Chromosomal Aberration Test: CAT

E-contents
prepared by

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Introduction

- The study of DNA damage at the chromosome level is an essential part of genetic toxicology.

- Different types of chemicals and radiations have been reported to be responsible for production of various types of aberrations in the structure and or number of chromosomes.
- By studying the metaphase structure after exposing the organisms to any chemical or physical agent, the clastogenic properties can be detected.

Know these terms

- A clastogen is an agent that induces chromosomal aberrations.
- An aneugen (aneuploidogen) is an agent capable of inducing aneuploidy.
- Chromatid-type aberrations are aberrations that involve one sister chromatid of any one chromosome or more chromosomes.
- Chromosome-type aberrations involve the same locus both sister chromatids on one or multiple chromosomes. (Albertini et al., 2000).

Types of Chromosomal Aberrations

- 1. Structural chromosomal aberrations -result from:
- (A) direct DNA breakage; (B) replication on a damaged DNA template; (C) inhibition of DNA synthesis and other mechanisms (e.g., topoisomerase II inhibitors) (Albertini, 2000).
- Very few agents (e.g., ionising radiation, bleomycin) induce direct DNA breakage.
- These agents induce, at the time of exposure, chromosome-type chromosomal aberrations (involving both chromatids of a chromosome) in cells in the G_0/G_1 phase of the cell cycle and chromatid-type (involving only one chromatid of a chromosome) chromosomal aberrations in cells in S/G_2 phase.
- Operationally, these agents are classified as S-phase-independent clastogens.

- 2. Numerical chromosomal aberrations (i.e., aneuploidy, polyploidy) refer to changes in chromosome number that occur due to abnormal cell division (results from damage in mitotic spindle and associated elements or damage to chromosomal sub-structures, alteration in cellular physiology, and mechanical disruption).

- Structural and numerical chromosomal aberrations are most commonly scored in proliferating cells arrested at metaphase using a tubulin polymerisation inhibitor (e.g., Colcemid, colchicine).

Principle of the assay.....

- The study of chromosomes involves arresting chromosomes at metaphase stage, hypotonic treatment, fixation, dropping the cells onto microscope slides for chromosome spreading, staining, microscopy and measurements/analysis.

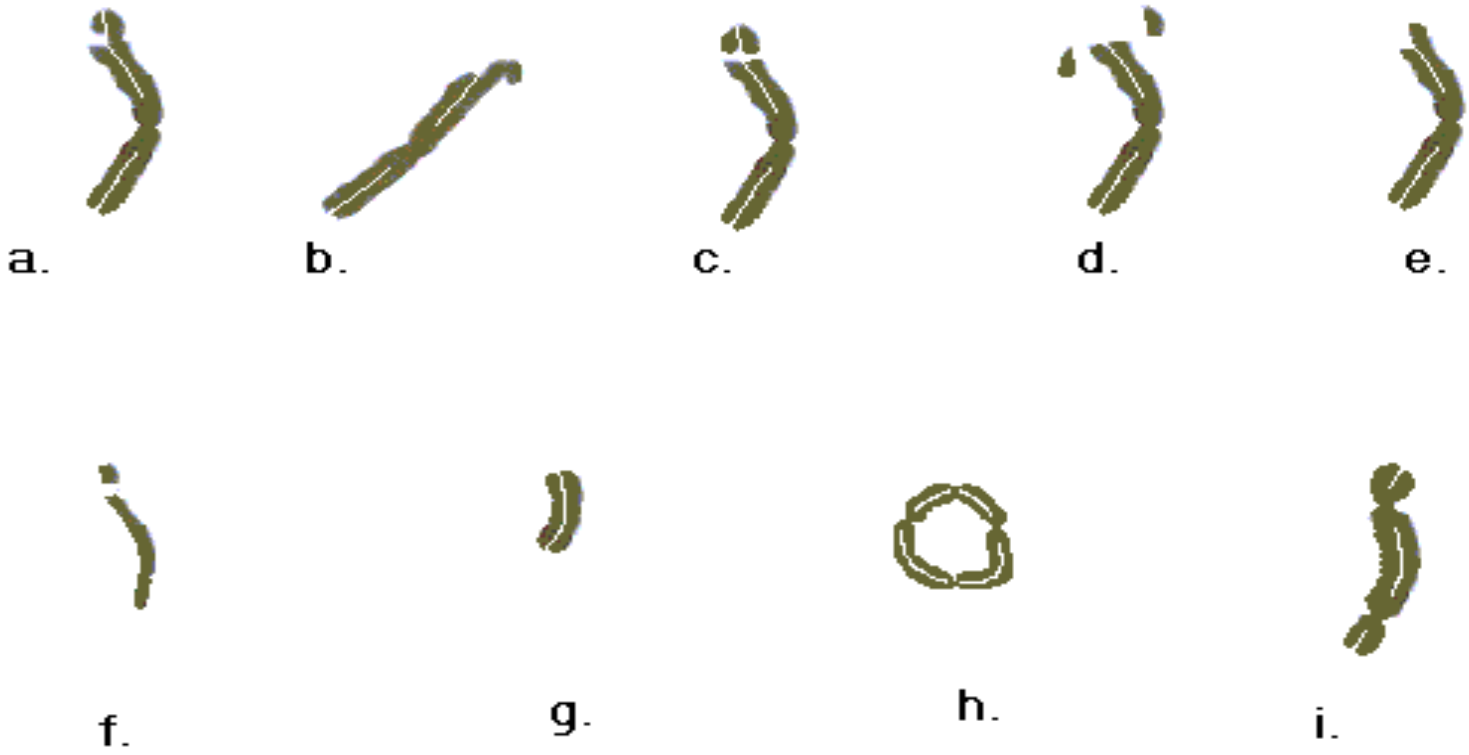
- The mitotic chromosomes from fish can be studied from the rapidly dividing cells. The chromosomes can be prepared directly from the dividing tissue (*in vivo*) as well as from the cultured cells (*in vitro*).
- The direct chromosome preparations are carried out from the cells with a large proportion of dividing stages such as cells of kidney and gills in fishes which can be arrested at metaphase stage by injecting colchicine, a spindle inhibitor.

- Colchicine is remarkable in the sense that though highly water soluble, it is very active at an extremely low concentration.

Common types of chromosomal Aberrations: (NBFGR, TOGAIF,2005).

Term	Definition
• **a.** Chromatid gap	An achromatic region in one chromatid, the size of which is equal to or smaller than the width of the chromatid
• **b.** Chromatid break	An achromatic region in one chromatid larger than the width of the chromatid; it may be either aligned or unaligned
• **c.** Chromosome gap	Same as (**a**), only in both chromatids
• **d.** Chromosome break	Same as (**b**), only in both chromatids
• **e.** Chromatid deletion	Deleted material at the end of one chromatid
• **f.** Fragment	A single chromatid without an evident centromere
• **g.** Acentric fragments	Two aligned (parallel) chromatids without an evident centromere

Common type of chromosomal aberrations….



a. Chromatid gap; **b.** Chromatid break; **c.** Chromosomal gap; **d.** Chromosomal break; **e**. Chromatid deletion; **f**. Fragment; **g**. Acentric fragment; **h**. Ring; **i**. Di-centric.**(NBFGR, TOGAIF,2005).**

Protocols for chromosome preparation

Requirements

Equipments

- Microscope with photographic attachment
- BOD incubator
- Water bath
- Electronic balance
- Hot-air oven
- Table top centrifuge
- pH meter
- Magnetic stirrer

Chemicals

- Colchicine (spindle inhibitor)
- Potassium chloride (hypotonic solution)
- Methanol
- Acetic acid
- Giemsa stain
- Glycerol
- Di-sodium hydrogen orthophosphate (Na_2HPO_4)
- Potassium di-hydrogen orthophosphate (KH_2PO_4)
- DPX mountant
- Xylene

Laboratory wares

- Sterile disposable syringes (1ml)
- Dissection box
- Petri plates
- Tissue grinder
- 15 ml graduated centrifuge tubes
- Test tube stand
- Pasture pipettes
- Microscopic slides (1mm thick)
- Slide stand
- Spirit lamp
- Coplin jar
- Filter paper
- Cover slip (No.1)
- Wash bottle

In vivo chromosome preparation from kidney / gill tissues:

- Collect healthy fish specimens (preferably weighing 20-100 gm.)

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- Inject 0.05% colchicine intramuscularly @ 1ml per 100 g of body weight

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- Keep fish specimen alive for 1- 2 hr. after the injection of colchicine

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- Anaesthetize fish specimen with ethylene glycol and dissect out the Kidney/gill tissues in a Petri dish and cut into small pieces.

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- Homogenize tissues in 6- 8 ml hypotonic solution (0.56% KCl) in glass tissue grinder to prepare cell suspension

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- Pour the cell suspension in 15 ml centrifuge tube and incubate it for 20-25 minutes at room temp for swelling.

In vivo chromosome preparation from kidney / gill tissues: .....

- Stop the hypotonic action by adding 1.0 ml freshly prepared chilled Carnoy's fixative (Methanol: Acetic acid 3:1 ratio) mix it gently with pasture pipette

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- Centrifuge cell suspension at 1200-1500 rpm for 10 min. at room temperature to get cell pellet at the bottom

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- Remove supernatant with a pipette and slowly overlay 6-8 ml freshly prepared chilled fixative. Keep the tubes in refrigerator for half an hour for thorough fixation

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- Mix the contents and centrifuge cell suspension at 1200-1500 rpm for 10 min. at room temperature.

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- Remove the supernatant without disturbing cell pellet at the bottom and add fresh fixative.

In vivo chromosome preparation from kidney / gill tissues: .....

- Repeat steps 13 -14 three times till clear transparent cell suspension is obtained

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- Take small quantity of cell suspension in pasture pipette and drop it onto grease free, pre-cleaned glass slide from a height of 1-1.5 feet

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- Allow the slide to air / flame dry.

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- Keep the slide for ageing for 1-3 days in dust free place

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- Stain it with 4-5% Giemsa in phosphate buffer (pH 6.8) for 15-20 minutes

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- Wash with DD water thoroughly.

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In vivo chromosome preparation from kidney / gill tissues: .....

- Air dry and store the slides in a slide box.

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- Observe metaphase spreads in bright field microscope to ascertain the quality of staining

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- Make the slides permanent by mounting in synthetic neutral mountant e.g. D.P.X.

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- Screen the slides for good spreads and take photographs of metaphase spreads under oil immersion objective (100X)

In vivo chromosome preparation from kidney / gill tissues: .....

- **For preparation of karyotype:** cut individual chromosomes from the photo prints

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- Group the chromosomes into four categories - metacentric (m);submetacentric (sm) c. subtelocentric (st) & telocentric (t)

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- Paste the chromosomes on ivory sheet in decreasing order of size within the group(centromeres of all chromosomes should be aligned pasted in each row)

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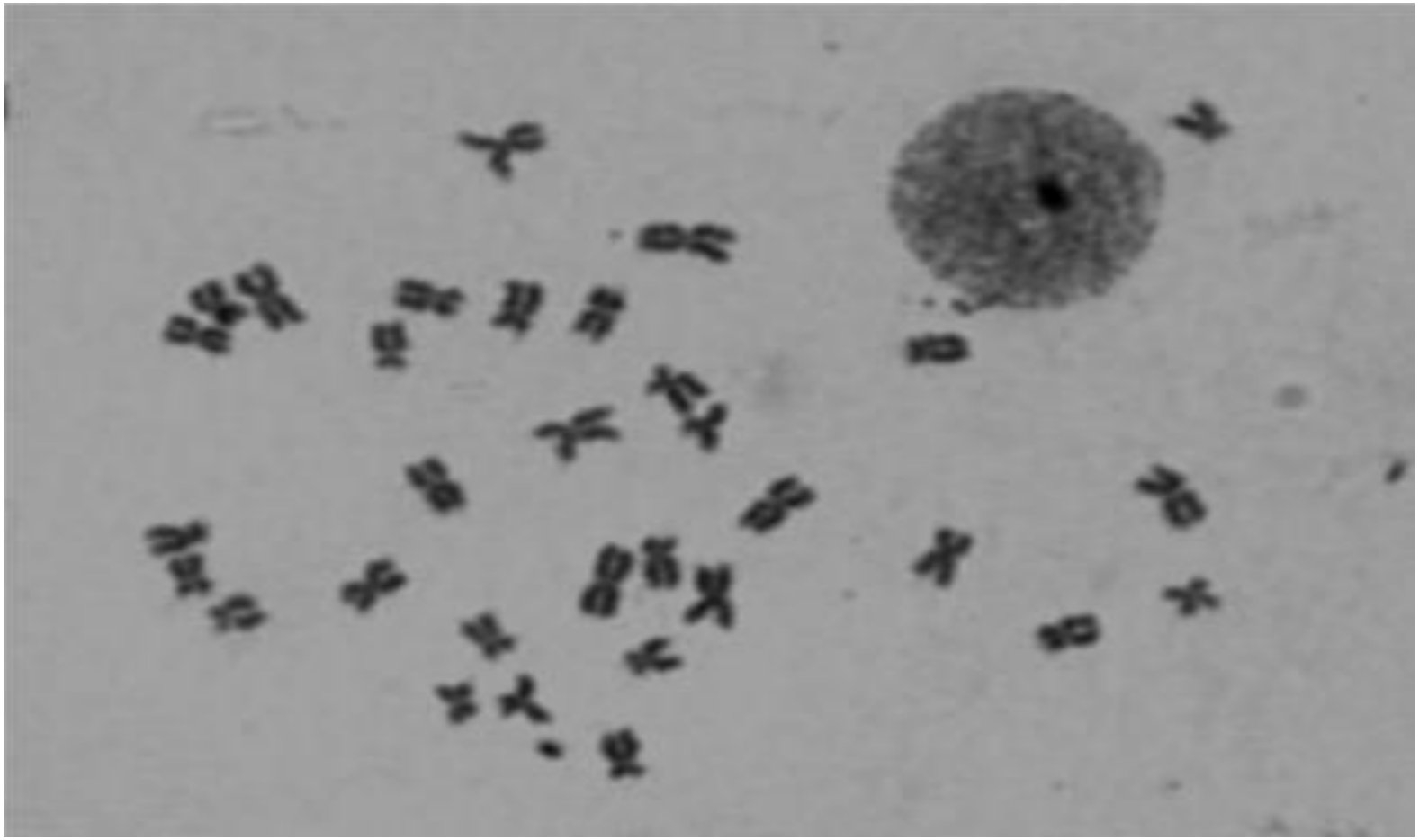
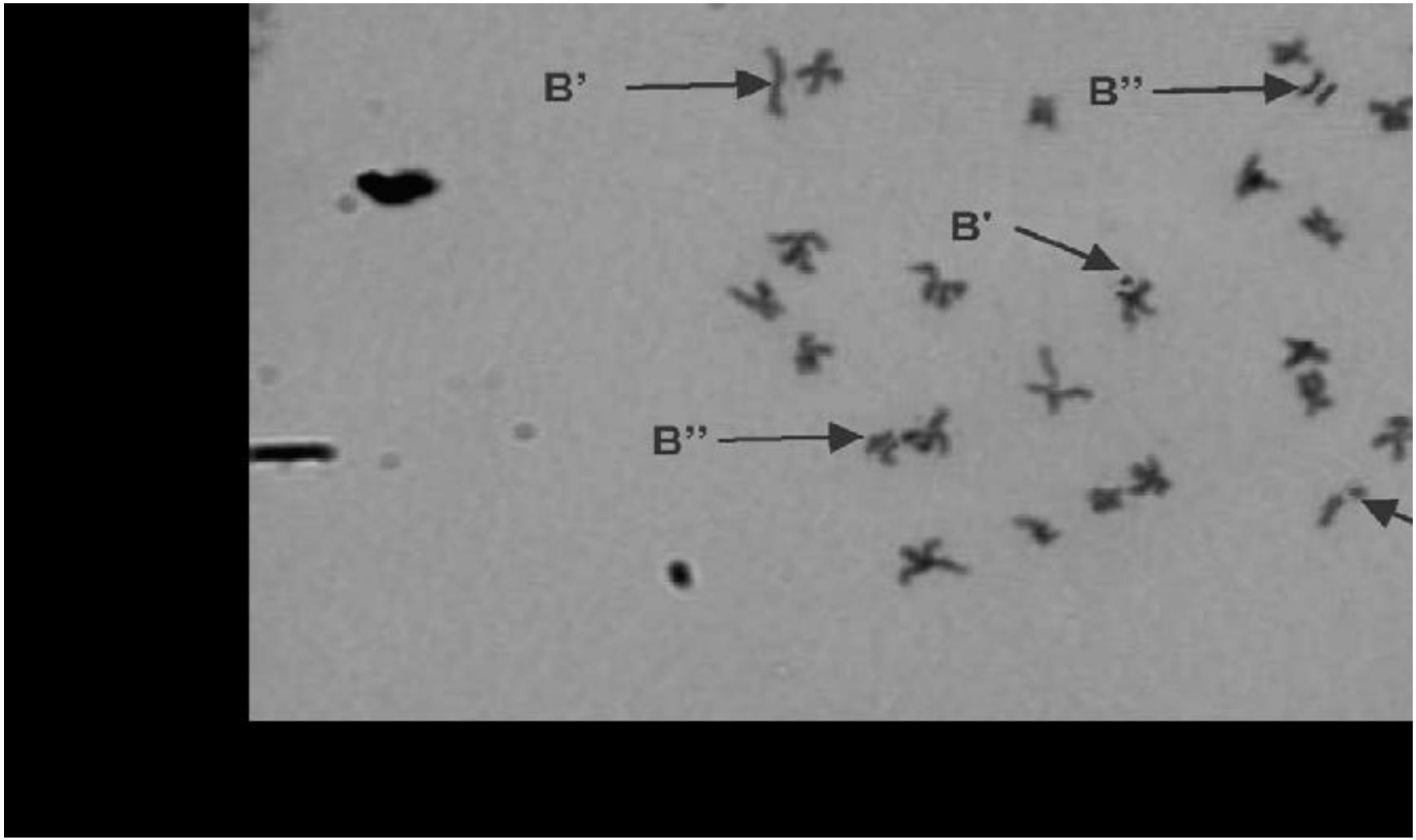
- Photograph the karyotpe, which can be used as base line data for detection of Chromosome aberrations.

- **Note**: Unused cell suspension can be stored for further use in refrigerator up to six months without marked deterioration in quality.

Normal Chromosome spread at Metaphase in *Channa punctatus,* Yadav & Trivedi, 2009.



Metaphase spread showing chromatid breaks(B’) and chromosome breaks
(B’’) induced by arsenic (72 h exposure), Yadav 7 Trivedi, 2009



Recent Research Publication based on CAT & MNT from Environmental Toxicology & Bioremediation Laboratory (ETBL)

Contents lists available at ScienceDirect

Mutation Research/Genetic Toxicology and Environmental Mutagenesis

journal homepage: www.elsevier.com/locate/gentox
Community address: www.elsevier.com/locate/mutres

Chromosomal aberrations in a fish, *Channa punctata* after *in vivo* exposure to three heavy metals

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