ACGS Association for Clinical Genomic Science Best Practice Guidelines

ACGS Best Practice Guidelines for Constitutional Karyotype Analysis and Targeted Chromosome Analysis

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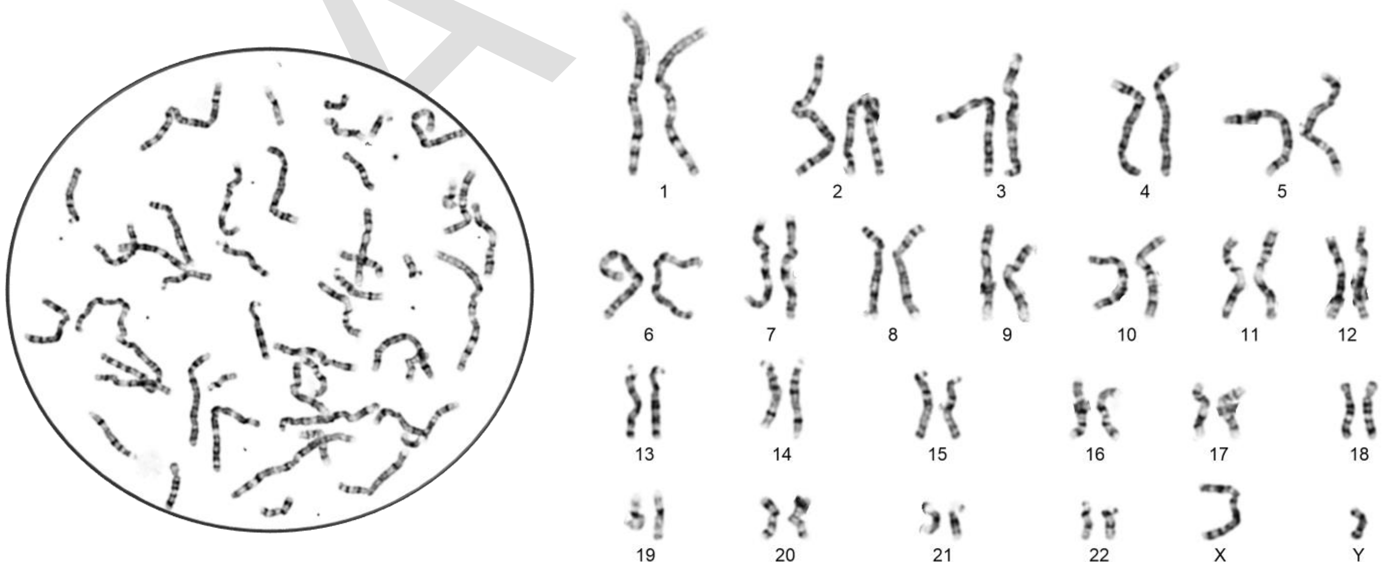
An ACGS working group was formed in October 2023 to review, consolidate and replace with technique based best practice guidelines (BPGs), the ACC (Association for Clinical Cytogenetics) General BPGs (2007), Postnatal BPGs (2007), Prenatal diagnosis BPGs (2009), Constitutional postnatal chromosomal microarray BPGs (2011), Solid tissue BPGs (2010), Maternal cell contamination (MCC) in prenatal samples for molecular studies BPGs (2008) and ACGS BPGs for use of Quantitative Fluorescence-PCR for the detection of aneuploidy v4 (2018). The guidelines below address chromosome analysis of G-banded metaphase chromosomes in prenatal, pregnancy loss, solid tissue, and postnatal samples for Rare Disease referrals.

Recommendations ratified by ACGS Quality Subcommittee on 20/08/2024



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1 Document version history

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3 Scope and introduction

Since the 1950s, when the human chromosome number was first reported to be 46 (Tijo & Levan, 1956), conventional chromosome analysis of metaphase chromosomes (karyotyping) has been a core test approach for the diagnosis of cytogenetic rearrangements and abnormalities. The history, development and evolution of human cytogenetics is comprehensively reviewed by Ferguson-Smith, 2008, and Ferguson-Smith, 2015, emphasising its influence on the emergence of modern medical genetics. Although many historical applications have been superseded by more modern technologies, chromosome analysis continues to have an important role in constitutional genomic analyses, specifically in determining the location/positioning of sizable imbalances and the detection of balanced structural rearrangements.

In this guide, conventional chromosome analysis is divided into **karyotype analysis (KA),** which specifies the analysis of an individual’s complete set of metaphase chromosomes and has been the mainstay of historical cytogenetic evaluation, and the more recent **targeted chromosome analysis (TCA)** which limits examination to specific metaphase chromosomes.

These recommendations should be used in conjunction with other relevant ACGS guidelines. They assume that the analytical process takes place in an appropriate, accredited laboratory setting, where routine aspects of good laboratory practice such as sample tracking and record keeping are in place. They incorporate the standards required by the United Kingdom Accreditation Service (UKAS) and the current Medical Laboratories – Requirements for quality and competence standard (ISO 15189:2022) and by statute (Clinical Governance), whilst considering current practice in the UK.

These guidelines describe the **minimum standards** for the application of chromosome analysis that are necessary for United Kingdom (UK) ACGS affiliated laboratories. Professional judgement is of paramount importance and should be exercised in their application.

Within this document, the use of:

- **'shall'** or **'must'** are **compulsory requirements**,
- **'should'** is the **best practice desirable or expected situation**,
- **'may'**, '**might'** or '**could'** are **optional recommendations**,
- **'acceptable'** highlights an area where **more than one approach is satisfactory**,
- **'unacceptable'** indicates practice where the **quality of the service may be compromised**.

Where there appears to be contradiction between available recommendations/guidelines, the most recently published should be taken to apply to all. All diagnostic laboratories shall be accredited to nationally or internationally accepted standards. Laboratories shall participate in External Quality Assessment (EQA) relevant to the services that they provide.

Where possible, and appropriate, these guidelines have been aligned with the European Guidelines for Constitutional Cytogenomic Analysis (Silva *et al*., 2019), although some aspects have been updated to reflect evolving practice.

4 Test overview

4.1 Test principles

Conventional chromosome analysis involves the preparation of metaphase chromosome preparations, often from a synchronised cell culture, which are fixed to a microscope slide. The chromosomes are protease treated and stained to create a banding appearance which is visually surveyed by light microscopy, either by direct observation, or through image capture for computer-aided chromosome recognition, isolation, and classification systems. In the UK, G-banding by trypsin using Giemsa or Leishman (GTG or GTL banding technique) has been universally adopted. The chromosome constitution (the karyotype) is defined and described in accordance with the standardised International System for Human Cytogenomic Nomenclature (ISCN).

4.2 Test method definitions, test criteria, test eligibility and requesting specialities

In England, commissioned genomic testing is specified in the National Genomic Test Directory (NGTD), and in Scotland, by the Scottish Genetics Laboratory Consortium Genomic Test Directory, with both defining testing methodology as 'Karyotype'. At present, in Northern Ireland and Wales commissioned testing may also follow the NGTD. Application has been made to NHS England for the NGTD to incorporate 'TCA' as a specified test methodology.

The principal English NGTD clinical indications and testing criteria that are eligible for karyotype analysis are published on-line (https://www.england.nhs.uk/publication/national-genomic-test-

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directories/). These indications are usually associated with a sizeable structural chromosome rearrangement, such as a family history of a balanced chromosome rearrangement or a cytogenetic aetiology for infertility. The scope, test eligibility and permitted requesting specialties are defined, and assigned an R code, by indication. Equivalents for Scotland are published at https://www.nss.nhs.scot/publications/ssngm-rare-and-inherited-disease-test-directory/ .

4.3 Types of variant to be detected

As defined by the NGTD:

- Structural chromosome rearrangement
- Aneuploidy
- Chromosomal mosaicism

4.4 Standard operating procedures (SOPs)

The laboratory shall have clear protocols for all aspects of sample processing, analytical and reporting procedures in accordance with ISO 15189:2022. Policies and protocols must comply with best practice guidelines for service delivery, test provision and service quality, enabling patient experience to be consistent and standardised across the United Kingdom.

5 Assay details

5.1 Samples

Samples suitable for constitutional chromosome analysis are typically:

- postnatal blood samples: lithium heparin tubes* are the most appropriate blood collection vessels.
- prenatal samples: chorionic villus (CV) (in transport media), amniotic fluid (AF) (in a sterile universal) and fetal blood (in lithium heparin collection tube*).
- infrequently, solid tissue samples are referred for chromosome analysis. These are preferably collected in transport media or sterile saline to maintain cell viability. This is not necessary for solid tissue samples referred solely for DNA based investigations.

*NB: Blood samples in EDTA tubes are typically required for parallel DNA based tests.

Samples shall be handled aseptically and collected in sterile single use vessels. Samples shall not be frozen, fixed, or exposed to other extreme environmental conditions. As the preparation of metaphase chromosomes requires the induction of mitotic cell division in live cells, samples should be dispatched to the laboratory as soon as possible following collection and should be processed promptly.

Sample packaging should be secure and leakproof according to UN3373 shipment classification and packaging instruction P650 (https://www.gov.uk/government/publications/packaging-and-transport-requirements-for-patient-samples-un3373/packaging-and-transport-requirements-for-patient-samples-un3373)

If there is unavoidable delay between sample collection and despatch, samples may be temporarily stored, ensuring that they are not frozen (Bhargava *et al.*, 2018).

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Referral forms and/or laboratory website guidance should clearly convey sample requirements to the clinical teams collecting samples.

Laboratory generated identity labels shall not be placed directly over the sample's original patient identity labelling.

5.2 Sample rejection

Lithium heparin is the preferred collection tube for blood samples referred for karyotype analysis. It is acceptable to reject samples that are sent in unsuitable blood collection tubes. A report should inform the referrer of the error and request a repeat sample in a lithium heparin tube. Urgent samples sent in EDTA tubes should be processed and laboratories should have a SOP for processing them to achieve metaphase chromosome preparations.

Where repeat sampling is possible, it is acceptable to reject blood samples that are clotted, excessively delayed, or compromised. Samples without unequivocal sample identity, where familial genotype analysis is not available, may be rejected. Notification and discussion with the referrer are recommended in such situations.

Prenatal invasive samples, or other sample types that cannot be repeated (e.g. from a deceased patient), should not be rejected; identity confirmation by familial genotype analysis may be required. Notification and discussion with the referrer are essential in such situations.

5.3 Repeating previously analysed samples

Previously analysed referrals shall be repeated if the previous analysis did not meet the minimum quality standards.

5.4 Techniques

Laboratories must have the facilities, verified protocols and expertise to produce banded metaphase chromosome preparations of a quality sufficient for the reason for referral (see Section 6.1.4); a repertoire of additional techniques (e.g. FISH) should be available for further investigations as required. All procedures/techniques that are employed should be subject to internal quality appraisal/control.

Cell culturing, harvesting and banding methods should be employed, including synchronisation, cell cycle manipulation, chromosome spreading and slide drying techniques, to achieve metaphase chromosome preparations of appropriate length, spread, resolution and banding quality.

Conventional chromosome analysis optical microscopy, by either direct observation traditional microscopy, or digital imaging microscopy, is acceptable. For brightfield/phase contrast traditional microscopy, staff shall be competent in the set-up and operation of a light microscope for optimal visual acuity. For automated digital capture platforms (that often have image manipulation capability and semi-automated chromosome classification), staff shall be competent in system configuration to optimise metaphase capture performance, image quality and user interaction. Staff should have documented competency for use of the microscopy platform and imaging software.

The laboratory should have a policy covering onward referral to specialised centres, for cases for which it does not have the relevant expertise or appropriate facilities, including chromosome instability syndromes.

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6 Analysis standards

6.1 Chromosome analysis

Here, chromosome analysis is divided into **karyotype analysis (section 6.2),** which specifies the analysis of an individual's complete set of metaphase chromosomes, and **targeted chromosome analysis (section 6.3),** which denotes examination of specific metaphase chromosomes.

All analyses shall be performed by appropriately qualified professionals who have evidenced, documented competency. To perform targeted chromosome analysis staff shall have documented competency in karyotype analysis.

As the analysis of metaphase chromosomes involves qualitative analytical considerations, case analysis must involve two analysts performing independent analyses (historically referred to as the 'analysis' and 'check'); at least one analyst shall be a Health and Care Professions Council (HCPC) registered Clinical Scientist (further detail in sections 6.2.1 and 6.3.3).

Analytical and checking procedures shall be documented and specify the minimum staff level/band and experience, the scope of analysis for the different referral categories, the minimum quality standards, and whether the analysis is a karyotype or targeted chromosome analysis.

6.1.1 Table 1: G-banding quality evaluation score

Quality score (QA)	Bands°	Landmark bands*
0		No banding
1		Identification of some chromosomes by morphology and major landmarks
2 (POOR)	<300	Unequivocal identification from major landmarks
3	300	2 bands on 8p (**8p12** & **8p22**) 3 bands on 10q (**10q21**, **10q23**, **10q25** **20p12** visible **22q12** distinct
4 (MODERATE)	400	3 bands on mid-4q (**4q22-28**) 3 bands mid-5q (**5q14, 5q21, 5q23**) 2 bands on 9p (**9p21** & **9p23**) **13q33** distinct
5	500	**7q33** & **7q35** distinct 3 bands on 11p (**11p12, 11p14, 11p15.4**) **14q32.2** distinct 4 bands on 18q (**18q12.1, 18q12.3, 18q21.2, 18q22**)
6 (GOOD)	550	**5q31.2** distinct **8p21.2** visible 2 bands on 11pter (**11p15.2** & **11p15.4**) **22q13.2** distinct
7	700	**2p25.2** **2q37.2** distinct **10q21.1** and **10q21.3** resolve **17q22-q24** resolves into 3 bands

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8 (EXCELLENT)	850	**4p15.31** & **4p15.33** distinct **5p15.32** distinct **11q24.1** and **11q24.3** distinct **19p13.12** and **19p13.2** distinct

*At least three of the criteria to be obtained to apply banding scores 3-8
°Number of bands in haploid set (22 autosomes plus X and Y) based on ideograms of G-banding patterns of different levels of resolution published in ISCN
NB: cytogenetic band resolution is a continuum, landmarks are approximate to band number level and banding quality can affect score assignment clarity; therefore, professional judgement, with reference to wider ISCN band level designations, should be exercised when quality scoring. Scoring system reproduced from retired ACGS (previously ACC) General Best Practice Guidelines (2007). Original source unknown, however, assumed to have been devised by the UK cytogenetics professional body.

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6.1.2 Case quality score

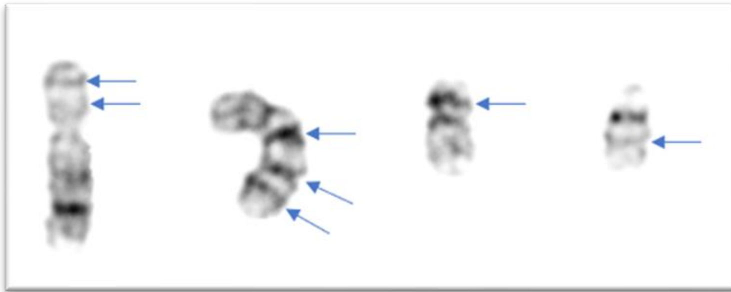
The **case quality** is defined from the **lowest quality cell** used for the analysis, or used to clear all the chromosomes (see section 6.2), to satisfy the minimum analysis requirement (see 6.1.4). For example, 2 cells cleared at QA6 and 1 cell cleared at QA5 corresponds to a case quality of QA5.

Additional cells beyond the minimum requirements may be lower quality without altering the overall case quality score.

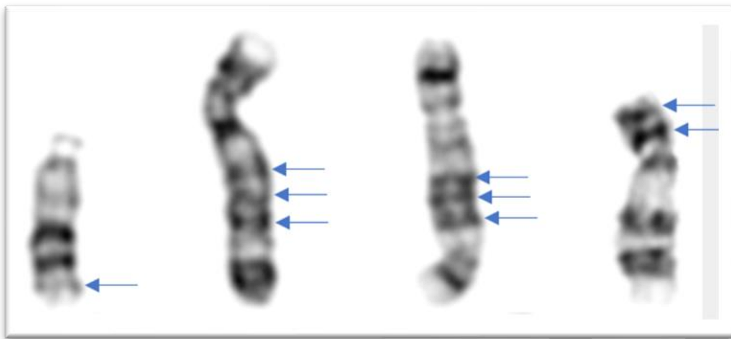
For TCA, a trained cytogeneticist should be able to discern cell quality without embarking on a full evaluation. Alternatively, quality can be determined on the relative band number on the target chromosome(s) with reference to ISCN ideograms. *For example, if a target chromosome 13 has four resolved bands, this equates to 400 bands in haploid set, which corresponds to QA4.*

6.1.3 Quality score landmarks - Quick guide

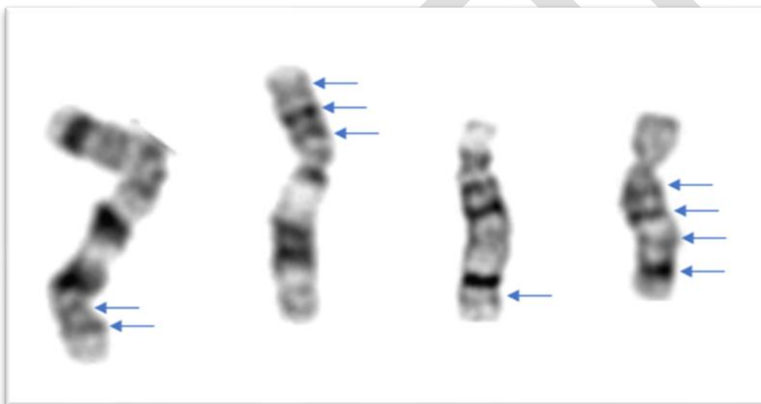
Quality 3



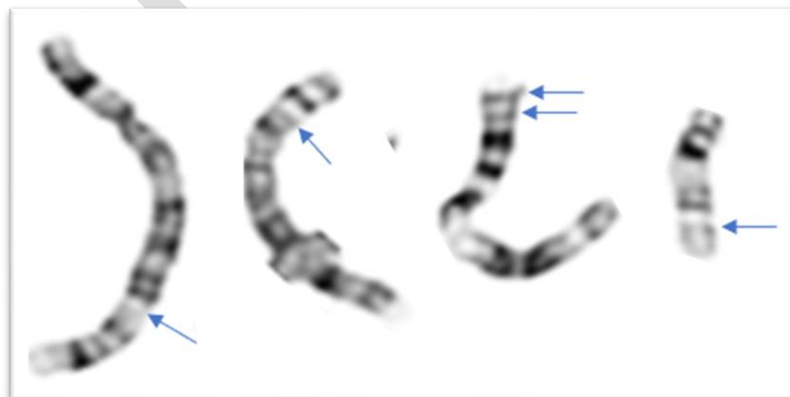
Quality 4



Quality 5



Quality 6



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6.1.4 Minimum G-banding quality

Professional judgement should be applied based on the referral reason. The best quality cells available should be used for analysis whenever possible; this is particularly relevant for delineation of breakpoints and assessing for unknown structural rearrangements or subtle known familial rearrangements (target QA6).

Table 2, below, provides the minimum recommended scores. These define the lowest standard acceptable for a given reason for testing without issuing a qualified report. Where this is not achievable, the test should either be failed, with a repeat sample requested, or a qualified report issued with the clinician invited to send a repeat sample. Prior to this, alternative test strategies and the results of parallel tests should be considered, and if an abnormality explaining the referral indication has been detected, report qualification alone may be sufficient.

Reason for Testing	Minimum G-band Quality (QA) score
Detection of nondisjunctional chromosomal aneuploidy and/or exclusion of involvement of whole arm rearrangement (e.g. Robertsonian translocation).	3
Exclusion/detection of known structural rearrangement.	*
Evaluation for unknown structural rearrangement+ **(e.g. referrals for infertility, recurrent miscarriage, possible sex chromosome abnormality, egg/sperm donors).**	5

* Professional judgement based on the structural rearrangement in question.
+Where breakpoint delineation is necessary, and when achievable, cells of higher banding quality should be used for accurate breakpoint assignment.

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6.1.5 Incomplete cells

An incomplete cell is defined as a cell that is not representative of the final reported karyotype (e.g. 43,X,der(12)t(12;22),-13,-22 cell, when karyotype is 46,XX,t(12;22)).

The analysis of incomplete metaphase spreads (that likely reflect excessive cell bursting during slide making fix evaporation) is discouraged but may be used to clear individual chromosomes that are overlapped in analysed cells. Incomplete cells should be avoided in mosaicism screens for numerical abnormalities.

6.1.6 Female age-related X chromosome loss

The loss of one X chromosome to give an occasional 45,X cell line is a normal characteristic of aging in the 46,XX female (Zietkiewicz *et al.*, 2019).

Low level monosomy X mosaicism should be interpreted on a case-by-case basis, considering the reason for referral, the age of the patient and the level, relative to that expected for age-related loss (Russell *et al.*, 2007). Mosaicism screening is defined in sections 7.2 and 7.3. A reference graph for use by diagnostic laboratories to illustrate the %X chromosome loss (XCL) that would be regarded as normal, in PHA-stimulated blood cultures, is reproduced in figure 1. On this basis, if the mosaicism

is interpreted as likely age-related loss of no clinical significance, it should not be reported to the clinician.

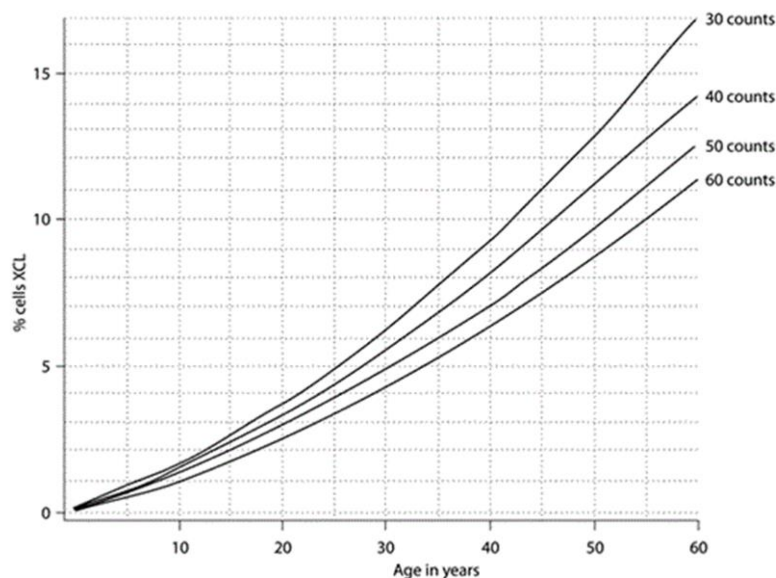

Figure 1. Reference graph to illustrate the % X chromosome loss (XCL) that would be regarded as normal at a given age (Russell *et al.*, 2007).
[Reproduced with permission of the first author, and the publisher, S. Karger AG, Basel, Switzerland]

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6.1.7 Normal chromosomal variation - cytogenetic heteromorphisms

Cytogenetically visible polymorphic heteromorphisms that are documented to be benign should not be reported but should be documented in the patient's laboratory record. These include (Gardner and Amor, 2018):

- Marked heterochromatic size variants, including: 1qh+/-,9qh+/-,16qh+/-, acrocentric p+/- ,Yqh+/-
- Acrocentric short arm variants resulting from the Yq heterochromatin translocation and satellited Y chromosome
- Pericentric inversions with the breakpoints in the heterochromatic region, such as: inv(1)(p11q12), inv(1)(p12q12), inv(9)(p11q12), inv(9)(p11q13), inv(16)(p11q11)
- Pericentric inversions that have been described as presumed harmless variants: inv(2)(p11.2q13), inv(3)(p11q11), inv(3)(p11q12), inv(3)(p13q12), inv(5)(p13q13), inv(10)(p11.2q21.2)

inv(Y)(p11q11) is a recognised normal population variation of no clinical significance (Gardner and Amor, 2018). One study has reported heterogeneity in the inv(Y) breakpoints and speculated that an inv(Y)(p11.2q11.223), which they define as a 'type III' submicroscopic Yq breakpoint, may be associated with fertility impairment (Knebel et al, 2011); however, genotype-phenotype correlation was inconclusive. Therefore, until there is further clarity on this premise, it is recommended that this rearrangement is treated like other cytogenetic heteromorphisms and not reported. When detected in the context of male infertility, reporting with explanation of current understanding could be considered and paternal carrier status may assist interpretation.

Benign G-banding detectable euchromatic heteromorphisms, including 8p23, 8q21.2, proximal 9p, insertion of euchromatin into 9qh, proximal 9q, 15q11.2 and 16p11.2 are recognised (Barber, 2005;

Gardner and Amor, 2018). Further investigations, literature review and/or family studies should be employed to confirm the benign genomic composition of such findings.

Euchromatic duplications and deletions detectable by chromosome analysis and suspected to be those documented without apparent phenotypic effect, as inferred from the observation of transmission from a phenotypically normal parent to a normal child, should be further investigated by microarray to prevent misinterpretation. Decision on whether to report should be based on professional judgement.

Care should be taken to distinguish clinically silent heteromorphisms from pathogenic euchromatic imbalances. It is recognised that cases may require further laboratory investigations and/or family studies for clarification and certainty; this is subject to professional judgement.

6.1.8 Detection of, or investigation for, mosaic abnormalities

These are defined in the application specific sections of these guidelines (see Sections 7, 8 and 9).

6.1.9 Preparation of a karyogram

The preparation of a digital karyogram or partial karyogram is encouraged for abnormal cases if helpful or requested to assist patient genetic counselling. This should clearly illustrate the cytogenetic abnormality with annotations and contain multiple patient identifiers such as patient name and a unique laboratory number. Convention is for the abnormal chromosome to be positioned to the right of the normal homologue.

6.2 Karyotype analysis (KA)

For constitutional cytogenomics a karyotype analysis is employed to establish and designate the patient's chromosome constitution. This is described using the international standard for human cytogenomic nomenclature (ISCN) core nomenclature.

6.2.1 Karyotype analysis definitions (minimum standards)

The **karyotype analysis of a metaphase cell** shall consist of a **chromosome count**, the establishment of the **sex chromosome complement**, and **comparison of every band for every set of homologues**, including sex chromosomes, with banding pattern integrity matched to the ISCN ideogram**.** If one homologue pair is involved in an overlap ('crossover') with itself or another chromosome, or is otherwise obscured, the homologue pair (or arm, or region) that is concealed, must be analysed (cleared) in an alternative cell.

A **case karyotype analysis** shall involve a **minimum of 3 different metaphase cells** at the minimum quality level appropriate for the referral reason (Section 6.1.4). The **analyst shall analyse at least 2 cells** and, with other cells if required, ensure every homologue pair is cleared in full twice. **The second analyst shall analyse at least 1 cell** ensuring every homologue pair is cleared in full once. If the analyst has only analysed the minimum two cells, then the second analyst must analyse a different, further cell. If the analyst has analysed more than two cells it is acceptable for the second analyst to use one of the analyst's original cells.

In mosaic cases, **both analysts** should examine and corroborate the chromosomal constitution **in at least one cell from each cell line**.

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In mosaicism screens (e.g. 27 additional cells examined), structural abnormalities should have the target chromosome(s) cleared in all cells examined. For aneuploidy screens the target chromosome should be unequivocally discernible but does not have to be free of overlaps.

6.3 Targeted chromosome analysis (TCA)

A targeted analysis of metaphase chromosomes is appropriate where the investigation of a specified chromosome abnormality is indicated (see Section 6.3.2). The prospective ISCN 2024 edition will provide nomenclature for targeted chromosome analysis and define consideration for its application to normal and abnormal result outcomes (personal correspondence, ISCN Editor, Dr R Hastings).

6.3.1 TCA Terminology

The term ‘karyotype’ must not be used in either the report headings, labels, or body text of a targeted chromosome analysis report. It is discouraged from being used in analysis documentation. The terms ‘targeted chromosome analysis’, or equivalent, should be used. This is to prevent inadvertent interpretation of a targeted analysis as a karyotype analysis by the clinical report recipient, future professionals accessing the report, or laboratory staff citing archived analysis records.

The report should clearly state that a targeted chromosome analysis has been performed, which chromosome(s) the analysis is limited to, and the degree of analytical evaluation (e.g. restricted to presence/absence of targeted structural abnormality).

6.3.2 Appropriate TCA referral situations

(i)	**Cytogenetic characterisation of a chromosome imbalance(s) detected by another technique (e.g. QF-PCR, FISH, microarray, WGS).** **Gain / complex imbalances are likely to require karyotype analysis to determine the location of the extra material.** *Example: Following the detection of an imbalance by array/WGS which has a straightforward cytogenetic structural rearrangement characterisation in the proband to inform interpretation and inheritance/transmission risk (e.g. derivative chromosome from a reciprocal translocation, ring chromosome, ESAC, etc.).*
(ii)	**Following aneuploidy detection, to detect/exclude an underlying structural rearrangement to inform the recurrence risk.** *Example: To exclude in proband, or parent, an acrocentric trisomy arising from a Robertsonian translocation.*
(iii)	**Family history of known chromosome structural rearrangement when the abnormality is evidenced by a report from an accredited laboratory.** *Example: To determine whether an individual carries a defined balanced familial translocation.*
(iv)	**Assessment for a specific mosaic chromosome abnormality indicated by clinical phenotype or request.** *Example: Request to screen for trisomy 21 mosaicism in an individual with suggestive phenotype where alternative tests are negative.*

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(v)	**Assessment of sex chromosomes indicated by clinical phenotype or request (see section 7.4).** *Example: To confirm biological sex (prior to further investigation (e.g. NGS panel))* *Example: Referral for Turner syndrome (to include a mosaicism screen).*

6.3.3 TCA definitions (minimum standards)

TCA analysis will consist of the **target chromosome(s) homologues being cleared in full in each cell examined.** If regions of the target chromosome(s) are obscured in overlaps, these must be cleared in an alternative cell.

A case TCA shall involve analysis of a **minimum of 3 different metaphase cells**, at the minimum quality level appropriate for the referral reason (Section 6.1.4). The **analyst shall clear the target chromosome(s) in at least 2 cells** and **the second analyst in at least 1 cell.** If the analyst has only used the minimum two cells, then the second analyst must clear the target chromosome in a different, further cell. If the analyst has used more than two cells it is acceptable for the second analyst to use one of the analyst's original cells.

In mosaic cases, **both analysts** should examine and corroborate the chromosomal constitution **in at least one cell from each cell line**.

In mosaicism screens (e.g. 27 additional cells examined), structural abnormalities should have the target chromosome(s) cleared in all cells examined. For aneuploidy screens the target chromosome should be unequivocally discernible but does not have to be free of overlaps.

A chromosome count may be undertaken for each assessed cell; this will be dependent on the reason for testing (e.g. suspected mosaicism for an aneuploidy). A discrepancy in chromosome number must trigger an extended examination or reflex to a karyotype analysis. Acknowledgement of the sex chromosomes may be undertaken as a quality control measure. The count and sex chromosome recognition do not need to be disclosed on the targeted chromosome analysis report, unless related to the targeted investigation or the resulting diagnosis.

6.3.4 Detection of an incidental/unexpected abnormality during TCA

An incidental/unexpected finding (IF) is a clinically relevant finding not related to the primary referral reason and that was not actively sought. Pre-genomic test counselling should include the possibility of such findings that may be relevant to the patient or to their family members.

The incidental detection of an abnormality not related to the targeted chromosome analysis should reflex a full karyotype analysis to elucidate the finding. Consideration of whether to report incidental finding should follow relevant ACGS and NHS guidance, such as the NHSE Rare Disease Interpretation and Reporting Guidelines, April 2024, https://future.nhs.uk/connect.ti/NHSgenomics/view?objectId=154355013 (NB FutureNHS registration required), and BSGM guidance on incidental findings, June 2024, https://bsgm.org.uk/healthcare-professionals/guidance-on-incidental-findings/ .

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Table 3. Examples of incidental findings detected during TCA.

Incidental finding	Actions/Outcome	I&R document v2.0 (April 2024)
XX or XY DSD	Report with clinical genetics involvement	✓
Sex chromosome aneuploidy	Report	✓
Balanced structural rearrangement	Report if reproductive implications for proband or family	
Unbalanced autosomal structural rearrangement	Report if classified as pathogenic	

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7 Application specific to postnatal diagnosis

7.1 Urgent referrals

Referrals submitted for chromosome analysis shall be prioritised according to urgency.

Prioritised urgent samples shall include:

- Indeterminate sex at birth.
- Neonates with a suspected chromosome abnormality.
- Urgent microarray referrals that require cytogenetic characterisation.
- Parents of a structural abnormality detected during prenatal diagnosis.
- Pregnant or partner pregnant with a family history of a chromosome abnormality or structural rearrangement.
- Critically ill patients where results may inform patient management.

7.2 Recommended number of cells to examine

A base (standard) analysis comprising of **3** cells analysed by TCA or karyotype.

A mosaicism screen of **30** cells, comprising of 3 cells analysed and 27 counted/screened. This excludes the presence of mosaicism at a level of 10% or greater, with 95% confidence (Hook, 1977).

An extended mosaicism screen of **60** cells, comprising 3 cells analysed and 57 counted/screened. This excludes the presence of mosaicism at a level of 5% or greater, with 95% confidence (Hook, 1977).

7.3 Detection of, or investigation for, mosaic abnormalities

A mosaic screen of 30 cells should be performed in cases referred for primary amenorrhoea or premature ovarian failure (POF), or other clinical presentation associated with potential mosaicism (e.g. short stature female, ?mosaic Turner syndrome), or where the referral is to rule out the presence of an autosomal aneuploidy or structural rearrangement with a specific association with mosaicism (e.g. trisomy 21 mosaicism, +i(12)(p10)).

A mosaic screen should be employed to accurately characterise an imbalance detected by other technologies (e.g. microarray, QF-PCR) that could indicate mosaicism (e.g. X/XX or X/XY) or, where clinically indicated, exclude the potential masking of a mosaicism due to a balanced state (e.g. X/XXX or X/XYY).

If during routine analysis a cell, or cells, with a discrepant karyotype is detected, which may indicate a constitutional mosaicism, the number of cells examined should be increased to 30*.
[*excluding recurring benign rearrangements involving T-cell receptor or Ig loci in lymphocyte stimulated blood samples, unless indicative of a chromosome breakage disorder or haematological malignancy].

If the clinical presentation is highly indicative of a mosaicism, or a disorder is evidenced with low level mosaicism incidence (e.g. ring (20) Peron *et al.*, 2020) the number of cells examined may be increased to 60 cells, at professional discretion.

Statistical modelling (table in Hook, 1977) should be used for reference when citing details of the level of mosaicism excluded and confidence limits.

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A request for a second tissue may be appropriate when the referral is for a syndrome with known tissue specific mosaicism (i.e. Pallister Killian Syndrome) or a potentially relevant mosaicism has been detected at a level below reporting confidence levels.

Chromosomal mosaicism screening by SNP microarray should be considered as an alternative first-line mosaicism test as this is generally performed on DNA prepared from a more heterogenous cell population, has high sensitivity for the detection of minority cell lines and the SNP microarray B-allele frequency (BAF) profile provides corroborative evidence of a likely mosaic finding.

We note that cytogenetic mosaicism screening is recommended for complete AZFc or Yq terminal deletions (Krausz *et al.*, 2024). Our recommendation is to limit this to 30 cells in the first instance.

7.4 Investigation for sex chromosome abnormality

For referral reasons where a sex chromosome abnormality is suspected, targeted sex chromosome analysis or QF-PCR or FISH analysis, (using sex chromosome markers/probes including the *SRY* locus), are acceptable front-line test methods. These methods provide information regarding the presence of a Y chromosome, sex chromosome aneuploidy, including mosaicism, and in the case of TCA, any structural rearrangement involving the sex chromosomes.

Where the presenting phenotype may be associated with a chromosome rearrangement involving the autosomes (e.g. Robertsonian translocation in male infertility referral), karyotype analysis should be performed.

Table 4. Example test approaches.

Referral type	Analysis	Mosaicism screen
Clinical suspicion of Turner syndrome	TCA	✓
Primary amenorrhoea	TCA	✓
Secondary amenorrhoea, POF	TCA	✓
Delayed puberty	TCA	✓
Clinical suspicion of Klinefelter syndrome	TCA /QF-PCR	-
Ambiguous genitalia, ?DSD	TCA	✓
Female with inguinal hernia – to exclude AIS (XY female)	QF-PCR	-
Primary infertility	KA	-
Secondary infertility	KA	-
Azoospermia, oligospermia, OAT, male sub-fertility, etc.	KA	-
Recurrent miscarriages (see NGTD R297 for eligibility)	KA	-

Abnormal QF-PCR / FISH results may be **followed-up** by TCA, FISH analysis, KA, or microarray testing, as appropriate. Examples where follow-up should be carried out include:

- Results indicative of imbalance related to a sex chromosome structural rearrangement.
- Where mosaicism for sex chromosome aneuploidy is detected/inferred (include 30 cell screen).
- Male XX DSD with *SRY* present, to confirm position of *SRY*.
- Male XX DSD with *SRY* absent (include 30 cell screen).
- Female XY DSD (include 30 cell screen).

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8 Application specific to prenatal diagnosis

8.1 Prenatal diagnosis from invasive samples

Prenatal diagnosis from invasive sampling is predominantly performed on amniotic fluid (AF) or chorionic villus (CV) samples, although fetal blood (FB) samples or other prenatal samples such as fetal urine, fetal pleural effusion may also be referred. The laboratory shall have standard operating procedures for processing all potential sample types.

Processing usually requires (prepared) samples to be divided between direct DNA based tests and cell culture (where required). In addition to providing dividing cells for chromosome analysis, cell culture can provide back-up material should direct DNA preparation be unsuccessful, or DNA test results are equivocal.

8.2 Preparation

8.2.1 Chorionic villus (CV) samples

Prior to DNA preparation or cell culture set-up, CV samples shall be examined using inspection microscopy to ensure the sample is of sufficient size, of conceptus origin and suitable for processing. Detectable maternal tissue (decidua), blood clots and material of questionable tissue origin should be removed by dissection. This procedure should be checked by a second competent operative.

The laboratory shall have a documented policy for situations where no conceptus tissue is present.

Direct/short term CV culture techniques and/or karyotyping of direct preparations should not be undertaken for routine analysis.

CV must be disaggregated by enzymatic and/or physical chopping to produce a single dissociated heterogenous cell population that is used for both DNA preparation and cell culture set-up (where carried out): DNA prepared from a heterogenous cell population minimises the risk of misdiagnosis from confined placental mosaicism (CPM) (Waters *et al.*, 2006; Waters *et al.*, 2007).

8.2.2 Amniotic fluid (AF) samples

Collection vessels should be inspected for structural integrity (cracks) prior to centrifugation.

Sample and pellet conditions, such as blood staining, tissue contamination, fluid and cell pellet appearance, cell density etc. should be recorded in the case notes to assist result interpretations.

8.3 AF and CV culturing

The laboratory shall have a documented policy for the apportioning of samples for the different tests (e.g. direct DNA extraction and cell culture). Application of this will be dependent on sample/referral circumstances and test requirements and should be based on professional judgement. Whenever possible, adequate sample shall be allocated for cell culture to facilitate back-up test material, even if cultures are not established.

Where culture is carried out, multiple independent primary cultures shall be set-up from the initial sample. A minimum of two cultures is recommended, although this is dependent on sufficient sample, the need for direct DNA extraction and professional judgement.

To minimise the risk of the cultures from any one patient being lost due to equipment failure, cultures should be propagated across at least two separate incubators. Similarly, the cultures from any one

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patient shall be propagated using separate culture media preparations that are prepared from different batches of basal media and supplements.

To minimise the risk of microbial cross contamination, prenatal cultures should be incubated separate to other laboratory samples.

Cultures should be retained until the final genetics report is issued where appropriate. Risk can be mitigated in cases requiring further testing by ensuring there is sufficient DNA stored prior to disposal of cultures.

DNA from uncultured and cultured prenatal samples should be retained in accordance with the Royal College of Pathologists guidance G031, Wilkins, 2015. It is recommended that DNA from prenatal samples follow the retention guidance of ‘at least 30 years if needed for family studies in those with genetic disorders’ (Section 139).

For prenatal samples referred with ultrasound anomalies, additional testing may be indicated either during or post-pregnancy. Where DNA yields are low and/or DNA has been required for multiple tests, additional DNA extraction from cultures may be required; this should be reflected in laboratory procedures.

8.4 Culture failure

When karyotyping is pertinent to a diagnostic result, the laboratory should consider informing the referring clinic if no culture growth is evident within 10 days. Expected culture failure preventing the establishment of a karyotype result should be communicated if no cell growth is discernible by 14 days.

8.5 Chromosome analysis of prenatal samples

Chromosome analysis of direct CV preparations, with its quality and accuracy limitations, shall not be undertaken for analyses.

It is recommended that the application of chromosome analysis in prenatal samples is limited to the follow-up of abnormal results obtained from QF-PCR and array analysis, as appropriate. Targeted chromosome analysis is appropriate in these circumstances, to provide information regarding the nature of the chromosome imbalance and/or infer recurrence risks or to follow-up mosaic results.

For parental carriers of balanced rearrangements, it is recommended that prenatal testing is limited to determining unbalanced translocation status by microarray and that balanced carrier status is not determined, unless balanced status, which cannot be determined by microarray, has clinical significance or benefit for disclosure for the current pregnancy (breakpoint disrupts a gene or involves the X chromosome). This aligns with the recommendation regarding genetic testing for carrier status in childhood (Clarke *et al.*, Section C9.5, RCP, RCPath and BSGM, 2022). Access to balanced translocation carrier status testing shall be offered at an appropriate age postnatally and this requirement must be referred to in the prenatal report.

The minimum G-banding quality shall be in accordance with that defined in Section 6.1.4.

Where it is not possible to achieve the minimum quality level for the reason for referral, the report should be qualified and any suitable reflex tests (e.g. microarray) initiated. Repeat invasive procedures that are not clinically appropriate should not be requested or inadvertently encouraged.

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8.6 Number of cells to examine

The standard analysis for long-term cultures shall include that defined by the minimum karyotype analysis or targeted chromosome analysis (Sections 6.2.1 and 6.3.3).

The total number of cells examined will be dependent on the analysis context.

Targeted chromosome analysis (e.g. establishment of nondisjunctional trisomy 21) shall involve a minimum of 3 cells.

Mosaicism screens, should be in accordance with the guidance in Sections 8.8 and 8.9, involve the base evaluation of a minimum of 10 cells from each of two independent cultures or 15 colonies from at least two independent *in situ* cultures (in accordance with Hsu *et al.,* 1992; Hsu and Benn, 1999), and be subject to professional judgement.

For *in situ* harvested preparations, each cell analysed should be from a separate colony.

8.7 Need for second cultures

It is acceptable to report a chromosome analysis result on a **single** culture when:

- The result is **male**.
- The result is **consistent with a QF-PCR or microarray diagnostic result**.
- The result is **consistent with the transmission of an unbalanced form of a known familial structural rearrangement**.
- **A female result which is different to that of the mother** (e.g. balanced structural rearrangement inherited from the father).

Second/multiple cultures should be examined for:

- Results **discordant to a QF-PCR or microarray result**.
- **Female results with no other parallel test result.**
- The **detection or investigation of mosaicism**.

8.8 Follow-up to QF-PCR/array detected mosaicism or increased risk of mosaicism

Work-up should be in accordance with Hsu and Benn guidelines, 1999 (see Appendix Tables 5 and 6).

An **extensive** targeted chromosome analysis work-up shall be undertaken to screen for the presence of a second cell line in long-term cultures for:

- CV with a biallelic trisomy QF-PCR result in the absence of ultrasound anomalies consistent with the detected trisomy.
- Detected/inferred mosaicism in a CV or AF sample.

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8.9 Detection and interpretation of culture incidental mosaicism

The primary use of DNA-based analysis minimises the finding of pseudomosaicism. However, where chromosome analysis is applied, culture incidental mosaicism may be identified. The detection of single or multiple abnormal cells, or normal cells on a background of chromosome abnormality, in CV or amniotic fluid cultures shall necessitate work-up to determine whether the finding is likely to reflect true mosaicism. Work-up should be in accordance with Hsu and Benn guidelines, 1999 (see Appendix Tables 5 and 6).

These guidelines should be used as a minimum basis to attempt to establish true mosaicism. The reason for referral, and whether the pregnancy is still ongoing, should also be taken into consideration when deciding on the level of work-up. Professional judgement should be exercised in situations where a work-up cannot be fully achieved. The guidelines assume that the analysis is from primary cultures. Microarray and QF-PCR results from uncultured DNA and/or from individual cultures should also be taken into account.

Level I mosaicism; a single abnormal cell after additional work-up, is likely to reflect pseudomosaicism.

Level II mosaicism; (two or more cells from a dispersed culture from a single vessel or a single abnormal colony from an *in situ* culture that is not detected in other independent cultures) is likely to be pseudomosaicism. However, if additional work-up was inadequate, if fetal anomalies had been identified, or in the case of certain chromosome abnormalities which are well recognised as existing in the mosaic state, it may be appropriate to disclose the finding and advise a follow-up amniocentesis if found in a CV. This should be considered on a case-by-case basis using professional judgment.

Level III mosaicism; two or more cells with the same chromosome abnormality, distributed over two or more independent cultures, is likely to reflect true mosaicism and this would be expected to be reflected in the QF-PCR and/or microarray result (NB. this does not differentiate between confined placental mosaicism (CPM) and genuine fetal mosaicism).

[Adapted from Gardner and Amor, 2018]

8.10 Follow-up to detection of true mosaicism

Where level III mosaicism is found in a CV, a follow-up amniocentesis or fetal blood sampling, and detailed ultrasound scan assessment, should be advised.

Where mosaicism is reported, reference should be made to the level detected not necessarily reflecting the proportion, or the tissue distribution, in the fetus.

Mosaicism considered to be an artefact by application of Hsu and Benn, 1999, guidelines should usually not be mentioned in the report. Particular care should be taken in interpreting level II mosaicism for a clinically significant aneuploidy. It may be appropriate to mention the finding in the report and to advise a follow-up amniocentesis if found in a CV.

8.11 Maternal cell contamination (MCC)

Where maternal contamination is suspected this should be investigated, for example, using genotype analysis or SNP microarray.

Prenatal report qualifiers should include reference to MCC considerations (e.g. unless otherwise specified, this result assumes that the sample consists only of tissue originating from the conceptus).

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8.12 Uniparental disomy (UPD) studies

Laboratories shall have a documented policy for the suitability and application of UPD investigations in a prenatal setting.

Prenatal UPD testing **should be carried out** for:

- cases with prenatal ultrasound findings indicative of mosaic upd(11p15.5)pat or upd(14)pat (Dawson *et al*., 2011, del Gaudio *et al*., 2020, Eggermann, 2020).

Further indications for prenatal UPD testing following prenatal cytogenomic analysis, are aligned with the ACMG position statement on testing for UPD (del Gaudio *et al*., 2020). See this publication for primary citations providing evidence.

Prenatal UPD testing is **recommended** for:

- A *de novo* balanced Robertsonian translocation involving chromosomes 14 or 15 and der(14;14)(q10;q10) and der(15;15)(q10;q10).
- A balanced microarray/QF-PCR in a prenatal case referred for a parental Robertsonian translocation involving chromosomes 14 or 15.
- A *de novo* ESAC (extra structurally abnormal chromosome), derived from an imprinted chromosome with no apparent euchromatin present.
- Level II or level III mosaicism for trisomy 7, 14 or 15 on CV, followed by a normal AF result.
- SNP array BAF profile indicative of mosaic segmental UPD of 11p15 and duplications of 11p including the 11p15.5 imprinted region.

Prenatal UPD testing should be **considered** for:

- A confirmed *de novo* or a familial isochromosome 14 or 15 in a balanced state.
- Level II or level III mosaicism for trisomy 6 or 20 on CV, followed by a normal AF result. This should be subject to professional judgement as upd(6)pat in majority of reported cases is due to paternal isodisomy of chromosome 6, consistent with monosomy rescue and similar accounts for upd(20)pat.
- A non-Robertsonian translocation between any chromosomes known to carry imprinted genes and at risk for 3:1 disjunction in which trisomy or monosomy rescue or gamete complementation could occur. However, in theory every chromosome abnormality that increases the occurrence of nondisjunction would increase the risk for UPD of the chromosomes involved, and evidence indicates that only very few cases have ever been reported.
- Non-mosaic upd(11), either paternal or maternal is unlikely to be associated with viability of the fetus, therefore level II or level III mosaicism for trisomy 11 on CV, followed by a normal karyotype in AF is not a recognised risk factor for upd(11) disorder.

It is recognised that some of the above UPD scenarios involving *de novo* rearrangements may not arise when first-line test is limited to microarray.

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8.13 Prenatal diagnosis follow-up

There is no statutory or clinical requirement to confirm prenatal diagnosis results. Confirmation and/or further investigation should be undertaken if the prenatal diagnosis result and the infant's postnatal phenotype appear inconsistent or discordant.

Where appropriate, the report should carry a statement recommending that a copy of the prenatal result/report should be transferred to the infant's medical notes at delivery.

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9 Application specific to solid tissues and reproductive loss

In general, karyotype analysis is not recommended for the genome-wide testing for reproductive loss referrals. The NGTD defines the appropriate test method, which is currently common aneuploidy testing and microarray testing.

The NGTD defines when parental karyotyping is acceptable following unsuitable samples, failed collection, or failed testing of pregnancy loss samples.

Targeted chromosome analysis of pregnancy loss samples may be employed instead of parental chromosome examination to establish recurrence risk for imbalance identified by other techniques, although this approach will involve initial culture of pregnancy loss referrals and its adoption will be dependent on local cost modelling and/or clinical preference considerations.

9.1 Selection and preparation of solid tissues

Maternal cell contamination can be a complicating factor, extensively in placental tissue samples. Care should be taken to remove maternal decidua from placental tissue. A dissecting microscope should be used to select and clean samples, especially those from products of conception. Gentle scraping of the cord and membranes may remove adherent maternal cells. Genotype analysis (QF-PCR / SNP microarray) should detect MCC, if a mixture of maternal and conceptus genotypes is present (although differentiating the minor and major genotypes in maternal and normal female conceptus mix is not possible).

Placental tissue must be disaggregated by enzymatic and/or physical chopping to produce a single dissociated heterogenous cell population that is used for both DNA preparation and cell culture set-up (where carried out): DNA prepared from a heterogenous cell population minimises the risk of misdiagnosis from confined placental mosaicism (CPM).

The operative responsible for selecting tissues for genetic testing must be competent in the recognition of the various tissue types that may be encountered. Observations of tissue types and sample condition should be recorded in the case notes to assist result interpretations.

The laboratory must have a policy for situations where there is no material originating from the conceptus.

9.2 Guidance on the handling and disposal of pregnancy remains

The laboratory must have a policy and practice for the sensitive handling and disposal of pregnancy remains that is in accordance with the English Human Tissue Authority (HTA) guidance (2015) England https://www.hta.gov.uk/guidance-professionals/guidance-sector/post-mortem/guidance-sensitive-handling-pregnancy-remains or Human Tissue (Scotland) Act 2006 Human Tissue (Scotland) Act 2006 (legislation.gov.uk).

9.3 Detection of or investigation for mosaic abnormalities

The investigation for mosaicism in solid tissue biopsies may warrant chromosome investigation, although other technologies (e.g. microarray) are validated to detect unbalanced mosaicism to high resolution. Chromosome analysis may be employed to detect the structural nature of a suspected mosaicism detected by another technique (e.g. +i(12)(p10) associated with Pallister-Killian syndrome).

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10 Reporting Standards

Reports shall be constructed in accordance with the ACGS General Laboratory Reporting Recommendations (See https://www.acgs.uk.com/quality/best-practice-guidelines/ for current version).

10.1 Multiple cytogenetic techniques

When multiple cytogenetic techniques have been used to diagnose and characterise a cytogenetic abnormality, these should be combined on a single report, apart from tests with differing reporting timelines (e.g. rapid QF-PCR and microarray).

10.2 Balanced rearrangements

The use of the word 'abnormal' should not be used in describing a balanced rearrangement.

10.3 Terminology and nomenclature for 'differences of sex development'

Differences of sex development (DSD) nomenclature shall be used to describe results of sex chromosome abnormalities (Hughes, 2008; https://www.nhs.uk/conditions/differences-in-sex-development/).

10.4 Clinical Genetics referral

Recommending referral to Clinical Genetics should be based on local policy. It is not generally required for the common aneuploidies, but is advised for other cytogenetic abnormalities, atypical results (e.g. mosaicism at prenatal diagnosis) and incidental findings.

10.5 Limitations of chromosome analysis

Reports should clearly state the limitations of conventional chromosome analysis with regard to not being able to exclude subtle chromosome rearrangements (or subtle imbalances if microarray is not performed), or mosaicism, and state the limitations of targeted analysis. This is particularly pertinent for prenatal diagnosis where, although very rare, CPM and/or false positive/negative diagnoses are possible.

10.6 Report qualifiers

Chromosome analysis reports shall include a report footnote (qualifier) that details the level and the limitations of the analysis performed. This may include technical information and reference to compliance with best practice guidelines.

The use of report qualifiers will not defend the lack of detection of an abnormality if an independent assessor determines that the abnormality should have been detected and therefore provides no legal protection.

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Example of report qualifier for **karyotype analysis**:

Methodology and Technical Information:
Chromosome analysis of the complete set of GTG-banded peripheral blood metaphase chromosomes has been undertaken on 3 cells at a minimum resolution level of 550-bands, with a further 27 cells examined for chromosome number and sex chromosome complement only, in accordance with ACGS best practice guidelines. Cells were digitally captured, and chromosomes paired, using the ***** software. An evaluation of 30 cells excludes the presence of mosaicism at a level of 10% or greater, with 95% confidence (Hook *et al.*, 1977, Am J Hum Genet, 29:94-97). Subtle or cryptic structural rearrangements/imbalances may not have been detected and this result may not reflect the karyotype of cells in other tissues. Cytogenetic heteromorphisms that reflect normal chromosome variation are not reported. To avoid error and/or misinterpretation, transcription of all or part of this report is inadvisable.

Examples of report qualifier for **targeted chromosome analysis**:

Methodology and Technical Information:
Targeted chromosome analysis of specific GTG-banded peripheral blood metaphase chromosomes, as detailed in the report, has been undertaken on 3 cells at a minimum resolution level of 300-bands, in accordance with ACGS best practice guidelines. Cells were digitally captured, and chromosomes paired, using the ***** software. This limited examination of G-banded metaphase chromosomes has been performed to confirm a nondisjunctional trisomy and exclude a structural rearrangement involving the trisomic chromosome. Cytogenetic heteromorphisms that reflect normal chromosome variation are not reported. Incidental findings of no clinical actionability may not be reported. To avoid error and/or misinterpretation, transcription of all or part of this report is inadvisable.

Methodology and Technical Information:
Targeted chromosome analysis of specific GTG-banded peripheral blood metaphase chromosomes, as detailed in the report, has been undertaken on 3 cells at a minimum resolution level of 550-bands, in accordance with ACGS best practice guidelines. Cells were digitally captured, and chromosomes paired, using the ***** software. This limited examination of G-banded metaphase chromosomes has been performed to investigate the targeted structural rearrangement. Cytogenetic heteromorphisms that reflect normal chromosome variation are not reported. Incidental findings of no clinical actionability may not be reported. To avoid error and/or misinterpretation, transcription of all or part of this report is inadvisable.

10.7 Cytogenetic structural rearrangement transmission risk assessment

Where there are reproductive risks associated with a chromosome structural rearrangement this should be stated on the report. Reproductive risk assessment should, at minimum, consider:

- Mode of ascertainment of the family.
- Personal segregation analysis from family history, if available.
- The predicted type of segregation leading to potentially viable gametes.
- The sex of the transmitting parent.
- Review of literature describing viability for the specific imbalances, and/or established empirical risk figures (Gardner and Amor, 2018).
- For unbalanced segregants with relatively small imbalances, where breakpoints have been accurately defined previously by microarray or FISH, and gene content is minimal, application of the CNV interpretation pathogenicity scoring guidelines can assist risk assessment.

Risk assessments shall be based on citable evidence and appraised critically. Where there is well-established risk information from published data, specific figures can be used, and the literature source cited, however, they should not give a false sense of precision.

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11 Appendix

Table 5. Prenatal *in situ* culture mosaicism work-up (Hsu and Benn, 1999).

FINDING	ADDITIONAL WORK-UP
Autosomal trisomy for chromosomes 2,5,8,9,12,13,14,15,16,18,20,21 or 22	
Partial Colony	***BASIC***
Single Colony	***EXTENSIVE***
Multiple Colony	***EXTENSIVE***
Autosomal trisomy for chromosomes 1,3,4,6,7,10,11,17, or 19	
Single Colony	***MODERATE***
Multiple Colony	***MODERATE***
Marker Chromosome	
Partial Colony	***BASIC***
Single Colony	***MODERATE***
Multiple Colony	***EXTENSIVE***
Extra Sex Chromosome or 45,X	
Partial Colony	***BASIC***
Single Colony	***MODERATE***
Multiple Colony	***MODERATE***
Unbalanced Structural Rearrangement	
Single Colony	***MODERATE***
Multiple Colony	***EXTENSIVE***
Balanced Structural Rearrangement	
Single Colony	***BASIC***
Multiple Colony	***MODERATE***
Monosomy 18,21,22	***MODERATE***
Single Cell Abnormalities if a 'free' cell	**As single colony work-up**

***In Situ* culture work-up definitions**

	Examination of:
BASIC	**15 colonies** from two independent cultures
MODERATE	**12 colonies** from cultures that did not contain the original finding
EXTENSIVE	**24 colonies** from at least 2 independent cultures that did not contain the original finding

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Table 6. Prenatal suspension culture mosaicism work-up (Hsu and Benn, 1999).

FINDING	ADDITIONAL WORK-UP
Autosomal trisomy for chromosomes 2,5,8,9,12,13,14,15,16,18,20,21 or 22 Or a normal cell in a background of trisomy Single Cell Multiple Cell	 ***EXTENSIVE*** ***EXTENSIVE***
Autosomal trisomy for chromosomes 1,3,4,6,7,10,11,17 or 19 Single Cell Multiple Cell	 ***MODERATE*** ***MODERATE***
Marker Chromosome Single Cell Multiple Cell	 ***MODERATE*** ***EXTENSIVE***
Extra Sex Chromosome or 45,X Single Cell 45,X Single Cell Extra Sex Chromosome Multiple Cell	 ***BASIC*** ***MODERATE*** ***MODERATE***
Unbalanced Structural Rearrangement Single Cell Multiple Cell	 ***BASIC*** ***EXTENSIVE***
Balanced Structural Rearrangement Single Cell Multiple Cell	 ***BASIC*** ***MODERATE***
Monosomy 18,21,22	***MODERATE***
Other Single Cell Abnormalities	***BASIC***

Suspension culture work-up definitions

	Examination of:
BASIC	Total **20** cells from **2** cultures (**ideally 10+10**)
MODERATE	**20** cells from an independent culture
EXTENSIVE	**20** cells from each of **2** independent cultures

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12 Definition of terms and abbreviations

Case: a single genetics referral episode usually recorded as a separate entity on LIMs**.**

Karyotype analysis (KA): to count metaphase chromosomes and compare every chromosome, band for band, with its homologue and to verify the banding pattern of the X and Y chromosomes in male karyotypes.

Targeted chromosome analysis (TCA): to assess specific metaphase chromosomes for the presence or absence of a chromosome abnormality.

Chromosome analysis: general reference to both karyotype analysis and targeted chromosome analysis.

Clear: to confirm that a chromosome, or region of a chromosome, is normal by comparison with its homologue.

Count: to enumerate the total number of chromosomes in any given metaphase.

Karyotype: the normal, or abnormal, complete chromosomal constitution of an individual, tissue, or cell line (ISCN, 2020).

Karyogram: systemised array of the chromosomes prepared either by digitised imaging or photography (ISCN, 2020).

Ideogram: idealized graphic (schematic) representation of a karyotype, often depicting the landmarks and numbered regions, bands, and sub-bands (ISCN, 2020).

AF: amniotic fluid sample

CV: chorionic villus sample

CPM: confined placental mosaicism

ESAC: Extra Structurally Abnormal Chromosome

FISH: Fluorescence *in situ* hybridisation

LIMS: Laboratory information management system

MCC: maternal cell contamination

NGTD: National Genomics Test Directory

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16 Review of this procedure

The frequency of review will be controlled and managed by the Quality Subcommittee of the Association for Clinical Genomic Science.

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