**Giemsa Staining**

**DESCRIPTION:** The Giemsa stain is a member of the Romanowski group of stains, which are defined as being the black precipitate formed from the addition of aqueous solutions of methylene blue and eosin, dissolved in methanol.  The variants of the Romanowski group differ in the degree of oxidation (polychroming) of the methylene blue stain prior to the precipitation.

The stain class was originally designed to incorporate cytoplasmic (pink) staining with nuclear (blue) staining and fixation as a single step for smears and thin films of tissue (spread preparations of omentum).  Minor modifications of working stain concentration and staining time have been made over the years for fixed tissue sections.

The Romanowski stains are extremely tedious to prepare, and are best purchased as the commercially available pre-made stock stain.

**Technical Points**

1.   (step 2) - Usually the staining is performed at room temperature overnight, however, increasing the stain temperature shortens staining time.  Sections stained at 37°C for several hours, (staining time assessed by microscopical examination), produce better results than sections stained at 60°C for a shorter period.  The higher the staining temperature, the greater the intensity of blue staining, but without the equivalently increased red staining - see technical point 2 below.

2.   Differentiation with acetic acid will vary according to the staining time and temperature, but it is generally achieved within 30 secs.  The differentiating agent removes only the blue dye component, thus increasing the apparent intensity of the red component.

**Method**

1. Dewax in Xylene – 2 mins
2. Dewax in Xylene – 2 mins
3. Dewax in Xylene – 2 mins
4. Wash in Absolute Alcohol – 2 mins
5. Wash in Absolute Alcohol – 2 mins
6. Wash in 90% Alcohol – 2 mins
7. Wash in 70% Alcohol – 2 mins
8. Wash in Running Water – 2 mins
9. Stain with diluted Giemsa's stain made up fresh (see technical point 1)
10. Rinse in distilled water
11. Differentiate with 0.5% aqueous acetic acid (see technical point 2)
12. Wash rapidly in 70% ethanol
13. Wash rapidly in 95% ethanol
14. Wash rapidly in 100% ethanol
15. Wash rapidly in 100% ethanol
16. Wash rapidly in 100% ethanol
17. Clear in Xylene – 2 mins
18. Clear in Xylene – 2 mins
19. Clear in Xylene – 2 mins
20. Mount slides with coverslips using DePeX

**Results**

          bile pigments.........................................................green

          collagen, muscle, bone..............................................pale pink

          micro-organisms, fungi, parasites.................................purplish-blue

          starch granules, cellulose............................................sky blue

          pigments (native colour is yellow/brown, or if fixed in dichromate containing fixative)...green

          nuclei..................................................................dark blue to violet

          erythrocytes..........................................................salmon pink

cytoplasm…………………...............................................varying light blue shades

**Reagent Formulae**

1.        Giemsa's stain, stock solution – obtain from commercial sources.

                    Giemsa reagent improves with age, expiry is unimportant.

                                   Giemsa stain, working solution

Giemsa stock solution ------ 40 drops

Distilled water --------------- 40 ml

The diluted stain keeps well, but is best made up fresh each time.

2.      Acetic acid 0.5%