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Rapid and Inexpensive Method of Diluting Giemsa Stain for Diagnosis of Malaria and Other Infestations by Blood Parasites

For more than a century, Giemsa stain (2) has been used for the staining of blood parasites. The fixation of blood smears in methyl alcohol or the use of the May-Grunwald staining solution (1) is followed by the use of Giemsa stain for 25 to 30 min. Giemsa stain (3 ml) is diluted with buffered distilled water (100 ml) and is the stain of choice for demonstrating the presence of parasites in blood smears.

Distilled water was used for dilution purposes many years ago by chemists because of its purity. Since distilled water is slightly acid, it was necessary to buffer it to obtain a pH of 7.0 to 7.4, with the optimum pH being 7.2 to 7.4. This buffered distilled water-Giemsa stain solution is excellent for the staining of blood parasites and particularly for use in cases of malaria, where Maurer's clefts, characteristic of *Plasmodium falciparum*, are readily evident in blood smears (4).

The preparation of buffered water, however, is time-consuming. Certain spring waters, without organic matter but with their own characteristic mineral composition, have buffering power. Some of the commercially available spring waters have pH values between 7.0 and 7.4; among them is the Evian brand (5) which is easily found in many countries (Table 1).

Twenty-nine bottles of Evian water were purchased in Paris, France, in seven different shops, in lots as follows: 4 bottles containing 300 ml of water each, 10 bottles containing 500 ml of water each, 3 bottles containing 1,000 ml of water each, and 12 bottles containing 1,500 ml of water each. The mean pH measured, after the bottles were opened, with a blood gas pH meter (Instrumentation Laboratory B. G. Electrolytes) was 7.36, with a standard deviation of 0.05. There was no significant variation among the lots.

This pH of 7.36 is about the same as the pH of human arterial blood. Other spring waters from various countries also have pH values suitable for staining procedures. With reference to blood smear staining procedures, the advantages of these spring waters are that they are ready to use, remain stable for weeks after opening, can be kept at room temperature, and have a low cost compared to chemicals for preparation of appropriate buffers.

TABLE 1. Mineral composition in Evian water in France[a]

Mineral(s)	Concn (mg/liter)
Bicarbonates	357
Calcium	78
Chlorides	4.5
Dissolved solids	309
Magnesium	24
Nitrates	3.8
Potassium	1
Silica	13.5
Sodium	5
Sulfates	10

[a] Water source was Cachat, France. In the United States, the mineral composition is slightly different, with concentrations as follows (according to bottle labeling): silica, 14 mg/liter; chlorides, 4 mg/liter; and nitrates, 60 mg/liter. Bottles from both countries have a listed pH of 7.2.

Use of bottled water for diluting Giemsa stain has proven reliable for the detection of malaria-causing parasites on thin and thick blood smears. Importantly, it allows for the demonstration of Maurer's clefts (3). With this procedure we have been able to show these characteristic clefts in 90% of 205 cases of malaria caused by *P. falciparum*.

Maurer's clefts are slow to appear during the morphological stages of *P. falciparum*. Only red blood cells containing older trophozoites show dots that later become the typical spots. The dots can be round or irregular in shape, triangular, or loop-like and have a red-brown or even purplish color. They are always few in number (1 to 20 dots per cell) and easy to count. They are very important for the diagnosis of severe *P. falciparum* infestation. Schuffner's stippling (6) also appears when staining is carried out in cases of *Plasmodium vivax* or *Plasmodium ovale* infestation.

Diagnosis of malaria can also be done by molecular biology techniques involving PCR or immunochromatographic testing (ImmunoChromatic test (Amrad ICT, Sydney, Australia). However, several tests are necessary to detect the four species of *Plasmodium*, microfilariae, and trypanosomes. Giemsa stain diluted with spring water is very useful not only for the detection and study of blood parasites (5) but also for studies of blood cytology.

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