HAEMATOLOGY & SEROLOGY

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The Hong Kong Institute of Medical Laboratory Sciences Quality Assurance Programme (HKIMLSQAP), formerly named Medical Technology Association Quality Assurance Programme (HKMTAQAP), of Haematology & Serology was introduced in 1990.

Four batches of survey materials were distributed to participants each year at quarterly intervals. Each batch included 2 commercially acquired Complete Blood Count (CBC) control samples for measurement of haemoglobin (Hb), red blood cells (RBC), mean cell volume (MCV), white blood cells (WBC) and platelets; 2 lyophilised plasma samples for prothrombin time/International Normalized ratio (PT/INR), and activated partial thromboplastin time (APTT); 2 Romanowsky stained peripheral blood films for differential leukocyte count % and blood cell morphology; 2 sets of red cell suspensions and serum samples for ABO and Rh(D) groupings.

Specific codes were assigned to reagents, methods and instruments used. Participants were requested to enter these codes in the Return Form together with the results. The results were then compiled and mean, standard deviation (SD), standard deviation index (SDI) and coefficient of variation (CV) were analysed statistically.

In order to eliminate the significant weight of the "much-away" outliers on the statistical data, results beyond 3 SD were excluded from statistical analysis. Only those within 3 SD were compiled to give the statistical mean, SD, SDI and CV. Finally results, which fell out of 2 SD, were highlighted and bold printed to alert the participants.

For differential leukocyte count %, blood cell morphology and ABO & Rh groupings, consensus values from the Haematology and Serology panel were provided.

I. Survey Report

The aim of HKIMLSQAP is to offer inter-laboratory comparison and provide objective and constructive comments on the survey performance of participants. The survey report comprises of an individual report and a common report.

Both CBC and INR/APTT have no target values set. However, participants could realise their performance by comparing their results with those of their peer groups.

On account of the diversity of the laboratory methods/instruments and reagents employed, each CBC parameter is further stratified by method/instrument, participants could then compared each parameter against the mean, SD, CV yielded from just their peer groups or all participants (all methods). The total return for analysis by method/instrument groups was 68 in year 2009.

Similarly for INR/APTT, survey data derived from participants were interpreted with reference to the reagent group and method/instrument group. The total return for analysis by reagent groups was 51 in year 2009.

Consensus values from the Haematology and Serology panel were provided for differential leukocyte count %, blood cell morphology and ABO & Rh groupings. Besides, comparing own results with the panel's consensus, the "mode" values of the results from all participants were also provided for reference.

In the common report, for each blood film, the panel will write up the related laboratory and clinical findings concerning the case in "Blood Film Remarks" for educational purpose. Comments made by participants for the blood film were included in the "Notes on Participants' Returns". Individual remark would be incorporated into a participant's survey report if the performance of a particular parameter deviated much from the peer. Tables and figures were presented as much as possible to facilitate easy reading, comparison and analysis.

II. Summary of Year 2009 QAP Results

The total number of participants in year 2009 was 52. Profiles of participating laboratories include: government (1), public hospital (14), private hospital (10), private sector (27) and with none from overseas.

The total number of returns of each dispatch is below illustrated:

	Survey 1 (Feb)	Survey 2 (May)	Survey 3 (Aug)	Survey 4 (Nov)
No. of Returns	50	48	49	47

A. Complete Blood Count (CBC)

The CBC sample is commercially obtained from R&D systems Inc. Because of different measuring principles employed with different CBC instruments, therefore comparison by instruments among peer group would better reflect the performance the laboratory.

Listed below are tables showing the "Overall CVs of CBC parameters for all instruments" and examples of respective CVs by instrument group for Hb and WBC.

Table 1. Overall CVs of CBC parameters for all instruments in 2009

	HS0911	HS0912	HS0921	HS0922	HS0931	HS0932	HS0941	HS0942
Hb	1.57	1.73	1.38	1.68	1.44	1.32	1.47	1.70
RBC	2.19	2.16	2.21	1.97	2.20	2.18	1.95	2.27
MCV	5.89	6.42	5.48	6.07	5.79	6.28	5.72	5.92
HCT	6.08	6.53	5.43	6.41	6.11	6.33	5.69	6.02
WBC	4.74	7.76	4.35	7.87	3.16	6.39	5.90	7.93
PLT	12.35	8.95	7.85	9.29	7.76	7.10	9.43	8.30

Table 2. CV of Hb by instrument group (numbers of participants in bracket)

	HS0911	HS0912	HS0921	HS0922	HS0931	HS0932	HS0941	HS0942
All methods	1.57 (68)	1.73 (68)	1.38 (67)	1.68 (67)	1.44 (65)	1.32 (64)	1.47 (62)	1.70 (62)
Advia	0.82 (5)	1.30 (5)	1.23 (7)	1.07 (7)	1.31 (6)	1.32 (6)	2.66 (5)	1.16 (5)
Cell-Dyn	1.26 (18)	1.40 (18)	1.21 (16)	1.60 (16)	1.60 (15)	1.61 (15)	1.32 (16)	2.05 (16)
Cell-Dyn 3200	2.25 (10)	2.29 (10)	1.78 (8)	2.81 (8)	1.77 (6)	2.08 (6)	2.43 (6)	2.79 (6)
Coulter	0.84 (5)	1.19 (5)	0.76 (4)	1.63 (4)	0.46 (3)	1.43 (3)	1.26 (3)	1.29 (3)
Coulter LH	0.98 (16)	1.06 (16)	0.84 (18)	1.26 (18)	1.14 (18)	1.01 (18)	1.25 (17)	1.22 (17)
Sysmex	1.64 (14)	1.67 (14)	1.38 (14)	1.18 (14)	1.44 (14)	1.25 (17)	1.10 (15)	1.28 (15)

Table 3. CV of WBC by instrument group (number of participants in bracket)

	HS0911	HS0912	HS0921	HS0922	HS0931	HS0932	HS0941	HS0942
All methods	4.74 (65)	7.76 (67)	4.35 (67)	7.87 (67)	3.16 (63)	6.39 (65)	5.90 (62)	7.93 (62)
Advia	16.47 (5)	14.23 (5)	4.30 (7)	12.00 (7)	12.13 (6)	10.41 (6)	5.61 (5)	4.13 (5)
Cell-Dyn	4.65 (18)	6.31 (18)	4.36 (16)	6.00 (16)	4.56 (15)	5.52 (15)	5.17 (16)	6.30 (16)
Cell-Dyn 3200	4.99 (10)	7.69 (10)	3.19 (8)	4.55 (8)	1.95 (6)	2.19 (6)	14.73 (6)	16.18 (6)
Coulter	2.50 (5)	3.67 (5)	0.94 (4)	3.41 (4)	1.79 (3)	3.18 (3)	0.00 (3)	1.78 (3)
Coulter LH	1.91 (16)	1.93 (16)	1.81 (18)	1.91 (18)	2.28 (18)	2.03 (18)	1.82 (17)	2.44 (17)
Sysmex	2.76 (14)	3.57 (14)	3.57 (14)	4.23 (14)	2.70 (17)	3.49 (17)	3.49 (15)	4.42 (15)

B. INR/APTT

From year 2006 survey onwards, participants were required to report only INR results.

Listed below are tables showing the "overall CVs of INR/APTT by all reagents and all methods" and respective CVs of INR and APTT by reagent group.

Table 4. Overall CVs of INR/APTT in 2009

	HS0913	HS0914	HS0923	HS0924	HS0933	HS0934	HS0943	HS0944
INR	4.90	6.98	4.85	11.56	4.90	8.85	5.88	10.97
APTT	6.03	9.34	6.05	13.38	4.95	10.13	5.48	17.33

Table 5. CV of INR by reagent group (number of participants in bracket)

	HS0913	HS0914	HS0923	HS0924	HS0933	HS0934	HS0943	HS0944
All reagents	4.90	6.98	4.85	11.56	4.90	8.85	5.88	10.97
	(51)	(50)	(51)	(52)	(50)	(50)	(49)	(49)
Recombiplastin	3.92	6.73	2.88	7.41	4.95	7.69	4.85	8.36
	(22)	(22)	(23)	(23)	(22)	(22)	(20)	(20)
Neoplastin CI+	2.91	2.59	4.72	18.66	5.77	21.76	6.80	7.92
	(4)	(4)	(4)	(4)	(4)	(4)	(4)	(4)
Thromborel S	5.94	6.91	6.00	9.16	4.85	6.63	6.86	7.75
	(25)	(24)	(25)	(25)	(24)	(24)	(25)	(25)

Table 6. CV of APTT by reagent group (number of participants in bracket)

	HS0913	HS0914	HS0923	HS0924	HS0933	HS0934	HS0943	HS0944
All reagents	6.03	9.34	6.05	13.38	4.95	10.13	5.48	17.33
	(51)	(51)	(50)	(51)	(48)	(49)	(48)	(48)
Actin	5.15	12.65	5.00	16.67	5.05	16.06	5.77	15.74
	(20)	(20)	(20)	(20)	(18)	(19)	(20)	(19)
Automated	3.33	3.93	4.09	5.84	2.14	3.91	2.19	6.86
APTT	(13)	(13)	(13)	(13)	(13)	(13)	(11)	(11)
CK Prest	4.49	4.28	5.88	11.78	5.39	2.07	6.53	2.72
	(3)	(3)	(3)	(3)	(3)	(3)	(3)	(3)
Synthasil IL	5.25	3.27	2.98	9.34	3.88	4.00	3.46	5.15
	(15)	(15)	(14)	(15)	(14)	(15)	(14)	(13)

C. Differential Count and Red Cell Morphology

Although two blood films were mainly assessed for differential leukocyte count % and RBC morphology, participants also had to comment on the WBC morphology, platelet morphology and whether malaria parasites were found.

In the survey report, participants can then compare their results with the "mode" values of all participants and the consensus of the panel.

In order to broaden the educational role of HKIMLSQAP and to share with participants the concerned haematological disorder, the Haematology panel would write up a "Blood Film remark" for each slide with reference to the clinical and laboratory data. Below are the Blood Film remarks issued for year 2009 blood films.

HS 0915

The film belongs to an infant of 26 days old suffering from prolonged neonatal jaundice. The red cells are round or oval, a moderate number of macro-ovalocytes is also seen. Apart from mild anisocytosis and poikilocytosis, the most prominent feature is the presence of a large number of stomatocytes, the stomas of which are longitudinal, transverse, V-shaped or Y-shaped. These are characteristic features of South-east Asian ovalocytosis. Regarding to the age of the patient, the white cell differential and morphology are unremarkable and there is moderate thrombocytosis. In view of the relatively low haemoglobin level and the prolonged neonatal jaundice in this case, the morphology alone may not be sufficient to definitely categorize the red cell disorder as "hereditary ovalocytosis", which is usually asymptomatic, or "hereditary stromatocytosis", which is mostly associated with haemolytic anaemia resulting from abnormal cation flux.

HS0916

The most striking feature of this film is the presence of large number of eosinophils which constitute to around 40% (absolute count of $5.2x10^9$/L) of the white cell population. The red cell morphology and platelet count are normal. The clinical history shows that the patient has suffered from exertional shortness of breath (SOB) for one month and there is consistent eosinophilia in the complete blood count within the period. This is probably a case of reactive eosinophilia associated with lung disease.

HS0925

The blood film was prepared from a 10-year old girl who has been genetically confirmed suffering from $--^{SEA}$ α thalassaemia. The RBC count is increased. The white cell population displays a normal differential distribution and the platelet count is adequate. The blood film shows a marked degree of anisocytosis, hypochromia and microcytosis. Moderate numbers of poikilocytes, target cells, spherocytes and schistocytes are present. Mild polychromasia is also noted. Supra-vital staining demonstrates the characteristic "golf-ball' Hb-H inclusions in around 5% of the red cell population. Haemoglobin electrophoresis on agarose gel at alkaline pH demonstrate Hb-A and a faint fast moving Hb-H band, which can only be revealed by using a fairly concentrated haemolysate.

Hb-H disease results from a variety of genetic abnormalities. The most common cause is the compound heterozygosity for α^0 thalassaemia (deletion of both α genes, e.g. $--^{SEA}$ as in this case) and α^+ thalassaemia (deletion of one α gene, e.g.-$\alpha^{3.7}$). The former is relatively common in southern Chinese and South-East Asians. There is a severe degree of hypochromic and microcytic anaemia with the haemoglobin concentration ranges from 3 to 10 g/dL. The characteristic Hb-H inclusions are usually detected in a relatively large proportion of red cells accounting for >35%. However, a less likely occurrence is evident when there is a concomitant episode of iron deficiency or a non-deletional α thalassaemia. The α^0 thalassaemia trait normally results from the deletion of both α genes on same chromosome. There is a mild anemia with a slightly deranged haemoglobin concentration. Hb-H inclusions, though detected, are present in a small proportion of red cells.

In conjunction of the basic haematological analyses, the molecular study would play an important role to elucidate the genetic make-up and etiology of α thalassaemia. Table 1

shows the haematologic and molecular profile of HS0925 with reference to the classification of Hb-H disease and α^0 Thal trait.

Table 1. Differential diagnosis of Hb-H disease and α^0 thalassaemia trait

	Barbara Bain Haemoglobinopathy Diagnosis, 2001		
Classification	Hb-H disease	α^0 Thal trait	HS0925
Molecular Abnormalities	--/ ≥1 deletional or non-deletional mutation	--/ ≥2 normal alleles	$--^{SEA}$/??
Hb (g/dL)	3-10	Slightly Deranged	11.0
RBC (x 10^{12}/L)	↑	↑	7.07
MCV (fL)	50-65	↓ MCV	49.4
MCH (pPg)	15-20	↓ MCH	15.6
Hb-H inclusions	35-90%	rare	~5%

HS0926
The film is prepared from a 99-year old female hospitalized for fracture bone. The cell counts are adequate and no remarkable feature is noted in the three lineages of red cells, white cells platelets. It is essentially a normal blood picture.

HS0935
The prominent features of the film are marked leucocytosis and thrombocytopenia. The number of blasts accounts for approximately 93% of nucleated cells. They exhibit coarse chromatin, inconspicuous nucleoli, irregular nuclear clefts and cytoplasmic blebs. Cytoplasm is scanty, slightly basophilic, agranular and no Auer rods are seen. Cytochemical staining demonstrated that blasts are negative for Sudan Black B and myeloperoxidase.

Immunophenotyping revealed the expression of CD10, CD19, CD20, CD22, CD34 and TdT on blasts, whereas T-lineage markers (CD2, CD3, CD5 and CD7) and myeloid markers (CD13, CD33, CD117 and cMPO) are not evident. Cytogenetic analysis demonstrated Philadelphia chromosome resulting from translocation of t(9;22)(q34;q11.2). Molecular study displayed the chimeric transcripts of *BCR/ABL1* at the major breakpoint cluster region b2a2. Data suggests B-lymphoblastic leukaemia with t(9;22)(q34;q11.2).

HS0936
The film is evident with eye-catching morphological irregularities of red cell, white cell and platelet lineages. The dimorphism is noted featuring the co-existence microcytic, hypochromic red cells and normocytic, normochromic counterparts and is attributed to the

transfusion of two units of red cells two weeks prior to blood sampling for blood count and film preparation. There are slight to moderate number of bizarre poikilocytes, polychromatic red cells, target cells, Howell-Jolly bodies and nucleated red cells account for approximately 28% with some display defected haemoglobinization, which may result from splenectomy years ago. Leucocytosis with neutrophilia and thrombocythaemia are remarkable having undergone 4-day G-CSF injection with mobilization of haemopoietic stem cells. The automated white cell count needs to be corrected for the interference of a significant number of nucleated red cells.

HS0945

The film was prepared from an African boy of seven years of age who has been confirmed to be suffering from sickle-cell anaemia. The blood film shows a reduced number of red cells with a moderate degree of anisocytosis, poikilocytosis, hypochromia and polychromasia. Many target cells are present with a few spherocytes and schistocytes. Meticulous microscopy can reveal sickle cells in boat constructs with points at both ends and inclusions of Howell-Jolly bodies. The white cell population is normal and the platelet count is adequate. The patient first visited the clinic at the age of three. The analysis of haemoglobin pattern showed a predominance of 80.8%-Hb-S, 13%-Hb-F, 3.6%-Hb-A_2 and little Hb-A. The findings were consistent with homozygous Hb-S disease.

Sickle cell anaemia is an inherited β-chain haemolytic disorder mostly found in black people. It is caused by the homogenous expression of the variant haemoglobin, Hb-S, which GAG encoding glutamic acid in the sixth position of the β-chain is mutated to GTG encoding valine. Hb-S is prone to polymerize in the deoxygenated state causing red cells to sickle and precipitate. As a result, small blood vessels would be obstructed by the deformed red cells leading to tissue infarction. The presence of inclusions of Howell Jolly bodies and Pappenheimer bodies and numerous target cells are morphologic anomalies frequently encountered in adolescents with hypo-splenism.

The demonstration of sickle cells in whole blood, Hb-S solubility test and ELISA assay using specific monoclonal antibodies are diagnostic for sickle cell anemia. Nonetheless, electrophoresis and HPLC are normally used for confirmation. Hb-S exhibits a slow mobility cathodic to Hb-A in alkaline electrophoresis. Hb-S predominates with Hb-F at 2-15% and minimally elevated Hb-A_2. Hb-A is not evident. Molecular analysis is helpful in pre-natal and neonatal diagnosis.

HS0946

The patient was infected with erythromycin- and penicillin-sensitive *Streptococcus sanguis* three weeks prior to the blood sampling. She was diagnosed suffering from infective endocarditis. The white cell differential shows 50%-neutrophils, 32%-lymphocytes, 9%-monocytes and 9%-eosinophils. It is essentially a normal blood picture with adequate cell counts and no remarkable feature.

D. Blood Group Serology

Listed in below table are the consensus results of ABO Rh(D) grouping and reaction scores from the Haematology panel.

Table 7. Consensus on ABO Rh(D) groupings and reaction scores.

Samples	Blood Group	Anti-A	Anti-A,B	Anti-B	A cells	B cells	O cells	Anti-D	Rh ctl
HS0917	A+	12	12	0	0	12	0	12	NA
HS0918	O+	0	0	0	12	12	0	12	NA
HS0927	AB+	12	12	12	0	0	0	12	NA
HS0928	A+	12	12	0	0	12	0	12	NA
HS0937	B+	0	12	12	12	0	0	12	NA
HS0938	O+	0	0	0	12	12	0	12	NA
HS0947	B-	0	12	12	12	0	0	0	NA
HS0948	AB+	12	12	12	0	0	0	12	NA

ABO grouping

A standard ABO grouping procedure for an adult sample (>4 months old) should include both forward (cell) grouping and reverse (serum) grouping. The reaction patterns should be complementary to one another and the reaction scores should be clear cut and indisputable in order to arrive at a definite ABO grouping result. The panel recommends the inclusion of "O" cells in the reverse grouping to identify the possible Bombay/Para-Bombay phenotypes. Although these phenotypes are rare, their identification is of utmost importance particularly when blood transfusion is required. Mistyping of Bombay as Group "O" would possibly lead to haemolytic transfusion reaction due to the presence of warm reactive atypical anti-H in these individuals.

Rh (D) typing reagents

Several types of anti-D reagents are available in the market. The test conditions of these test reagents should follow the manufacturer's direction before use.

When a high-protein reagent is used in Rh D typing, an appropriate control test shall be included according to manufacturer's instructions. A negative Rh control test can exclude the false-positive reactions due to the high protein levels and the macromolecular additives in the reagent.

Low-protein Rh reagents which are formulated predominately with monoclonal antibodies are recently employed in many laboratories as routine anti-D typing reagent.

- IgM anti-D monoclonal antibodies require no potentiator and agglutinate most D+ red cells in a saline system but they may fail to agglutinate red cells of some partial D categories. Moreover, these reagents cannot be used for weak D detection.
- Blends of anti-D monoclonal regents are cocktails of IgM anti-D monoclonal antibodies and a small amount of IgG anti-D and they can be used in all routine typing methods to detect weak D.

These IgM anti-D monoclonal antibodies and blends of anti-D monoclonal reagents, prepared in a low-protein medium seldom require a separate control test. False-positive reaction may occur if the serum contains cold autoagglutinins or a protein imbalance causing rouleaux formation for unwashed tested red cells. Spontaneous agglutination can be excluded by absence of agglutination by anti-A and /or anti-B in the cell grouping for ABO. If the tested red cells show agglutinations in all tubes i.e. group AB, D+, a control should be performed according to manufacturer's instructions. A suspension of the patient's red cells with autologous serum or with 6% bovine albumin may serve as an adequate control.

Anomaly in Rh(D) Typing

If problems in D typing arise, the patient should be given D-negative blood until the problem is resolved. Testing a recipient's red cells for weak D is not necessary, because giving D-negative cells causes no harm to recipients of the weak D phenotype. Omitting the test for weak D prevents misinterpretations arising from the presence of a positive direct antiglobulin test (DAT). Routine testing for other Rh antigens is not required.

The antiglobulin test should not be used for D-grouping patient samples for the purposes of transfusion. Patients should not be classified as D positive on the basis of a weak positive result using a single anti-D reagent, or a pool of more than one reagent. If clear-cut positive results are not obtained, it is safer to classify the patient as D-negative until confirmation of D status is carried out by reference laboratory. However, in the testing of donor's D grouping where the D group is in doubt it is safer to classify such donors as D positive.

ABO and Rh (D) typing in pregnancy

In July 2008, BCSH Guideline for Blood Grouping and Antibody Testing in Pregnancy recommended the ABO and D grouping in maternal samples must be performed in accordance with the guidelines for compatibility procedures in blood transfusion laboratories.

II. Some Commonly Encountered Errors among Participants

APTT

- The clinical assessments (Normal/Borderline/Prolonged) for APTT are not entered onto the Return form.

Differential Leukocyte Count

- The total percentage differential count does not sum up to 100.
- The total percentage differential count sums up to more than 100.
- The number of nucleated RBC is included into the total percentage differential count.
- Contradiction on RBC morphology - "normal" is regarded but graded simultaneously with abnormal features.

ABO blood group serology

- Reverse grouping is omitted. It is necessary to perform both forward (cell) and reverse (serum) groupings in order to detect any ABO anomalies.
- Group O cells are not used in reverse grouping. The O cells act as a negative control in order to arrive at an indisputable ABO blood grouping interpretation.

- Positive reactions are reported for auto/negative control. Positive reaction of (Rh control/"O" cells) invalidates the ABO/Rh(D) blood grouping interpretation and requires further investigation.
- Abnormally weak reaction scores.

Figure 1 shows the frequency of the most common errors encountered among participants.

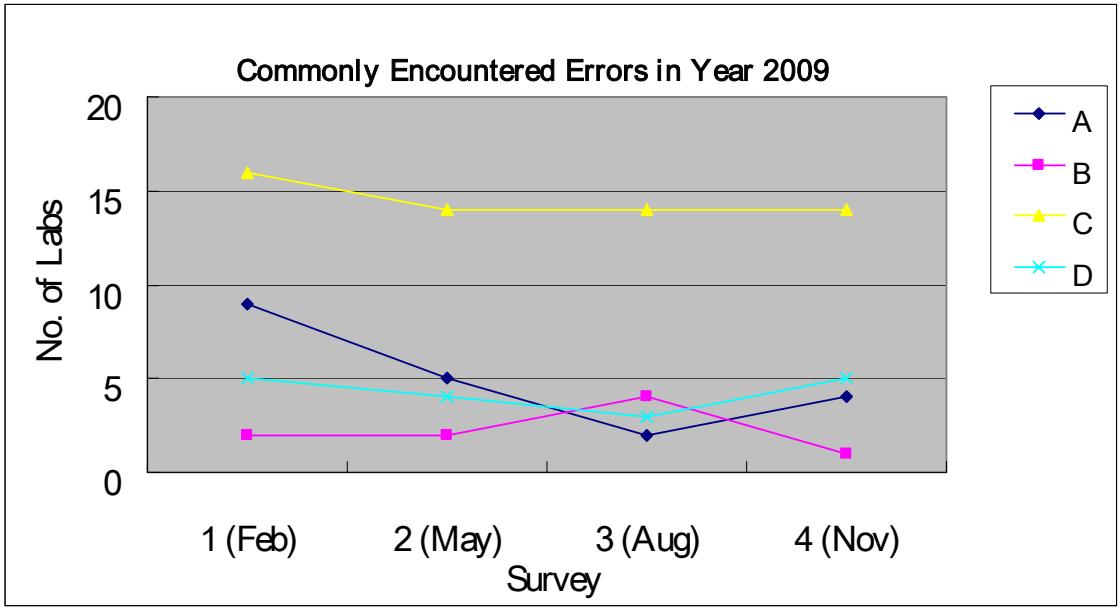

Figure 1. Commonly encountered errors. A: Clinical assessments of APTT are missing; B: Total % differential count sums up to > 100 or <100; C: In ABO blood group serology, reverse grouping is not done and/or O cells are not used in the reverse grouping and D: Other ABO blood group serology problems.

III. Conclusion

Participation in external quality program is one of the critical items required by the accreditation bodies. Demand on proficiency testing provider local or overseas is therefore indispensible. We are also working to obtain accreditation from the HKAS in the future so as to achieve a recognized standard for a proficiency testing provider. We hope not only to entertain our local demand but also to cater the needs from overseas. Our goal can only be achieved with the continuous support from our participants.

References

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