



# *-SPUTUM-*

## *THE BREAD AND BUTTER OF THE TB LABORATORY*

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# *Sputum Quality*



# *Guidelines and Algorithms for Sputum Collection*



**GUIDELINES**

# Phases of Testing

Preamerical



Analytical

Postanalytical

# Specimen Quality

- *The accuracy of laboratory results are directly related to the quality of the specimen*
- **GOOD sputum**
  - Recently-discharged material from the bronchial tree, with minimal amounts of upper respiratory tract secretions

# Specimen Quality

- **Sputum Collection**
  - Requires trained, dedicated individuals
- **Direct observed collection (DOC)**
  - Assures optimal specimens
  - Aids with “uncooperative” patients
    - Patients who want to avoid isolation
    - Cultural issues
- **Sputum induction for patients who cannot produce sputum**
  - Induced sputum resembles saliva
    - Important to label as “induced”

# Collection Kits



# WSLH Sputum Collection Kit



# Storage and Transport

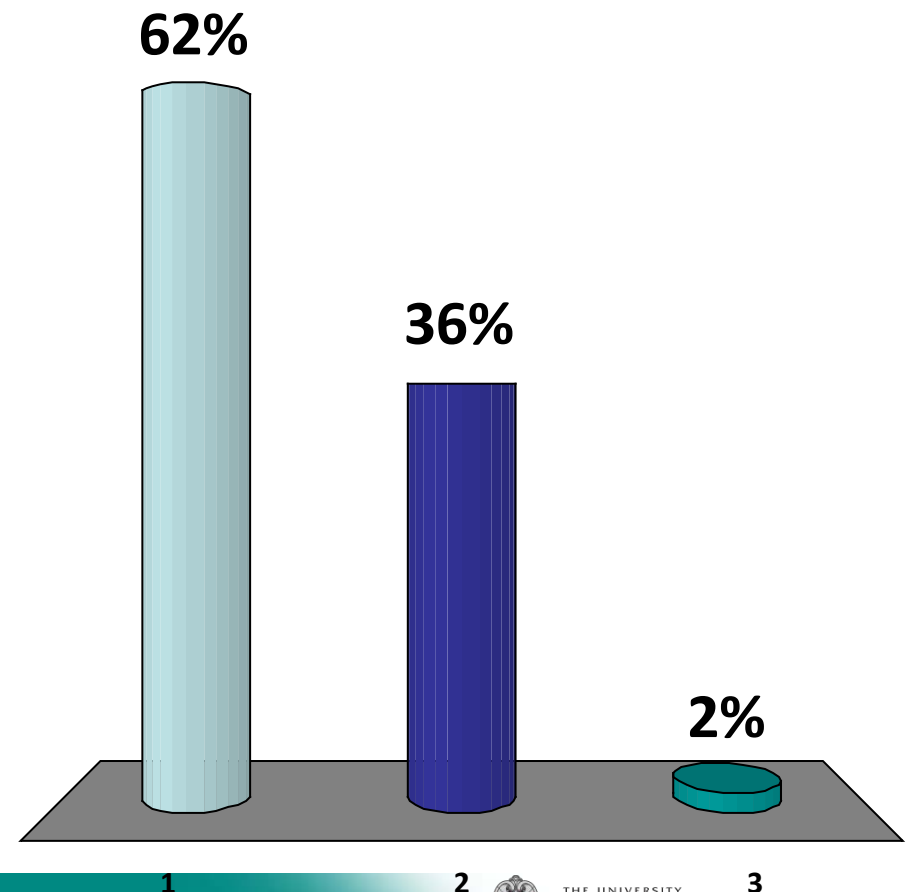
- Specimen storage
  - Refrigerate if not transported to the lab within 1 hour
- Transport
  - Package according to current DOT regulations
  - Ship with refrigerant (e.g. Cool Paks)
  - Preferably overnight delivery

# Laboratory Role in Specimen Quality

- Establish a working relationship with the health care personnel who collect patient specimens
- Provide detailed instructions in collection kits
  - **Appropriate number of specimens**
  - **Explain difference between saliva and sputum**
  - **Need for deep productive cough**
  - **Rinsing the mouth prior to collection**
  - **Specimen volume—Optimal 5-10 ml; not less than 3 ml**
  - **Avoid contamination of the outside of the tube**
  - **Seal container tightly**

# Our laboratory asks that specimens be...

1. Shipped with refrigerant
2. Shipped at ambient temperature
3. Shipped by pony express



# Specimen Quality Evaluation in the Laboratory

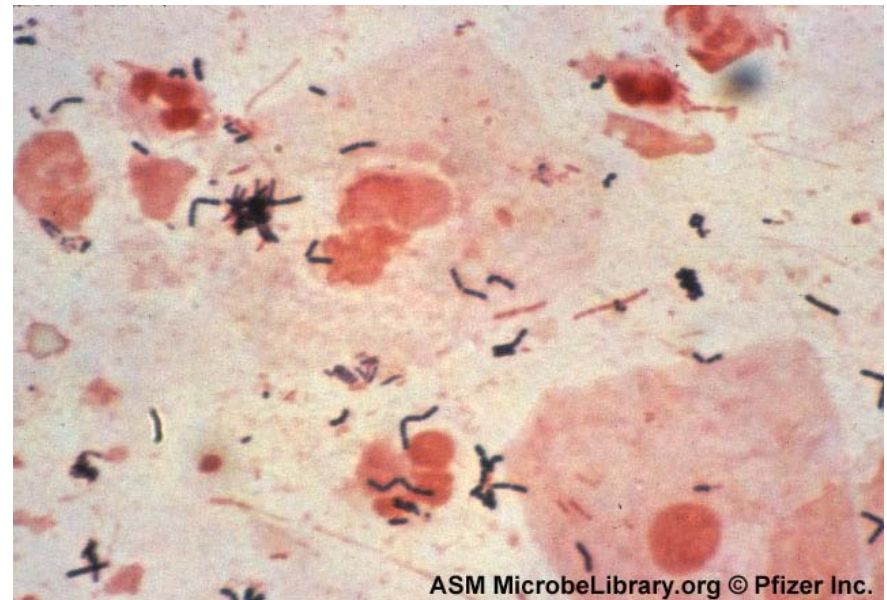
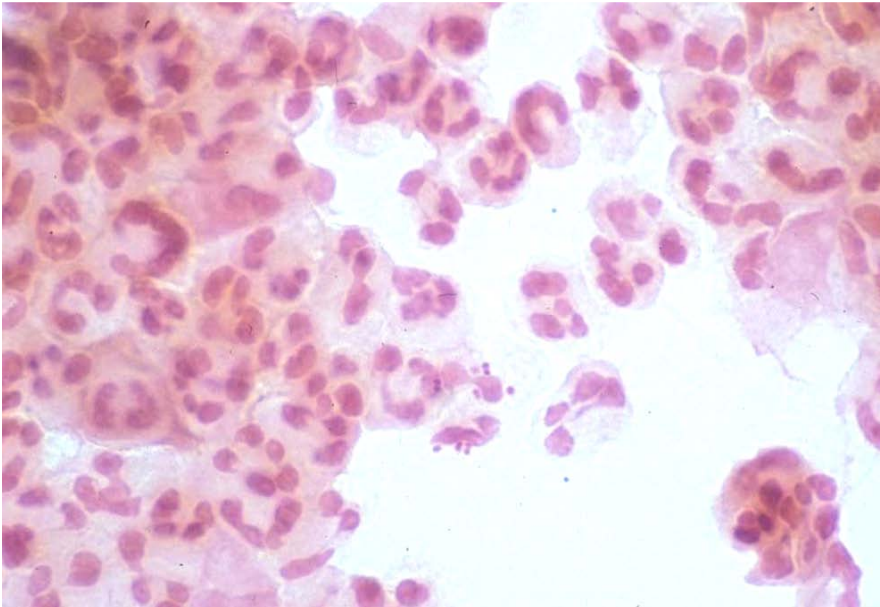
## SPIT-ON-A-STICK



# Specimen Quality Evaluation in the Laboratory

- Assess volume
  - >3 ml
- Time between collection and receipt
  - Should not be > 7 days
- Saliva vs Sputum?????????

# Can we evaluate using the Gram Stain?



# Gram Stain Evaluation of Sputum Specimens for Mycobacterial Culture

No. of Specimens	Score	M. tb Isolated	NTM Isolated
70	0	2	1
115	1	3	1
29	2	3	1
77	3	5	4
39	4	6	2
70	5	6	0
<b>Total 400</b>		<b>25</b>	<b>9</b>

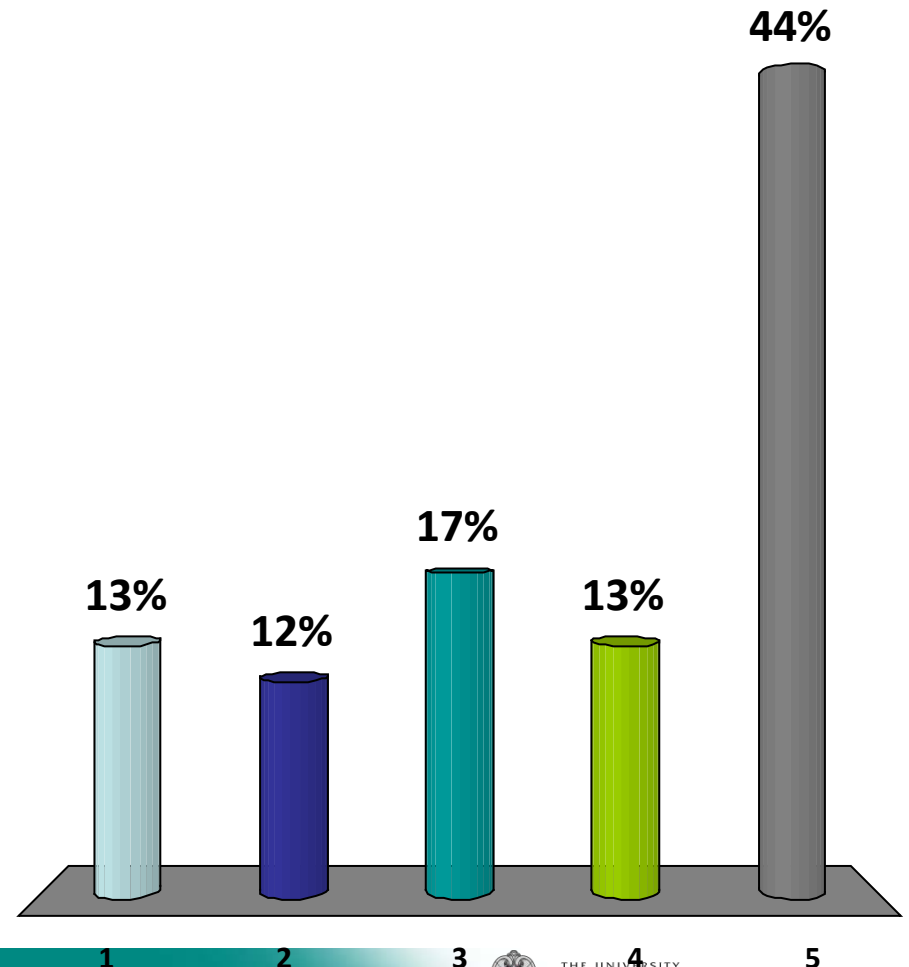
## Scores

- 0** Not sufficient cells or mucus to make a judgment
- 1 or 2** Salivary in origin
- 3 to 5** Representative of material from the lower respiratory tract

C.J. Curione, Jr. et. al J Clin Micro 5:381-382, 1977  
 Contra Costa County Public Health Laboratory

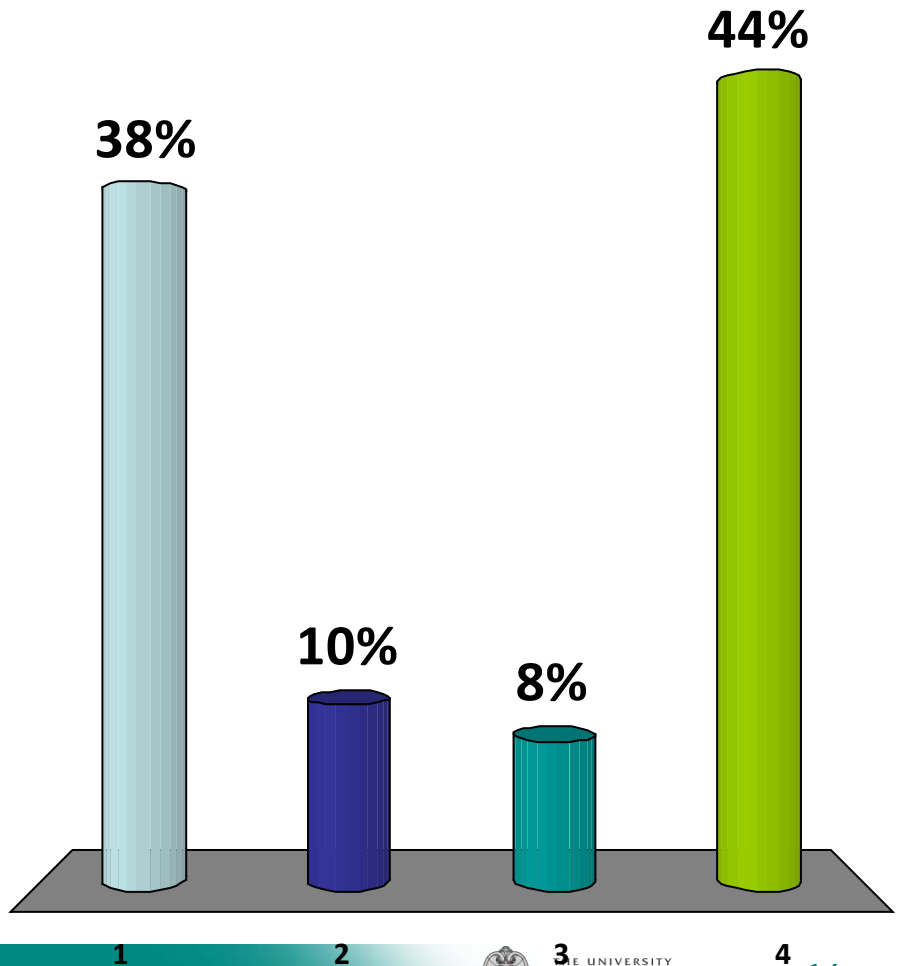
# Does your laboratory reject specimens that have a volume of...

1. Less than 3 ml
2. Less than 2 ml
3. Less than 1 ml
4. Less than 0.5 ml
5. If we can see it, we process it



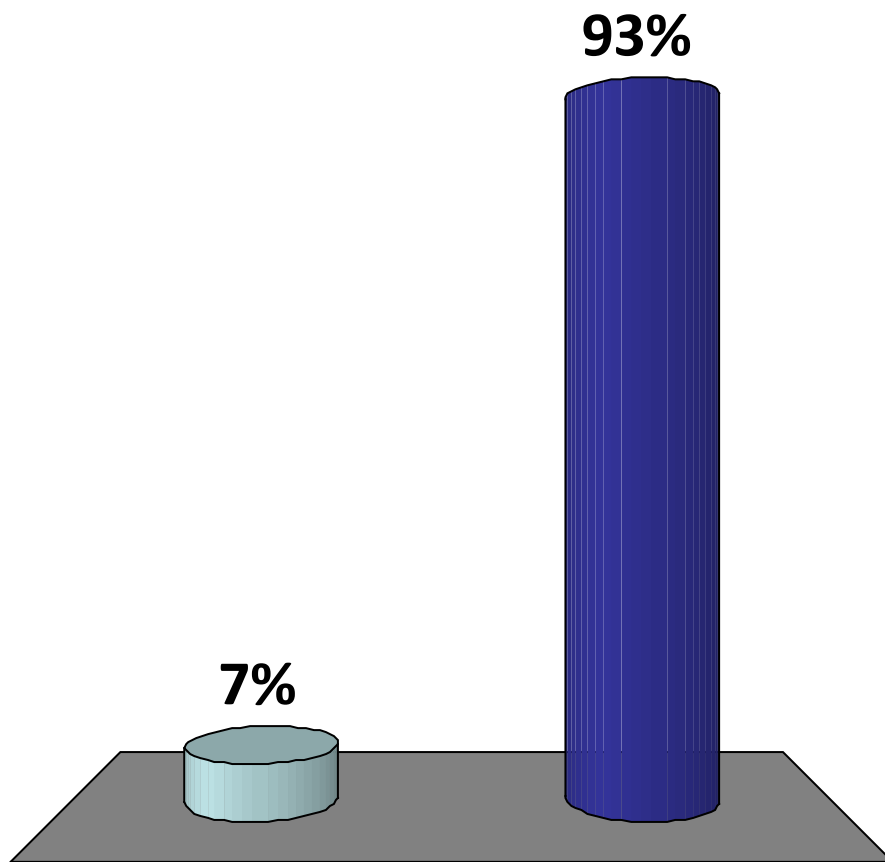
# Does your laboratory reject sputa that are...

1. More than 7 days post-collection
2. More than 5 days post-collection
3. Other time criteria
4. Don't reject sputa based on time post-collection



# Does your laboratory reject sputa based on gross appearance i.e. if they look like saliva

- 1. Yes
- 2. No



# Sputum Collection Guidelines



## GUIDELINES

# Initial Diagnostic Specimens—CDC Guidelines

- 3 sputum specimens
  - 8-24 hours apart
  - At least one of which is an early morning specimen
  - Additional specimens may be needed if specimens are smear negative
- Optimally, collect prior to initiation of drug therapy
- Test by both concentrated smear and culture
  - Report smear results within 24 hours

# How Many Sputum Specimens are Enough?

**TABLE 2.** Frequency distribution of the first positive specimen for patients from whom three or more specimens were collected for AFB smear and culture

Collection order of specimen	No. of patients (%) that were:			
	Either <sup>a</sup>	Smear positive <sup>b</sup>		Smear negative
		Smear	Culture	
First	80 (67)	41 (73)	41 (73)	39 (61)
Second	33 (28)	8 (14)	11 (20)	22 (34)
Third	7 (5)	4 (7)	4 (7)	3 (5)
Fourth or later	0 (0)	3 (6)	0 (0)	0 (0)

<sup>a</sup> Numbers are for the first culture–positive specimens for all patients, both smear positive and smear negative.

<sup>b</sup> At least one specimen obtained from each member of this group of patients was smear positive.

S.M. Nelson et. al J.Clin.Micro 36:467-69, 1998

# NAA Testing

- CDC guidelines
  - NAA testing on first sputum of all patients suspected of TB for whom the test result would alter case management or TB control activities
    - NAA should not be ordered routinely when clinical suspicion of TB is low.
- *Can NAA reduce the number of sputum specimens needed?*

# NAA Testing and Culture

Processing: 5 days; NAAT 4 days; broth medium monitored 7 days

NAAT (first specimen) – AFB smear and culture (3 specimens) – 797 pt  
[81 TB] All induced sputa

Assay	Sens	Spec	PPV	NPV	Mean TAT
AFB Smear	70	98	79	96.7	1
NAAT	90	100	100	98.9	2
Culture x 3	96	100	100	99.6	18

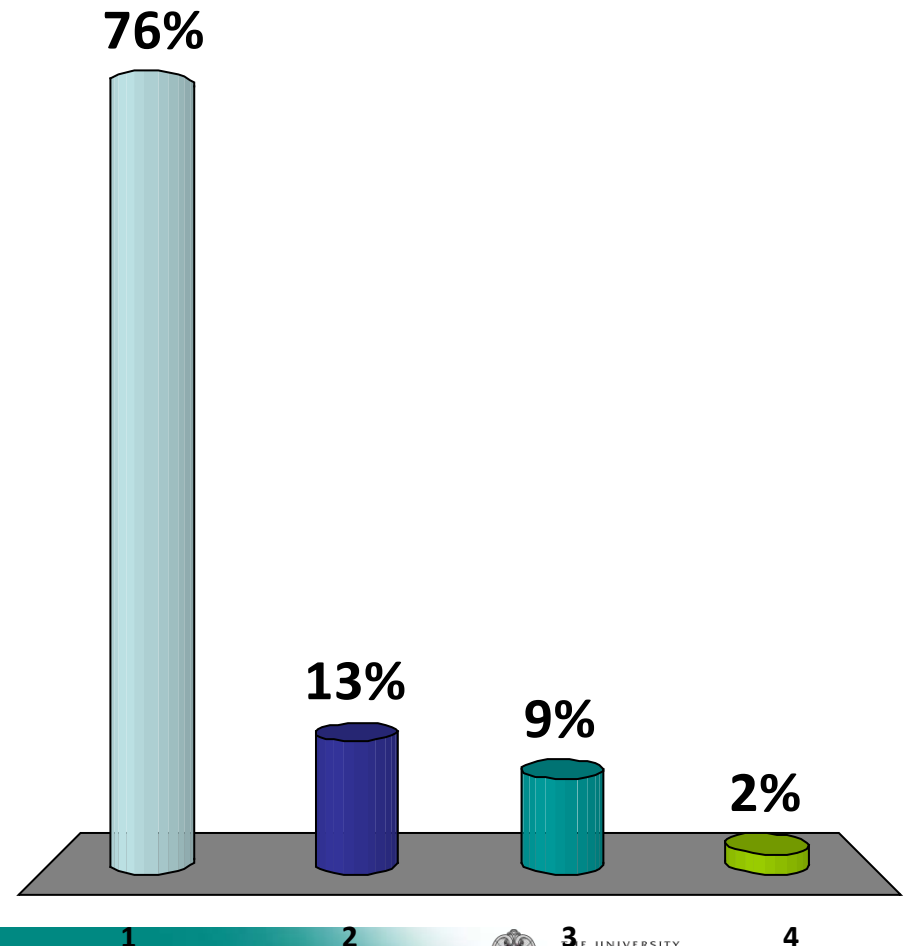
Moore et al - DMID 52:247-254(2005)

# Testing for Release from Isolation

- Criteria for release from isolation
  - Adequate course of therapy for minimum of 2 weeks
  - Clinical evidence of improvement
  - 3 consecutive **SMEAR NEGATIVE** sputa or bronchial secretions collected on different days
    - Collect at 8-24 hour intervals, with at least one sputum being an early morning specimen
- Performance of culture is not necessary on these specimens

# Our laboratory offers a test that is smear only (without culture)...

1. No
2. Yes, but it is not utilized
3. Yes, and it is utilized
4. Yes, but it's often ordered incorrectly



# Monitoring the Course of Treatment

- Obtain specimens at least monthly (for patients with positive cultures before therapy) until the cultures convert to negative
  - If sputum culture becomes contaminated, request a new specimen to avoid gaps in patient monitoring

# Monitoring the Course of Treatment

- MDR-TB patients
  - Perform cultures monthly for the entire course of treatment
  - Do not release from isolation until 3 consecutive sputa are **CULTURE NEGATIVE**

# Monitoring the Course of Treatment

- Patients whose smears or cultures have not become negative or symptoms do not resolve after 3 months therapy should be reevaluated for potential drug-resistance as well as for potential failure to adhere to the treatment regimen
  - Repeat DST
- Consider consultation with the Regional Training and Medical Consultation Center

# Monitoring the Course of Treatment

- Second-line DST should be considered ASAP if patient:
  - Has had prior therapy
  - Contact of patients with MDR-TB
  - From a country with endemic MDR-TB
  - Isolate resistant to rifampin or other first-line drugs
  - Has positive cultures after 3 months or more of therapy

# Acknowledgements

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