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## RAPID STAINING METHOD OF BLOOD SMEARS

J.S.B. (Jaswant SINGH and BHATTACHARJI) Stain

This method requires two solutions which are prepared as follows:

Solution I

Medicinal methylene blue*	0.5 gm.
Potassium Dichromate ( $K_2Cr_2O_7$ )	0.5 gm.
Sulphuric acid ( $H_2SO_4$ ) (1% by volume)	1.75 cc. (50 minims)
Water	500 cc

Methylene blue is first dissolved in water. Sulphuric acid is mixed with it and then the chrome salt is added. This will result in the formation of a heavy amorphous purple coloured precipitate of methylene blue chromate. Place the solution in an autoclave at a temperature of 100 to 109°C for three hours (the pressure is maintained between 0 and 5 pounds). The solution should have turned blue by the end of this period indicating nearly complete polychroming of methylene blue. Should it appear greenish, heating for another hour or so may be required. The temperature must not be permitted to rise above 110°C., since this may result in carrying the oxidation of the methylene blue too far.

When the solution has become blue after three hours or so of boiling, it is allowed to cool at room temperature. Ten cc. of 1% KOH or NaOH is then added drop by drop, very gradually, the flask being continuously shaken meanwhile. Transfer half the contents of the flask to another flask of same capacity, and

\* Medicinal methylene blue differs from the commercial product in being zinc-free.

both are shaken for fifteen minutes more, after which the two are again combined. In this way, the precipitate which has formed is gradually dissolved, and the solution will take on a deep blue colour with a violet iridescence. After being allowed to stand for forty-eight hours at room temperature, the solution is filtered through a soft filter paper, and is ready for use.

Solution II

This is prepared by dissolving 1 gm. of water soluble yellow eosin in 500 cc. of water.

Staining technique for both thick and thin smears involves the following steps:

1. Thin smears are fixed with methyl alcohol as in other methods (but alcohol should not be allowed to come into contact with the thick film.)
2. Dry, preferably by waving in air.
3. Immerse slide (both thick and thin films) in Solution I for 30 seconds.
4. Wash in a jar of acidulated tap water for 2 to 3 seconds (pH.6.2 to 6.6)
5. Stain in solution II for 1 second.
6. Wash in same jar (No.4) for 4 seconds.
7. Immerse again in solution I for 30 seconds.
8. Wash as above for 10 seconds, or until the smear gives a pink background (No.4)
9. Stand slide on end to dry.

The above procedure is capable of modification in that the thick and thin smears may be treated as follows:

1. )
2. ) as above
3. Immerse slide in solution II for 2-3 seconds.
4. Wash as in 4 above for 2-3 seconds.
5. Stain in solution I for 45 seconds.

6. Wash as in 8 above.

7. Stand slide on end to dry

For staining thick smears alone the process may be slightly different:

1. Immerse slide in solution I for 10 seconds.
2. Wash in acidulated water (pH as above) for two seconds.
3. Stain in solution II for one second.
4. Wash (as in No.2) for 5 seconds.
5. Immerse again in solution 1 for 10 seconds.
6. Wash as before for two seconds, or until the smear shows a pink background.
7. Stand slide on end to dry.

Thus for thick smears the total time does not exceed half a minute.

The following advantages are claimed in favour of this method:

1. Ingredients are in common use in laboratory work.
2. The staining solutions can be prepared in the laboratories and improve with age.
3. Technique is simple and rapid.
4. Thick and thin smears can be stained on the same slide and can be kept as permanent preparations.
5. Clear differentiations of chromatin, cytoplasm and stripling is obtained as with standard stains.
6. Stained smears do not fade unless continuously exposed to intense light for long periods.
7. Initial costs of ingredients and the fact that hundreds of slides can be stained in the same solution without any waste as in the standard Romanowsky stains make this method most economical.
8. It has given satisfactory results in the staining of other organisms such as LD bodies, trypanosomes and also for routine differential leucocyte counts.