New Method Implementation in a Clinical Laboratory: Challenges and Opportunities

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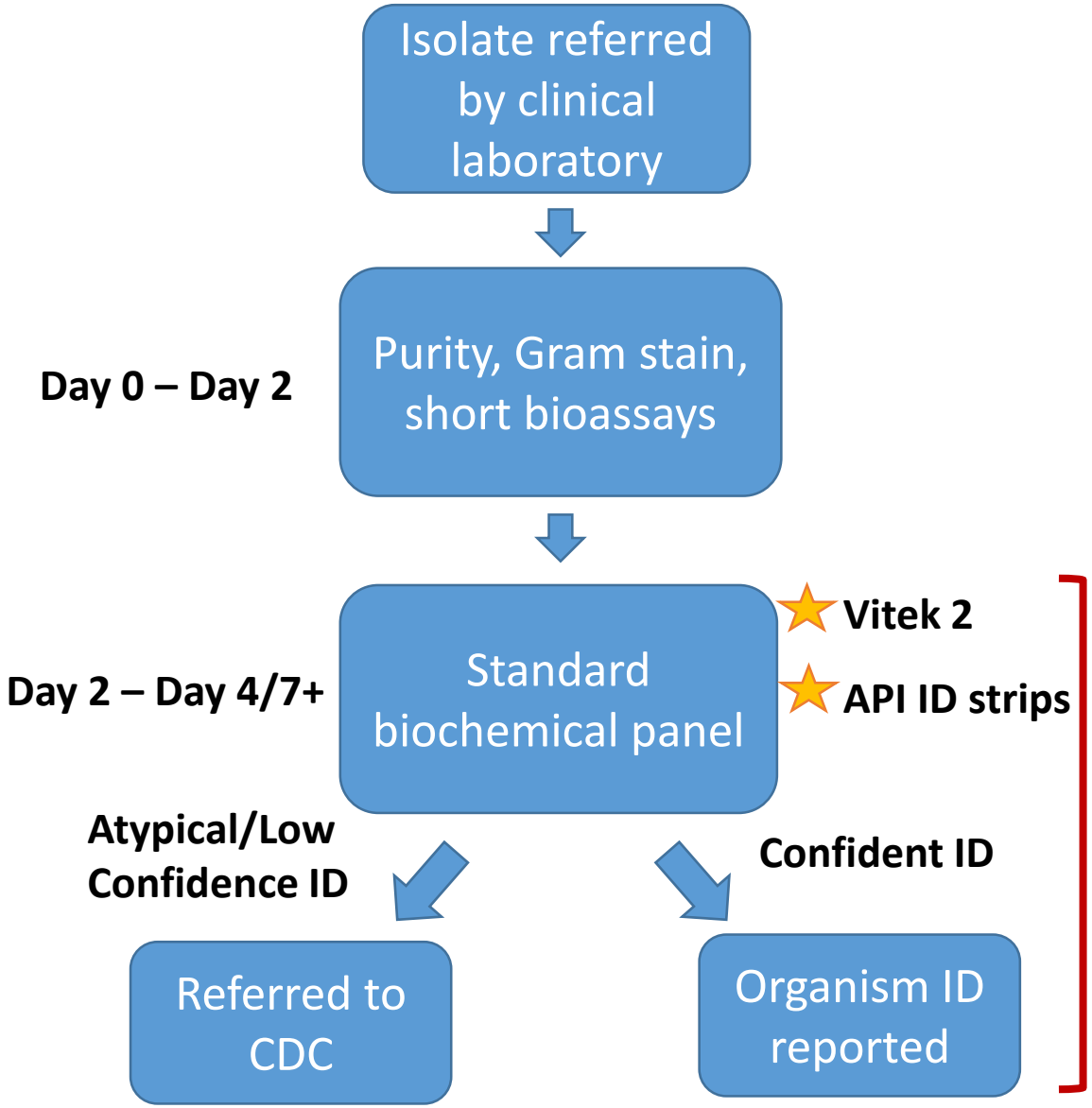
Conventional Approaches for Bacterial Identification

- Panels assays with numerous biochemical tests/tube bioassays
  - Challenges:
    - Taxonomic limitations
    - Developed for typical isolate biochemical profiles
    - Expertise and resource demands for test set-up, reading and interpretation
    - Standard panels may include 30 – 50 bioassays

Gram-Negative Rods/Coccobacilli	Biochemicals/Tube Bioassays
Strong glucose-fermentation	47+
Weak glucose-fermentation	27+
Non-fermenting Gram-negative bacilli	36+
Oxidase-positive, strong fermentation	50+

Biochemical Identification Workflow

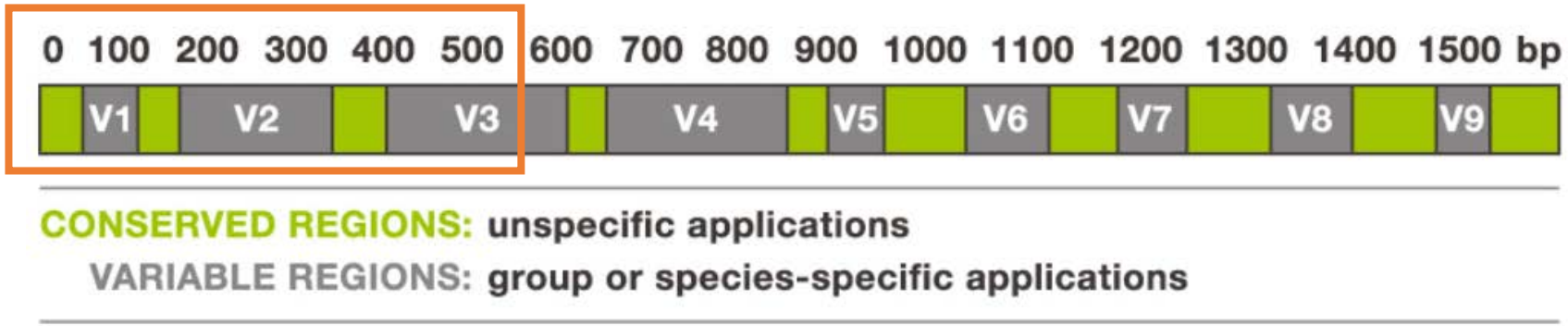


Opportunities for new technologies:

- **Reduce time and labor**
- **Increase sensitivity**
- **Reduce cost**
- **Improve turn-around time to result**
- **Reduce referrals to CDC**

New Technologies for Clinical Pathogen Identification: 16S rDNA Sequencing

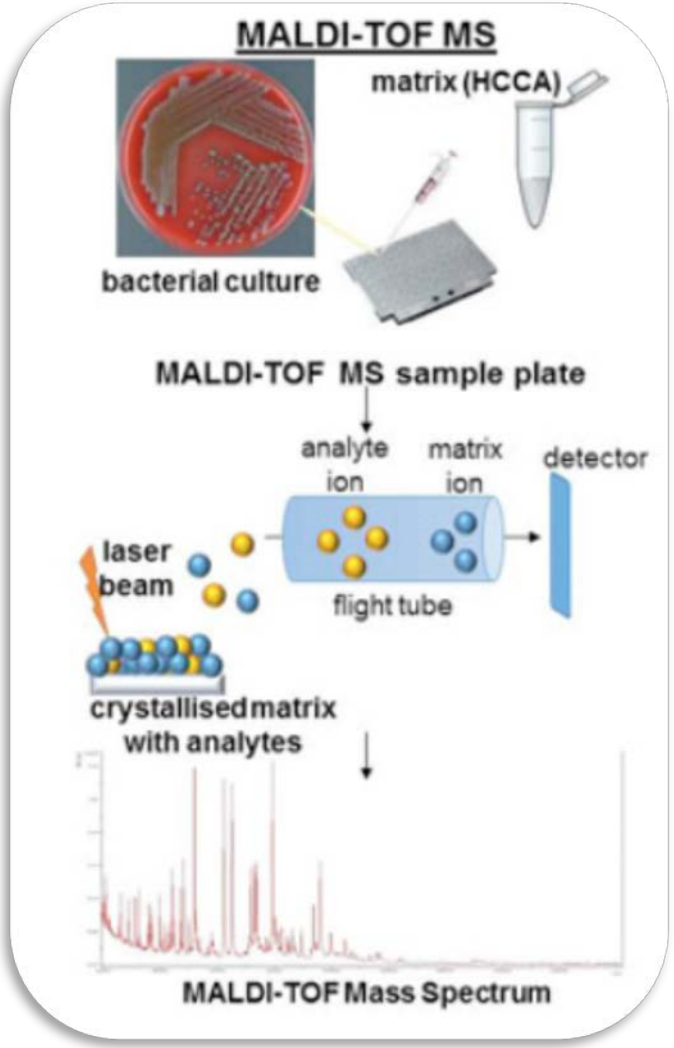
- **Molecular testing: 16S rDNA gene sequencing**
  - PCR amplification and comparative DNA sequencing of the bacterial 16S region (first 500 bp of rDNA)
  - Target is ubiquitous in bacteria
  - Reference sequence databases identification
  - Utilizing databases expanded to reflect updated taxonomy
  - **Lab benefit: Improved accuracy, reduced turnaround-time, ease of interpretation**



New Technologies for Clinical Pathogen Identification: MALDI-TOF MS

- **MALDI-TOF MS:**
  - Spectral analysis of bacterial biomass or extracted proteins
  - Reference database comparison of ribosomal protein spectra
    - Bruker Biotyper: Clinical applications (CA) and research use only (RUO) Databases
  - **Lab benefit: Minimal processing time, CA FDA approved, significant cost and TAT reductions**
  - One publication:
    - "Time and labor cost reductions by 56.9% for one laboratory testing 952 isolates (824 bacterial and 128 yeast) = $102,424"
    - "TAT to identification reduced by 1.45 days vs. standard protocols"

J Clin Microbiol. 2012 Oct; 50(10): 3301–3308



http://dx.doi.org/10.14440/jbm.2016.125

Challenges to Adoption of New Technologies

- Cost
- Validation
- Training
- Implementation and workflow integration
- Management of data records
- Expanding application of new technologies

Overcoming Cost Challenges

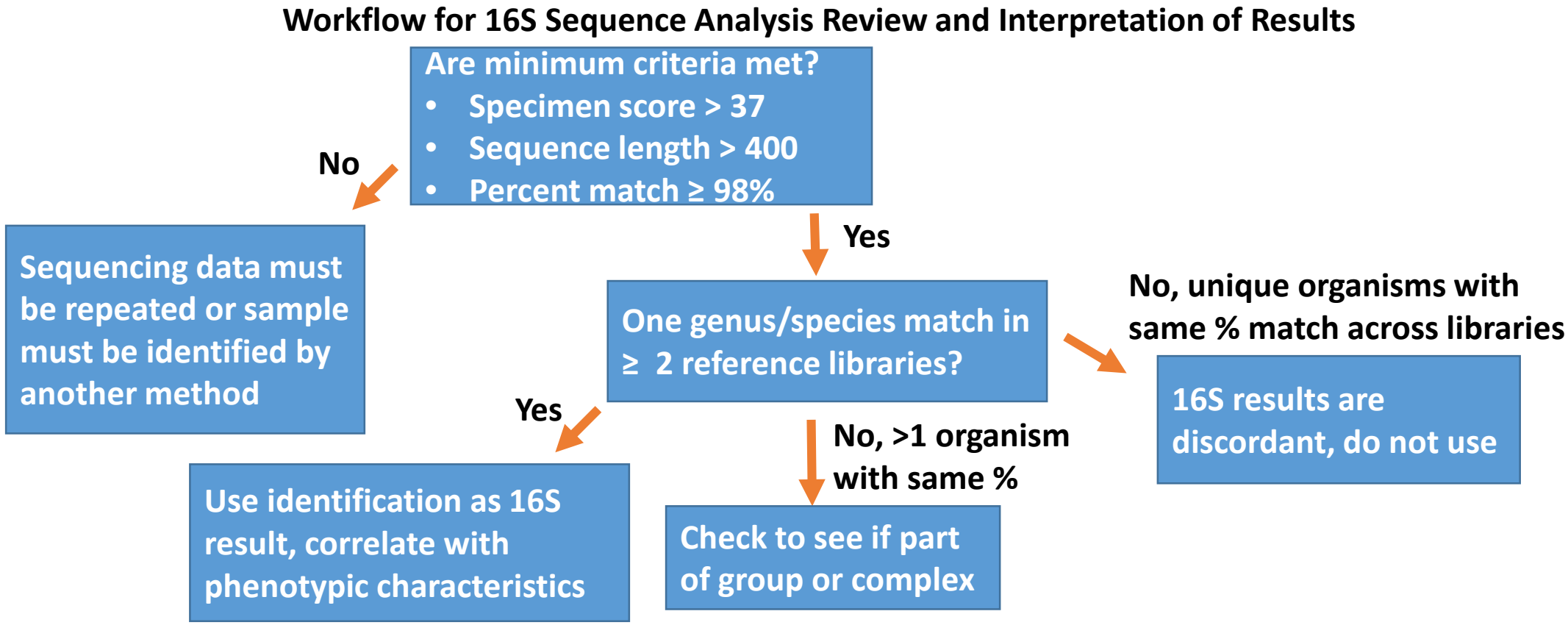
- Costs associated with procurement new instrumentation
  - MALDI-TOF MS: ~$250K
    - With broad implementation, savings in time and reagents ultimately offset the initial investment
  - 16S rDNA sequencing: ~$175K for ABI3500xl
    - Applicable across numerous assays
- Funding (grants, state appropriations, carryover)
- Justifications for investment:
  - Reference laboratory responsibilities
  - Improved accuracy
  - Cost offset by reduced personnel time, biochemical reagents and kits
  - Improved turnaround-time, public health response

Performance Verification of Broad Application Tests

- Impossible to perform an exhaustive validation of all organisms
- DCLS' Strategy:
  - Verification of a subset of organisms based on typical diversity
    - Gram-negative, Gram-positive, mycobacteria
    - Previously identified pathogens, ATCC strains, PT strains
  - Include all libraries that will be utilized in the production workflow
  - Typical growth and storage conditions and culture media used
  - Anticipated sample preparation procedures:
    - 16S DNA extraction method
    - MALDI: Direct spot and full extraction
  - Establish interpretation criteria
  - Investigate discrepancies between methods

Lab Validated Interpretation Criteria

- "Interpretation of 16S sequencing results must be used in conjunction with phenotypic characteristics, including Gram stain, colony morphology, and biochemical reactions, to determine the appropriate isolate identification."



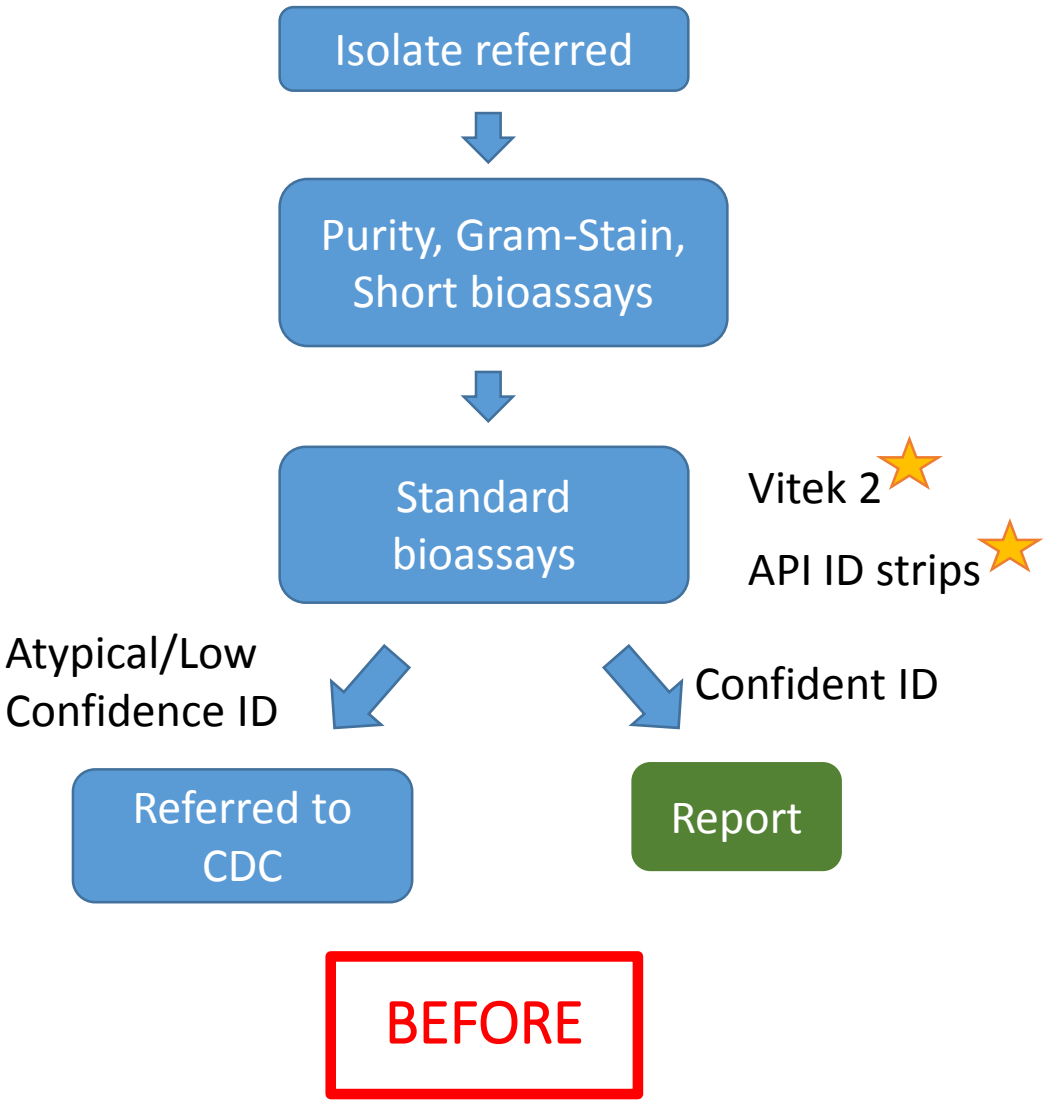
Training Best Practices

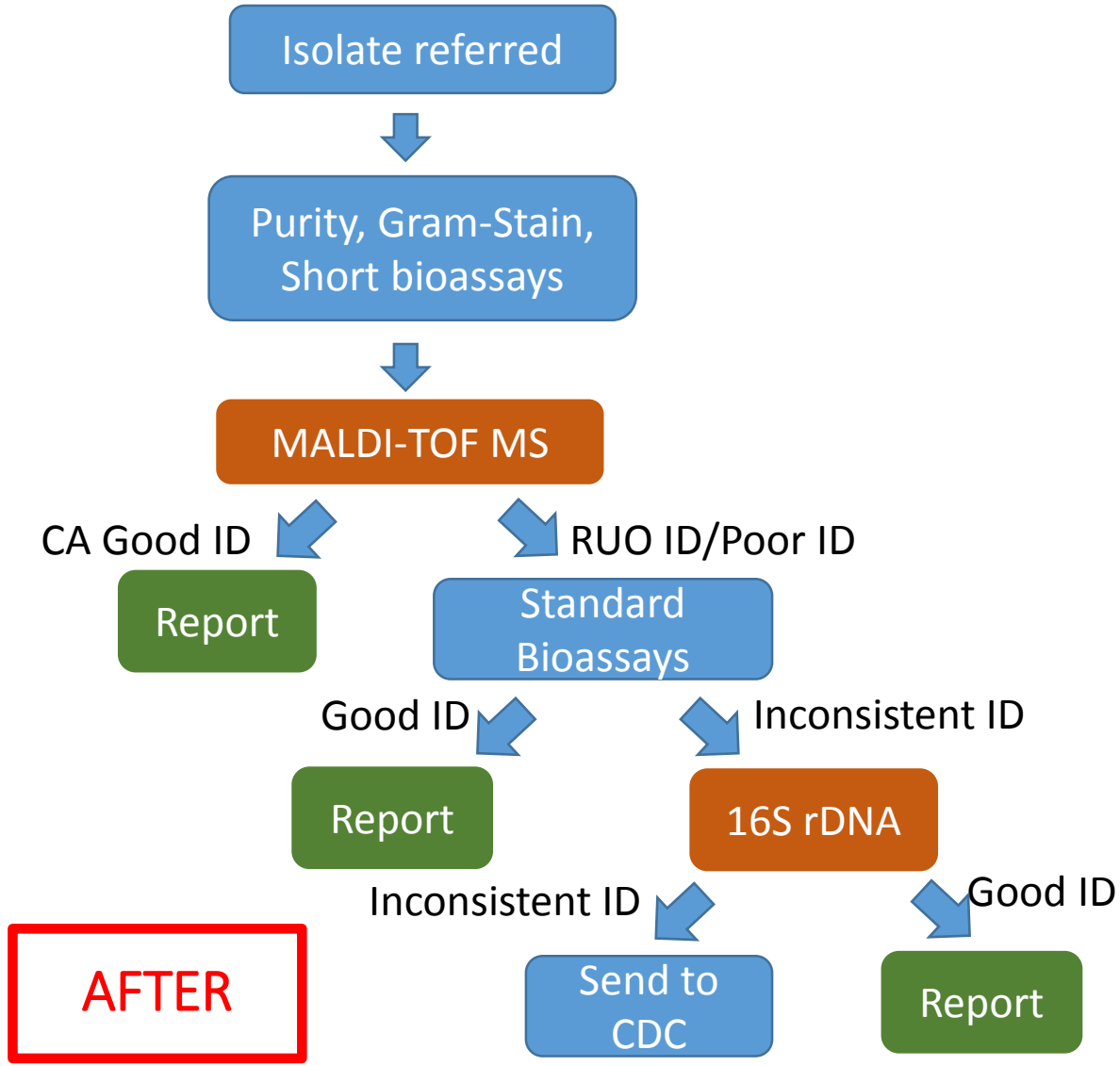
- Standardize training regardless of skill level and background
- Incorporate the follow elements:
  - Technical document review
  - Observation test performance
  - Hands-on observed and independent testing
  - Blinded-samples
  - Challenge questions and real-life scenarios

- Consider supplemental resources for conceptual development
  - CDC TRAIN videos, manufacturer videos, recorded webinars, articles
- Assess method competency at 6 months and annually

Integration of New Technologies

- Incorporate new methods with interpretation criteria guiding reflex testing





Reporting and Data Management

- Retain hardcopy library comparisons and instrument reports with the date of comparison and version utilized documented
- Incorporate appropriate disclaimers for FDA versus non-FDA approved lab validated tests
- Data retention:
  - Per CLIA raw data, isolate, worksheets, reports kept 2 years + current
  - Spectra and fasta file backup (hard drives, data servers, and/or magnetic tape)

Takeaways

- Prepare detailed justification for new technology with selling points like cost benefits, reduced turnaround times and improved emergency/threat response
- Consider funding streams beyond general state appropriations
- Prepare validation parameters to reflect the intended scope of testing, sample handling and storage conditions
- Prepare detailed criteria for results and disclaimers
- Develop a training plan for molecular and non-molecular scientists
- Consider needs for future expansion

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