A NEW AND RAPID TRIPLE STAIN.¹

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Two years ago I published² an account of a new process of triple staining, in which I combined the stains saffranin, methyl-violet, and orange G in such a way that the saffranin selected the cytoplasmic structures of the tissues, the methyl-violet all chromatic substance, and the orange G the fibrous groundwork.

The process, though yielding good results, was somewhat lengthy, and I subsequently modified it, first, by substituting pyronin for saffranin, and second, by making a mixture of the two principal stains. By these means simplicity of method and certainty have been attained, and the time required has been reduced from an hour to five minutes.

The process is as follows :—

SOLUTIONS.

1. Methyl-violet	.	.	.	.	.	.	0.25 gm.
Pyronin	.	.	.	.	.	.	1.0 "
Distilled water	.	.	.	.	.	.	ad 100 c.c.

N.B.—Make the solution by heat and filter,—label, “Methyl-violet-pyronin solution.”

2. To 100 c.c. of acetone *add slowly, drop by drop, from a drop bottle, a 2 per cent. aqueous solution of orange G (made by heat and filtered).*

Whilst adding the solution of the stain keep stirring and rubbing the mixture with a glass rod. When the fluid has attained a pale yellow colour a faint cloudiness appears. Further addition of the stain increases this cloudiness until a flocculent precipitate is formed. Continue the addition of the orange G *drop by drop*, and the precipitate presently redissolves.

Immediately this has taken place, filter into a bottle and label “Orange-acetone.”

N.B.—It is important to add the orange G solution very slowly, otherwise the precipitate does not appear. After twenty-four hours a crystalline precipitate appears which augments with time, but the efficacy of the solution is not impaired. After keeping for some weeks, however, its action may be reinforced by the addition of a few drops of the watery solution of orange G.

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² In *Lancet*, London, January 27, 1906, p. 221; *Virchow's Archiv*, 1906, Bd. clxxxv. S. 59.

METHOD.

1. Fix material in acetic alcohol (glacial acetic acid, F.P., 14°7–15° C., 1 part; absolute alcohol, 2 parts), alcohol, or sublimate solution. *Chromates and formalin render the method useless* unless subsequent to staining the sections are treated by oxidising agents.

2. Stain for two minutes in the methyl-violet-pyronin solution.

3. Wash rapidly in water, and *wipe dry all the slide* around the section.

4. Flood the slide with orange-acetone—a colour cloud comes out. Pour off and flood again—when no more colour comes out.

5. Wash rapidly in pure acetone, and

6. Transfer to xylol and mount.

N.B.—Acetone being very volatile, *be careful the slide does not dry*. The acetone should be kept in a stoppered jar.

NOTES.

The whole process should not take more than five minutes, and may be done in less. If the preparation be over- or understained, return it *through acetone to water* and proceed as before until the requisite tints are obtained.

All chromatic substance is stained by the methyl-violet, mitotic figures and nucleoli showing very distinctly, and lymphocytes, in which the nucleus is wholly chromatic, are rendered very prominent.

Keratin, either of surface epithelium or occurring in cell nests in carcinoma, is also stained violet.

The cytoplasm of all cells is stained varying degrees of red by the pyronin. The bodies of plasma cells in particular are vividly stained, and so also are those of epithelial cells, especially when belonging to the deeper layers of a squamous epithelium. The cytoplasm of fixed tissue cells and endothelial cells is less strongly tinted.

The connective tissue framework is stained yellow by the orange G, as is also the intercellular substance between epithelial cells.

The net result is a marked colour differentiation of the individual elements of the tissues, which is particularly appreciated when examined by high magnification.

The method, on account of its rapidity and simplicity, can be well used in routine work, instead of hæmatoxylin, over which it presents marked advantages. It is quite permanent, and may be combined with Weigert's elastic tissue stain if desired. As a test of plasma cells it is equal to Pappenheim's stain, but it does not differentiate mast cells so strikingly. On the other hand, it shows great contrasts between other types of tissue cells which Pappenheim's stain does not. I have tried it for blood films, but without success.

When working with artificial light, the best results are attained if a blue screen be used.