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). Similarly for INR/APTT, survey data derived from participants were analyzed with reference to the reagent group and method/instrument group.

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 2008 QAP Results

The total number of participants in year 2008 was 50.

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 peer group (by instrument) comparison 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 2008

	HS0811	HS0812	HS0821	HS0822	HS0831	HS0832	HS0841	HS0842
Hb	1.78	1.88	1.73	1.82	1.72	1.80	1.57	1.67
RBC	2.00	2.20	2.00	3.21	2.19	2.31	1.99	1.99
MCV	5.00	5.63	5.54	5.85	5.46	5.91	5.72	6.20
HCT	5.06	5.84	5.40	5.63	5.58	6.02	5.82	6.17
WBC	4.90	8.23	5.67	7.61	6.21	6.94	4.39	6.33
PLT	7.50	9.17	8.68	7.17	7.41	6.91	7.85	7.29

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

	HS0811	HS0812	HS0821	HS0822	HS0831	HS0832	HS0841	HS0842
All methods	1.78 (62)	1.88 (63)	1.73 (66)	1.82 (66)	1.72 (65)	1.80 (65)	1.57 (63)	1.67 (63)
Advia	1.03 (6)	1.24 (6)	1.48 (6)	1.12 (6)	4.44 (7)	4.15 (7)	3.24 (6)	3.29 (6)
Cell-Dyn	1.24 (15)	1.29 (15)	1.42 (16)	1.62 (16)	1.33 (14)	1.53 (14)	1.34 (14)	1.78 (14)
Cell-Dyn 3200	3.81 (6)	2.45 (6)	2.26 (8)	1.94 (8)	2.69 (7)	2.53 (7)	2.75 (8)	2.41 (8)
Coulter	1.13 (7)	1.38 (7)	1.28 (7)	1.57 (7)	0.68 (7)	1.82 (7)	1.13 (6)	0.87 (6)
Coulter LH	0.97 (16)	1.20 (16)	1.44 (16)	1.72 (16)	1.20 (18)	1.19 (18)	1.20 (18)	1.50 (18)
Sysmex	2.03 (13)	1.71 (13)	2.03 (13)	1.52 (13)	2.16 (13)	1.68 (13)	1.42 (12)	1.11 (12)

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

	HS0811	HS0812	HS0821	HS0822	HS0831	HS0832	HS0841	HS0842
All methods	4.90 (61)	8.23 (63)	5.67 (64)	7.61 (66)	6.21 (64)	6.94 (66)	4.39 (62)	6.33 (64)
Advia	12.80 (6)	15.17 (6)	17.19 (6)	12.62 (6)	12.65 (7)	10.01 (7)	13.07 (6)	9.03 (6)
Cell-Dyn	4.16 (15)	6.91 (15)	4.16 (16)	4.17 (16)	4.09 (14)	4.84 (14)	4.43 (14)	5.57 (14)
Cell-Dyn 3200	4.42 (6)	6.65 (6)	3.37 (8)	2.50 (8)	4.18 (7)	3.02 (7)	3.29 (8)	4.07 (8)
Coulter	0.96 (7)	1.75 (7)	1.96 (7)	2.14 (7)	2.42 (7)	2.47 (7)	2.09 (6)	2.60 (6)
Coulter LH	2.29 (16)	4.81 (16)	2.45 (16)	2.59 (16)	1.99 (18)	2.31 (18)	2.01 (18)	3.54 (18)
Sysmex	5.56 (13)	3.34 (13)	2.96 (13)	2.12 (13)	3.09 (13)	3.13 (13)	3.11 (12)	4.36 (12)

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 2008

	HS0813	HS0814	HS0823	HS0824	HS0833	HS0834	HS0843	HS0844
INR	4.72	7.11	4.85	9.09	4.95	5.09	4.90	9.42
APTT	6.74	10.09	7.88	13.56	6.73	9.84	6.64	12.97

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

	HS0813	HS0814	HS0823	HS0824	HS0833	HS0834	HS0843	HS0844
All reagents	4.72	7.11	4.85	9.09	4.95	5.09	4.90	9.42
	(48)	(48)	(45)	(45)	(46)	(46)	(46)	(46)
Recombiplastin	4.72	6.44	3.92	8.79	2.94	5.63	3.88	6.58
	(20)	(20)	(18)	(18)	(18)	(18)	(19)	(19)
Neoplastin CI+	3.70	3.17	**	**	**	**	11.71	12.89
	(3)	(3)	(2)	(2)	(2)	(2)	(3)	(3)
Thromborel S	5.66	6.48	4.85	6.38	5.94	5.05	4.90	6.69
	(25)	(25)	(25)	(26)	(26)	(26)	(25)	(25)

** CV not available as total number of participants is < 3.

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

	HS0813	HS0814	HS0823	HS0824	HS0833	HS0834	HS0843	HS0844
All reagents	6.74	10.09	7.88	13.56	6.73	9.84	6.64	12.97
	(46)	(46)	(45)	(44)	(45)	(45)	(46)	(46)
Actin	4.10	10.52	5.93	22.48	5.71	12.44	4.01	19.99
	(16)	(17)	(19)	(19)	(20)	(20)	(19)	(19)
Automated	4.50	5.26	3.20	10.48	3.64	6.85	4.15	5.29
APTT	(15)	(15)	(13)	(13)	(13)	(13)	(14)	(14)
CK Prest	4.95	10.39	**	**	**	**	**	**
	(3)	(3)	(1)	(1)	(1)	(1)	**(2)**	(2)
Synthasil IL	4.69	11.09	5.00	5.10	2.81	3.33	4.07	4.56
	(12)	(12)	(12)	(12)	(11)	(11)	(11)	(11)

** CV not available as total number of participants is < 3.

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 2008 blood films.

HS0815

The film is from a 6-year-old boy with febrile illness presented with haemoglobinuira. The prominent features are erythrophagocytosis and toxic granulations. There is moderate leukocytosis and the white cell differential shows neutrophilia with left shift. The red cell population exhibits moderate spherocytosis. Mild polychromasia and anisocytosis are also noted. Platelet count is adequate. Intravascular haemolysis is evident. These features are suggestive of paroxysmal cold haemoglobinuria (PCH) during a haemolytic episode.

PCH is characterized by a sudden onset of hemoglobinuria either spontaneously or after exposure to cold due to the presence of cold reacting autoantibody. This autoantibody is a polyclonal immunoglobulin G (IgG) known as Donath-Landsteiner autoantibody. The event triggering autoantibody formation may be attributable to the exposure of a microorganism

antigen mimicking the P antigen in the red blood cell (RBC) membrane. The Donath-Landsteiner autoantibody is a biphasic haemolysin which binds to the RBCs at cooler temperatures and complement lysis occurs at warmer temperatures. These characteristics have thus formed the basis of the Donath-Landsteiner test which is diagnostic for the disease. Direct Coombs' test, if performed, can be positive for poly-specific AHG and anti-C3d, but negative for anti-IgG because of dissociation of IgG from the RBC surface at warm temperatures. The disease is usually associated with a recent respiratory or other viral infection in children. The illness is transient and self-limiting particularly if the primary infection can be adequately treated. Children who have an acute post-infection onset usually have an excellent outcome as the illness is transient and self-limiting.

HS00816

The most remarkable feature of the smear is the presence of intra-cellular red cell inclusions, malaria parasites, in the red cell population. The malaria parasites are almost purely ring forms (trophozoites), possessing delicate bluish cytoplasm with one to two small reddish chromatin dots. Some red cells are multiply infected with 2-3 ring forms. Occasional appliqué (accolle) forms are seen. A few schizonts are also found. There is no red cell enlargement. The white cell differential is predominated by neutrophils. They are hyposegmented with some showing Pelger-Huet anomaly. Marked thromobocytopenia is also evident.

The first diagnosis is Plasmodium falciparum infection. Staining of the blood film in pH7.2 may help reach a definite classification of the parasites. A few participants reported a mixed infection with *Plasmodium malariae*. This is probably due to the presence of a few schizonts with a low merozoite count. The schizont of *Plasomodium falciparum* only fills up two-third of an RBC, while that of *Plasmoidum malaraie* occupies the whole RBC. The numbers of merozoites in a mature *Plasmodium falciparum* are 8-24 while in a mature Plasmodium malaraie are 6-14. The numbers of merozoites are even less in an early schizont. Although rare, schizonts of *Plasmodium falciparum* can also be seen in peripheral blood when the infection is severe.

Pelger-Huët anomaly (PHA) is a benign but dominantly inherited defect of terminal neutrophil differentiation, secondary to mutations in the lamin B receptor (*LBR*) gene. The characteristic features of the blood picture include leukocytes with dumbbell-shaped bi-lobulated nuclei; a reduced number of nuclear segments; and a coarse clumping of the nuclear chromatin in neutrophils, lymphocytes, and monocytes. Acquired or pseudo Pelger-Huët anomaly has been associated with blood disorders such as anemia, leukemia and myelodysplastic syndromes, drug therapy with colchicines or ibuprofen, infections such as mononucleosis and malaria. It is therefore important to distinguish the congenital benign anomaly from the acquired form. The latter need further investigation of the primary disease. The marked thromobocytopenia together with the Pelger-Huët anomaly encountered in this case should arouse more attention to look for other underlying causes.

HS0825

The film was prepared from a 72-year old lady having JAK2 mutation. RBC morphology is normal. Elevated haemoglobin concentration and packed cell volume are unapparent, excluding polycythaemia vera. White blood cells display a mild degree of basophilia. The most prominent feature of the blood smear is a marked degree of thrombocytosis. Besides,

giant platelets are easily encountered. The patient is likely suffering from myeloproliferative disorder of essential thrombocythaemia.

HS0826
The film was prepared from a male patient of 49 years of age diagnosed of acute myeloid leukaemia (FAB classification – M1) shortly after systemic chemotherapy. Platelet count is severely reduced. The prominent feature is marked leukocytosis with over 80% of medium-sized round blasts of scanty to moderate cytoplasm, fine chromatin and small nucleoli. There was no evidence of faggot cells. Cytochemical staining illustrated SBB-positive but NSE-negative blasts. Immunophenotyping demonstrated that the blasts were positive for CD13, CD33 and cMPO, but negative for T- and B-lymphoid markers. Coagulation studies revealed a slight increase of INR to 1.34. The D-Dimer was severely deranged to 5589 ng/mL FEU as compared to normal reference value of <500 ng/mL FEU. Molecular study showed no known chimeric transcripts of *PML/RARA* or *AML1/ETO*.

HS0835
The film was prepared from a 2-year baby girl. The most prominent feature is the presence of blasts accounting for 25%. They are medium to large in size with deep basophilic cytoplasm, fine chromatin and 2-4 prominent nucleoli. Occasional azurophilic granules are seen. A few blasts show cytoplasmic vacuoles and cytoplasmic projections. There is marked thrombocytopenia.

The red blood cells show slight poikilocytosis and anisocytosis, with a few macro-ovalocytes occasionally present. Some participants graded the red cell population with microcytosis and macrocytosis simultaneously. It should be noted that the terms “normocytosis”, “microcytosis” and “macrocytosis” are used to reflect the average size of the entire red cell populations, only one of them should be used in respective with the Mean Cell Volume (MCV). On the other hand, the term “anisocytosis” means variation in red cell size, it describes the simultaneous presence of normocytes, microcytes and macrocytes in a blood film.

The blasts are positive for some myeloid markers (CD13, CD33 and CD117) and negative for MPO, HLA-DR and CD34 on immunophenotyping by flow cytometry. In addition, they also express CD7 aberrantly. Other lymphoid markers and TdT are negative. The findings are consistent with Acute Myeloid Leukaemia.

HS0836
The film was prepared from a 65-year healthy female seeking medical consultation in a health clinic. Complete blood counts of WBC, RBC and platelet and haemoglobin level are within normal ranges. The WBC differential shows a normal distribution. RBC and platelet morphology are unremarkable.

HS0845
The film was prepared from a 57-year-old man seeking urologic consultation for painful haematuria. Blood film examination confirms the automated blood counts and normal differential of white cells. The morphology and count of platelets are unremarkable. The complete blood picture is essentially normal.

HS0846
The film was prepared from a 38-year healthy female hospitalized for investigations of fever, fatigue, weight loss and shortness of breath with exertion. Leukocytosis with more than 20% of blast cells and immature monocytoid cells are noted in the peripheral smear. They are medium to large in size with a moderate amount of basophilic cytoplasm. Some of them have cytoplasmic granules but no Auer rod. Fine nuclear chromatin and prominent nucleoli are seen in round or folded nuclei. The white cell differential shows that there is a mild degree of neutropenia. Myelocytes are present occasionally. The red cells display a mild extent of anisocytosis, whereas the platelets are morphologically normal.

The bone marrow is hypercellular and diffusely infiltrated with over 80% blast cells with morphology similar to those observed in the peripheral blood. Cytochemical staining shows that the blast cells are Sudan black B-negative, while non-specific esterase is positive with fluoride sensitivity. Flow cytometric analyses demonstrate that the blast cells are negative for myeloperoxidase. They are, positive for the myeloid antigens CD13, CD14, CD15 and CD33. Besides, they aberrantly express T-cell and B-cell markers of CD5 and CD20, respectively. The findings are consistent with acute monoblastic leukaemia (AML-M5a).

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 Control
HS0817	O+	0	0	0	12	12	0	12	NA
HS0818	B+	0	12	12	11	0	0	12	NA
HS0827	AB+	12	12	12	0	0	0	12	NA
HS0828	O+	0	0	0	12	12	0	12	NA
HS0837	O+	0	0	0	11	11	0	12	NA
HS0838	B+	0	12	12	10	0	0	12	NA
HS0847	AB+	12	12	12	0	0	0	12	NA
HS0848	A-	12	12	0	0	12	0	0	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

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. The HKIMLS Haematology and Serology QAP panel has strived to improve the quality and scope of this program by introducing the Interpretive QAP in Haematology (co-organized with Hong Kong College of Pathologists) since year 2005. 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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