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Use of Liquid TB Culture and Drug Susceptibility Testing (DST) in Low and Medium Income Settings

Summary report of the

Expert Group Meeting on the use of liquid culture media,

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Use of Liquid TB Culture and Drug Susceptibility Testing (DST) in Low and Medium Income Settings

The international TB laboratory experts and representatives of partner organizations recommend the use of TB liquid culture and DST in low income settings. Liquid culture systems are the standard of care for TB diagnosis and patient management in industrialized countries. Demonstration projects conducted by the Foundation for Innovative New Diagnostics (FIND) have shown that liquid culture and DST systems are feasible for implementation in lower income settings to improve diagnosis of multidrug-resistant (MDR) TB and AFB smear-negative pulmonary TB. Consistent with the large body of published literature on liquid culture, the projects have found a higher rate of mycobacterial isolation and a shorter time to detection when compared to culture of the same specimens on solid media. (see list of references in Annex 1).

Certainly, liquid culture and DST systems are more complex and sensitive than solid culture and DST media. Increased bacterial contamination and an increased frequency of nontuberculous mycobacterial (NTM) isolation must be addressed. A rapid method to differentiate *M. tuberculosis* complex from other mycobacterial species is essential. The higher cost of liquid culture systems compared to solid media is being addressed by significant reductions in commercial prices for the public health sector in lower income countries.

FIND expects to have a much larger body of liquid culture and DST performance data, as well as detailed costing and patient impact data, available in the next year that will be provided to WHO. In the meantime, the following recommendations are well-supported by the available data.

Documentation of need

The decision by a national Ministry of Health (MOH) to implement a liquid culture system, with or without DST, should only be made in the context of a comprehensive and detailed country plan for TB laboratory capacity strengthening and expansion to address the need for increased access to TB culture and DST. Many countries have developed or are in the process of developing such plans, and resources are becoming available for the provision of technical assistance for this planning process. Such plans should be based on a strong network of quality-assured microscopy that remains the cornerstone for TB diagnosis.

With the increased emphasis on expansion of TB laboratory services, it is expected that entities outside of the MOH, e.g., NGOs, will also wish to implement this technology. Ideally, this should be done in collaboration with the MOH to help ensure that country needs are being addressed.

Guaranteed financial support

Although available cost data suggest that at the favorable pricing negotiated by FIND for its projects the total cost per test for liquid culture may be only marginally higher than that for solid media, there should be some assurance of continued financial support for the culture media and other consumables before expensive equipment is purchased. There are too many examples of commercial liquid culture automated readers sitting idle in laboratories unable to pay for the needed reagents. This consideration especially applies to countries largely dependent on outside funding for TB control activities. A commitment by a manufacturer to provision of liquid culture materials at an "affordable and sustainable price" would also be helpful in assessing the longer-term financial requirements for the system. This should be a preamble in the commercial sales contract.

Infrastructure

Due to the biosafety, contamination, and logistical issues associated with the use of liquid culture, a number of infrastructure recommendations should be considered before implementing such a system in any laboratory. Most importantly, for the protection of the laboratory staff, aerosols produced during specimen processing and culture inoculation need to be minimized and contained. Specimen processing for culture purposes has to be performed in Biological Safety Cabinets (BSCs), at least in Biosafety Level 2 (BSL2) facilities. However, culture manipulation (conventional identification, subculturing and DST activities) must be performed in BSL3 facilities (see Annex 2 for a brief summary of the minimal requirements for a BSL3). For most high burden countries, there are major constraints to the successful establishment, staffing and maintenance of BSL3 laboratories. Upgrading to BSL3 should be planned, financed and implemented according to a short-term plan, a responsibility of the country.

In case of power failure, infectious particles are no longer trapped in the HEPA filter and flow back to the open front, thus constituting a major bio-hazard for the personnel.
Thus, BSCs should be connected to a suitable UPS system with capacity for at least 15 minutes of extra running time, along with an inverter in any location where electricity supply may be discontinued. Again for staff protection, the high-speed centrifuge should be equipped with aerosol-containing canisters that are opened in a BSC. The centrifuge should also be refrigerated so that specimens do not become too hot during centrifugation, which can lead to unwanted mycobacterial killing.

Liquid culture automated readers require a continuous power supply. In all settings, the instrument should be connected to a UPS to avoid loss of culture viability and data in the event of a power outage. In laboratories where power is frequently lost for extended periods of time, it is advantageous to use multiple UPS devices or to connect the instrument

to a back-up generator. Because the instrument is constantly using power, it is also continuously releasing heat into the room. When added to the other laboratory equipment, the ambient temperature in the laboratory can become significantly increased. Extreme temperatures, whether resulting from the equipment or the climate, can affect the stability of reagents, viability and contamination of specimens, and all the electronic control mechanisms of certain lab equipment. All of the existing automated culture instruments have a maximal operating temperature of 28-30°C, thus, the laboratory should be equipped with some type of air-conditioning unit(s).

Taking into account all of the equipment required to perform solid or liquid culture and DST, it is important that the physical space in the laboratory is sufficient to accommodate the machines and the laboratory staff performing the procedures.

Prevention procedures of cross contamination should be strictly followed. It has been shown that in some settings in low-incidence countries, cross-contamination rates could be higher than 10%. In high-incidence countries, as more positive specimens are processed, the risk for carryover of TB bacilli from positive to negative specimens may be even greater. Sufficient equipment, space, and personnel should be provided in order that separate BSCs can be dedicated to the processing of specimens while others are used for manipulation of cultures (e.g., for DST, subcultures). In addition, culture processing in the batch mode should not be done, and the order of processing should proceed from smear negative to smear positive. These methods have been successfully implemented in high-incidence settings (e.g., South Africa).

Human resources

The success of liquid culture and DST is highly dependant on the laboratory staff performing the procedures. With training and experience, initially high culture contamination rates can be decreased to acceptable levels within a short period of time (1-2 months). Off-site training in a laboratory which is experienced in performing liquid culture and DST exposes the visiting laboratorians to habits and techniques that can aid them in implementing liquid culture in their own laboratory. Because each setting is different, however, not all laboratories have access to the same resources. Thus, it is recommended that even if off-site training is performed, on-site training is also made available as described in the customer support plan. Such training by a laboratory expert will help to streamline the workflow and implementation process. For liquid culture and DST systems, provision of a procedure manual that contains more detailed guidance than laboratory standard operating procedures (SOPs) can be helpful.

It is important to note that with the increased sensitivity and decreased time to detection associated with liquid culture, there also comes an increase in the laboratory workload. The use of automated systems requires daily removal of vials which will need AFB smear and evaluation, species identification, purity plate inoculation and evaluation, and sub-

culturing. These processes translate to increased human resource needs that should be evaluated prior to implementing liquid culture and DST into the laboratory.

Commercial sales contract

The commercial sales contract should address the following issues:

Whether implementing or maintaining a liquid culture and DST system, it is important to have access to an ample supply of consumables and reagents well within their expiration dates (> 6 months after arrival in the laboratory). Failure to do so may cause unnecessary interruptions in the use of the system or may impact the validity of the results, both of which are can be detrimental for the patient.

Because liquid culture and DST manufacturers may not have distributors near the laboratory using the system, ordering, shipping, and receiving/customs logistics should be well-coordinated between the manufacturer and the customer in advance to facilitate customs clearance.

The manufacturer and/or shipping service should guarantee optimal shipment conditions to the end user, taking any temperature/humidity sensitivities of the goods into consideration.

The customer is responsible for timely reporting of any shipping or reagent quality control (QC) concerns to the manufacturer.

The contract should include a detailed customer plan (see below) addressing training of the staff, maintenance and reparation issues, and claim procedures. Communication channels should be clearly identified and specified.

Customer support plan

In addition to performance and cost considerations, a purchaser should consider the logistical and technical support that is provided. For this it is imperative that a commercial entity provide its potential customers with a customer support plan outlining specific training and scientific/technical support procedures.

In order to ensure proper implementation and ongoing successful use and performance of the product, the customer support plan should provide country-by-country descriptions of measures that guarantee device installation and maintenance, as well as device repair or exchange, as needed. Because maintenance and repair often will be performed by a third party, the customer support plan should include instructions on how to achieve contractual agreements with suitable organizations/institutions/ companies to carry out these functions. Maintenance of the equipment and spare part replacement should be scheduled and detailed

in the contract. An emergency system for repairing should be in place in case of malfunction or break event.

Due to the complexity of liquid culture and DST systems, the customer support plan must detail the measures which guarantee product (assay/device) training country–by–country. These measures are to include the preparation of training materials (handbooks, troubleshooting) and identification of appropriate trainers (including contractual agreements). The manufacturer should also detail the scope of scientific/technical inquiry support they will provide, as well as how this support will be provided (i.e., call centers). Once the support mechanisms are defined, the manufacturer should make the customer aware of how information will flow between the inquirer and the support staff.

To address potential customer concerns, the manufacturer should define who will be responsible for claim handling in each country (including contractual agreements), and describe the measures in place to guarantee claim resolution. There must also be defined procedures in place for the exchange and distribution of lot-specific information to all affected customers. To maintain customer satisfaction and continually improve upon the customer support processes, the manufacturer should provide a description of how to guarantee efficiency of customer support activities in each country. This guarantee should include the procedures in place for a quality assurance monitoring system, as well as who is responsible for this system, and strategies for permanent improvement of customer support efficiency.

Specimen Collection, Storage, and Transport

Implementing liquid culture and achieving and maintaining acceptable contamination rates is not the sole responsibility of the laboratory. Its success depends on political commitment and may require programmatic adjustments, specifically in the organization and conditions of specimen transportation to the culture laboratory from the peripheral laboratory. It is important to note that liquid culture methods are not compatible with cetylpyridium chloride (CPC), and its use should be avoided for specimens to be cultured in liquid media. Rather than relying on CPC to control contamination, specimens should be kept refrigerated and sent to the laboratory within no more than 4 days from the time of collection. Often this will require investing in a rapid and reliable specimen transportation system. Doing so will not only increase the quality of culture results, but also afford the opportunity to offer culture services to TB suspects and patients in more remote areas. If transport times are prolonged, specimens must be maintained at 2-6 ºC which can be achieved for little cost with the use of cold boxes and reusable ice packs.

Recording and Reporting

In order for patients and TB programs to benefit from the decreased time to detection and increased sensitivity associated with liquid culture, clinicians and patients must receive the

results in a timely manner. Therefore, it is important that the laboratory puts into place a consistent and expedient recording and reporting system. Cultures identified as positive for *M. tuberculosis* and DST results indicating drug resistance, especially MDR, should be reported to the appropriate clinician as soon as possible. This can be achieved in a variety of ways, including telephone, fax, computer, or messenger. Then, once the clinician receives a patient's results, he or she must make every effort to locate the patient quickly in order to get the patient on appropriate therapy and halt further transmission.

In order to streamline these processes, it is useful to measure the time taken for each step from specimen collection through appropriate treatment initiation. Identifying and addressing any unnecessary delays will ultimately benefit individual patients as well as the TB program as a whole.

Phased implementation

As noted above, the decision to implement a liquid culture and DST system should be based on need and be consistent with a country's plan for TB laboratory capacity strengthening and expansion. Such plans should be considered only in countries with a strong network of quality-assured microscopy.

In most circumstances, the first priority would be to implement the system in the NRL, assuming that the NRL is currently supervising quality-assured (QA) microscopy of the laboratory network and performing QA TB culture and DST. This would provide valuable experience that could be applied if a decision were made to scale up the system. In those countries where a liquid culture and DST system is currently in use at the NRL, the decision to scale up should be informed by the accumulated experience at the NRL with the liquid culture system.

Subsequent expansion of liquid culture and DST capacity would logically be to regional TB culture and DST laboratories. The extent of scale up should be determined by need and availability of funding, and again consistent with a national laboratory plan.

There has been limited experience on implementing liquid culture (with or without DST) in a laboratory that has not already established TB culture on solid media. However, it is expected that this experience will rapidly accumulate and that a general recommendation on this practice can be made in the near future.

Choice of a liquid culture and DST system

FIND's demonstration projects of liquid culture and DST have involved one commercial system. It is likely that most of the findings would also apply to the other commercially available systems, although they are not identical. Automated liquid culture systems decrease workload and result in faster time-to-detection, as cultures are read constantly. However, automated systems are more costly and subject to malfunction. The choice

between an automated system and manual reading is dependent upon available resources and anticipated workload. Depending on labor costs, laboratories processing fewer than 50 cultures per week may find it more cost-effective to use manual liquid culture. In addition to overall costs and training requirements, provision of a comprehensive and credible customer support plan is essential.

Consideration of liquid culture only

Current international guidelines recommend that all specimens that are cultured on liquid media also be inoculated on solid media. As noted in FIND's report, several laboratories have abandoned routine solid culture after establishing and validating liquid culture. The preliminary data from the FIND demonstration projects would support this policy. However, at this time a global recommendation on this practice cannot be made. Such a decision would also be based on locally developed data, including cost data. If such a practice is adopted, solid culture will continue to be needed for work-up of positive liquid cultures and as a back-up system should liquid culture be interrupted.

Species identification

It is imperative that all mycobacterial isolates be speciated at least to the level of *M. tuberculosis* complex vs. NTM. When using liquid culture, with the expectation that time-to-detection will be significantly reduced, it is also imperative that a rapid method of species identification be used. Molecular tests and other methods (e.g., HPLC), while highly accurate and rapid, are expensive. FIND evaluation studies of a lateral flow immunochromatographic slide assay found that the test was easy to use and accurately identified *M. tuberculosis* complex in cultures within five minutes. At a fraction of the cost of other tests for rapid species identification, this assay should be considered for general use. Where identification of NTM is needed, standard biochemical tests or other methods can be considered.

Quality Management Systems (QMS) and SOPs

SOPs for liquid culture and DST must be put into place and carefully followed. SOPs developed for FIND's DST demonstration projects are included in the FIND report. In order for laboratory results to be of use to the clinician and patient, it is imperative that the laboratory have QMS in place for pre-analytical, analytical, and post-analytical phases of testing, including indicators for monitoring and evaluation of the laboratory diagnostic services. These systems must include not only internal quality control protocols, but also external quality assurance (EQA) for smear microscopy, and proficiency testing for culture, identification, and DST. Ideally, ongoing outside technical assistance should be available as needed.

Future information for WHO use to support countries

Detailed costing data and cost-effectiveness analyses are included in the FIND projects. Preliminary cost data were included in the FIND report. FIND expects to have a detailed report available for WHO and partners use in 2008.

Data on improved patient management resulting from more rapid case detection and MDR TB diagnosis will also be presented to WHO in 2008. This includes results from two liquid culture demonstration projects that are evaluating the new WHO algorithm for the diagnosis of AFB smear-negative TB in high HIV prevalent settings. Partner organizations within SLCS will also monitor implementation and scale up of liquid culture and DST in low income settings. Information collected during the coming year will be presented to WHO in 2008.

During the coming year, the SLCS will prepare materials to assist laboratories in implementing and strengthening TB culture and DST. These include performance indicators for internal quality assurance and procedures for EQA for TB culture.

ANNEX 1 : List of references

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8. Systematic review of fully automated liquid culture tests. Chapter 14 In "A systematic review of rapid diagnostic tests for the detection of tuberculosis infection" J. Dinnes (ed), Health Technology Assessment 2007;vol. 11:No 3.

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ANNEX 2. Brief summary of WHO recommendations for BSL3 (from Laboratory biosafety manual, Third edition, WHO, 2004).
Some illustrative examples are indicated for practical application. Brief summary of WHO recommendations (Laboratory Safety Manual, Third edition) with some illustrative examples for practical application.

	BIOSAFETY LEVEL		
	1	2	3
Isolation[a] of laboratory	No	No	Yes
Room sealable for decontamination	No	No	Yes
Ventilation:			
— inward airflow	No	Desirable	Yes
— controlled ventilating system	No	Desirable	Yes
— HEPA-filtered air exhaust	No	No	Yes/No[b]
Double-door entry	No	No	Yes
Airlock	No	No	No
Airlock with shower	No	No	No
Anteroom	No	No	Yes
Anteroom with shower	No	No	Yes/No[c]
Effluent treatment	No	No	Yes/No[c]
Autoclave:			
— on site	No	Desirable	Yes
— in laboratory room	No	No	Desirable
— double-ended	No	No	Desirable
Biological safety cabinets	No	Desirable	Yes
Personnel safety monitoring capability[d]	No	No	Desirable

[a] Environmental and functional isolation from general traffic.
[b] Dependent on location of exhaust (see Chapter 4).
[c] Dependent on agent(s) used in the laboratory.
[d] For example, window, closed-circuit television, two-way communication.

1. The containment BSL3 laboratory must be separated from the areas that are open to unrestricted traffic flow within the building. Additional separation may be achieved by placing the laboratory at the blind end of a corridor, or constructing a partition and door or access through an anteroom (e.g. a double-door entry or basic laboratory – Biosafety Level 2), describing a specific area designed to maintain the pressure differential between the laboratory and its adjacent space. The anteroom should have facilities for separating clean and dirty clothing.

2. Surfaces (walls, floors, ceilings) should be of non-porous and seamless surface to be easily cleaned. Glossy paint can be used as a means to achieve this.

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3. The laboratory room must be sealable for decontamination. Sealing can either be part of the design or by the use of thick plastic sheets, placed appropriately to create the non-permeable condition.

4. Windows must be closed, sealed and break-resistant.

5. There must be a controlled ventilation system that maintains a directional airflow into the laboratory room. BSCs ducted to the outside ensure such a negative pressure when operating.
A visual monitoring device with or without alarm(s) should be installed so that staff can at all times ensure that proper directional airflow into the laboratory room is maintained. A small piece of light tissue can be used for this purpose. To facilitate air entry into the containment room, the access door may be equipped with grids at the bottom (preferably with pre-filters to eliminate dust, at least partially). The visual indicator should be placed at this level and be flapping up when BSCs are on as evidence of efficient airflow circulation.

5. The building ventilation system must be so constructed that air from the containment laboratory – Biosafety Level 3 is not recirculated to other areas within the building. When exhaust air from the laboratory (other than from biological safety cabinets) is discharged to the outside of the building, it must be dispersed away from occupied buildings and air intakes.

6. The exhaust air from Class I or Class II biological safety cabinets, which will have been passed through HEPA filters, must be discharged in such a way as to avoid interference with the air balance of the cabinet or the building exhaust system. All cabinets must be vented to the outside, either with hard duct or with thimble connection.

7. An autoclave for the decontamination of contaminated waste material should be available in the containment laboratory. If infectious waste has to be removed from the containment laboratory for decontamination and disposal, it must be transported in sealed, unbreakable and leakproof containers according to national or international regulations, as appropriate.

8. At Biosafety Level 3, manipulation of all potentially infectious material must be conducted within a biological safety cabinet installed according to the Table below.

9. Three factors are important to define BSL3, including i) physical requirements as mentioned above and sustained maintenance of the equipment and installation, ii)

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adherence to BSL3 practice using protocols minimizing aerosol production and spill, iii) adequate training of staff and safety plans practiced.

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Table 9. ***Differences between Class I, II and III biological safety cabinets (BSCs)***

BSC	FACE VELOCITY (m/s)	AIRFLOW (%)		EXHAUST SYSTEM
		RECIRCULATED	EXHAUSTED	
Class I[a]	0.36	0	100	Hard duct
Class IIA1	0.38–0.51	70	30	Exhaust to room or thimble connection
Class IIA2 vented to the outside[a]	0.51	70	30	Exhaust to room or thimble connection
Class IIB1[a]	0.51	30	70	Hard duct
Class IIB2[a]	0.51	0	100	Hard duct
Class III[a]	NA	0	100	Hard duct

NA, not applicable.
[a] All biologically contaminated ducts are under negative pressure or are surrounded by negative pressure ducts and plenums.