Report on Cameroon laboratory network technical assistance visit

Dr. Armand Van Deun, Lab Consultant The Union, 21 May to 5 June, 2010

This visit was made in the context of the TBCAP project, "Optimising the functioning of the SRLN on the African continent". It concerned a first assessment of a good candidate new Supra-National TB Reference Laboratory (SRL) for Africa, one of the key objectives of the project.
In an earlier phase of the project, a questionnaire had been sent out to all TB national reference labs (NRL) and national TB programmes (NTP) in Africa, with the aim of assessing the current status of their laboratory services in-country and their suitability to be upgraded to regional or supra-national reference laboratories. Analysis showed that most laboratories declared themselves to be candidates, but also revealed serious weaknesses for most of them.
The objective of this assessment was to evaluate the suitability of the TB laboratory of the Pasteur Institute Yaounde (CPC), the Cameroon NRL, to be upgraded to SRL status, eventually after a tutoring period linked to an established SRL.
Most of the visit time was thus spent assessing the CPC TB laboratory. However, to be able to function as a SRL, a NRL must be comfortable in its own country regarding laboratory services essential to the NTP. These include the microscopy network, including training, logistical and quality assurance (internal and external, EQA) aspects under guidance and/or with support of the NRL, besides continuous drug resistance surveillance (DRS) and MDR-TB management with ideally full country coverage. The latter require good culture and drug susceptibility testing (DST) services, including rapid DST. Overall, well functioning intermediary level laboratories supporting the NRL will be needed to make this possible. For these reasons, the assessment included also up-country visits to selected intermediary level and smear microscopy laboratories, mainly targeting those doing LED fluorescence microscopy (FM), EQA, culture and DST.

Calendar of the visit:

- Friday, 21 May: travel to Yaounde
- Saturday, 22 May: work with Dr. Sara Eyangoh, Head of CPC TB lab
- Sunday, 23 May: study NRL standard operating procedures (SOPs)
- Monday, 24 May: local holiday
    - review of CPC infrastructure and equipment
    - review of NRL lab methods, techniques and performance
- Tuesday, 25 May
    - work with Dr. Sara Eyangoh, Head of CPC TB lab: assessment questionnaire
    - meeting with Dr. Juergen Noeske, German Cooperation and Advisor to NTP
- Wednesday, 26 May
    - further review of NRL lab methods, techniques and performance
    - meeting with Dr. André Gotingar, NTP Manager, and staff
    - collection of information at NTP (Mr. Ndi, laboratory focal point)
    - travel to Douala
- Thursday, 27 May, Douala
    - visit Baptist Hospital CEBEC Bonaberi: evaluation of culture and rapid DST, LED FM microscopy, microscopy EQA, lab infrastructure and equipment
    - visit Barcelone H. Center: LED FM microscopy; reagent preparation
- Friday, 28 May, Douala
    - study NRL and NTP documents

- Saturday, 29 May: travel Douala - Kumba
    - visit Limbe SW Region regional Hospital lab: EQA microscopy; LED FM performance; preparation staining solutions and quality control
- Sunday, 30 May: travel Kumba – Bamenda (NW Region)
- Monday, 31 May: Bamenda
    - visit Mezam polyclinic: regional culture and DST laboratory
    - discussions regarding expansion of surveillance / programmatic management of MDR-TB
- Tuesday, 1 June: Bamenda; travel to Yaounde
    - visit NW Region regional hospital: EQA, ZN performance, preparation staining solutions and quality control
    - ESTHER project: plans new TB culture/DST laboratory at regional hospital
- Wednesday, 2 June: Yaounde
    - visit Jamot national hospital laboratory: LED FM
    - functionality of sputum referral for culture / DST to CPC
- Thursday, 3 June
    - visit CPC laboratory: completion of assessment review
    - debriefing preparations
- Friday, 4 June
    - meeting with Permanent Secretary for Health
    - debriefing at NTP
    - report writing
    - return flight

A detailed assessment regarding the NTP, its laboratory services and the suitability of CPC Pasteur as candidate new SRL has been drawn up during the visit with the help of Dr. Eyangoh and Mr. Ndi, using the by WHO prescribed format. This assessment has been handed over to Dr. Eyangoh for submission to WHO together with the other documents requested meantime by Dr. Gilpin from WHO HQ.

Main findings and conclusions

Overall, the CPC TB lab is a good candidate new SRL. The SRL tasks fit entirely in the Institute's mission, infrastructure and equipment are excellent, all major techniques recommended by WHO are available and regularly used (except for liquid culture in the BACTEC MGIT 960 system, which is expected to be installed under the UNITAID EXPAND-TB project), and technical performance is very good. The Head, Dr. Sara Eyangoh, PhD, is of consultant level and already trained as such with support from TBCAP. Moreover, the TB laboratory network in the country is relatively well developed, with ongoing decentralization of guidance, microscopy quality assurance and logistical responsibilities to regional level and availability of culture/DST as well as LED fluorescence microscopy (FM) already at a few of these regional laboratories. Continuous drug resistance surveillance (DRS) and systematic management of MDR-TB covers by now a reasonable part of the country, with plans for expansion.

However, there are a few major obstacles to overcome, in particular the status of CPC as perceived by the government and the support it receives from that side. Although it considers itself as a national institution, government sees CPC as private or at best a parastatal. It receives support from government, but guaranteeing government salaries at CPC is highly problematic. Technical staff at the TB lab, 3 technicians besides the Head, is absolutely insufficient even with present workload; under the 9th GFATM round two more technicians

will be added, but this will not solve the deficiencies and increased demand in case of SRL status on the management side. Already now CPC has problems to deliver up to the expectations of several key players (particularly for DRS and rapid DST). A second major obstacle is the objection of the government to set up liquid culture/DST by MGIT (at least outside CPC) because of non-sustainability, and possibly also because of the second proposed location where it would need to fund costly infrastructural works (negative pressure and uni-directional airflow) to make this possible. Both other functional culture/DST laboratories, and particularly that at Douala, lack the high security level and partly also the equipment needed. This is blocking the signature of the EXPAND-TB agreement, which foresees the need for two MGITs and two P2/P3 labs, also at CEBEC Douala (a private hospital), besides CPC where this facility already exists.

In spite of the overall good or very good performance and level of development / coverage by the TB laboratory services, there remains of course scope for improvement. Here below we will elaborate mainly on the weaknesses observed.

Observations

- The NTP lacks vision and clear planning regarding improvement and expansion of the laboratory network for TB microscopy, DRS and MDR-TB management:
    - prospects for use of LED FM: the visit and an earlier assessment by CPC made it clear that the technique was very well received and gives excellent results, with much reduced workload and more positives being detected. Procurement of supplies is ongoing. However, all available instruments have not been installed as yet, and no plan for further expansion was made.
    - referral of patients for culture and DST. These services now cover 3 major cities (Yaounde, Douala and Bamenda) and part of the coast and N. West regions. In absence of clear instructions from the NTP, they remain too much dependent on referral of cases by a few interested physicians and organization / cost coverage by the German Technical Cooperation (GTZ). The Rd 9 GFATM plan foresees expansion of DRS coverage to 5 regions (Centre, Littoral, West, N. West and North) by 4 functional culture/DST laboratories (CPC Yaounde, CEBEC Douala, Mezam Bamenda and Pasteur Garoua).
    - indications for culture and DST, by method: it is not clear where and for which type of patients the various available slow or rapid techniques are going to be used. So far the since one year available rapid techniques have been under-utilised (for slide DST also since it has not yet been recommended by WHO; evaluation at CPC Yaounde is now almost complete, and shows highly satisfactory results)
- There has been too little coordination between NTP and NRL, and NTP has given too little support to NRL in terms of staffing and other running costs. This should improve once Rd 9 GFATM funds are available, with budget lines for 2 technicians and some running costs for CPC besides one technician each for the 4 other culture / DST labs, where staff is insufficient in numbers
- Staff turn-over at microscopy labs is high, with many NTP non-trained staff involved, sometimes (i.e. Bamenda regional hospital) leading to poor results. Training and supervision of the microscopy network are too much ad hoc, due to obstacles for the planned use of available budgets.
- SOPs and technical guidelines exist for all routine techniques, but need to be corrected / completed / updated. At intermediate level, SOPs for FM were not conform with the

technique used; also those for preparation of staining reagents and their quality control (QC) need to be improved

- Registration and reporting at culture/DST labs need to be improved:
    - often essential patient / sample information is missing, mainly due to lack of a good request form (except at Bamenda)
    - at least at CPC a computer database and automated, more complete analyses and reporting are also hampering complete and regular analyses for internal QC and epidemiological monitoring and reporting
    - manual analysis and reporting by the two other functional culture/DST labs at CEBEC Douala and MEZAM Bamenda is partial and it is not clear how the results are used for epidemiological / operational purposes
- Procurement of lab supplies is very irregular, with excessively largely quantities being ordered at too long intervals. This is due to delayed availability of approved government funds, and the difficulty to assure quality products (tender regulations). Decentralised preparation and distribution of Ziehl-Neelsen (ZN) staining reagents works well, but intermediate labs lack essential small equipment and supplies, i.e. balance, glassware, distilled water. Procedures (SOPs) don't seem to be uniform. Preparation and distribution of FM staining reagents, now in too large quantities from CPC or Douala regional coordination, need to be revised in view of their very short shelf-life. The same destaining reagent as for ZN has been used because of lack of alcohol, seemingly with acceptable results.
- Procurement of culture/DST supplies depends entirely on the individual laboratories, causing problems for slide DST at CEBEC. Costs have to be covered mainly by the patients and institutions; a regular NTP budget will be available only under GFATM Rd 9.
- The microscopy network is somehow covered by EQA, but the system is highly dependent on supervision visits and availability of funding. Main problem is lack of procedure and data uniformity, compilation, analysis and feedback for quality improvement, due to changing instructions over the years and too little coordination from the central level. Internal controls, i.e. on prepared staining solutions, have started but are not quite satisfactory. A LED FM quality assurance system needs to be developed. There is very incomplete and heterogeneous reporting of microscopy network routine performance and particularly of its EQA; available data are under-utilised.
- Culture internal quality assurance is only partially done, and lacks analysis of false negative cultures (versus contamination). Although proficiency testing with the Antwerp SRL has been done with good results annually for several years now, DST quality assurance needs to be completed by periodical rechecking of NRL strains at the SRL, besides internal validation and monitoring of profiles. DST quality assurance inside the country starts to be organized.

Recommendations

Overall strategy:
CPC will need more support to be able to assume the tasks of a SRL as well as to continue functioning as NRL, as outlined below. Moreover, considering the demand and opportunities, some re-organization seems advisable to relieve the workload at CPC. With more culture and (rapid) DST laboratories, also at Jamot hospital, CPC responsibilities could be shifted more towards coordination, training, quality assurance and logistical support of the networks. The current plan for expansion of culture/DST thus seems appropriate, although a better location may be needed for these labs in Douala and Bamenda. MGIT may better be installed at CPC (no problem) and at Bamenda regional hospital in absence of a suitable location at Douala and because of expected infrastructure improvements at Bamenda. It is recommended to use MGIT for detection of smear-negative TB among patients presenting at Jamot / Bamenda hospital only. MGIT is highly performant for TB detection, but not for its DST where it is known to have problems (more rifampicin as well as ethambutol false results), at least as long as the EXPAND-TB project provides the consumables. Rapid DST should be used routinely: slide DST can be installed at all these laboratories considering its low safety requirements and running costs, to be used preferentially for fresh or cold-chain sputum; otherwise sputum preserved in ethanol with killing of the TB bacilli should be sent for testing at CPC only, which can yield sufficiently fast results with excellent logistics. LJ DST should be used in parallel for the same patients as a backup and for quality assurance; it should only be done at labs with a sufficient safety level (P2); otherwise, a safe and simple culture method and acidified Ogawa with forwarding of strains to CPC can be used.
Intermediate level laboratories at the regional hospitals should be reinforced by expanding LED FM in all regions, by giving them the necessary small equipment for preparation and quality control of staining reagents, and by regular support for EQA and supervision visits. SOPs for FM and reagent preparation need to be harmonized.

Specific points:

- Staffing at the NRL needs to be reinforced considerably, at least with an epidemiologist / data manager and a technical supervisor besides the two technicians from GFATM. Training for NRL key staff will be needed for SRL preparations: TB control course and joint consultancy missions for the Head; data management course.
- Additional culture & DST equipment as well as infrastructural works will be needed mainly at regional sites, to meet the increased needs for expansion of DRS and MDR-TB management.
    - Decentralization to 4 culture/DST labs (Douala, Garoua, Bamenda and a new one soon to be started at Jamot Hospital) is planned under GFATM.
    - An EXPAND-TB MGIT machine should be installed at CPC, with TA for training. The second machine foreseen under the project would better be installed at Bamenda regional hospital where prospects for the installation of a negative pressure laboratory with support of the ESTHER project / GTZ are much better, and where this machine has far better chances to be useful than at a facility such as CEBEC in Douala. The MEZAM culture/DST lab should then be moved to the regional hospital, as has been already considered.
    - Simple culture technique on acidified Ogawa medium is recommended for laboratories with low safety level such as CEBEC.
    - Recommended equipment (replacement / new) for Douala and Bamenda labs is listed in Annex 1. A list of materials and supplies needed for new slide DST labs (Bamenda, Jamot, also Garoua?) is in Annex 2.

- The SOPs for various tests need to be revised, updated and completed. Remarks and suggestions for CPC SOPs are given in Annex 3. A SOP for bulk preparation and quality control of staining solutions (for ZN, for FM) should be adopted and distributed. An example is found in Annex 4.
- Quality assurance needs to be strengthened:
    - microscopy EQA needs a uniform protocol, refresher training, improved reporting and analysis at central level. The globally recommended analysis workbook should be used for compilation of reported data and more refined analysis.
    - rechecking for FM should be organized from all LED FM laboratories. It may best be done at CPC; internal positive controls for FM need to be used consistently. In the large laboratories, such as Jamot, where keeping all slides is problematic, the Head of the lab should select a slide from the bench randomly one day a week, adding it to the sample box for collection by the regional lab supervisor.
    - a uniform system of internal quality control of cultures should be adopted by all culture labs
    - DST rechecking should be organized with the SRL for the NRL, and with the NRL for the regional labs. Slide DST quality assurance will mainly be done by internal monitoring of results, besides comparison with final LJ DST on the parallel specimen.
- A national strategy for DRS and early detection of MDR-TB needs to be developed:
    - definition of suspects to be screened, screening and referral procedures. Analysis of the pilot data from systematic screening of retreatment cases from the Littoral shows that the highest proportion MDR is found among failures (70%), the lowest among defaulters (5%); the percentage among relapses is modest, around 20%, but this groups brings the highest number of cases. It might thus be most efficient to focus on rapid DST of failures and relapses, already after first treatment to avoid toxicity of streptomycin.
    - at intermediary level labs with functional LED FM (and electrical power supply not too bad), FDA vital staining could be used systematically for MDR suspect screening; only those positive in the test should then undergo rapid DST. If well accessible, patients / sputum should be referred for slide DST; from areas with difficult access, and for MDR-TB suspects failing this DST, sputum killed in alcohol should be referred for molecular testing. With good organization (sample transport; prompt testing and feedback of results) the latter technique needs to be available only at the NRL.
    - DRS and MDR-TB management coverage needs to be expanded to the whole territory gradually, following development of facilities for detection and correct DOTS treatment. Under GFATM planned expansion is adequate: Bamenda covering NW and W region; Garoua covering N and later also Extreme N. and Adamoua.
- FM at intermediary level should be expanded to:
    - reduce the routine workload at these often overloaded laboratories
    - make FDA vital staining possible
    - free time for rechecking of ZN smears from other labs in the region

  Of the two LED FM instruments that still remain at CPC, one should be given to Bamenda Regional Hospital instead of for instance Bafia, considering overload and poor ZN results at Bamenda RH.

- Regional labs must be further upgraded with minimal equipment for (bulk) preparation of staining solutions. At least they will need a balance, magnetic stirrer / hot plate, suitable glassware (large conical flasks; measuring cylinders) and large containers (jerrycans); cf. list in Annex 4. For FM, consider using staining jars at all labs (also Jamot) to improve quality, sometimes done too fast now. Make sure the solutions are renewed at least twice weekly, and that they remain protected from light.
- Develop / improve registration and reporting for culture/DST:
    - adopt and disseminate a proper request form, sufficiently detailed to allow internal monitoring of cultures / DST as well as epidemiological and operational analyses. The globally recommended format is found in Annex 5.
    - standardize the registers, conform with international recommendations
    - install a computer database at least at CPC
    - introduce standardized analyses for internal monitoring and reporting, conform with international standards (Annex 6)
- Develop a culture/DST supply system for all culture/DST/molecular labs. The responsibility should be given to the NRL, and it is highly recommended to give them also the responsibility for use of funds under this GFATM budget line:
    - LJ and/or acidified Ogawa to be prepared at CPC (also for Garoua and Jamot); and at Bamenda also for Douala
    - animal serum for slide DST to be collected, distributed and prepared by CPC, for intermediary labs having difficulty with local procurement
    - consider CPC preparing stock solutions of FDA and DST antibiotics with regular supply of freshly prepared aliquots to DST labs, before freezing, to prevent excessive losses of frozen stocks in DST labs with serious power-cuts
    - CPC to coordinate and execute procurement and distribution of culture / DST supplies from abroad

Dr. Armand Van Deun

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Annex 1 Recommended equipment (additional / replacements) for intermediary level culture/DST labs

The equipment proposed is based on observations at the CPC Yaounde, CEBEC, MEZAM and Jamot labs. It supposes that only FDA staining, slide DST and simple culture on acidified Ogawa (without centrifugation) are performed.

CPC Yaounde:

- a larger coagulateur for egg-based media
- more practical equipment for preparation of staining solutions (magnetic stirrer; large conical flasks..) as proposed for the intermediary labs

Douala:

- 1 wider safety cabinet: at least 120 cm; or locally made extraction hood (without filters, as is the case now), with a strong extraction fan mounted in an outer wall, airtight tubing and the exhaust as high as possible
- 1 dry oven or waterbath
- 1 second large pressure cooker or small autoclave for clean materials
- (1 larger incubator would be nice)

Bamenda:

- 1 sensitive electronic balance (0.1 mg)
- 1 larger autoclave
- if more cultures / DST is done on LJ: 1 larger centrifuge and 1 larger inspissator
- 1 surge protector / large UPS (indispensable for MGIT)

Jamot:

- 1 safety cabinet at least 120 cm; or locally made extraction hood (without filters, as is the case now), with a strong extraction fan mounted in an outer wall, airtight tubing and the exhaust as high as possible for the fan power
- 1 sensitive electronic balance (0.1 mg)
- 1 small autoclave; large pressure cooker
- 1 dry oven or waterbath
- 1 incubator 400 L

Annex 2 List of small materials and supplies for slide DST labs

Item	Specifications	Measuring unit	Supplier & Ref. no.	Reference	Pack unit	Pack size in measuring units	Price (€) per pack	Number of packs ordered	Cost Euro
Para benzoic acid	Para benzoic acid (4-Nitrobenzoic acid),	grammes	Acros	128461000	bottle	1000	11,6	1	11,6
7H9 medium base powder ready-mix	Middlebrook 7H9 medium base powder, ready-mix	grammes	Becton Dickinson		bottle	500	220,0	1	220,0
Glass cutter, steel wheel type	Glass cutter, steel wheel type	pc	Bohle Benelux BV, Nieuweweg Noord 314 B-20 NL-3905 LX Veenendaal.D35 T +31 318 553151 / F +31 318 519859 / info@bohle.nl		pc	1	10,8	1	10,8
reagent bottle 250 ml	250 mL thick glass bottles with screw cap (Schott ® type), autoclavable (up to 135°C), suitable for freezing,	pc	Boom, The Netherlands - Lab Logistics Group	9.071.992	pc	1	10,6	12	127,2
reagent bottle 500 ml	500 mL thick glass bottles with screw cap (Schott ® type), autoclavable (up to 135°C), suitable for freezing,	pc	Boom, The Netherlands - Lab Logistics Group	9.071.995	pc	1	12,8	12	154,0
Autoclave tape	Autoclave tape, 55mx19mm, per roll	roll	Boom, The Netherlands - Lab Logistics Group	38030195	roll	1	9,7	6	58,4
Filter paper	Filter paper, 470x570 mm	sheet	Boom, The Netherlands - Lab Logistics Group	20000001	pack	100	15,0	2	30,1
Micropipette	Micropipette, variable volume , 50-200 µL, autoclavable	pc	Boom, The Netherlands - Lab Logistics Group	13731148	pc	1	274,0	1	274,0
Petri dish	Petri dish, in glass, d=120mm h=20mm	pc	Boom, The Netherlands - Lab Logistics Group	23755510	pc	1	1,9	100	186,0
Pi pump 2500	Pi pump 2500, for 10 mL pipette (green)	pc	Boom, The Netherlands - Lab Logistics Group	15125200	pc	1	9,5	2	19,1
Pincers, stainless steel	Pincers, stainless steel, 105 mm lenght, for coverglass, straight	pc	Boom, The Netherlands - Lab Logistics Group	LLG9160205	pc	1	4,7	2	9,4
Pouring rings	pouring rings, blue, PP, thread 45	pc	Boom, The Netherlands - Lab Logistics Group	25242280	pc	1	40,0	1	40,0
Graduated pipette 10 mL	Graduated pipette, glass 10 mL, autoclavable, with constriction for cotton plug	pc	Boom, The Netherlands - Lab Logistics Group	40111437	pc	1	3,3	12	39,0
Insulin syringe 100 Unit =1mL	Insulin syringe 100 Unit =1mL	pc	Boom, The Netherlands - Lab Logistics Group	38745019	pack	100	11,5	5	57,7
Screwcap,autoclavable	Screwcap , autoclavable, blue, thread 45	pc	Boom, The Netherlands - Lab Logistics Group	25239280	1	1	1,1	40	42,0
Standard tips	Standard tips, 2-200 µl, Eppendorf, per 1000, non sterile, autoclavable	pc	Boom, The Netherlands - Lab Logistics Group	13730001	pc	1000	28,6	2	57,2
Microscopy slides	Slides26x76mm, frosted-end 20mm, packaging of 50 slides	pc	Boom, The Netherlands - Lab Logistics Group	38026762	pack	50	2,4	20	47,6
Staining dish	Glass staining dish 105x85x70 mm, pressed glass, with lid , per 10	pc	Chem Lab (Marienfeld)	MA 42 000 04	pc	1	6,7	10	67,0
Staining tray	Staining tray for 10 slides, insert for staining dish, pressed glass , per 10	pc	Chem Lab (Marienfeld)	MA42 000 05	pc	1	4,8	5	24,0
Wire handle for staining tray	Wire handle for glass staining tray , per 10	pc	Chem Lab (Marienfeld)	MA66 110 00	pc	1	1,2	5	6,0
Markerpen, indelible, fine point, black	Permanent marker designed to indelibly lable microscope slides and tissue cassettes, black ink provides high contrast, in boxes of 12 pcs.	pc	Chem Lab (Marienfeld)	61 306 03	pack	12	30,0	2	60,0
Microscopy slide blue coloured label	Slide 72x26 mm, colour labels (white, yellow, blue, green, pink, orange), packets of 50, for slide DST. Order: Marienfeld Unimark slides, per 5000 of 1 colour	pc	Chem Lab (Marienfeld)	07 043 02	carton	5000	220,0	1	220,0
Microscopy slide green coloured label	Slide 72x26 mm, colour labels (white, yellow, blue, green, pink, orange), packets of 50, for slide DST. Order: Marienfeld Unimark slides, per 5000 of 1 colour	pc	Chem Lab (Marienfeld)	07 042 02	carton	5000	220,0	1	220,0
Microscopy slide orange coloured label	Slide 72x26 mm, colour labels (white, yellow, blue, green, pink, orange), packets of 50, for slide DST. Order: Marienfeld Unimark slides, per 5000 of 1 colour	pc	Chem Lab (Marienfeld)	07 045 02	carton	5000	220,0	1	220,0
Microscopy slide pink coloured label	Slide 72x26 mm, colour labels (white, yellow, blue, green, pink, orange), packets of 50, for slide DST. Order: Marienfeld Unimark slides, per 5000 of 1 colour	pc	Chem Lab (Marienfeld)	07 044 02	carton	5000	220,0	1	220,0
Microscopy slide white coloured label	Slide 72x26 mm, colour labels (white, yellow, blue, green, pink, orange), packets of 50, for slide DST. Order: Marienfeld Unimark slides, per 5000 of 1 colour	pc	Chem Lab (Marienfeld)	07 040 02	carton	5000	200,0	1	200,0
Microscopy slide yellow coloured label	Slide 72x26 mm, colour labels (white, yellow, blue, green, pink, orange), packets of 50, for slide DST. Order: Marienfeld Unimark slides, per 5000 of 1 colour	pc	Chem Lab (Marienfeld)	07 041 02	carton	5000	220,0	1	220,0
universal bottle 28 ml	28 mL universal bottle, heavy duty glass, wide opening without narrowing at the neck; with aluminium screw cap with rubber lining inside	pc	Defrance, Wemmel	BS 150-15	carton	144	142,4	10	1423,6
rack for 48 universal bottles	rack for 48 universal bottles 28 mL , plastified metal	pc	Defrance, Wemmel	CJC3001	pc	1	40,0	10	400,0
Autoclave brown paper, 50 x 35 cm, 70 g, per 25 Kg, 4 packs of 6,25 Kg	Autoclave brown paper, 50 x 35 cm, 70 g, per 25 Kg, 4 packs of 6,25 Kg	pack of 6.25 kg	Epacar n.v. Duffelsesteenweg 160 B-2550 Kontich	8117	carton	4	85,5	1	85,5
Falcon tube, 50 ml	50 ml, plastic, sterile, conical with screwcap	pc	Greiner	Cellstar 227.261	carton	500	120	1	120,0
Falcon tube 15 ml	15 mL sterile conical tube (Falcon® type), plastic	pc	Greiner	cellstar 188271	carton	1000	137,9	1	137,9
Mitchison selective cocktail	Mycobacteria selectatab (Kirchner), 10 tablets, 1 tablet per 500 mL	pc	Lucron, St. Martens Latem	MAST MS24	bottle	10	70,0	5	350,0
Acetone	Acetone, ACS reagent, ≥99.5%	ml	Sigma Aldrich	179124-1L	bottle	1000	20,0	1	20,0
N-acetylcysteine	N-acetylcysteine, 100 grammes	grammes	Sigma Aldrich	A7250-100G	bottle	100	79,6	1	79,6
DMSO	Dimethyl sulfoxide (DMSO), ACS reagent, ≥99.9%	ml	Sigma Aldrich	472301-1L	bottle	1000	49,8	1	49,8
Glycerol	Glycerol, ReagentPlus®, ≥99.0% (GC)	ml	Sigma Aldrich	G7757-1L	bottle	1000	46,1	2	92,2
Nicotinamide	Nicotinamide , ≥98% (TLC), powder	grammes	Sigma Aldrich	N3376-100G	bottle	100	17,4	1	17,4
Tween 80®	Tween 80® (Polyoxyethylene Sorbitan Monooleate) , viscous liquid, 500 mL	mL	Sigma Aldrich	P1754-500ML	bottle	500	28,1	1	28,1
Fluoresceine diacetate	Fluorescein diacetate	grammes	Sigma Aldrich	F7378-5G	bottle	5	31,2	1	31,2
Ofloxacin	Ofloxacin	grammes	Sigma Aldrich	O-8757-5G	bottle	5	25,0	1	25,0
Kanamycin	Kanamycin sulfate from *Streptomyces kanamyceticus*	grammes	Sigma Aldrich	K1377-5G	bottle	5	106,0	1	106,0
Rifampicin	Rifampicin, plant cell culture tested, ≥97% (HPLC), crystalline , 1 g	grammes	Sigma Aldrich	R7382-1G	bottle	1	94,7	1	94,7
PBS tablets pH 6.8		tab	Sigma Aldrich		bottle	100	10,0	1	10,0
								TOTAL COST	**5644,9**

Annex 3 Remarks on existing guidelines and SOPs

Remarques Guide Technique Microscopie

- colorants utilisés : fuchsine 0,3%, acide sulphurique 25% ne sont plus utilisés par le PNT.
- p. 63, interprétation d'un résultat rares BAAR : pas correcte qu'on devrait considérer 1-3/100 ch comme négatif. La recommandation actuelle est de considérer positif même avec un seul BAAR dépisté pour la lame (à condition d'un contrôle de qualité régulier)
- p. 64 : il n'est pas recommandé de garder les frottis positifs et négatifs dans des boîtes différentes ; pour assurer une relecture en aveugle, il est mieux de les mélanger et de garder toutes les lames ensemble en ordre de numérotation
- en plus, d'après les observations sur le terrain : mieux de filtrer pendant la coloration, la fuchsine phéniquée aussi bien que l'auramine. Doter les CDT d'un petit entonnoir et de papier filtre.

Remarques SOPs CPC Yaounde

1. Coloration et examen microscopique

- pas clair où se fait la coloration (pont sur un évier?); comment on rince les lames ? (robinet + tuyau pas recommandé, plutôt bécher propre)
- controle de qualité manque; conservation des lames
- utilisation des microscopes pas décrite ; utilisation huile d'immersion pas décrite
- ne pas laisser le choix entre 2 réactifs pour la décoloration
- formulation réactifs:
    - acide sulfurique ¼, 5% chlorhydrique pour fluorescence? On recommande 0,5% chlorhydrique
    - bleu méthylène fluorescence 0,2% / Ziehl non-spécifié ? On recommande 0,1% pour Ziehl et 0,3% fluorescence
- grossissement fluorescence 500X : plus efficace de faire le screening à 200X, confirmer à 500X
- bacilles en jaune en fluorescence : plutôt verdâtres avec le LED ; différentiation d'artéfacts ?

2. Instruction d'enregistrement des échantillons

- manque les formats des différents supports et mode d'emploi
- nécessité d'un fichier informatique (échantillons mis en culture / identification / ATB) ?

3. Procédure de recueil des prélèvements

- par. 5, dernière ligne : bromure de céthyl pérymidium: ?? Il manque la description précise des indications et de l'utilisation du milieu de transport (chlorure de cétylpyridinium, CPC ?)
- nécessité d'y ajouter les modèles des bons de demande des examens (seulement microscopie, modèle PNT ; culture/ID/ATB/moléculaire) avec des instructions claires

4. Procédure d'hygiène et sécurité

- je pense à refaire en entièreté : trop de généralités, trop peu axé sur la pratique au CPC ; accidents non-traités

- utilisation anses métalliques / usage unique / bambous : pas clair ; contradictions avec autres SOPs
- utilisation gants ? différents antiseptiques ?
- examens médicaux personnel ?

5. Préparation des solutions antibiotiques
- en général : considérer la préparation de solutions de stock plus concentrées (100 mg / 10 ml) qu'on peut aliquoter, congeler et pré-tester
- faut-il continuer de tester PAS ? TCH ?
- qui est chargé de la préparation de ces solutions ?
- conservation des poudres (et annotations dates réception, ouverture..)
- conduite à tenir envers la péremption des produits ?
- pas clair comment on corrige pour la potence (mais fait, p.ex. SM 50 mg pesés donnent concentration finale de 5µg/ml mais le SOP dit que c'est 4 µg/ml sans expliquer comment ; OK vu que la potence est +/- 80%)
- rifampicine : dissoudre dans l'éthanol à 95% ?? garder un mois à 4°C ?? Attention évaporation (tubes !!). DMSO peut être plus pratique (aussi bon pour les fluoroquinolones et PNB)

6. Préparation des réactifs de décontamination
- matériel à utiliser pas bien spécifié
- préparation soude 4% pas décrite

7. Préparation des réactifs de coloration
- matériel à utiliser ?
- solutions de décoloration décrites 2 fois
- solution fuchsine : mieux sans sol. mère de fuchsine (fuchsine + alcool + phénol + 100 ml d'eau, bien mélanger le tout)
- recommandé pour Ziehl : 1% fuchsine ; 0,1% bleu méthylène
- étiquetage ? durée de vie ?

8. MO réalisation de l'antibiogramme
- manque tube PNB ; utilité PAS et TCH ? utilité double série ?
- matériel à utiliser trop peu spécifié
- concentration Ofx, Km ?
- ajustement opacité : définition du témoin opacimétrique ? on n'attend pas pour laisser sédimenter les amas ?
- dilutions 1/10 : nouvelle pipette chaque dilution ?
- gouttes / ml ensemencées ?
- tubes à ensemencer pas très clairs pour -4 et -5
- lecture à 4 semaines ou 6 semaines : on laisse trop de liberté ; la lecture peut être définitive à 4 semaines seulement pour les produits montrant une résistance
- description comparaisons et calcul / décision pourcentage pas claire
- 10% pour 2ème ligne n'est pas recommandé pour les concentrations critiques utilisées, aussi 1%
- nombre de colonies à noter pour chaque tube (ATB, contrôles) ; tableau de quantification
- PNB manque !! et son interprétation !!
- utilisation souches de contrôle ??

9. Décontamination des prélèvements
- matériel à utiliser trop peu spécifié
- mieux de vortexer après ajout du décontaminant de façon que l'intérieur du tube vienne en contact avec la solution partout
- temps de contact avec soude 4% trop long (15 minutes pour agir ; puis 20+ minutes centrifugeuse) : mieux de diluer avant de centrifuger
- quantités à ajouter peuvent varier avec viscosité (et quantité) du produit
- procédure à suivre pour échantillons qui arrivent en CPC ?
- Coletsos = pyruvate ??

10. MO mise en culture
- matériel à utiliser trop peu spécifié
- solution antiseptique eau de javel : concentration ? à renouveler tous les jours !
- notification de la croissance devrait être plus détaillée (quantifiée à chaque lecture)
- pas clair quand et pour quoi on fait les Ziehl des cultures positives : noter aspect ? % de non-BAAR (et alors indications de sous-culture avec décontamination préalable) ?

11. MO préparation technique des échantillons
- SOP inutile, combine des instructions déjà (partiellement) présentes dans d'autres SOPs
- en grande partie à combiner avec SOP 2 & 3 (aussi 9 & 10)

12. Fiche technique traitement de l'échantillon
- ce n'est pas un SOP, plutôt une aide à étaler dans le labo

13. Préparation des panels de lames
- mentionner qu'au moins pour les première étapes on doit travailler dans une hotte
- partie validation (à la fin) très importante trop peu développée
- méthode assez lourde, résultats souvent pas très satisfaisants : utilité ?

14. Contrôle qualité des colorants
- à ajouter au mode opératoire de leur préparation ?
- uniquement fiche, méthode pas décrite : choix des lames de contrôle positive ? technique de coloration / lecture ? notification des résultats ?
- attitude en cas de résultats aberrants ?
- mentionner nombre attendu de BAAR du lot de lames contrôle pos. utilisé

15. CQ milieu de culture et réactif & CQ antibiogrammes
- à ajouter au mode opératoire de leur préparation ?
- uniquement fiche, méthodes pas décrites : contrôle stérilité ? activité ? souches de contrôle utilisées ?
- définitions et attitude en cas de résultats aberrants ?

16. Elimination des déchets
- produits toxiques non-contaminés (réactifs) manquent
- % eau de javel ??

17. Procédure de validation des résultats
- trop général, mais OK

- devrait être complété par description des points important pris en compte pour la validation de chaque examen (p.ex. croissance suffisante des tubes contrôle d'un ATB ; souches de contrôle interne…)

18. Procédure de conservation des souches
- matériel à utiliser trop peu spécifié
- types de souche à conserver : pourquoi pas toutes ?
- système de numérotation pas clair
- tube à choisir s'il y en a plusieurs avec la même souche (ATB) ?
- lait écrémé : préparation (en fait stérilisation difficile ; pas le meilleur milieu)

19. MO identification biochimique & Préparation des réactifs de l'identification
- manque PNB ! utilité des autres tests lorsqu'on a les moyens d'identification moléculaire ? limiter leur nombre ?
- préparation suspension bactérienne pas bien décrite
- test niacine : décrire danger d'acidification & mesures de sécurité
- test catalase : tampon phosphate Sorensen est à 1/15 M chaque sel, pas 15M ; sol. stock Na2HPO4 à 9,47 g incorrect s'il s'agit du di-hydrate, doit être 11,87 g
- test nitratase : interprétation de la confirmation au zinc pas claire
- tableau interprétation : pousse au-delà de 3 semaines bien possible pour MTB ; nitratase +/- pour les atypiques (?? très rare)
- conservation et période maximale

20. Préparation des milieux de culture
- matériel à utiliser trop peu spécifié
- comment ajuster le pH de la solution sels LJ ?
- coagulation : four pré-chauffé ? tubes vissés ?
- conservation LJ (et pyruvate) : 1 semaine ou 1 mois, frigo ou t° ambiante ??
- contrôle de qualité à décrire en bien plus de détail

21. Procédure de traitement de l'échantillon
- nécessaire ?

22. MO préparation technique des échantillons
- version plus récente du no. 11 ?

23. Règles de sécurité au laboratoire de mycobactéries
- déjà compris dans no. 4

Antibiogramme sur lames:

Je n'ai pas vu le SOP, à développer. Il sera mieux d'utiliser dorénavant 7H9 au lieu de Sula, les autres ingrédients (glycérine, sérum animal, Selectatabs) restent les mêmes.

Faire attention à l'interprétation :

- des non-TB : surtout croissance sur PNB, morphologie des colonies TB varie et la morphologie est donc moins fiable.
- de la croissance avec RMP 0,5 µg/ml : ignorer les colonies de taille 1+ ou 2+ si les contrôles sont bien plus nombreux et/ou d'une taille 3+/4+

CPC pourrait faire d'autres essais :

- plusieurs lames contrôle : en colorer une après 10-12 jours, et si les colonies sont bien développées alors procéder à la coloration et lecture de la série. Sinon, continuer de suivre le développement des contrôles tous les 2-3 jours, tuer et colorer la série au meilleur moment.
- réduire la quantité de milieu par bouteille à la moitié (bien que de cette façon les frottis peuvent rester partiellement au dessus) : moindre consommation ; lecture plus facile ?
- utiliser l'objectif 4X (avec oculaire 10X) pour la lecture: il est mieux de ne pas voir les très petites microcolonies de taille 1+ ; lecture plus facile et rapide

Annex 4 Bulk preparation of staining solutions

Specific equipment needed as a priority

- 1 magnetic stirrer / hot plate, large surface area of the plate; with 3 large cylindric stirring bars and one magnetic bar for removal of the stirrers
- 6 conical flasks, 3 L capacity
- 3 conical flasks 1 L capacity
- 2 measuring cylinders polypropylene 100 ml
- 2 measuring cylinders polypropylene 500 ml
- 2 measuring cylinders polypropylene 1000 ml
- plastic jerrycans 4-5 liters, HDPE (chemical resistant): plenty, for staining solutions distribution
- plastic bottles 1 liter, HDPE (chemical resistant): plenty, for staining solutions distribution
- at least 6 plastic jerrycans 20-30 liters, HDPE (chemical resistant), with wide filling opening and screwcap, with drainage tap at the bottom, cylindric shape, for preparation and storage of staining solutions and distilled water
- 6 ordinary jerrycans (without tap; box-shape) of 20 L
- 4 large size plastic funnels, long stalk (for filling the jerrycans)

PS: a balance is not essential if the SOPs below are followed, but it will be needed if smaller quantities need to be prepared. If any is provided, it should be a sturdy model with sufficient capacity and low sensitivity (i.e. mechanical, 0.01 g sensitivity, at least 300 g)

Procedure for preparation of large quantities of stains

1. Carbolfuchsin stain

The equipment in the list supposes that 10 liters are prepared at a time, but that storage and lot definition is done per 20-30 L jerrycan. This allows for better mixing.

- liquefy a full container of phenol (500 g) by adding 50 ml of distilled water to it a few days before
- pour the phenol and 1 liter of alcohol (ethanol or methanol) in a 3 L conical flask on the magnetic stirrer containing a stirring rod; add 200 ml of distilled water
- slowly add 100 g of basic fuchsin (to arrive at 1% final concentration; no weighing required, just empty 4 complete bottles of 25 g or one of 100g)
- let swirl on the mixing plate till all powder seems to be dissolved (no heating needed)
- remove the magnetic bar using a second one and sliding it upwards with the flask tilted as much as possible
- empty the contents in a 20-30 L jerrycan, and add the remaining 8.25 L of distilled water (total water used should be 8.5 L)
- mix by manual shaking of the closed jerrycan
- transfer this ready stain to another 20-30 L jerrycan through a big funnel (without filter paper)
- repeat the preparation as needed, each time for another 10 L, filling the storage jerrycan completely
- label the jerrycan with stain name and date of preparation; target consumption within 12 months by the final user

2. Decolourising solutions

Use cold alcohol / water if possible.

A. 3% hydrochloric acid (acid alcohol) for ZN staining: preparing 3 L at a time, filling up a 20-30 L jerrycan gradually.

- fill 2.9 L of alcohol (ethanol or methanol; pure or 70%) in a 3 L conical flask with stirring rod on the magnetic stirrer
- slowly add 90 ml of hydrochloric acid (fuming, 37%), while mixing strongly
- remove the stirring rod
- transfer the mixture to a 20-30 L jerrycan without filtration
- repeat till the jerrycan is full
- label the jerrycan with stain name and date of preparation

Alternative (only for hydrochloric in alcohol): prepare directly in a jerrycan
- fill 9.7 L of alcohol in a 20 L jerrycan
- slowly add 300 ml of acid
- close the jerrycan and mix manually
- repeat this in another 20 L jerrycan and add this amount to the first one
- label the jerrycan with stain name and date of preparation

B. 0.5% hydrochloric acid for FM staining: prepare directly in a jerrycan
- fill 9.95 L of alcohol in a 20 L jerrycan
- slowly add 50 ml of acid
- close the jerrycan and mix manually
- repeat this in another 20 L jerrycan and add this amount to the first one
- label the jerrycan with stain name and date of preparation

C. 20% sulfuric acid
- fill 2.4 L of cold distilled water in a 3 L conical flask with stirring rod on the magnetic stirrer
- slowly add 600 ml of sulfuric acid (technical quality, about 96%), while mixing strongly; avoid overheating: if needed, add the acid at intervals
- remove the stirring rod
- transfer the mixture to a 20-30 L jerrycan without filtration
- repeat till the jerrycan is full
- label the jerrycan with stain name and date of preparation

3. Methylene blue 0.125% (for ZN) or 0.25% (for FM)

Prepare directly in a 20 L jerrycan.
- empty one (for ZN final conc. 0.125%) or two (for FM, final conc. 0.25%) vials of 25 g methylene blue in the jerrycan
- add 10 L of distilled water, and mix well manually
- check if all powder is dissolved
- now add the remaining 10 L of distilled water; transfer one or two times to another 20 L jerrycan for better mixing
- label the jerrycan with stain name and date of preparation

4. Auramine O stock solution / phenol stock solution

The concentrated auramine stock solution keeps much longer (about one year) than the diluted staining solution ready for use with max. 3 months shelf life. The two stocks are distributed as such, and mixed 1 volume auramine stock for 9 volumes phenol stock to obtain the working solution, in quantities that are sure to be consumed within a short time (one month is recommended).

1. Phenol stock solution
- transfer the contents of a complete bottle (500 g originally) of liquefied phenol into a 20L jerrycan. Add 15 L of distilled or purified water, best in 2-3 parts to make good mixing. Mix well by swirling manually.
- label the jerrycan as "3.3% phenol stock solution" and date of preparation. This is the complete phenol stock solution with shelf life several years.

2. Auramine stock solution
- slowly add about half an auramine O 50 g vial to 2.5L ethanol 95% in a 3 L conical flask; mix well with a stirring rod on the magnetic stirrer
- after complete dissolution of the auramine, transfer the solution to a 10 to 20L jerrycan
- slowly add the remaining half of the auramine O 50 g vial to a second volume of 2.5L ethanol 95% in a 3 L conical flask; mix well with a stirring rod on the magnetic stirrer
- after complete dissolution of the auramine, add the solution to the already earlier prepared solution in the 10 to 20L jerrycan
- mix well by swirling manually
- label the jerrycan as "1% auramine stock solution" and date of preparation.

3. Working solution
Mix 100 ml of the auramine stock with 900 ml of the phenol stock (or accordingly smaller or larger quantities, as appropriate). Keep in the dark.

Procedure for quality control (QC) of newly prepared stains

This is necessary to make sure that the stains (especially carbolfuchsin) work well, and that they do not contain contaminating AFB (especially methylene blue). For the first purpose, two low positive smears will be used, for the second two negative or fake smears. The stain preparing lab should control each batch prepared, so testing per jerrycan of 20-30 L. It must keep careful records in a logbook. In the logbook, the batches will be identified by name of reagent and preparation date. Any jerrycan or bottle filled from this batch will be labeled with the same information.
QC is done using one or more freshly prepared stains and a shortened staining procedure for the positive controls. It is best to stain negative controls three times before examination. This is to make the contaminants in acid or methylene blue visible by exposing them again to carbolfuchsin after the first staining cycle, and to give possibly present contaminants more chance to stick to the smears. Positive controls must be stained only once, and the controls will be more sensitive when not the best technique is used. A good strong staining solution will still give quite good results with once heating of the carbolfuchsin covered smear till steam arises, and leaving it for 5 minutes only before rinsing. The destaining step is as per routine (3 minutes, repeat is necessary), as is the counterstaining (methylene blue 0.125% for 1 minute).
Examine all controls carefully for number, completeness and intensity of red color of AFB, as well as color and complete destaining of background with absence of crystals. Record the results in a stain QC register as in the example below.
In case a lot of carbolfuchsin does not seem to work well, the positive controls can be repeated using other smears, making sure that the staining technique is respected. If also these are unsatisfactory, the lot must be discarded.

AFB-positive and -negative unstained control smears.

The positive are best 1+, the negative are best made from an acute pneumonia patient's sputum. Sputa from smear-negative TB suspects are more risky for negative controls, since AFB might have been missed. Make as many slides as possible from the same low positive sputum, after letting it stand one day and mixing carefully (container closed); check average nr. of AFB in 6 stained smears, and note this number in your logbook. Keep fixed unstained smears protected from dust and sunlight, best in a slidebox.

Example of a logbook for quality control of stains

Batches checked on date 5/1/02: Carbolfuchsin (CF) batch 5/1/02, Sulphuric acid batch 5/1/02, Methylene blue batch 5/1/02 Average grading positive controls: no. 345 = 30/100 fields ; no. 411 = 22/100 fields				
Control Slide	AFB color	AFB number	Destaining	Decision
345/12	strong red	20/100 F	OK	Accept CF
411/25	strong red	50/100 F	OK	Accept CF
NEG	NA	none	OK	Accept others
NEG	NA	none	OK	Accept others
Batches checked on date 15/1/02: CF batch 15/1/02, Sulfuric acid batch 15/1/02 (+ old methylene blue) Average grading positive controls: no. 345 = 30/100 fields ; no. 411 = 22/100 fields				
Control Slide	AFB color	AFB number	Destaining	Decision
345/13	weak red	2/100 F	OK	Reject CF
411/26	NA	0/100 F	OK	
345/14	weak red	6/100 F	OK	
345/15	NA	0/100 F	OK	
411/27	weak red	1/100 F	OK	
411/28	weak red	2/100 F	OK	
NEG	NA	none	OK	Accept others
NEG	NA	none	OK	Accept others
Note: this batch of carbolfuchsin is bad, all has been discarded. Sulfuric solution is OK.				

Annex 5 Recommended format culture/DST request form

Bon de demande et réponse culture et antibiogramme (ATB) TB

Identification du malade (ID):

No. registre TB:_____ No. precedent registre TB:_____ No. registre MDR:_____

Nom et postnom du malade:_____ Age (ans):_____ Sexe:____

Salle / Service: _____ Adresse: _____

*Statut VIH: Pos / Nég / Inconnu _____

Type de TB et antécédents de la maladie

Site: □ pulmonaire
□ extrapulmonaire (préciser):_____

Antécédents: □ nouveau (jamais traité pour ≥1 mois)
□ rechute
□ échec
□ retour après abandon
□ chronique
□ contact MDR
□ incertain

Traitement antérieur: □ Cat.1
□ Cat.2
□ Cat.4 (2ème ligne)
□ Autre _____

Origine de la demande:

Région ID:_____ District ID:_____ Laboratoire local ID:_____

Date spécimen collecté: ____/____/20____ Spécimen ID no.:_____

Laboratoire local: résultat frottis: 1er____ 2ème____ 3ème____ spécimen
technique de microscopie utilisée: □ Ziehl-Neelsen à chaud □ frottis direct
□ à froid □ frottis concentré
□ fluorescence

Demande de tests à effectuer au labo de référence :

Raison: □ diagnostic
□ suivi à …. mois du traitement
□ suivi à …. mois après le traitement

Spécimen: □ crachats
□ crachats en préservatif, type ……
□ autre (spécifier):_____

Tests demandés: □ microscopie (type _____) □ culture □ ATB (première / seconde ligne)

Personne demandant les examens: Nom:_____ Position:_____

* Information non-obligatoire ID = no. ou code d'identification

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Résultats du laboratoire de référence:

Date réceptionné au Laboratoire de Référence _____/_____/20_____ Spécimen ID Labo de Référence:_____

Résultat examen microscopique: rapporté déjà le _____/_____/20_____

ID #	Nég	1-9	1+	2+	3+

□ Ziehl-Neelsen à chaud □ à froid □ fluorescence
□ frottis direct □ frottis concentré

Résultat culture: rapporté déjà le _____/_____/20_____ □ à suivre

ID #	Conta-minée	Nég	Mycobacterie non-TB (espèce)	Mycobacterium tuberculosis complex			
				1-9 colonies (nombre)	10 – 100 col 1+	>100 - 200 col 2+	>200 col 3+

Résultat de l'antibiogramme de *M. tuberculosis*: □ à suivre

□ méthode phénotypique utilisée _____
□ méthode génétique utilisée _____

ID # _____			Légende: S = sensible; R = résistante; C = contaminée; NF = non-fait											
	INH		Rifampicine		Ethambutol		Streptomycine		Pyrazinamide		Ofloxacin		Kanamycine	
µg/ml														
résultat														

Date: _____/_____/20_____ Signature:_____

Annex 6 Recommended culture/DST reporting formats

RAPPORT TRIMESTRIEL **Trimestre / Année** .....

CULTURES ET ANTIBIOGRAMMES FAITS POUR TB

LABO ..... **DISTRICT** ..... **REGION** .....

Rapport sur les cultures ensemencées 3-6 mois avant

Résultats des frottis	Résultats des cultures									
	Suspects, pas en traitement					Suivis (malades en traitement)				
	Contaminé	Négatif	Positif	Autre	Total	Contaminé	Négatif	Positif	Autre	Total
Négatif										
Positif										
Rares BAAR										
Inconnu										
Total										

Rapport sur les antibiogrammes et tests d'identification ensemencés 3-6 mois avant

Résultat	Type de malade (no.)						
	Nouveau	Rechute	Echec	RAA	Chronique	Autre	Total
Total ensemencé							
Contaminé							
Non-poussé							
Non-TB Mycobactéries							
Total M. tuberculosis complexe							
Sensible aux produits 1ère ligne							
MR résistant							
HRES							
HRS							
HRE							
HR							
HR, E et/ou S inconnu							
MR + Km (ou Cm ou Ak)							
MR + Fluoroquinolone (FQ)							
MR + autre 2ème ligne							
MR + FQ + injectable (XDR)							
Résistant à 3 produits non-MR							
HES							
RES							
Résistant à 2 produits non-MR							
HE							
HS							
ES							
RS							
RE							
Résistant à 1 seul produit							
H							
R							
E							
S							

Légende

RAA retour après abandon
H isoniazide
R rifampicine
E ethambutol
S streptomycine

MR multi-résistant
XDR extensivement résistant
Km kanamycine
Cm capreomycine
Ak amikacine
FQ fluoroquinolones