**UW Medicine - Pathology**

400-02-01-09

Fanconi's Anemia Breakage Study

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| Adopted Date: 09/04/91  Review Date: 06/12/09  Revision Date: 01/28/2013 |

PURPOSE

This procedure uses Mitomycin C and Diepoxybutane (DEB) in culture to induce chromosome breakage. This test can differentiate between individuals with Fanconi's anemia (FA) and those with an idiopathic aplastic anemia; most of the FA patients show a significant increase in chromosome breakage over the control on the order of 10-fold.

***Note****:* This procedure is done on postnatal blood specimens only.

PROCEDURE

### Specimen Requirements

Freshly drawn peripheral blood in Sodium Heparin tube is required (not more than 24 hr old for best results) and should be drawn prior to patient exposure to radiation or chemotherapy (2-5ml minimum). A control blood is also needed (age matched if possible).

### Material and Equipment

See Peripheral Blood Cultures procedure, SOP number 400-02-01-03.

### Reagents and Solutions

* + - 1. Mitomycin C (Sigma, Cat. #M-0503).
      2. HCl (diluted to 50%).
      3. Diepoxybutane (DEB) (Sigma, Cat. #20,253-3).

### Procedure

***Note***: No mouth pipetting. Adhere to sterile techniques. Use tissue culture hood [Biosafety Hood (BioGard)]. Use gloves and lab coat.

Accession specimen and log into PB book. Set up cultures according to blood procedure and the table below. The control specimen is accessioned as a RE (research) case. Log into RE book. Use the following cultures, additives and conditions.

1. **Culture Initiation**

***Note****:* Cultures E, F, and G should be wrapped with aluminum foil to protect against light degradation of clastogens.

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| **Fanconi's Anemia Breakage Study** | |  | Add: |
| A—72 hr | 4.5 ml RPMI + 0.5 ml blood | 15 ml tube | Nothing |
| B—72 hr | 9 ml RPMI + 1 ml blood | T25 flask | 0.3 ml of 1% thymidine after 48 hr |
| E—72 hr | 4.5 ml RPMI + 0.5 ml blood | 15 ml tube (foil wrapped) | 25 l-0.1 g/ml DEB (after 24 hr) |
| F—96 hr (4 day) | 4.5 ml RPMI + 0.5 ml blood | 15 ml tube (foil wrapped) | 12.5 l-20 ng/ml mitomycin C (add during set-up) |
| G—96 hr (4 day) | 4.5 ml RPMI + 0.5 ml blood | 15 ml tube (foil wrapped) | 25 l-40 ng/ml mitomycin C (add during set-up) |
| **Control (same age and sex)** | |  |  |
| E—72 hr | 4.5 ml RPMI + 0.5 ml blood | 15 ml tube (foil wrapped) | 25 l-0.1g/ml DEB after 24 hr |
| G—96 hr (4 day) | 4.5 ml RPMI + 0.5 ml blood | 15 ml tube (foil wrapped) | 25 l-40 ng/ml mitomycin C (add during set-up) |

1. Mitomycin C is made according to the mitomycin C serial dilution chart (below).\*
2. The stock solution can be kept frozen and in the dark for six months. DEB is made according to DEB serial dilution chart (below).\* Pure DEB solution is good for one year when refrigerated.
3. Make dilution of MMC (working solution) fresh each time using the frozen stock solution.
4. ***Note*:** Mitomycin C is a mutagen ‑ use extreme caution! Inactivate spills, discard dilutions, plastic and glassware with 50% HCl.
5. **Mitomycin C Dilution Chart**

Dilution I 2 mg (in vial) + 5 ml dH2O (0 .4 mg/ml)

Dilution II 0.5 ml Dilution I + 2 ml PBS (80 µg/ml)

Dilution III 0.5 ml Dilution II + 4.5 ml PBS (8. µg/ml) (working solution.)

Add this much Dilution III G F

to 5.0 cc culture 25 µl 12.5 µl

to get final concentration. 40 ng/ml 20 ng/ml

\*Mitomycin C is added to cultures when the case is set up\*

1. **DEB Dilution Chart**

Dilution I 5 ml PBS + 5 µl DEB (pure)

Dilution II 3 ml PBS + 2 ml Dilution I

Dilution III 2.25 ml PBS + 0.25 ml Dilution II

(working solution)

Add 12.5 µl Dilution III to 5 cc culture D to get final concentration.

Add 25 µl Dilution III to 5 cc culture E to get final concentration.

\*DEB is added to culture after it has incubated 24 hr.\*

1. **Harvest**

Cultures A, B, E for patient and for control are harvested at 72 hr (3 days), and cultures F, G are harvested at 96 hr (4 days). Harvest procedures are the same as routine for Peripheral Blood Cultures in Hanabi Harvester.

1. **Slide Making and Analysis -**

Make slides from A, B, E, G for both patient and control slides are stained with Wright-stained (do not use trypsin). 50 cells are scored from 40 ng/ml MMC culture G and DEB culture E. If an adequate number of metaphases is not obtained from 40 ng/ml MMC culture, go to 20 ng/ml MMC culture F. Score the A and B cultures (no clastogens) only if MMC & DEB cultures are positive. Take sample photographs of some "pertinent" cells. Results are tabulated in the table shown below. Reference ranges for radial figures are: Normal: 0-4%, Fanconi: 30-100%

REFERENCES

1. Auerbach, A.D., Adler, B., and Chaganti, R.S.K. Prenatal and postnatal diagnosis and carrier detection of Fanconi anemia by a cytogenetic method. *Pediatrics* 67:128-135, 1981.
2. Cervenka, J., and Hirsh, B.A. Cytogenetic differentiation of Fanconi Anemia, idiopathic aplastic anemia, and Fanconi anemia heterozygotes. *Am. J. Med. Genet.* 15:211-223, 1983.
3. Schroeder-Kurth, T.M., Auerbach, A.D., and Obe, G., eds. *Fanconi Anemia: Clinical, Cytogenetic, and Experimental Aspects*. Berlin: Springer-Verlag, 1989.

APPENDIXES

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| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| *Lab Number* | *Clastogen* | |  |  |  |  |  |  |  |  |  |  |  |
|  | *0* | *1* | *2* | *3* | *4* | *5* | *6* | *7* | *>=8* | *Radials* | *% Cells w/*  *radials* | *Total #*  *Cells* |
| Proband | No clastogen |  |  |  |  |  |  |  |  |  |  |  |  |
|  | 20 ng/ml MMC |  |  |  |  |  |  |  |  |  |  |  |  |
|  | 10 ng/ml MMC |  |  |  |  |  |  |  |  |  |  |  |  |
|  | 0.01 µg/ml DEB |  |  |  |  |  |  |  |  |  |  |  |  |
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| Control | No clastogen |  |  |  |  |  |  |  |  |  |  |  |  |
|  | 20 ng/ml MMC |  |  |  |  |  |  |  |  |  |  |  |  |
|  | 10 ng/ml MMC |  |  |  |  |  |  |  |  |  |  |  |  |
|  | 0.01 µg/ml DEB |  |  |  |  |  |  |  |  |  |  |  |  |
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| MMC=Mitomycin C | |  |  |  |  |  |  |  |  |  |  |  |  |
| DEB - Diepoxybutane | |  |  |  |  |  |  |  |  |  |  |  |  |

**Breakage/Radial Analysis**

Written By: Director Approval:

(Signature and Date) (Signature and Date)

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Cytogenetics Supervisor