Reviewer's report

Title: Evaluation of bleach-sedimentation for sterilizing and concentrating M. tuberculosis in sputum samples

Version: 1 Date: 10 April 2010

Reviewer: Sara Irène EYANGOH

Reviewer's report:

* Major Compulsory Revisions

I have two major concerns in the study design and results interpretation which do not allow supporting conclusions of this study.

1- Bleach sterilization assessment.

The authors stated that "this study defined the concentrations and exposure time needed to sterilize M. tuberculosis in sputum" this is not quite exact since the authors have not assessed the quality of bleach use and do not determine the % of free chlorine content (active ingredient) of the solution; there is no possibility to know exactly what is the chlorine concentration in the different dilution 3%, 6%, 10% and 15% bleach. The term "bleach" is a generic term that encompasses numerous chemical oxidation products, and the use of term bleach without due characterization has led to confusion in the scientific literature. The methodology presents is not sufficiently informative to allow replication of the experiment; results presented here give neither answer to the concentration nor to the exposure time since the two parameters are linked.

2- Bleach sedimentation assessment

Authors stated that "our quantitative evaluation demonstrated that bleach sedimentation caused a significant decrease in the number of AFB visualized". Authors should use the standard scale gradation of AFB microscopy to validate this observation (exact number/ 100 field, 1+, 2+, 3+). E.g: data given in the paper could not be significantly different 146 BAAR/ 100 fields (mean of 1.5 AFB/field) is not different from 346 AFB/ 100 fields (mean of 3.5 AFB / field), because according to the standard scheme of recording and reporting the two are graded 2+. The conventional gradation should be use instead of terms as paucibacillary or multibacillary (see WHO/IUTLAD table below)

Finding Recording
No AFB found in at least 100 fields negative
1–9 AFB per 100 fields exact figure/100
10–99 AFB per 100 fields +
1–10 AFB per field (count at least 50 fields) ++
More than 10 AFB per field (count at least 20 fields) +++
Fig 3 is incomprehensive for the non-statistical expert. Perhaps should be removed if results are expressed using standard scale.

- Minor Essential Revisions

Methods
The authors should elaborate clearly this part.

Study design:
The authors should give details of their sampling strategy. How do they estimate the minimal sample size (N= 72 sputum samples) to test all hypothesis? What was the duration of study?

On page 10, "selection of bleach-sedimentation technique" authors should give more details on the five gravity bleach –sedimentation techniques to make easier understanding. Results should not be given in methods part. "These 3 techniques gave similar results" which one? These should be clearly presented to give support in their selection of the method described by Gebre-Selassie.

Details on ZN coloration process are not necessary. The important parameter is the fuchsin concentration, 1% or 0.3% this could explain further discordances in reading if AFB are not strong red colored.

In sedimentation process specify if the sputum stand at room temperature.

. Needless to give details on decontamination process.

Results:
"Bleach sterilization assessment" authors mentioned a control, but the rest of the paper however does not indicate the result of this control and its usefulness.

Fig1 should be completed by adding the control used during the decontamination process and the 16 samples split up into two set of triplicate smears.

Fig2: add the control result

- Discretionary Revisions

In Abstract, background section, on the first line, authors could replace the term "insensitive" by "low sensitive".

Second paragraph of introduction, on the first line, please correct ref [o]

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

**Quality of written English:** Needs some language corrections before being
published

**Statistical review:** Yes, but I do not feel adequately qualified to assess the statistics.

**Declaration of competing interests:**

I declare that I have no competing interest