Reviewer's report

Title: An improved microtiter assay for evaluating anti-HIV-1 neutralizing antibodies from sera or plasma

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Reviewer: David Montefiori

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The authors describe a minor variation of standard neutralization assays for HIV-1 that aimed to minimize the cellular toxicity and non-specific antiviral activity of serum and plasma samples. The MTT assay was preferred over the neutral red assay because of fewer steps involved and problems encountered with the solubility of neutral red. The authors noted a growth advantage of C8166 cells in the presence of low dilutions of serum and plasma test samples. They also noticed cellular toxicity in some cases. Both are common occurrences in these assays. They modified the assay procedure to remove the serum or plasma samples by a series of washes several hours after the addition of virus and cells to minimize these effects. This modification was shown to be effective in both cases.

Growth advantage of high serum concentration is minor in most cases and rarely affects the outcome of neutralization measurements. This would have become apparent to the authors had they tested normal human serum for neutralizing activity. Although they conclude that growth advantages over-estimate neutralizing activity, the converse may also be true. That is, the decreased neutralization titer detected after their wash-out procedure could be interpreted as an underestimate of neutralizing activity, since antibody was absence as non-neutralized progeny virions were produced.

The growth-inhibitory properties of their samples is almost certainly due to the presence of anti-coagulants in plasma samples. Most anti-coagulants (e.g., EDTA, heparin, ACD) are toxic to cells to a plasma dilution of about 1:60 when standard tubes are used for blood collection. This again is a well-known phenomenon that can be avoided by either collecting serum (no anticoagulants), testing plasma at higher dilutions or performing a washout procedure similar to what was described in this report (and as described elsewhere).

In summary, the authors appear to have made significant progress adopting standard neutralization assays in their laboratory. However, the modification described here is minor and, in fact, generally practiced by other laboratories.

What next?: Reject because too small an advance to publish in any journal

Level of interest: Too insignificant to warrant publication in any journal
Quality of written English: Needs some language corrections before being published

Statistical review: No

Declaration of competing interests: None