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A Survey of HIV Proficiency Testing Program in Taiwan

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Abstract

Laboratory report is an important basis for clinical diagnosis of human immunodeficiency virus (HIV) infection. Assuring the test quality is an important index for laboratories to provide a reliable report. To effectively control HIV in Taiwan, one of the most important parts is to maintain a good quality of laboratory test besides the full cooperation between relevant offices in governmental and non-governmental organizations. Only a laboratory with good analytical quality will provide an accurate result, and only on the basis of accurate laboratory test, an appropriate control policy can be established and HIV infection sources can be controlled effectively. The medical institutions performing HIV testing in Taiwan show large differences in scale and size and lack of an overall assessment on quality of laboratory test. In order to assess the quality of HIV analysis and, through the assessment, to promote the performance of HIV testing in Taiwan, we initiated a survey on the current situation of HIV testing at different levels of medical institutions and a proficiency testing program. In total, 368 institutions have participated in the proficiency testing program for HIV antibody assay and 22 institutions for HIV nucleic acids assay. The results show that all of the 240 institutions applying EIA technique correctly detected HIV antibody from six proficiency panel samples. For 156 institutions adopting PA assay for HIV antibody analysis, the concordance of the results was 98.7%. These data shows that HIV analytical ability of medical institutions performing EIA and PA method has reached a comparably high level. In addition, all participating institutions accurately identify a HIV-2 positive specimen. This means that reagents

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currently used in medical institutions in Taiwan can actually detect HIV-2 infected cases although the HIV-2 case has never been reported in this country. As to the proficiency testing for HIV nucleic acids assay, all 22 participating laboratories which affiliated with hospitals at or higher than regional level in scale or research institutes, the concordance of test results was 100%. This implies that the HIV nucleic acids assay in the large hospitals and research institutes in Taiwan stayed in a very high level of quality. HIV case identification and control policy making needs reliable laboratory data which is also a key factor for successful HIV control. Therefore, for HIV control, it is necessary to keep monitoring the quality of HIV screening test so that a correct analysis could be obtained.

Keywords: medical institution, HIV, AIDS, laboratory test quality, proficiency testing

Introduction

Human immunodeficiency virus (HIV) is primarily spread among humans through infected body fluids, such as blood and semen. After infection, HIV can reach everywhere in the body through blood circulation, and then infects and destroys CD4+ cells, leading to impairment of immunologic function and occurrence of opportunistic infection, such as *Pneumocystis jirovecii* pneumonia, candidiasis, cryptococosis, and cryptosporidiosis. The disease caused by the decrease of immunologic function is called acquired immunodeficiency syndrome (AIDS), a fatal opportunistic infection may occur due to the condition of cellular immunodeficiency [1-4]. AIDS was first identified in the USA in 1981, and has gradually become a pandemic disease. The statistics in 2007 shows that a total of 25,000,000 AIDS cases have died and 33,000,000 people have been infected with HIV [5]. In Taiwan, the first imported AIDS case was notified in 1984 and the first native HIV-infected case was reported in 1986. Since then, the number of HIV-infected cases was gradually increasing every year. Based on data released by Taiwan CDC, an accumulated of 19,260 HIV-infected cases have been identified by May 2010 [6].

The most commonly used methods for HIV screening test are antibody detection by enzyme immunoassay (EIA) and particle agglutination (PA), and then followed by a confirmation test using either the Western blot to detect HIV antibody, or real-time reverse transcription-polymerase chain reaction (real-time RT-PCR) to detect HIV-1 nucleic acids. The results of laboratory test are

important for AIDS control since activities to effectively prevent HIV from further spreading and to control AIDS, such as identifying infection sources rapidly and correctly, and administering health education and medical treatment appropriately, will need the accurate test results. Since medical facilities conducting HIV test at different levels are not the same size in Taiwan, and the analytical procedures and apparatus used for HIV test are usually different, the specificity and sensitivity are therefore different. In order to assure the quality of test results, the National Institute of Preventive Medicine (the antecedent of Taiwan Centers for Disease Control, Taiwan CDC) constituted a program to monitor the quality of HIV test performed by hospitals for general public and foreign labors in 1995. Up to 2006, the program was entrusted to the Taiwan Society of Laboratory Medicine by Taiwan CDC to keep monitoring test quality and helping laboratories upgrade test quality. This report describes the survey, based on the program, on current situation of HIV test in Taiwan and the results of HIV proficiency testing, to provide a reference for planning future activities on monitoring and promotion of test quality.

Materials and Methods

A. Institutions and proficiency testing

The institutions include hospitals or laboratories providing HIV testing in Taiwan, such as laboratories in local health departments, contracted hospitals responsible for foreign labor physical examination or pregnant woman HIV and hepatitis B virus screening program, hospitals working for anonymous HIV testing, hospitals serving for military conscript physical examination, laboratories affiliated with drug abuse monitoring authority, private laboratories, and laboratories affiliated with blood donation and blood usage. A questionnaire was sent to these institutions in 2009 to collect the current information on HIV testing and their willingness to participate in the proficiency testing program. Among the institutions performing HIV antibody analysis, a total of 724 have responded a valid questionnaire, 368 institutions agree to participate in the proficiency testing program, including 22 hospitals working for anonymous HIV testing. Of the 22 hospitals, 20 are contracted hospitals and 2 are non-contracted hospitals. Among the institutions performing HIV-1 nucleic acids analysis, 38 have responded a valid questionnaire, and 22 of them agree to participate in the proficiency testing program.

B. Proficiency testing

A commercial quality control panel was used as proficiency testing samples for hospitals or laboratories where EIA or PA, and Western blot were applied for HIV antibody assay. Each testing panel has six samples, positive, negative, and weak positive, and others, and these were randomized mixed and sent to participants. Another commercial quality control panel bought abroad was provided in a randomized fashion for participants who performed RT-PCR assay for HIV nucleic acid detection. This commercial panel contains four samples, a negative and three concentrations of virus as positives. The virus concentration of the positive samples is 1,069, 1,511 and 120,546 copies/mL, respectively. To ensure the quality and stability of the proficiency testing

samples, all of them were sent to reference laboratories located at northern, central, and southern Taiwan to make sure that they met the stability and uniformity standards seven days earlier before mailing out to participants. The samples were first placed in a specimen box, then put into a plastic ziplock bag, and wrapped with an anti-vibration airbag. Following these steps, the samples together with ice pack, a sheet describing proficiency test scheme, and a sheet for recording test results were packed within a polyurethane foam box. After double-checking that the samples and relevant documents were packed correctly, the boxes were distributed to the participating hospitals or laboratories under cooling conditions at the same day, but the samples for HIV nucleic acids proficiency testing was transported under freezing conditions. At the same time, information on the assay methods, reagents and expiration date, and lot numbers of the reagents used by the participating institutions was also collected. The participating institutions were required to return the test results within seven days after receiving proficiency testing samples.

Statistical analysis

To determine the results of proficiency testing, the correct percentage was calculated for each participating institution. When the percentage of concordance is larger than 80% then the results of the panel samples will be determined. A participating institution is considered as meeting the testing requirements when the results obtained are 100% concordant to all of the proficiency testing samples. For institution that the concordance of the HIV antibody proficiency testing was not satisfactory, a retesting, telephone instruction, or onsite instruction was provided to identify the problems, correct the drawback, and improve the quality of HIV antibody assay. The criteria for evaluation of HIV nucleic acids proficiency testing are the mean value obtained from all participating institutions plus/minus three standard deviations. An institution is considered as meeting the testing requirements when the test result is 100% within the range. For institutions that the results were not satisfactory, a retesting, telephone instruction, or onsite instruction was provided to identify the problems, correct the drawback, and improve the quality. The analysis of the data is performed by using Fisher's exact test, chi-square test with Yates' correction, and Student's t-test. The results are considered to have a statistically significant difference when p value is less than 0.05.

Results

A. Current situation of HIV antibody assay

This survey has collected the name and contact information from a total of 2,318 medical institutions and received 724 (31.2%) valid questionnaires from laboratories affiliated with these institutions. Of the 724 laboratories, 391 (54.0%) providing HIV testing service for general public, 368 (50.8%) agreed to participate in the proficiency testing program. Eleven provided HIV testing service but was unable to participate in the proficiency testing due to either the testing service has shifted to other counterpart institutions, going to move to a new office, the service has transferred to headquarter institution, the institutions provided only rapid

testing service, or the institutions has terminated the service because of cost-benefit consideration. Of the 368 laboratories participating in the proficiency testing program, 22 laboratories offering the anonymous HIV testing service met the proficiency testing requirements. A total of 296 (80.4%) laboratories used single method in HIV testing service and the rest of the participating laboratories applied two or more methods. The number of laboratories applying Western blot assay for confirmation of HIV testing was 33, accounted for 9.0% of the participants. Among the institutions participating in the survey program, 148 (40.2%) institutions are private laboratories, 78 (21.2%) are local hospitals, 85 (23.1%) are regional hospitals, and 23 (6.3%) are medical centers.

B. Proficiency testing for HIV antibody assay

The most commonly used HIV screen testing among participants in the survey program was EIA which recorded 240 (67.2%) laboratories, followed by PA testing, with 156 (43.7%) laboratories. The concordance for proficiency testing samples A, B, C, and E analyzed by PA and EIA methods was 100%. However, the concordance for samples D and F analyzed by PA was 99.4%, although it was 100% by EIA method (Table 1). The number of laboratories performing Western blot assay for confirmation of HIV antibody test was 33, including 26 using the NEW LAV BLOT I (Bio-Rad) and 7 using the HIV BLOT 2.2 (MP Diagnostics). The results analyzed by these two methods for testing samples were shown in Table 2. According to the currently adopted HIV screening procedures, the laboratory will first conduct EIA or PA assay for samples B and E. When the result is negative, a further confirmation by Western blot assay will be unnecessary. Therefore, some participants did not perform Western blot assay for samples B and E. The concordance of samples B, C, D, E, and F analyzed by either NEW LAV BLOT I or HIV BLOT 2.2 was 100%. The sample A analyzed by NEW LAV BLOT I was positive in one laboratory but indeterminate in the rest of the participating laboratories. The ratio of positive to indeterminate for all samples was no significant difference between the methods of NEW LAV BLOT I and HIV BLOT 2.2.

Table 1. Statistics of proficiency testing for HIV antibody screening assay

Samples code	Analytical methods [a]	Total number [b]	Number of institutions [c]			
			positive	%	negative	%
A	PA	156	156	100	0	0
	EIA	240	240	100	0	0
B	PA	156	0	0	156	100
	EIA	240	0	0	240	100
C	PA	156	156	100	0	0
	EIA	240	240	100	0	0
D	PA	156	155	99.4	1	0.6
	EIA	240	240	100	0	0
E	PA	156	0	0	156	100
	EIA	240	0	0	240	100
F	PA	156	155	99.4	1	0.6
	EIA	240	240	100	0	0

[a] PA: particle agglutination, EIA: enzyme immunoassay
[b] Total number: Number of medical institutions participating in proficiency testing
[c] Number of institutions: Number of medical institutions reporting positive or negative results

Table 2. Statistics of proficiency testing for HIV antibody analysis by Western blot assay

Samples code	Analytical methods [a]	Number of institutions [b]			Total number [c]
		Positive	Indeterminate	Negative	
A	HIV BLOT 2.2	0	7	0	7
	NEW LAV BLOT 1	1	23	0	24
B	HIV BLOT 2.2	0	0	3	3
	NEW LAV BLOT 1	0	0	5	5
C	HIV BLOT 2.2	7	0	0	7
	NEW LAV BLOT 1	26	0	0	26
D	HIV BLOT 2.2	7	0	0	7
	NEW LAV BLOT 1	26	0	0	26
E	HIV BLOT 2.2	0	0	3	3
	NEW LAV BLOT 1	0	0	5	5
F	HIV BLOT 2.2	7	0	0	7
	NEW LAV BLOT 1	26	0	0	26

[a] Reagents used in HIV antibody Western blot assay
[b] Number of institutions: Number of medical institutions reporting positive, indeterminate, or negative results
[c] Total number: Number of medical institutions participating in proficiency testing

C. Proficiency testing for HIV nucleic acids assay

The proficiency testing results from the 22 institutions performing HIV nucleic acids assay for HIV confirmation were within the acceptable range, and the concordance of test result reached 100%. None has obtained different results from other participants.

Discussions

The questionnaire-based investigation conducted in 2009 found that 391 laboratories providing HIV antibody testing service for general public, and 368 (94.1%) of them, participated in the proficiency testing program. This implies that medical institutions have a very high interest to take part in the proficiency testing in order to know whether their test capability and quality is compatible to other peer institutions in HIV testing. The types of institutions with the most participants were private laboratories, with 148 joined the survey program. This reveals that primary testing institutions were also actively involved in the HIV control. Since the primary testing institutions, the private medical laboratories, have deeply cultivated relationship with local people and knew more about local culture and residents' characteristic, the AIDS control program should consider to increase their participation in terms of the AIDS case investigation and screening test, to maximize the control efficiency.

Among the participating laboratories, 240 performed EIA assay with the concordance of 100%; 156 used PA assay for HIV antibody testing but the concordance was 98.7%. The institutions not meeting the proficiency testing requirements are mostly small hospitals and private laboratories. The main reasons for not meeting requirements include filling test results in a wrong space and mistaking in reading the test results. These data show that the quality of HIV antibody testing in institutions using EIA and PA assay have reached a comparable level. For the 33 laboratories participating in HIV antibody confirmatory test (Western blot assay), the concordance of test result for samples B-F (with negative and positive) was excellent (100%), while the concordance for sample A

(weak positive) varied (95.8%-100%) slightly. This suggests that the education and training for Western blot assay should be strengthened. In addition, sample A was a HIV-2 positive sample, which can be detected by laboratories adopting EIA or PA testing. This means that reagents currently used in laboratories of Taiwan can actually detect HIV-2 cases when it occurred although the HIV-2 case has never been reported in this country. All the 22 laboratories participating HIV nucleic acids proficiency testing were those affiliated with hospitals at or higher than regional level in scale or research institutes. The concordance of test results was 100%. This indicates that the HIV nucleic acid assay in the large hospitals and research institutes in Taiwan stayed in a very high level of quality.

HIV case identification and control policy relies on laboratory data which is also a key factor for successful HIV control. Therefore, to control HIV, it is necessary to continue monitoring the quality of HIV screening test so that a correct analysis could be offered.

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Development and Evaluation of a New Questionnaire on AIDS-Related Knowledge

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Abstract

In 2004, there was an AIDS outbreak among Taiwanese injecting drug users (IDUs). A questionnaire on AIDS-related knowledge was thus needed, but the existing questionnaire was outdated. We designed a new questionnaire and evaluated its reliability and validity.

Many psychometric properties were evaluated, including expert validity, construct validity for known group difference, item analysis and internal consistency, and test-retest reliability. Study sample were enrolled from school adolescence, drug users arrested by police, IDUs who received MMT, and public health workers.

The ratings from 9 content experts were all higher than 3.2 (maximum 5), indicating acceptable content validity. During 2005-2007, 3,130 adolescents, 1,162 arrested drug users, 204 IDUs with MMT, and 116 public health workers completed the questionnaire. Public health workers had the highest rate of correct answers, followed by IDUs with MMT, the arrested drug users and school adolescents implying a good known group difference of construct validity. The difficulty index was within the acceptable range, and the discrimination index was >25%. Cronbach's α was 0.60-0.82, indicating good internal consistency. Regarding test-retest reliability, the correlation coefficient for 83 individuals who completed the questionnaire twice was 0.59, showing good stability over time. Our questionnaire has an acceptable psychometric property, including good expert validity, construct validity and test-retest reliability.

Keywords: AIDS, knowledge, questionnaire, reliability, validity

Introduction

In 2004, there was an AIDS outbreak among injecting drug users (IDUs) in Taiwan [1]. In December 2005, a total of four Taiwanese counties and cities began a trial of a harm reduction program. In July 2006, a nationwide Clean Needles Program was implemented in Taiwan. And, in December 2006, the country began a nationwide methadone maintenance treatment (MMT) program.

The Public Health Bureau of Taoyuan County promoted a series of prevention activities to control human immunodeficiency virus (HIV) transmission in IDUs. These efforts included (1) helping medical institutions establish MMT clinics [2], (2) distributing clean needles and collecting used needles in public health facilities and community pharmacies [3], (3) surveying current knowledge, attitudes, and practices (KAP) regarding AIDS prevention, and providing relevant education to adolescents [4] and groups at a high risk for AIDS (such as inmates, arrested drug users, and sex workers and their customers). A questionnaire on AIDS-related knowledge was needed to implement the KAP survey and to provide focus for health education for AIDS prevention.

A review of the literature showed that the questions used for assessment of AIDS-related knowledge in past research were very similar [5-10]; they tended to focus on how HIV is transmitted (via blood, unsafe sex, and from mother to child). However, there were a number of misconceptions in Taiwan regarding HIV infection (Can it be prevented by taking or injecting medicine?

Can we determine whether individuals have HIV/AIDS from their appearance?). Studies revealed that many people gave incorrect answers to questions on HIV/AIDS, such as items related to the window period and symptoms of infection [6-10]. In addition, investigators predominately used true-false options in past questionnaires on AIDS-related knowledge [5-10]. With true-false items, there is a greater chance of guessing an answer correctly and of misinterpreting questions, which results in low reliability and poor discrimination [11].

Therefore, we designed a questionnaire on AIDS-related knowledge by combining our understanding of the recent AIDS epidemic in Taiwan with findings from the relevant literature. Information evaluated on the questionnaire included: groups recently at high risk for HIV infection in Taiwan [1], whether HIV can be transmitted by sharing needles/syringes and diluents [12], what HIV testing services are provided by the government [4], the punishment for intentionally spreading HIV [13], the window period, the incubation period [14], and the longest survival time for HIV in a used syringe with residual blood at room temperature [15]. Outdated questions were deleted and multiple-choice items with one correct answer were used. After we drafted the questionnaire, content experts were invited to rate the appropriateness of the content and to provide opinions, after which the questionnaire was amended in accordance with their advice. We then administered the questionnaire to various groups and made minor changes. In this article, we describe the process of developing this questionnaire on AIDS-related knowledge, and evaluate its reliability and validity. We hope that our questionnaire can be used by the Taiwan government and in future academic research. In addition, other investigators may be able to save time and resources in developing their own similar questionnaires [16]. Finally, if this or similar questionnaires were widely used, the findings could be compared.

Methods

A. Questionnaire Design

There were 16 items on the first version of the questionnaire. The content experts suggested removing two questions, after which two new questions were added. We used multiple-choice questions with four answers, one correct answer, and the option of answering "I don't know". Higher scores indicated greater AIDS-related knowledge (Table 1).

B. Reliability and Validity

A total of 9 experts from different fields were invited to score the appropriateness, relevance, and clarity of the questionnaire (1 to 5 points). A score of 3 points indicated that partial modification was necessary; a score of ≤2 points indicated a need for deletion or substantial revision.

We used the known group difference method to evaluate construct validity. The questionnaire was distributed to four groups among which we expected to see differences in AIDS-related knowledge. The four groups were school adolescents, drug users arrested by the police, IDUs who received MMT, and public health workers. The construct validity of the questionnaire would be confirmed [17], if there were differences in AIDS-related knowledge among these groups.

For item analysis, we used the item discrimination index and item difficulty index [18]. A higher item difficulty index indicates that the question is easier. Ideally, the item difficulty index should be between 40% and 70%. A higher item discrimination index indicates better item discrimination; the index should be ≥25% [11]. Cronbach's α was used to evaluate internal consistency; the optimal value is 0.70-0.90 [17]. For test-retest reliability, the Pearson correlation coefficient was used to indicate the extent of agreement for the rate of correct answers from individuals who completed the questionnaire twice in a short period of time; the optimal value is >0.75 [17].

C. Study Sample

1. School adolescents

Stratified multistage cluster random sampling was used to obtain the sample. The four strata were junior high school, senior high school, vocational school, and night school in Taoyuan. The Public Health Bureau of Taoyuan County sent an official letter to the selected schools to request participation in the study. The students in selected classes were then asked to answer anonymously the questions on AIDS-related knowledge. To assess test-retest reliability, students in a senior high school, a vocational school and a "cram school" for grades 7 through 12 in the Taoyuan area were asked to complete the same questionnaire twice within two weeks (the names of the test-takers were recorded).

2. Drug users arrested by the police

All drug users arrested in the Taoyuan area were asked to complete the questionnaire (with recording of names) during July 2006 and December 2007. Test-retest reliability test was assessed among drug users who were arrested twice and completed questionnaires twice on the same day.

3. IDUs who received MMT

During October 2006 and November 2007, IDUs who received MMT were asked to complete the questionnaire (with recording of names) in MMT clinics in Taoyuan.

4. Public health workers in Taoyuan county

Public health workers from the Public Health Bureau of Taoyuan County or from Public Health Centers in Taoyuan City were invited to complete the questionnaire anonymously from October to November 2007.

D. Data Processing and Statistical Analysis

To evaluate content validity, we used the mean rating given by the content experts. Internal consistency was assessed using Cronbach's α, and test-retest reliability was assessed using the Pearson correlation coefficient. Known group difference was expressed using ANOVA and Scheffe's post-hoc comparison. The significance level was 0.05.

Results

A. Expert Validity

The mean rating from the nine experts was >3.2, indicating good content validity. Minor modifications were made accordingly (Table 1).

The options "DNA virus" and "RNA virus" were combined because the general public probably would not understand the difference between them (#1). "Sexual behavior" was changed to "unprotected sex" (#3). Experts suggested that there was no need to include the term of imprisonment in the item, "What is the punishment for intentionally infecting someone with HIV/AIDS in Taiwan?" (#13).

Later, a physician suggested that "blurred

Table 1. Expert validation of the questionnaire

		Appropriateness	Relevance	Clarity
(1)	What is the pathogen responsible for AIDS? 1☐ Bacterium 2☐ Fungus 3☐Parasite 4☐ Virus*	3.6	4	4
(2)	Which of the following is a medium for spreading HIV? 1☐ Air 2☐ Body fluids* 3☐ Soil/dirt 4☐ Food	4.4	4.6	4.4
(3)	Which of the following is not a route for spreading HIV? 1☐ Unprotected sex 2☐ Needle sharing 3☐ Eating together* 4☐ Blood transfusion	4.2	4.4	4.4
(4)	In Taiwan, which of the following groups is not at high risk for AIDS? 1☐ IDUs 2☐ Homosexuals/bisexuals 3☐ Hemophiliacs* 4☐ Newborn of a mother with HIV/AIDS	3.6	3.8	3.4
(5)	Over the past year, which group at risk for AIDS experienced the greatest increase in Taiwan? 1☐ IDUs* 2☐ Homosexuals/bisexuals 3☐ Hemophiliacs 4☐ Newborn of a mother with AIDS	4.8	4.8	4.8
(6)	Which body system is destroyed by the AIDS pathogen? 1☐ Digestive system 2☐ Immune system* 3☐ Endocrine system 4☐ Respiratory system	4.6	4.6	4.6
(7-1)	The early symptoms of HIV/AIDS are hard to distinguish. Which of the following is not one of them? (school adolescents, arrested drug users)† 1☐ Blurred vision* 2☐ Unexplained weight loss 3☐ Swollen lymph 4☐ Weakened immunity	3.2	3.2	3.2
(7-2)	Which of the following is not a common symptom of AIDS? (IDUs who received MMT, public health workers)† 1☐ Loss of smell* 2☐ Unexplained weight loss 3☐ Swollen lymph 4☐ Weakened immunity			
(8)	Which of the following is not true of HIV/AIDS treatment? 1☐ There is no vaccine 2☐ There is no cure 3☐ Cocktail therapy has excellent results 4☐Radiotherapy is effective*	4.6	4.4	4.4
(9)	Which of the following does not transmit the AIDS pathogen? 1☐ Blood 2☐ Semen 3☐ Saliva* 4☐ Vaginal discharge	4.4	4.4	4.4
(10)	After being infected with the AIDS pathogen, there is a period of time during which blood tests will remain negative but it remains possible to infect others. This is called the "window period". How long can this period last? 1☐ 5-7days 2☐ 2-6 weeks 3☐1-3 months* 4☐ 5-7 months	4.4	4.4	4.4
(11)	After infection with HIV, there are no symptoms during a period of time that is called the incubation period. How long can this period last? 1☐ 1-3 years 2☐ 4-5 years 3☐ 6-7 years 4☐ 8-10 years*	3.8	3.8	3.8
(12-1)	Which of the following can prevent HIV/AIDS infection? (school adolescents, arrested drug users)† 1☐ Not sharing needles/syringes* 2☐ Taking anti-HIV drugs 3☐ Having sex with strangers 4☐Using a condom before inserting the penis into the vagina.	4.8	4.8	4.8
(12-2)	Which of the following is a correct way for preventing HIV/AIDS infection? (IDUs who received MMT, public health workers)† 1☐ Not sharing needles/syringes, diluents, and wash water with others* 2☐ Taking medicine to enhance the body's immunity 3☐ Having sex and not using a condom with strangers 4☐ Using a condom before inserting the penis into the vagina; it is not necessary to wear a condom for genital contact only			
(13)	What is the punishment for intentionally infecting someone with HIV/AIDS in Taiwan? 1☐ No punishment 2☐ A fine 3☐ Prison sentence* 4☐ Lifetime imprisonment	4.4	4.8	4.8
(14)	Which HIV testing service is not currently provided by the government? 1☐ Free HIV testing 2☐ Anonymous HIV testing 3☐ HIV testing as part of blood donation* 4☐ HIV testing with results in 5-7 working days.	4.4	4.4	4.4
Added questions				
(15)	Which of the following can transmit the AIDS pathogen? 1☐ Shared toilet seat 2☐ Shared diluents and wash water* 3☐ Shared tableware 4☐ Shared towel			
(16)	At room temperature, what is the longest period of time that the AIDS pathogen can survive in residual blood in a used syringe? 1☐ A few minutes 2☐ A few hours 3☐ A few days 4☐ A few weeks*			

*Correct answer †Question for specific groups

vision" is a possible early symptom of AIDS, therefore "early" symptom of AIDS (#7-1) was changed to "common" symptom of AIDS (#7-2) and option "blurred vision" was changed to "loss of smell". For item 12, "the correct way for preventing HIV/AIDS infection", "not sharing diluents and wash water (#12-2)" was added to the option of "not sharing needles/syringes" (#12-1). The option of "taking anti-HIV medicine (#12-1)" was removed because it was suggested that taking such medication might have a small chance of preventing HIV infection. Hence, this option was changed to, "Taking medicine to enhance the body's immunity" (#12-2).

B. Known Group Difference of Construct Validity, Item Analysis, and Internal Consistency

Among the school adolescents, 3,130 out of 3,182 completed questionnaires used for this study—a response rate of 98.4%. The questionnaire was also completed by 1,162 out of 1193 (97.4%) drug users arrested by police, by 204 out of 217 (94.0%) IDUs receiving MMT, and by 116 public health workers (100%).

In the analysis of construct validity using the known-group method, the rate of correct answers regarding AIDS-related knowledge, the order from low to high was school adolescents, arrested drug users, IDUs who received MMT, and public health workers. There were statistically significant differences among the groups (Table 2).

Table 2. Rate of correct answers on questionnaire items, by group

Item no.	School adolescents (n=3,130)	Arrested drug users (n=1,162)	IDUs who received MMT (n=204)	Public health workers (n=116)	p
1	69.3%	65.0%	71.5%	97.4%	<.001
2	93.0%	97.0%	93.6%	100.0%	<.001
3	90.0%	90.8%	90.6%	99.1%	.004
4	54.3%	29.5%	59.7%	72.3%	<.001
5	35.5%	66.4%	82.1%	88.6%	<.001
6	79.8%	70.3%	85.7%	98.3%	<.001
7	35.0%	23.8%	40.1%	89.5%	<.001
8	12.8%	24.1%	33.8%	85.0%	<.001
9	74.2%	88.2%	74.7%	86.6%	<.001
10	17.3%	26.1%	21.8%	56.5%	<.001
11	22.2%	28.0%	28.9%	71.3%	<.001
12	70.9%	62.0%	77.5%	96.3%	<.001
13	23.7%	60.9%	64.5%	77.7%	<.001
14	17.8%	13.4%	28.3%	86.6%	<.001
15			81.4%	92.9%	.003
16				3.5%	
All questions	49.6%[A]	53.1%[B]	61.0%[C] (questions 1-14)	86.0%[D] (questions 1-14)	<.001
			62.4% (questions 1-15)	87.0% (questions 1-15)	<.001
				81.7% (questions 1-16)	

[ABCD:] Different letters indicate significant differences in the rate of correct answers (Scheffe's post-hoc test).
IDU: injecting drug users; MMT: methadone maintenance treatment

On item analysis, the mean difficulty index percentages for the items on AIDS treatment (#8) and the window period (#10) were low among school adolescents, arrested drug users, and IDUs who received MMT; however, the discrimination index among school adolescents still > 25%. The public health workers answered most questions correctly; thus, the discrimination index was relatively low, except for the item on survival time of the AIDS pathogen (#16; difficulty index =0.9%; Table 3).

Regarding internal consistency, most values for Cronbach's α or item-deleted α were higher than 0.55, indicating that there was good internal consistency among questions (Table 4).

Table 3. Results of item analysis

Item no.	School adolescents (n=3,130)		Arrested drug users (n=1,162)		IDUs who received MMT (n=204)		Public health workers (n=116)	
	Difficulty	Discrimination	Difficulty	Discrimination	Difficulty	Discrimination	Difficulty	Discrimination
1	62.4%	66.5%	62.0%	66.7%	66.7%	62.8%	95.9%	8.3%
2	87.3%	24.9%	60.3%	69.4%	87.5%	25.0%	100.0%	0.0%
3	82.8%	32.7%	84.2%	15.7%	83.4%	33.3%	98.6%	2.8%
4	49.7%	65.3%	86.2%	26.6%	56.2%	65.4%	70.8%	33.0%
5	34.0%	41.9%	34.0%	36.2%	72.7%	47.4%	84.3%	31.4%
6	69.5%	59.2%	60.7%	61.5%	74.5%	51.0%	97.2%	5.6%
7	37.6%	58.4%	92.2%	14.0%	42.7%	81.3%	84.3%	31.4%
8	17.7%	29.4%	57.4%	59.0%	43.7%	83.6%	80.9%	34.6%
9	65.7%	57.7%	95.4%	9.3%	69.2%	50.3%	81.8%	22.3%
10	19.7%	32.3%	15.2%	9.8%	22.3%	17.2%	51.0%	79.8%
11	26.4%	45.0%	69.7%	51.8%	31.9%	59.8%	62.7%	56.8%
12	64.3%	52.5%	27.5%	34.6%	67.7%	53.7%	95.9%	4.7%
13	26.9%	34.6%	27.3%	20.5%	59.2%	63.4%	72.8%	39.8%
14	21.3%	35.4%	27.1%	23.1%	31.9%	51.7%	86.1%	20.6%
15					69.3%	57.8%	91.2%	17.6%
16							0.9%	1.8%

IDU: injecting drug users; MMT: methadone maintenance treatment

Table 4. Internal consistency (Cronbach's α) of questionnaire items

Item no.	School adolescents (n=3,130)	Arrested drug users (n=1,162)	IDUs who received MMT (n=204)	Public health workers (n=116)
1*	0.68	0.55	0.80	0.63
2	0.68	0.59	0.80	—†
3	0.68	0.57	0.80	—†
4	0.69	0.58	0.81	0.66
5	0.71	0.56	0.80	0.62
6	0.67	0.57	0.79	0.63
7	0.69	0.58	0.80	0.58
8	0.70	0.58	0.80	0.62
9	0.68	0.60	0.81	0.62
10	0.71	0.61	0.83	0.60
11	0.70	0.60	0.81	0.61
12	0.69	0.55	0.80	0.64
13	0.71	0.57	0.81	0.64
14	0.70	0.60	0.81	0.64
15			0.80	0.62
16				0.67
All questions	0.71	0.60	0.82	0.65

*indicates Cronbach's α after the question was deleted. †Cronbach's α could not be calculated due to the absence of variability.
IDU: injecting drug users; MMT: methadone maintenance treatment

C. Test-Retest Reliability

Eighteen junior high school students from a cram school, 7 senior high school students, 45 vocational school students and 13 arrested drug users (a total of 83) completed the questionnaire twice; the response rate was 100%. The correlation coefficient was 0.59 ($p<.0001$). The kappa of all items was between 0.26 and 0.66, indicating good short-term stability (Table 5).

Discussion

Although the questionnaire on AIDS-related knowledge had good reliability and validity, there were too many items. Items not associated with the spread of the virus were deleted, and items related to the transmission route of the virus were retained (including prevention, early symptoms of AIDS, AIDS vaccine, screening, law, window period, incubation period, and virus survival time). In addition, the time interval for items regarding the window period, incubation period, and survival period of the virus were lengthened to reduce their difficulty and increase discrimination. There were 6 questions in the final version (Table 6).

Table 5. Test-retest reliability of questionnaire items

Item no.	School adolescents and arrested drug users (n=83)		
	First	Second	
	Correct (%)	Correct (%)	Kappa/ r
1	71.1%	73.5%	0.34
2	97.6%	96.4%	0.38
3	79.5%	79.5%	0.63
4	61.4%	59.0%	0.60
5	41.0%	42.2%	0.33
6	81.9%	78.3%	0.66
7	25.6%	31.3%	0.26
8	12.0%	8.4%	0.41
9	80.7%	85.5%	0.32
10	21.7%	19.3%	0.48
11	21.7%	18.1%	0.66
12	63.9%	71.1%	0.45
13	21.7%	25.3%	0.57
14	19.3%	14.5%	0.66
All	50.5±16.4	50.2±16.9	0.59

Table 6. Suggested version of the questionnaire

(1) Which of the following does not transmit the AIDS pathogen?
1☐ Blood 2☐ Semen 3☐ Saliva* 4☐ Vaginal discharge 9☐ I don't know

(2) Which of the following can prevent HIV/AIDS transmission?
1☐ Not share syringes, needles, diluents, or wash water with others*
2☐ Taking medicine to enhance the body's immunity 3□ Having sex with strangers
4☐ Use a condom before insertion of the penis into the vagina; it is not necessary to wear a condom for genital contact only
9☐ I don't know

(3) Which of the following is not correct regarding HIV infection?
1☐ The early symptoms of AIDS are unexplained weight loss and reduced immunity
2☐ There is no available vaccine or medicine that can prevent HIV infection or cure the disease
3☐ People can donate blood to find out whether they are infected with HIV or not*
4☐ In Taiwan, intentionally spreading HIV can result in a prison sentence 9☐ I don't know

(4) After being infected with the AIDS pathogen, there is a period of time during which blood tests will remain negative but it is still possible to infect others. This is called the "window period". How long can this period last?
1☐ 5-7 days 2☐ 2-3 weeks 3☐1-3 months* 4☐ 1-2 years 9☐ I don't know.

(5) After infection with HIV, there are no symptoms during a period of time called the incubation period. What is the longest time this period can last?
1☐ 1-3 weeks 2☐ 2-6 months 3☐ 1-2 years 4☐ 5-10 years* 9☐ I don't know.

(6) What's the longest time the AIDS pathogen can survive in a used syringe with residual blood?
1☐ A few minutes 2☐ A few hours 3☐ A few weeks* 4☐ A few months 9☐I don't know

* indicates correct answer. Note: the questions and options differ slightly from those in Table 1.

A. HIV Transmission

In Taiwan, both the rate of sex between adolescents [19] and the rate of addictive drug use [20] are increasing. Items on safer sex and the dangers of sharing needles, diluents, and wash water were added to the suggested version of the questionnaire. We expect that this version of the questionnaire will more accurately assess the extent of individual knowledge regarding the transmission and prevention of HIV/AIDS.

B. Early Symptoms of AIDS and the AIDS Vaccine

The early symptoms of AIDS, the AIDS vaccine, screening tests, and relevant legal issues were combined into a single question because the rates of correct answers for the original items were not very high. In regards to the early symptoms of AIDS, we hope that individuals realize that unexplained loss of body weight and reduced immunity are early symptoms of AIDS, so that they can pay more attention to physical changes in themselves and their partners. Early screening and prompt treatment are thus more likely. Regarding vaccine options, we hope that individuals at risk recognize that there is no vaccine or medicine that can prevent or cure HIV infection. As a result, individuals might be more careful in avoiding HIV infection.

C. AIDS Screening and the Law

In this study, respondents were mostly unaware of AIDS screening provided by Taiwan government. In the suggested version of the questionnaire, the answer, "people can donate blood to find out whether they are infected with HIV or not" was retained because we hoped that people at risk of infection would not donate blood for the purpose of determining their infection status.

In Taiwan, people who knowingly spread HIV to others are subject to imprisonment [13]. We thus retained item answers about AIDS law in the suggested version of the question, with the hope that people will recognize the consequences of spreading HIV.

D. Infectiousness of HIV (Window Period, Incubation Period, Survival)

The rates of correct answers on items pertaining to the window period, incubation period, and survival of HIV were relatively low. In our questionnaire, we hoped to teach subjects about the window and incubation periods. Furthermore, we expected that individuals might better appreciate the dangers of HIV infection when they understand that the window period is one to three months, that the incubation period can last eight to ten years, and that HIV can survive for several weeks in residual blood in a used syringe at room temperature.

E. Limitations

Despite the use of stratified random sampling (for school adolescents) and census (for arrested drug users, IDUs who received MMT, and public health worker), the representativeness of the sample is still problematic because the study was limited to Taoyuan. Our findings may thus not reflect AIDS knowledge in other areas of Taiwan.

In addition, some questions were valid only for a certain year (#5) or only for Taiwan (#4, #5, #13, #14). Hence, caution is advised when citing this study's rate of correct answers regarding AIDS-related knowledge.

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