

**Multidrug Resistant Tuberculosis:
Emerging Global Challenge**
International Science and Technology Center
Moscow, Russia

**Specimen Collection for TB Bacteriological Confirmation
and Drug-Susceptibility Testing**
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Carlos M. Perez-Velez, MD
Grupo Tuberculosis Valle-Colorado (GTVC)
Infectious Diseases Service
Clínica Leon XIII
Universidad de Antioquia
Medellin, Antioquia, Colombia

Factors in Bacteriological Confirmation

- Specimen collection
- Bedside processing
- Transportation from POC to laboratory
- Laboratory processing
- Microbiological test

- Most diagnostics for TB developed to test sputum
- Most young children (< 6-8 y.o.) unable to produce good quality sputum
- Gastric aspirate frequently used

Gastric Aspiration or Lavage

- Poorly accepted by children
- Requires hospitalization or repeated visits (x 3 days)
- Trained staff
- Low bacteriological yield
- Specificity?
- Test inhibitors

Gastric Aspirate (GA)



Sputum Induction

Arch Dis Child 2000;**82**:305–308

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Sputum induction for the diagnosis of pulmonary tuberculosis in infants and young children in an urban setting in South Africa

H J Zar, E Tannenbaum, P Apolles, P Roux, D Hanslo, G Hussey

- Ambulatory clinics
- Yield at least as good as sequential GA
- Single IS had same cumulative yield as 3 GA

Sputum Induction

Requirements:

- Bronchodilator via inhalation
- Nebulizer
- Hypertonic saline via nebulization
- Well-ventilated rooms

Limitations:

- In younger children who cannot expectorate with coaching, requires nasolaryngeal suctioning

Induced Sputum (IS)



Induced Sputum (IS)



Induced Sputum (IS)



Sputum Induction

Original article

Induced sputum or gastric lavage for community-based diagnosis of childhood pulmonary tuberculosis?

M Hatherill,^{1,2} T Hawkrige,^{1,2,5} H J Zar,² A Whitelaw,^{3,4} M Tameris,^{1,2} L Workman,^{1,2} L Geiter,^{5,6} W A Hanekom,^{1,2} G Hussey^{1,2}

- IS and GA
- 191 culture-positive by IS or GA
 - IS+ = 108
 - GA+ = 127
- Single IS (38%) = single GA (42%)
- One-day IS + GA = 67%
- 2 samples GA = 66%
- 2 samples IS = 55%

Sputum Induction vs. Gastric Lavage

Indian J Pediatr. 2011 May 26. [Epub ahead of print]

Microbiological Diagnosis of Pulmonary Tuberculosis in Children: Comparative Study of Induced Sputum and Gastric Lavage.

Qureshi UA, Gupta AK, Mahajan B, Qurieshi MA, Altaf U, Parihar R, Bhau KS.

- 65 children (SI possible in 60 children)
- IS+ = 13.3% (8/60)
- GA+ = 7.7% (5/65)

Gastric Aspiration & Sputum Induction

OPEN ACCESS Freely available online

PLoS one

Multiple Sampling in One Day to Optimize Smear Microscopy in Children with Tuberculosis in Yemen

Nasher Al-Aghbari¹, Najla Al-Sonboli², Mohammed A. Yassin^{3*}, John B. S. Coulter³, Zayed Atef², Ali Al-Eryani², Luis E. Cuevas³

- 13.6% (29/213) children culture-positive
- Smear microscopy:
 - 3xGA = 34.5% (10/29)
 - 3xIS = 20.7% (6/29)
- Culture:
 - 3xGA = 9.4% (19/203)
 - 3xIS = 15.9% (13/82)

Nasopharyngeal / Nasotracheal Aspiration (post-nebulization with HTS)



Nasopharyngeal Aspirate

- Peru: Culture yield from NPA (19/64; 30%) was similar to that of GA (24/64; 38%).
- Uganda: Yield of culture from a single NPA sample (24%) was similar to that from an IS sample (22%).
- Peru: Culture yield from stool, NPA and gastric aspirate specimens amongst 165 children:
 - Stool (4/292; 1.4%)
 - NPA (12/314; 3.8%)
 - GA1 (22/321; 6.8%)
- Yemen: Single NPA and three consecutive GA and sputum (induced or expectorated) samples
 - NPA 7%
 - GA 9%
 - Expectorated sputum 8%
 - Induced sputum 14%

String Test

- Hyperabsorbent nylon string
- Via: orogastric or nasogastric
- Recovered 1 hour to 8 hours (i.e., left overnight) later
- Acceptability: well accepted by older children [Chow et al, 2006 BMC Infect Dis].
- Yield reported to be as good as IS in HIV-infected, sputum-scarce adult patients with probable pulmonary TB.

String test (ST)



String test (ST)



String test (ST)



Stool

- Children swallow sputum
- AFB resist digestion , survive intestinal transit
- Detection of bacilli or of their DNA (5 studies)
 - Smear microscopy (children) [Donald, 1996]
 - 8.1% (5/62) [Donald et al, 1996]
 - Culture (children)
 - 4.8% (3/62) [Donald et al, 1996]
 - 4.7% (9/192) [Cadmus et al, 2010]
 - 1.4% (4/292) [Oberhelman et al, 2006]
 - NAAT (adults)
 - [Cordova, 2010]
 - [Ramadass, 2010]
- Yield: lower than culture of GA or sputum

Stool

Setting: South Africa

Donald et al, 1996; *J Trop Pediatr.* 42:311

Results of investigations in 62 children with probable pulmonary tuberculosis

Non-diagnostic CR	Diagnostic CR	Contact	Mantoux positive	Culture of gastric aspirate positive	Stool	
					Culture positive	AFB
2	—	—	—	2	—	1
8	—	8	—	1	—	—
1	—	—	1	1	—	—
4	—	4	4	1	—	—
—	28	—	2	8	1	2
—	6	6	—	3	2	2
—	6	—	6	1	—	—
—	6	6	6	2	—	—
—	—	—	—	1*	—	—
15 (24%)	46 (74%)	24 (39%)	19 (31%)	20 (32%)	3 (5%)	5 (8%)

CR = chest radiograph; AFB = acid fast bacilli.

* This child has a normal CR and a negative Mantoux test, and was investigated because of severe malnutrition.

Fine needle aspiration biopsy (FNAB)

TB lymphadenitis

- Most common EPTB presentation
- 25% of children with unresolving cervical lymph nodes have TB adenitis

Advantages

- Yield: yield can be higher than culture of respiratory specimens.
- Invasiveness: Minimal
- Risk: low
- Ambulatory
- Often smear-positive
- Sterile specimen: culture & NAAT
- Other indications: Also useful for histopathological study of other causes of lymphadenopathy (e.g., lymphoma, squamous cell carcinoma, neurofibroma, lipoma, cysticercosis, HIV infection)

Fine needle aspiration biopsy (FNAB)

Author	Country	Age Group	# Samples	Confirmed
Cadmus, 2011	Nigeria	Adults/Children	1120	285 (25-45%)
VanWyk, 2011	S. Africa	Children	182/121	Cult 84 (69%)
				FM 48-52%
Sharma, 2010	India	Adults/children	150	49 (33% by culture, ZN, PCR)
Lakhey, 2009	Nepal	Adults/Children	122	71 (58% AFB)
Sharma, 2010	India	Adults/Children	622	84.2% (cytology)
Balaji, 2009	India	Children	135	46 (34%)
Wright, 2010	S. Africa	Children	104	54 (51.9%)
Annam, 209	India	Adults/Children	93	31 (33%) ZN
				59 (44%) bl-ZN

Standardization of Protocols for Optimization

Gastric Aspiration

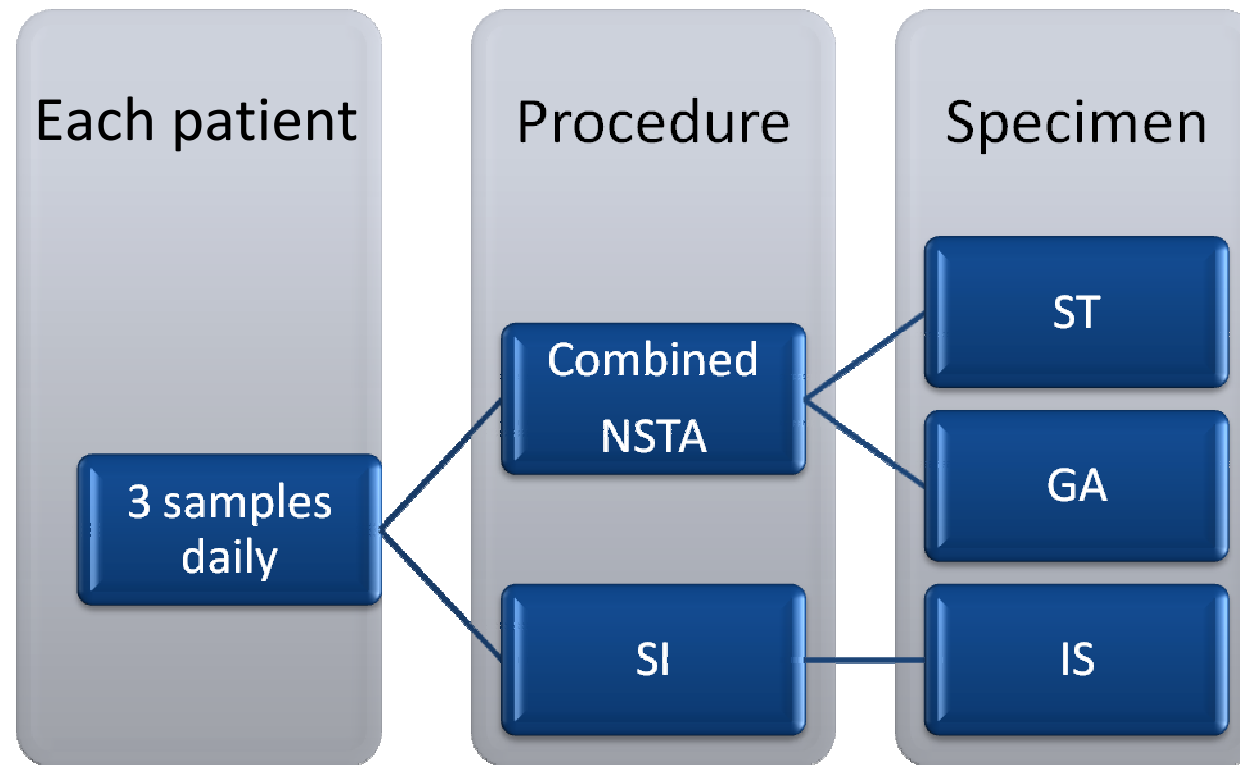
- Place NGT ahead of time (e.g., at night) vs. at time of collection (e.g., in morning)
- Add saline solution or sterile water (“lavage”) vs. avoidance of lavage (may contain preservatives)
- Neutralize
 - by titration vs. by predetermined volume of buffer solution (NaOH)
 - Within 30 minutes or at time of processing in lab

Factors affecting the Relative Yield of Specimen Collection Methods

- Patient population
- Technique
- Method(s) for detecting *M. tuberculosis*

Methods

Interventions



Each procedure was carried out every morning for 3 consecutive days:
total of 9 samples were collected from each patient

Yield of Combinations of Specimens

Specimens	Culture ¹		PCR ²		Culture or PCR ³	
	Patients (%) n= 245	CI	Patients (%) n= 86	CI	Patients (%) n=86	CI
GA	20 (8.2)	Ref	28 (32.6)	Ref	31 (36.0)	Ref
ST	17 (6.9)	-0.063 - 0.039	44 (51.2)	0.030 - 0.342*	45 (52.3)	0.005 - 0.321*
IS	22 (9.0)	-0.045 - 0.062	30 (34.9)	- 0.130 - 0.176	31 (36.0)	-0.155 - 0.155
GA or ST	23 (9.4)	-0.042 - 0.066	59 (68.6)	0.209 - 0.511*	61 (70.9)	0.198 - 0.500*
GA or IS	25 (10.6)	-0.031 - 0.080	44 (51.2)	0.030 - 0.342*	48 (55.8)	0.040 - 0.355*
ST or IS	23 (9.8)	-0.038- 0.071	55 (64.0)	0.161 - 0.467*	56 (65.1)	0.136 - 0.445*
GA or ST or IS	28 (11.4)	-0.024 - 0.089	64 (74.4)	0.272 - 0.566*	66 (76.7)	0.260 - 0.554*

¹Positive by one or more samples. ²Positive by pooled sample. ³Positive by any sample or pooled sample, by either detection method.

⁴95% confidence interval for the difference between two proportions (GA as reference). *Fisher's exact test with 2-sided *p* value < 0.05

Conclusions

- ❑ The combined NSTA is a feasible procedure, “*proof of concept*”.
- ❑ In those who met WHO criteria for the diagnosis of probable TB, bacteriological confirmation by culture (even using both solid and liquid media) of 3 samples of 3 different specimens (a total of 9 samples) is still remarkably low (11.0%).
- ❑ By culture methods, no individual or combination of specimens had a significantly greater yield (all CIs overlapped 0).

Conclusions

- ❑ However, by PCR, the ST alone had a much greater yield than GA or IS alone (51.2% vs 32.6% vs 34.9%).
- ❑ The combination of ST and GA had the highest yield (68.6%).
- ❑ PCR had a significantly greater sensitivity than culture methods for detecting MTC in any type of specimen.
- ❑ Nonetheless, culture isolation complemented PCR as there were specimens that were positive only by culture and negative by PCR.

Specimen Collection:

Key Points

- DR-TB is bacteriological diagnosis
- Bacteriological confirmation is dependent on specimen sampling
- There are good alternatives to spontaneous sputum and to gastric aspirate
- Combinations of specimens appear to have a higher yield than a single specimen
- The increased yield of combinations may allow for decreased days of sampling.