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Title	Development of an Immunochromatography Assay (QuickNavi-Ebola) to Detect Multiple Species of Ebolaviruses
Author(s)	Yoshida, Reiko; Muramatsu, Shino; Akita, Hiroshi et al.
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Supplementary Table 1. Absence of cross-reactivity of the IC assay to various human viral and bacterial pathogens.

			Interpretations[a]	
Pathogen	**Strain/Type**	**Titer**	**VLP (–)**	**VLP (+)**
Chikungunya virus	SL10571	6.0×10^{8} PFU/mL	– – –	+ + +
West Nile virus	NY99-6LP	1.4×10^{9} PFU/mL	– – –	+ + +
Rabies virus	CVS	1.0×10^{8} PFU/mL	– – –	+ + +
Junin virus	Candid#1	1.5×10^{5} PFU/mL	– – –	+ + +
Lassa virus	Josiah	5.0×10^{5} $TCID_{50}$/mL	– – –	+ + +
Crimean-Congo hemorrhagic fever virus	10200	2.0×10^{5} $TCID_{50}$/mL	– – –	+ + +
Influenza A virus	H1N1 pdm	5.0×10^{6} PFU/mL	– – –	+ + +
Influenza B virus	B7 Shandong/7/97	1.0×10^{5} PFU/mL	– – –	+ + +
Adenovirus	NIH Adeno-3	3.2×10^{8} $TCID_{50}$/mL	– – –	+ + +
Respiratory virus type A	A2-003	3.2×10^{7} $TCID_{50}$/mL	– – –	+ + +
Respiratory virus type B	CH-18-2	3.2×10^{4} $TCID_{50}$/mL	– – –	+ + +
Dengue virus serotype 1	Mochizuki	8.9×10^{4} FFU/mL	– – –	+ + +
Dengue virus serotype 2	16681	1.8×10^{5} PFU/mL	– – –	+ + +
Dengue virus serotype 3	H87	2.1×10^{5} PFU/mL	– – –	+ + +
Dengue virus serotype 4	H241	2.5×10^{5} PFU/mL	– – –	+ + +
Rotavirus	unknown	1.0×10^{8} $TCID_{50}$/mL	– – –	+ + +
Streptococcus pneumonia	Type 3	1.0×10^{8} CFU[b]/mL	– – –	+ + +
Haemophilus influenza	Type A	1.0×10^{8} CFU/mL	– – –	+ + +
Haemophilus influenza	Type B	1.0×10^{8} CFU/mL	– – –	+ + +
Staphylococcus aureus	ATCC25923	1.0×10^{8} CFU/mL	– – –	+ + +
Salmonella Typhimurium	unknown	1.0×10^{8} CFU/mL	– – –	+ + +
Citrobactor freundii	unknown	1.0×10^{8} CFU/mL	– – –	+ + +
Shigella sonnei	unknown	1.0×10^{8} CFU/mL	– – –	+ + +
Vibrio cholerae	O1 Ogawa	1.0×10^{8} CFU/mL	– – –	+ + +
Plasmodium falciparum	3D7 strain	7% parasitemia[c]	– – –	+ + +

[a] The indicated viruses and bacteria were diluted, with (+) or without (-) EBOV VLP, in the human plasma or whole blood at the indicated titers, and then applied to the IC device The results were recorded after 10 minutes. [b] Colony-forming unit, [c] 50% Ht. Each experiment was performed in triplicate.

Supplementary Table 2. Performance evaluation of the IC assay under adverse ambient environmental conditions

VLP dilution[a]		Low temp.	Low humidity				High humidity		
	Temperature	4℃	25℃	45℃	50℃	55℃	25℃	45℃	50℃
	Relative humidity	69.0%	26.6%	26.6%	20.8%	26.6%	92.8%	92.0%	91.0%
x1280		+++[b]	+++	+++	+++	+++	+++	+++	+++
x2560		+++	+++	+++	+++	+++	+++	+++	+++
x5120		+++	+++	+++	+++	−−−	+++	+++	−−+
x10240		−−−	−−−	++−	−−−	−−−	−−−	−−−	−−−
No VLP		−−−	−−−	−−−	−−−	−−−	−−−	−−−	−−−

The IC devices were removed from the packaging under the above mentioned conditions and then were immediately used. [a] The EBOV VLPs were diluted in human plasma, and then 30 μl of the diluted sample was applied to the IC device, and the results were recorded after 10 min. [b] Three IC devices were used for each indicated dilution and condition; hence one + or − symbol indicates one device.

Supplementary Table 3. Effect of exposure duration to adverse conditions on the IC performance using human plasma

VLP dilution[a]		Mild		Tropical	Severe				Control[c]
	Temperature	23℃		35℃	46-51℃				
	Relative humidity	62%		88%	90-98%				
	Exposure time[b]	30 min	60 min	65 min	10 min	15 min	20 min	25 min	
x1280		+++[d]	+++	+++	+++	+++	+++	+++	+++
x2560		+++	+++	+++	+++	+++	+++	+++	+++
x5120		+++	+++	−−−	+++	+++	+++	+++	+++
x10240		−−−	−−−	−−−	−−−	−−−	+++	−−−	−−−
No VLP		−−−	−−−	−−−	−−−	−−−	−−−	+++[e]	−−−

[a] The EBOV VLPs were diluted in human plasma and then 30 μl of the diluted sample was applied to the IC device, and the results were recorded after 10 min. [b] The IC devices were removed from the packaging and exposed to the indicated conditions, and then used for the detection of VLPs at various concentrations. [c] The samples were tested at room temperature. [d] Three IC devices were used for each indicated dilution and condition; hence one + or − symbol indicates one device. [e] Under the severe condition (46-51 °C temperature, 90-98% humidity), antibody-conjugated latex in the conjugate pad, one of the critical component of the IC test strip, might be nonspecifically aggregated due to high humidity, causing this false-positive reaction.

Supplementary Table 4. Effect of exposure time to adverse ambient conditions on the IC performance using human whole blood

VLP dilution[a]		Mild	Tropical
	Temperature	23℃	35℃
	Relative humidity	62%	97%
	Exposure time[b]	65 min	65 min
x160		+ + +	+ + +
x320		+ + +	+ + +
x640		+ + +	+ + +
x1280		– – –	– – –
No VLP		– – –	– – –

[a] The EBOV VLPs were diluted in human whole blood and then 10 μl of the diluted sample was applied to the IC device, and the results were recorded after 10 min. Note that the applied volume of the whole blood samples was less than those of the plasma samples shown in Supplementary Table 3 and that the whole blood samples only contained approximately 50% volume of plasma. Thus, the number of VLPs applied with the whole blood samples were approximately 1/6 of that with the plasma samples. [b] The IC devices were removed from the packaging and exposed to the indicated conditions, and then were used for the detection of VLPs at various concentrations. Three devices were used for each indicated dilution and condition; hence one + or – symbol indicates one device.

Supplementary Table 5. Effect of potential interfering substances on the IC performance

Interfering Substance	Concentration Tested	Solvent	Interpretation					
			VLP (–)			VLP (+)		
Hemoglobin	25 g/dL	DW	–	–	–	+	+	+
	12.5 g/dL	DW	–	–	–	+	+	+
Conjugated Bilirubin	32 mg/dL	DW	–	–	–	+	+	+
	0.2 mg/dL	DW	–	–	–	+	+	+
Free Bilirubin	29 mg/dL	0.1N NaOH	NT[a]	NT	NT	–	NT	NT
	25 mg/dL	0.1N NaOH	–	–	–	+	+	+
	15 mg/dL	0.1N NaOH	–	–	–	+	+	+
	1 mg/dL	0.1N NaOH	–	–	–	+	+	+
Intrafat	10%	Plasma	–	–	–	+	+	+
Triglyceride	1500 mg/dL	Plasma	–	–	–	+	+	+
EDTA (Short Draw)	50 mM	Plasma	–	–	–	+	+	+
	5 mM	Plasma	–	–	–	+	+	+
RF 3063-146-2 (2712.5 IU/mL)	1:1 dilution	–	+	NT	NT	NT	NT	NT
	1:4 dilution	Plasma	+	NT	NT	NT	NT	NT
	1:8 dilution	Plasma	–	–	–	+	+	+
RF2 (746 IU/mL)	1:1 dilution	–	+	NT	NT	–	NT	NT
	1:2 dilution	Plasma	–	–	–	+	+	+
RF3 (649 IU/mL)	1:1 dilution	–	+	NT	NT	–	NT	NT
	1:2 dilution	Plasma	–	NT	NT	–	NT	NT
	1:3 dilution	Plasma	–	–	–	+	+	+
Doxycycline	19.5 mM	DW	NT	NT	NT	–	NT	NT
	1.95 mM	DW	NT	NT	NT	–	NT	NT
	195 μM	DW	–	–	–	+	+	+
Ibuprofen	4.85 mM	0.1N NaOH	–	–	–	+	+	+
Ribavirin	8 mg/mL	DW	–	–	–	+	+	+
Promethazine	3.9 mM	0.1N NaOH	–	–	–	+	+	+
Ampicillin	1.24 mM	DW	–	–	–	+	+	+
Acetaminophen	300 μg/mL	0.1N NaOH	–	–	–	+	+	+
Quinine	500 μg/mL	DW	–	–	–	+	+	+
Ciprofloxacin	20 mg/mL	DW	NT	NT	NT	–	NT	NT
	10 mg/mL	DW	–	–	–	+	+	+
Aspirin	20 mg/mL	0.1N NaOH	NT	NT	NT	–	NT	NT
	15 mg/mL	0.1N NaOH	–	–	–	+	+	+

[a] Not tested. Each sample was tested in triplicate. Samples testing "+" in VLP (-) samples suggest false-positives, and samples testing "-" in VLP (+) samples suggest false-negative results.

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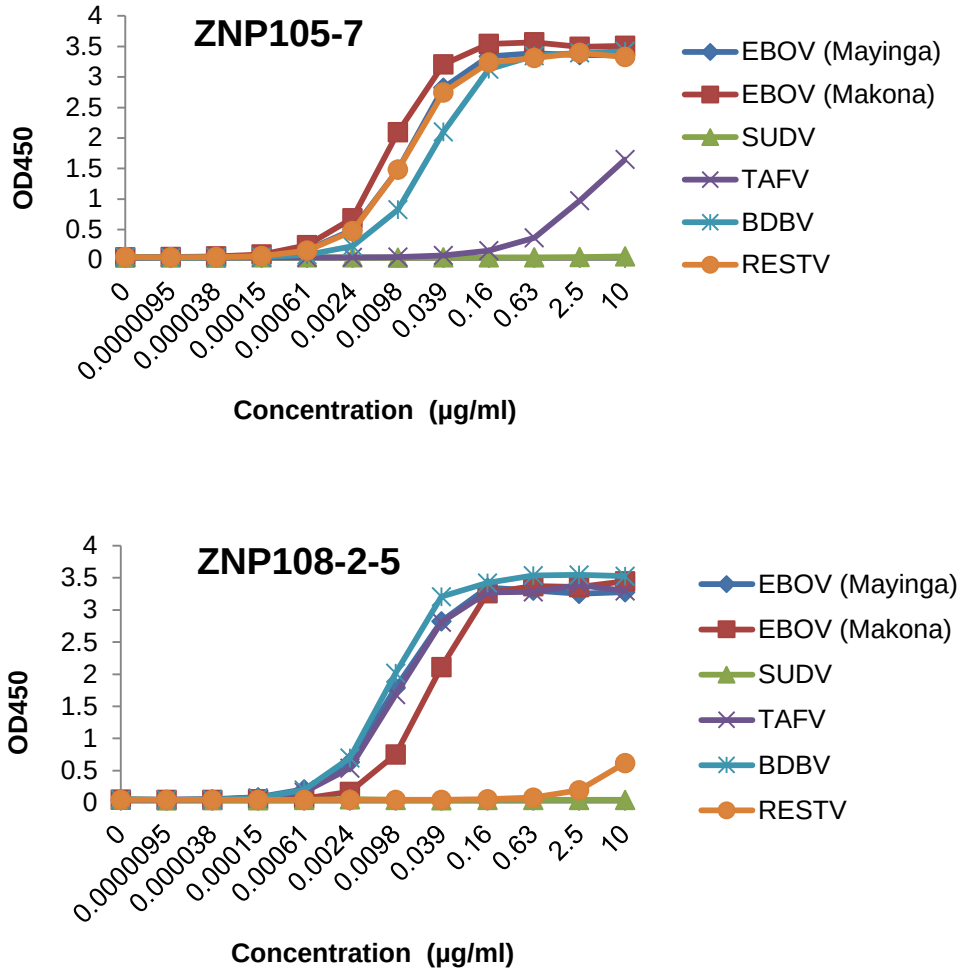

Supplementary Figure 1. Binding activities of anti-NP mAbs. ZNP105-7 and ZNP108-2-5 were examined by ELISA as described in Materials and Methods. Purified rNPs derived from the representative viruses of each ebolavirus species were used as antigens.

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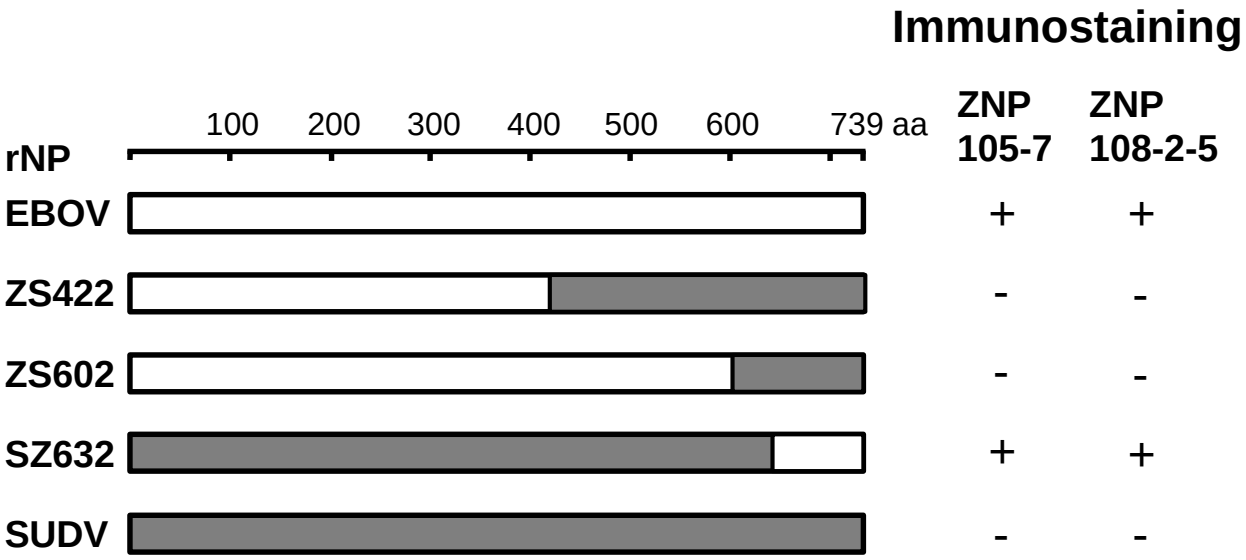

Supplementary Figure 2. Binding activities of mAbs to chimeric NPs. The expression plasmids encoding wild-type or chimeric proteins between EBOV (Mayinga) and SUDV NPs, ZS422(EBOV 1-421 and SUDV 422-739), ZS602 (EBOV 1-601 and SUDV 602-739) and SZ632 (SUDV 1-631 and EBOV 632-739), were constructed by joining the multiple overlapping DNA fragments in a single-tube isothermal reaction (Gibson). 293T cells were transfected with these plasmids. Twenty-four hours later, cells were fixed with methanol for 20 minutes, washed with PBS, and then blocked with 1% bovine serum albumin (BSA) in PBS. Primary antibodies diluted at 1 μg/m1 in 0.5% BSA-PBS were added to the fixed cells, incubated at RT for 1 h, and washed with PBS. Then an HRP-conjugated anti-mouse IgG (H+L) secondary antibody was added to the cells and the plates were incubated at RT for 1 h. After washing, the substrate was added to each well to visualize the antigens.