Laboratory

Recommendations of the Task Force about transportation and processing of sputum samples, smear microscopy and culture procedures

1. How many sputum samples should be collected from each suspect?
   At least 2. Samples of 3 to 5 ml, with macroscopic presence of mucopurulent viscous material are considered to have good quantity and quality.

2. What is the best way to collect the samples in terms of guarantying a good participation and the best positivity rate (early morning collection at home x collection at the field site)?
   Most surveys collect the first sputum at the field site (on the spot), right after the X-ray is taken and considered to be positive. This sample should be collected under supervision.
   The second sample is collected in the early morning, immediately after the suspect wakes up, and before he/she engages in any other activity, like having breakfast. The early morning collection represents the pulmonary secretions accumulated overnight, and therefore it usually has a higher positivity. This is even more relevant in a prevalence survey rather than in a clinical setting, because of the bacterial load is expected to be smaller. Patients should be advised to take their samples to the field site / laboratory as soon as possible.
   If it is considered that it is unfeasible for one or more patients to collect the early morning samples and take them to the field site or to the laboratory (because the suspect lives too far away, for example), then an alternative would be to collect both samples at the field site, in which case it would be best if the collection of the samples was done with an interval of one or two hours in between them.
   Educational material for patients and field staff with clear instructions and figures explaining the sputum collection procedures should be made available.

3. What bio-safety requirements should be available at the field site in order to avoid transmission of TB to the staff survey team?
   Sputum samples should be collected in an open ventilated space. The staff supervising the collection should not stand in front of the patient, because of the risk of aerosol infection.

4. What should be done if a suspect cannot produce a sputum sample? Should we accept a sample of saliva? Should we try to stimulate the production of sputum? If so, how can this be done?
   It is not recommended to induce the production of sputum in a survey. The patient should receive a full explanation on how best to produce a sample (most countries have brochures about that, which are used for the routine NTP activities).
   If a sputum sample cannot be obtained, a sample of saliva collected after the suspect coughs is acceptable (better than nothing).
5. Should we chase defaulted suspects and suspects that cannot produce a sputum sample at the site in their homes/work places?
   Ideally yes, but logistic considerations will determine its feasibility. In most surveys up to now, this was not considered to be possible. The first sputum soon after CXR should guarantee at least one specimen from every suspect.

6. What type of container should be used?
   Containers should wide-mouthed, unbreakable, leak-proof and screw-capped. Containers should have a volume capacity of 50 ml and made of translucent material in order to observe specimen volume and quality without opening the container. It is essential for all samples to be appropriately labelled with two patient identifiers and the date of sputum collection.

7. Should all samples be put in a special transport specimen box with ice packs or in a refrigerator (cold chain) immediately after collection? What is the maximum interval of time that a sample can be left out of the transport specimen box? If a sample is collected at home at 6 am and the patient can only hand it in at 6 pm, is it still valid, even if it was not kept in any transport specimen box?
   It is very important to deliver all specimens to the laboratory as soon as possible after collection. However, survey samples are usually not discarded, even if they arrive at the central lab many hours after collection.
   As a rule, transport of specimens collected from suspects at home is done without any refrigeration. However, it would be better if these samples should be put in a small cooler box. Cold conditions should be in place for transportation of specimens from the field site to the central level. The exception is for specimens preserved with CPC which should not be refrigerated, because CPC crystallizes at low temperatures.

8. What is the preferable smear microscopy technique: light microscopy with Ziehl-Neelsen staining (ZN) or fluorescence microscopy (FM)?
   The use of light vs. fluorescence microscopy should be carefully considered. These techniques have similar sensitivity (are able to find similar number of positive cases) when performed by skillful lab staff. However, the fact that FM is performed in a much faster way (typically 100 slides per staff/day against 20 slides per staff/day for the light microscopy) could be very useful in a survey where the anticipated workload is very big. One important consideration is that FM should not be implemented immediately before or during the survey, or exclusively for the survey, because it takes time for the staff to be competent in performing this technique.

9. Is it OK if in a survey a country uses ZN in some labs and FM in others? Or should the same technique be used for every sample (even if some samples are submitted to both techniques so as to compare their yield)? Should FM results be confirmed by ZN?
   For the survey results to be comparable across clusters, it is preferable to use only one type of microscopy technique. FM does not need to be confirmed by light microscopy.
10. Should we try to optimize the sputum smear microscopy by processing the sputum samples with bleach or sodium hydroxide, or sedimentation (there is extensive literature in support of these procedures)? Does this depend on the capacity of the labs performing the smear examinations?

Smear optimization procedures should only be used if these techniques are already implemented in the routine. If the survey staff is not perfectly comfortable in applying such procedures, they should not be implemented just for the survey. In practice, this means that these techniques are not relevant for prevalence surveys. The same procedures should be used across the clusters.

11. What culture method should be used (liquid x solid, with and without decontamination procedures)? Should the choice of method be guided by the length of the interval from sputum collection to processing? Please explain the choice of method in terms of culture yield, availability of trained staff and bio-safety facilities, distance/time from the field sites to the laboratories.

Solid culture should be used (LJ or Modified Ogawa have comparable sensitivity). If liquid culture is to be used, then a separate tube should be concomitantly inoculated using solid media. In a survey setting in which samples are not fresh, contamination is expected to be much higher in liquid culture than in solid culture media.

12. Is it preferable to process all cultures in a central lab? If cultures are to be processed in a peripheral lab, what would be the minimum requirements of such lab?

Ideally, only one central laboratory should be used for all examinations, so as to avoid contaminations of specimens, to assure quality of the work and to avoid inter facility bias. However, considering the capacity to treat a large amount of specimen and the required time to send all specimens to one central lab, using regional reference lab(s) should be considered in some countries.

However, regional lab(s) often do not have a capacity and infrastructure to do more than primary culture. In such conditions, positive culture tubes must be sent to the Central Lab without opening caps in the regional lab. The regional labs must not touch colonies. This practice avoid contaminations of specimen and bio-hazard to the staff. Identification, sub-culture and further examinations should be done in the BSL3 lab.

When smear examination is done in a local lab because of the need to provide quick feed-back to the patients (diagnostic purpose), it is not recommend to re-cap and send the same specimens to the central lab for culture. Specimens for culture should not be opened after the collection to avoid contamination. All smear slides prepared and read by local lab should be sent to a designated upper lab for re-checking. At least one specimen should be kept untouched until it arrives in a culture lab.

When it is not possible to avoid using the same specimen for smear examination at the local lab and culture, direct inoculation to a culture media should be done at the local lab.

13. If two samples are collected from every patient, which sample should be sent for culture (the one collected early in the morning or the one collected at field site)? Please explain.
The best procedure is to send both samples for culture, and to compare their results. However, if just one of the samples is to be cultivated, then it should be the early morning one, provided that the quality of the morning specimen equals or is superior to the quality of the spot specimen.

14. Is it true that sputum specimen containers for culture examinations should not be opened for smear examination at the site lab? Is it OK to use the same specimen for field smear examinations and culture if direct inoculation is done in the same lab?

As a rule, just open the sputum containers in the central or regional labs. This is important to avoid contamination (including cross-contamination from one sample to the other) of the samples. It is preferable not to use the same specimen for smear and culture if these procedures are to be done in different laboratories, because if you open the container to do the smear examination at the field/local lab, there is a higher chance that the sample will be contaminated when it comes to the culture inoculation performed in the central or regional labs.

15. Should a concentration method be adopted for all cultures? Or does this depend on the volume of sputum that was collected?

Again, this depends on the previous capacity of the staff performing the cultures. On the one hand, centrifugation and other concentration methods increase sensitivity, on the other hand they require much more manipulation of the samples, which increases the risk contamination of the samples and infection of the staff. In a survey, it is always best to opt for the more simple methods which can be performed in a comparable way across the clusters, particularly when there is no or uneven previous experience with the new technologies/procedures.

16. Is it OK to use different methods for different clusters in an attempt to maximize the yield as much as possible? For example, should CPC be added only to the samples coming from clusters that are far away from the central lab? What are the consequences of doing this in terms of the results of the survey?

It is best to use the same protocol for every cluster, but there will be exceptional circumstances that need to be anticipated and carefully planned for. Optimal conditions for transport conditions are the shortest delay between collection of specimen and processing for culture and cold conditions during that time. Treatment with Cetylpyridinium chloride/bromide (CPC) is used for decontamination of samples, but it should be used only if delays are expected to be longer than 1 week. As mentioned above, CPC crystallizes at low temperatures, so that samples with CPC are expected to be kept at room temperature.

17. What is the danger of the survey for the routine lab activities? Should the survey use local lab staff or should new staff be hired? How can the country guarantee that the laboratories adapt to the increased workload during the survey, without loosing quality?

It is essential to maintain the routine activities of the NTP mycobacteria laboratories during the survey. Recruitment of additional staff with previous experience in performing the routine activities is usually necessary during the
survey. Another limiting factor is not staff, but incubator capacity. The number of cultures expected to arrive in the lab during the survey field work weeks has to be carefully calculated not to surmount the incubator capacity.

Provision should be made for retraining during the survey to ensure that staff follow procedures correctly. Survey supervisors will guarantee that the same methods and standards are used in all the survey labs.

18. What methods should be used for species identification? Can the Capillia TB assay be used instead of more conventional methods?

During a survey, mycobacteria should be discriminated in between TB and not-TB, using the conventional method available in the country labs. The Capillia TB assay is very easy and practical, and therefore highly recommended for surveys settings. However, it is expensive and the method has to be officially registered to be used in the country. Even when there is no possibility to use the Capillia assay for every culture because of budget constraints or because the test is not registered in the country, it is recommended to randomly separate some cultures to be confirmed by the Capillia assay (for research purpose, there is usually no need for the method to be registered, whereas registration is required for diagnostic purpose).

19. Should we do drug sensitivity testing (DST) for all culture positive samples?

Positive cultures from a survey of the prevalence of TB disease should be tested for drug susceptibility, provided that patients who are found to have drug resistance will be able to access appropriate treatment. Including DST in a survey of the prevalence of TB disease is especially relevant for countries that have not yet implemented a representative drug resistance survey. However, it should not be a substitute for conducting such a survey. The overall number of drug-resistant cases found in a survey of the prevalence of TB disease (which typically identifies a total of around 100–300 TB cases) is likely to be too small to provide precise estimates of the prevalence of drug-resistant TB (although the data will help to calculate the sample size needed for a drug resistance survey and may provide an initial estimate of the prevalence of drug resistance).

20. How can the quality of smear microscopy and culture be assured? Should laboratories without quality assurance be excluded from the survey?

It is of course better to use quality assured labs. If such labs are not available in the country, it may still be possible to run a survey if the survey lab staff is properly trained, and if quality assurance procedures are implemented and adequately maintained and recorded during the survey.

a. Smear microscopy performed in the field should always be double-checked at regional or central labs.

b. All positive smear slides and a sample of 10-20% of the negative ones should be double-checked (re-read by a second microscopists). If a single false-negative is detected, all slides from the cluster should be re-examined.

c. Culture positive / smear negative samples should have the smear slides reread and the culture reassessed.

d. Low-positive cultures (except those from patients under treatment) should be reassessed to differentiate false-positive
cultures from genuine clinically significant low-positive cultures. Smear negative subjects with only a single culture positive tube with less than 5 colonies should have all results including CXR re-assessed by a central panel of reviewers to decide if they are TB cases or not.

e. Cultures positive for Mycobacteria other than TB should be reassessed.

f. Contamination rate should be from 3 to 5%. Zero contamination should also be verified, as it is not expected.

21. Is it true that the survey definition of a smear positive case may be different from the NTP's clinical definition? Please explain.

TB cases and TB suggested cases will be advised to initiate treatment or will be promptly submitted to further examinations as early as possible for the benefits of patients. Survey participants may initiate treatment based on the results of a single smear exam performed by field laboratories. However, after completion of laboratory works, the survey central panel will review all the available data: presence of symptoms, smear positive, culture positive or CXR suggestive results. The central panel will decide the final categorization of prevalent cases and feed back such information to the local doctors: TB or non-TB; smear positive, smear negative/culture positive, or CXR suggestive; new or previously treated. Some patients who were initially advised to initiate treatment according to their smear results may be categorized as Non-TB cases by this process, as the results of culture and identification tests become available a few months later. Final individual results for the prevalence study could be different from the clinical decision made for case management purposes.