Improving the Diagnosis of Tuberculosis through Optimization of Sputum Microscopy

Expert Consultation, WHO Headquarters
Geneva 1-2 September 2005

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Chair: John Ridderhof, CDC, USA

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The meeting was held in parallel with an Expert Consultation on Improving the Diagnosis of Tuberculosis through Diagnostic Algorithms. Both meetings were organized by WHO Stop TB Department, the UNICEF/UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases (TDR), and the Foundation for Innovative New Diagnostics (FIND). The parallel meetings came together for two joint sessions.

The Expert Consultation on Improving the Diagnosis of Tuberculosis through Optimization of Sputum Microscopy had the following objectives:

1. To review and determine the strength of existing data, identify knowledge gaps, and define a research agenda regarding the role of sputum concentration methods to improve smear microscopy;
2. To review and determine the strength of existing data, identify knowledge gaps, and define a research agenda regarding the wider use of fluorescence microscopy;
3. To review and determine the strength of existing data, identify knowledge gaps, and define a research agenda regarding the number of sputum smears per suspect;

The meeting was centred on three systematic reviews commissioned by TDR and FIND in the context of their joint work-plan, and conducted by a team at the University of California, San Francisco, USA:

- Sputum processing methods to improve the sensitivity and yield of smear microscopy for tuberculosis: A systematic review. KR Steingart, V Ng, M Henry, PC Hopewell, A Ramsay, J Cunningham, R Urbanczik, MD Perkins, MA Aziz, M Pai.

- Fluorescence vs conventional sputum smear microscopy for tuberculosis: A systematic review. KR Steingart, M Henry, V Ng, PC Hopewell, A Ramsay, J Cunningham, R Urbanczik, MD Perkins, MA Aziz, M Pai.

- Yield of serial sputum smear examinations in the evaluation of pulmonary tuberculosis: A systematic review. SR Mase, V Ng, M Henry, PC Hopewell, A Ramsay, J Cunningham, R Urbanczik, MD Perkins, MA Aziz, M Pai.

These reviews will be submitted for publication in peer-reviewed journals.

The agenda for the meeting is provided in Annex I.

Background

These two Expert Consultations, one on sputum microscopy and one on diagnostic algorithms, held 1-2 September 2005 in Geneva, were organized to address a fundamental issue of growing importance – the relatively large numbers of tuberculosis patients whose diagnosis is delayed or missed when conventional diagnostic approaches are used.

Two characteristics of sputum microscopy, poor sensitivity and the requirement for significant labour and training, result in missed cases, reduced access to diagnostic services, and heavy workload to already overstretched health systems. This makes it difficult for health centres to offer high quality diagnostic services, and for patients, particularly the poor, or those with early tuberculosis to get a rapid examination and diagnosis. These difficulties are reflected in elements of the *Global Plan to Stop TB 2006-2015*, as noted below.

Performance characteristics of direct sputum smear microscopy

The Global Plan to Stop TB 2006 - 2015 recognizes the limitations of direct sputum smear microscopy, and calls for efforts for improvements and eventually substitution of this technique. In most resource-limited countries sputum smear microscopy is the primary technique for microbiological diagnosis of TB for the foreseeable future. Direct sputum smear microscopy is specific and inexpensive, but relatively insensitive. In some populations (e.g. HIV co-infected) the contribution of direct sputum microscopy to TB

diagnosis is particularly limited. Therefore, possibilities of improving the sensitivity of smear diagnosis need to be explored urgently.

Poverty, diagnostic pathways and access to DOTS

The Global Plan to Stop TB 2006 - 2015 recognizes that TB patients from poorer sections of society may not have access to DOTS services. At national level, even where DOTS programmes are well-established, patients with TB may face substantial costs prior to diagnosis because care-seeking pathways are long and involve many consultations with different providers. Wide-spread poverty is a factor contributing to the uncontrolled epidemic in Africa. Work in Malawi has shown that a significant proportion of smear positive TB patients attending a district hospital drop out of the diagnostic pathway before their results can be communicated to them and treatment initiated.[1] Simplifying and abbreviating diagnostic pathways can be expected to result in providing effective treatment to more patients. As a result, ways of shortening the time required for patients to receive a TB diagnosis need to be explored urgently.

Human resource crisis in health systems, particularly in Africa

The draft Global Plan to Stop TB 2006 - 2015 recognizes that human resources for health is among the greatest challenges in TB control especially in Africa. Staff shortages combined with increasing numbers of requests for sputum smear microscopy are creating unmanageable workloads with detrimental effects on the quality of laboratory services. Ways of reducing the laboratory workload associated with direct sputum smear microscopy need to be explored urgently.

Building Quality into Optimization

Quality management has been shown to improve the reliability of diagnostic laboratory services. Any new technologies or methodologies that are implemented should be accompanied by efforts to strengthen laboratory quality management systems. Quality management should include elements of training, standards, monitoring visits, internal quality control (including specimen quality) and external quality assessment.

The Consultation on sputum microscopy evaluated three classes of modifications to conventional sputum microscopy to accelerate diagnosis and treatment. It was recognized that these modifications are not mutually exclusive and that combinations of interventions may be necessary to optimize this technique.

1. Using fluorescent rather than light microscopes
2. Processing sputum with chemical additives and centrifugation or gravity sedimentation.
3. Decreasing the number of slide examinations required in a diagnostic series

FINDINGS AND RECOMMENDATIONS

FLUORESCENCE MICROSCOPY (FM)

Recommendations:

i. FM may be considered at all levels of the health system in high HIV prevalence countries seeking to improve the sensitivity of sputum microscopy, shorten time to diagnosis and reduce laboratory workload;

ii. This technology has been demonstrated to be effective in high volume settings. Countries wishing to implement FM at the peripheral level, in lower volume settings, should do so within the context of operational research to best determine models for implementation and to explore issues of cost, feasibility and sustainability;

iii. Research in this area should follow a coordinated and standardized approach[2], both to strengthen the country-specific evidence base and to permit comparison with data from different settings.

iv. NTPs may consider building operational research on fluorescence microscopy into their planning and applications for financial support.

v. The feasibility of developing fluorescent microscopes that overcome the limitations of existing equipment, particularly capital cost and maintenance needs, should be investigated.

The above recommendations are based on the evidence of technology performance and on consideration of feasibility, as detailed below.

Assessment of evidence on performance of FM

There is strong evidence, presented in the systematic review, that:

a) fluorescence microscopy is on average 10% more sensitive for the detection of pulmonary tuberculosis than conventional light microscopy (LM);

b) the specificity of FM for detection of acid fast organisms in sputum is comparable to that of LM;

c) the increased sensitivity of FM is greatest in low grade positives. The proportion of low grade positives in the population served may thus determine the relative sensitivity of the method over LM in any particular setting;

d) Fluorochrome-stained smears take less time to examine than those stained with the Ziehl-Neelsen (ZN) method (25% of the time taken to examine ZN-stained smears).

There is limited evidence:

a) on the use of FM to diagnose TB in HIV co-infected patients (however, the available evidence (two studies) suggests FM may be promising);
b) comparing different fluorescent staining techniques (however, based on a very few studies, there appears to be little difference in sensitivity or specificity between auramine and auramine-rhodamine stained smears);
c) on the use of FM in combination with sputum processing methods (however, based on very few studies the advantages of FM would remain when sputa are processed with bleach).

Feasibility of implementation of FM

The literature review did not include an assessment of the feasibility of FM implementation. It was determined that data on the use of FM outside of reference centres was inadequate.

Advantages in sensitivity warrant the recommendation to use FM outside of heavy workload settings. The caveat for recommendation would be "where appropriate and feasible". However, the settings where FM would be "appropriate and feasible" remain to be defined by further research and are likely to differ from country to country.

The capital costs associated with fluorescence microscopy have decreased in recent years. Simple and relatively inexpensive equipment for FM are available (e.g. Paralens, Fluorolens, LED-based systems), which may serve as alternatives to expensive fluorescent microscopes.

Several concerns remain about the implementation of FM at the peripheral level in resource-poor settings:

- The feasibility and sustainability of FM in settings with irregular electricity supply, limited human and financial resources, and inadequate training;
- The impact of irregular supply on FM bulb life;
- The lack of internationally-agreed EQA methods for blinded rechecking of fluorescent smears;
- Uncertainty about the stability of FM reagents under field conditions;
- Uncertainty about the acceptability of enclosed dark rooms to microscopists in tropical settings;
- That further research may be needed to clarify whether re-staining positive slides with ZN is required for confirmation.

Implementation is, therefore, likely to be complex and models for implementation are not available. Operational research through demonstration projects is needed.

SPUTUM PROCESSING

Recommendations - centrifugation with bleach:

i. The implementation of methods utilizing bleach and centrifugation for case-finding are not recommended at any level of the health system. This is because:
   - methods lack standardization;
   - large-scale implementation is likely to be very problematic;
   - of safety concerns surrounding the centrifugation of liquefied infectious sputum in rudimentary laboratories,
   - of large variations and inconsistencies in the results obtained using the methods, and the difficulty in distinguishing the effects of chemical from physical processing methods.

ii. While choosing not to recommend bleach + centrifugation methods, the committee recognizes that other authorities have recommended them. It also recognizes that some countries with high HIV prevalence, seeking to improve the yield of smear microscopy, are either already implementing these techniques, or are considering implementing them, even at peripheral level. In such circumstances, the committee wishes to advocate for a research component to implementation.

iii. Research is needed to assess the performance characteristics of the various bleach + centrifugation methods under carefully controlled conditions. After performance is determined and a standardized method selected, additional operational research will be needed to evaluate the feasibility, safety, sustainability, cost, and case-finding impact of using this sputum processing method.

iv. Research on the performance and implementation of bleach centrifugation should follow internationally-coordinated and standardized approaches, both to strengthen the country-specific evidence base and to permit comparison with data from elsewhere.[2]

v. As standardized methods become available, NTPs may consider building operational research on the performance and implementation of bleach centrifugation methods into their planning and applications for financial support.

Recommendation - gravity sedimentation with bleach:

i. There is not enough evidence to support the implementation of bleach sedimentation methods until results from further studies become available.

ii. Multi-centre studies are required to investigate the performance of these techniques, particularly those involving long sedimentation times, against the reference standard method. These studies would be most useful if they followed an internationally-coordinated and standardized approach.[2]

The above recommendations are based on the evidence of technology performance and on consideration of feasibility, as detailed below.

Assessment of Evidence (Sputum processing)

Centrifugation with any chemical processing method

There is moderate evidence supporting the use of centrifugation with various chemical methods. The 14 studies on the use of centrifugation in sputum processing with various chemicals showed an 18% mean increase in sensitivity of smear (range, -3% to +39%) . Most studies used a Relative Centrifugal Force (RCF) of at least 2000 x g.

Centrifugation with bleach

There is a moderate evidence base for the use of bleach with centrifugation in sputum processing prior to microscopy (6 studies using comparable methodologies and mycobacterial culture as reference, 13 studies examining incremental yield). The mean increase in sensitivity reported was approximately 15% (range, +1% to +38%). One of the studies using culture comparison provides the only evidence related to alternative sputum processing methods in patients with documented HIV infection. In that study of 96 patients, bleach + centrifugation increased sensitivity by 11%.

Gravity sedimentation with any chemical processing method

The evidence base for gravity sedimentation in processing sputum is weak. The 8 studies that used mycobacterial culture as reference used a variety of chemical agents and different sedimentation times. Four studies used short sedimentation times of less than 1 hour; the remaining four used long sedimentation times, at least 12 hours or overnight.

In 4 studies using long sedimentation times and a variety of chemicals, there was a 23% mean increase in sensitivity (range +2% to +34%); the increase in sensitivity was noted in all four studies. In 5 studies using long sedimentation times without culture, there was a 5% mean increase in incremental yield (range, +1% to +17%), an increase in yield was noted in all 5 studies.

The data for the studies using short sedimentation times is inconsistent. The 4 studies with culture as the reference standard demonstrated sensitivity increases ranging from 0 to 36%. A further 3 studies, without a culture comparison, found incremental yields of -4%, +5%, and +8% over direct microscopy.

Gravity sedimentation with bleach

Four studies compared gravity sedimentation and bleach against culture by splitting specimens. Of these, one study using a long sedimentation time noted a 33% increase in sensitivity, and 3 studies using short sedimentation times reported no, or minimal increase in sensitivity (0, 0, 1%).

Seven studies used sedimentation and bleach without culture, and examined the incremental yield over direct microscopy. Of these, 4 studies using long sedimentation times showed a 6% mean increase in incremental yield (range, +1% to +17%); 3 studies using short sedimentation times reported variable incremental yields (4%, +5%, and +8%).

Considering the current evidence from studies using sedimentation and a variety of chemicals, and considering the specific advantage of avoiding a centrifugation step, the overnight sedimentation studies with bleach and other chemicals (although inconsistent) show promise and merit further investigation.

Feasibility of implementation (Sputum processing)

Centrifugation with bleach:

Experience to date of implementing these techniques in resource-poor settings, as a means of improving case-detection, raises concerns about feasibility and sustainability. These include, concerns about:

- the introduction of further processing steps that may result in delays in producing results for patients;
- the process compromising the use of mycobacterial culture on the same specimen;
- the need to standardize EQA procedures;
- the technical complexity of the procedures;
- capital cost of adequate specification centrifuges (performance, capacity, safety) and the power required to run such machines;
- recurrent costs (centrifuge tubes).

Furthermore the presumed reduction in biohazard associated with bleach-processing of sputa has not been established.

Gravity sedimentation with bleach:

Although the evidence base is weak, this appears to be a promising processing method which is likely to be more feasible for implementation at the peripheral level. However, further research using appropriate study designs which identify the optimal bleach concentration and sedimentation times is required. Studies are also required to investigate the effects of bleach storage conditions on performance of the test. Such designs should be guided by the evidence presented in the Systematic Review and would be prepared by TDR in partnership with academic and technical agencies.

INCREMENTAL YIELD OF SECOND AND THIRD SPECIMENS IN THE 3 SPUTUM SMEAR STRATEGY

Recommendations:

i. There is strong evidence that the mean incremental yield of the 3rd sputum smear is only 2-5%. However, evidence on incremental yield is derived from an analysis in which one positive smear would define a smear positive TB case. This differs from the currently internationally recommended requirement for at least 2 positive smears to define a smear positive case. A separate analysis would be required to evaluate the evidence base for the current international case definition.

ii. The advantages which could accrue from omitting the third specimen in serial sputum examinations are pre-empted by the requirement for 2 positive smears to define a smear positive case as described in (i).

iii. Omitting the third smear could alleviate the overwhelming workload of laboratories, particularly in Africa. It could be expected that a 2 smear strategy based on a revised case definition would have, either, negligible adverse impact on case-finding or actually improve case-finding through improved quality of service (including a shortened time to diagnosis).

iv. Because of the limitations imposed by the standard case definition, countries should not consider the introduction of a 2 sputa strategy until the key research questions laid out in (vi) below are addressed ;

v. The requirement for 2 positive smears to define a smear positive case should be urgently reviewed by the relevant authorities.

vi. In countries with high microscopy workloads and human resources crises, NTPs may have to choose between examining 3 sputum specimens per person screened, or screening more people through reducing the number of specimens examined per patient. The committee recognizes that some countries are either already implementing, or are considering implementing, a 2 smear strategy. In such circumstances, the committee wishes to advocate for a research component to implementation. Wherever possible this should attempt to address at least some of the issues in (vii) above as well as operational concerns. Adequate surveillance of case-notification rates must be maintained;

vii. Multi-centre studies, which should follow one or more internationally-coordinated well-designed protocols[2], are required to:

- Determine the sensitivity and specificity (compared with culture) of a revised case definition with one positive smear result;
- Prospectively evaluate the relative yield of serial sputum specimens;
- define the optimal timing of specimen collection that minimizes delay in the diagnostic pathway.

vii. Operational research should address the cost-effectiveness of a 2 smear strategy in promoting case-finding, as well as strategies for reinvesting time saved in improving diagnosis through improved sputum collection, smear microscopy and the patient-orientation of service. These studies would be most useful if they followed an internationally-coordinated and standardized approach, both to strengthen the country-specific evidence base and to permit comparison with data from elsewhere [2] NTPs may consider building operational research on 2 sputa strategies into their planning and applications for financial support

The above recommendations are based on the evidence presented in the review, and on consideration of feasibility, as detailed below.

Assessment of evidence (2 vs 3 smears)

The systematic review presents strong evidence that, overall (ZN and FM studies combined), the mean incremental yield in sensitivity (compared to culture) of the 2nd smear is 9% (12 studies) and that of the third smear is 4% (12 studies). The mean incremental yield in smear positive results (in the absence of a culture standard) of the 2nd smear is 13% (19 studies) and that of the 3rd smear is 4% (19 studies).

Using ZN stain, there is strong evidence that the mean incremental yield in sensitivity (compared to culture) of the 2nd smear is 15% (3 studies) and that of the third smear is 5% (3 studies). The mean incremental yield in smear positive results (in the absence of a culture standard) of the 2nd smear is 14% (12 studies) and that of the 3rd smear is 4% (12 studies).

Using FM, there is strong evidence that the mean incremental yield in sensitivity (compared to culture) of the 2nd smear is 5% (5 studies) and that of the 3rd smear is 2% (5 studies). The mean incremental yield in smear positive results (in the absence of a culture standard) of the 2nd smear is 7% (4 studies) and that of the 3rd smear is 2% (4 studies).

The studies reviewed did not distinguish between 1st on-the-spot, early morning, and 2nd on-the-spot sputum specimens. Neither can it be assumed that the 3 smears represent these specimens in some order or other, since those studies conducted in Europe/North America are as likely to be based on 3 morning specimens.

Approximately half of the studies were retrospective laboratory register analyzes, presumably unblinded. It is uncertain whether, or to what extent, this might bias the results.

Feasibility of implementation (2 vs. 3 smears)

Clearly, omitting the third specimen and reducing workload does not create significant difficulties. When considering a two sputa strategy a national programme will need to consider and balance the following to make a decision:

- The potential for a decrease in diagnostic yield (although this may be compensated for by improved quality of microscopy resulting from the decreased workload)
- The human resources available for smear microscopy
- The potential decrease of patients dropping-out of the diagnostic pathway.
- The potential decrease in smears required for blinded rechecking EQA programmes
- The potential saving in time and costs that could be potentially diverted to improving quality of microscopic examination, or specimen collection procedures.
- Gaps in knowledge (see Recommendation vii below)

The decision is likely to be determined by the circumstances in individual countries.

Opportunities for synergistic combinations of interventions

Since the incremental yield of the 3rd specimen is only 2% in series of auramine-stained smears (compared with 5% in ZN-stained smears) opportunities may exist for synergistic combination of fluorescent microscopy and 2 sputa strategies. Since FM would seem to be unaffected by prior bleach processing of sputum specimens there may even be opportunities for synergism between all three interventions. Further research is required. These studies would be most useful if they followed an internationally-coordinated and standardized approach, both to strengthen the country-specific evidence base and to permit comparison with data from elsewhere.[2]

A G E N D A

Agenda for the meeting on optimization of TB microscopy methodologies
1-2 September 2005, Geneva, Switzerland

Background:

In most resource limited countries AFB smear microscopy is the primary technique for microbiological diagnosis of TB. The technique is simple and inexpensive. However, it is labour intensive and in smear negative population (e.g. HIV co-infection, paediatric, extrapulmonary TB cases) contribution of AFB microscopy in TB diagnosis is limited. Therefore, it is essential to explore possibilities of improving the TB diagnosis to address these challenges.

During the aforementioned meeting the review of the existing evidence and data addressing smear microscopy and diagnosis of smear negative pulmonary and extrapulmonary TB will be discussed and recommendations issued.

In order to allow the optimum coverage of the subject matter, the meeting will be held in two parallel sessions (one with focus on microscopy issues and the other on smear negative diagnosis). For the most part of the meeting the respective groups will work separately; however, joint sessions will also be scheduled for interactive discussion.

Please refer below to the specific objectives and expected outcomes for the microscopy group sessions:

Objectives:

1. To review existing data addressing methodological questions in microscopy (i.e. concentration methods and reagents, light vs. fluorescence microscopy, number of smears per suspect) to determine the strength of the data, to identify knowledge gaps, and to define a research agenda.

2. Define the quality of data and the degree of benefit required to drive policy change for AFB microscopy.

Expected Outcomes:

1. Clarification of the nature and strength of all recently published data on microscopy methods.

2. Formulate specified technical recommendations on the use of alternative microscopy methods and the risks and possible benefits of their early introduction to the following entities:
    1) Laboratory Capacity Strengthening Subgroup (SLCS) of the DOTS Expansion WG,
    2) STB Partnership and STAG,
    3) TB/HIV Working Group,

3. A prioritized research plan, including, to the degree possible, funding sources, study sites, endpoints, study objectives, and timelines for each element of the plan

AGENDA

Chair: J Ridderhof **Rapporteur**: A. Ramsay		
SEPTEMBER 1, 2005		
Time	**Topic**	**Presenter**
9:00-9:15	Opening and welcome	L. Blanc
9:15-9:30	Objectives of the meeting: **Joint plenary session**	H.Getahun, A. Ramsay
9:30-10:00	Systematic review methodology	M. Pai
10:00-10:30	COFFEE	
10:30-11:00	Literature review results: light vs. fluorescent microscopy	K. Steingart
11:00-12:45	Discussion and development of recommendations	
12:45-13:45	LUNCH	
13:45-14:15	Literature review results: concentration vs. direct methods	K. Steingart
14:15-15:30	Discussion	
15:30-15:50	COFFEE	
15:50-17:30	Discussion and development of recommendations on concentration vs. direct method	
SEPTEMBER 2, 2005		
9:00-10:30	Session provisionally allocated to further development of recommendations on concentration vs. direct method	
10:30-11:00	COFFEE	
11:00-11:30	Literature review results: 2 vs. 3 smears per TB suspect	S. Mase
11:30-12:45	Discussion and development of recommendation on 2 vs. 3 smears per suspect	
12:45-13:45	LUNCH	
13:45-15:00	Review of recommendations to be made at the joint session	
15:00-15:30	COFFEE	
15:30-18:00	**Joint plenary session** Presentation and discussion of joint recommendations and next steps (with the algorithm group)	Chair-R. O'Brien
18:00	Closing of the meeting	M. Aziz

[1] Squire SB, Belaye AK, Kashoti A, Salaniponi FM, Mundy CJ, Theobald S, Kemp J. 'Lost' smear-positive pulmonary tuberculosis cases: where are they and why did we lose them? Int J Tuberc Lung Dis. 2005;9:25-31

[2] TDR takes the responsibility for convening the partnerships required (FIND, STB, NTPs, other technical agencies and academic institutions), developing protocols, identifying funding and coordinating activities around core aims, objectives and deliverables.