VIRUS SEROLOGY

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The external quality assessment programme of Virus Serology of the Hong Kong Institute of Medical Laboratory Sciences Quality Assurance Programme Limited was launched in 1991. The testing involved anti-HIV, HBsAg/anti-HBs, and anti-HCV. Totally there were four surveys in 2012. Five anti-HIV, five HBsAg/anti-HBs 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 participating in the programme in 2012 are shown in Table 1. Statistics of laboratories returning survey data of anti-HIV, HBsAg/anti-HBs and anti-HCV are shown in Table 2.

Table 1. Types of laboratories

Participants (n = 42)	Local	Overseas	Percent
Government Laboratories	1	2	7.1
Public Hospital Laboratories	6	-	14.3
Private Hospital Laboratories	7	2	21.4
Private Laboratories	23	-	54.8
Other	1	-	2.4

Table 2. Number of laboratories returned results within deadline

Test items	Survey				Total
	1st	2nd	3rd	4th	
Anti-HIV	35 / 37	38 / 38	36 / 39	40 / 41	149 / 155 (96.1%)
HBsAg	36 / 38	39 / 39	37 / 40	41 / 42	153 / 159 (96.2%)
Anti-HCV	29 / 30	31 / 31	30 / 32	34 / 34	124 / 127 (97.6%)

II. Results

Results were scored by using the scheme as 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

The statistics of participants getting full score for anti-HIV, HBsAg and anti-HCV in 2012 are shown in Table 4.

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Table 4. Numbers of participants getting 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	169 / 175	183 / 190	174 / 180	192 / 199	718 / 744 (96.5%)
HBsAg	152 / 180	171 / 195	153 / 185	184 / 205	660 / 765 (86.3%)
Anti-HCV	118 / 145	123 / 155	93 / 120	133 / 170	467 / 590 (79.2%)

Intended results and scoring for all survey specimens for anti-HIV, HBsAg and anti-HCV in 2012 are summarized in Table 5. The intended Western blot results of all anti-HIV survey specimens are shown in Table 6.

Table 5. Score statistics

Specimen	Test	Intended Result	Score				Total Number of Participants
			2	1	0	-1	
VS1201	Anti-HIV	Pos	33	2	0	0	35
VS1202	Anti-HIV	Neg	35	0	0	0	35
VS1203	Anti-HIV	Pos	33	2	0	0	35
VS1204	Anti-HIV	Neg	35	0	0	0	35
VS1205	Anti-HIV	Pos	33	2	0	0	35
VS1206	HBsAg	Pos	29	7	0	0	36
VS1207	HBsAg	Pos	29	7	0	0	36
VS1208	HBsAg	Pos	29	7	0	0	36
VS1209	HBsAg	Pos	29	7	0	0	36
VS1210	HBsAg	Neg	36	0	0	0	36
VS1211	Anti-HCV	Pos	20	9	0	0	29
VS1212	Anti-HCV	Pos	20	9	0	0	29
VS1213	Anti-HCV	Neg	29	0	0	0	29
VS1214	Anti-HCV	Pos	20	9	0	0	29
VS1215	Anti-HCV	Neg	29	0	0	0	29
VS1216	Anti-HIV	Pos	36	2	0	0	38
VS1217	Anti-HIV	Pos	36	2	0	0	38
VS1218	Anti-HIV	Neg	38	0	0	0	38
VS1219	Anti-HIV	Neg	38	0	0	0	38
VS1220	Anti-HIV	Pos	35	2	0	1	38
VS1221	HBsAg	Pos	33	6	0	0	39
VS1222	HBsAg	Pos	33	6	0	0	39
VS1223	HBsAg	Pos	33	6	0	0	39
VS1224	HBsAg	Pos	33	6	0	0	39
VS1225	HBsAg	Neg	39	0	0	0	39
VS1226	Anti-HCV	Pos	23	8	0	0	31
VS1227	Anti-HCV	Pos	23	8	0	0	31
VS1228	Anti-HCV	Neg	31	0	0	0	31
VS1229	Anti-HCV	Pos	23	8	0	0	31
VS1230	Anti-HCV	Pos	23	8	0	0	31
VS1231	Anti-HIV	Pos	34	2	0	0	36
VS1232	Anti-HIV	Neg	36	0	0	0	36
VS1233	Anti-HIV	Pos	34	2	0	0	36
VS1234	Anti-HIV	Neg	36	0	0	0	36
VS1235	Anti-HIV	Pos	34	2	0	0	36
VS1236	HBsAg	Neg	37	0	0	0	37
VS1237	HBsAg	Pos	29	8	0	0	37
VS1238	HBsAg	Pos	29	8	0	0	37
VS1239	HBsAg	Pos	29	8	0	0	37

Specimen	Test	Intended Result	Score				Total Number of Participants
			2	1	0	-1	
VS1240	HBsAg	Pos	29	8	0	0	37
VS1241	Anti-HCV	Pos	20	9	0	1	30
VS1242	Anti-HCV	Pos	21	9	0	0	30
VS1243	Anti-HCV	Pos	22	8	0	0	30
VS1244	Anti-HCV	Neg	30	0	0	0	30
VS1245	Anti-HCV	Pos	NS	NS	NS	NS	30
VS1246	Anti-HIV	Pos	38	1	0	0	39
VS1247	Anti-HIV	Neg	40	0	0	0	40
VS1248	Anti-HIV	Pos	38	2	0	0	40
VS1249	Anti-HIV	Pos	38	2	0	0	40
VS1250	Anti-HIV	Pos	38	2	0	0	40
VS1251	HBsAg	Neg	40	0	1	0	41
VS1252	HBsAg	Neg	41	0	0	0	41
VS1253	HBsAg	Pos	35	6	0	0	41
VS1254	HBsAg	Pos	34	6	0	1	41
VS1255	HBsAg	Pos	34	7	0	0	41
VS1256	Anti-HCV	Pos	25	9	0	0	34
VS1257	Anti-HCV	Pos	25	9	0	0	34
VS1258	Anti-HCV	Neg	34	0	0	0	34
VS1259	Anti-HCV	Pos	25	9	0	0	34
VS1260	Anti-HCV	Pos	24	9	0	1	34

Remarks: Pos: Positive; Neg: Negative; NS: Not scored

Table 6. Western blot results of anti-HIV specimens

Specimen	Intended Result	Western Blot Results
VS1201	POS	p24, p31, gp41, p51, p55, p66 & gp120/160
VS1202	NEG	No band
VS1203	POS	p24, p31, gp41, p51, p55, p66 & gp120/160
VS1204	NEG	No band
VS1205	POS	p24, p31, gp41, p51, p66 & gp120/160
VS1216	POS	p24, p31, gp41, p51, p66 & gp120/160
VS1217	POS	p24, p31, gp41& gp120/160
VS1218	NEG	No band
VS1219	NEG	No band
VS1220	POS	p24, p31, gp41, p66 & gp120/160
VS1231	POS	p24, p31, gp41, p51, p66 & gp120/160
VS1232	NEG	No band
VS1233	POS	p24, p31, gp41, p51, p66 & gp120/160
VS1234	NEG	No band
VS1235	POS	p24, p31, gp41, p51, p55, p66 & gp120/160
VS1246	POS	p24, p31, gp41, p51, p66 & gp120/160
VS1247	NEG	No band
VS1248	POS	p24, p31, gp41, p51, p66 & gp120/160
VS1249	POS	p24, p31, gp41, p51, p66 & gp120/160
VS1250	POS	p24, p31, gp41, p51, 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)
VS1206	POS	4	VS1236	NEG	Not detected
VS1207	POS	39	VS1237	POS	>250
VS1208	POS	>250	VS1238	POS	23
VS1209	POS	39	VS1239	POS	>250
VS1210	NEG	Not detected	VS1240	POS	>250
VS1221	POS	117	VS1251	NEG	Not detected
VS1222	POS	53	VS1252	NEG	Not detected
VS1223	POS	>250	VS1253	POS	>250
VS1224	POS	2	VS1254	POS	13.5
VS1225	NEG	Not detected	VS1255	POS	104

Remarks**:** POS: Positive; NEG: Negative

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

Specimen	Expected Concentration (mIU/mL)	Range of Reported Concentration (mIU/mL)
VS1210	581.8	454.9 to 704.4
VS1225	21.4	16 to 40.1
VS1236	100	75 to 204.6
VS1251	214.4	101 to >500
VS1252	366.3	280.5 to >500

Table 9. Intended results of anti-HCV

Specimen	Intended Result of Anti-HCV
VS1211	POS
VS1212	POS
VS1213	NEG
VS1214	POS
VS1215	NEG
VS1226	POS
VS1227	POS
VS1228	NEG
VS1229	POS
VS1230	POS
VS1241	POS
VS1242	POS
VS1243	POS
VS1244	NEG
VS1245	Weakly POS (NS)
VS1256	POS
VS1257	POS
VS1258	NEG
VS1259	POS
VS1260	POS

Remarks: POS: Positive; NEG: Negative; NS: Not scored

III. Methods and assay kits

The commercial kits used by the participants in 2012 for testing of anti-HIV, HBsAg/anti-HBs and anti-HCV are summarized in Tables 10 to 13.

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

Name of Anti-HIV Assay	Method*	Survey				Total
		1st	2nd	3rd	4th	
Abbott ARCHITECT HIV Ag/Ab Combo	CMIA	22	24	22	24	92
Abbott AxSYM MEIA AHIV	MEIA	1	2	2	2	7
ACON HIV 1+2		0	1	0	1	2
Alere Determine Inverness Medical HIV-1/2	ICA	3	3	3	5	14
Bioline HIV SCAN 1/2 3.0	ICA	1	1	3	2	7
bioMérieux VIDAS HIV DUO QUICK	ELFA	2	2	2	2	8
bioMérieux VIDAS HIV DUO ULTRA	ELFA	4	3	2	3	12
bioMérieux Vironostika HIV	EIA	2	2	2	2	8
Diasorin Murex HIV Ag/Ab Combination	EIA	1	1	1	1	4
EY HIVSCAN TM 1+2 (2.1)	ICA	1	1	0	0	2
Innogenetics, INNO-LIA HIV I/II Score	LIA	1	1	1	1	4
Ortho-Clinical Diagnostics Anti-HIV 1+2	ECLIA	1	2	2	2	7
MP Diagnostics HIV Blot 2.2	WB	2	2	2	2	8
Roche HIV Combi	CHLIA	6	7	7	9	29
Siemens ADVIA Centaur EHIV	CHLIA	1	1	1	2	5
Standard Diagnostics SD HIV 1/2 3.0	ICA	2	2	3	2	9

Table 11. Commercial assays for HBsAg used by participants

Name of HBsAg Assay	Method*	Survey				Total
		1st	2nd	3rd	4th	
Abbott ARCHITECT HBsAg	CMIA	5	5	3	4	17
Abbott ARCHITECT HBsAg Qualitative	CMIA	20	22	22	24	88
Abbott AxSYM HBsAg (V2)	MEIA	1	2	2	2	7
Alere Determine HBsAg	ICA	2	2	2	5	11
bioMérieux VIDAS HBsAg	ELFA	3	3	2	3	11
Diasorin Murex HBsAg Ver. 3	EIA	2	2	2	2	8
Ortho Clinical Diagnostics HBsAg	ECLIA	3	2	3	3	11
Roche HBsAg II	ChLIA	6	7	7	9	29
Siemens ADVIA Centaur HBsAg	ChLIA	4	3	3	4	14
Standard Diagnostics SD HBsAg	ICA	2	2	2	1	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	25	26	24	26	101
Abbott AxSYM AUSAB	MEIA	0	1	0	0	1
bioMérieux VIDAS Anti-HBs	ELFA	1	1	1	1	4
Diasorin ETI-AB-AUK-3	EIA	0	0	1	1	2
Ortho Clinical Diagnostics Anti-HBs	EIA	1	1	1	2	5
Roche Cobas Core Anti-HBs	ChLIA	6	7	7	8	28
Siemens ADVIA Centaur Anti-HBs	ChLIA	2	2	1	2	7

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	18	19	20	21	78
Abbott AxSYM MEIA anti-HCV	MEIA	2	2	1	2	7
Biokit Bioelisa HCV 4.0	EIA	1	0	0	0	1
Diasorin Murex HCV ver. 4.0	EIA	1	1	1	1	4
INNO-LIA HCV Score	LIA	0	0	1	2	3
Ortho CHIRON RIBA HCV 3.0	RIBA	1	2	1	1	5
Ortho Clinical Diagnostics Anti-HCV	ECLIA	3	4	2	4	13
Ortho HCV Ver 3.0 ELISA	EIA	1	1	1	1	4
Roche HCV 3.0	ChLIA	2	2	2	4	10
Siemens ADVIA Centaur anti-HCV	ChLIA	3	3	3	3	12
Standard Diagnostics SD HCV	ICA	3	4	4	4	15
WONDFO HCV	EIA	0	0	1	1	2

Abbreviations:*

ChLIA: Chemiluminescence Immunoassay
CMIA: Chemiluminescent Microparticle Immunoassay
ECLIA: Electrochemiluminescence Immunoassay
EIA: Enzyme Linked Immunoassay
ELFA: Enzyme-linked Fluorescence 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 2012, the majority of the participants (85.9%) indicated referral of screened positive specimens to a reference laboratory for confirmation of anti-HIV. There were two laboratories using a single assay for anti-HIV without a supplementary testing or referral for positive specimens (Table 14).

Table 14. Anti-HIV testing

Testing strategy	Survey			
	1st	2nd	3rd	4th
Using a single assay	22	25	24	26
Using two assays	10	9	9	11
Using a supplementary testing	3	4	3	3
With indication of referral	30	30	31	35
Without supplementary testing or referral	2	2	2	2

For hepatitis B serology, majority of the participants (62.7%) confirmed positive results. Up to eight laboratories performed a single assay without supplementary testing or referral for HBsAg positive specimens (Table 15).

Table 15. HBsAg testing

Testing strategy	Survey			
	1st	2nd	3rd	4th
Using a single assay	21	14	8	6
Using two or more assays	18	25	24	29
With indication of referral	11	8	5	6
Without supplementary testing or referral	7	6	8	6

For anti-HCV testing, majority of participants (49.2%) indicated sending specimens with reactive results to the reference laboratory for confirmation. Up to nine laboratories performed a single assay without supplementary testing or referral for anti-HCV positive specimens (Table 16).

Table 16. Anti-HCV testing

Testing strategy	Survey			
	1st	2nd	3rd	4th
Using a single assay	23	24	24	25
Using two or more assays	6	7	6	9
With indication of referral	14	16	15	16
Without supplementary testing or referral	9	8	9	9

V. Unexpected issues observed in 2012

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

Table 17. Unexpected issues observed in the surveys in 2012

Category	Observation	Involved test/assay
Incorrect result (n = 5)	In Survey One, a participant (048) indicated a false positive anti-HBs result for VS1208.	Roche Anti-HBs
	In Survey Four, two participants (236 & 898) obtained false reactive HBsAg results for VS1251 (Lab 236 reported an overall negative result after confirmation).	Abbott Architect HBsAg-Qualitative II
	In Survey Four, a participant (336) reported a false negative HBsAg result for specimen VS1254.	Abbott Architect HBsAg-Qualitative II
	In Survey Four, a participant (336) reported a false negative anti-HCV result for specimen VS1260.	Roche Elecsys Anti-HCV
Outlier of sample result (n = 4)	In Survey Four, a participant (561) reported readings of greater than mean-3SD for all reactive HBsAg survey samples (VS1253 to VS1255).	bioMerieux VIDAS HBsAg-Ultra
	In Survey Four, two participants (236 & 878) reported results of anti-HBs with readings greater than mean-3SD for specimen VS1252.	Abbott Architect Anti-HBs
	In Survey Four, a participant (898) reported results of anti-HCV with readings greater than mean ±3SD for all specimens (VS1256 to VS 1260).	Abbott Architect Anti-HCV
Sensitivity of assay (n = 4)	In Survey Three, a participant (683) reported a false negative anti-HCV result for VS1241 using only a rapid immunochromatographic assay for test.	Standard Diagnostics SD-HCV
	In Survey Three, three participants (002, 508 & 683) obtained initial false negative anti-HCV results for VS1245 using the same rapid kit as mentioned above.	
Use of expired assay for testing (n = 2)	In Survey Four, two participants (642 & 036) used expired assays for anti-HIV and anti-HCV testing respectively.	Abbott Architect HIV Ag/Ab-Combo Ortho Clinical Diagnostics-Anti-HCV
Transcription error (n = 3)	In Survey One, a participant (336) returned results after the deadline of submission and reported a false positive anti-HCV result for VS1204 with test reading below the cutoff value.	Abbott Architect HIV Ag/Ab-Combo
	In Survey Two, a participant (898) reported a result with reactive anti-HIV sample reading for VS1220, but an overall false negative result.	Abbott Architect HIV Ag/Ab-Combo
	In Survey Four, a participant (336) reported a negative anti-HCV result for specimen VS1260 with a positive OD ratio.	Roche Elecsys Centaur Anti-HCV

VI. Recommendations

The improvement areas noted in the four survey exercises of 2012 can be categorized into five different entities namely, incorrect result, outlier of sample result, sensitivity of assay, use of expired assay for testing and transcription error.

Quality assurance of clinical testing is important to ensure the accuracy and precision of test results. Errors in clinical tests may lead to spurious diagnosis, delayed treatment or increased costs associated with re-testing. In order to minimize errors, every step from pre-analytical to post-analytical procedures should be handled prudently. Laboratory automation, training of laboratory personnel and adoption of quality control programmes are main factors that govern the quality assurance of a clinical laboratory. For quality measures, analyzers should be calibrated according to the instruction given by the suppliers. Expired assays should not be used. Results of test runs should be validated using controls of the assay, reference materials and quality control from other external sources on routine testing. Daily monitoring on the reading of the quality control samples and the scattering in the control charts are good ways to identify deviations of tests. 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 charts are indications of performance deterioration. The swapping of specimens at time of testing or transcription errors made at time of result reporting is not uncommon among participants in the survey exercises. Counterchecking samples and test results may help to minimize such errors. Action should be taken to find out the root causes of any nonconformity and prevent re-occurrence.

Simple and rapid tests have existed for years. Although they can generate results in a short period of time, they may be relatively less specific and sensitive as compared to conventional assays. Difficulty in the interpretation of test results may increase especially for those specimens with 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. If rapid kits are to be used for screening, their characteristics and performance should be understood and thoroughly evaluated with respect to sensitivity and specificity. Incorrect false negative results are recurrently observed with test results obtained using certain rapid kits in this external quality assurance scheme in the past few years. For example, from the submitted data in 2012, a few laboratories have obtained negative results using SD Bioline HCV (Standard Diagnostics, Inc.) for some intended positive samples [VS1241 & VS1245 (Survey 3)] which may suggest its lower sensitivity as compared to other conventional assays

used by other participated laboratories. It is further evidenced by the WHO evaluation report (Phase 1) of SD Bioline HCV, which stated that the assay gave an initial and repeat sensitivity value of 94.1% (95% CL [85.6% to 98.4%]) and an initial specificity value of 100% (95% CL [98.1% to 100%]) from 257 evaluated samples. The main criterion for an anti-HCV screening assay is to have excellent sensitivity in combination with the best specificity as far as possible. The performance of an assay should also be reviewed if questionable results are repeatedly noted, and in such cases, reporting based on a single assay result should be avoided. Should a confirmatory test or a supplementary test be non-available in an individual laboratory, the specimen with initial reactive result has to be referred to a reference laboratory for confirmation. In addition, a regular mechanism of kit evaluation will also help to ensure use of quality commercial assays.

VII. Reference

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- End -

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