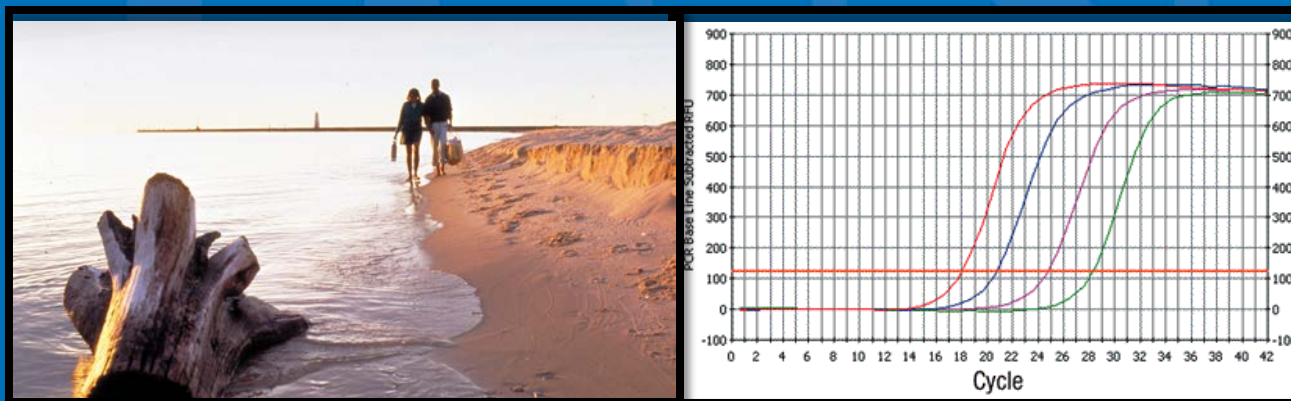


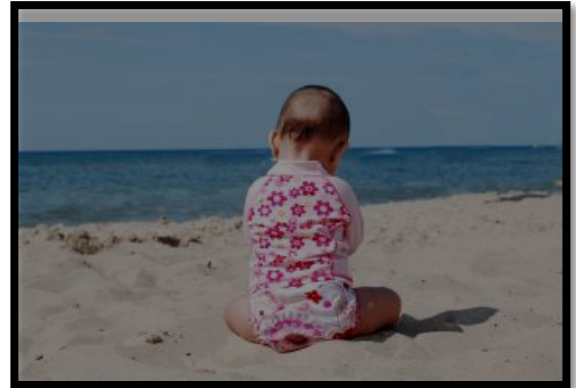
# Use of Microbial Source Tracking Tools in Waterborne Disease Outbreak Response

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# Presentation Overview

1. Microbial Source Tracking Overview
2. EPA Method Implementation Activities
3. CDC Waterborne Disease Outbreak Response
4. Case Study: CDC Response using Microbial Source Tracking



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# Fecal Pollution is a Nationwide Problem

- Fecal microbes are a common biological contaminant in U.S. surface waters
- Public health, economic, and ecological impacts

## U.S. Annual Trends

~4B surface water recreation events

~90M illnesses

~\$2.9B economic burden



DeFlorio-Barker et al. (2018) Environmental Health 17:3



# Three EPA Responsibilities

## 1. Protect and Restore Waters for Recreational Use

- Clean Water Act 1972

## 2. Risk Assessment of Beach Contaminants

- BEACH Act 2000
- Recommendation of Ambient Water Quality Criteria

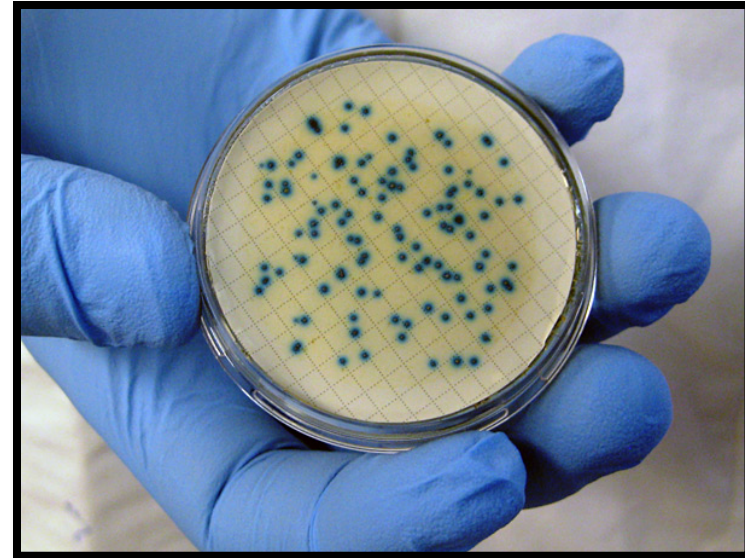
## 3. Management of Point and Non-Point Pollution Sources

- Total Maximum Daily Load programs
- National Pollutant Discharge Elimination System programs
- Combined Sewer Overflow consent decrees



## Current Fecal Pollution Management Tools

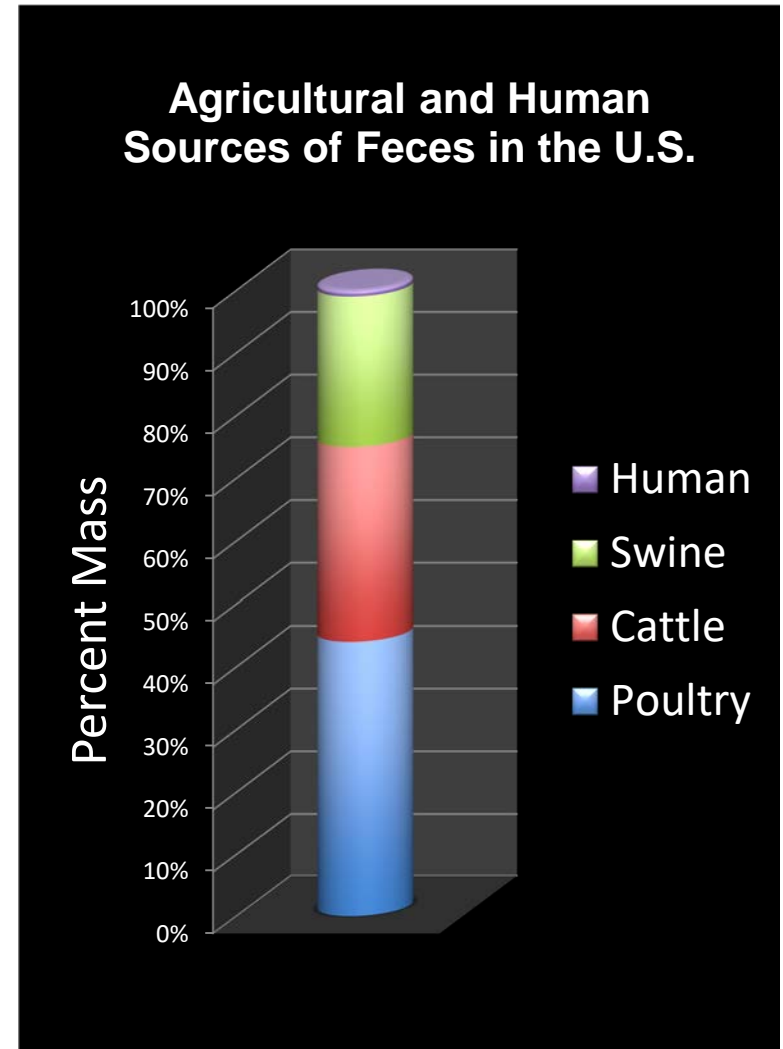
- Based on [general fecal indicators](#)
- Measure of total fecal pollution
- Presence in water is a warning signal of public health risk
- Do not discriminate between sources





# Source of Fecal Pollution is Important

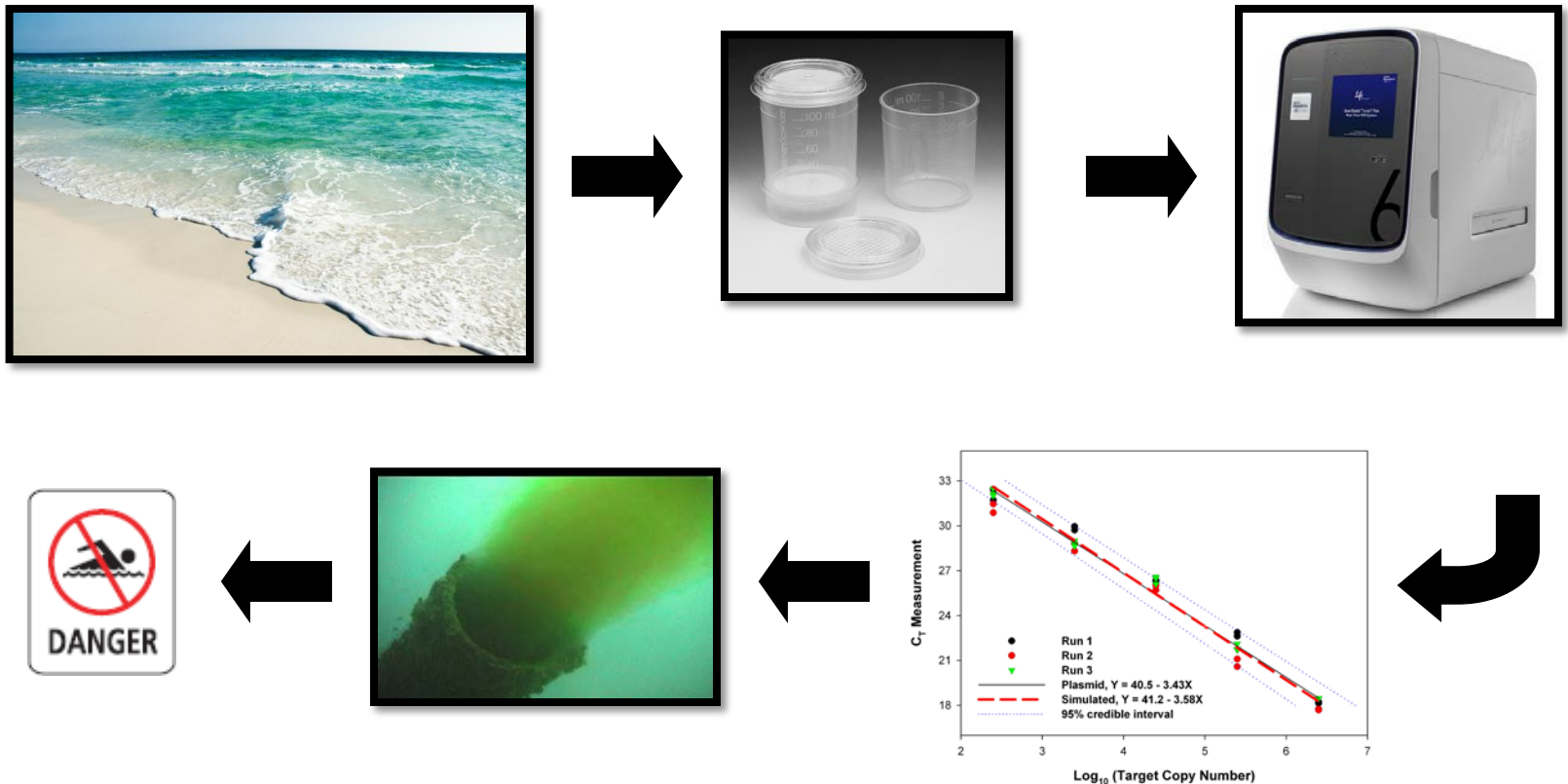
- Estimated  $1 \times 10^9$  tons of fecal material produced in U.S. each year
  - Human (0.01%)
- Public health risk can vary by source
- Mitigation strategies can vary by source



Estimated  $1 \times 10^9$  tons of fecal material produced in U.S. each year (human, ~0.01%). RL Kellogg, CH Lander, DC Moffitt, N Gollehon - NRCS and ERS GSA Publ. No. NPS00-0579. Washington, DC: USDA, 2000

# The Microbial Source Tracking Concept

**SOLUTION** ... Method designed to collect, isolate, identify, and measure a host-associated identifier from an environmental sample



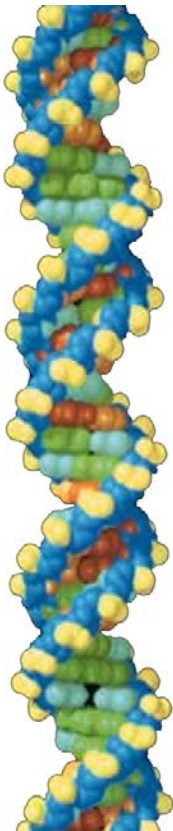
# Many Potential Applications

- Total Maximum Daily Load support tool
- Impaired site prioritization for remediation
- Evaluation of a best management practices
- Stormwater discharge management support
- Combined sewer overflow monitoring
- Waterborne disease outbreak response investigative tool





## Microbial Source Tracking Implementation: Status

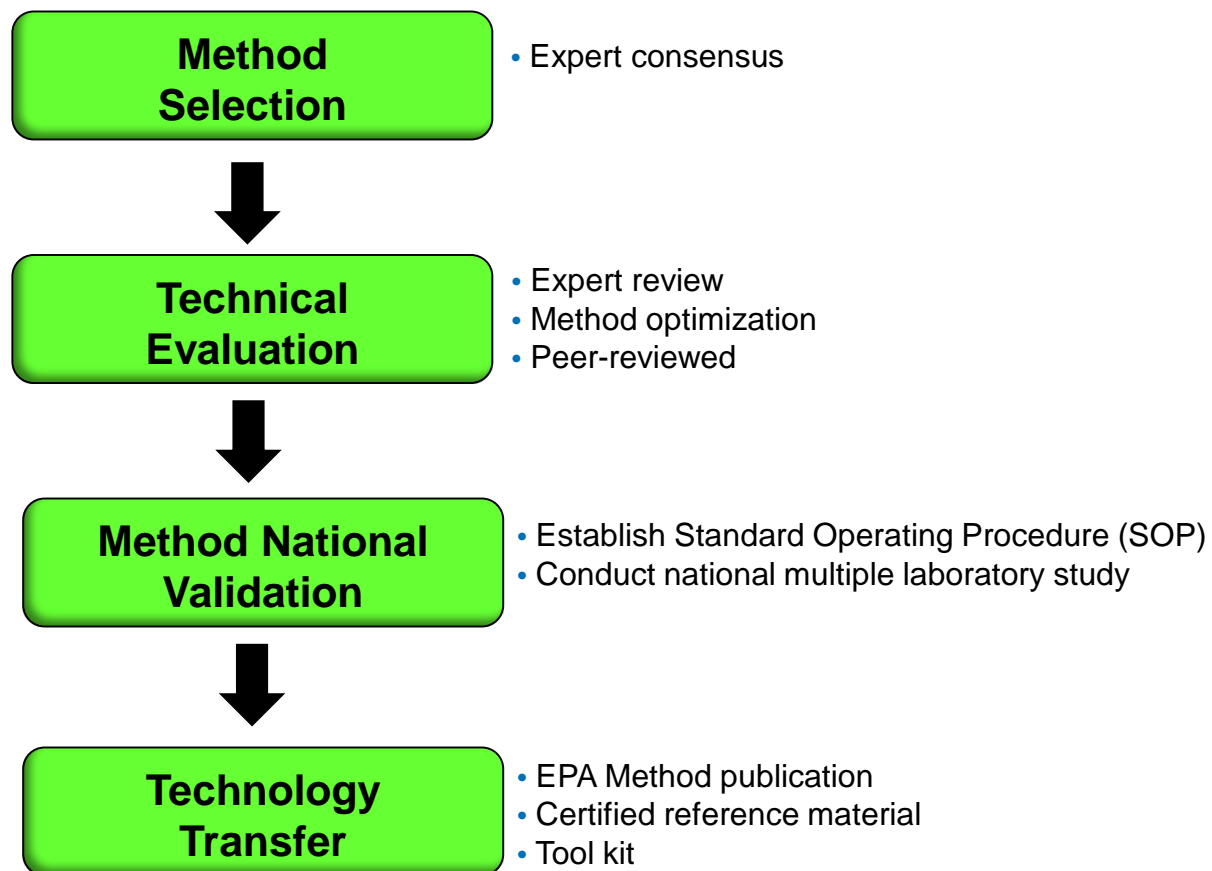


- Intensively studied in scientific arena
- Official Technical Report (#804) for use in California
- No standardized method recognized on federal level
- No official federal guide on use
- Public interest in use rapidly growing

## Microbial Source Tracking Tool Implementation Wish List

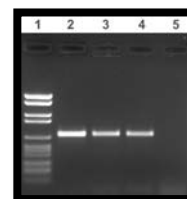
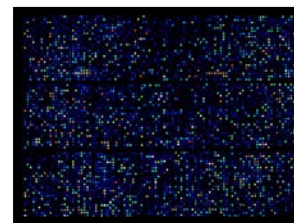
Goal	Description
Clear Host Association	Strong evidence of close link with a specific pollution source
Quantitative Metric	Identifier concentrations are accurate and reproducible
Expert Consensus	Agreement among majority of experts
Standardization	Complete standard operating procedure
National Validation	Multiple laboratory confirmation that the method adequately meets application needs
Technology Transfer Kit	Application guidance, training tools, easy to use kit, and reference materials

## Human Microbial Source Tracking Method Implementation Strategy



## Many Microbial Source Tracking Methods Available

- Microarray
- Next generation sequencing
- End-point PCR
- Quantitative real-time PCR
- Digital PCR
- Immuno-magnetic separation
- Terminal restriction fragment length polymorphism
- Selective bacterial culturing
- Antibiotic resistance profiling
- Chemical detection
- Canine scent detection



## Human Method Selection By Expert Consensus



- Source Identification Protocol Project

- 5 organizations formed technical lead team
- Public challenge via blinded study
- 27 expert laboratories
- 41 methods



- Majority of experts (>90%) favor a **PCR-based technology**

- PCR-based methods are highly reproducible across labs only when protocols are **standardized**

- Identification of top human-associated method  
**HF183 qPCR**

Boehm et al. (2013) Performance of forty-one microbial source tracking methods: a twenty-seven lab evaluation study. *Water Research* 47: 6812-6828.

Ebentier et al. (2013) Evaluation of the repeatability and reproducibility of a suite of PCR-based microbial source tracking methods. *Water Research* 47: 6839-6848.

Layton et al. (2013) Performance of human fecal anaerobe-associated PCR-based assays in a multi-laboratory method evaluation study. *Water Research* 47: 6897-6908.

Stewart et al. (2013) Recommendations following a multi-laboratory comparison of MST methods. *Water Research* 47: 6829-6838.

## HF183 qPCR Technical Evaluation via Peer-Review



- Administered by team of experts
  - Government and academic sectors
- Rigorous laboratory assessment subject to peer-review
- Protocol adherence to Minimum Information for Publication of qPCR Experiments (MIQE)
- Optimization to reagents custom designed for environmental samples

**Bustin, S. A. et al.** (2009). The MIQE Guidelines: minimum information for publication of quantitative real-time PCR experiments. *Clinical Chemistry*. 55: 611-622.



## HF183 qPCR EPA Method National Validation - Overview

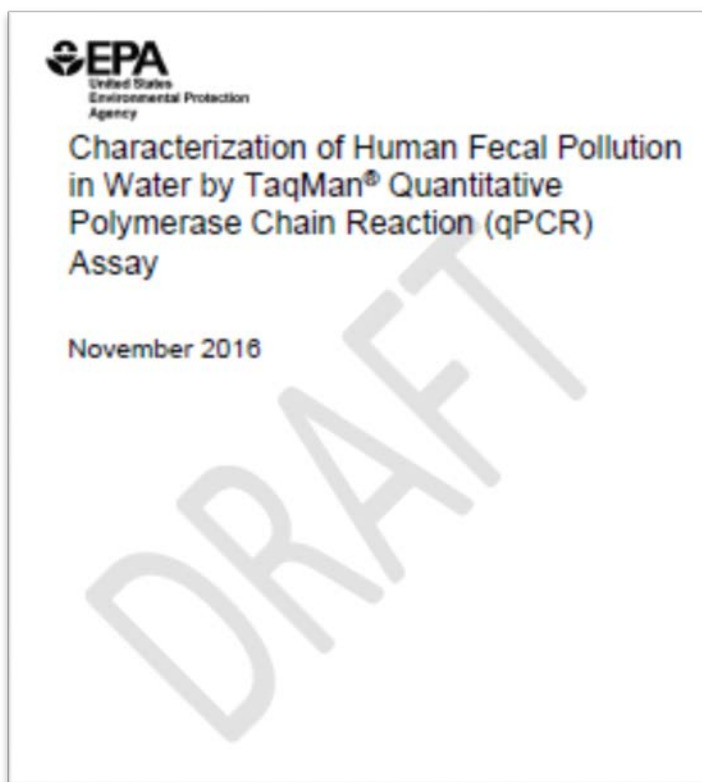


- Formal study conducted by EPA
  - Office of Water
  - Office of Research & Development
- HF183 qPCR
- 14 Laboratory Participants
  - Fresh and marine water matrices
- Supplied with:
  - Standard protocols
  - Reference DNA materials
  - Sewage spike material
  - Blinded filter set (n = 18)
  - All reagents and consumables

## Customized HF183 qPCR Data Acceptance Metrics

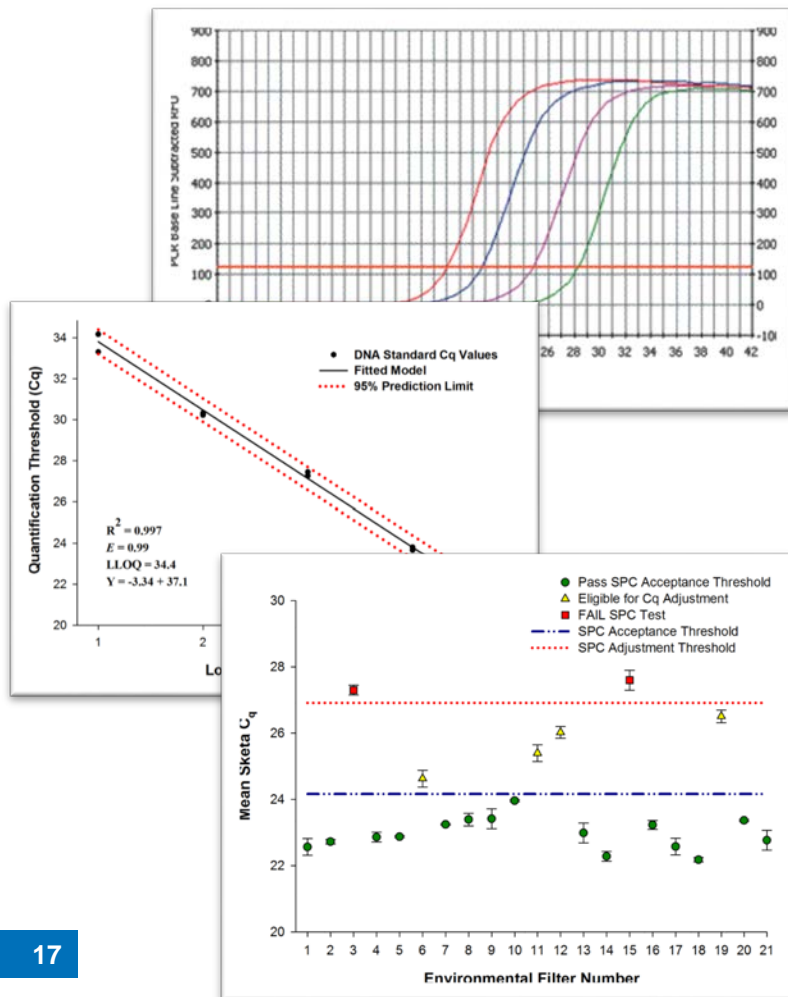
Type	Metric
Calibration Curve Model	R <sup>2</sup> Amplification efficiency (E)
Extraneous DNA	No-template controls (NTC) Method extraction blank (MEB)
Matrix and Amplification Control Proficiency	Internal amplification control proficiency Sample processing control proficiency
Test Sample	Inhibition screen with IAC Matrix interference with SPC Lower limit of quantification (LLOQ)

## Draft HF183 qPCR EPA Method Content Overview

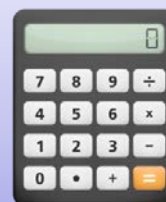


- Safety
- Laboratory organization
- Equipment, reagents, and supplies
- Sample collection, handling and storage
- Standardized laboratory procedures
- Quality controls
- Data analysis and calculations

# HF183 qPCR Automated Data Analysis Tool



- Simplify complex calculations
- Ensure standardized analysis
- Implement data acceptance metrics
- Concentration estimates with error



- Microsoft Excel Workbook
- Standardized data input
- Summary report

## Self-Administered HF183 qPCR Method Proficiency Test

- Prior to environmental sample testing
- After new reference material preparations
- Six metrics based on:
  - 14 multiple laboratory study
  - Reagent manufacturer recommendations
  - qPCR experts
- Training and management tool



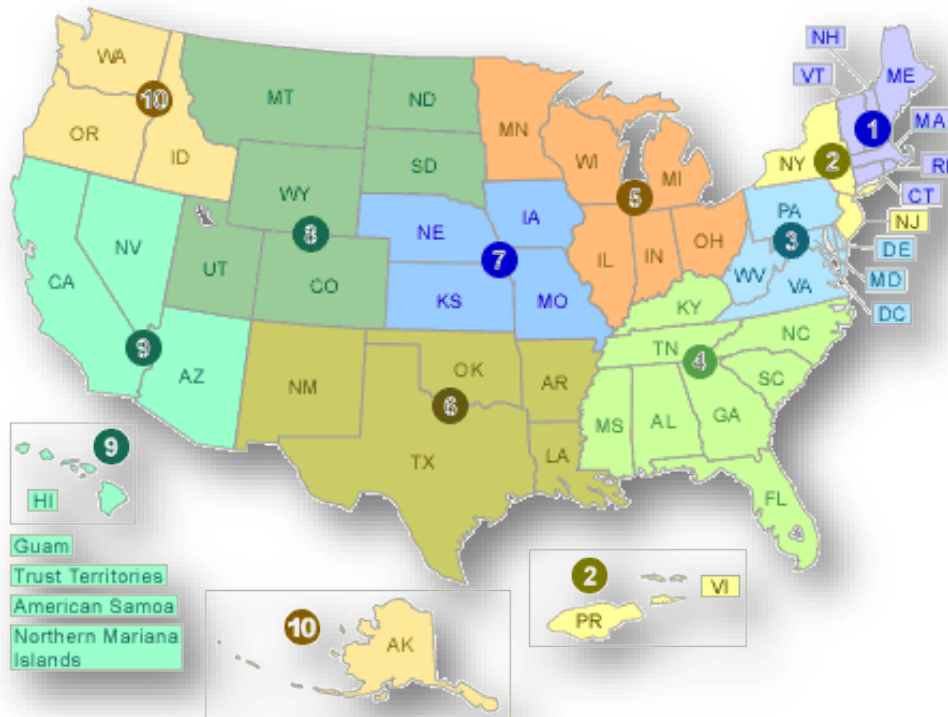
## HF183 qPCR Standardized Reference DNA Material Development

- National implementation requires high quality reference DNA material
- Centralized and standardized source
- Not feasible for EPA to manufacture and distribute
- Interagency Agreement with National Institute of Standards and Technology

**NIST**  
**National Institute  
of Standards  
and Technology**



## HF183 qPCR Technology Transfer: Ongoing Activities



- Technical support network
- Communication tools
- Training opportunities
- Application guides
- Cooperative partnerships
  - Association of Public Health Laboratories MOU
  - States and local labs
  - Federal agencies