**UW Medicine - Pathology**

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G-Banding by Trypsin Using Wright's Stain (GTW)

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| Adopted Date: 09/04/91  Review Date: 06/12/09  Revision Date: 04/13/11 |

PURPOSE

Banding patterns, consistent with ISCN chromosome descriptions, will permit the identification of chromosomes and allow karyotyping. Patient slides containing metaphase spreads are enzymatically digested in a solution of trypsin and PBS, followed by staining with a solution of Wright's stain. This is the standard banding method used at the University of Washington Cytogenetics and Genomics Laboratory.

PROCEDURE

### Materials and Equipment

* 1. Dry heat oven.
  2. 3 Coplin jars.
  3. Light microscope.

### Reagents and Solutions

* 1. Trypsin (Difco Trypsin 250 Cat. #215240).
  2. Dulbecco Phosphate Buffered Solution (DPBS) without Ca+2 or Mg+2 (Gibco Cat. #14190-136).
  3. Fetal bovine serum (discarded from tissue culture use).
  4. Wright's stain (Sigma Cat. #W-3000, or Fisher W35-100).
  5. pHydrion buffer capsules ‑ pH 6.8 (VWR Cat. #34175-231).
  6. Trypsin Solutions:
     1. Trypsin solution #1 -For solid tissue, chorionic villi, tumor, and amniotic tissue mass cultures slides

**Stock solution**: Weigh 0.25g trypsin, reconstitute with 22 ml DPBS and put in 2 ml cryotube aliquots. Expires in 1 yr kept at -20°C.

**Working solution**: 2 ml trypsin in a Coplin jar with 50 ml of DPBS. Expires in 12 hours at room temperature.

* + 1. Trypsin solution #2 -For blood and bone marrow slides

**Stock solution**: Weigh 2g trypsin. Reconstitute with 50 ml DPBS and put in 1 ml cryotube aliquots. Expires in 1 yr kept at -20°C.

**Working solution**: 1 ml trypsin in a Coplin jar with 50 ml of DPBS. Expires in 12 hours at room temperature.

* 1. Buffer

**Stock solution**: 1 capsule pHydrion (pH 6.8) in 100 ml of distilled H2O. Expires in 6 mo kept at room temperature.

**Working solution**: 5 ml of stock pHydrion buffer in 95 ml of distilled H2O. Expires in 3 months at room temp.

* 1. Wright's stain

**Stock solution**: 0.6 g Wright's stain in 200 ml absolute methanol. Swirl in bottle for at least 10 min then filter with Whatman's #1 filter paper into a dark bottle. Expires in 6 months at room temperature.

**Working solution**: Mix 1 part stock Wright's stain with 4-5 parts 5% working pHydrion buffer just prior to staining. Expires in 1 day.

### Procedures

1. Heat fresh slides in a 95°C oven for 30-60 min. Slides, which are over 4-5 days old, may not need to be heated.
2. Dip slide into room temperature trypsin working solution using the following times as guidelines: Note times vary depending a variety of factors including slide type cell density

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| **Type of slide** | **Trypsin time (seconds)** | **Stain time (seconds)** |
| Amniotic Fluid Mass culture | 21-28 | 70-75 |
| Amniotic Fluid In-situ | 15-20 | 70 |
| Chorionic Villi | 30 | 65 |
| Tumor/Solid Tissue | 15 | 70 |
| Peripheral Blood | 60\* | 60 |
| Bone Marrow | 8-16\* | 50 |

\* use trypsin solution #2

1. Rinse slide briefly in 50 ml PBS containing 5 drops of fetal calf serum.
2. Rinse slide briefly in PBS.
3. Stain slides immediately for recommended seconds above in Wright's stain mixed one part to four parts 5% Hydrion buffer, pH 6.8 (use 1 ml to 4ml for 5 ml per slide). Mix the stain and buffer just prior to staining, and apply to a horizontally positioned slide.
4. Rinse slide with distilled H20 and air dry. Check in microscope for quality of banding. Adjust trypsin time, if needed.

REFERENCES

1. Modified from Seabright M, A rapid banding technique for human chromosomes. Lancet, ii:971-972, 1971.
2. Modified from Chapter 6, Barch MT, Knutsen T, Spurbick JL The AGT Cytogenetics Laboratory Manual. Raven Press, 3rd edition, 1997.
3. ISCN (2005): *An International System for Human Cytogenetic Nomenclature*: Recommendations of the International Standing Committee on Human Cytogenetic Nomenclature, eds., Shaffer, L.G., Tommerup, N., S. Karger, Basel, Switzerland, 2005.

Written By: Director Approval:

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Cytogenetics Supervisor