Don’t Stop IDin’: Mycobacterial Identification Strategies

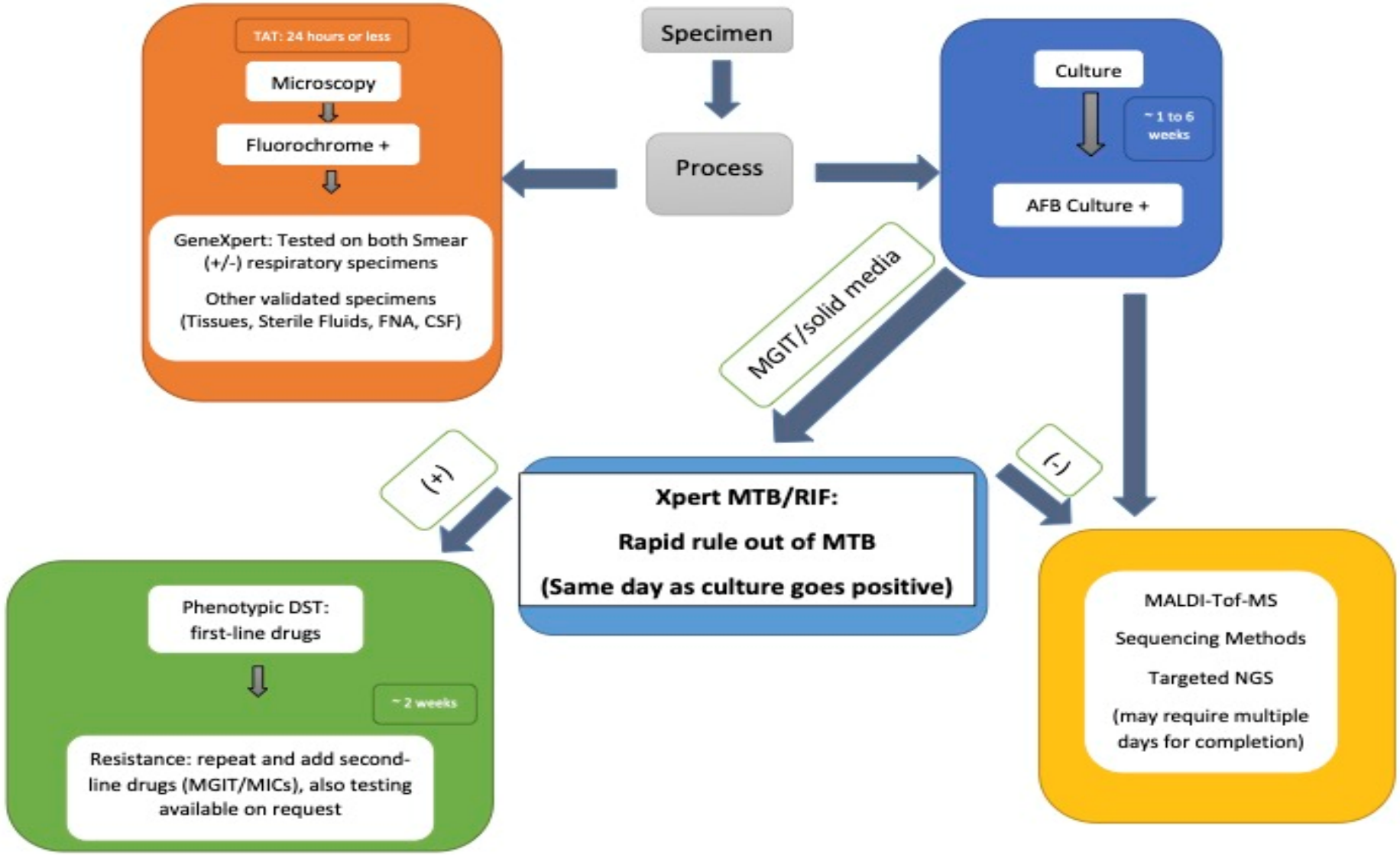
Expansion of GeneXpert testing:
JHU Clinical Mycobacteriology Laboratory

JOHNS HOPKINS
SCHOOL of MEDICINE

Gap: Rapid (Same-day) rule-out of TB on Non-respiratory samples and AFB(+) cultures (liquid/solid)

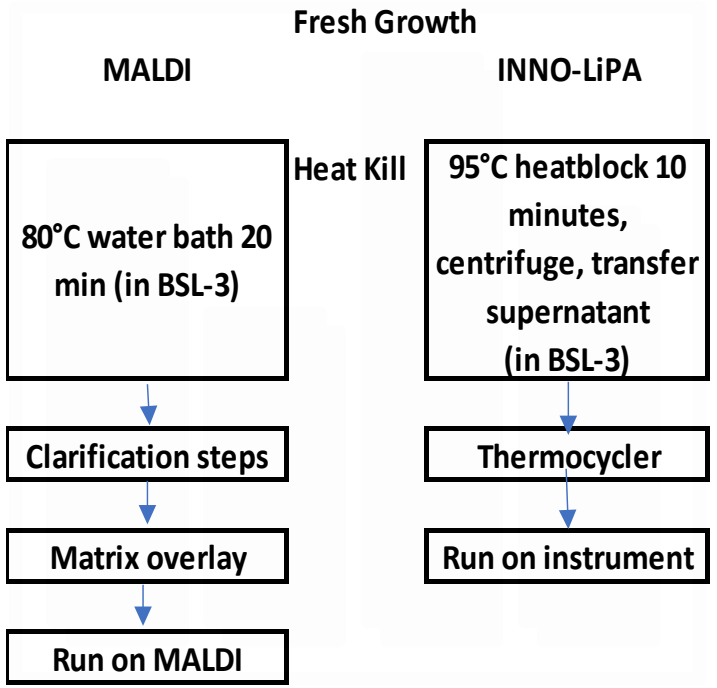
- **Solution:** Validation of GeneXpert MTB/RIF (Already in-house)
  - Processed EPTB samples (Either Smear positive or by physician request/Lab Director approval)
    - Tissues, Sterile Fluids, CSF, FNA
  - AFB(+) Cultures
    - Machine-flagged (+) MGIT tubes, confirmed AFB Smear (+) by ZN
    - LJ (+), confirmed AFB Smear (+) by ZN
- **Process**
  - Validation testing (Accuracy, Precision)
    - Mixture of clinical AFB(+) MGITs and spiked MGITs-MTBC (RIF-R and RIF-S)
    - Reproducibility, Cross-reactivity (NTM, mixed) assessed
    - Comparator: MALDI-TOF and Accuprobe™, Reference strains from JH archive and ATCC
  - Implementation after tech training, QA/QC setup and physician communication
  - Testing occurs according to Package Insert (3:1 Reagent to Sample ratio) inside BSL3
  - Cartridge cost: ~$60 / test

Sample Diagnostic Algorithm (JHU)



TN TB identification

- MALDI is the primary method- in place since 2016



- INNO-LiPA secondary method for mixed culture or NTM from sterile sites that failed identification on MALDI.

	MALDI	INNO-LiPA
TAT	Same day	Overnight
COST	$45	$113

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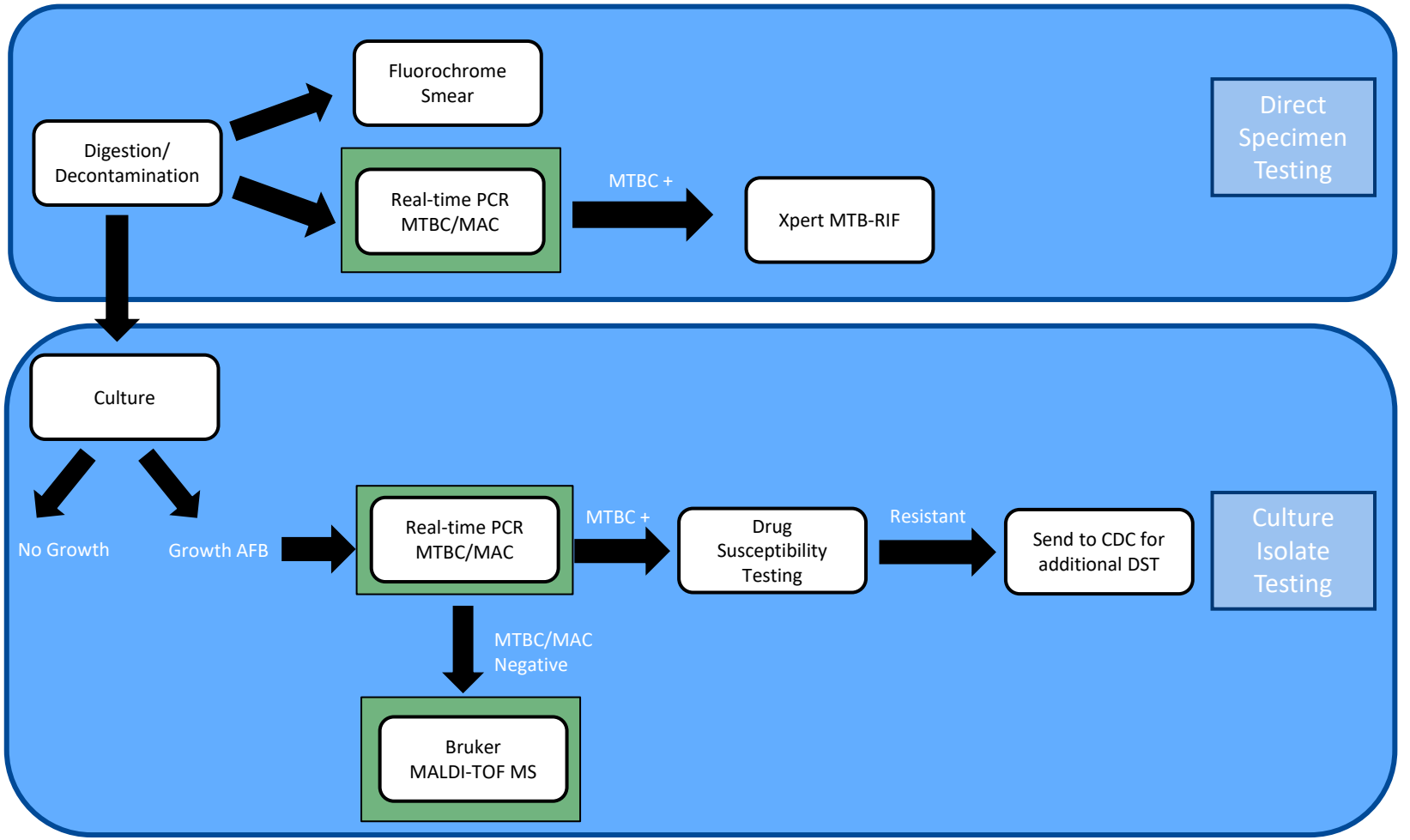
Mycobacteria Testing



Inactivated prior to testing performed in BSL-2

PCR: 300 µL sputum sediment (Direct) or 1 mL suspension (Isolate) heated at 80°C for 1 hour
MALDI: 300 µL suspension heated at 95-100°C for 30 minutes

Cost

PCR = ~ $10/test
MALDI = ~ $2/test

TAT

PCR = 48-72 hours
MALDI = batched weekly

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Test	Schedule of Testing	Test Information
Digestion/ Decontamination	• Performed daily (Monday – Friday) • TAT: 24 hrs from specimen receipt	• Optimal volume 5 mL sputum • Store refrigerated; Send to lab within 24 hours of collection • Contact lab for guidance on other sample types
Fluorochrome Smear	• Performed daily (Monday – Friday) • TAT: 24 hrs from specimen receipt	• Results: Negative; Positive (with quantitation)
Real-time PCR MTBC/MAC	• Performed daily (Monday – Friday) • TAT: 72 hrs (Direct testing) • Sputum only	• Direct testing performed on all patient samples unless previously positive MTBC sample; Culture testing to identify isolates • Results: MTBC Detected/Not Detected; MAC Detected/Not Detected • Positive Direct MTBC will reflex to Xpert MTB-RIF
Xpert MTB-RIF	• Performed daily as needed (Monday – Friday) • Sputum only	• Results: Rifampin Resistance Detected/Not Detected/Indeterminate • Not approved for pediatric patients (< 18 years); patients treated with anti-tuberculosis treatment for ≥ 3 days* • If resistance detected, will reflex to MDDR testing
Culture	• Read weekly • Negative cultures incubated for 6 weeks	• Preliminary report (issued if culture growth): AFB isolated, identification to follow • Reflex to Real-time PCR and/or MALDI-TOF for identification • Identification of MTBC will reflex to drug susceptibility testing
MALDI-TOF MS	• Performed weekly	• Preliminary or Final identification result
Drug Susceptibility Testing	• Performed twice per week • 4 - 21 days for results (from identification) • MGIT 960 method	• Results: RIPE, high INH, MOX Sensitive/Resistant • Resistant result will reflex to send out susceptibility testing at CDC • Performed on MTBC isolate from each patient (every 3 months from collection date)

Color indicates Molecular Testing

MTBC: *Mycobacterium tuberculosis* complex; MAC: *Mycobacterium avium* complex; MDDR: molecular detection of drug resistance; AFB: acid-fast bacilli; MALDI-TOF: matrix-assisted laser desorption ionization – time of flight; RIPE: rifampin, low isoniazid, pyrazinamide, ethambutol; INH: isoniazid; MOX: moxifloxacin

*Samples that are not approved for the Xpert MTB-RIF testing may qualify to be tested for MDDR at CDC.
DCLS TB Laboratory (804)648-4480 ext.255

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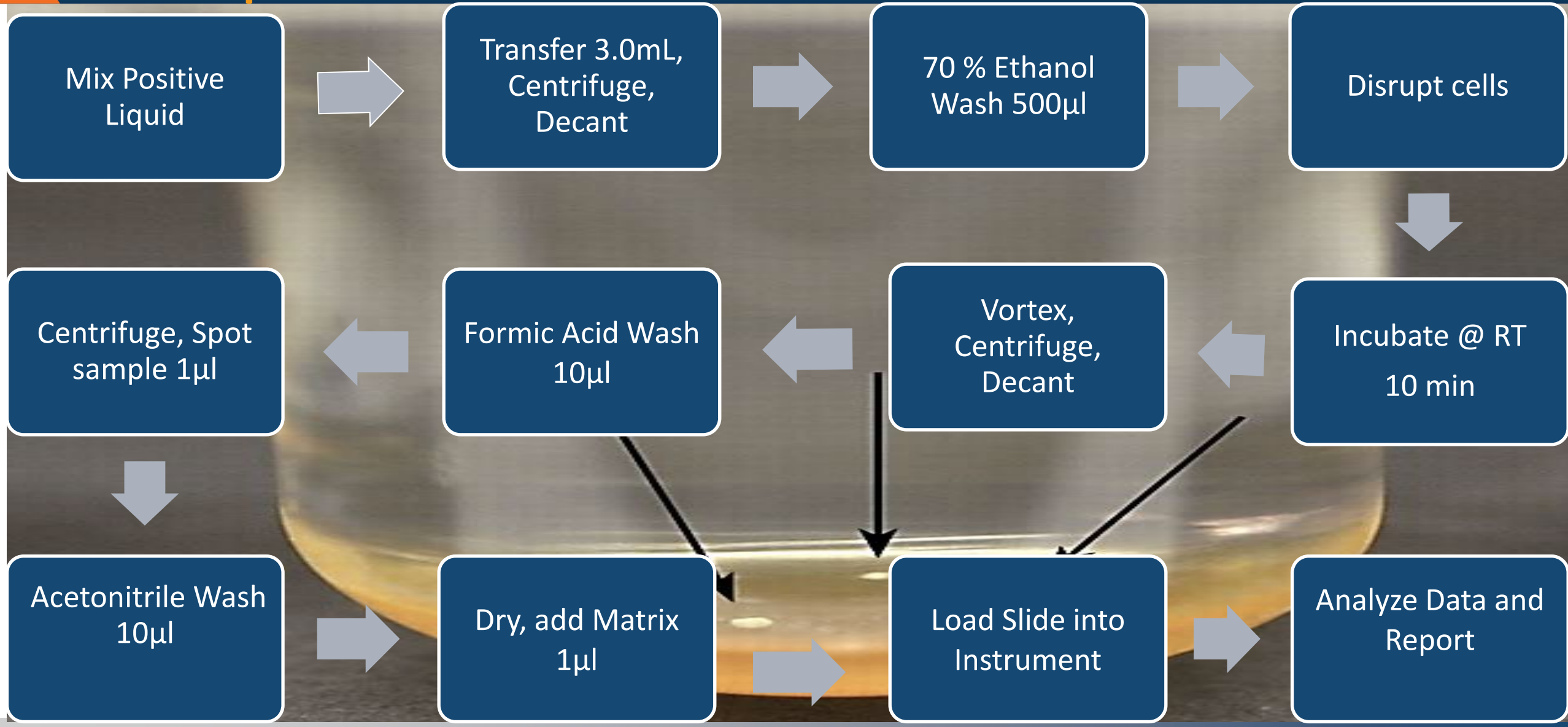
Mycobacteria Sample Preparation

From Liquid Media

HOUSTON HEALTH DEPARTMENT



#1: What did you take into consideration when choosing which method to select for your laboratory?

#2: What was your approach to validation or verification?

Please discuss the reference/comparator methods and number and type of strains used, including how isolates were obtained.

#3: Please explain your approach to validation of a kill step, if applicable.

#4: What roadblocks did you encounter during your validation or verification? How did you solve these problems?

#5: Are there issues that you have encountered after implementation?

#6: Is the method you are using sustainable, or will your laboratory potentially be looking for additional methods in the future?

#7: What kind and extent of training was required for testing personnel?

#8: Why did you choose this workflow for TB and/or NTM testing in your lab and what do you perceive as advantages and disadvantages?

#9: What is the cost per sample for the methods you utilize (with or without technical time)?

Questions?