DCLS

Division of

Consolidated Laboratory Services

DGS

DEPARTMENT OF GENERAL SERVICES

Validation and Implementation of Real-time PCR of MTBC/MAC: Impacts to Virginia's Public Health Laboratory and Department of Health

Rana Mehr

9/29/2022

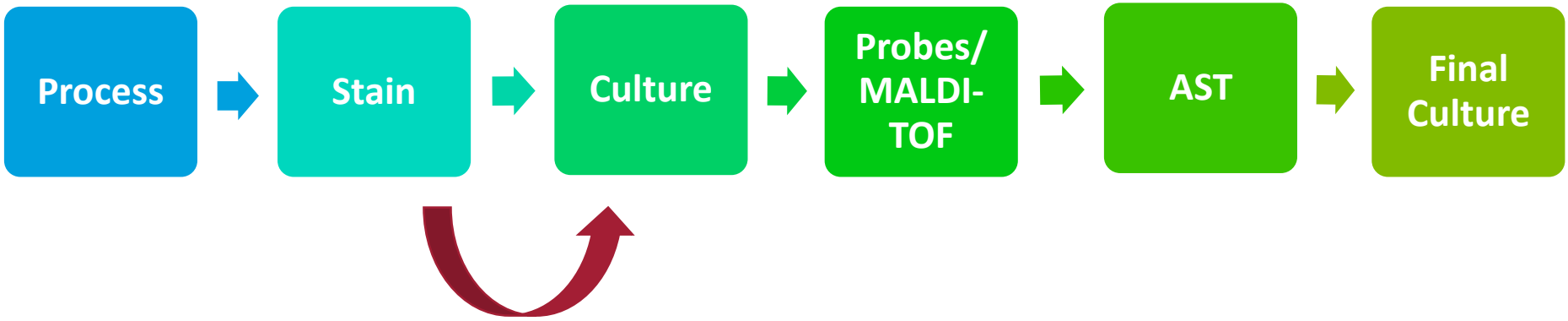
Objectives

- ✓Recognize the different mycobacterial nucleic acid amplification testing (NAAT) performed at DCLS
- ✓Identify the benefits of the DCLS TB Laboratory's newly implemented testing algorithms
- ✓Summarize the impacts of mycobacterial NAAT on individual patients and public health

Keywords and Abbreviations

- **MAC:** *Mycobacterium avium* complex
- **MTBC/MTB:** *Mycobacterium tuberculosis* complex/*Mycobacterium tuberculosis*
- **RIF:** Rifampin
- **PCR/Polymerase Chain Reaction:** Gene-specific primers are used to select segments within a genome to amplify many copies of DNA
- **Extraction:** Method used to purify DNA by using physical and/or chemical methods to separate DNA from other cellular components
- **AST:** Antimycobacterial susceptibility testing

Previous TB Laboratory Workflow



Xpert® MTB/RIF Advantages and Disadvantages

Advantages

- Test directly from processed Sputa
- Detection of target sequences
  - MTBC
  - RIF resistance
- Rapid results (within 2-3 hours)
- Increased sensitivity over AFB stain

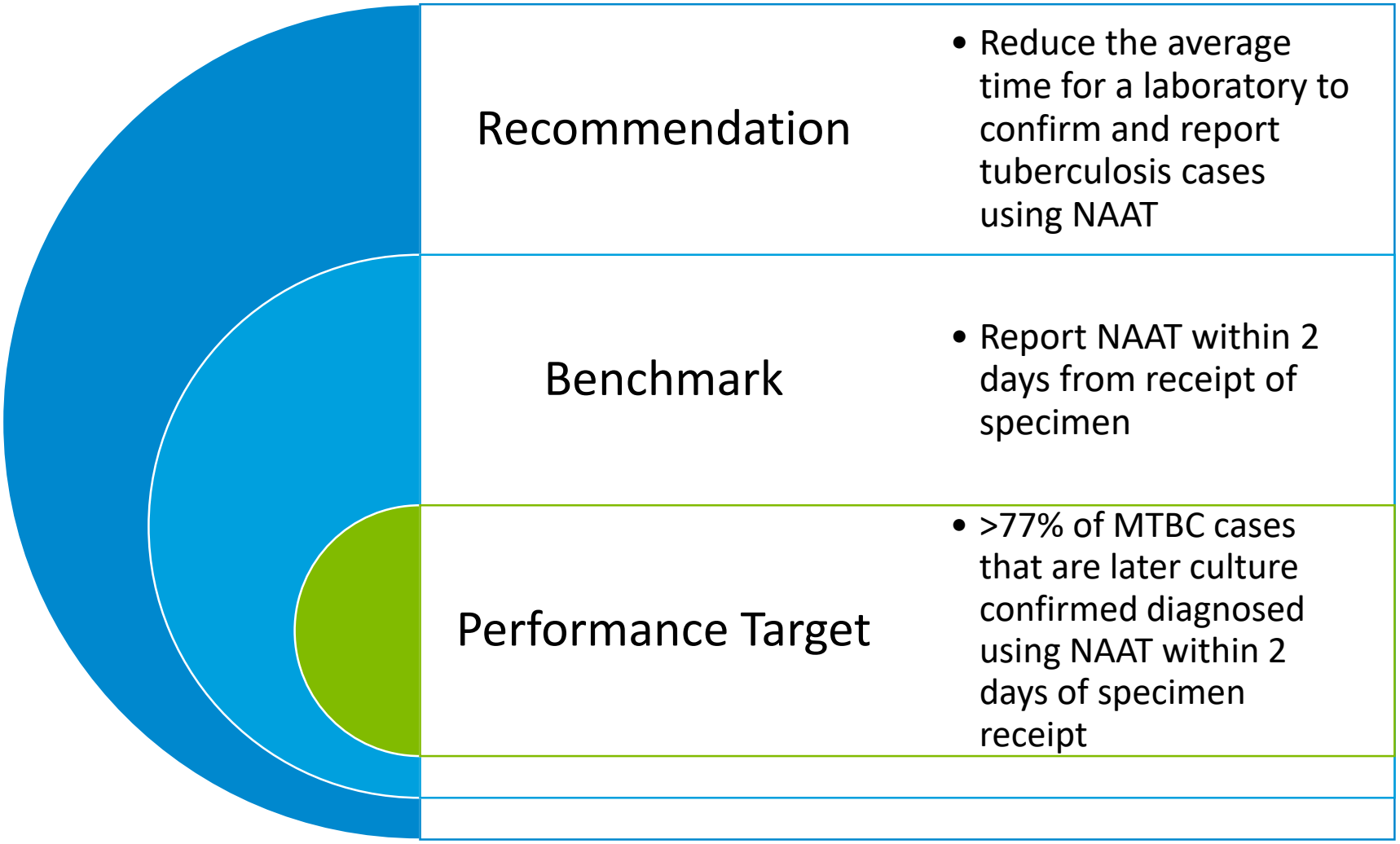
Disadvantages

- Specimen may contain amplification inhibitors (false negative)
- Detects non-viable MTBC
- Negative test does not rule out possibility of isolating MTBC from sputum culture
- Xpert has not been validated for samples from pediatric patients

Previous Xpert® MTB/RIF Testing Criteria

- Expectorated or induced sputum
- Patient on less than 3 days of anti-tuberculosis therapy
- Cannot be performed on pediatric patients
- First time smear Fluorochrome positive patients only
- Not available for Fluorochrome-negative patient specimens
  - Limited DCLS' performance against the CDC Healthy People NAAT benchmark

Healthy People 2020/2030 Background



NAAT Criteria for Direct Sputa

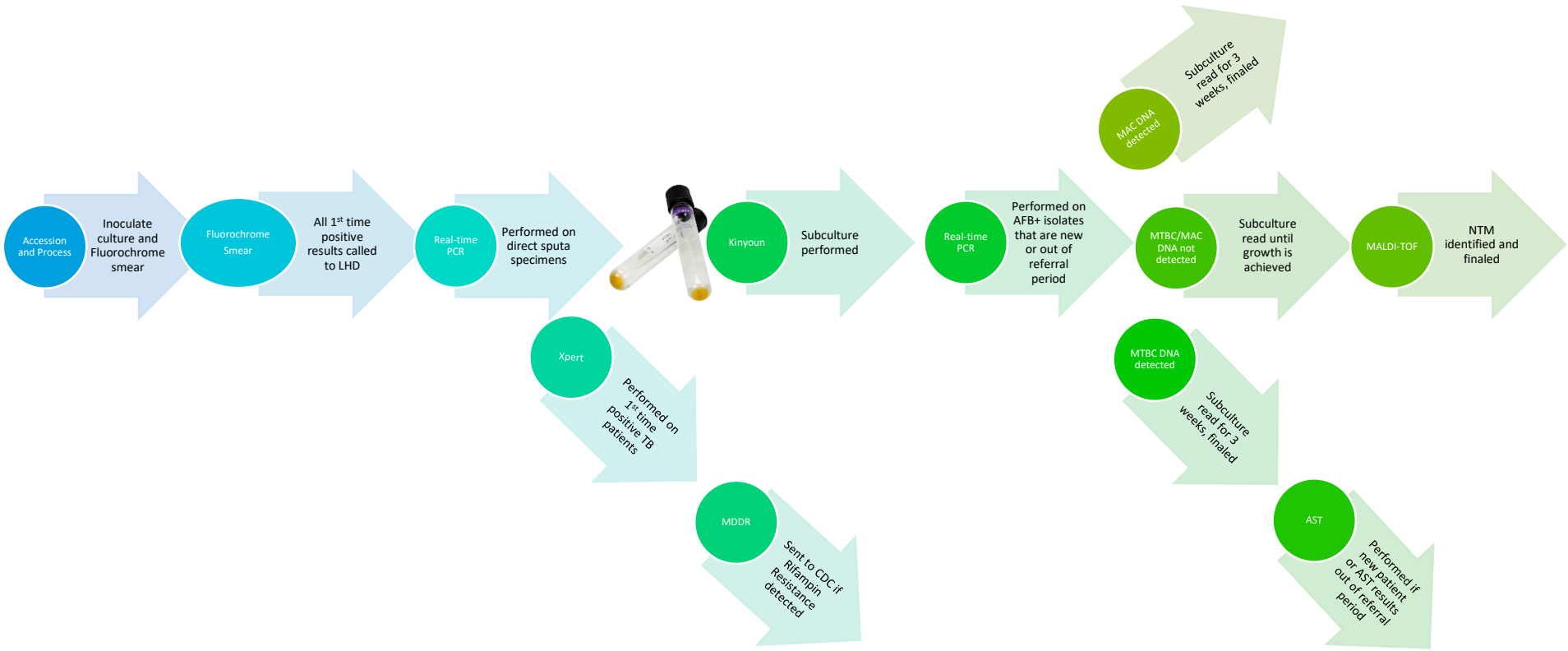
Real-time PCR

- Patients currently not on anti-tuberculosis therapy
- Patients without a previous positive MTBC result (NAAT and/or culture) within the past 12 months

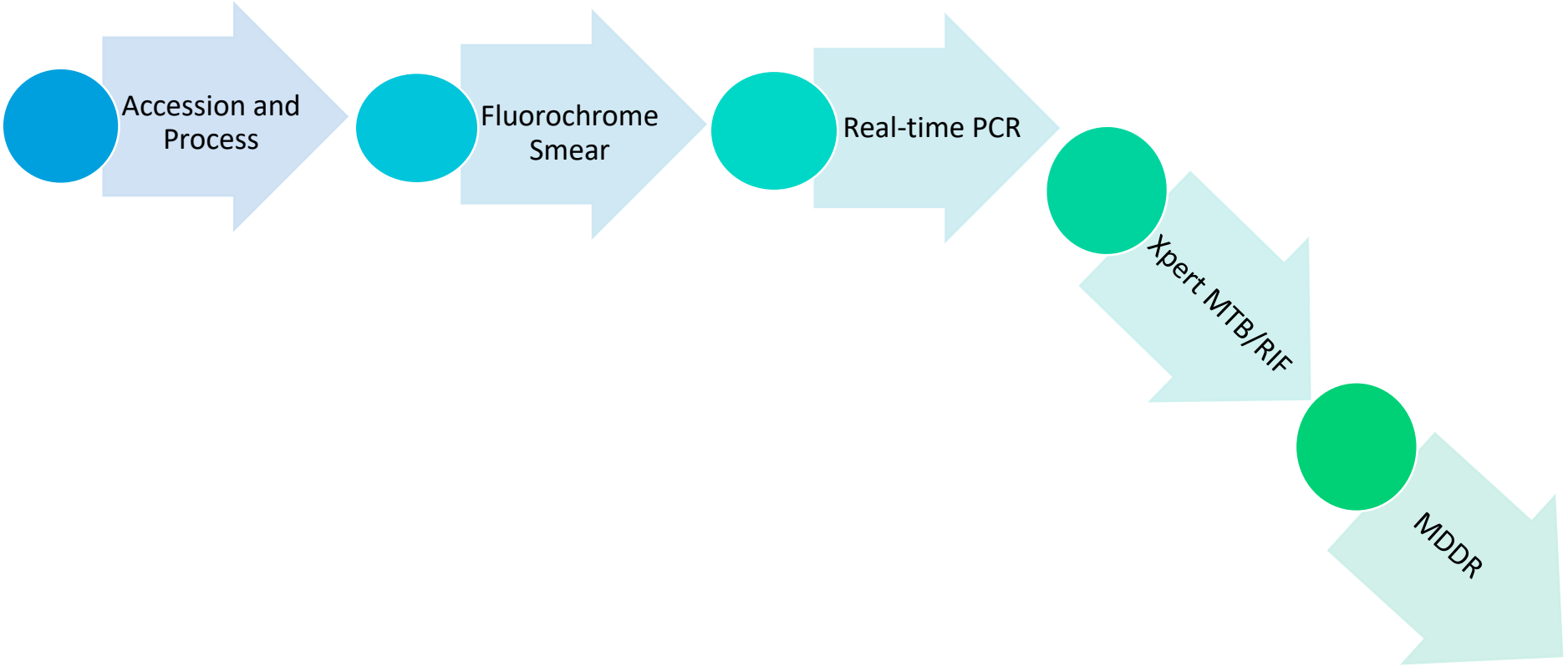
Xpert MTB/RIF

- Patient on anti-tuberculosis therapy for less than 3 days
- Sufficient volume
- Non-pediatric patients

TB Laboratory Testing Algorithm



NAAT Algorithm for Sputa



NAAT Algorithm for Sputa with Previous Positive

Accession and Process

Fluorochrome Smear

Kinyoun and Subculture

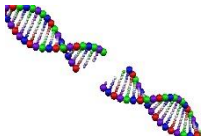
Subsequent Testing

Final Report

Real-time PCR Method

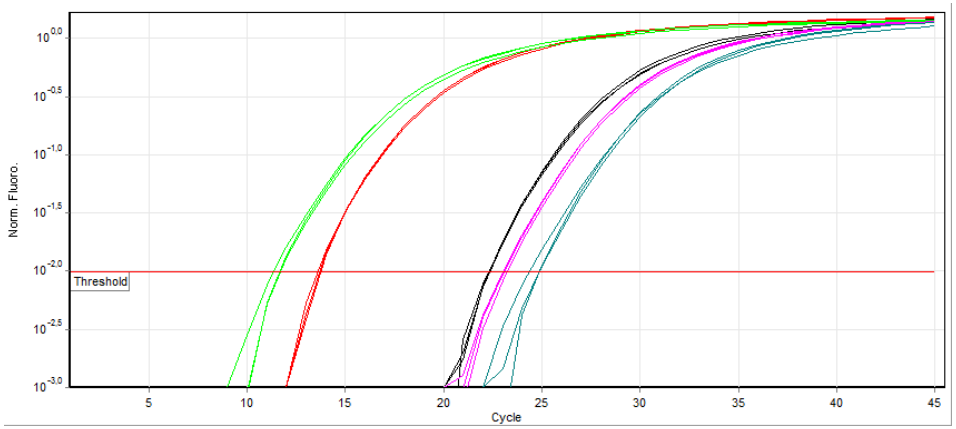
Direct sputa and positive AFB cultures are heat inactivated

DNA extraction



Mastermix and samples are added to 96 well plate

Real-time PCR occurs on ABI



Samples are analyzed for detection of DNA

Direct Real-time PCR Results

- Both MTBC and MAC results are released via preliminary report
  - MTBC
    - DNA Detected
      - LHD notified via phone if initial positive result
    - DNA Not Detected
    - Inconclusive
    - Inconclusive due to inhibition
  - MAC
    - DNA Detected
    - DNA Not Detected
    - Inconclusive
    - Inconclusive due to inhibition

PRELIMINARY REPORT

Direct PCR M. tuberculosis complex/M. avium complex **Date Released:**

Mycobacterium avium complex DNA by real-time PCR: DETECTED
Mycobacterium tuberculosis complex DNA by real-time PCR: Not Detected
Disclaimer: This test has not been cleared or approved by the U.S. Food and Drug Administration. The performance characteristics of this test have been validated by DCLS. The results from this assay should not be used independently to make decisions regarding the management of patient care or public health.

Implications of Direct Sputa Real-time PCR for Tuberculosis Diagnosis and Treatment

- **Advantages:**
  - Rapid results
  - Earlier treatment initiation
  - Improved patient outcomes
  - More rapid opportunities to disrupt transmission
  - Effective public health intervention
- **Disadvantages:**
  - May detect non-viable MTBC
  - Specimen may contain inhibitors (false negative)
  - A negative result (not detected) does not exclude possibility of isolating MTBC from sputum culture

DCLS Healthy People Benchmark

Prior to Real-time PCR

Benchmark reporting: 19.2% (2021 data)

Real-time PCR in April 2022

Benchmark reporting: 76.9% (April-June 2022)

DCLS TB Lab Real-time Facts

- 13 cases of MTBC from April- June 2022
  - 10/13 were reported within 48 hours of specimen receipt
    - 2 were PCR negative but later culture positive
    - 1 patient was not tested in 48 hours due to holiday weekend

- Of the 13 MTBC+ patients
  - 8 were Fluorochrome positive
  - 5 were Fluorochrome negative
    - 3/5 were reported MTBC+ within 48 hours

- From April- July 2022 DCLS performed real-time PCR on 521 sputa sediments

Questions?

Rana Mehr, MS
(804)648-4480 ex. 231
rana.mehr@dgs.virginia.gov