Anisol - a Convenient Immersion Medium for Microscopy

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During the surveillance phase of malaria eradication the examination of blood for malaria parasites is of paramount importance. In the early stage of surveillance the amount of work connected with the collection and examination of very numerous blood slides is so great that it often constitutes a major bottle-neck of the programme. The re-examination of all or a proportion of positive blood films by a central laboratory is an accepted feature of a well-organized programme of malaria eradication. Any improvements in the microscopical technique of blood examination are therefore of considerable value.

One of the most common immersion media used for the examination of blood slides with high-power lenses is cedar oil, obtained from the juniper tree (*Juniperus virginiana*), commonly known by the wrong name of " red cedar ". The thickened oil has a refractive index of 1.515, which is very close to that of the glass of which high-powered objectives are made. The refractive index of the glass used for slides and coverslips is about 1.520. There are slight differences in the refractive indices of cedar oils produced by different manufacturers, and some manufacturers have produced proprietary oils of standard composition which are less subject to drying than cedar oil itself.

Several microscopists have recommended other immersion media, such as mixtures of resin and linseed oil, castor oil, glycerin, etc. Rowntree [a] advised the use of heavy mineral oil, otherwise known as liquid paraffin or petrolatum, which has a lower refractive index (1.470) and should, for best observation, contain 12%-18% α-bromonaphthalene (Jensen[b]). Neutral mineral oil can be gently wiped away and preserves the stain, but has the drawback of being messy and collecting dust on the slide (Wilcox[c]).

The major disadvantages of an ordinary immersion oil are its messiness and the fact that if the slide has to be re-examined the oil must be removed by xylene soon after the examination. It frequently happens that the available xylene contains a trace of resin and leaves on the slide an iridescent film which interferes greatly with the re-examination of the blood film.[d] Moreover, the repeated use of xylene discolours the Giemsa stain.

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[a] Rowntree, W. (1909) *J. Path. Bact.*, 13, 28

[b] Jensen, V. (1921) *C. R. Soc. Biol. (Paris)*, 84, 424

[c] Wilcox, A. (1950) *Manual for the microscopic diagnosis of malaria in man*, Washington, D.C. (National Institutes of Health Bulletin, No. 180)

[d] " Gummy " xylene can be redistilled quite easily and safely by the following improvised method, used in Nigeria by the author. A Pyrex flask containing the xylene should be heated over a 100-watt electric bulb fitted into a short chimney made out of a piece of a drain pipe. The xylene vapours pass into a glass tube, about 50 cm long, fitted into the flask and bent so that the condensation takes place in the far end of the tube, whence the liquid can be collected in a bottle. The distilled xylene should be crystal clear, without a tinge of yellow.

In tropical areas the cedar oil thickens quite quickly and often the xylene will not dissolve the oil completely. On re-examination the stained blood film has a granular, bi-refringent layer which makes the identification of parasites quite impossible. Wilcox[c] suggests that this trouble can be dealt with by covering the slide with some mineral oil and heating gently for ten minutes. This method does work at times, but in our own experience is not always foolproof.

The use of cedar oil causes inconvenience in the mass cleaning of slides needed for future re-use. In tropical areas the boiling of slides in a Lysol solution might be necessary to remove the dry cedar oil. An alternative is the use of a detergent or of a sulfuric-acid bichromate solution (Field[e]).

The above disadvantages of ordinary immersion oil have led some microscopists to prefer other media, such as methyl benzoate and methyl phenyl ether.

1. *Methyl benzoate*, $C_6H_5COOCH_3$, also known by the more romantic name " oil of Niobe ", is a methyl ester of benzoic acid. Its specific gravity is 1.093, its refractive index is 1.518, and its boiling-point is 199°C. It has a pronounced aromatic smell and is used as a solvent in the paint and rubber industries. It has been advocated for laboratory use as a dehydrating medium before embedding. Its greater fluidity is also an advantage for the examination of living cells under the coverslip. (Gatenby & Beams;[f] Cowdry[g]).

2. *Methyl phenyl ether*, $C_6H_5OCH_3$, is known commonly as anisol or anisole (Karrer[h]). Its specific gravity is 0.9878, its refractive index is 1.515, and its boiling-point is 156°C. It has a distinctive, not unpleasant smell, and is used in perfumery and as a solvent (Becher[i]).

Both compounds are soluble in alcohol, ether and toluene. Anisol is somewhat more fluid than methyl benzoate, evaporates a little faster and is less " oily ".

The use of these compounds for immersion microscopy was first described in 1925 by Becher, a professor of zoology in Giessen. Becher[i] drew attention to the many advantages of both compounds, but particularly anisol, over the classical cedar oil. Anisol is not greasy or sticky, and does not harden on the slide, but volatilizes slowly. A drop of anisol disappears from the slide in 20-30 minutes or can be removed immediately by touching it with a piece of filter- or blotting-paper. Anisol is not ionized and does not interfere with Romanowsky stains as shown by Lentze,[j] although its repeated use on the same slide may produce some fading. Its refractive index is very close to that of cedar oil.

It has two minor drawbacks. It is more fluid than cedar oil and hence may flow somewhat when the stage of the microscope is inclined at more than 45°. It has a higher chromatic aberration and reduces slightly the colour

[e] Field, J. W. (1948) *The microscopic diagnosis of human malaria*, Kuala Lumpur (Institute for Medical Research, Federation of Malaya, Study No. 23)

[f] Gatenby, J. B. & Beams, H. W. (1950) *The microtomist's vademecum*, London

[g] Cowdry, E. V. (1943) *Microscope technique in biology*, Baltimore, Md.

[h] Karrer, P. (1947) *Organic chemistry*, Amsterdam

[i] Becher, S. (1925) *Z. wiss. Mikr.*, **42**, 16

[j] Lentze, H. (1928) *Z. wiss. Mikr.*, **48**, 200

correction of apochromatic objectives, especially when an immersion condenser is used. Thus anisol should not be used for high-definition photomicrography; for any ordinary laboratory microscopy, however, this disadvantage of anisol is irrelevant.

Gatenby & Beams[f] pointed out that methyl benzoate may damage the cement used in *some* microscope lenses and it is possible that anisol, which has similar chemical properties, should not be used with the old makes of microscopes. On the other hand, several makers of microscopes (Leitz, Zeiss, Cooke, Troughton and Simms) stated in their replies to our inquiry that anisol would not affect the lenses of modern instruments.

Anisol has been used for many years by several workers and it is surprising that it has not become better known. Kobayashi[k] stated that it is equally good if not superior to cedar oil and other proprietary media. Professor Raffaele has been using anisol for several years with excellent results, and it has become a standard immersion medium at the Institute of Malariology in Rome. A brief experience with anisol in Nigeria convinced the writer of the suitability of this medium for large-scale blood examinations under tropical conditions.

Finally, a word should be said about the immersion-oil containers for permanent use on the laboratory bench. The classical glass cedar-oil bottle has many drawbacks and was replaced several years ago, at the Parasitology Department of the London School of Hygiene and Tropical Medicine, by a plastic oilcan such as is used for lubricating oil. These small plastic oilcans are now available in most hardware shops and department stores and cost about $0.10 - $0.20. They are very handy, clean, unbreakable, well-stoppered and free from dust, and enable one to squeeze out the exact amount of immersion medium needed. They are ideal for the purpose and should replace the classical cedar-oil bottle everywhere.

[k] Kobayashi, J. (1953) *Chem. Abstr.*, 47, 5638

Measurement of Adsorption of Residual Insecticides using Flowing Chromatography

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When residual insecticides are applied on mud walls, which are so common in tropical areas, sooner or later they will penetrate below the sprayed surface. This phenomenon is known as sorption. As a consequence, the insecticide is out of reach of the mosquito alighting on the wall and therefore cannot exert its full toxic effect. In the case of BHC, which acts mostly as a fumigant, sorption is an advantage over the short term, whereas in that of DDT and dieldrin, which are longer lasting, it is a definite inconvenience.