ACID-FAST AND H&E STAININGS CAN BE COMBINED BETTER THAN IN THE TRIFF METHOD

Sir,

The TRIFF staining for *Mycobacterium leprae*[1] is usually considered convenient and ideal for demonstration purposes.[2,3] In some institutions this method is the main tool of the histological

diagnosis of leprosy. Compared with *M. tuberculosis* the *M. leprae* is much less acid and alcohol fast,[4] thus any treatment with alcohol and acid should be minimal. However, the TRIFF method requires six treatments with acid alcohol or alcohol as follows:

1 differentiation of the carbol fuchsin
2 differentiation of the Harris' haematoxylin
3 dehydration after eosin
4 staining in alcoholic saffron
5 rinsing after saffron
6 final dehydration before mounting

All these steps contribute to the removal of the carbol fuchsin accounting for the difficulties experienced in controlling the method. In our hands the TRIFF staining is rather capricious and invariably gives poor results on granular bacilli. For diagnostic purposes the separately stained H&E and modified Wade–Fite stainings[5] have not been surpassed. If one opts for the use of a single slide, then we would recommend an alternative stain. This method is a combination of the H&E and acid-fast stainings and it has been aimed at simplicity and reliability.

1	Dewax sections in a mixture of xylene and vegetable oil, preferably clove oil (2:1)	15 min
2	Blot, dry till opaque and wash in running water	5 min
2/a.	Remove mercury/formol pigment, if applicable	
3	Stain with filtered carbol fuchsin	25 min
4	Wash in running water	5 min
5	Differentiate in 0·5% acid alcohol or in 10% sulphuric acid	1–20 sec
6	Wash in running water	5 min
7	Stain in Mayer's or preferably Carazzi's haematoxylin[6]	20 sec
8	Blue in tap water or in tap water substitute if necessary	
9	Stain in 1% aqueous eosin	60 sec
10	Differentiate eosin in running water	1–5 min
11	Blot, dry in oven at 45°C	30 min
12	Clear in xylene and mount	

This method eliminates the last five alcoholic treatments of the TRIFF staining. The use of a progressive haematoxylin makes the second acid alcohol unnecessary, which is a major drawback of the TRIFF staining. The staining with alcoholic saffron has entirely been left out as it gives little additional information. Finally, the alcoholic dehydration has been substituted by the blot and dry method.

Following dewaxing the sections should be carefully blotted several times and then dried. Breaking this rule results in prolonged differentiation time, moreover the desirable decolourization of the background may not be reached. The only critical step is the differentiation of the carbol fuchsin. Usually every batch contains a few slides which have to be returned into the differentiating agent for a few more dips or seconds. The progress and the final result of the decolourization should be checked under the microscope. However, as experience grows the pale pink hue of the sections is a good indicator of the proper differentiation. The times for steps 7 to 10 may vary widely depending on the actual solutions and the pathologist's preference. A relatively long differentiation of the eosin is important as the strong eosin can obscure the bacilli. The rule of thumb is that the hues of both the haematoxylin and the eosin should be lighter than those of the ordinary H&E staining.

It cannot be overemphasized that each batch of slides to be stained by any acid-fast method for either diagnostic or research purposes *must* include a positive control section,[7] as false negative results are much more common than it is usually supposed. The control specimen of choice is an old regressing lepromatous case predominated by granular bacilli of decreased stainability.

The information on the bacteria furnished by this method is fully comparable with that of the

modified Wade–Fite staining.[5] On the other hand the staining may not be equal to a neatly prepared H&E due to the slight fuzziness of the cellular details resulting from the oily dewaxing.

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