Reviewer’s report

Title: Sensitivity of direct versus concentrated sputum smear microscopy in HIV-infected patients suspected of having pulmonary tuberculosis

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Reviewer: Maryline Bonnet

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General comments:
This study is important because it evaluated the performance of concentrated smear microscopy in HIV TB suspects. This group of TB suspects are likely to benefit a lot from an improved smear microscopy for diagnosis of tuberculosis, mostly in peripheral setting. Also, few authors reported that sputum concentration methods are likely to be more effective in patients with poor quality specimen, such as HIV co-infected patients. The study methodology uses different and strong Gold Standards to define a TB case and ensures a good blindness of the technicians’ smear reading.

Unfortunately, the study population of hospitalised patients in a referral national hospital is a serious limitation of this study. Indeed, sputum concentration methods are an alternative to more sensitive diagnostic methods, such as rapid TB culture for peripheral health care clinics where the majority of TB suspects seek cares. Therefore, the level of TB suspicion and the clinical presentation of the study patients are likely to be different compared with outpatients B suspects at a peripheral setting. This limitation was already very well pointed out in the recent meta-analysis of specimen processing methods by Steingart et al (Lancet Infect Dis 2006) and is one of the reasons why the results between different studies evaluating concentration method are difficult compare.

We can also regret that this study didn’t use the sodium hydroxide, which was used in most of the studies and is easily available. The authors decided to compare direct smear with concentrated smear obtained after specimen decontamination and centrifugation, which is the specimen preparation before culture. This can simplify a lot the study procedure and be an advantage in culture setting but this is not the case of laboratories where the concentration method would be the most suitable. Again the use of different concentration methods across studies makes comparison between study results very difficult.

The decision to limit the evaluation to the morning specimen can also be discussed. In peripheral clinics of resource poor settings, patients’ dropout between the 1st sputum specimen collected at the 1st consultation and the 2nd morning specimen collected at home is frequent, mostly when patients live far from the diagnostic centre. Therefore, a smear-microscopy approach based on the examination of the morning specimen only could miss part of the patients. Also, the morning specimen is known to be of better quality and to have a higher
smear diagnostic yield than the on-spot specimen (see meta-analysis of Mase et al, Int J Tuberc Lung Dis 2007). Therefore, it is likely that this specimen will benefit less from the specimen concentration.

Major Compulsory Revisions

In Methods:
- Under “Study population”, could the authors precise if any consecutive eligible patients were enrolled?
- Under “Patient evaluation”, could the authors describe the criteria used by the treating ward physician to initiate TB treatment?
- Under “Laboratory methods”, the level of performance of direct smear microscopy should be better documented with results of the internal and external quality controls during the study period. As clearly pointed pout by the authors, the quality of the direct smear microscopy influence the results of the comparison with concentrated smear microscopy.
- Under “Outcome definition”, could the authors precise the criteria use to define patients’ clinical improvement, mostly when the evaluation was conducted through telephone interview.

In Results
- Could the authors precise the reasons why 10% of patients did not submit a morning specimen, and why 6% had no concentrated smear. There is an inconsistency between the text and figure1 regarding the number of sputum non-concentrated.
- Could the authors present the reasons of patients’ hospitalisation and the baseline clinical presentation? This could be added in table 1.

In Discussion
- The authors should further address the study limitations discussed above in General Comments (study population, concentration method, morning specimen).
- The authors should further discuss the reasons, which could explain that 19 culture positive negative concentrated smears were positive with >10AFB/100 HPF using direct smear microscopy.

Minor Essential Revisions

In Methods, under “Laboratory methods”, the sample collection from the concentrated specimen for the smear preparation should be further described.

In Results:
- Could the author add the numbers used for the calculation of the overall concordance? I found an overall agreement of 80% (224/279) when I include both culture positive and negative results. 75% is limited to culture positive results.
- In table 3, the scanty result of patient 9 is not at the right place. It should be in the column “concentrated” of smear result.

- Table 4 presents AFB density for direct and concentrated smears regardless of the results (negative or positive). The first sentence of the chapter “Density of Acid-Fast Bacilli” should be revised accordingly.

In discussion:

- In the 2nd chapter, it would be interesting that the authors compare also their results with studies using similar in-patients population and similar concentration method (NALC-NaOH and centrifugation).

- Page 11, in the last chapter of the discussion, the authors should also discuss the operational benefit of using direct + concentrated smear of 1 single specimen compared to direct smear on 2 specimens in term of the potential reduction of the number of visit to the health centre and the delay to initiate treatment. I agree that this should be further evaluated in a cost effectiveness study.

In Conclusion:

Could the authors precise “HIV-infected hospitalised adult population” in the 1st sentence.

Discretionary Revisions

The description of the quality of the morning specimen is missing. This information would be very useful to maybe understand why there was no difference between direct and concentrated smear microscopy in this study. Indeed, if most of the morning specimen were purulent, then the benefit of the concentration method is likely to be minimal.

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Acceptable

**Statistical review:** Yes, and I have assessed the statistics in my report.

**Declaration of competing interests:**

I declare that I have no competing interests