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Development of quantified HIV-1 antigen panel for evaluating HIV Ag/Ab combination tests using the RT-qPCR method

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ABSTRACT

We established a human immunodeficiency virus type 1 (HIV-1) antigen (Ag) panel from culture supernatants of 27 HIV-1 isolates, including 11 HIV-1 subtypes, circulating recombinant forms (CRFs), and groups (HIV-1 types), to evaluate the HIV-1 Ag detection sensitivity and HIV-1 type specificity of three HIV-1 Ag/antibody (Ab) combination tests approved in Japan. The HIV-1 copy numbers were quantified by the reverse transcription quantitative polymerase chain reaction (RT-qPCR) method. They were diluted to four different copy numbers and used in this evaluation. Enzygnost HIV Integral IV gave HIV-positive results in nearly all samples, with the single exception being an HIV-negative result in a case with a value just below the cut-off in a CRF08_BC member (100,000 copies/mL). Genscreen HIV Ag-Ab ULT showed low sensitivity to HIV-1 group O members, but this is not an urgent problem as no HIV-1 group O infection cases have been reported in Japan. The detection sensitivity of Determine HIV Early Detect was lower than that of the aforementioned two tests by ten-to hundred-fold, indicating that the kit may have limited performance in the acute phase of HIV-1 infection. Our HIV-1 Ag panel is useful for evaluating the HIV-1 Ag sensitivity of HIV-1 Ag/Ab combination tests.

1. Introduction

Early human immunodeficiency virus (HIV) diagnosis and treatment is important in the control of the HIV-1 epidemic. HIV testing plays a key role in the prevention and control of HIV infection. Since the introduction of HIV antigen (Ag) and antibody (Ab) combination assay for the HIV screening test, the window period has been reduced in comparison with anti-HIV Ab assay.

All HIV in vitro diagnostics (IVDs) for screening tests approved in Japan consist of antigen/antibody (Ag/Ab) combination tests, with the exception of Genedia HIV-1/2 mix PA (particle agglutination assay, Fujirebio, Tokyo, Japan). These tests can detect both anti-HIV-1/2 IgG and IgM and HIV-1 p24 Ag. Because elevation of HIV-1 p24 Ag due to initial viral replication starts before seroconversion in the acute phase of HIV-1 infection, HIV Ag/Ab combination tests can detect HIV-1 infection earlier than HIV Ab tests can, thus reducing the window period [1].

The performance of acute HIV-1 infection (AHI) is generally evaluated using seroconversion panels. However, almost no seroconversion panels specify the HIV-1 subtypes, circulating recombinant forms (CRFs), or groups (HIV types) that they are intended to detect, which means that we cannot evaluate the HIV-1 type-specificity with which these panels detect HIV-1 p24 Ag, in particular HIV-1 types. In this study, we established a new HIV-1 antigen panel composed of 11 HIV-1 types isolates, and examined its sensitivity

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Table 1
Results of HIV IVDs using HIV-1 Ag panel.

Isolate	Type	Enzygnost HIV Integral IV			Genscreen HIV Ag-Ab ULT			Determine HIV Early Detect			
		COI[a]			COI			Decision of Ag line			
		2,500,000	500,000	100,000	2,500,000	500,000	100,000	10,000,000	2,500,000	500,000	100,000
93RW_024¶	Subtype A	13.09	7.28	2.18	10.61	8.41	3.28	+	–	–	–
00 KE_KNH1144¶	Subtype A	11.06	4.81	1.51	10.4	7.6	2.72	+	+	–	–
00 KE_KNH1207¶	Subtype A	11.41	4.38	1.35	10.27	7.32	2.69	+	+	–	–
94US_33931 N¶	Subtype B	10.01	3.82	1.72	10.03	6.63	2.72	+	+	–	–
84US_MNp¶	Subtype B	12.44	5.53	1.8	10.34	8.07	3.07	+	+	–	–
96 TH_NP1538¶	Subtype B	11.78	5.62	1.73	10.45	8.11	3.22	+	+	–	–
02 ET_288¶	Subtype C	12.67	6.21	2.14	10.45	8.23	3.23	+	+	–	–
93IN101	Subtype C	13.44	8.28	2.67	>10.62	9.27	4.36	+	+	–	–
96USNG31	Subtype C	12.80	7.78	2.72	10.31	7.23	2.58	+	+	–	–
93UG_065¶	Subtype D	11.15	5.59	1.72	10.48	7.95	2.99	+	+	–	–
00 KE_NKU3006¶	Subtype D	9.28	3.15	1	10.31	7.28	2.71	+	+	–	–
BZ163	Subtype F	9.62	3.37	1.13	10.17	6.21	2.15	+	+	–	–
BCI-R07	Subtype F	11.20	4.77	1.37	9.6	4.57	1.5	+	+	–	–
BCF-DIOUM	Subtype G	11.88	4.57	1.48	10.07	5.83	2.15	+	+	–	–
96 TH_M02138¶	CRF01_AE	12.62	7.23	2.17	10.62	8.33	3.04	+	+	+	–
90 TH_CM244¶	CRF01_AE	12.32	6.39	1.88	10.17	6.74	2.37	+	+	–	–
98 TH_NP1251¶	CRF01_AE	12.65	6.8	2.01	11.49	6.99	2.14	+	+	–	–
01CM_0005BBY¶	CRF02_AG	8.4	2.83	1	11.12	6.15	1.97	+	–	–	–
02CM_1970LE¶	CRF02_AG	9.59	3.05	1.14	11.28	7.14	2.21	+	–	–	–
91DJ_263¶	CRF02_AG	10.44	4.26	1.47	10.95	6.26	1.99	+	+	–	–
99CN013	CRF07_BC	11.91	5.22	1.63	11.55	8.99	3.09	+	+	–	–
HH043†	CRF07_BC	10.99	4.14	1.35	11.83	9.27	3.41	+	+	–	–
HH040†	CRF08_BC	8.68	2.74	0.98	11.68	8.02	2.69	+	+	–	–
DL001†	CRF08_BC	>13.44	9.97	3.4	>11.83	10.93	8	+	+	+	–
QJ001†	CRF08_BC	9.09	3.01	1.09	11.68	7.97	2.7	+	–	–	–
BCF06	Group O	>13.44	10.67	6.11	11.82	7.82	2.53	+	+	+	+
I–2478B	Group O	10.54	3.47	1.22	6.33	1.47	0.85	+	+	–	–

[a] Cut-off index, ¶: Reference [2], †: Reference [3].

to HIV-1 p24 detection in HIV Ag/Ab combination tests.

2. Materials & methods

2.1. HIV-1 Ag panel

Twenty-seven HIV-1 isolates, including members of 11 HIV-1 subtypes/CRFs/groups (Table 1), were propagated in phytohemagglutinin -stimulated human peripheral blood mononuclear cells. The copy number contained in the supernatant was determined using the in-house reverse transcription quantitative polymerase chain reaction (RT-qPCR) method (Supplementary Table 1) and diluted to four different copy numbers (10,000,000, 2,500,000, 500,000, 100,000 copies/ml [cp/ml]) from the virus stock using Basematrix 53 (SeraCare Life Sciences, Milford, MA, USA).

2.2. HIV IVDs

Detection sensitivity to HIV-1 p24 Ag was examined in three IVDs, namely, Enzygnost HIV Integral IV (enzyme immunoassay [EIA], Siemens Healthcare Diagnostics, Marburg, Germany), Genscreen HIV-1 Ag-Ab ULT (EIA, Bio-Rad Laboratories, Hercules, CA, USA), and Determine HIV Early Detect (immunochromatography assay [ICA], Abbott Diagnostic Medical, Chiba, Japan). All tests were used according to their respective manufacturers' instructions. To confirm the copy number of the HIV-1 p24 Ag panel members after preparation, COBAS TaqMan HIV-1 v2.0 assay (Roche Diagnostics, Indianapolis, IN, USA) was used.

3. Results

We evaluated three HIV IVDs, and the results are shown in Table 1. Enzygnost HIV Integral IV gave HIV-positive results in nearly all samples, with the single exception being an HIV-negative result in a case with a value just below the cut-off in HH040, a CRF08_BC member, at 100,000 cp/ml (Table 1). When Genscreen HIV-1 Ag-Ab ULT was applied to I–2478B, an HIV-1 group O member, it yielded a low cut-off index (COI) at 2,500,000 and 500,000 cp/ml and missed at 100,000 cp/ml (Table 1). The results of I–2478B were almost the same as those of other samples using Enzygnost HIV Integral IV and Determine HIV Early Detect, but the HIV-1 RNA copy number was lower (Fig. 1).The evaluation of I–2478B was divided among IVDs. All samples with 10,000,000 cp/ml and 23/27 (85.2%) samples with 2,500,000 cp/ml gave positive results in Determine HIV Early Detect (Table 1). 96 TH-M02138 and DL001 were positive until 500,000 cp/mL, and BCF06 was Ag-positive until 100,000 cp/ml. DL001 and BCF06, however, showed a high COI in Enzygnost HIV Integral IV (Table 1). To confirm the HIV-1 RNA copy numbers in "100,000 cp/ml" samples, the copy numbers of these samples were higher than in other samples (Fig. 1).

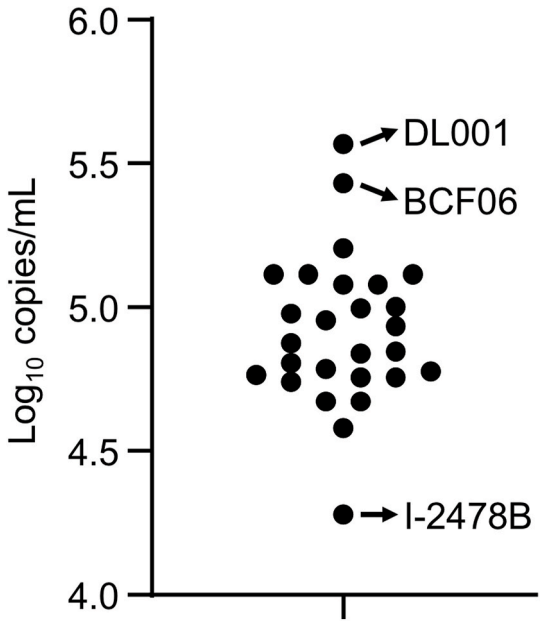

Fig. 1. Twenty-seven HIV-1 Ag panel members, including 100,000 cp/ml of HIV-1 RNA, were analyzed by COBAS TaqMan HIV-1 Auto Ver2.0 and the obtained copy numbers were plotted on a graph.

4. Discussion

We report the establishment of an HIV-1 Ag panel for the evaluation of HIV Ag/Ab combination tests using the RT-qPCR method. HIV-1 Ag panels containing 10,000,000, 2,500,000, 500,000, and 100,000 cp/ml HIV-1 were made and used to evaluate the three IVDs currently available in Japan. The COI of the panel samples with 100,000 cp/ml of HIV-1 RNA was near the cut-off value by Enzygnost HIV Integral IV and Genscreen HIV Ag-Ab ULT. Qiu et al. examined the antigen detection limits for four AHIs using three HIV Ag/Ab combination tests [4] with ranges of 21,000 to 36,700 cp/mL (ARCHITECT HIV Ag/Ab Combo assay, Abbott Laboratories, Abbott Park, IL, USA), 31,200 to 125,000 cp/mL (ADVIA Centaur HIV Combo, Siemens Healthcare Diagnostics), and 25,600 to 143,000 cp/mL (Bioplex HIV Combo, Bio-Rad Laboratories). Brennan et al. reported that the median RNA viral load of AHIs at the cut-off of the ARCHITECT HIV Ag/Ab Combo assay was 57,900 cp/mL (range: 26,400–102,000 cp/mL) [5]. These values were consistent with other AHI data [1,6]. According to the package inserts accompanying the ARCHITECT HIV Ag/Ab Combo assay and two HIV EIA tests used in this study, the analytical sensitivity for HIV-1 Ag is at almost the same level (10–20 pg/mL). This demonstrates that the HIV-1 Ag panel made according to our new protocol can be used to accurately evaluate a kit's sensitivity for HIV-1 Ag detection.

Genscreen HIV-1 Ag-Ab ULT gave a low COI for I–2478B, an HIV-1 group O member. Although the copy number of another HIV-1 group O sample, BCF06, was higher than those of other panel samples according to the COBAS TaqMan HIV-1 v2.0 assay, its COI was almost the same as those of the other panel samples. These results indicate that the HIV-1 Ag detection sensitivity of Genscreen HIV-1 Ag-Ab ULT to HIV-1 group O is lower. It should be noted, however, that the epidemic of group O infections is limited to central Africa, and that no HIV-1 group O–infected case has ever been reported in Japan [7]. Thus, this result does not indicate an urgent problem.

The detection sensitivity of Determine HIV Early Detect, however, was lower than that of the EIA tests by ten-to hundred-fold. It was reported that the HIV-1 antigen detection sensitivity of Determine HIV Early Detect was greater than that of Determine HIV-1/2 Ag/Ab Combo, the former product of Determine HIV Early Detect, but inferior to that of Genscreen HIV-1 Ag-Ab ULT [8–10]. Our results were consistent with these reports, and demonstrate that our HIV-1 Ag panel is a useful tool to deduce the performance of HIV IVDs as screening tests for detecting HIV-1 infection in the acute phase.

Determine HIV Early Detect can be used in a testing facility where it is difficult to set up a specific analyzer. It is necessary to understand the characteristics of each IVD and select an appropriate testing flow depending on the purpose and the size of the laboratory.

5. Conclusions

In this study, we established a method of generating a new HIV-1 Ag panel for the evaluation of HIV Ag/Ab combination tests without a complicated procedure and at low cost. We proved that the panel can be useful to deduce these tests' Ag detection sensitivity across several HIV-1 subtypes.

Ethical approval

Not required.

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Author contributions

SK designed the study. SK and MT prepared the HIV-1 virus panel, and SK performed laboratory analyses and analyzed the data. MT and IH supervised the projects. All authors approved the final manuscript.

Declaration of competing interest

The authors declare that they have no competing interests.

Data availability

No data was used for the research described in the article.

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Abbreviations

HIV-1	human immunodeficiency virus type 1
Ag	antigen
Ab	antibody
IVD	in vitro diagnostics
PA	particle agglutination assay
AHI	acute HIV-1 infections
CRF	circulating recombinant form
HIV-1 type	HIV-1 subtype/CRF/group
RT-qPCR	reverse transcription quantitative polymerase chain reaction
cp/mL	copies/mL
EIA	enzyme immunoassay
ICA	immunochromatography assay
COI	cut-off index.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.plabm.2022.e00301.

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