

[54] HEMATOLOGICAL STAIN SYSTEM

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1965, abandoned.

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[58] Field of Search 424/3, 7, 75

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[57] ABSTRACT

A hematological stain system comprising a solution of
Wright's stain, a modified buffer and a thiocarbamyl fixative
glycerine and a lower alkanol.

3 Claims, No Drawings

HEMATOLOGICAL STAIN SYSTEM

CROSS REFERENCE TO RELATED APPLICATION

The present application is a continuation-in-part of application Ser. No. 517,139 filed Dec. 28, 1965, now abandoned, for "Hematological Stain System."

BACKGROUND OF THE INVENTION

I. Field of the Invention

This invention relates to blood staining systems and more specifically to an improved Wright's stain solution.

II. Prior Art

The use of blood stain system employing various dyes selected to provide differentiation to the constituents of a blood sample for microscopic inspection is well established.

The dye most commonly employed in these systems is a compound of eosin, methylene blue and allied dyes, termed Wright's stain, which was first described by J. H. Wright in the Journal of Medical Research, volume 7, p. 138, 1902.

In order to employ such a stain it is usually necessary to prepare a solution of Wright's stain powder in methyl alcohol and to apply that solution to a blood smear or the like. Next, a buffer solution is added dropwise to the stained smear until visible physical change occurs. The buffer is intended to develop the pH range (between 5.8 and 7.0) and to stabilize the pH value of the stain solution against large changes in cases where the blood sample itself is strongly acidic or alkaline. Then, the stained sample must stand for approximately 5 minutes to allow proper fixation of the dye to the blood groups in the smear. The time will differ with every batch of stain solution and may vary between 2 and 6 minutes. The stained blood smear is then washed with distilled water. During this process care must be taken to insure that the Wright's stain stays in solution with the buffer and does not precipitate out. If the Wright's stain does precipitate out, the smear will be ruined.

If a properly stained blood smear is obtained, then rapid examination of that smear must be made since the stain is unstable and fades quickly when under the influence of light.

It is readily apparent that the standard Wright's stain system and the procedure associated therewith has inherent pitfalls. To improve such a system and procedure it is incumbent that:

1. the time required to stain a sample blood smear be reduced;
2. the buffer must prevent the precipitation out of solution of the Wright's stain; and
3. the stain must not fade rapidly.

These and other improvements, such as improved differentiation of the blood constituents and the elimination of the need for distilled water as a rinse agent, are afforded by the present invention as will be more fully explained hereinafter. It is noteworthy, also, that the present invention now makes possible the commercial availability of a stable pre-bottled Wright's stain system affording the above improvements.

SUMMARY OF THE INVENTION

The present invention contemplates the addition of a thiocarbamyl compound, preferably thiourea, to a Wright's stain solution prepared with a buffer which comprises the salts of weak acid and a strong base and a weak acid. The thiocarbamyl compound fixes the stain to provide a differentiation of the blood groups that is inherently more pronounced than that obtained with heretofore known Wright's stain solutions while stabilizing the stained blood smear to prevent fading of the slide.

Additionally, it has been found that the thiocarbamyl compound makes possible the use of ordinary tap water as a rinse agent since the chlorine and other oxidizing agents present in tap water are reduced or eliminated by reaction with the thiocarbamyl compound.

Thus it is a broad object of the present invention to provide a blood staining solution that gives better differentiation of the

blood constituents by positive fixation of the dyes to the constituents.

It is another object of the present invention to provide a blood stain solution that will not fade rapidly.

It is still another object of the present invention to provide a blood staining solution that eliminates the need for distilled water as the rinsing agent needed for preparation of a blood smear.

Other objects and advantages of the present invention will become readily apparent to those skilled in the art from the preferred embodiments of the present invention to be more fully described hereinafter.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

The present invention takes the form of a solution of both Wright's stain and a properly proportioned buffer to which is added a fixative. The solution rendered thereby may be added to a blood smear without any particular cautions and will produce a properly fixed and buffered stain in a small percentage of the time required by the heretofore known techniques and with differentiation of the blood constituents heretofore unobtainable.

Although the present invention will work well with the standard hematological staining buffer of monobasic potassium phosphate and dibasic sodium phosphate dissolved in water, to make the solution commercially successful, a modified buffer consisting of the salt of a strong base (the reaction product of a strong base and weak acid) and a weak acid with a pH lying in the range of 4.7-8.0 is employed.

The salts contemplated by the present invention generally include a sodium citrate anhydrous, sodium acetate anhydrous, sodium cacodylate, sodium lactate and the reaction product of tris (hydroxy-methyl) amino methane and sodium hydroxide, preferably sodium citrate anhydrous, sodium acetate anhydrous, and the reaction product of tris (hydroxy-methyl) amino methane and sodium hydroxide and mixtures thereof.

The weak acids of the present invention generally include, citric acid anhydrous, maleic acid, glacial acetic acid, cacodylic acid and lactic acid, preferably, citric acid anhydrous, maleic acid and glacial acetic acid.

The buffers derived from these salts and acids generally comprise the following mixtures; sodium citrate anhydrous-citric acid anhydrous; sodium acetic anhydrous-glacial acetic acid; sodium cacodylate-cacodylic acid; sodium lactate-lactic acid; the reaction product of tris (hydroxy-methyl) amino methane and sodium hydroxide-maleic acid, and sodium acetate anhydrous-citric acid anhydrous. Preferably, the buffers are selected from the mixtures of sodium citrate anhydrous-citric acid; sodium acetate anhydrous-glacial acetic acid; the reaction product of tris (hydroxy-methyl) amino methane and sodium hydroxide-maleic acid, or sodium acetate anhydrous-glacial acetic acid.

It is to be noted that the modified buffers employed in this Wright's stain solution are substantially insoluble in alkanols but are completely soluble in glycerine which is in turn completely soluble in an alkanol. Thus, the glycerine is utilized as a vehicle to promote the solubility of the buffer in the alkanol. It is apparent from the preceding that the buffers that can be employed in the present invention are restricted only by their solubility in glycerine and the pH range the buffer will develop. To be suitable for the present invention the buffers must develop a pH anywhere in the range of 4.7 and 8.0 and preferably 6.0 to 6.5.

I have found that small quantities of a thiocarbamyl compound when introduced into a Wright's stain solution of an alkanol and a modified buffer, as described above, stabilize and fix the stain for long periods of time and thereby inhibits fading of the prepared smear.

The thiocarbamyl compound by fixing the stain provides a means of differentiation of the pigmentation within leukocytes and eosinocytes that is inherently more pronounced than that

accomplished with heretofore known hemotological stain solutions.

Not only are the cytoplasmic periphery of the neutrophils clearly lilac, the eosinophiles bright red, erythrocytes orange or pink and the platelets purplish, but the nuclei and the cell walls surrounding these blood constituents are clearly defined.

The stabilization provided to the system by the thiocarbamyl compound maintains this clear architecture of the cells for long durations of time even when exposed to the diluterious effect of light; thus allowing easier comparative analysis of blood samples.

Because of the availability of thiourea, it is the most feasible thiocarbamyl compound to use, although any thiocarbamyl compound and their respective homologs will work with equal efficiency.

Following, are but four examples representing preferred embodiments of the present invention, and their methods of preparation all of which has been found to produce the desired results, but which in no way limit the scope of the invention:

EXAMPLE I

In an atmosphere purged of carbon dioxide and water dissolve 5.0 g. Wright's stain powder, 4.1 g. sodium acetate anhydrous, 0.44 g. citric acid anhydrous and 5.8 g. thiourea in 869.66 g. methanol absolute and 115.0 g. glycerine (99.5 percent assay min.). Place the components in a flask equipped with coils and reflux components. Boil the solution slowly for three hours. Cool to room temperature and filter. The liquid thereby obtained is then ready for use.

In the solution formed by this process, the sodium acetate reacts with any acidic constituent of the blood sample to produce products having a pH value of approximately 6.2 which is also the pH value of the buffer itself. The citric acid reacts with any basic constituent of the blood sample in a similar manner. Thus, within a wide pH range of the blood the solution will fix the stained blood sample at the highly desirable pH value of 6.2 whenever a sufficient amount of the solution is placed over the blood sample.

EXAMPLE II

Dissolve 5.0 g. Wright's stain powder, 6.77 g. tris (hydroxymethyl) amino methane, 5.37 g. maleic acid, 1.645 g. sodium hydroxide and 5.8 g. thiourea in 98.6 g. glycerine and 876.815 g. methanol as in example 1. In this solution, the tris (hydroxymethyl) amino methane and sodium hydroxide reaction product represents the salt of a strong base with which the maleic acid forms a buffer having an ideal pH 6.4.

EXAMPLE III

Dissolve 5.0 g. Wright's stain powder, 1.133 g. sodium acetate anhydrous, 0.0465 g. glacial acetic acid anhydrous and 5.8 g. thiourea in 99.5 g. glycerine and 888.521 g. absolute methanol as in example 1.

The buffer yields a pH value of 6.0.

EXAMPLE IV

Dissolve 5.0 g. of Wright's stain powder, 12.38 g. sodium citrate anhydrous, 1.545 g. citric acid anhydrous and 5.8 g. thiourea in 98.6 g. glycerine and 876.675 g. absolute methanol in accordance with the procedure of example 1.

With the Wright's stain solutions of the present invention no special smear preparation procedures or preparations are required as were heretofore required. By merely placing a quantity of the solution on a blood sample and allowing it to set from 25 to 40 seconds (until a cupric oxide like colored scum appears on the surface) a readied smear is prepared. The smear can be washed with ordinary tap water since it has been found that the thiourea will react with any oxidizers in the water to eliminate their presence.

The blood smear samples that are hereby produced with the present invention will not fade and will produce a differentiation of the blood constituents that has not been heretofore associated with Wright's stain solutions. The Wright's stain solution that is hereby disclosed is stable for long periods of time thereby making it suitable for commercial production and availability since it is storable.

Having thus described my invention, I claim:

1. The method of preparing a solution for differentially staining blood smears comprising dissolving in a mixture of glycerine and an alcohol selected from the group consisting of methanol and ethanol, in an atmosphere purged of carbon dioxide and water, an anhydrous buffer soluble in glycerine to develop a pH for said solution of between 4.7 and 8.0, Wright's stain powder and a fixative, thiourea, the ratio of said stain to said fixative being 5 to 5.8 parts by weight, and boiling the resulting stain solution for three hours, cooling and filtering.

2. The method of claim 1 wherein said buffer comprises a mixture of the salt of a strong base selected from the group consisting of sodium acetate anhydrous, sodium cacodylate, sodium citrate anhydrous, sodium lactate and the reaction product of tris (hydroxy-methyl) amino methane and sodium hydroxide, and a weak acid selected from the group consisting of lactic acid, cacodylic acid, glacial acetic acid, maleic acid and citric acid anhydrous.

3. The stain solution prepared by the method of claim 1.

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