Letters to the Editor

Pseudoparticle Neutralization Assay for Detecting Ebola-Neutralizing Antibodies in Biosafety Level 2 Settings

To the Editor:

Ebola virus is currently causing an unprecedented outbreak in West Africa. The pathogen is classified as a biosafety level 4 (BSL-4)[1] pathogen and should be handled only in high-level biosafety settings *(1)*. Virus neutralization is considered to be the gold standard serological assay for infections, but in the case of Ebola, this test requires culturing of live virus in a BSL-4 facility. Because of these stringent biosafety requirements, options for clinical diagnosis or epidemiological studies of Ebola cases are limited. For this reason, we developed a pseudoviral particle neutralization (PPNT) assay that can detect neutralizing antibody to Ebola virus in BSL-2 containments.

Ebola glycoprotein (GP) is a major target of neutralizing antibodies in humans. The pseudoviral particle generated in this study is a lentiviral vector carrying the GP of *Zaire ebolavirus* detected in the current outbreak *(2)*. Methods for the pseudoviral particle production and PPNT assay were essentially identical to those for Middle East respiratory syndrome (MERS) and influenza, as previously described *(3, 4)*. Briefly, a codon-optimized GP sequence was chemically synthesized (Genscript) and subcloned into a protein-expressing vector, pcDNA3.1+. The resulting pseudoviral particle is capable of achieving only a single-round infection and contains a luciferase reporter gene that can be expressed in infected cells. Anti-GP neutralizing antibodies can inhibit the entry of this pseudoviral particle into cells, thereby inhibiting the expression of luciferase in the assay. For each Ebola pseudoviral particle production, the preparation was titrated and diluted to a level that could produce 10000–50000 counts/s in a positive infection control reaction in the luciferase assay (Steady-Glo Luciferase Assay System, Promega). In contrast, mock-infected cells (i.e., cells treated in the absence of pseudoviral particle) yielded background signals in the assay (<1% of the positive infection control) (Table 1).

For a typical PPNT assay for serological screening, 5 μL heat-treated serum sample (56 °C, 30 min) was incubated with the diluted pseudoparticles in a 100-μL reaction at 4 °C for 60 min before inoculation onto Vero cells *(3, 4)*. The luciferase activities of infected cells were examined 72 h postinfection. Based on our previous experiences with similar assays *(3, 4)*, the cutoff value for a positive reaction was set to be 10% of the value deduced from the positive infection control (see above). For determining the neutralization antibody titer of a serum sample, 2-fold serially diluted samples of a specimen (starting dilution 1:20) were used in the PPNT assay. Thus, a sample with a neutralizing antibody titer of 1:20 was considered a positive specimen.

To determine whether the PPNT assay was reactive to Ebola virus, we tested 2 neutralizing monoclonal antibodies specific for *Z. ebolavirus* GP, mAb133/316 and mAb266/8.1 *(5)*. Both antibodies could neutralize the pseudoviral particle in a dose-dependent manner (Table 1). We also evaluated this test using serum samples collected from humans and animals (Table 1). All of these serum samples were tested at a screening dilution of 1:20 (1 part in 20 parts) and were negative in the assay (i.e., >10% of the value deduced from the positive infection control). The studied human samples were collected from healthy individuals in Hong Kong where no Ebola case has been reported in history. In addition, the studied animal species are not known to be susceptible hosts for Ebola viruses. Overall, these results suggest that this PPNT assay was specific to neutralizing antibodies against Ebola virus.

This study has some limitations, and further evaluations of the assay are needed. In particular, we were able to use only 2 Ebola virus–specific neutralizing monoclonal antibodies and negative human/animal serum samples for our evaluations. The studied monoclonal antibodies target 2 different conserved regions of *Z. ebolavirus* GP. One of these antibodies (mAb226/8.1) is known to bind to a conformational epitope of *Z. ebolavirus* GP *(5)*, indicating that the GP of the pseudoviral particles is correctly folded. We were not able to obtain convalescent sera from Ebola patients to evaluate our assays, however; such convalescent human serum samples are difficult to obtain because of limited availability, legal issues, and biosafety concerns. Nonetheless, it is important to evaluate this assay in the future using convalescent sera known to be IgG- or IgM-positive for Ebola virus.

Our assay has potential applications in studies related to seroepidemiology, passive immunotherapy, and vaccine immunogenicity. To avoid laboratory-acquired infections, we emphasize that all serum samples from confirmed or suspected cases must be fully inactivated before downstream processing *(1)*.

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Table 1. Characterization of Ebola virus–specific neutralizing monoclonal antibodies, human sera, and animal sera in the Ebola PPNT assay.[a]

Sample and dilution	Luciferase activity, %[b]	
	Mean (SD)	Range
Positive infection control	100	
Mock-infection control	0.10 (0.03)	0.13–0.07
mAb133/316		
640	0.92 (0.06)	0.88–0.99
5120	1.79 (1.56)	0.88–3.60
10240	3.18 (2.66)	0.86–6.09
20480	10.59 (5.01)	6.31–16.10
40960	16.88 (9.20)	7.11–25.36
81920	56.03 (35.26)	29.97–95.61
163840	79.93 (46.38)	31.92–124.50
327680	113.28 (14.33)	96.78–122.61
mAb266/8.1		
640	0.82 (0.03)	0.80–0.86
5120	1.05 (0.56)	0.73–1.70
10240	7.13 (7.50)	0.75–15.39
20480	11.17 (3.57)	7.51–14.65
40960	13.02 (3.64)	9.42–16.70
81920	49.40 (5.33)	43.75–54.33
163840	110.54 (51.12)	53.22–151.41
327680	84.36 (36.1)	42.76–107.34
Human (n = 61)	55 (21)	19–113
Buffalo (n = 5)	34 (9)	22–46
Camel (n = 5)	30 (6)	23–39
Goat (n = 5)	80 (18)	52–100
Sheep (n = 5)	68 (16)	52–90

[a] Each sample was tested in triplicate, and the mean value of the triplicate reactions was used in the subsequent analysis. Dilution is expressed as fold dilution in final reaction volume. Human, buffalo, camel, goat, and sheep were all at 20-fold dilution.

[b] Luciferase activity is expressed relative to cells treated with pseudoviral particle only (positive infection control).

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Efficient Detection of *BRAF* Mutation in Plasma of Patients after Long-term Storage of Blood in Cell-Free DNA Blood Collection Tubes

To the Editor:

The improvement of sensitive techniques has allowed tumor-specific aberrations in circulating tumor DNA (ctDNA)[1] to be detected, in-

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