Author’s response to reviews

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

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PDF covering letter
The response to reviewer's comments (original comments are listed in parenthesis)
The revision in manuscript has been performed according to the reviewer’s advice.

1. For Reviewer 1 (David Montefiori)
   As he indicated, the MTT assay that we used was preferred over the neutral red assay that the reviewer used to recommend.

2. For Reviewer 2 (Ruengpung Sutthent)
   1) (Normal human sera/plasma control on viability of cells)
      Sorry, we didn’t test the normal human sera/plasma.
   2) (HIV positive sera/plasma used in the tested from IDUs, that always consist of inhibitors.)
      Yes, we agree. In present study, the plasma from non-IDU and IDU were tested. Our experimental results demonstrated that elimination of these inhibitors’ effects by washout procedure is necessary for microtiter neutralization assay.
   3) (Are these infected HIV-1 individuals on any treatment that might effect viability of cells.)
      No, they were not treated.
   4) (For end-point neutralization assay by p24 Ag measurement, the normal procedure normally use 10-50 TCID50. What is the % level of inhibition that used for cutoff for protection (50% or 90%)? Why don't use 4 days for p24 AG detection similar to MTT assay.)
      We generally used 90% as the cutoff for protection. 50% was used for the cutoff by other researchers. The microtiter neutralization assay was established on the base of the quantification of virus-induced extensive cytolysis of host cells. The P24 antigen assay was based on detecting the reduction of p24 antigen production caused by NAbs. Therefore, the p24 AG detection should be performed at the initial phase of virus production. If p24 AG detection is performed at the time point of MTT assay, cytolysis occurred in approximately 80% of C8166 cells in virus control wells and less virus production would result in inaccuracy of HIV-1 neutralization.
5) (For discussion part, the authors should mention that more will be used on one replication cycle NT assays that will eliminate prolong time exposure cells to virus and sera.)

The discussion on the point has been added in page 9 of the manuscript.

3. For Reviewer 3 (Preston A. Marx)

1) (The principal weakness is the lack of an HIV-1 seronegative plasma/serum as a control in the experiments)

Sorry, we did not use HIV-1 seronegative plasma as controls.

2) (What are the results with serum from a recent seroconvertor?)

The neutralizing antibody positive control plasma PC-YN06 is screened from a blood donor, and is considered as a recent seroconvertor.

3) (Figs 2 and 3 present an index of cell proliferation rather than % cell proliferation. The label on the Y axis is therefore confusing.)

The label on the Y axis in Fig.2 was revised, but for Fig.3, Y axis shows the neutralization protection of plasma.

4) (The manuscript will benefit from revision by a native English speaker.)

We revised our manuscript again.

5) (It was not made clear whether plasma or sera was used in a particular test. Did sera and plasma yield different results in Figures 2 and 3?)

We did not perform the experiment using human sera, because both plasma and sera contain those unknown factors that affected cell growth, and sera and plasma would yield similar results in Figures 2 and 3.