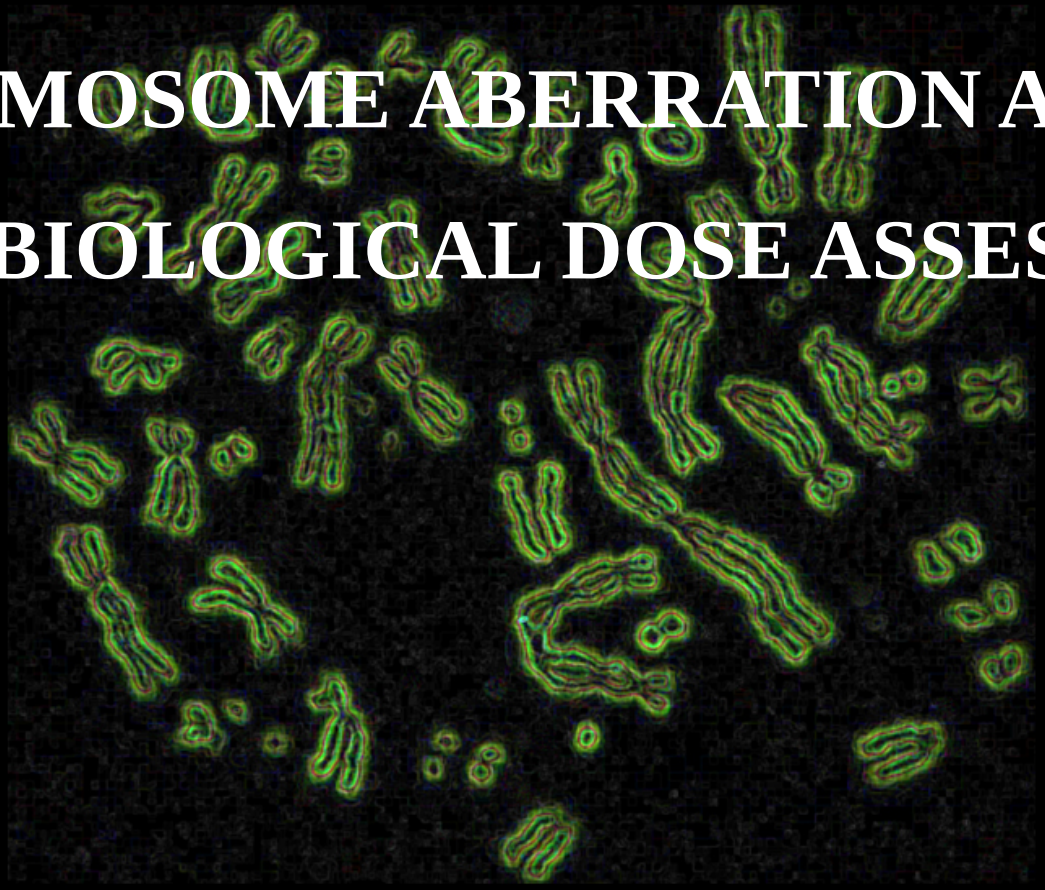
LABORATORY METHODS:
CHROMOSOME ABERRATION ANALYSIS FOR BIOLOGICAL DOSE ASSESSMENT



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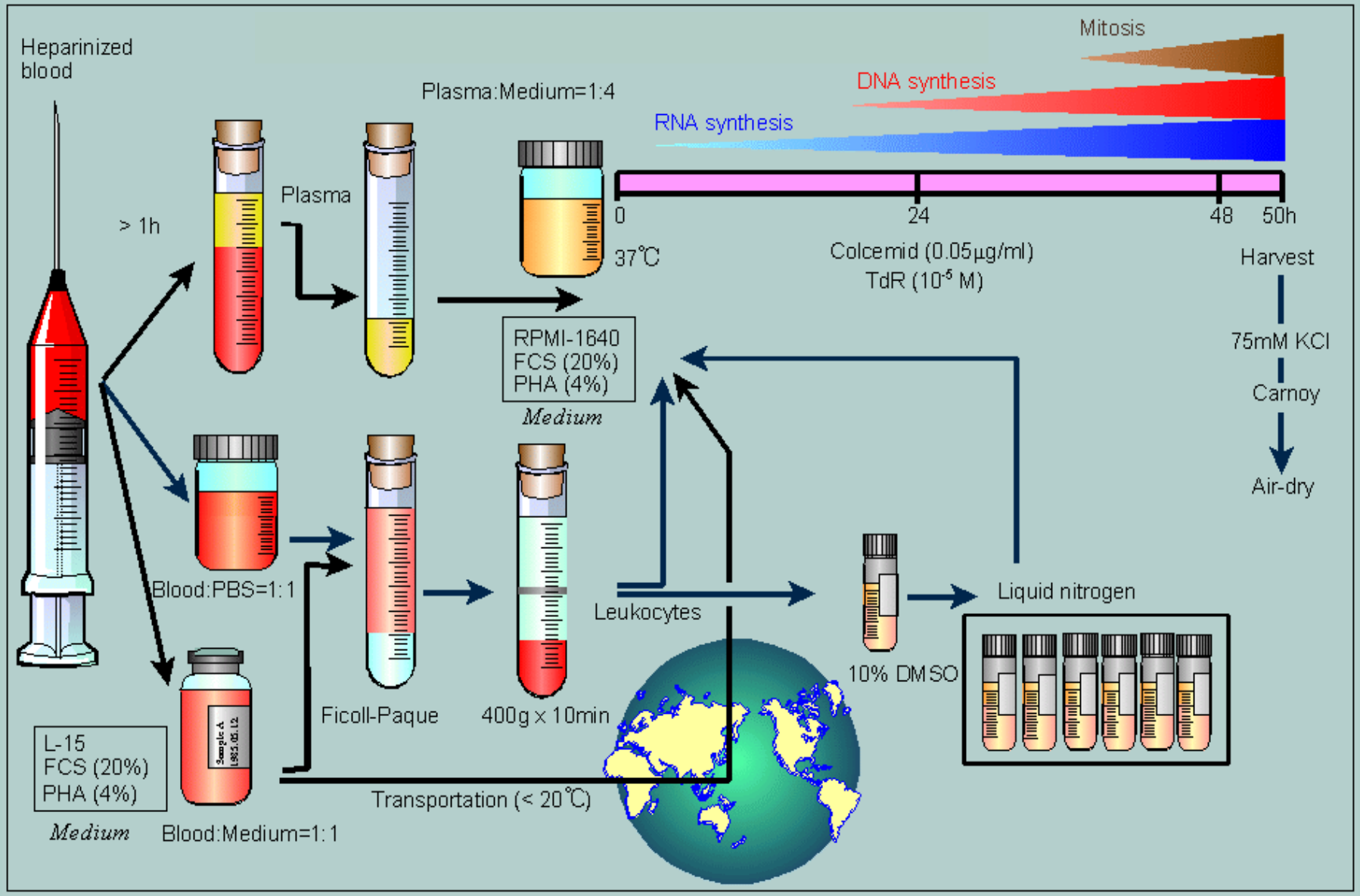
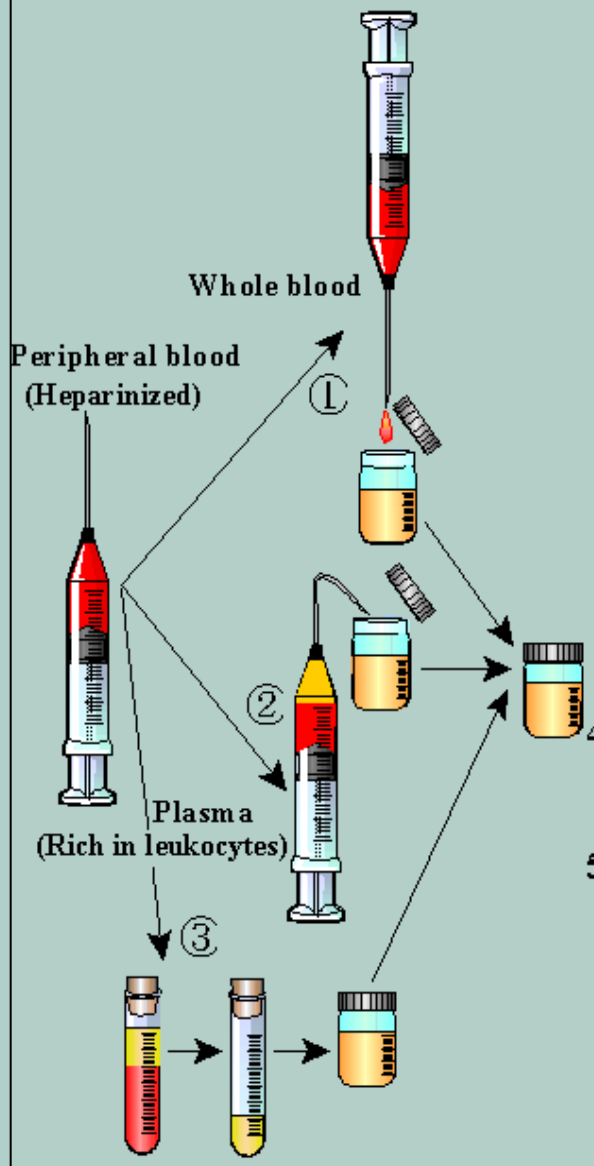
Chromosome Aberration Analysis in Peripheral Blood Lymphocytes

Outline



Blood Sampling and the Establishment of Culture



1. Blood sampling

Draw asceptically 3-5ml venous blood with a syringe containg 0.1ml heparin (1,000U/ml)

2. Culture medium (freshly prepared complete medium)

Medium RPMI-1640 (with L-glutamine and $NaHCO_3$)
Fetal bivine serum 20%
Phytohemagglutinin (PHA) 4% . . . . . *stimulation of T lymphocytes*

3. Culture procedures

Any methods of lymphocyte culture may be made, but either whole blod microculture (1) or cultures of leukocyte-rich plasma (2, 3) is recommended in the study of radiation-induced chromosome aberrations. In any case, the cultures shoud be setup shortly after exposure or irradiation. This is particularly important in the irradiation with high LET radiations, where the radiation dose imparted to the cells is not homogeneous and hence more heavily irradiated cells are selectively lost by interphase death.

4. Whole blood microculture

Blood 0.5ml in 4.5ml complete medium.

5. Plasma leukocyte culture

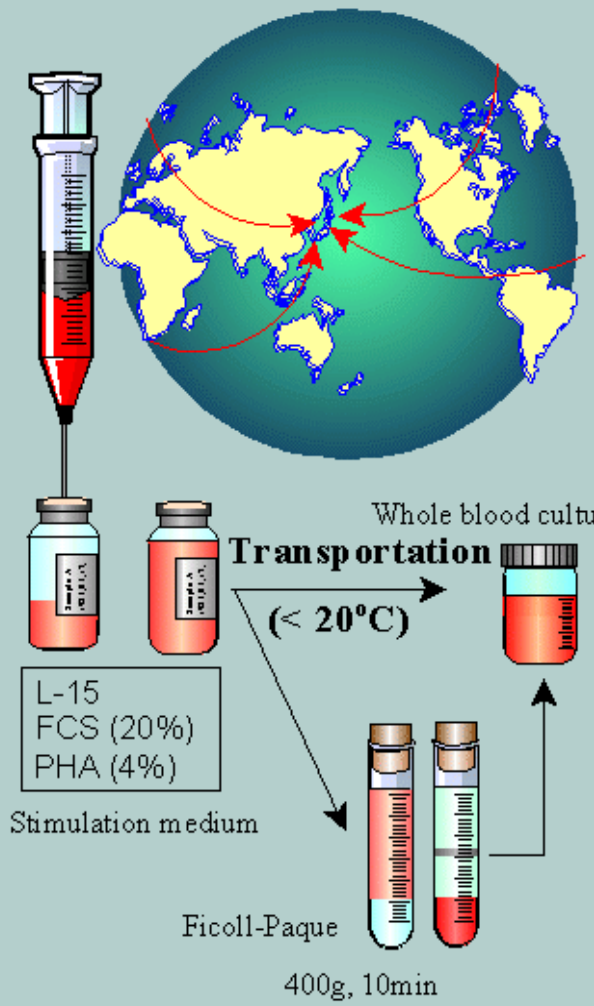
Plasma 1ml in 4ml complete medium.
(Incusion of a s*mall amount of red blood cells may enhance cell growth*)

6. Incubation of culture

Any types of culture vessels may be used, 20ml vials, 5cm plastic dishes or 50ml plastic bottles. The cultures should be incubated at 37 °C, either under the airation with 5% CO_2 and 95% air or under sealing with cap tight.

Transportation of the Remote Samples

"Cold" or "Chilled" Stimulation Method



The establishment of lymphocyte cultures may not neccesarily be possible at the site, and sometimes it takes days or weeks to carry to or send tothe laboratory for chromosome aberration analysis. The blood samples may be kept for a few days in the ice cold but only for a limited use, where the frequency of mitoses is severely reduced.

This problem is solved by "**cold stimulation**" at the site (Sasaki, IAEA Research Co-ordination Meeting, Budapest, Hungary, 1998). The method includes a mixture of blood sample with PHA-containing medium and keep, mail or transport to the laboratory. With this technique, the lymphocytes can be kept viable for more than 10 days without significant reduction of mitotic activity.

1. Prepare a sampling vial containing stimulation medium (see below).
   - 5ml **Leibovitz's L-15** medium (Gibco, BRL)
   - Fetal bovine serum 20%
   - PHA 4%
   - Penicillin 200U/ml

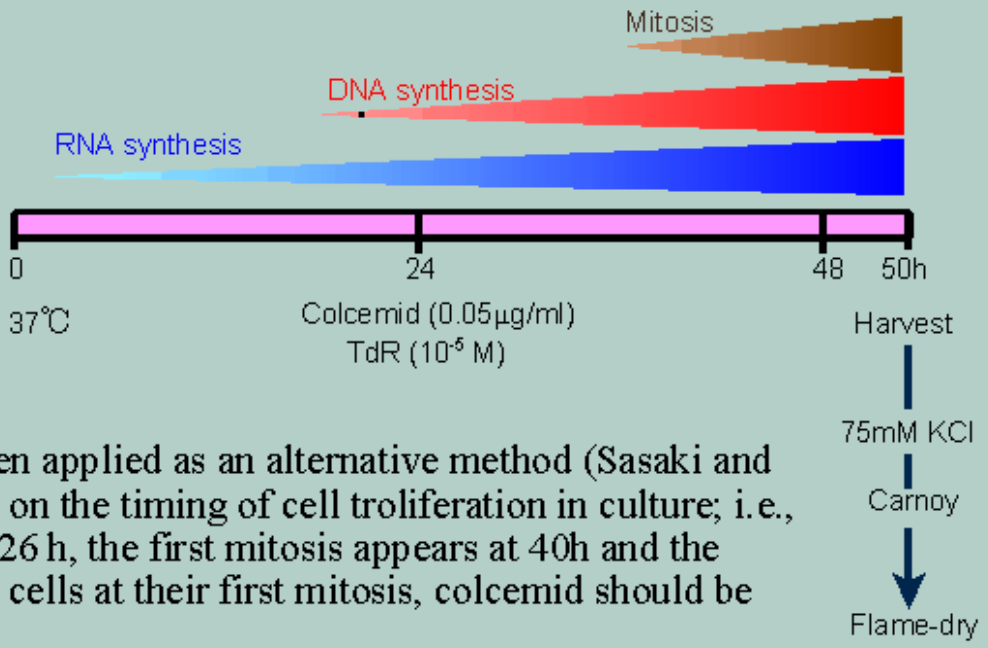
Leibovitz's L-15 medium is essential. (Amino acid-buffered medium)

2. Mix 3-5ml blood with the stimulation medium.
3. Keep at a temperature **below 20°C** (do not freeze) until culture setup.
4. Establishment of culture
   - (1) Whole blood microculture: 1ml cold blood mix and 4ml medium
   - (2) Culture of isolated lymphocytes

Slowly overlay cold mix on 3ml Ficoll-Paque (pharmacia Biotech) and centrifuge at 400g for 10min.
Take lymphocyte layer pasteur pipette, wash with PBS (Ca-, Mg -free) and culture in complete culture medium.

Mitotic Progression of T-lymphocytes and Culture Time

Chromosome aberrations should be studied in the first mitosis since the initiation of culture because unstable aberrations are lost when the cells attempt at mitosis. The exclusion of the second or more advanced mitoses can be made by labeling chromosomal DNA with BrdU. However, BrdU-mediated differential staining is tedious and often lose the sharpness of chromosome morphology.



Long-term colcemid treatment

The long-term mitotic arrest by colcemid has been applied as an alternative method (Sasaki and Norman, Nature 210:913-914, 1966). This is based on the timing of cell troliferation in culture; i.e., DNA synthesis becomes tetectable in some cells at 26 h, the first mitosis appears at 40h and the second mitosis appears at 48h. In order to arrest all cells at their first mitosis, colcemid should be added at 40h or earlier.

Procedure

At 24 h of culture,

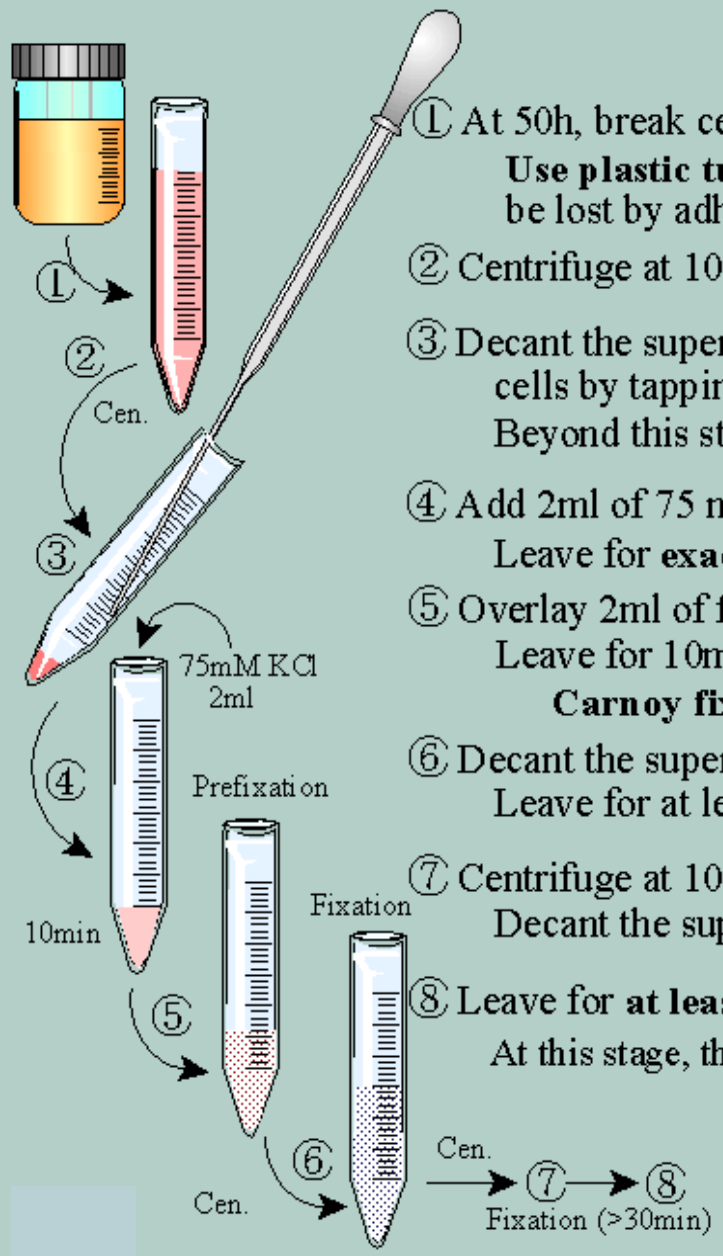
Replace 1ml culture medium with freshly prepared complete culture medium (prewormed at 37°C)
Add **thymidine** (prepared in Hanks solution) at a final concentration of 10^{-5} M.
Add **colcemid** at a final concentration of 0.05 μg/ml

(*Addition of fresh medium and TdR enhance the mitotic activity*)

At 50 h of culture,

Harvest the cells

Cell Harvest and Fixation



① At 50h, break cell klumps by pipetting, and transfer the culture into a centrifuge tube.
Use plastic tube. Glass centrifuge tube is not suitable, in which the fixed cells will be lost by adharing to the glass walls.

② Centrifuge at 1000 rpm for 10 min.

③ Decant the supernatant leaving small amount of medium (about 0.1ml). Suspend the cells by tapping the bottom of tube and further completely by Pasteur pipette.
Beyond this step, **strong pipetting should not be made.**

④ Add 2ml of 75 mM KCl (4°C) and suspend the cells with gentle pipetting.
Leave for **exactly 10min**.

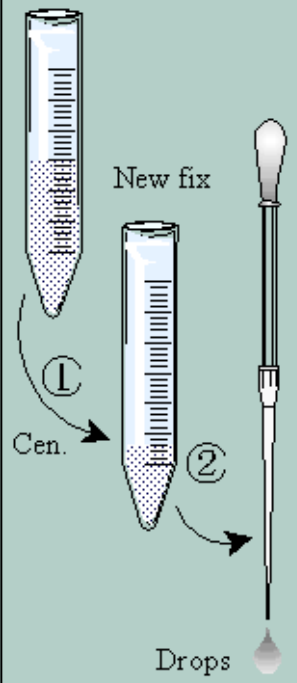
⑤ Overlay 2ml of freshly prepared Carnoy fixative, mix with gentle pipetting.
Leave for 10min and centrifuge at 1000rpm for 10min.
Carnoy fixative: 3:1 mixture of methanol (analytical grade) and acetic acid

⑥ Decant the supernatant, and resuspend the cells in 5ml fixative.
Leave for at least 10min.

⑦ Centrifuge at 1000rpm for 10min.
Decant the supernatant and resuspend the cells in 5ml fixative.

⑧ Leave for **at least 30min** before making chromosome preparations.
At this stage, the cells are ready for preparation or can be stored in refrigator for years.

Chromosome Preparation and Staining



① Centrifuge at 1000rpm for 10min.

② Decant the supernatant, and suspend the cells in freshly prepared Carnoy fixative.
The amount of the fixative is dependent on the amount of cells (usually 1-2ml).
(Adjust the cell density by checking the cell density on a pilot slide under phase-contrast microscope)
Use plastic pipette (e.g., autosampler tip AXL-1000C) to avoid cell adhesion.

③ Preparation of microscopic slide
Wash the microscope slides in warm detargent (e.g., 7X), wash in running tap water, rinse in distilled water, and store in 50% ethanol in refrigator.

④ Take out the **chilled slide**, and drain the excess ethanol (do not dry or wipe out).

⑤ Drop the cell suspension onto the wet slide (3-4 drops).

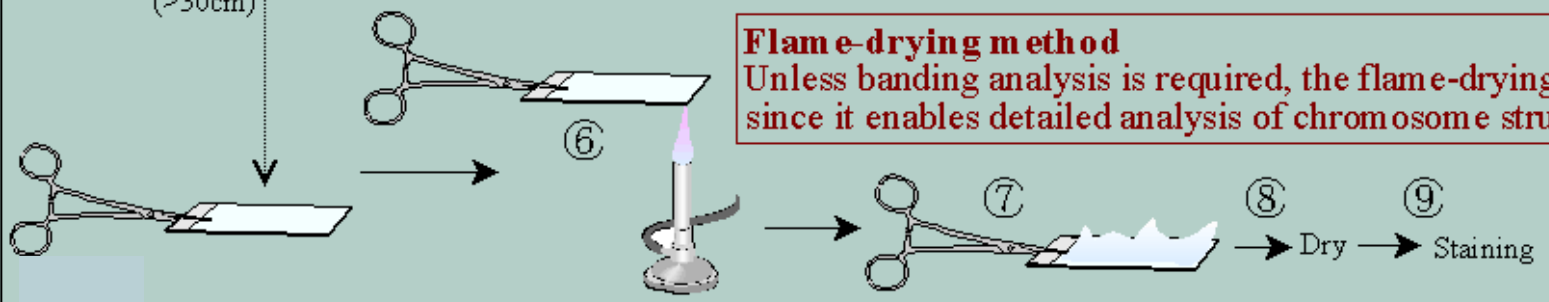
⑥ While holding the slide horizontally, ignite the slide by touching a flame (**only for ignition**).

⑦ Remove the slide as soon as ignited, and hold the slide **horizontally** until extinguised.

⑧ Drain the excess water, mark the slide, and **leave overnight**.

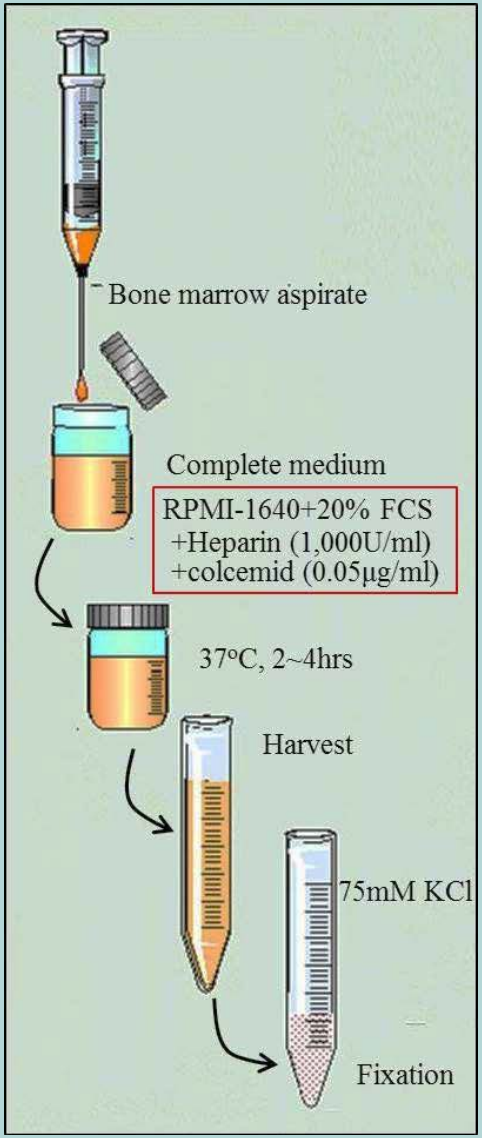
⑨ Next day, stain the slides in 3% Giemsa solution (**pH 6.4**) for 20min.

⑩ After complete drying, mount with cover-slips (No. 1).
(Put a mounting medium (e.g., Eukitt), cover-slips, then leave on hotplate (prewarmed at 50°C)



Flame-drying method
Unless banding analysis is required, the flame-drying is recommended since it enables detailed analysis of chromosome structural alterations.

Chromosome Preparation for Bone Marrow Cells and Leukemia Cells in Peripheral Blood



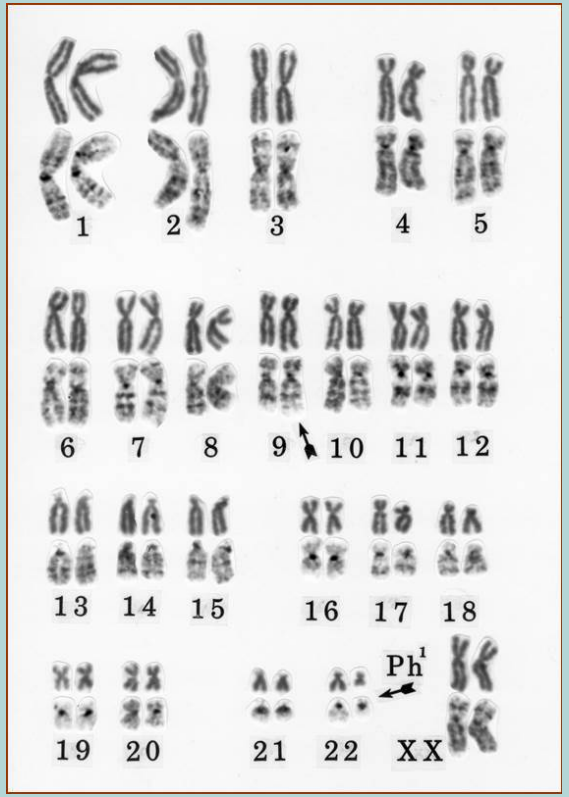
Usually, bone marrow cells and leukemia cells in circulating blood are progressing mitosis. Chromosome preparations may be made directly with these mitotic cells.

Procedure

(1) Bone marrow samples: Mix about 0.5ml BM aspirate with 5ml complete medium, and incubate 2~4 hrs at 37°C. Hypotonic treatment, fixation, and slide preparation my depend on chromosome analysis method concerened.

(2) Usually leukemic cells are dividing in the circulating blood. Mix the cells in leukocyte-rich buffy layer with complete medium, and proceed similarly to the method for BM cells.

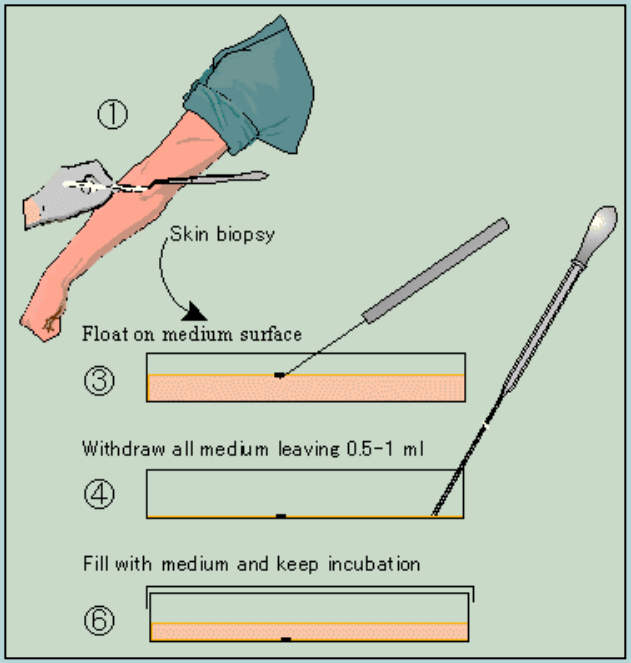
** The cells in mitosis are scarce due to time lapse after sampling, the cells can be cultured in culture medium (without colcemid) and harvested as is the case for culture cells.*

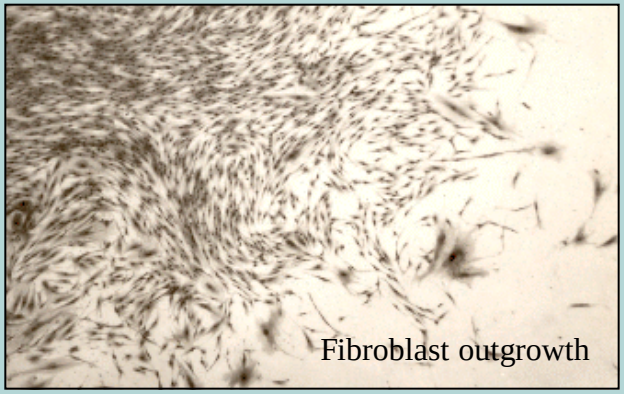


CML cells in bone marrow: Normal Giemsa staining (upper) and G-banding (lower)

Cell Culture from Tiny Skin Sample

Procedure




Fibroblast outgrowth

Minute skin sampling and cell culture. Sampling is painless and does not require anesthesia.

(1) Disinfect all apparatus including skin of concern with alcohol. Pick up the skin with sharp hooked forceps, and cut 1-2mm part beneath the forceps with surgical knife or razer blade. *(Dissection does not result in bleeding)*.

(2) Sample (1~2mm) should be put into culture medium, or placed on gauze wet with physiological saline. *(Prevent drying)*

(3) Prepare 5cm Petri dish with 5ml culture medium.

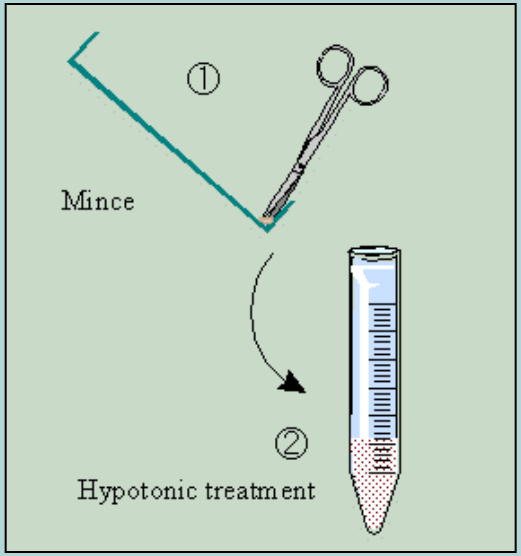
(4) Pick up the skin fragment on a tip of a fine needle. And, thus float the skin fragment on the surface of culture medium with the skin surface side up. If not successful, try again until floating with the skin surface side up and dermis side down.

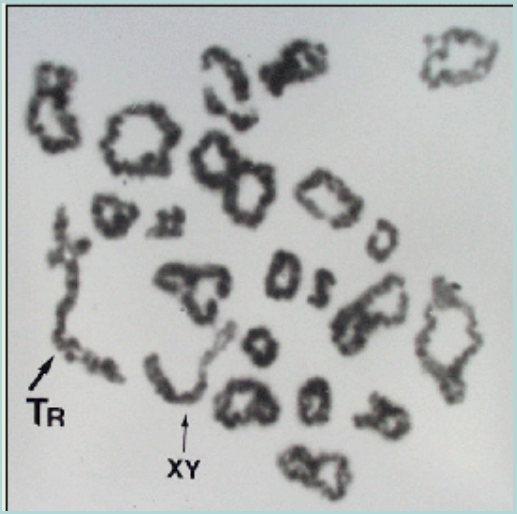
(5) Withdraw culture medium leaving approximately 1ml, which is small enough not to allow moving but provides thin layer to provide nutrient to the dermis side. (Leave in CO_2 incubator).

(6) After 2-3 day incubation, keratinocytes will first migrate out. At this stage, the culture dishes can be fed with fresh medium.

(7) Approximate 1 w after, outgrowth of fibroblasts is seen. The culture is then available for chromosome analysis or or further propagation by cell transfer.

Chromosome Preparation for Meiotic Cells (Testis)





Human male meiotic metaphase I

Testis samples are usually very small (needle biopsy). An, moreover, the cells are easily lost during preparation due to their sticky nature to the apparatuses. To avoid the cell loss, handling apparatuses should be coatd with serum before handling (Petri dishes, scissors, pipette, centrifuge tubes.

Procedure

(1) Mince the biopsy sample (usually 1-2mm) with fine-point scissors together with serum.

** In the case of mouse testis sample, the cells may be released by needles under dissection microscope. But, it can not be done for human testis samples.*

(2) Add 1~2ml 75mM KCl, and gently pipette in a centrifuge tube. (tube and pipette should be coated with serum in advance).

(3) Leave for 20 mins at room temperature.

(4) Overlay freshly prepared Carnoy fixative (3ml), gently mix, and leave for 10 min.

(5) Change the fixative by centrifugal sedimentation, and leave for at least 30 min before making slide preparation.

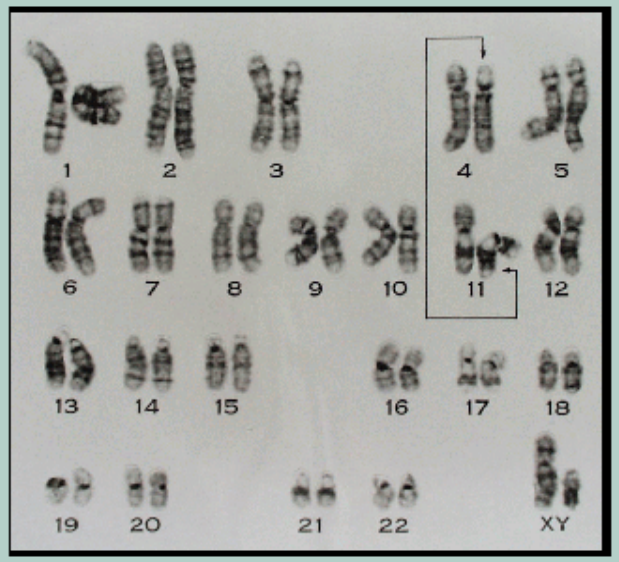
Technical Modifications for Aberration Analysis

(1) G-band Analysis

Since unstable-type aberrations (dicentrics, rings, fragments) are lost when the cells attempt at mitosis, the comventional dicentric analysis can not be applied to the dose assessment for the past and prolonged exposure. The alternative methods are the use of the frequencies of cells with only stable-type aberrations (translocations and/or inversions). Currently two methods are available for the identification of stable-type aberrations; one is Giemsa banding (**G-banding**) or quinacrine fluorescence banding (**Q-banding**), and another is fluorescent in situ hybridization (**FISH**) technology. The rationale of the use of stable-type aberrations is that translocations and inversions are stablly inherited by daughter cells at mitosis when they are not associated with unstable-type aberrations (Cs-cells) and hence reflect the dose and dose-accumulation irrespective of the elapsed time after exposure or duration of dose accumulation.

Procedures

For the G-band analysis, several modifications are needed in the chromosome preparation procedures. The cell culture method is essentially the same as that for dicentric analysis, but the following modifications are needed.



1) Colcemid treatment should be minimized, only for the last1 or 2 hours of the 24h or 50 hr of total incubation time.

2) Slow air-dy is essential, and frame dry should be avoided.

①Drop the fixed cell suspension onto chilled wet slide.

②Leave the slide in the moisture atmosphere (put the wet paper tawel on hotplate (45°C), then leave the slide on the warm paper towel until dry (high humidity).

③Leave the slides at room temperature for 1 or 2 days (aging).

3) Trypsin G-banding

④Immerge the slide in 0.25% trypsis solution for 10-12 sec.

⑤Rinse in Hanks solution with 5% calf serum.

⑥Rinse in Hanks solution.

⑦Rinse in phosphate buffer saline (pH 6.8).

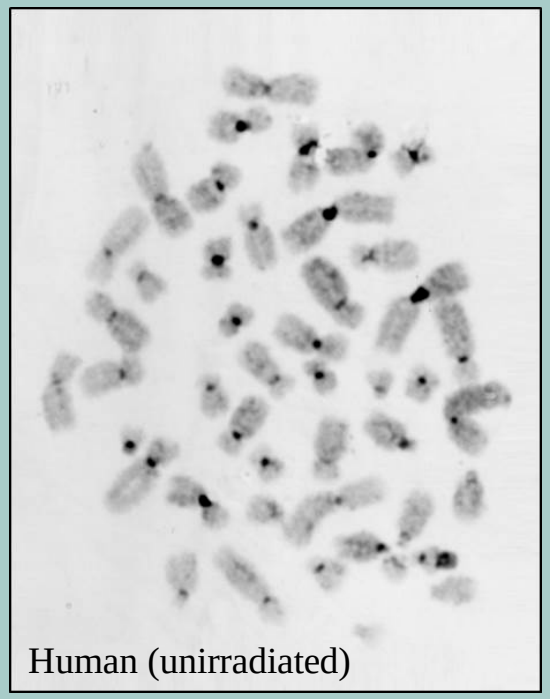
⑧Stain in 3% Geimsa solution (pH 6.8) for 30 min.

Technical Modifications for Aberration Analysis

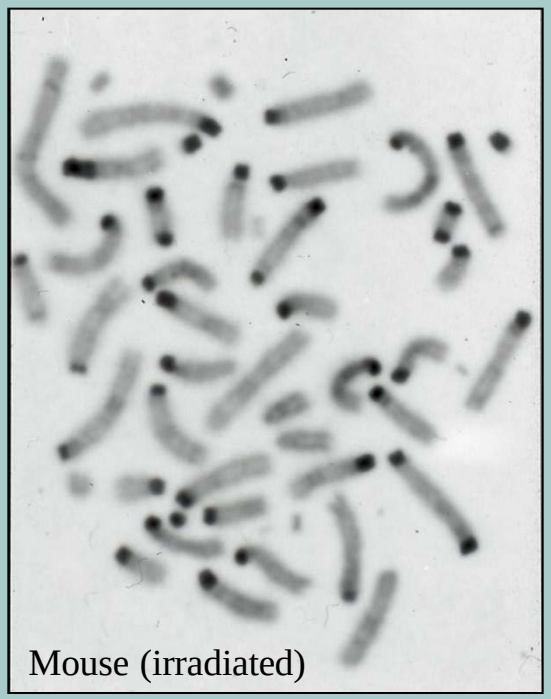
(2) C-band Analysis

C-banding is a simple Giemsa staining method for identification of pericentromeric heterochromatic regions of chromosomes. The method is not routinely used for the chromosome aberration analysis in human radiation cytogenetics. However, the method is highly efficient in identifying centromere position, namely identification of dicentric aberrations in some mammalian cells (*e.g.*, mouse cells). In human cells, the identification of the centromeric regions are currently possible by the use of FISH method applied to labelling with DNA or peptide probes specific to the centromeric regions.

Procedure



Human (unirradiated)



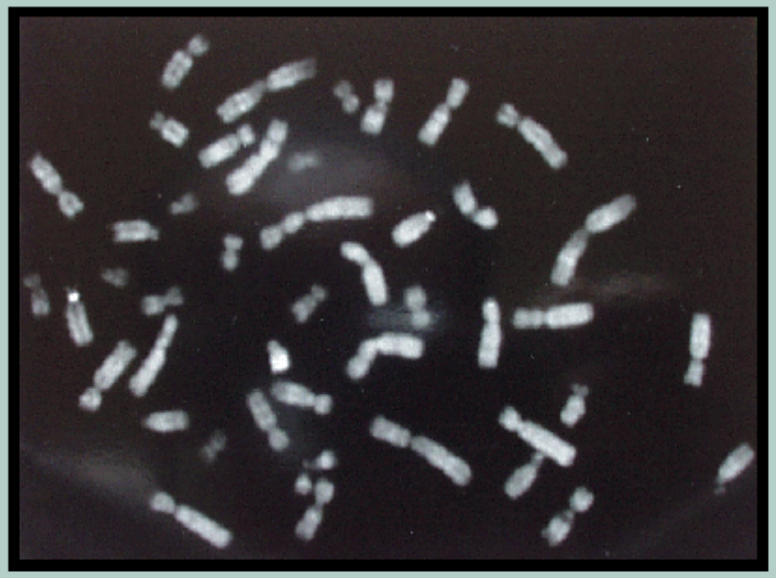
Mouse (irradiated)

C-banding

1. Use fresh slide
2. 1.0 N HCl 5 min
3. 0.2 N HCl Briefly immerse and air dry
4. 5% $Ba(OH)_2$ 6 min
5. 0.2 N HCl Immerse
6. 2 × SSC 60°C, 30 min
7. Rinse in phosphate buffer (pH 6.8)
8. Stain in Giemsa (6%, pH 6.8), 30 min

Technical Modifications for Aberration Analysis

(3) Q-band (R-band) Analysis



Quinacrine dihydrochloride is an acridine dye which binds to DNA either by intercalation or external ionic binding. The Q-babd patterns strongly resemble those of G-banding except for heterochromatic region of chromosomes 1, 9 and 16, and the acrocentric satellite regions. The polymorphic variation of Y chromosome is often seen with this staining.

Procedure

(1) Prepare Quinacrine dihydrochloride solution by dissolving 0.5 g of quinacrine in 100 ml distilled water. The solution should be stored in foil-covered container in the refrigator.

(2) Stain the slides (aged overnight) in quinacrine stain solution for 10 min.
(3) Rinse in tap water to remove excess stain.
(4) Place in McIlvaine buffer (pH 5.6) and mount with the same buffer using thin (No.1) coverslip.
(5) Remove the excess buffer by gently squeezing the slide between paper towels.
(6) Seal the coverslip with nail varnish.
(7) Scan the slide immediately under fluorescence miroscope with appropriate filter combination.

McIlvaine's buffer (pH 5.6)

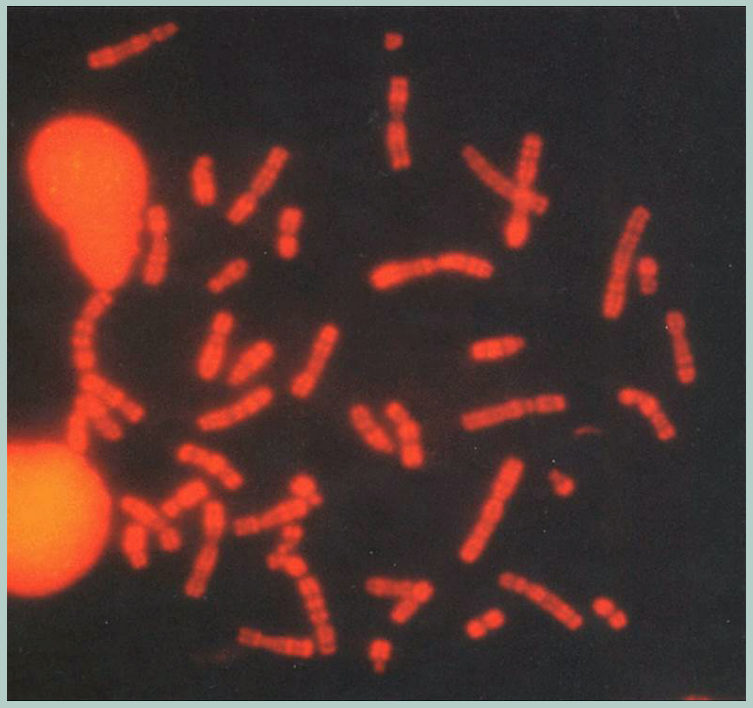
Solution A: 0.1 M anhydrous citric acid (19.2 g/l)
Solution B: 0.4 M anhydrous sodium phospate dibasic (Na_2HPO_4)(56.8 g/l)
Mix 92 ml of solution A and 50 ml of solution B.

Technical Modifications for Aberration Analysis

(4) Analysis of Replication Pattern

In the G-banding, G-positive (dark) regions are gene poor regions and replicate in the later stages of S-phase. Conversely, G-negative (light) regions are gene rich and replicate in the earlier stages of S-phase. When BrdU is incorporated into DNA only in the late stages of S-phase, the chromosomes are differentially marked (stained) after appropriate treatment. The banging pattern is called replication banding, but is reverse of the G-band. The followings are culture procedures using peripheral blood lymphocytes.

Procedure



(1) Establish PB lymphocyte culture to be harvested at 72nd hrs.

(2) Add thymidine (TdR, 300μg/ml) at 24hs before harvest.

(3) At -8h, wash the cells with TdR-free medium, and continue culture in medium with bromodeoxyuridine (BrdU, 25μg/ml).

(4) Add colcemid (0.05μg/ml) 2h before harvest.

(5) Harvest (0h) and make chromsome preparation by air-drying method.

(6) Expose the preparations to black light and treat with 2x SSC at 62^{o}C.

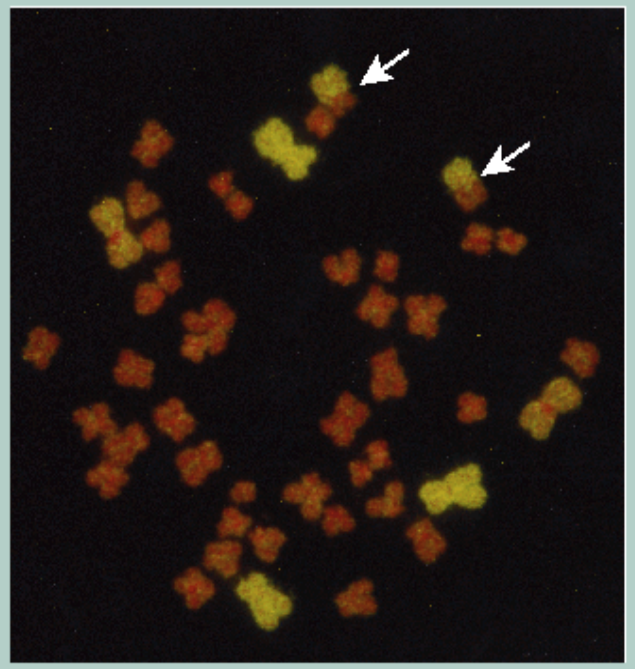
(7) Stain either with Giemsa, acridine orange, or propidiumiodide.

If the TdR and BrdU treatments are reverted, inverse images may be obtained.

Technical Modifications for Aberration Analysis

(5) Chromosome Painting (FISH) Analysis

Fluorescence in situ hybridization (FISH) is the method to visualize specific chromosomes. Chromosomal DNA is hybridized with fluorescent dye-conjugated chromosome-specific DNA probes. In conditions where the dicentric chromosomes are rarely ocurring, FISH method is a powerful method for identification of translocations, and hence often used for retrospective dosimetry, where the sampling is made long time after exposure.



Conversion from the observed translocation frequency (F_P) to the genome-equivalent frequency (F_G): $F_G=F_P/2.05p(1-p)$, where p is relative amount of the painted chromosomes.

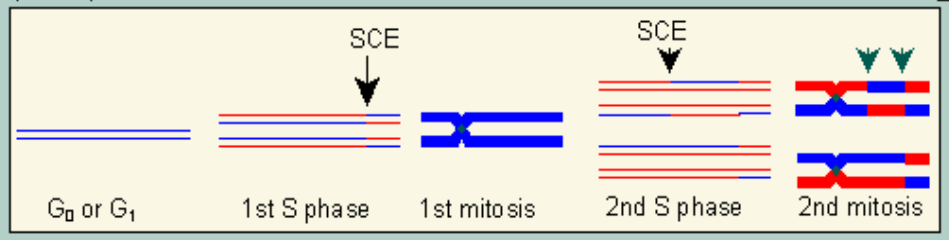
① Prepare slide with one drop of chromosome suspension and air dry. The mark the position on the other side of slide by diamond mark pen.
② Prepare whole chromosome painting probe (Chrom. 1, 2, 4) (A kit contains the hybridization buffer)
③ Mix each 1μl of probes 1, 2 and 4 with 7μl hybridization buffer.
④ High speed centrifuge for 1-3 sec. Incubate for 5 min at 73°C.
⑤ Centrifuge for 1-3 sec. Leave at 45°C .
⑥ Aging the slide in 2X SSC/0.1% NP-40 at 37°C for 30 min.
⑦ Dehydrate in 70%, 85%, 100% ethanol for 2 min each, then air dry.
⑧ Denaturation in 70% folmamide/2X SSC at 75°C for 5 min.
⑨ Dehydrate in 70%, 85%, 100% ethanol for 2 min each at 4°C, air dry.
⑩ Place the probe mix on marked area, put 20mm x 20mm coverslip.
⑪ Seal the edge of the coveslip with nail paint.
⑫ Incubate the slide at 42°C in humidified box for overnight (5-16h).
⑬ Remove the coveslip, gently wash in 50% formamide/2X SSC (45°C) .
⑭ Three washes in 50% formamide/2X SSC (45°C) for 10 min each.
⑮ Wash in 2X SSC (45°C) (10 min), 2X SSC/0.1% NP-40 (5 min) (45°C).
⑯ Wash in 2X SSC (room temp) for 10 min.
⑰ Counter stain with 10 μl PI (1μg/ml DABCO) and place the coverslip. (DABCO: Dissolve 1.25g DABCO in 10ml PBS(-), then add 90ml glycerol. Adjust pH8.7-8.8)

Technical Modifications for Aberration Analysis

(6) Sister Chromatid Differentiation for SCE Analysis

Sister chromatid exchange (SCE) is a reciprocal exchange of chromatid with its sister, being formed when DNA damage is carried over to DNA replication. Although the SCE-inducing capacity varies among types of DNA damage and DNA repair capcity, the SCE is one of the cytological manifestation of DNA damage.

When the cells are grown for two replication cyles in the presence of 5-bromodeoxyuridine (BrdU), one of the chromatids contains DNA which is bifilarly substituted with BU and another contains DNA of unifilar substitution. These two types of chromatids are distinguished by differential staing methods, such as fluorescence plus Giemsa (FPG) method, hot alkaline Giemsa method, acridine orange staining method, etc.



FPG Method for Sister Chromatid Differentiation

1) Establish the whole blood culture in medium:
   RPMI-1640 with 20% fetal bovine serum
   Phytohemagglutinin (PHA-M, 4%)
   BrdU, 20 μM (5μM for cultures of isolated lymphocytes)
2) Incubate the cultures at 37°C for 50-72 hrs. (Do not add thymidine)
   (Add colcemid (0.05μg/ml) 2 hrs before harvest).
   (Handing of cultures should be carried out under the yellow flurescent light (FL40S.Y-F) or photographic safety light (e.g., Kodak Wratten Series 2. Incubate the cultures in the dark, e.g., by wrapping with aluminium foil).
3) Cell harvest and fixation is the same as described in Section 5.
4) Preparation of slides should be made by air-drying method.
5) Stain the fresh slides (aged for 1 hr to overnight) with 33258 Hoechst (1μg/ml in McIlvane buffer, pH 7.0) for 1 hr.
6) Rinse in DW, mount in 2× SSC with coverslip, and expose to fluorescence light (Toshiba FL20BL) at 2- 3cm distance.
7) Remove the coverslips and incubate in 2× SSC at 62oC for 1 hr.
8) Rinse in phosphate buffer (pH 6.8), and stain the slides in 3% Giemsa (pH 6.8) for 20 mins.

Number Cells to be Scored

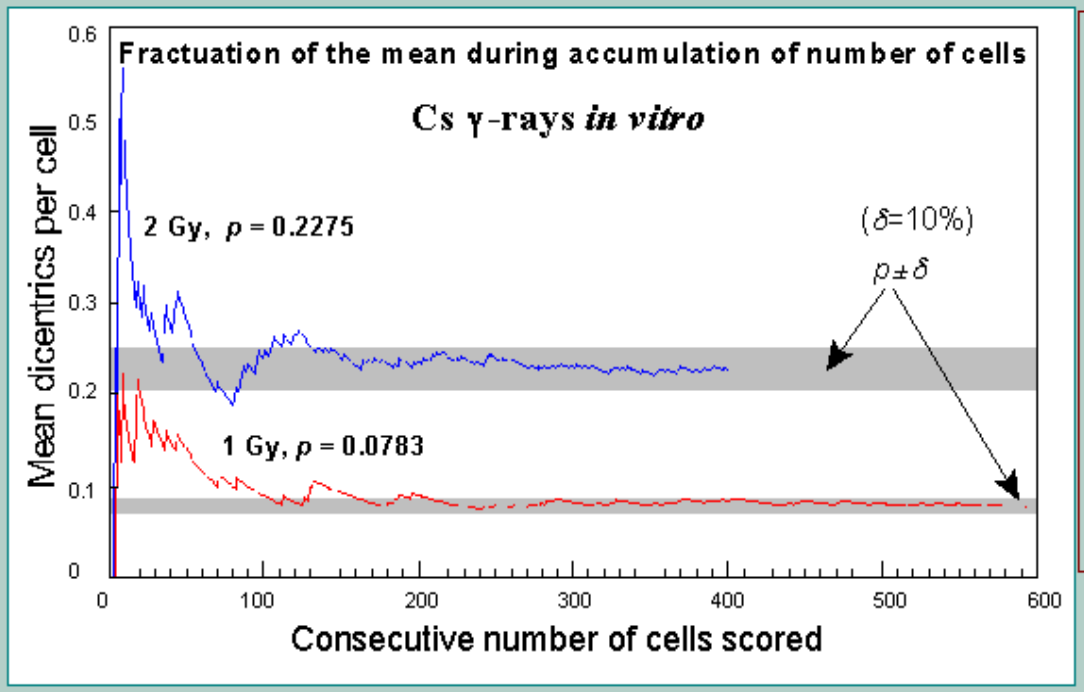
Number of cells to be scored is one of the important factors which determine the confidence level of the dose estimation. A rough estimate of the minimum sample size, N_0, may be made as follows.

Chromosome aberrations in the ith cell, X_i, is a stochastic valiable, and a chance to have r aberrations is expressed by a Poisson statistics in the cells population with the true mean, μ.

$p(r)=(e^{-\mu}\mu^r)/r!$, the variance is $V[X]=[\sum(x_i-\mu)^2]/(N-1)=\mu$ and the standard error is $\delta=(V[X]/N)^{1/2}$.

For the observed mean, p, to be in a range of $(\mu-\delta)\leq p\leq(\mu+\delta)$ at 95% confidence level, N_0 should satisfy $z_{0.05}(V[X]/N_0)^{1/2}\leqq\delta$ or $N_0\geqq[(z_{0.05})^2p]/\delta^2$, where $z_{0.05}=1.96$ for 95 % confidence level.

(*Note: For binomial distribution, use V[X]=p(1-p) for vriance. For* N_0 value only based on the error size, use $N_0\geq p/\delta^2$).



Examples:

When p=0.3 is expeced and the standard error is suppressed at 10% (i.e., $\delta=p\times0.1$) , N_0 should be larger than 1281 cells.

At p=0.3 and 20% standard error, $N_0\geq320$.

At p=0.3 and 30% standard error, $N_0\geq143$.

At p=0.1 and 10% standard error, $N_0\geq3842$.

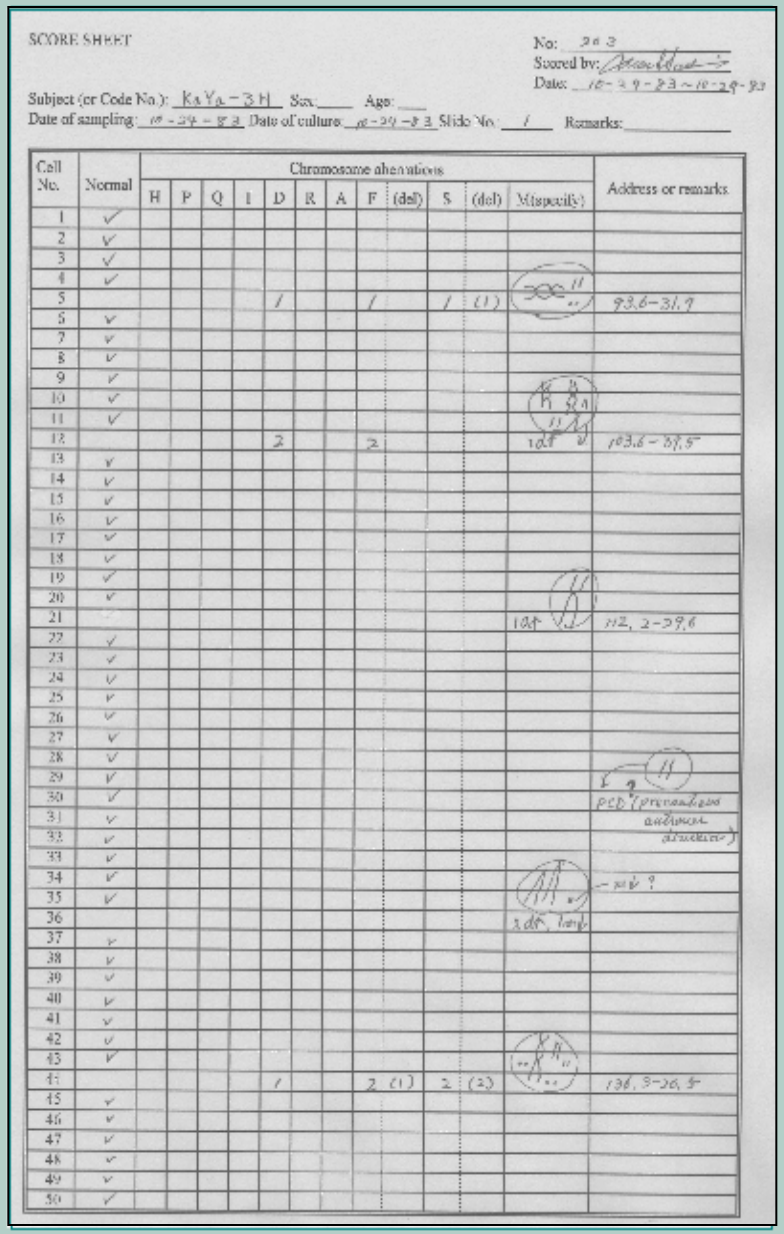
At p=0.1 and 20% standard error, $N_0\geq960$.

At p=0.1 and 30% standard error, $N_0\geq427$.

At p=0.1 and 50% standard error, $N_0\geq154$.

With this N_0, the mean may fall within this error range at 95% probability.

Scoring of Chromosome Aberration in Score Sheet



SCORE SHEET

No: 203
Scored by:
Date: 10-29-93~10-29-93

Subject (or Code No.): KaYa-3H Sex: Age:
Date of sampling: 10-24-93 Date of culture: 10-29-93 Slide No: 1 Remarks:

Cell No.	Normal	Chromosome aberrations											Address or remarks
		H	P	Q	I	D	R	A	F	(del)	S	(del)	M(specify)	
1	✓													
2	✓													
3	✓													
4	✓													
5						1			1		1	(1)		93.6-31.9
6	✓													
7	✓													
8	✓													
9	✓													
10	✓													
11	✓													
12						2			2					103.6-31.5
13	✓													
14	✓													
15	✓													
16	✓													
17	✓													
18	✓													
19	✓													
20	✓													
21														112, 2-29.6
22	✓													
23	✓													
24	✓													
25	✓													
26	✓													
27	✓													
28	✓													
29	✓													
30	✓													
31	✓													
32	✓													
33	✓													
34	✓													
35	✓													
36														
37	✓													
38	✓													
39	✓													
40	✓													
41	✓													
42	✓													
43	✓													
44						1			2	(1)	2	(2)		138.3-20.5
45	✓													
46	✓													
47	✓													
48	✓													
49	✓													
50	✓													

D: dicentric chromosome

Other polycentric chromosomes include hexacentric (H), pentacentric (P), tetracentric (Q) and tricentric (T) chromosomes. (Chromosome with ***n*** centromeres should be counted as ***n***-1 dicentric chromosomes)

R: centric ring

A: acentric ring

F: acentric fragment

F(del): acentric fragment not associated D or R

S: minute or dot

Acentric round chromosome with its chromatid diameter smaller than the width of chromatid.

S(del): minutes not associated with D or R

M: abnormal monocentric chromosome

According its apparent morphology, describe as

a ↑ : similar shape as group A but apparently larger.

b ↑ : similar shape as group B, but apparently larger.

d ↑ : similar shape as group D, but apparently larger.

f ↓ : similar shape as group F, but apparently smaller.

g ↓ : similar shape as group G, but apparently smaller.

m : small chromosome of unidentified morphology.

Scoring of Chromosome Aberration by Binary Gigits

Recording of the number and types of chromosome aberrations may facilitate the data processing and further statistical treatment. Such computer program is available in the Program <**Score**>. For each cell, the following data are recorded in a binary file.

R$: remarks

C: slide number

X, Y: location of the metaphase (automatic reading of the position of stage through a serial port, such as RS-232C, RS-422 or USB)(optional). In the program, manual input of X-Y location is adopted.

A$: chromosome aberrations in a single strings of 1 byte characters
(Either upper or lower cases can be used. But, they are automatically converted to upper cases for chromosome-type aberrations and lower cases for chromatid-type aberrations)
e.g., **A$="QDDDDRAFFFFFSSSSbbgi"**
(= 1 tetracentric, 4 dicentrics, 1 centric ring, 1 acentric ring, 5 acentric fragments, 4 minute dots, 2 chromatid breaks, 1 chromatid gap, and 1 iso-chromatid gap)
For "no aberration", just hit RETURN key, which is converted to A$="-" .
The data analysis can be made by character reading of a character string, A$.

The data processing includes,

in summary output,

(1) Total number of cells scored.
(2) number of normal-, Cu- and Cs-cells.
(3) number and frequenciy of each type aberration (with standard errors).
(4) number and frequency of dicentric equivalent (with standard errors).
(5) calculation of deletions, F(del) and S(del) for terminal and interstitial deletions, respectively.
(6) distribution of aberrations among cells (for dicentrics, dicentrics+rings, deletions, all chromosome-type aberrations, chromatid-type aberrations, and all chromosome- and chromatid- type aberrations).

in dump list,

All data are listed in a form of score sheet including the summary data.

International System for Human Cytogenetic Nomenclature

Historical perspectives

(*Discovery of normal human chromosomes, Tjio JH and Levan A: Hereditas, 42:1-16, 1956.)*

1. Denver Report: A proposed standard system of nomenclature of human mitotic chromosomes. Ann Hum Genet, 24:319-322. 1960.
2. London Conference: London Conference on the Normal Human Karyotype. Cytogenet Cell Genet, 2:264-268, 1963.
3. Chicago Conference: Standardization in Human Cytogenetics. Birth Defects Original Article Series II(2), National Foundation, New York, 1966.
4. Paris Conference (1971): Standardization in Human Cytogenetics. Cytogenet Cell Genet, 11:313-362, 1972.
   (G-, Q-, R- and C-banding)
5. Paris Conference (1971), Supplement (1975): Standardization in Human Cytogenetics. Cytogenet Cell Genet, 5:17-21, 1975.
   (Banding homology among human, chimpanzee, gorilla, and orangutan)
6. ISCN (1978): An International System or Human Cytogenetic Nomenclature (1978). Cytogenet Cell Genet, 21:309-404, 1978.
   (Nomenclature for acquired aberrations)
7. ISCN (1981): An International System or Human Cytogenetic Nomenclature. High-resolution banding (1981). (1978). Cytogenet Cell Genet, 31:1-23, 1981.
   (High resolution banding: 400-, 550- and 850-band level)
8. ISCN (1985): An International System or Human Cytogenetic Nomenclature (1985). Birth Defects Original Article Series XXI(1). National Foundation, New York, 1985.
   (Nomenclature for human meiotic chromosomes)
9. ISCN (1991): Guidelines for Cancer Cytogenetics. Supplement to An International System or Human Cytogenetic Nomenclature. F. Mitelman (ed). S. Karger, Basel, 1991.
   (Proposed nomenclature for chromosomes in cancer)
10. ISCN (1995): An International System or Human Cytogenetic Nomenclature. F. Mitelma (ed). S. Karger, Basel, 1995.
11. ISCN (2005): An International System or Human Cytogenetic Nomenclature (2005). LG Shaffer and N Tommerup (eds). Karger, Basel, 2005.

(……): Newly highlighted issues

END

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