MINISTRY OF INVESTMENT, TRADE AND INDUSTRY
DEPARTMENT OF STANDARDS MALAYSIA

STR 2.8 - SPECIFIC TECHNICAL REQUIREMENTS FOR ACCREDITATION OF CYTOGENETICS LABORATORIES

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(Supplementary to MS ISO 15189)

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LABORATORY ACCREDITATION SCHEME OF MALAYSIA

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Introduction

This document describes the specific technical requirements to be complied by cytogenetics laboratories. This document should be read in conjunction with the MS ISO 15189 *Medical laboratories - Requirements for quality and competence* and other accreditation criteria documents published by the Department of Standards Malaysia (JSM). The clause numbers correspond to those in the standard but since not all clauses require supplementary requirements, the numbering may not be continuous.

Compliance with this document does not in any way exempt laboratories from or diminish their responsibilities in observing/complying with existing national laws and regulations/guidelines currently enforced in the country.

1 Scope

The areas for accreditation of Cytogenetics Testing include the following:

1.1 Constitutional Cytogenetics (Prenatal & Postnatal)
1.2 Cancer Cytogenetics
1.3 Fluorescence In Situ Hybridisation (FISH)

2 Normative references

i) MS ISO 15189 - Medical Laboratories - Requirements for quality and competence
ii) SC 2 - Specific Criteria for Accreditation in the Field of Medical Testing

3 Terms and definitions

3.1 Analyse
To evaluate each chromosome in a cell, either by comparing the homologues band for band through the microscope, on a high-resolution digital display, or by using photographs.

3.2 Count
To enumerate the number of chromosomes in a cell. The chromosomes need not be banded. A count should include comment on any obvious structural aberrations.

3.3 G-banding
A technique that, following various pretreatments of chromosome preparations and staining with Giemsa (or similar) stain, produces characteristic alternating dark and pale bands along each chromosome. G-banding is the standard technique for chromosome identification in human cytogenetics.

3.4 Karyotype
To arrange the banded chromosomes of a single cell in the standard arrangement (International System for Human Cytogenomic Nomenclature, ISCN). The arrangement may be achieved by physically cutting up a photograph or by using an image analysis system.

3.5 Score
To enumerate the presence or absence of a specific cytogenetics feature.

4 General requirements

Same as MS ISO 15189 and SC 2.

5 Structural and governance requirements

Same as MS ISO 15189 and SC 2.

6 Resource requirements

6.2 Personnel

6.2.1 General

a) Workload

i) Cytogenetics is currently a labour intensive field, but with increasing automation, the staff to workload ratio may be affected. The number of specimens processed will depend on the other duties among staff members, such as the experience of that person, the degree of laboratory automation and the complexity of the analyses.

ii) The following ranges can be considered as a basis for calculating annual workload per staff**:**

a. 250 - 350 lymphocyte cultures; or
b. 150 - 250 bone marrow cultures; or
c. 250 - 350 prenatal cultures; or
d. 400 - 500 metaphase/interphase FISH tests; or
e. 150 - 220 specialised FISH tests (i.e. multiple subtelomere); or
f. 50-100 solid tissue FISH tests (i.e. paraffin-embedded tissues).

However, the annual workload can be considered in cumulative numbers with minimum of 150 samples per staff.

iii) A laboratory is recommended to process no less than 250 samples annually (including all types of samples) to maintain staff competency.

6.2.2 Competence requirements

a) Personnel qualifications

i) A **Cytogeneticist** shall be a person with appropriate qualifications and experience as follows:

a. A qualified pathologist registered with the Malaysian Medical Council (MMC) with postgraduate in the field of genetics/medical genetics or subspecialty qualification in cytogenetics recognised by the Government of Malaysia and/or registered with the National Specialist Register (NSR); or

b. A medical practitioner registered with the MMC with at least four (4) years of experience in laboratory cytogenetics; or

c. A Doctor of Philosophy (PhD) degree in a subject relevant to the field of genetics/human genetics with at least one (1) year of experience in laboratory cytogenetics post-degree training; or

d. A Masters of Science Degree in a subject relevant to the field of genetics/human genetics with at least three (3) years of experience in laboratory cytogenetics post-degree training; or

e. A Bachelor of Science Degree or equivalent qualification in a subject relevant to the field of genetics/human genetics with at least seven (7) years of experience in laboratory cytogenetics post-degree training.

ii) A **Scientist** shall be a person with a minimum Bachelor of Science or Biomedical Science Degree or equivalent qualification and experience relevant to the laboratory operation to directly supervise diagnostic work in the cytogenetics laboratory.

iii) A **Medical laboratory technologist** shall be a person with at least a Diploma in Medical Laboratory Technology or equivalent and who has undergone supervised training in laboratory cytogenetics.

c) Personnel resuming duties after an absence of more than six months are required to undergo reassessment and, if necessary, retraining.

6.3 Facilities and environmental conditions

6.3.2 Facility controls

i) The laboratory shall have sufficient and safe spaces allocated for processing and analysis of the different types of specimens and also a dedicated area for culture, harvesting, slide preparation, staining and FISH procedures.

ii) The cytogenetics laboratory shall also have designated areas for receiving specimens, FISH analysis, reagent preparation, microscopy, washing & sterilisation, specimen aliquoting (for archival storage) and storage of consumables.

iii) The laboratory in which cultures are set up shall have a facility for storage of sterile containers and other accessories required for tissue culture in order to avoid repeated movement of staff.

iv) Cell culture reagents shall have dedicated areas for storage in the refrigerator.

v) Work areas in which the analysis is done should preferably be separated from all other laboratory procedures/operations.

6.5 Equipment calibration and metrological traceability

i) Incubator

a. Prenatal and non-prenatal samples shall be incubated separately to minimise the risk of microbial cross-contamination.

b. If amniotic fluid/chorionic villi/products of conception (POC) specimens are being processed, the specimen shall be divided between at least two independent incubators.

c. Incubators shall be connected to independent emergency electrical circuit, backup gas sources (where relevant), and alarm system.

d. All incubators shall have monitoring records for the temperature, gas levels (where relevant) and relative humidity to protect the viability of the cultured cells.

ii) Biosafety Cabinet

a. Appropriate biosafety cabinets should be used for the containment of biological materials.

b. The maintenance policy for the cytogenetics cell culture laboratory shall include infection control of the laminar flow hoods at regular intervals and checking of CO_2 incubator temperature & humidity.

iii) For image capture system, all image capture systems should be maintained regularly with software upgrades.

iv) For automated systems (i.e. slide scanner, robotic harvester), a contingency backup system shall be in place in case the automated device breakdown.

v) Critical equipment shall have a system to monitor breakdowns or downtime of machine when necessary.

vi) Calibration records shall be maintained for pH meters, balances and pipettes.

6.6 Reagents and consumables

Upon receipt of media, reagents and/or kits the laboratory shall check the expiration date and inspects for any signs of contamination and/or evidence of exposure to extreme temperatures.

7 Process requirements

7.2 Pre-examination processes

The laboratory shall ensure that all measures are implemented to minimise failure rate of cell / tissue culture. The laboratory shall check the sterility of medium before use. For in-house preparation of cell culture medium the protocol given by the manufacturer shall be followed. For samples of bone marrow / peripheral blood / solid tissues / amniotic or other fluids, more than one culture shall be set up (overnight / 24 / 48 / 72 hours / direct / long term culture) with proper labeling of culture flasks / tubes.

Every staff member working with tissue culture shall be familiar with sterile technique, preparation, handling of culture media and safe laboratory practices.

7.2.6 Sample receipt

7.2.6.1 Sample receipt procedure

f) The laboratory should have a written policy for reporting time. Recommended report times for 90% of the referrals are as stated below:

i) 28 days for lymphocytes cultures
ii) 21 days for bone marrows cultures
iii) 7 days for urgent lymphocyte, bone marrow or cord blood cultures
iv) 15 days amniotic fluid/CVS cultures
v) 7 days FISH
vi) 28 days for product of conception

7.3 Examination processes

7.3.1 General

i) Cultures

a. Prenatal cultures

1. Prenatal specimens such as Amniotic fluid, Chorionic villi (CVS), Product of Conception (POC) and Percutaneous Umbilical Blood Sampling (PUBS) shall be processed under sterile conditions using appropriate aseptic cell culture techniques.
2. Each prenatal specimen shall be divided and cultured in two separate incubators and maintained with independent cell culture media and other reagents.
3. All cultures should be retained until the final results are released.
4. For chorionic villus specimens, careful examination should be carried out to reduce the risk of maternal cell contamination (villi free from maternal decidua, blood vessels and unidentified tissue). Chorionic villus karyotype studies should include analysis of long-term cultures.

5. In the presence of fetal chromosome abnormality, where parental studies are required, it is recommended that these studies should be carried out in the same laboratory as the prenatal sample.

b. Postnatal cultures

1. If possible, each postnatal specimen such as peripheral blood and fetal blood shall be established in two independent cultures.
2. When appropriate and upon request, molecular analysis/FISH is recommended to supplement cytogenetics analysis.

c. Bone marrow cultures

1. In bone marrow cultures, the likelihood of obtaining high-quality results shall be optimised by using direct, short-term and synchronised cultures. Where necessary, suitable mitogens or growth factors may be used.
2. Processing of specimens for hematological malignancy shall be consistent with the reason for referral or presumed clinical diagnosis.

ii) Banding

a. Karyotyping should be carried out using a G-banding technique. Other banding techniques may be carried out if necessary.

b. A standardised method for assessing banding quality shall be used (Refer **Appendix 1**), with an agreed minimum standard that may vary depending on the reason for referral. (Refer **Appendix 2**).

iii) Chromosome analysis

a. Each case should be independently analysed by two laboratory personnel. Additionally, a cytogeneticist shall review and verify each case by examining every chromosome, using direct microscopy, photographs, or digital images.

b. If artificial intelligence (AI) is used for digital karyotyping of metaphase chromosomes, the laboratory shall validate the AI function against manually captured images. Additionally, a structured training and competency program shall be established to ensure that personnel are adequately trained before using the AI system.

c. Normal chromosomal variants shall not be included in the ISCN karyotype description but should be documented in the patient's laboratory records.

d. The laboratory shall establish a written procedure outlining its chromosome analysis policy for prenatal, postnatal and cancer cytogenetics including the delineation of mosaicism, noting that individual cases require careful assessment and interpretation, and that the numbers of cells counted and analysed may need to be greater than the minimum guidelines.

e. Mosaicism or clonality can be confirmed by FISH analysis if a suitable probe is available.

1. Prenatal cytogenetics

a. For in situ preparations, metaphase cells should be analysed from as many different colonies as possible, ideally representing a minimum of two independent cultures.

b. A total of fifteen (15) banded metaphase cells should be examined from two independent in situ cultures. A minimum of five (5) cells should be analysed and an additional ten (10) cells should be counted.

c. If adequate in situ colonies are available for analysis, no more than two (2) metaphase cells should be examined from a single colony. If discrepancies are observed between the two (2) cells from the same colony, further analysis of that colony is warranted.

d. If an insufficient number of in situ colonies are available, or if only a single in situ culture is available for analysis, this limitation should be clearly noted in the report.

e. When analysing suspension harvests from sub-cultured cells, analysis should, whenever possible, be conducted using at least two (2) independent cultures, with an examination of approximately ten (10) banded cells from each culture.

f. For mosaicisms, in situ colony method is preferred to distinguish true mosaicism from pseudo mosaicism and for differentiating true chromosomal abnormalities from culture-induced artefacts.

2. Postnatal cytogenetics

a. A minimum of five (5) banded metaphase cells should be analysed, and an additional ten (10) metaphase cells should be counted for constitutional except mosaicism.

b. Where clinically relevant mosaicism is suspected in peripheral blood, examination of at least thirty (30) banded metaphase cells is recommended to exclude mosaicism of 10% at the 0.95 confidence level.

c. In cases with strong clinical suspicion (e.g. sexual development disorders), a more extended examination is recommended to exclude low level mosaicism.

d. An attempt should be made to analyse adequate metaphases to exclude structural abnormality before FISH analysis is recommended.

3. Cancer cytogenetics

a. If the initial analysis of five (5) banded metaphase cells reveals no clonal abnormality, a further five (5) cells should be fully analysed, and an additional ten (10) cells should be counted.

b. Analysis from more than one (1) culture should be performed, whenever possible, if no abnormal clone is identified.

c. Where an abnormal result is obtained at diagnosis, chromosome analysis of subsequent samples is appropriate.

- Normal result obtained: Minimum of twenty (20) metaphases should be analysed or scored for the diagnostic abnormality.
- Abnormal clone detected: Minimum analysis can be limited to 10 metaphases.
- Where there is evidence of clonal evolution, sufficient cells should be examined to elucidate the evolved clone.

d. If fewer metaphase cells are available for analysis, this limitation must be clearly stated in the report.

e. As abnormal metaphase cells often exhibit suboptimal morphology, laboratory personnel shall ensure that these cells are included in the analysis, including those selected by automated capture systems or artificial intelligence (AI).

f. If a karyotype result is not available (e.g. no growth / culture failure), alternative methods such as FISH, chromosome microarray, or other molecular tests should be considered, tailored to the clinical indication.

g. Where feasible, follow-up studies and the detection of measurable residual disease should preferably use real-time quantitative PCR (RQ-PCR), other molecular techniques, or multiparametric flow cytometry.

h. For post-transplantation cases, molecular techniques are preferred. FISH may be employed if a suitable diagnostic marker was identified at the time of diagnosis.

i. If clinical findings suggest relapse, refractory disease, or a secondary hematological neoplasm, repeat analyses are warranted, and samples should be processed following the same procedures as for initial diagnostic testing.

j. If a constitutional aberration is suspected, additional metaphases should be analysed to assess the presence of a normal cell line. If a normal cell line cannot be confirmed, a peripheral blood sample may be requested to establish a constitutional karyotype.

Note: Adapted from the ASDG best practice guidelines for the interpretation and reporting of karyotypes and FISH, Carey, Louise et al. Pathology, Volume 57, S82.

iv) FISH

a. When appropriate and upon request, FISH is recommended to supplement cytogenetics analysis. Confirmation of rearrangements using FISH may be appropriate to differentiate between an interstitial or a telomeric deletion or more complex rearrangement.

b. Adequate numbers of metaphases, or interphases cells shall be analysed to ensure the statistical validity of the result by two (2) independent readers.

c. Interphase and metaphase FISH, either as a single probe analysis, or using multiple chromosome probes, can give reliable results in different clinical situations.

d. For metaphase FISH analysis, clonality is defined according to ISCN guidelines. To demonstrate that an abnormality is clonal, it must be present in a minimum number of metaphases.

e. FISH scoring;

1. For locus-specific probes, a minimum of 5 metaphase cells for each probe should be scored to confirm an abnormality.
2. For interphase, a minimum of 50 cells for each probe should be scored.
3. For mosaicism or acquired clonal abnormalities, a minimum of 50 interphase cells should be scored to establish a significant clone. If no abnormality is found in the first 50 cells, score a minimum of 200 cells, if available.

f. Tissue FISH on Formalin-Fixed Paraffin-Embedded (FFPE);

1. The laboratory should consider the tumour tissue histology, tumour heterogeneity and truncation artefacts when analysing and interpreting signals for copy number, gene amplification, structural rearrangements and/or gene-fusion events
2. In tissues not expected to exhibit heterogeneity, a minimum of 50 cells should be scored. However, additional cells may be required to obtain a valid result if there is tumour heterogeneity or a low proportion of cells with a clonal abnormality in the designated region of interest.
3. In tissues that are expected to show heterogeneity, a minimum 100 cells should be scored. Further scanning of the entire area of hybridisation for abnormal signal patterns is suggested.
4. It is recognised that some FFPE material may be from small core biopsies with limited and minimal tumour, or necrosis with less cells available for examination. A valid abnormal analytical result should be above the laboratory's established measurement uncertainty for the probe, which will likely require further modification to establish cut-off threshold value that avoid false positive results due to signal loss from 'truncation artefacts'.

Note: Adapted from Section E9 of the American College of Medical Genetics technical standards and guidelines: Fluorescence in situ hybridisation.

7.3.4 Evaluation of measurement uncertainty (MU)

The laboratory shall have a documented procedure for uncertainty of measurement in FISH studies or other tests which may influence the management of patients based on the results obtained and quantitative results.

Note: Reference can be made to the Cancer Cytogenetics Methods and Protocols, Thomas S.K. Wan.

7.3.7 Ensuring the validity of examination results

7.3.7.1 General

The laboratory shall establish and monitor the success rate of acceptable performance. The recommended success rate based on specimen types as follow:

i)	Amniotic fluid specimens	99%
ii)	Chorionic villi specimens	98%
iii)	Percutaneous umbilical blood sampling	90%
iv)	Product of conception	60%
v)	Peripheral blood specimens	98%
vi)	Bone marrow specimens	90%

Where the success rate falls below the acceptable performance, the laboratory shall investigate and take the necessary corrective action.

7.3.7.2 Internal quality control (IQC)

The laboratory shall set, monitor and maintain laboratory quality control procedure.

7.4 Post-examination processes

7.4.1 Result reporting

7.4.1.2 Result review and release

i) All reports shall be reviewed, authorised and released by a qualified pathologist.

ii) In the absence of a qualified pathologist, a cytogeneticist can release the results with the following mandatory remark in the report: “Tests results shown on this report require clinical interpretation and comments by a qualified pathologist.”

iii) A minimum of 2 karyotypes/images shall be printed and archived.

iv) In prenatal cytogenetics, if maternal cell contamination (MCC) is suspected, further investigations may be required (i.e. QF-PCR) before reporting and this information should be available in the report.

7.4.1.6 Requirements for reports

Reports shall include the following:

i) Patient details (name, date of birth, gender, age)

ii) Name of requesting clinician and location

iii) Clinical indication/reason for referral

iv) Specific studies undertaken (if applicable)

v) Specimen type

vi) Date of specimen collection

vii) Date of specimen received

viii) Date of report (preliminary and final report)

ix) Laboratory/specimen identification

x) For karyotype, total numbers of cells, number of cells analysed and number of cells counted

xi) For FISH, total numbers of cells scored (interphase or metaphase)

xii) Banding method and level of resolution achieved

xiii) The description of test results shall follow the recent International System for Human Cytogenetics Nomenclature (ISCN)

xiv) An indication that the result is normal or abnormal (i.e. no abnormality has been detected or abnormality detected or the result is inconclusive)

xv) Where applicable, appropriate interpretative comment / clinical interpretation should be included correlating to the clinical information. A discussion of the significance of the findings should be included, when appropriate. Include guideline/literature to support the clinical interpretation, when appropriate

xvi) Name and signature of the authorised person who release the report. The signature may be generated electronically or manually; Password protected electronic signatures shall be used, when appropriate

xvii) Where applicable, recommendations for further investigations and genetic counselling should be indicated

xviii) Test limitations

7.4.2 Post-examination handling of samples

Storage of the primary specimen and other laboratory sample shall be in accordance with the national or international guidelines of retention period i.e. College of Pathologists, Academy of Medicine Malaysia Guideline on Retention of Pathology Records and Materials.

8 Management system requirements

Same as MS ISO 15189 and SC 2.

Appendix 1

Assessment of banding quality of cytogenetics slide preparations (Informative)

Assessment of banding quality of cytogenetic slide preparations

ISCN bands per haploid set (bphs)	Examples
Solid stained	Unequivocal chromosome pairing is not possible
150	Can distinguish 8s from 9s. Can distinguish 4s from 5s
400	Two distinct dark bands in 8p Two distinct dark bands in 9p Three distinct dark bands in 5q (5q14, 5q21, 5q23)
550	Four distinct dark bands in 18q 10q21, 10q23, 10q25 split 7q33 and 7q35 are clearly distinct 22q13.2 is visible
700	2p25.2 distinct 2q37.2 distinct 10q21.1 and 10q21.3 resolve 17q22-q24 resolves into 3 dark bands
850	4p15.3 splits 5p15.32 is clearly visible 10q11.22 is clearly visible 11p14.1 should resolve from 11p14.3 20p12.1 and 20p12.3 are clearly visible

Reference:
Adapted from the National Pathology Accreditation Advisory Council (NPAAC), Requirements for medical testing for human genetic variation (Third Edition, 2022)

Appendix 2

Recommended minimum banding quality (Informative)

The recommended banding resolutions given below are defined as the lowest standard acceptable for a given reason for referral without issuing a qualified report.

Reason for referral	**Banding Resolution (bphs)**
Routine prenatal diagnosis (e.g., for age or biochemical prescreens)	400
Aneuploidies and known large structural rearrangements	400
Expected small structural rearrangements, including their prenatal diagnosis	400 - 550
Possible small unknown structural anomalies (e.g., recurrent abortion, dysmorphic features, delayed development)	550
Microdeletion syndromes (FISH or microarray is the preferred method of analysis where available)	700 - 850

In practice, a range in the banding resolution will be apparent for the cells examined and the minimum band resolution applied to a laboratory report should be based on more than one cell, the confidence in the analysis and clinical context.

Reference:
Adapted from the National Pathology Accreditation Advisory Council (NPAAC), Requirements for medical testing for human genetic variation (Third Edition, 2022)

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