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Effect of a confirmatory testing algorithm on early acute HIV diagnosis in Korea

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Abstract

The 17 Provincial Institutes of Health and Environment (PIHEs) in Korea use HIV antibody, antigen, and Western blot assays for confirmatory testing of HIV infection. The Korea Disease Control and Prevention Agency (KDCA) has further included p24 antigen neutralization and nucleic acid tests (NATs) since 2015. Our study aimed to investigate the effect of this new testing algorithm on the confirmation rate of HIV infection.

Annual changes, from 2012 through 2017, in positive or indeterminate HIV confirmatory results were compared for the two algorithms between the PIHEs and the KDCA. Fiebig stages and Western blot p31 band were used to identify the diagnostic proportions of acute or early chronic HIV for the two algorithms.

The number of positive cases in the samples requested from PIHEs for reconfirmation by the KDCA has steadily increased from 10.3% in 2014 to 33.3% in 2017. However, the number of indeterminate cases dropped sharply, from 71.9% in 2014 to 14.0% in 2017. The results for the p31 reactive band were 27.4% and 88.4% for the KDCA and PIHEs, respectively. Of positive cases reported by the KDCA, 22.9% were in the early acute stage and Fiebig stages I to II.

The new testing algorithm has improved the diagnosis of HIV infections in the early acute stage. Early confirmatory diagnosis can prevent secondary transmission of HIV and provide early treatment opportunities for people living with HIV infection.

Abbreviations: KDCA = Korea Disease Control and Prevention Agency, NAT = Nucleic acid test, PIHE = Provincial Institutes of Health and Environment.

Keywords: algorithms, HIV antibodies, HIV infections, western blotting

1. Introduction

In Korea, the first case of the HIV infection was reported in 1985,[1] and more than 1000 new infections have occurred every year in the last 5 years. In 2019, 1222 new HIV infections were recorded, of which 1111 (90.9%) were in males and 111 (9.1%) were in females. The number of people living with HIV was 13,857. In addition, the highest percentage of cases was observed among individuals 20 to 29 years of age (438 cases, 35.8%), followed by the 30 to 39-year (341 cases), and 40 to 49-year (202 cases) age groups.[2]Among these cases, 99.8% of respondents reported sexual contact as the route of transmission.[3,4] Overall, the HIV prevalence is estimated to be <0.1% in Korea, making Korea one of the countries with low number of HIV cases.[5]

HIV screening tests are performed at primary testing sites, including public health centers (PHCs), hospitals, clinics, and blood centers. A positive screening test necessitates further testing at the 17 Provincial Institutes of Health and Environment (PIHEs), which are the confirmatory testing institutes. PIHEs use fourth- or fifth-generation HIV antigen/antibody, antigen, and Western blot tests to confirm HIV infection. In the case of indeterminate results at the PIHEs, the specimen is sent to the Korea Disease Control and Prevention Agency (KDCA) for final confirmation (Fig. 1).

Korea has a low HIV seroprevalence, and a social stigma is associated with HIV-positive patients.[6] Therefore, the HIV confirmation testing algorithm, which conservatively diagnoses HIV infection by enhancing the specificity, is used to remove a high false-positive rate in low-risk populations. The Western blot assay with high specificity has been used as the confirmation test. Recently, Western blot-indeterminate HIV results have been increasing at the PIHEs following the use of HIV-1/2 "combo" immunoassays at the screening sites.[7,8]

In 2012, the US Centers for Disease Control and Prevention (CDC) recommended the use of the non-antibody test, p24 antigen test, including a neutralization assay, and detection of the

Editor: Eduard Karamov.

The authors of this work have nothing to disclose. Funding for HIV/AIDS and STD studies was provided by the Korea Disease Control and Prevention Agency (grant 4800-4842-304).

Data availability statement: The datasets generated during and/or analyzed during the present study are available from the corresponding author on reasonable request.

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How to cite this article: Wang JS, Sim HJ, Park SJ, Park GR, Kang C, Chung YS. Effect of a confirmatory testing algorithm on early acute HIV diagnosis in Korea. Medicine 2021;100:17(e25632).

Received: 7 December 2020 / Received in final form: 7 March 2021 / Accepted: 31 March 2021

http://dx.doi.org/10.1097/MD.0000000000025632

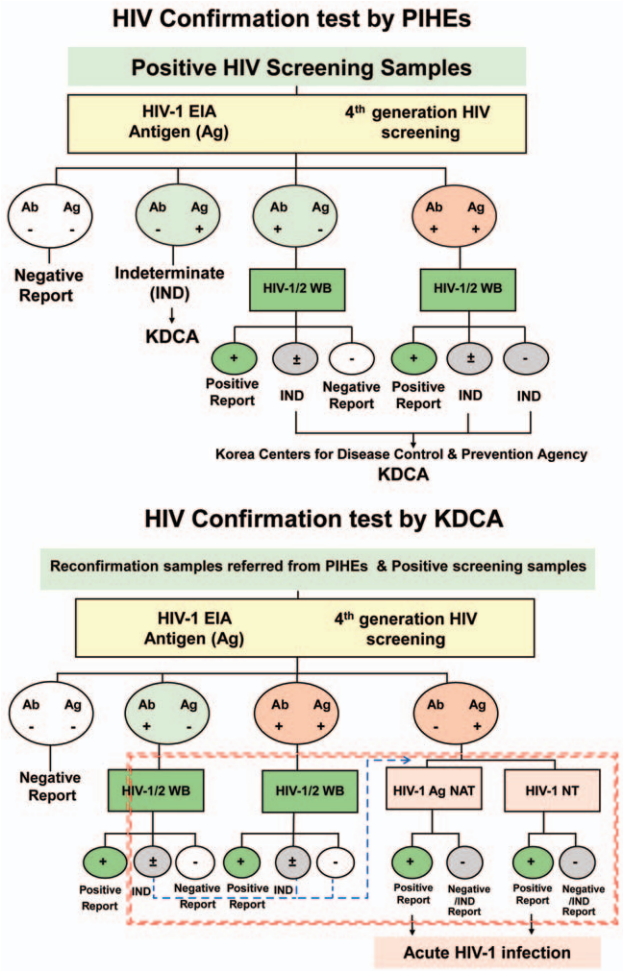

Figure 1. HIV confirmation test algorithm by the PIHEs and KDCA. KDCA = Korea Disease Control and Prevention Agency, PIHEs = Provincial Institutes of Health and Environment.

virus itself through HIV NATs (DNA or RNA) to confirm HIV infection when the screening test is positive. In June 2014, the US CDC also released a new HIV diagnosis algorithm and recommendations that provided a step-by-step account of the approach (step 1: fourth-generation HIV test, step 2: HIV-1/HIV-2 antibody differentiation immunoassay; step 3: NAT). The new protocol was intended to detect established infections as well as acute infections up to a month earlier than the previous antibody-based testing protocols by introducing the new technology, NAT, which could ease the diagnosis of acute HIV-1 infections.[9–11]

In the late 2000s, the number of new infections increased in Korea, and in recent years, HIV indeterminate and early infection cases have shown a similar trend.[12] The changing HIV dynamics have necessitated the introduction of an acute diagnosis system to offer a pre-emptive response to early stage HIV infections and prevent the secondary spread of HIV. In May 2015, the KDCA released a new HIV testing algorithm that added an HIV p24 antigen neutralization assay and NAT (viral load test) to the conventional testing methods for early diagnosis of acute HIV infections.

This study aimed to analyze and compare the results of the HIV confirmatory testing performed during 2012 to 2017 by the KDCA, which introduced the new testing algorithm with those of the PIHEs, which perform the confirmatory western blot assay. We sought to find out whether the diagnosis rate of people with acute HIV infection improved after applying the new HIV testing algorithm at the KDCA.

2. Methods

2.1. HIV confirmatory testing in Korea

HIV confirmatory tests are used to verify the positive results of HIV screening tests by combining several techniques, including the HIV antibody test, antigen test, neutralization test, Western blot, and NAT. The HIV indeterminate samples from the 17 PIHEs were sent to the KDCA for HIV reconfirmation. The PIHEs used an HIV-1/2 antigen/antibody (VIDAS HIV DUO Ultra [HIV5]; bioMérieux SA, Marcy l'Etoile, France), HIV-1 antigen (VIDAS HIV P24 II [P24]; bioMérieux SA), and HIV-1/2 Western blot method (HIV Blot 2.2 Western Blot Assay; MP Biomedicals Asia Pacific Pte Ltd, Singapore) for HIV confirmatory testing, whereas the KDCA used an HIV p24 antigen neutralization test (VIDAS HIV P24 II Confirmation; bioMérieux SA) and NAT test (Abbott RealTime HIV-1; Abbott Molecular Inc, Des Plaines, IL) in addition to the three tests by PIHE to augment the detection of early HIV infections. Since May 2015, the KDCA has performed HIV NAT and p24 antigen neutralization tests on indeterminate samples for acute infection (antigen-positive and antibody-negative or Western blot-indeterminate cases), false positive in immunoassays, and the final stage of patients with AIDS requested by the PIHEs.

2.2. Laboratory markers for the HIV infection period

In this study, acute HIV infection was defined as the phase of HIV infection occurring immediately after HIV transmission and is characterized by detectable HIV RNA or HIV p24 antigen and negative/indeterminate for antibody in the Western blot test but tests positive after a certain period. The acute infection covers Fiebig stages I through V. HIV Western blot p31 was recognized as a marker that appeared 100 days after HIV infection[13]; the Western blot p31 band was used to define the HIV infection period. The viral load changes according to patterns of the HIV antigen and antibody were analyzed.[14]

2.3. HIV test result analysis

We analyzed and compared results of HIV confirmatory tests obtained from an HIV confirmatory testing database performed by the KDCA and the 17 PIHEs. In particular, we focused on the annual changes in positive or indeterminate HIV results to find the impact of the new HIV testing algorithms by the KDCA from 2012 to 2017. We used Fiebig stages and the Western blot p31 band to identify the rate of acute or early chronic HIV infection diagnosed from the test results reported by the KDCA and PIHEs under different diagnostic algorithms from 2015 to 2017.

2.4. Statistical analysis

SAS version 9.4 (SAS, Cary, NC) was used to measure the equality of proportions test. The level of statistical significance was set at $P < .05$ (providing a 95% confidence interval). This research was not submitted for ethics board approval because it does not involve human or animal subjects.

Table 1

Increased HIV-positive results in samples requested for re-confirmatory testing from PIHEs.

Year	Total	Negative	Positive	Indeterminate
2012	460	146 (31.7%)	67 (14.5%)	247 (53.7%)
2013	435	91 (20.9%)	79 (18.2%)	265 (60.9%)
2014	398	71 (17.8%)	41 (10.3%)	286 (71.9%)
2015	213	68 (31.9%)	36 (16.9%)	109 (51.2%)
2016	220	98 (44.5%)	63 (28.6%)	59 (26.8%)
2017	207	109 (52.7%)	69 (33.3%)	29 (14.0%)
P			<.0001	<.0001

3. Results

3.1. Increased HIV-positive results in samples requested from PIHEs for reconfirmatory testing by the KDCA

The confirmatory tests to diagnose HIV have been strengthened after the introduction of the acute infection confirmatory testing algorithm at the KDCA. The number of positive cases in the requested samples from PIHEs for HIV status reconfirmation by KDCA steadily increased: 10.3% in 2014, 16.9% in 2015, 28.6% in 2016, and 33.3% in 2017 ($P < .001$) (Table 1). Following the inclusion of the HIV NAT and p24 antigen neutralization test, HIV diagnosis was confirmed to be ~18% more positive than that detected earlier (10.3% in 2014, 28.6% in 2016) compared with the previous testing approach. In contrast, the number of indeterminate pending reports dropped sharply from 71.9% in 2014 to 14.0% in 2017 ($P < .001$). Hence, the window period samples reported as undetermined in the Western blot test changed to positive after the introduction of the HIV acute infection confirmatory algorithm in May 2015.

3.2. Diagnostic proportions of acute HIV infection between KDCA and PIHEs under different diagnostic algorithms

We analyzed the results of the HIV confirmatory testing conducted by the KDCA and PIHEs from 2015 to 2017 using the Western blot p31 band. The results of the p31 reactive band were 27.4% (270/987) and 88.4% (5539/6266) in the KDCA and PIHEs, respectively ($P < .001$). The results from the KDCA showed that 72.6% (717/987) of the HIV-positive results were early HIV cases, as indicated by the non-reactive Western blot p31. Among the specimens reported as positive by the KDCA, 226 cases (22.9%, 226/987) were in the acute stage of infection, which showed negative HIV antibody, and ranged between Fiebig stages I and II: 10 [NAT (+)], 124 [NAT (+) and p24 Ag neutralization (+)], and 92 [p24 Ag neutralization (+)] (Table 2).

3.3. Changes in diagnostic markers: HIV viral load and Western blot p31 according to the progression of HIV infection

HIV results were divided into four groups: NAT (+); NAT (+) and p24 Ag neutralization (+); NAT (+), p24 Ag neutralization (+), and Ab (+); NAT (+) and Ab (+). The proportion of the HIV viral load of 10^6 copies/mL or more within each group were 0% (0/10), 66.1% (82/124), 26.3% (41/156), and 0% (0/293), respectively ($P < .001$). HIV RNA was detected at low levels of viral load in group 1 (Fiebig stage 1) but rapidly increased to 10^6 copies/mL or more in group 2 (Fiebig stages 2 and 3). HIV RNA began to decrease in group 3 (Fiebig stages 4, 5, and 6) and decreased more dramatically in group 4 (Fiebig stages 5 and 6). It was observed that HIV RNA and antigens were produced as the infection progressed, but they decreased as HIV antibodies formed. The p31 reactive proportions in groups 3 and 4 were 14.7% (23/156) and 49.2% (144/293), respectively.

4. Discussion

To prevent the secondary spread of HIV through early diagnosis and treatment, the KDCA, in May 2015, introduced and implemented an acute HIV confirmatory testing algorithm, comprising of the HIV p24 antigen neutralization test, NAT, and pre-existing Western blot assay. The HIV diagnostic paradigm is shifting from antibody-based assays to non-antibody NAT assays.

Table 2

HIV Western blot p31 non-reactive/reactive in the HIV-positive confirmation results from KDCA and 17 PIHEs laboratories, 2015–2017.

HIV confirmation sites	HIV results	WB p31(–)	WB p31(+)	Total
KDCA (Ag, Ab, WB, NAT, p24 neutralization)	NAT (+)	10	0	10
	NAT (+) and p24 Ag neutralization (+)	124	0	124
	p24 Ag neutralization (+)	92	0	92
	p24 Ag neutralization (+) and Ab (+)	83	9	92
	NAT (+) and p24 Ag neutralization (+) and Ab (+)	133	23	156
	NAT (+) and Ab (+)	149	144	293
	Ab (+)	126	94	220
	Total	717 (72.6%)	270 (27.4%)	987 (100%)
17 PIHEs (Ag, Ab, WB)	Ag (+) and Ab (+)	186	1076	1262
	Ab (+)	541	4463	5004
	Total	727 (11.6%)	5539 (88.4%)	6266 (100%)

Ab = antibody, Ag = antigen, KDCA = Korea Disease Control and Prevention Agency, NAT = nucleic acid test, PIHEs = Provincial Institutes of Health and Environment, WB = Western blot.

The underlying reasons for the early diagnosis of HIV infection are as follows. First, a person who is not aware of an HIV infection is 3.5 times more likely to spread HIV than an infected person who is aware of the infection.[15] The primary stage of HIV-1 infection is associated with high rates of transmission because of the high viral loads during this period.[16,17] Second, the rapid and timely treatment of HIV-infected persons can significantly reduce the risk of opportunistic infections and AIDS.[18] In addition, the treatment cost for HIV infection diagnosed early is much lower than that of a late infection, which can reduce the social and economic burdens of the disease.[19] For these reasons, the US CDC recommended the use of non-antibody tests, such as a NAT, antigen neutralization test, or virus isolation for confirmation diagnosis of HIV infection in the early stages. In 2014, HIV NAT was included in the recommendation for a new HIV testing algorithm by the US CDC.[9]

Accordingly, the KDCA has introduced and operated the HIV acute diagnosis system since 2015. Since HIV-2 cases in Korea are extremely low,[20] we did not include any additional HIV-1/HIV-2 antibody differentiation immunoassay in the new our testing algorithm except for the pre-existing HIV-1/2 Western blot assay. We noted that the HIV confirmatory system improved significantly.

First, our findings showed that the new HIV testing algorithm significantly reduced the proportion of indeterminate results, as follows: 71.9% in 2014, 51.2% in 2015, 26.8% in 2016, and 14.0% in 2017 (Table 1) ($P < .001$). There was an increase in positive results by >10% using the new algorithm compared with that using the previous testing method. Second, the new testing algorithm has enabled the KDCA to diagnose acute stage infections that are reported as indeterminate results by the PIHEs. The Western blot band profile can be used as a useful surrogate marker for HIV disease progression. The WB p31 band is a diagnostic index that appears 100 days after HIV infection. The proportion of WB p31 non-reactive results by KDCA was 72.6%, which was significantly higher than 11.6% reported by the PIHEs. Third, of the 987 positive confirmed results, 226 early acute infection cases corresponding to the Fiebig stages I to II period were confirmed.

Previous studies on new CDC HIV testing algorithm reported that of the 15 acute HIV infection patients, 8 were classified as Fiebig stage II or III, which was negative in the Western blot, and 7 were classified as Fiebig stage IV, which was indeterminate on the Western blot.[21] This new algorithm has improved the diagnostic capacity of early HIV infections. According to another report, the immunoassay for false-positive reactions can be resolved with NAT, and the viral loads detected in participants with acute HIV infection were very high, with 91% of patients having viral loads ≥100,000 copies/mL.[22]

Since the introduction of the acute HIV confirmatory testing algorithm in 2015, it has been positively evaluated for rapid identification of early acute HIV-infected subjects. However, some clinicians require a faster response to the diagnosis result. To solve the delay in reporting the confirmation test results due to the three-step diagnosis process in which the specimens reported as indeterminate by the PIHEs are sent to the KDCA, PIHEs are also preparing to conduct p24 antigen neutralization assay and NAT tests. When the diagnosis process is changed to a two-stage system, PIHEs promptly report the results without requesting an indeterminate sample to the KCDC. Confirmation test results are expected to be reported to the clinicians more quickly.

This study has some limitations. Since the KDCA performs five types of tests (HIV combo, antigen, antigen neutralization, Western blot, and NAT) for HIV confirmation, the amount of

Table 3

Classification of HIV-positive results conducted by the KDCA using NAT and Western blot p31.

Groups	Fiebig stages	HIV results	HIV RNA Copies	WB p31(–)	WB p31(+)	Total
I	I	NAT (+)	>100	2	0	2
			>1000	3	0	3
			>10,000	5	0	5
			>100,000	0	0	0
			>1,000,000	0	0	0
			Subtotal	10	0	10
II	II, III	NAT (+) and p24 Ag neutralization (+)	>100	0	0	0
			>1000	0	0	0
			>10,000	7	0	7
			>100,000	35	0	35
			>1,000,000	82	0	82
			Subtotal	124	0	124
III	IV, V, VI	NAT (+) and p24 Ag neutralization (+) and Ab (+)	>100	1	0	1
			>1000	0	0	0
			>10,000	23	7	30
			>100,000	73	11	84
			>1,000,000	36	5	41
			Subtotal	133	23	156
IV	V, VI	NAT (+) and Ab (+)	>100	16	10	26
			>1000	36	51	87
			>10,000	71	70	141
			>100,000	26	13	39
			>1,000,000	0	0	0
			Subtotal	149	144	293
			Total	416	167	583

Ab = antibody, Ag = antigen, NAT = nucleic acid test, RNA = ribonucleic acid, WB = Western blot.

sample required for the test is about 2 mL. In particular, ~1 mL of serum or plasma sample is required for the NAT test. However, some insufficient samples were reported as positive without NAT testing if they returned positive results in the p24 antigen neutralization test or Western blot. To reduce confusion when analyzing Table 3, 583 out of 987 results used in Table 2 were selected, whereas 404 results (92 p24 Ag neutralization [+], 92 p24 Ag neutralization [+] and Ab [+], and 220 Ab [+]) that were negative or did not have NAT were excluded. However, the proportions of early acute infection cases were similar, 22.9% (226/987) and 23% (134/583), in Tables 2 and 3, respectively.

In conclusion, the new testing algorithm improved the diagnosis of HIV infections in the early acute stage. Implementation of a new confirmation diagnosis system can contribute to the prevention of secondary transmission of HIV by people who are unaware of their positive status. It can also improve public health by providing early treatment opportunities and augment the quality of life of people living with HIV.

Acknowledgments

We thank Yu-Sang Lee for supporting the statistical analysis of the data.

Author contributions

Conceptualization: Jin-Sook Wang and Yoon-Seok Chung. Funding acquisition: Chun Kang and Yoon-Seok Chung. Supervision: Chun Kang and Yoon-Seok Chung. Investigation: Hyo-Jung Sim, Su-Jin Park, and Gye-Ryeng Park. Writing—original draft: Jin-Sook Wang. Writing—review and editing: Jin-Sook Wang and Yoon-Seok Chung. All authors edited and approved the final version of the manuscript.

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