Low volume HIV-1 neutralisation assay

**Issue**

The evaluation of vaccine efficacy in humans during clinical studies calls for assessing the presence of HIV neutralising antibodies in vaccinees. However, *in vitro* testing of precious biological samples available in limited quantity may be challenging. Here we describe an adaptation of our *in vitro* neutralisation assay to accommodate such low sample volumes.

**Solution**

In our modified format, antibody source (plasma, sera, ...) is first incubated with virus and reporter cells\(^*\) in a 20 µL volume for several hours. Afterwards, antibodies and viruses are removed, replaced with normal culture medium and cell culture is resumed for several days before to proceed to reporter quantitation.

\(^*\): In our reporter cell line (CD4\(^+\), CXCR4\(^+\), and CCR5\(^+\)) HIV replication is monitored by a quantitative reporter induction.

**Validation**

Neutralisation assay was performed using both normal and modified format. The inhibition parameters are reported in the table below:

<table>
<thead>
<tr>
<th></th>
<th>High volume</th>
<th>Low volume</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>EC(_{50})</td>
<td>EC(_{50})</td>
</tr>
<tr>
<td>2F5</td>
<td>6</td>
<td>165</td>
</tr>
<tr>
<td>4E10</td>
<td>3.4</td>
<td>233</td>
</tr>
</tbody>
</table>

The tested modification led to non-significant variations, within the assay limits. Therefore, it was further assessed using clonal viruses of different subtypes and plasma from HIV-infected long-term non-progressor patients (see figure 1).

![Figure 1](image)

**Figure 1.** Validation of the modified assay using HIV-1 clones of various subtypes and plasma from long-term non-progressors. Inhibition curves were fitted using Xlfit software and inhibition parameters derived from the fitted curves.

**Conclusion**

We have successfully adapted our HIV neutralisation assay to accommodate low sample volume. Using this format, 6 µL of biological samples allow for the testing of 4 recombinant viruses along with 2 specificity controls (dose-response curves).