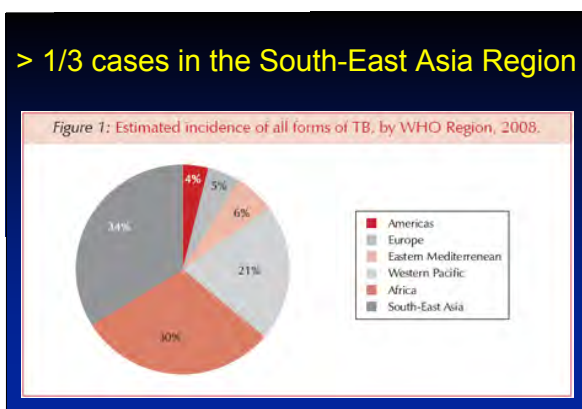
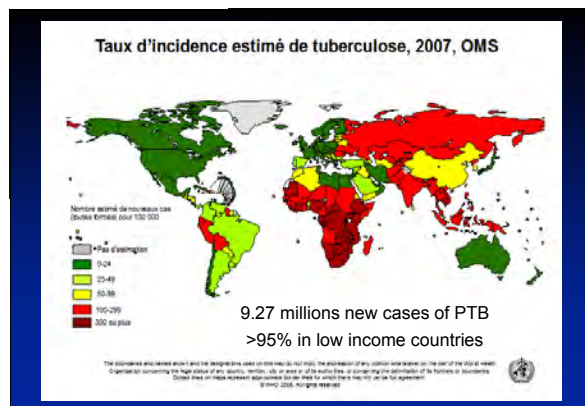



 8^{ème} Congrès international
 de la Société de Pathologie Exotique
Health challenges in Southeast Asia
 Vientiane 25-28 janvier 2010



**Improvement of microscopic screening
 of pulmonary tuberculosis
 in low_income countries**


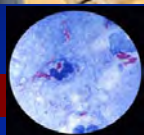
Pr Yves Buisson
 IFMT, Vientiane, RDP Lao



- DOTS strategy (1995)
- Directly Observed Treatment Short course*
- ➔ 5 key components:
- Government commitment to sustained TB control activities
 - Case detection by sputum smear microscopy among symptomatic patients self-reporting to health services
 - Standardized treatment regimen of 6-8 months
 - A regular, uninterrupted supply of all essential anti-TB drugs
 - A standardized recording and reporting system

Sputum smear microscopy

- Ziehl-Neelsen staining
- the most cost-effective method of pulmonary TB screening
- rapid, specific, inexpensive
- can detect AFB > 10⁴/ml sputum = highly infectious TB cases

but low sensitivity < 50%

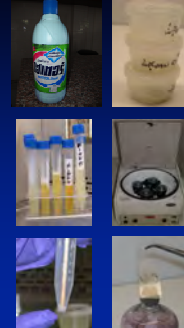
- Factors limiting the case detection of pulmonary TB in Lao PDR
- Sensitivity of sputum smear microscopy: 47% in 2007
 - Constraint of international criteria for positivity: ≥ 2 sputum samples/3 AFB+
 - Distance to laboratory screening
 - Quality problems: materials, techniques, technicians. Culture of mycobacteria not performed
 - Prevalence of HIV co-infection (3.3% of incident TB cases): decrease the sensitivity of smear microscopy < 20%

How to improve detection of pulmonary TB by 2015 in low income countries ?

- > **increase the number of samples:**
 - 3 consecutive sputum: sensitivity 2-5% _
 - 2 sputum on the same day: workload _ performances _
- > **increase the contrast:**
 - fluorescence examination: sensitivity 10% _
 - limits: costs and stability of reagents
- > **increase the concentration of AFB in sputum:**
 - liquefaction (sodium hypochlorite) + centrifugation

The bleach method

1. Add a solution of NaOCl 5% to the sputum (vol / vol) in the vial
2. Mix thoroughly and incubate for 15 min at room temperature
3. Transfer 2 to 15 ml in a conical tube with distilled water (vol / vol)_
4. Centrifuge at 2000 rpm for 15 min
5. put a drop of the pellet on a slide dry, fix and stain with Ziehl Neelsen



First study in Lao PDR, 2008

(612 patients – 1675 sputum samples)

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The bleach method improves the detection of pulmonary tuberculosis in Laos

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Design of the study

→ 3 months: central lab

Mahosot hospital

560 patients

1552 samples

1 month: peripheral lab

Attapeu hospital

52 patients

123 samples



Double blind reading



2 independent microscopists
20 mn/frottis (200 champs)_



matching of results

agreement

discrepancy

validation

3rd reading
contradictory

Semi-quantitative scale of the IUATLD



Number of AFB	Result
No AFB* on at least 100 HPF**	0
1-9 AFB / 100 HPF	±
10-99 AFB / 100 HPF	+
1-10 AFB / HPF on at least 50 HPF	++
>10 AFB / HPF on at least 20 HPF	+++

* AFB: acid-fast bacilli

** HPF: high-power microscopic field

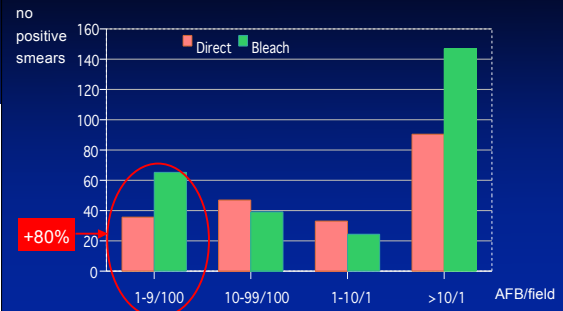
Qualitative results

Smear microscopy	Direct method		Bleach method	
	no	(%)	no	(%)
negative	1469	(87.7)	1400	(83.6)
positive	206	(12.3)	275	(16.4)
Total	1675	(100)	1675	(100)

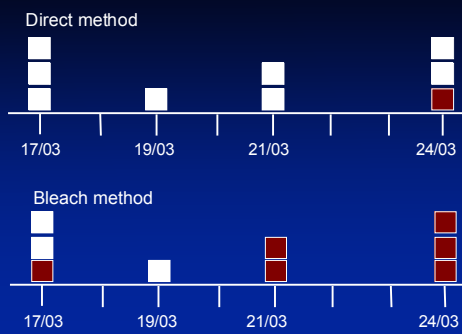
p = 0.0007

Quantitative results

(according to the semi-quantitative scale of UICTMR)



9 serial samples from a HIV+ patient



Second study in Lao PDR, 2009

Master en Médecine Tropicale et Santé Internationale
Mémoire de fin d'étude
IFMT-AUF-Université Nationale du Laos
9^{ème} Promotion 2007-2009

**Application de la méthode
de fluidification-centrifugation à l'eau de Javel
au dépistage de la tuberculose pulmonaire
chez les patients infectés par le VIH en RDP LAO**

Présenté par : Dr Chaysavanh THAMMAVONG

Gold standard: culture of Mycobacteria



First study
in Lao PDR
using culture
on Loewenstein
Jensen medium
for the diagnosis
of pulmonary TB



Provisional results

(60 patients – 121 samples)

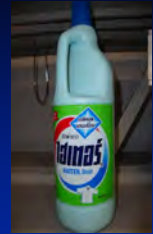
Culture	Smear microscopy				Total
	Direct method		Bleach method		
	neg	pos	neg	pos	
negative	100	0	100	0	100
positive	7	14	0	21	21
Total	107	14	100	21	121

Cost-effectiveness analysis

	direct method	bleach method
Effectiveness		
1 - suspect patients	60	60
2 - patients AFB+	7	11
Costs (US\$)		
3 - unit (one patient)	1	1.5
4 - total (60 patients)	60	90
5 - cost-effectiveness (4/2)	8.6	8.2
6 - marginal cost-effectiveness (\neq of 4/ \neq of 2)		7.5

Bleach method: advantages

- inexpensive, available everywhere
- easy to implement in peripheral and poorly equipped laboratory
- does not require special technical skills
- better sensitivity and negative predictive values_ than direct method
- improves the yield of case detection, especially for paucibacillary samples
- reduces the number of samples to examine
- reduces the risk of laboratory contamination



Bleach method: disadvantages

- **Lack of standardization:**
 - what concentration of the solution of NaOCl? 2 to 5%
 - how long the incubation period? 15 min \pm ?
 - how long and what speed centrifugation? 15 min, 2000 rpm \pm ?
- **Additional costs and delays:**
 - centrifuge and disposable (conical centrifuge tubes)_
 - preparation 30 min before staining
- **samples must be doubled if culture:**
(sputum treated with the bleach can not be then cultured)_
- **Technician Training & Quality Assurance**

Conclusion

The bleach method can improve the case finding of pulmonary TB in low income countries, especially

- when culture is not yet available
- when HIV co-infection is prevalent

Applied in all laboratories involved in the NTP, it might effectively strengthen the DOTS strategy provided that training and quality control are ensured

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