Dicentric Chromosome Assay

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Abstract

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Materials

- Potassium Chloride by Contributed by users
  Acetic acid, glacial 537020 by Sigma Aldrich
  1L Giemsa Stain Stock Solution 786-1067 by G-Biosciences
- Methanol 141091.1214 by Contributed by users
  Colcemid 10 µg/ml in PBS 47253.01 by Serva, Germany
  Phytohemagglutinin, M form (PHA-M) 10576015 by Gibco - Thermo Fischer
  RPMI 1640 (with L-glutamine and sodium bicarbonate) R8758 by Sigma Aldrich
  Fetal Bovine Serum (South America), Ultra low Endotoxin FB-1101/500 by Biosera

Protocol

Step 1.

Peripheral blood samples were collected into Li-heparinized tubes (Vacuette, Mundelein, IL, USA) and immediately after collection placed into fridge at temperature from +4°C to +8°C.

Step 2.

Samples were transported to the laboratory using by cooling bag at temperature from +4°C to + 8°C and they were processed for subsequent cytogenetic analysis within 2 h after collection.

Step 3.

Whole peripheral blood in amount 0.8 mL was applied into cultivation tube with 7.5 mL medium (RPMI 1640, 20% FBS, 2% PHA), gently mixed in hand and placed into incubator at 37°C for 48 h.

Step 4.

At 48th h 200 µL of Colcemid solution (stock concentration 10 µg/ml) was added to the culture, gently

mixed in hand and incubation continued for next 2 h (to 50th h).

Step 5.

At 50th h the cultures were harvested using by centrifugation (RCF 300 g, 3 min), treated with 10 mL of prewarmed (37°C) hypotonic KCl solution (0.55g KCl in 100mL distilled water, 10 min), fixed in first step by 10 mL of fixation solution consisting of 92 mL distilled water, 5 mL glacial acetic acid and 3 mL methanol, in second step by 10 mL of methanol and in third step by 10 mL methanol:glacial acetic acid (3:1). There was gently mixing using by pipette, centrifugation (RCF 300 g, 3 min) and removal of every solution above the pellet by pipette between every step. The fixation was performed in room temperature.

Step 6.

After fixation the cells were dropped onto chilled humid slides and left to dry overnight in room temperature.

Step 7.

Slides were stained with 5% Giemsa (5 min) next day. The slides were analyzed on microscope (Optika Microscopes, B-383PLi, Italy). In each blood sample, 100 of mitotic sets were evaluated at 100-fold original magnification and immersion oil. We determined number of dicentric chromosomes and structurally aberrant cells.