.

Discussion

Serum, plasma or dried blood spots are the most commonly used specimens for HIV diagnosis in Zimbabwe and the rest of the world. These specimens are difficult to collect from children, especially infants. OMT collection is less invasive than blood collection, may have fewer culturally linked misconceptions, and may therefore be more acceptable than DBS, serum and plasma.26 It could therefore increase the participation rate among children.

Sensitivity, specificity and other diagnostic parameters of OMT were assessed using DBS as the gold standard specimen. Overall sensitivity of OMT specimens was very low at 48.8%, but specificity was high (98.5%). While low sensitivity for children has also been observed in another study,4 sensitivities above 80% among infants were found in two studies.5,7 The prevalence of HIV infection in the latter was much higher (about 11%) than that observed in this study (3.2%), so the low HIV prevalence could have influenced the sensitivity of the OMT specimens.13 The low sensitivity of OMT specimens in HIV testing in children in this study could have resulted from the children moving during specimen collection, so that saliva instead of OMT may be a good specimen for HIV testing. The reasons for the low sensitivity include dietary factors and oral hygiene. It is worth noting that the test efficiency, which refers to the total effectiveness of an assay, was high (96.8%).

Conclusion

The overall sensitivity of OMT specimens for HIV testing in children using ELISA was low. Stratifying the analysis by sector showed that OMT may be a good specimen for HIV testing. The reasons for the sectoral and age differences could be technical or biological, and require further investigation. It is important to note that factors such as low HIV prevalence in our study population, quality of the OMT, diet and oral hygiene may have influenced the results.

We sincerely thank the participants and the local authority leaders, without whom this study could not have been successful. We also thank the W K Kellogg Foundation for funding the study, and the Human Sciences Research Council, South Africa, the Biomedical Research and Training Institute and the National Institute of Health Research of the Ministry of Health and Child Welfare, Zimbabwe, for the resources they provided. Special mention goes to Mrs J Mutsangwanga, Mrs J Magwenzi,

<table>
<thead>
<tr>
<th>Sector</th>
<th>Communal</th>
<th>Resettlement</th>
<th>LSC</th>
<th>SSC</th>
<th>Urban</th>
<th>Overall</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>629</td>
<td>313</td>
<td>152</td>
<td>63</td>
<td>117</td>
<td>1 274</td>
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<tr>
<td>DBS neg. + OMT neg.</td>
<td>602 (95.7)</td>
<td>305 (97.4)</td>
<td>145 (95.4)</td>
<td>62 (98.4)</td>
<td>98 (83.8)</td>
<td>1 213 (95.2)</td>
</tr>
<tr>
<td>DBS pos. + OMT pos.</td>
<td>9 (1.4)</td>
<td>5 (1.6)</td>
<td>4 (2.6)</td>
<td>1 (1.6)</td>
<td>2 (1.7)</td>
<td>21 (1.7)</td>
</tr>
<tr>
<td>DBS pos. + OMT neg.</td>
<td>9 (1.4)</td>
<td>0 (0.0)</td>
<td>1 (0.7)</td>
<td>0 (0.0)</td>
<td>8 (6.8)</td>
<td>18 (1.4)</td>
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<tr>
<td>DBS neg. + OMT pos.</td>
<td>9 (1.4)</td>
<td>3 (1.0)</td>
<td>2 (1.3)</td>
<td>0 (0.0)</td>
<td>9 (7.7)</td>
<td>22 (1.7)</td>
</tr>
</tbody>
</table>

LSC = large-scale commercial; SSC = small-scale commercial; neg. = negative; pos. = positive.
Mrs M Manyema, Mr W Soko, Mr H Bariri and the research assistants for their assistance in collecting the data and specimens as well as the testing.

Table III. Sensitivity of OMT according to age group and sector

<table>
<thead>
<tr>
<th>Sector</th>
<th>Sensitivity (% (95% CI))</th>
<th>Specificity (% (95% CI))</th>
<th>PPV (%)</th>
<th>NPV (%)</th>
<th>Test efficiency (% (95% CI))</th>
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<tr>
<td>Overall</td>
<td>48.8 (33.3 - 64.5)</td>
<td>98.5 (97.7 - 99.1)</td>
<td>52.4</td>
<td>98.3</td>
<td>96.8 (95.8 - 97.8)</td>
</tr>
<tr>
<td>Age group</td>
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<td></td>
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<tr>
<td>2 - 5</td>
<td>38.5 (13.9 - 68.4)</td>
<td>99.0 (97.2 - 99.8)</td>
<td>52.4</td>
<td>98.3</td>
<td>96.6 (94.6 - 98.6)</td>
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<tr>
<td>6 - 8</td>
<td>77.8 (40.0 - 97.2)</td>
<td>98.3 (95.2 - 99.7)</td>
<td>74.1</td>
<td>98.6</td>
<td>97.3 (95.0 - 99.6)</td>
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<tr>
<td>9 - 11</td>
<td>42.9 (9.9 - 81.6)</td>
<td>100 (97.7 - 100.0)</td>
<td>100</td>
<td>99</td>
<td>97.6 (95.3 - 99.9)</td>
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<tr>
<td>12 - 14</td>
<td>42.9 (17.7 - 71.1)</td>
<td>97.9 (96.4 - 98.9)</td>
<td>39</td>
<td>98.2</td>
<td>96.5 (95.0 - 97.9)</td>
</tr>
<tr>
<td>Sector</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Communal</td>
<td>52.9 (27.8 - 77.0)</td>
<td>98.5 (97.2 - 99.3)</td>
<td>50.9</td>
<td>98.6</td>
<td>97.3 (96.0 - 98.6)</td>
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<tr>
<td>Resettlement</td>
<td>62.5 (24.5 - 91.5)</td>
<td>100.0 (98.8 - 100.0)</td>
<td>100</td>
<td>99.3</td>
<td>99.0 (98.0 - 100.0)</td>
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<tr>
<td>LSC</td>
<td>66.7 (22.3 - 95.7)</td>
<td>99.3 (96.2 - 100.0)</td>
<td>76.9</td>
<td>98.9</td>
<td>98.0 (95.8 - 100.2)</td>
</tr>
<tr>
<td>SSC</td>
<td>100 (25.0 - 100.0)</td>
<td>100 (94.2 - 100.0)</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Urban</td>
<td>18.2 (2.3 - 51.8)</td>
<td>92.5 (85.7 - 96.7)</td>
<td>19.2</td>
<td>92</td>
<td>85.5 (79.1 - 91.9)</td>
</tr>
</tbody>
</table>

PPV = positive predictive value; NPV = negative predictive value; LSC = large-scale commercial; SSC = small-scale commercial.

References


Accepted 9 August 2010.