VIRUS SEROLOGY

K.L. Tong, M.C. Wong, Y.K. Ho, S.Y. Kong, W.W. Choy, W.L. Lau, W. C. Lee

The external quality assessment programme of Virus Serology of the Hong Kong Institute of Medical Laboratory Science Quality Assurance Programme Limited was launched in 1991. The testing involved anti-HIV, HBsAg/Ab, and anti-HCV. Totally there were four surveys in 2011. Five anti-HIV, five HBsAg/Ab and five anti-HCV specimens were distributed in each survey. The testing of anti-HBs was optional and results were not scored. Participants were invited to record anti-HBs results for those specimens negative for HBsAg.

I. Participants

The overall number of participants and types of laboratories participated in the programme in 2011 are shown in Table 1. Statistics of laboratories returning survey data of anti-HIV, HBsAg/Ab and anti-HCV is shown in Table 2.

Table 1. Types of laboratories

Participants (n = 37)	Local	Overseas	Percent
Government Laboratory	1	1	5.4
Public Hospital Laboratories	6	-	16.2
Private Laboratories	19	-	51.4
Private Hospital Laboratories	8	1	24.3
Other	1	-	2.7

Table 2. Number of laboratories returned results in time

Test items	Survey				Total
	1st	2nd	3rd	4th	
Anti-HIV	33 / 33	33 / 33	34 / 34	31 / 35	131 / 135 (97.04%)
HBsAg	36 / 36	35 / 36	36 / 37	32 / 37	139 / 146 (95.21%)
Anti-HCV	27 / 27	27 / 27	27 / 27	24 / 28	105 / 109 (96.33%)

II. Results

Results were scored by using the scheme shown in Table 3.

Table 3. Scoring scheme of survey results

	Intended Result	
Participant report	Positive	Negative
Positive, to be confirmed or referred	2	0
Positive, not confirmed or referred	1	-1
Equivocal, to be confirmed or referred	1	2
Equivocal, not confirmed or referred	0	0
Negative	-1	2

Prepared by Mr Kwok-Leung Tong, Virus Serology Panel Head and authorized by Dr Kent Tsang, Chairman, HKIMLSQAP
Flat 1711, 17/F, Block C, Bell House, 525-543, Nathan Road, Yaumatei, Kowloon, Hong Kong
Phone: (852) 2499 0015, Fax: (852) 2124 2798, E-mail: qap_info@hkimls.org, URL: http://www.hkimls.org
Date: 13th October, 2012

Scores of two were given to the correct results of the reactive specimens of anti-HIV, HBsAg and anti-HCV with adequate confirmation and referral, while one for the reactive results without confirmation or indication of referral. The statistics of participants getting full score for anti-HIV, HBsAg and anti-HCV in 2011 are shown in Table 4.

Table 4. Numbers of participants getting the full score of 2

Test item	Ratio of total number of samples that attained full score to the total number of samples with results returned in time for data analysis for each test				Total (%)
	Survey One	Survey Two	Survey Three	Survey Four	
Anti-HIV	157 / 165	157 / 165	164 / 170	146 / 155	624 / 655 (95.3%)
HBsAg	159 / 180	154 / 175	162 / 180	152 / 164	627 / 699 (89.7%)
Anti-HCV	117 / 135	119 / 135	96 / 108	83 / 96	415 / 474 (87.6%)

Intended results and scoring for all survey specimens for anti-HIV, HBsAg and anti-HCV in 2011 are summarized in Table 5. The intended results of western blotting of all survey specimens are shown in Table 6.

Table 5. Score statistics

Specimen	Test	Intended Result	Score				Total Number of Participants
			2	1	0	-1	
VS1101	Anti-HIV	Pos	31	2	0	0	33
VS1102	Anti-HIV	Pos	31	2	0	0	33
VS1103	Anti-HIV	Pos	31	2	0	0	33
VS1104	Anti-HIV	Neg	33	0	0	0	33
VS1105	Anti-HIV	Pos	31	2	0	0	33
VS1106	HBsAg	Pos	29	7	0	0	36
VS1107	HBsAg	Neg	36	0	0	0	36
VS1108	HBsAg	Pos	29	7	0	0	36
VS1109	HBsAg	Neg	36	0	0	0	36
VS1110	HBsAg	Pos	29	7	0	0	36
VS1111	Anti-HCV	Pos	21	6	0	0	27
VS1112	Anti-HCV	Neg	27	0	0	0	27
VS1113	Anti-HCV	Pos	21	6	0	0	27
VS1114	Anti-HCV	Neg	27	0	0	0	27
VS1115	Anti-HCV	Pos	21	6	0	0	27
VS1116	Anti-HIV	Pos	30	3	0	0	33
VS1117	Anti-HIV	Neg	33	0	0	0	33
VS1118	Anti-HIV	Pos	30	3	0	0	33
VS1119	Anti-HIV	Neg	33	0	0	0	33
VS1120	Anti-HIV	Pos	31	2	0	0	33
VS1121	HBsAg	Pos	28	7	0	0	35
VS1122	HBsAg	Pos	28	7	0	0	35
VS1123	HBsAg	Neg	35	0	0	0	35
VS1124	HBsAg	Neg	35	0	0	0	35
VS1125	HBsAg	Pos	28	7	0	0	35
VS1126	Anti-HCV	Pos	22	5	0	0	27
VS1127	Anti-HCV	Neg	27	0	0	0	27
VS1128	Anti-HCV	Pos	21	6	0	0	27
VS1129	Anti-HCV	Neg	27	0	0	0	27
VS1130	Anti-HCV	Pos	22	5	0	0	27
VS1131	Anti-HIV	Pos	32	2	0	0	34

Specimen	Test	Intended Result	Score				Total Number of Participants
			2	1	0	-1	
VS1132	Anti-HIV	Pos	32	2	0	0	34
VS1133	Anti-HIV	Pos	32	2	0	0	34
VS1134	Anti-HIV	Neg	34	0	0	0	34
VS1135	Anti-HIV	Neg	34	0	0	0	34
VS1136	HBsAg	Pos	30	6	0	0	36
VS1137	HBsAg	Pos	30	6	0	0	36
VS1138	HBsAg	Pos	30	5	0	1	36
VS1139	HBsAg	Neg	36	0	0	0	36
VS1140	HBsAg	Neg	36	0	0	0	36
VS1141	Anti-HCV	Pos	20	6	0	1	27
VS1142	Anti-HCV	Neg	27	0	0	0	27
VS1143	Anti-HCV	Pos	22	5	0	0	27
VS1144	Anti-HCV	Pos	NS	NS	NS	NS	27
VS1145	Anti-HCV	Neg	27	0	0	0	27
VS1146	Anti-HIV	Pos	29	2	0	0	31
VS1147	Anti-HIV	Neg	30	0	1	0	31
VS1148	Anti-HIV	Pos	29	2	0	0	31
VS1149	Anti-HIV	Pos	29	2	0	0	31
VS1150	Anti-HIV	Pos	29	2	0	0	31
VS1151	HBsAg	Pos	28	4	0	0	32
VS1152	HBsAg	Neg	32	0	0	0	32
VS1153	HBsAg	Pos	28	4	0	0	32
VS1154	HBsAg	Neg	32	0	0	0	32
VS1155	HBsAg	Pos	32	4	0	0	32
VS1156	Anti-HCV	Pos	20	4	0	0	24
VS1157	Anti-HCV	Neg	24	0	0	0	24
VS1158	Anti-HCV	Pos	19	5	0	0	24
VS1159	Anti-HCV	Pos	NS	NS	NS	NS	24
VS1160	Anti-HCV	Pos	20	4	0	0	24

Remarks: Pos: positive; Neg: negative; NS: Not scored

Table 6. Results of anti-HIV-1derived from western blotting

Specimen	Intended Result	Result of western blotting
VS 1101	POS	p24, p31, gp41, p51, p55, p66 & gp120/160
VS 1102	POS	p24, p31, gp41, p66 & gp120/160
VS 1103	POS	p24, p31, gp41, p51, p55, p66 & gp120/160
VS 1104	NEG	No band
VS 1105	POS	p24, p31, gp41, p51, p66 & gp120/160
VS 1116	POS	p24, p31, gp41, p51, p55 & p66, gp120/160
VS 1117	NEG	No bands
VS 1118	POS	p24, p31, gp41, p66 & gp120/160
VS 1119	NEG	No band
VS 1120	POS	p24, p31, gp41, p66 & gp120/160
VS 1131	POS	p24, p31, gp41, p51, p55, p66 & gp120/160
VS 1132	POS	p24, p31, gp41, p51, p55, p66 & gp120/160
VS 1133	POS	p24, p31, gp41 & gp120/160
VS 1134	NEG	No band
VS 1135	NEG	No band
VS 1146	POS	p24, p31, gp41, p51, p55, p66 & gp120/160
VS 1147	NEG	No band
VS 1148	POS	p24, p31, gp41, p51, p55, p66 & gp120/160
VS 1149	POS	p24, p31, gp41, p51, p55, p66 & gp120/160
VS 1150	POS	p24, p31, gp41, p51, p55, p66 & gp120/160

Remarks: POS: positive; NEG: negative

The intended results and concentrations of HBsAg are summarized in Table 7. The concentrations of anti-HBs are shown in Table 8, while the intended results of anti-HCV are summarized in Table 9.

Table 7. Intended results and concentrations of HBsAg

Specimen	Intended result	Concentration (IU/mL)	Specimen	Intended result	Concentration (IU/mL)
VS 1106	POS	74	VS 1136	POS	0.85
VS 1107	NEG	Not detected	VS 1137	POS	104
VS 1108	POS	>250	VS 1138	POS	66
VS 1109	NEG	Not detected	VS 1139	NEG	Not detected
VS 1110	POS	13	VS 1140	NEG	Not detected
VS 1121	POS	33	VS 1151	POS	87.5
VS 1122	POS	102	VS 1152	NEG	Not detected
VS 1123	NEG	Not detected	VS 1153	POS	13.2
VS 1124	NEG	Not detected	VS 1154	NEG	Not detected
VS 1125	POS	15	VS 1155	POS	110

Remarks**:** POS: positive; NEG: negative

Table 8. Concentrations of the anti-HBs in the specimens negative for HBsAg

Specimen	Concentration (mIU/mL)	Range of Reported concentration (mIU/mL)
VS 1107	404	280.90 to 804.60
VS 1109	36	12 to 44.16
VS 1123	27	17 to 35.3
VS 1124	>600	438.9 to >1,000
VS 1139	33	18.3 to 62.7
VS 1140	nil	Not applicable
VS 1152	566.5	208 to >1,000
VS 1154	36.2	25.9 to 76.21

Table 9. Intended results of anti-HCV

Specimen	Intended Result of Anti-HCV
VS1111	POS
VS1112	NEG
VS1113	POS
VS1114	NEG
VS1115	POS
VS1126	POS
VS1127	NEG
VS1128	POS
VS1129	NEG
VS1130	POS
VS1141	POS
VS1142	NEG
VS1143	POS
VS1144	POS
VS1145	NEG
VS1156	POS
VS1157	NEG
VS1158	POS
VS1159	POS
VS1160	POS

Remarks: POS: positive; NEG: negative

III. Methods and assay kits

The commercial kits used by the participants in 2011 for testing of anti-HIV, HBsAg/Ab and anti-HCV are summarized in Tables 10 to 13.

Table 10. Commercial assays for anti-HIV antibodies used by participants

Name of Anti-HIV Assay	Method	Survey				Total
		1st	2nd	3rd	4th	
Abbott ARCHITECT HIV Ag/Ab Combo	CMIA	21	21	22	20	84
Abbott AxSYM HIV 1/2 gO	MEIA	2	1	2	2	7
Abbott AxSYM HIV Ag/Ab Combo	MEIA	2	2	1	1	6
Abbott Murex HIV Ag/Ab Combination	EIA	1	1	1	1	4
ADVIA Centaur EHIV	ChLIA	2	2	2	0	6
Bioline HIV SCAN 1/2 3.0	ICA	0	0	1	1	2
bioMérieux VIDAS HIV DUO QUICK	ELFA	2	2	3	2	9
bioMérieux VIDAS HIV DUO ULTRA	ELFA	2	2	3	4	11
bioMérieux Vironostika HIV	EIA	2	2	2	2	8
Determine Inverness Medical HIV-1/2	ICA	2	2	1	2	7
EY HIVSCAN TM 1+2 (2.1)	ICA	0	0	1	1	2
Innogenetics, INNO-LIA HIV I/II Score	LIA	1	1	1	1	4
MP Diagnostics HIV Blot 2.2	WB	2	2	2	2	8
Ortho Anti-HIV Reagent	EIA	1	1	1	1	4
Roche HIV Combi	ChLIA	5	5	5	6	21
Standard Diagnostics SD HIV 1/2 3.0	ICA	3	3	2	2	10
Trinity Biotech Uni-Gold HIV	ICA	1	0	0	0	1

Table 11. Commercial assays for HBsAg used by participants

Name of HBsAg Assay	Method	Survey				Total
		1st	2nd	3rd	4th	
Abbott ARCHITECT HBsAg	CMIA	8	6	6	4	24
Abbott ARCHITECT HBsAg Qualitative	CMIA	17	18	20	19	74
Abbott AxSYM HBsAg (V2)	MEIA	4	5	4	3	16
ADVIA Centaur HBsAg	ChLIA	3	3	3	2	11
bioMérieux VIDAS HBsAg	ELFA	2	2	2	2	8
Determine HBsAg	ICA	4	4	4	5	17
Johnson & Johnson HBsAg	ECLIA	3	2	2	3	10
Murex HBsAg Ver. 3	EIA	1	1	2	1	5
Ortho HBsAg	EIA	1	1	1	1	4
Roche HBsAg II	ChLIA	5	5	4	6	20
Standard Diagnostics SD HBsAg	ICA	2	1	2	2	7

Table 12. Commercial assays for anti-HBs used by participants

Name of Anti-HBs Assay	Method	Survey				Total
		1st	2nd	3rd	4th	
Abbott ARCHITECT anti-HBs	CMIA	22	22	25	22	91
Abbott AxSYM AUSAB	MEIA	4	5	4	3	16
ADVIA Centaur Anti-HBs	ChLIA	2	2	2	0	6
bioMérieux VIDAS Anti-HBs	ELFA	1	1	1	0	3
Ortho Anti-HBs	EIA	1	1	1	3	6
Roche Cobas Core Anti-HBs	ChLIA	4	4	4	5	17

Table 13. Commercial assays for anti-HCV used by participants

Name of Anti-HCV Assay	Method	Survey				Total
		1st	2nd	3rd	4th	
Abbott ARCHITECT anti-HCV	CMIA	17	17	17	15	66
Abbott AxSYM MEIA anti-HCV	MEIA	2	2	2	2	8
Abbott Murex HCV ver. 4.0	EIA	1	1	1	1	4
ADVIA Centaur anti-HCV	ChLIA	3	3	3	3	12
Biokit Bioelisa HCV 4.0	EIA	0	0	1	0	1
CHIRON RIBA HCV 3.0	RIBA	0	1	1	1	3
Ortho HCV 3.0	EIA	4	4	4	2	14
Roche HCV 3.0	ChLIA	1	1	1	1	4
Standard Diagnostics SD HCV	ICA	4	4	4	3	15

Abbreviations:
ChLIA: Chemiluminescence Assay
CMIA: Chemiluminescent Microparticle Immunoassay
ECLIA: Electrochemiluminescence Immunoassay
EIA: Enzyme Linked Immunoassay
ELFA: Enzyme-linked Fluorescent Assay
ICA: Immunochromatographic Assay
LIA: Line Immunoassay
MEIA: Microparticle Enzyme Immunoassay
RIBA: Recombinant Immuno-Blot Assay
WB: Western Blot

IV. Testing strategies used by participants

In 2011, a majority of the participants (93.1%) conveyed the survey specimens to a reference laboratory for confirmation of anti-HIV. There were three laboratories using a single assay for anti-HIV without a supplementary testing or referral (Table 14).

Table 14. Anti-HIV testing

Testing strategy	Survey			
	1st	2nd	3rd	4th
With indication of a referral	31	30	32	29
Without a supplementary testing or referral	2	3	2	2
Using a single assay	17	20	23	18
Using two assays	13	13	12	10
Using a supplementary testing	3	3	3	3

For hepatitis B serology, majority of the participants (82.7%) confirmed positive results. Up to seven laboratories performed a single assay without supplementary testing or referral for HBsAg (Table 15).

Table 15. HBsAg testing

Testing strategy	Survey			
	1st	2nd	3rd	4th
With indication of a referral	29	28	30	28
Without a supplementary testing or referral	7	7	6	4
Using a single assay	22	22	25	18
Using two or more assays	14	14	12	14

For anti-HCV testing, majority of participants (81.0%) indicated sending specimens with reactive results to the reference laboratory for confirmation. Up to six laboratories performed a single assay without supplementary testing or referral for anti-HCV (Table 16).

Table 16. Anti-HCV testing

Testing strategy	Survey			
	1st	2nd	3rd	4th
With indication of a referral	21	22	22	20
Without a supplementary testing or referral	6	5	5	4
Using a single assay	23	23	23	19
Using two or more assays	4	4	6	5

V. Unexpected issues observed in 2011

Non-conformities encountered commonly in the returned results are categorized in Table 17.

Table 17. Unexpected issues observed in the surveys in 2011

Category	Observation	Involved test/assay
Incorrect result	In Survey Two, a participant indicated a false positive HBsAg result for VS1124. The participant reported a final equivocal result with indication of a referral confirmation for the specimen.	ADVIA Centaur HBsAg
	In Survey Four, a participant reported a positive anti-HIV result for VS1147.	Abbott Architect HIV Ag/Ab Combo
	In Survey Three, a participant reported an incorrect false negative result of HBsAg for VS1138.	Abbott Architect HBsAg Quali
Transcription error	In Survey One, a participant obtained a non-reactive HBsAg result for VS1110, but submitted a reactive result in the result form.	
	In Survey Three, a participant reported a negative HBsAg result for VS1136 based upon a positive readout. With reference to the result of another assay, the participant submitted a positive result finally.	
Use of Insensitive assay	In Survey Two, a participant reported weakly positive results for VS1126 and VS1130 by using a rapid immunochromatographic assay.	Standard Diagnostics SD HCV
	In Survey 3, two participants reported a weakly positive and a negative anti-HCV result for VS1141 by using a rapid immunochromatographic assay.	Standard Diagnostics SD HCV
Outliers	In Survey One, a participant reported readings of greater than 3SD for all reactive anti-HIV survey samples.	Abbott Architect HIV Ag/Ab Combo
	In Survey Three, three participants reported ratios of sample reading to the cutoff reading being greater than SD for anti-HIV, HBsAg and anti-HCV.	Abbott Architect HIV Ag/Ab Combo Abbott Architect HBsAg Quali Abbott Architect Anti-HCV
Use of expired assays	In Survey Three, two participants used expired assays for testing anti-HIV and anti-HCV.	Abbott Architect HIV Ag/Ab Combo Abbott Architect Anti-HCV

VI. Recommendations

The flaws noted in the four survey exercises of 2011 can be categorized into five different entities namely, incorrect result, transcription errors, use of insensitive assays, outliers of sample results and the use of expired assays for testing.

Quality assurance of clinical testing is important to ensure the accuracy and precision of test results. Analyzers should be calibrated according to the instruction given by the suppliers. External quality controls are used to ensure accuracy of detection on a daily basis in order to minimize errors in a clinical test which may lead to spurious diagnosis, delayed treatment or increased costs associated with re-testing. For quality measures, expired assays should not be used as the performance of the assay may reduce. Results of test runs should be validated by using controls of the assay and/or reference materials from other external sources. Daily monitoring on the reading of the quality control samples and the scattering in the distribution charts is a good way to identify deviations that may exist in the testing procedures. The examination of the graphics can help reveal clues of inferior performance. The occurrence of outliers significantly deviated from the mean value by 3 standard deviations and the existence of trends of positive or negative bias of readouts in the quality control plots are suggestive of performance deterioration. Action should be taken to find out the root causes. The performance of an assay should be reviewed if questionable results are repeatedly noted.

Errors which were encountered in the participating laboratories are classified into pre-analytical, analytical and post-analytical. Laboratory automation, training of laboratory personnel and adoption of quality control programmes can reduce substantially the occurrence of analytical errors. The swapping of specimens at time of testing and transcription errors made at time of result reporting are evident among participants in survey exercises. Laboratories experiencing transcription errors should review the testing and reporting procedures meticulously to identify the root causes and prevent the re-occurrence. Counterchecking samples and test results is also helpful to minimize errors.

Simple and rapid tests have existed for years. They can generate results in a short period of time. However, they are relatively less specific and sensitive compared to the conventional assays. Errors in the interpretation of readouts increase especially in specimens of a low reactivity. For laboratory diagnosis, a sensitive assay for initial testing and a more specific assay for the confirmation of an initial reactive result should be used. Should a confirmatory test or a supplementary testing be non-available in an individual laboratory, the specimen with initial reactive result has to be conveyed to a reference laboratory for affirmation

Using diagnostic assays beyond the expiration date is not recommended as they may compromise test results.

VII. Reference

Immunoassays for the diagnosis of HIV: Test Quality & Laboratory Quality Assurance: http://www.medscape.com/viewarticle/715166_5

HIV Testing Algorithm: A Status Report: A publication from the Association of Public Health Laboratories and the Centers for Diseases Control and Prevention. April 2009: http://www.aphl.org/aphlprograms/infectious/hiv/Documents/StatusReportExecutiveSummary.pdf

Point-of-Care Rapid Tests for HIV Antibody: http://www.cdc.gov/hiv/topics/testing/resources/journal_article/J_Lab_Med_20031.htm

Guidelines for Laboratory Testing and Result Reporting of Antibody to Hepatitis C Virus: http://www.cdc.gov/MMWR/PREVIEW/mmwrhtml/rr5203a1.htm

Tong, K.L, Cheng, K.C., Wong, M.C, Ho. Y.K., Foo S.Y., Szeto, T.C., V. Lee, Virus Serology. In Hong Kong Medical Technology Association Quality Assurance Programme. Annual Report 1995:45-52.

Tong, K.L, Cheng, K.C., Wong, M.C, Ho. Y.K., Kong S.Y. Virus Serology. In Hong Kong Medical Technology Association Quality Assurance Programme. Annual Report 2008.

UK National External Quality Assessment Service for Microbiology

Acknowledgements

The HKIMLSQAP would like to thank Hong Kong Red Cross Blood Transfusion Service for supplying plasma samples and medical laboratories participating in the establishment of consensus readouts of the survey materials in year 2011.

- End -

Prepared by Mr Kwok-Leung Tong, Virus Serology Panel Head and authorized by Dr Kent Tsang, Chairman, HKIMLSQAP
Flat 1711, 17/F, Block C, Bell House, 525-543, Nathan Road, Yaumatei, Kowloon, Hong Kong
Phone: (852) 2499 0015, Fax: (852) 2124 2798, E-mail: qap_info@hkimls.org, URL: http://www.hkimls.org