References

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4 de Kantor I, Laszlo A, Vazquez L, Reniero A, Latini O, Urbanczik R. Periphery to centre quality control of sputum smear microscopy and 'rapid fading' of Ziehl-Neelsen staining. [Correspondence] Int J Tuberc Lung Dis 2000; 4: 887–888.
5 Van Deun A. Periphery to centre quality control of sputum smear microscopy and 'rapid fading' of Ziehl-Neelsen staining. [In reply] Int J Tuberc Lung Dis 2000; 4: 888–889.
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7 World Health Organization. Laboratory Services in Tuberculosis Control. Part II: Microscopy. WHO/TB/98.258. Geneva, 1998: pp 27–28.
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In reply

I agree with Dr de Kantor and colleagues that recommendations regarding quality control (QC) of smear microscopy can have a huge impact and thus should be based on carefully analysed evidence. However, this principle seems to have been accepted only recently. Where is the evidence for the old and at one time universally accepted recommendation to re-check 10% of negatives and all positives? Or, for the rule that slides should be re-checked without re-staining?

As to the specific points raised: "The additional workload and expense because of re-staining not justified by any discernible benefit."

Dr de Kantor and colleagues correctly state that improper staining and other causes of false negative results will have to be investigated through direct supervision. However, for this to be possible, first of all the staining problem has to be detected. The point, already brought up in my former reply and which has not been commented upon by the correspondents, is that deficiencies in stains or staining techniques may lead to false negative smears in which the bacilli cannot be seen at all or only in very low numbers. It is also possible that poorly stained bacilli fade more easily, but I have no proof of that. Unless the slides have undergone proper re-staining first, QC controllers may thus not find false negative errors in a situation that needs urgent correction. From what I have seen in various developing countries, this is not a rare occurrence, but the correspondents seem to be of a different opinion. However, what is needed here is not opinions but facts.

The difference in false negative errors detected before and after re-staining all slides has been documented recently in trials done at the Research Institute of Tuberculosis (RIT), Seoul, Korea. Clearly more false negative errors were found after re-staining of 669 reportedly negative slides (2.8% compared to 0.9%; data courtesy Dr Sang Jae Kim, September 2000).

Another example comes from a seminar on QC that I conducted in Laos some months ago. Just to have a rough idea of the performance in the different provinces, participants were asked to come with positive and negative recent smears from their own laboratory, only 2–3 of each. They could select these themselves, it was only asked that the negatives should be follow-up smears at 2 months of treatment. Smears were re-checked before and after re-staining. The results, shown in the Table below as laboratories failing because of more than one error in these very few slides, were totally different before and after re-staining. Without re-staining, several centres seemed to have false positives (fading, as I could see in some of the still weakly positive smears), while there were only few false negatives. The conclusions and necessary actions were totally different after re-staining: not a single false positive was left, but a few technicians now had far too many false negatives, raising the suspicion of deficient staining. To me this suggests that it may be better to aim at thorough re-checking, rather than at high numbers, if it comes to limitations because of workload.

I have not disputed the value of quality assurance of AFB microscopy in areas of lesser TB prevalence. The statement I made was ". . . the justification not only of re-staining, but of the whole exercise (QC by re-checking AFB microscopy) . . .". It was thus meant to apply to the method of QC by re-checking of peripheral slides only, and I fully agree that a system of quality assurance remains necessary and potentially effective. Also in the examples cited by de Kantor and colleagues, another system (central to periphery) has been used as far as we could check (although we can not be aware of the details of his personal communication, Dr Küchler has published on results in Germany with a central to periphery system[1]).

	No. of laboratories with more than one false result (of a total of 14 laboratories)	
Type of errors / total smears re-checked	Before re-staining	After re-staining
High false positives / 35 smears	5	0
Scanty false negatives / 43 smears	0	1
High false negatives / 43 smears	2	4

The fading studies[2] were meant to solve operational problems (inexplicable rates of seemingly false positives) occurring at that time in the Bangladesh control project. A 1% carbolfuchsin solution was used there as routine, and so the investigation also used 1% solutions. At the same time, it was assumed (but indeed never proved) that a stronger stain might be less liable to fade quickly. Nevertheless, Engbaek and colleagues have shown that high carbolfuchsin concentrations yield superior results.[3] And I must admit that I do not understand the rationale behind the WHO/IUATLD recommendation to use a 0.3% fuchsin concentration.[4]

The following lines on page 888, paragraph 2, have urged me to correct the statements of de Kantor and colleagues in their previous letter: "Apparently, no control slides went without weekly or monthly reading and subsequent cleaning with xylene. . . . Repeated soaking could increase this loss, which could also be a very plausible cause for fading".[5] Earlier, in the paragraph cited by them now, they had indeed correctly stated that 100% relative humidity and highest temperature control slides had not been exposed to xylene before fading. However, the later phrase seemed to need a correction, since it was immediately followed by the suggestion that fading would have been caused by xylene.

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