



# Improvement of pulmonary tuberculosis case detection by the bleach microscopy method in Laos

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# INTRODUCTION

# How about the TB ?

- TB is a major health problem in most developing countries.
- 1/3 of the world popul. is infected with *M. tuberculosis* (WHO)
- In SE Asia: 36 million TB cases estimated (206/100 000), WHO in 2005
- 5 - 10% of infected people may develop the disease
- HIV multiplies by 50 the risk of developing TB

# TB in Laos

- TB ranks 7<sup>th</sup> of the death causes in Laos
- TB ranks 3<sup>th</sup> among outpatients, and 5<sup>th</sup> among inpatients in Attapeu province hospital
- TB control programme aims to identify and treat patients with infectious pulmonary TB according to DOTS
- TBP diagnosis in Laos: is based on sputum smear direct microscopy (AFB detection poorly sensitive 50 %)
- Culture of Mtb not available
- PPD skin test not recommended

# Improve the sensitivity of bacilloscopy

- Concentrating the AFB by centrifugation after liquefaction:  
bleach method ...
- ☺ This method may be appropriate for developing countries

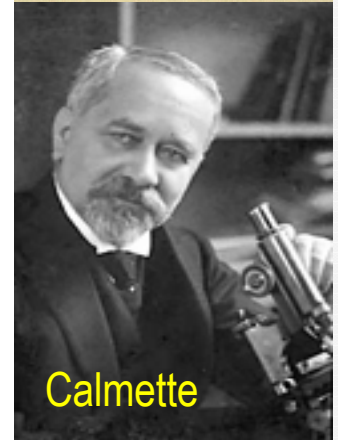
# Bleach(sodium hypochlorite, NaOCl)

## History:

- In 1774, discovery of chlorine by **Scheele**
- In 1787, synthesis, French chemist **Berthollet** for whitening activity
- In 1793, employment by the surgeon **Percy**, to address the "rotting hospital"
- In 1820, use as disinfectant **Labarraque**
- In 1892, **Calmette** shows that the BK is destroyed by NaOCl
- In 1989, **A. Dodin** shows that bacteria are destroyed in 30 sc with NaOCl to 0.036% of chlorine
- Effective disinfectant: bactericidal, fungicidal, sporicidal, virucidal



Scheele



Calmette



Dodin



# Objectives

- General objective:

To improve the microscopy screening performance for pulmonary TB case finding

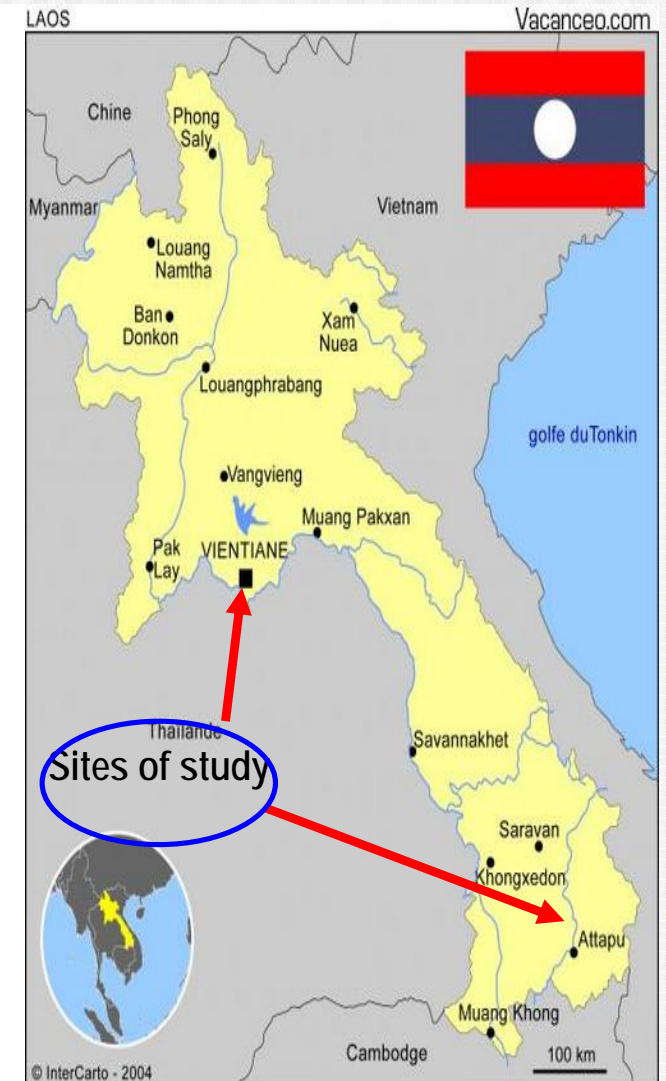
- Specific objective

1. To compare the bleach liquefaction / centrifugation method of sputum specimens and the standard method for microscopic AFB detection

2. To assess the feasibility of the bleach method in hospitals at the provincial level

# Methods

- **Comparative study:** duration for 4 months( march-june 2008)
- **Pop. study :** all patients suspected of pulm. TB
- **Inclus criteria :** all patients screened for AFB in sputum sample.
- **Exclus. criteria:** salivary sputum.
- **TTT of sputum samples:** each sample of sputum was treated by the direct smear method :
- The rest of the sample was used for the method of liquefaction/ centrifugation with NaOCl



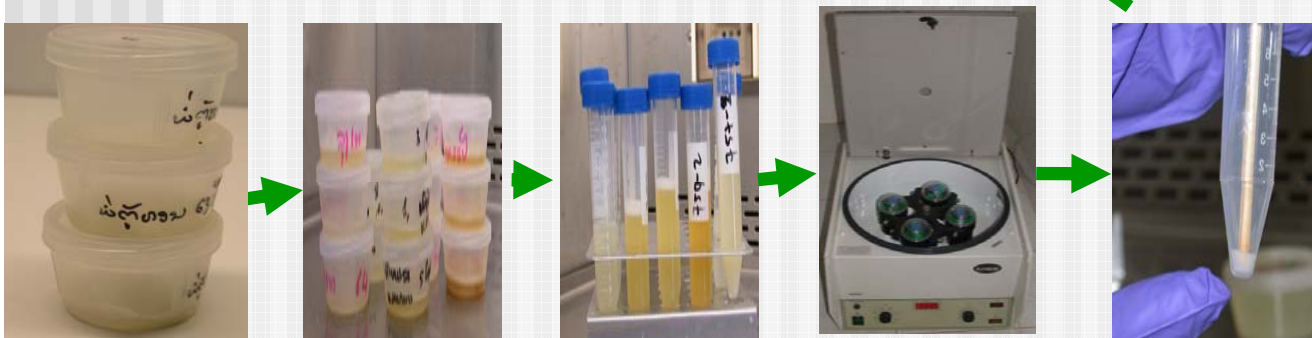


# Methods

## I. Standard smear method



## II. Bleach method



# Quantitative scale (IUATLD)

Number of AFB	Results
No AFB / 300 fields	0
1-9 AFB / 100 fields	±
1-99 AFB / 100 fields	1+
1-10 AFB /field minimum 50 fields	2+
> 10 AFB /field minimum 20 fields	3+

# 1675 sputum samples from 612 patients

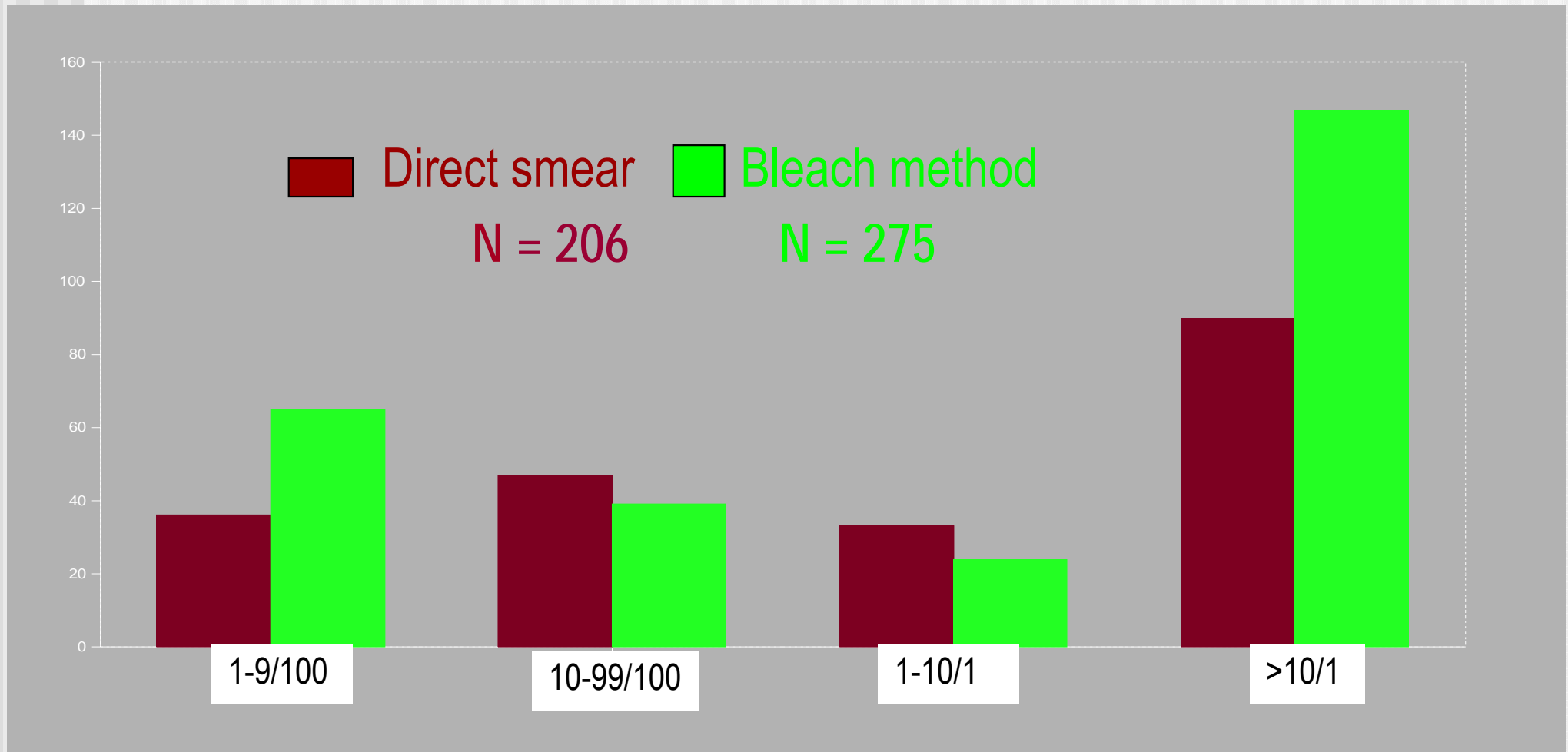
	Number of patient ( %)	Number of sputum sample (%)
sex		
Femal	261 ( 42,65 )	960 ( 53,31 )
Group of age(year		
<10	4	8
10-19	24	54
20-29	86	221
30-39	90	213
40-49	92	280
50-59	96	263
60-69	123	338
70	97	298
Average age 56,73 ± 18,50 (extreme 0-99)		
Region of prelement		
Vientiane	560	1552
Attapeu	52	123
Prelement for		
Diagnosis	600 ( 98.04 )	1625 ( 97.01 )
Control after TT	12	50
Serology		
Positive	1	9

# Qualitative and global results

	Standard method Nb (%)	Bleach method Nb (%)
Negative	1469 (87.70)	1400 (83.58)
Positive	206 (12.30)	275 (16.42)
Total	1675 (100)	1675 (100)

Chi<sup>2</sup> test; p=0.0007

# Distribution of positive samples AFB+ according to the quantitative scale of IUATLD



# Semi-quantitative results assessment of the two method (IUATLD scale)

UICTMR scale		<i>Standard methode</i>					Total
		<i>Negative</i>	$\pm$	<i>1+</i>	<i>2+</i>	<i>3+</i>	
<b>NaOCl method</b>	Negative	1400	0	0	0	0	1400
	$\pm$	51	14	0	0	0	65
	1+	14	16	9	0	0	39
	2+	3	4	15	2	0	24
	3+	1	2	23	31	90	147
	Total	1469	36	47	33	90	1675

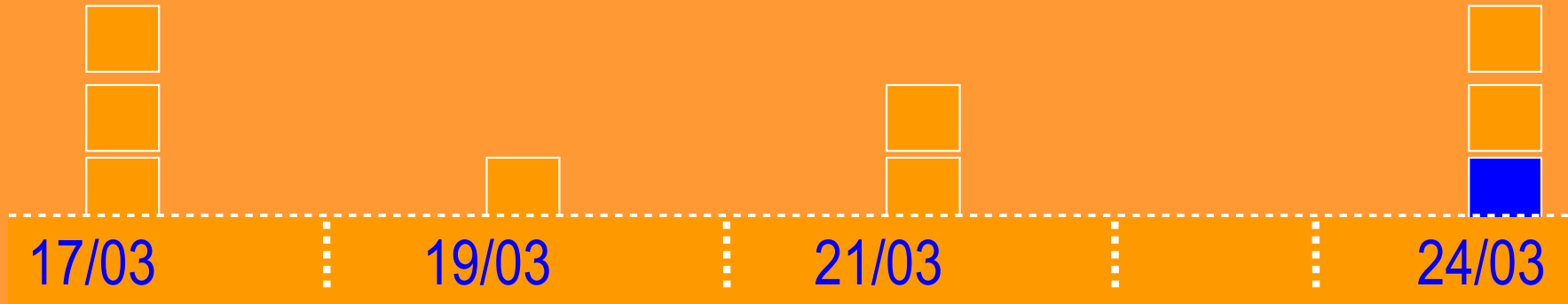
$\pm$  = 1-9 BAAR / 100 fields;      1+ = 10-99 BAAR / 100 fields

$\pm 2+$  = 1-10 BAAR / 1 field;      3+ = > 10 BAAR / 1 field

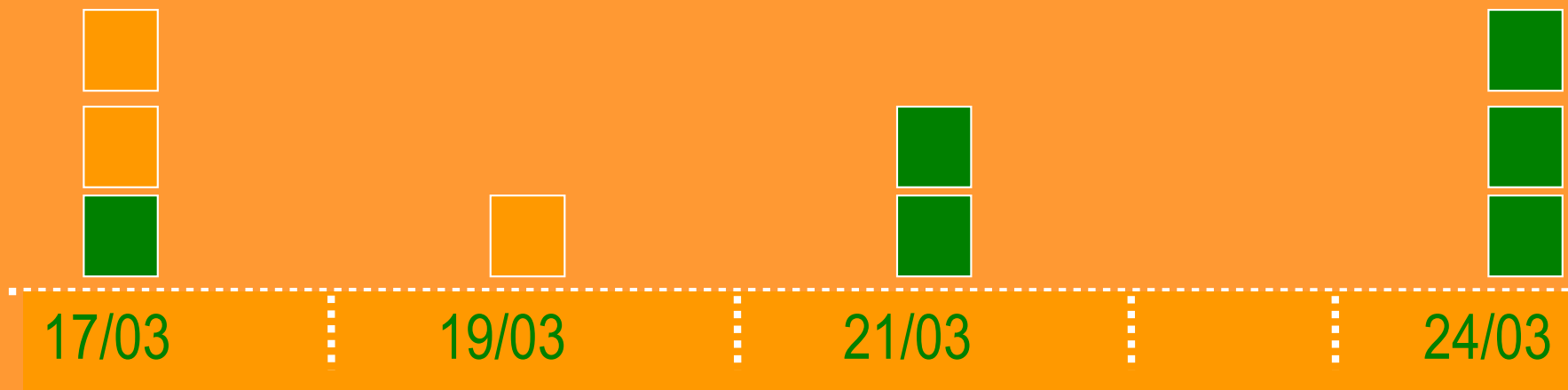
Chi<sup>2</sup>-test = 2 , p < 0.0001

# Results in one patient infected with HIV

## Standard method



## Bleach method



# Results comparing on 2 settings : Attapeu, Vientiane

Sites and Nb	Results	Standard methode	Bleach methode
Vientiane (1552)	+	195 (12.56 %)	255 (16.43 %)
Attapeu (123)	+	11 (8.94 %)	20 (16.20 %)

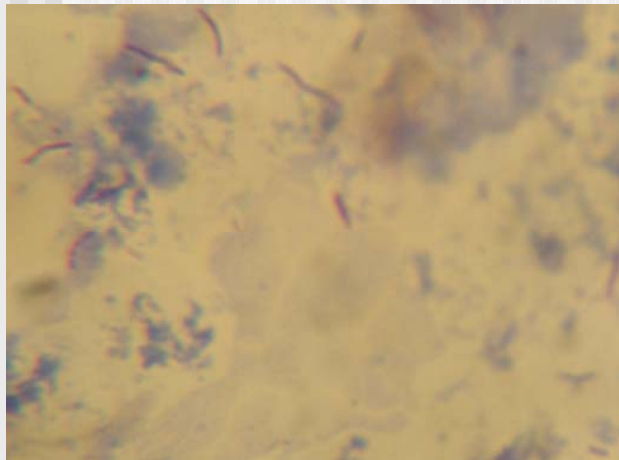


■ In the 2 sites, the NaOCl method could screen AFB+ more than the standard method (  $p = 0.005$  )



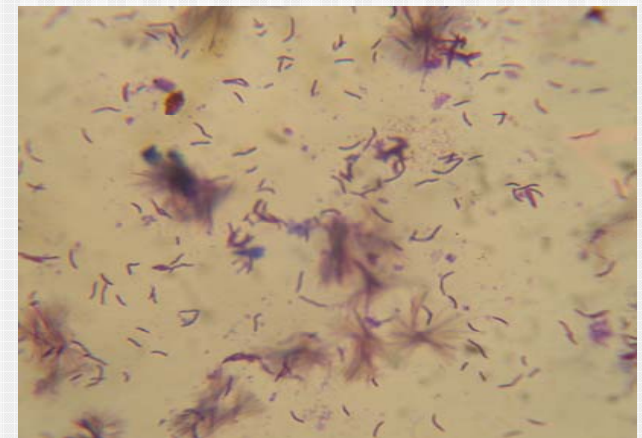
# AFB concentration compared with the two methods in the same sputum sample

Standard method



Naive Patient

Bleach method



Patient after TT

2 months

(-)

(+)



# Discussion

- This study aimed to compare the NaOCl method with the standard direct smear method for sputum microscopy screening for pulmonary TB
- NaOCl method offers an undeniable superiority: 16.42% positive (bleach) *vs* 12.30% (standard).
- Using the IUATLD semi quantitative scale showed the NaOCl method to be equally effective in Attapeu and in Vientiane
- 24 (3.9%) of 612 patients tested, could be detected AFB (+) by the bleach method only (not by standard)
- Two patients could be detected 5-7 days earlier by the NaOCl compared to standard method
- This study was easily carried out in 2 different sites, on a large number of samples.
- As the gold standard diagnostic method, the *Mtb* culture was unavailable, sensitivity and specificity could not be assessed

## Compared: bleach method / sandard method

Countries	N	Total positive	Total négative	SE %	SP %	p
Switzerland	3287 E	544/510	2743/2777	-	-	<0,0028
Sweden	303 E	-	-	62/47	99/99	0,003
Ethiopia	50 P	36/16	16/36	-	-	-
Ethiopia	100 E	36/16	64/84	70/31	100/100	<0,0001
Malaisia	324 p	48/29	276/295	-	-	<0,0001
Honduras	971 E	100/75	871/896	-	-	<0,0001
Myanmar	948 P	293/248	655/700	-	-	<0,0001
Ethiopia	500 E	71/39	429/461	-	-	<0,0001
India	103 E	35/16	68/87	-	-	<0,0001
Ethiopia	200 P	51/17	149/183	-	-	<0,0001
Ethiopia	200 P	60/17	160/183	-	-	<0,0001
Ethiopia	509 P	-	-	63/54	96/97	0,0015
Iran	430 E	-	-	83/50	99/99	<0,0001

# Conclusion - Recommendations

- The NaOCl method could significantly improve the effectiveness of sputum microscopy.
- It is security for laborantins, inexpensive, widely available, and easy to use
- It would be of great value to the national program against TB to increase the case detection rate.
- Its implementation could be considered in all field laboratories of TB units in developing countries where the culture is unavailable.

# Thank for your attention

