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.

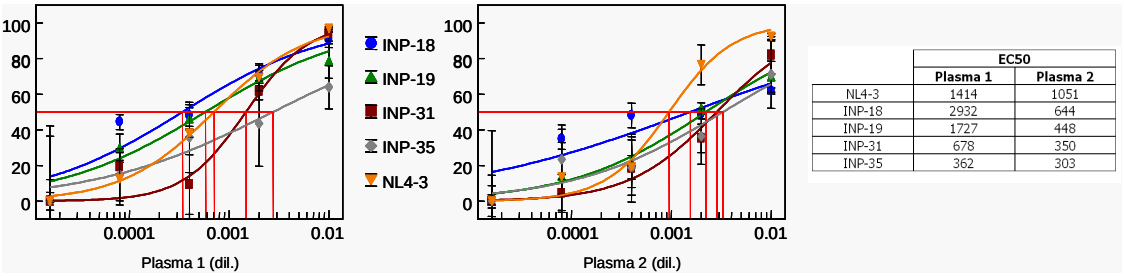
(*): In our reporter cell line ($CD4^+$, $CXCR4^+$, and $CCR5^+$) HIV replication is monitored by a quantitative reporter induction.

Validation

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

	High volume		Low volume	
	EC_{50}	EC_{90}	EC_{50}	EC_{90}
2F5	6	165	10	186
4E10	3.4	233	4.7	228

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-progessor patients (see figure 1).



	EC50	
	Plasma 1	Plasma 2
NL4-3	1414	1051
INP-18	2932	644
INP-19	1727	448
INP-31	678	350
INP-35	362	303

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 succesfully adapted our HIV neutralisation assay to accodomate 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).

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