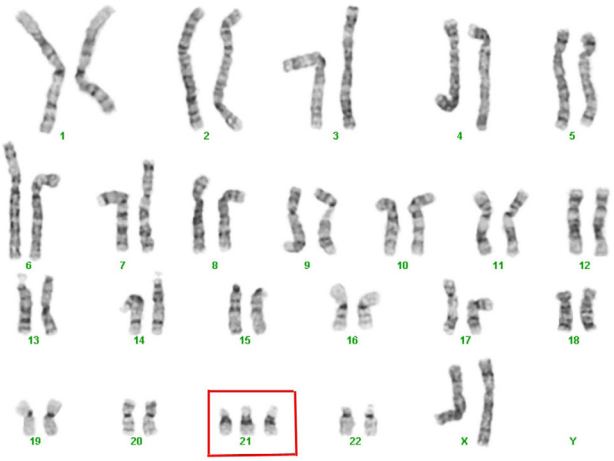
THE PATHCARE NEWS

CYTOGENOMIC TESTING



CYTOGENETICS TO CYTOGENOMICS

What is Cytogenomics?

CYTOGENOMICS is used as a general term that encompasses conventional karyotyping, as well as molecular cytogenetics (fluorescence in situ hybridization-FISH), microarrays and molecular-based techniques. With advancing technologies and consequent shift towards an increasing application of molecular genetic techniques with the potential for higher resolution, as well as the application of multiple testing strategies for the **diagnosis of chromosomal disorders,** it is important that testing laboratories keep up with technology and provide guidance to this ever-changing testing scenario. These will enable Cytogenetic laboratories to operate within acceptable standards and to offer a reliable, cost-effective service.

Guidelines for CONSTITUTIONAL Cytogenomic Analysis

Main clinical indications PRENATAL DIAGNOSIS	Main clinical indications POSTNATAL DIAGNOSIS
Abnormal foetal ultrasound	Abnormal clinical phenotype
High-risk maternal serum screening/NIPT result indicating an increased risk of a chromosomally abnormal foetus	Multiple congenital abnormalities
Parental chromosome rearrangement, mosaicism or previous aneuploidy	Clinically significant abnormal growth – short stature, excessive growth, microcephaly, macrocephaly
Previous livebirth/stillbirth with a chromosome abnormality	Primary or secondary amenorrhoea or ovarian insufficiency
Possible foetal mosaicism detected by prior prenatal study	Ambiguous genitalia
Familial monogenic disorder (i.e., CF, Noonan syndrome)	Infertility of unknown aetiology
	Sperm abnormalities – azoospermia or severe oligospermia
	A malformed foetus or stillbirth of unknown aetiology
	Third and subsequent miscarriage(s): products of conception
	Significant familial history of chromosome rearrangements
	Significant familial history of intellectual disability (ID) of possible chromosomal origin where it is not possible to study the affected individual
	Carrier-ship for an X-linked recessive disorder in a female.
	Microarray is the test method of choice for: Intellectual disability (ID), autism, neurodevelopmental disorders and/or congenital anomalies

TESTS AVAILABLE:

1. PRENATAL TESTING

TEST	DESCRIPTION	TAT	SAMPLE REQURIED
NIPT – Non-Invasive Prenatal Testing	NIPT is a screening test with a high specificity and sensitivity. All positive results should be confirmed by chromosomal analysis / FISH.	7-10 days	EDTA blood sample
AMNIOCENTESIS: FISH / QF-PCR	Fluorescent-in-situ-hybridization / Qualitative Fluorescent PCR – a method where chromosomal investigation of most common aneuploidies are analysed. i.e. 13,18,21, X,Y. Chromosomes 15 and 16 may be added for FISH / QFPCR performed on placental tissue.	3-5 days	Amniotic fluid Placental tissue / POC
KARYOTYPING- Chromosomal Analysis	Chromosomal analysis is performed on all 46 chromosomes. Can detect abnormalities >5mb. Can detect aneuploidies, translocations, and all re-arrangements	14 days	Lithium Heparin blood sample
CMA – CHROMOSMAL MICRO-ARRAY	Genome-wide microarray-based analysis (array) is used to detect chromosomal imbalances at a significantly higher resolution than routine cytogenetic analysis	10 – 14 days	EDTA blood sample Amniotic fluid Placental tissue / POC

2. POSTNATAL TESTING

TEST	DESCRIPTION	TAT	SAMPLE REQURIED
KARYOTYPING – Chromosomal Analysis	Chromosomal analysis is performed on all 46 chromosomes. Can detect abnormalities > 5mb Can detect aneuploidies, translocations, and all re-arrangements.	14 days	Lithium Heparin blood sample
CMA – CHROMOSOMAL MICRO-ARRAY	Genome-wide microarray-based analysis (array) is used to detect chromosomal imbalances at a significantly higher resolution than routine cytogenetic analysis.	10 – 14 days	EDTA blood sample
NGS – NEXT GENERATION SEQUENCING	Although widespread application is currently prohibited both by cost and the requirement for complex algorithms and data analysis pathways, it is predicted that this will become a standard approach for genetic diagnosis in coming years.		

- When deciding which methodology to apply, the specific referral indication and the advantages and limitations of each technique must be taken into account

Cytogenomic Technologies: CONSTITUTIONAL Postnatal Testing

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	KARYOTYPING	FISH / QF PCR	CMA – CHROMOSOMAL MICRO-ARRAY	NGS
Whole Genome Resolution	5 - 10 Mb	- 100 kb	20 - 200 kb	100 - 150 kb
TAT	14 days	5-7 days	21 days	variable
Targeted	ALL 46 chromosomes	Single probe or multiple chromosome probes can provide reliable results in different clinical situations.	Used to detect chromosomal imbalances at a significantly higher resolution than routine cytogenetic analysis.	NGS is a general term for a variety of methods and it has a number of applications, including NIPT for prenatal aneuploidy screening and whole-exome sequencing (WES)
Sample required	Blood / Placental tissue	Blood / placental tissue	Blood placental tissue	Blood / placental tissue
Reasons of clinical referral	Abnormal clinical phenotype Multiple congenital abnormalities Primary or secondary amenorrhoea or ovarian insufficiency; Ambiguous genitalia Infertility of unknown aetiology Sperm abnormalities – azoospermia or severe oligospermia Repeated miscarriages Significant familial history of chromosome rearrangements; Routine postnatal specimen preparations Haematological Oncology bone marrow – leukemic / lymphoma referrals	Interphase FISH Numerical abnormalities; Duplications; Deletions; Sex chromosome constitution; Mosaicism; Gene amplification. Metaphase FISH Marker chromosome; Unknown material attached to a chromosome; Identification and exclusion of small expected structural rearrangements; **QF PCR –** triploidy and mosaicism and genotypes the sample allowing for identification of MCC, twin pregnancies and chimeras. Haematological Oncology bone marrow – leukemic / lymphoma referrals	ID, autism, neurodevelopmental disorders and/or congenital anomalies - Carriership for an X-linked recessive disorder in a female. Abnormal clinical phenotype Identification and exclusion of small expected structural rearrangements; Haematological Oncology bone marrow – leukemic / lymphoma referrals	Significant familial history of intellectual disability (ID) of possible chromosomal origin where it is not possible to study the affected individual; - Carriership for an X-linked recessive disorder in a female. Haematological Oncology bone marrow – leukemic / lymphoma referrals
Aneuploidy	YES	YES	YES	YES
Balanced translocation	YES	YES	NO	YES
Unbalanced translocation	YES	YES	YES	YES
Polyploidy	YES	YES	YES	YES
Mosaicism	YES	YES	YES	YES
UPD	No	No	YES	YES
Copy Neutral LOH	No	No	YES	YES
Gene Fusions	YES	No	YES	YES

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Cytogenomic Technologies: CONSTITUTIONAL Prenatal Testing

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	AMNIOCENTESIS KARYOTYPING	AMNIOCENTESIS FISH / QF PCR	CMA – CHROMOSOMAL MICRO-ARRAY	NGS
Whole Genome Resolution	5 - 10 Mb	- 100 kb	20 - 200 kb	100 - 150 kb
TAT	14 days	5-7 days	21 days	variable
Targeted	ALL 46 chromosomes	Single probe or multiple chromosome probes can provide reliable results in different clinical situations.	Used to detect chromosomal imbalances at a significantly higher resolution than routine cytogenetic analysis.	NGS is a general term for a variety of methods and it has a number of applications, including NIPT for prenatal aneuploidy screening and whole-exome sequencing (WES)
Sample required	Amniotic fluid / Placental tissue/cord blood	Amniotic fluid / placental tissue / cord blood	Amniotic fluid / placental tissue / cord blood	Amniotic fluid / placental tissue / cord blood
Reasons of referral	Confirmation of aneuploidy; Exclusion of known large structural rearrangements; Identification and exclusion of small expected structural rearrangements; Routine prenatal specimen preparations; Prenatal specimen abnormal ultrasound referrals (in the absence of array-based analysis); Routine postnatal specimen preparations	Rapid prenatal FISH High risk of chromosome aneuploidy or recurrent microdeletion (e.g., abnormal ultrasound); Late gestation referral. Evaluation/ characterisation of interphase FISH Numerical abnormalities; Duplications; Deletions; Sex chromosome constitution; Mosaicism; **QF PCR** Detects triploidy and mosaicism and genotypes the sample, allowing for identification of MCC, twin pregnancies and chimeras.	To investigate the cause of miscarriage and foetal abnormality.	NIPT for prenatal aneuploidy screening approximately 85% of disease-causing variants have been identified in exons or at splice-junction boundaries in introns. In addition to sequence variants, structural variants or CNVs can also be detected in WES data, which increases the diagnostic yield. Although widespread application is currently prohibited both by cost and the requirement for complex algorithms and data analysis pathways, it is predicted that this will become a standard approach for genetic diagnosis in coming years.
Aneuploidy	YES	YES	YES	YES
Balanced translocation	YES	YES	NO	YES
Unbalanced translocation	YES	YES	YES	YES
Polyploidy	YES	YES	YES	YES
Mosaicism	YES	YES	YES	YES
UPD	No	No	YES	YES
Copy Neutral LOH	No	No	YES	YES
Gene Fusions	YES	No	YES	YES

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