ACCREDITATION SCHEME FOR LABORATORIES

Technical Note MED 002

Specific Criteria for Haematology Section

The SAC Accreditation Programme is managed by Enterprise Singapore

1. Introduction & Scope

1.1 This document describes the specific requirements for clinical haematology laboratory to be accredited.

1.2 The document shall be read in conjunction with ISO 15189 Medical laboratories – Requirements for quality and competence', SAC-SINGLAS documents, Proficiency Testing Technical Note 001, and other MEDICAL Series Technical Notes published by SAC-SINGLAS.

2. Laboratory Equipment

2.2 Automated Blood Cell Counting

2.2.1 The laboratory shall have a written, detailed procedure for calibration of automated instruments including indications from the quality control system for when calibration is needed.

2.2.2 Calibration techniques shall include the use of fresh whole blood specimens or stabilized commercial preparations.

Note: Assignment of values to calibrators either by primary reference methods or by verification of manufacturers' assigned values is essential to obtaining accurate results.

2.2.3 Where non-adjustable, pre-calibrated instruments are used, calibration must be verified with appropriate control materials.

Note: Commercial materials marketed as controls may lack the careful value assignments of those sold as calibrators.

2.2.4 Fluids used with blood cell counting instruments shall be periodically checked for contamination by performing background counts on the instrument. Appropriate count correction procedures shall be present as nucleated erythrocytes and blood megakaryocytes may have an additive effect on the instrument leukocyte count.

2.3 Automated Differential Counters

2.3.1 These instruments shall be carefully evaluated against prior patient-testing methods before being placed in service.

2.4 Automated Counters for Reticulocytes

2.4.1 Flow cytometric systems which are not using commercial kits traceable to International standards or validation protocol (e.g. FDA, NIST, etc.) shall have documented evaluation studies done on the strength and stability of the fluorescent dye binding to RNA or DNA. The laboratory shall have precision data for its automated method, based on analysis of commercial controls or comparison with manual methods. The laboratory shall have written policies and procedures for identifying samples that may give erroneous results due to interferences (e.g. Howell-Jolly bodies, nucleated RBCs, basophilic stippling, macrothrombocytes).

2.5 Body Fluids

2.5.1 There shall be a system for dealing with partially clotted specimens, cell clumps, or debris noted during haemocytometry or automated counting.

2.5.2 For instrument counts, the laboratory shall have documented procedures of linearity studies and defined lower limits below which instrument counts are not reliable.

2.6 Flow cytometry

2.6.1 The laboratory shall have document-controlled procedures for monitoring of optical alignment and the laser current of the flow cytometer. There shall be procedures for system performance quality control (at least daily or every time the flow cytometer is restarted) and tracking of photomultiplier tube voltages which are recorded.

2.6.2 The laboratory shall have records of the initial validation of new fluorochrome conjugated antibodies prior to the use for clinical test.

2.6.3 The laboratory shall have records of the validation of new lots of antibodies in comparison to the old lots.

3. Examination Procedures

3.1 Automated Blood Cell Counting

3.1.1 Procedures for daily control shall include any combination of the following three approaches, with tolerance limits defined:

a. Processing of stabilized commercial control materials. Two different concentrations (eg. normal and high) are required on each shift of patient testing. The laboratory shall plot standard Levy - Jennings graphs with control limits and applies at least some Westgard multi-rule criteria for determining if results are analytically acceptable. There is no requirement for three control levels and dilute low particle concentration controls are discouraged.

Note: It is important for the laboratory not to confuse package insert values for expected recovery range with ± 2 S.D limits based on their own instrument's between day imprecision.

b. Periodic reanalysis of at least two retained patient specimens is required. These shall be run in each shift if stabilized control material is not run.

Note: Standard deviation of duplicate pairs is an appropriate statistical approach to data evaluation.

c. Moving average algorithm for erythrocyte indices and other parameters may be used. The laboratory shall set limits that are sensitive to significant alterations in calibration status but insensitive to minor fluctuations in patient population values.

3.1.2 There also shall be protocols for common interference that may affect the accuracy of Full Blood Count data, such as lipemia, in-vitro haemolysis, microclots, cold agglutinin, cryoglobulins, etc. Patients results that exceed laboratory defined reportable limits shall be verified (e.g. cytopenic samples shall be checked against haemocytometry or blood film estimates) and documented.

3.2 Manual Complete Blood Count Methods

3.2.1 Where haemoglobin is quantified manually, at least four concentrations must be used to construct a calibration curve. Patient sample dilutions shall be checked for turbidity.

3.2.2 For microhaematocrits (packed cell volumes), packing studies shall be initially performed and then repeated when there is a change in the centrifuge timer or speed.

3.2.3 Where manual haemocytometry (WBC or Platelets) is performed, the laboratory shall carry out at least one cell count control during each shift of patient testing. Alternatively, a procedural control such as previously assayed patient sample or comparison with a blood film estimate shall be used.

Note: Because of the higher imprecision of chamber counts, the area sampled must be increased for cytopenic samples.

3.3 Manual Blood Films

3.3.1 There shall be written criteria for review of blood films with specified abnormalities by the laboratory head or qualified designee in haematological morphology.

3.3.2 The laboratory shall have a system that ensures that all personnel report microscopic morphology in a similar fashion.

Note : Suggested methods to accomplish this include:

I. Circulation of blood films with defined leukocyte differential distributions and specific qualitative abnormalities of each class of cells, and /or

II. multi-headed microscopy, and /or

III. use of blood or bone marrow photomicrographs with referee and consensus identifications.

3.3.3 For laboratories performing malaria blood film or any other parasitology tests, please refer to Technical Notes MED 002 Microbiology.

3.4 Bone Marrow Preparations

3.4.1 If fixed tissue sections and aspirates are independently evaluated by different sections of the laboratory, there shall be written policies of procedures to compare data and interpretations by pathologists or qualified haematologists. To assess technical adequacy, bone marrow slides for routine and cytochemical stains shall be reviewed.

3.5 Abnormal Haemoglobin Detection

3.5.1 If the laboratory detects abnormal haemoglobin on alkaline cellulose acetate/agarose gel electrophoresis, isoelectric focusing, capillary zone electrophoresis or HPLC, an alternative technique (as listed), as well as when applicable, acidic agar/citrate agar electrophoresis, sickle solubility test or any other tests as appropriate shall be used to verify all abnormal bands.

Note: It is emphasized that sickle solubility test alone is not appropriate as a stand-alone test for haemoglobinopathy screening or evaluation.

3.6 Body Fluids

3.6.1 For body fluid morphology, there shall be a written policy and personnel with written policy to ensure consistency of morphologic classification when multiple personnel are responsible for smear examination.

Note: Differentials shall always be performed on stained preparations, and use of cytocentrifuge is strongly recommended.

3.6.2 Body fluid preparations with suspected malignant cells shall be reviewed by a pathologist or other qualified physician shall review.

3.7 Coagulation Tests

3.7.1 If the laboratory is located within a hospital, there shall be sufficient list of tests available for routine and emergency testing. This may not apply to non-hospital laboratories.

Note: The tests available in a hospital location must be able to detect, evaluate, and monitor the progress and therapy of the common disorders of coagulation.

3.7.2 The test shall reflect coagulation factor deficiency, coagulation factor inhibitors, hyperfibrinolysis and the monitoring of Vitamin K antagonists or unfractionated heparin therapy. Patient results shall be reported with the accompanying reference ranges.

3.7.3 Appropriate control (at least two levels) shall be performed for all procedures. If factor assays are performed, the technical assessor shall examine sample assay data to determine that appropriate calibration points and dilutions of patient plasma are routinely used.

3.8 Transfusion Laboratory and Blood Processing/Testing Services

3.8.1 The goal of the transfusion service shall be safe transfusion of effective blood products to the patient. There should be a comprehensive and coordinated quality assurance programme in place which should include

a. measures to significantly decrease errors,
b. ensure credibility of test results,
c. implement process and system controls and
d. ensure continued product safety and quality.

3.8.2 There shall be written procedure manuals on handling of specimens, preparation, reagents and controls, maintenance of instruments, and verification and documentation of reagent performance.

3.8.3 All blood typing and compatibility procedures shall be followed in accordance with the procedures authorised by the procedure manual of the laboratory. Some of these are as follows:

a. Before transfusion, the ABO blood group of each whole blood or red blood cell unit and the RhD blood group of such units labelled as RhD negative shall be confirmed serologically on an integrally attached segment.

b. Pre-transfusion testing shall include ABO and RhD typing, and screening (and identification if screening positive) for unexpected red cell antibodies.

c. The method used for detection of unexpected red cell antibodies shall demonstrate clinically significant antibodies. The sample used for antibody detection shall be obtained from the patient within 3 days prior to the scheduled transfusion, if the patient has been pregnant or transfused with red cell-containing components within the last 3 months, or if history is uncertain.

d. There shall be a process to ensure that historical records of ABO/RhD typing, clinically significant red cell antibodies and special transfusion requirements are reviewed and compared to current results. Any discrepancy shall be investigated before the blood component is issued for transfusion.

e. There shall be a process to ensure that transfusion recipients are issued compatible blood components.

3.8.4 The laboratory shall follow authorised procedures, including correct patient and sample identification, and correct issue of blood component to the correct patient.

In case of discrepancy or doubt about the identifying information on the sample and request, another sample should be requested.

3.8.5 Blood and blood component records shall be documented and traceable from source to final disposition. It shall have identification and traceability for every unit, including quarantine, ultimate disposition, wastage, incineration and other records. For transfusion laboratory services, it shall record and retain the identity the patient receiving a given unit.

3.8.6 Records of regular maintenance and monitoring of equipment including blood storage refrigerators shall be documented and monitored. Storage facilities for blood and blood components shall have a system to monitor the temperature continuously; this shall include an alarm system.

3.8.7 If donors are drawn and/or blood products are processed at the facility, then there shall be written policies and procedures in the aspects below to be followed for the donated blood products:

a. the donor interview and selection,
b. the phlebotomy procedure
c. transport
d. testing
e. processing
f. storage,
g. release and quarantine procedures.
h. distribution (if applicable)

3.8.8 Blood and blood components shall be managed appropriately including handling, storage and transport conditions as detailed by the procedure manual of the laboratory. If blood components are prepared, a system must be in place to ensure that blood component specifications are met.

3.8.9 There shall be adequate and appropriate documentation of procedures if infectious disease testing is done in the premises, regardless of where in the facility this is performed. Results not satisfying specified acceptance criteria shall be clearly identified to ensure that blood components are held from release.

3.8.10 There shall be a system of quarantine for blood components to ensure that they cannot be released for issue until all the specifications for collection, processing, handling and testing have been met.

a. The ABO group of donor blood shall be determined for each donation by testing the red cells with anti-A and anti-B reagents and by testing the serum or plasma for unexpected antibodies with A1 and B reagent red cells.

b. The RhD type of donor blood shall be determined for each collection with anti-D reagent. If the initial test with anti-D is negative, the blood shall be tested using a method designed to detect weak D. When either test is positive, the label shall read “Rh Positive”. When the tests for both D and weak D are negative, the label shall read “Rh Negative”.

c. The ABO and RhD blood group must be verified on each subsequent donation and a comparison must be made with the historically determined blood group. If a discrepancy is found, the applicable blood components must not be released until the discrepancy is unequivocally resolved.

d. Serum or plasma from donors shall be tested for unexpected antibodies to red cell antigens by a method that can demonstrate clinically significant red cell antibodies.

3.9 Histocompatibility

3.9.1 The emphasis on this section is on proper procedure in specimen handling, and preparation of reagents and controls with verification and documentation of reagent performance.

3.9.2 Quality control requirements are similar to those in chemistry and diagnostic immunology in regard to procedure manuals and instrument maintenance.

3.10 Flow cytometry

3.10.1 Quality Control:

Performance of the antibodies shall be verified by using positive and negative controls. Two level controls shall be run for single/dual platform qualification of blood lymphocyte subsets or CD34 quantification. The results of controls shall be recorded and monitored. Corrective action shall be taken if the results are out of acceptable limits.

3.10.2 Sample integrity and storage:

There shall be documented procedures to monitor sample integrity. There shall be documented appropriate procedures for sample storage.

3.10.3 Blood lymphocyte subset quantification:

There shall be documented gating strategy to define and enumerate blood lymphocyte subsets.

3.10.4 CD34 stem cell quantification:

The laboratory shall use appropriate anti-CD34 monoclonal antibodies for stem cell enumeration. Viability of the sample shall be monitored. The maximum coefficient of variation for CD34+ cell counts should be 10%, hence, a minimum of 100 events shall be acquired for CD34+ events (*ISHAGE guidelines*). Sequential gating strategy (*ISHAGE recommendations*) shall be applied to define CD34+ events.

3.10.5 Leukemia and lymphoma immunophenotyping:

The laboratory shall use appropriate and robust panels based on published evidence to investigate clinical samples. Appropriate gating strategy shall be applied to identify the abnormal and normal cell populations. Internal controls, isotype controls or fluorescence minus one controls shall be used appropriately.

4. Ensuring the Validity of Examination Results

4.1 Automated Differential Counters

4.1.1 Quality control options include periodic comparisons with;

a. manual differentials or

b. processing of commercial control materials with at least two different classes of leukocytes or WBC surrogates.

4.1.2 The laboratory shall have written criteria for checking and reviewing leukocyte differential counter, histograms and /or blood smears which have clinically important results flagged by the automated counter.

4.2 Manual Reticulocytes

4.2.1 To reduce imprecision of microscopic enumeration, the reported reticulocyte concentration shall be based on a minimum sample size of 1,000 red cells.

5. Reporting of Results

5.1 For glycated haemoglobin (HbA1c) by HPLC, the presence of haemoglobin variant is reported when present.