



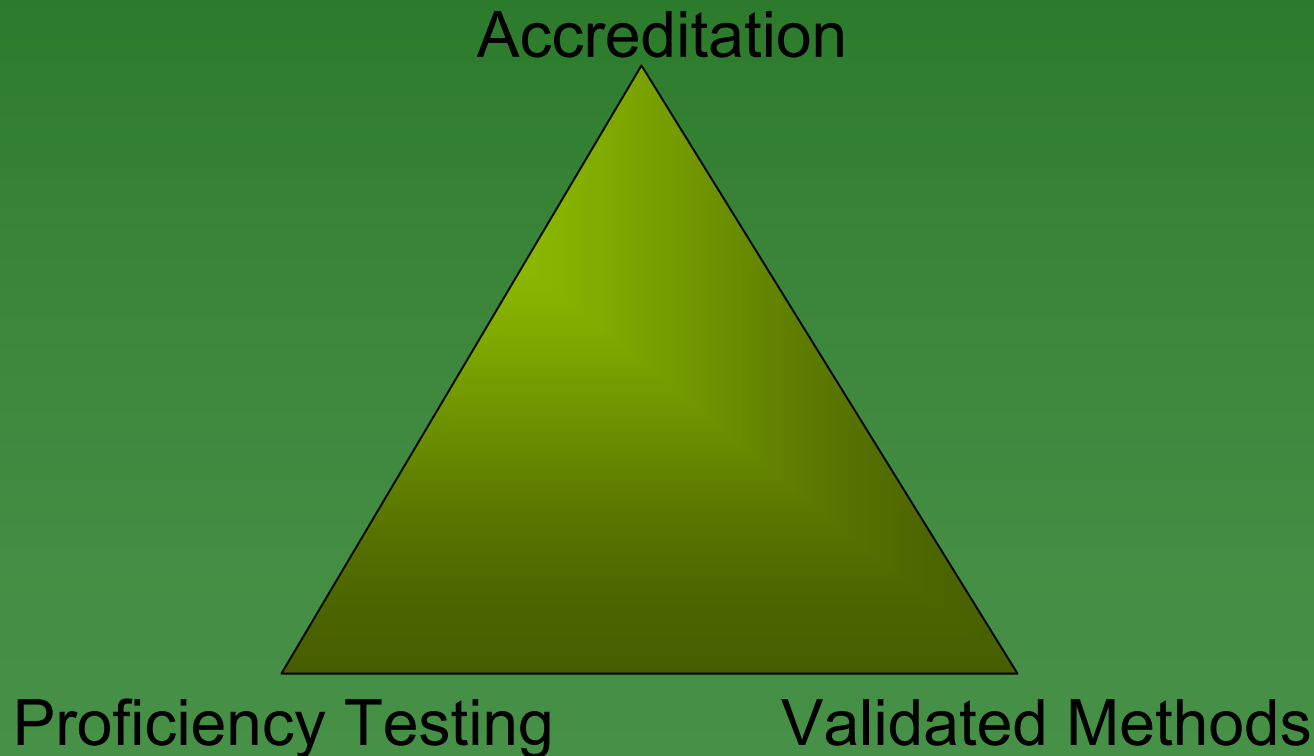
THE ORIGINAL GODZILLA MOVIE

# GODZILLA

*The Ultimate in Invasive Species*



# The Laboratory Reliability Triangle



# Definitions

## ISO

- Accreditation: Procedure by which an authoritative body gives formal recognition that a body or person is competent to carry out a specific task or test
- Certification: Procedure by which a third party gives written assurance that a product, laboratory work, process, or service conforms to specified requirements.

## NPPLAP

- Accreditation: The determination that a laboratory is capable of performing competent diagnoses.
- Certification: The determination that individual analysts from an accredited laboratory have undergone training and participate in a proficiency testing program for a specific diagnostic method.

# Why an accreditation program?

- **Enhanced Consistency of Test Results**
- **Access to Validated Procedures**
- **Defined Standards for Facilities and Equipment**
- **Staff Competence Independently Evaluated**
- **Defined Training Requirements**
- **Clear Chain-of-Custody and Documentation Requirements**
- **Regulatory acceptance of results**
- **Improved network capacity**
- **Increased reliability of results**
  
- **Leads to Continual Improvement!**

# Benefits – Improvement

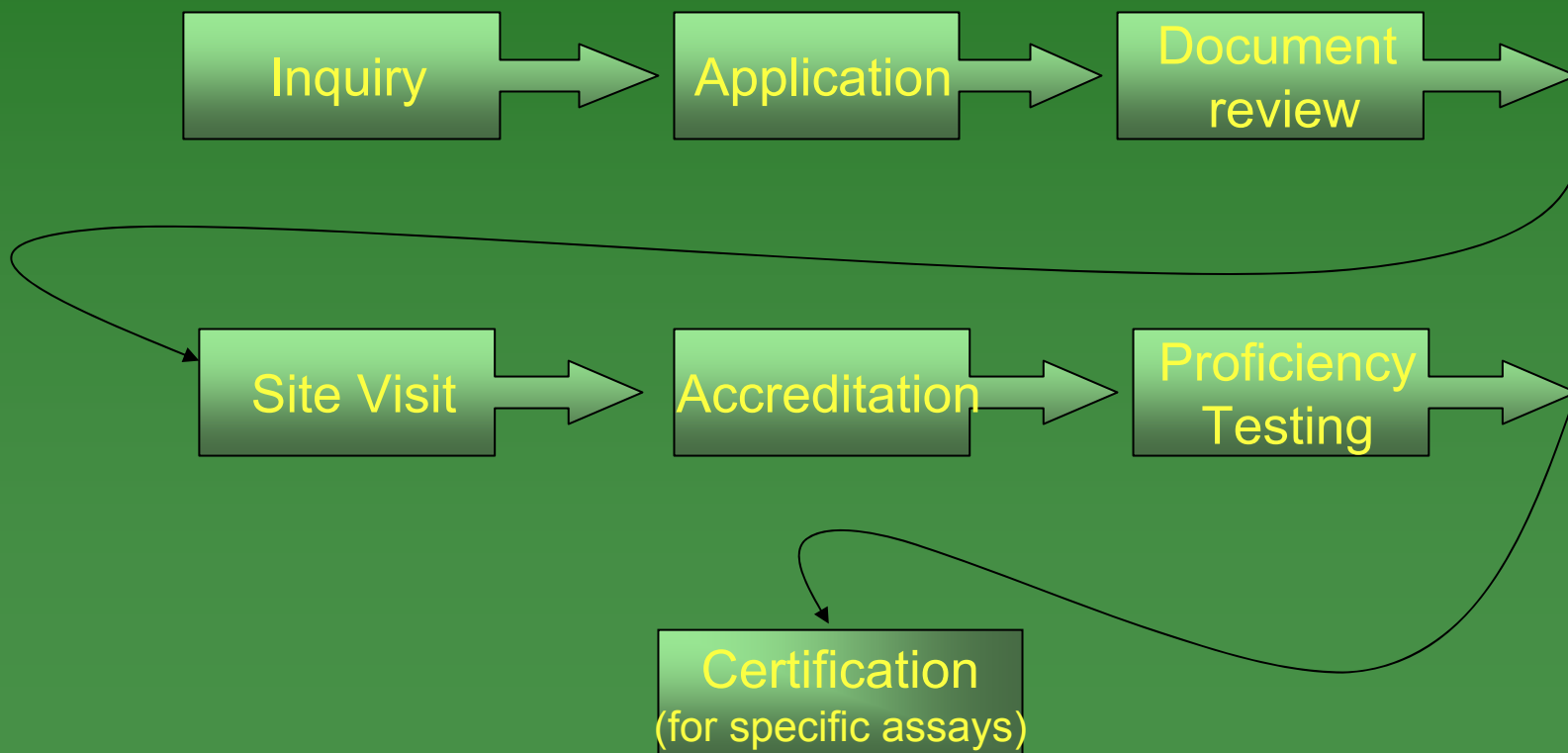
- **Internal Auditing**
  - Identifies training needs
  - Identifies problems with the system
  - Identifies opportunities for improvement
- **Corrective Actions**
  - Eliminates potentially flawed processes
  - Provides documentation of changes
- **Management Reviews**
  - ISO 17025:2005 underpinnings
- **Applicable to all Plant Diagnostic labs**

# Goals of NPPLAP Accreditation Standards

- Ensure that the highest quality tests are performed using validated methods and following defined standards of quality assurance
- Increase national laboratory capacity, capability and quality for diagnostic analysis of plant pests and pathogens of greatest regulatory concern
- Easy to use and understand, build and expand, revise and implement
- **Facilitate rapid and more accurate detection of plant pests and pathogens**



# Accreditation Path



# Why personnel should be certified?

- Assures laboratory management and clients that staff are competent and meet certain standards.
- Allows individuals to be confident that they meet recognized performance standards.
- Allows for staff changes with-in laboratories.
- Promotes continual improvement.
  - Establish Minimum Qualifications
  - Specify Training Requirements
  - Participate in Annual Competency Program (Proficiency Testing)

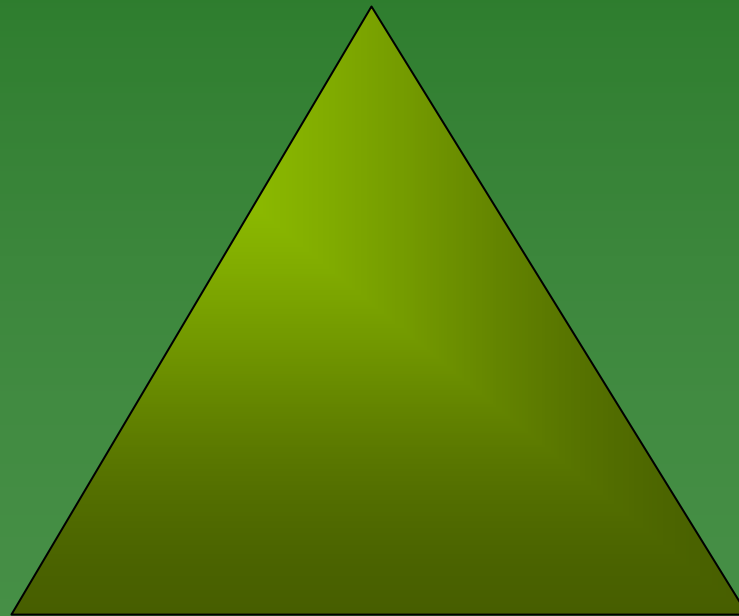
# Challenges of Accreditation

- Dissemination of regulatory samples
  - Inter- and Intra-state considerations
- Identification of exotic organisms
  - Are definitive methods in our arsenal
- Validation of test methods
  - Assure tests perform well across laboratories
- Proficiency Testing
  - Development and implementation



# The Laboratory Reliability Triangle

Validated Methods



Proficiency Testing

Accreditation

# Method Validation - Definition

***Validation – confirmation by examination and provision of objective evidence that the particular requirements for a specified end use are fulfilled***

# Method Validation

- Identify the scope and application of the method. (What is the method supposed to do?)
- Develop a procedure that will produce data consistent with the intended scope and application of the method.
- Establish appropriate quality control procedures and specifications to ensure the procedure is followed and the method generates data that will meet the established criteria.

# Partnering in Validation

- Phase I – Method Development
  - Purpose and Scope
  - Specificity
  - Confirm identity
  - Optimize
  - Ruggedness testing
  - Method description published

e.g. SOD Nested and Multiplex procedures

# Partnering in Validation

- Phase II – Single-lab Validation
  - Study protocol prepared
  - Develop performance characteristics
    - Limit of detection (Sensitivity)
    - Linearity
    - Precision/Repeatability estimates
  - Assess Stability
  - Calculate with-in lab precision
  - Prepare report

e.g. HLB Real-time and Conventional (Li, Hartung and Levy, 2007)

# Partnering in Validation

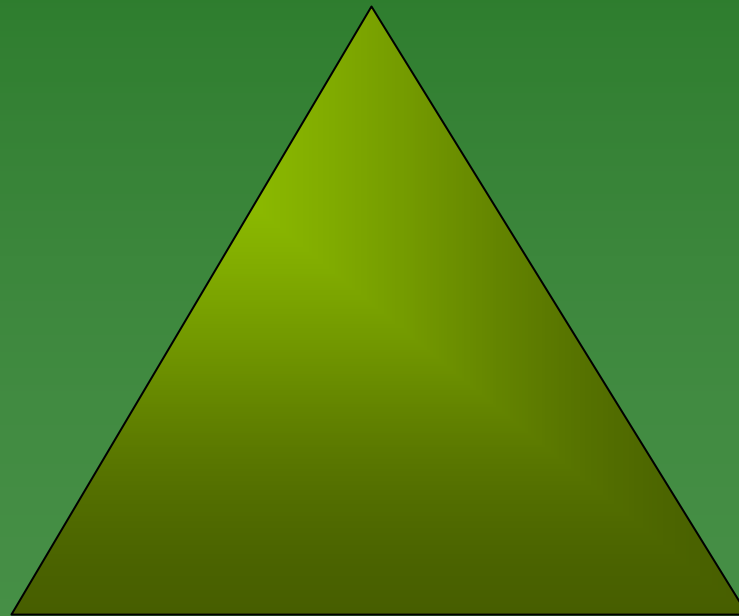
- Phase III – Multi-lab Validation
  - Study protocol prepared
  - Invite participation
  - Prepare study materials
  - Homogeneity testing
  - Test samples distributed
  - Precision/Reproducibility assessed
  - Prepare report

- Dossier Review

e.g. SOD Real-time PCR

# The Laboratory Reliability Triangle

Proficiency Testing



Validated Methods

Accreditation

# Proficiency Testing - Definition

**Proficiency Testing** - A periodic assessment of the performance of individual analysts, ... through the distribution ... of typical materials for unsupervised analysis by the participants (IUPAC).

# Proficiency Testing

- Allows a laboratory to monitor analyst performance
- Provides a feedback mechanism for identifying areas of concern or improvement
- Serves as a systems audit of the entire laboratory testing system
- Serves as a tool to educate and prepare laboratories and analysts for future challenges

## **2006 *P. ramorum* PT Program Results**

- Test material was dispatched between April and June
- 40 analysts received panels
- 18 laboratories participated
  - 2 Federal
  - 14 NPDN
  - 2 non-NPDN State Dept. of Ag. labs

## 2006 Conventional PCR Results

- 37 panels were analyzed and returned for scoring
- Failure resulted from one or more of the following reasons:
  - Nested PCR
    - 1 failure due non-detection of a positive
    - 1 failure due to primary contamination of water
    - 1 failure due to contamination of non-target species.
    - 2 failures due to contamination of a water dilution
    - 2 failures due to faulty interpretation
  - Multiplex PCR
    - 3 failures due to water contamination
    - 2 failures due to positive control failure

# Definitions

**Diagnostic Sensitivity** - Proportion of known infected materials that test positive in the assay; infected materials that test negative are considered to have false-negative results.

**Diagnostic Specificity** - Proportion of known uninfected materials that test negative in the assay; uninfected materials that test positive are considered to have false-positive results.

# Conventional Results

Expected

		Expected		Total
		+	-	
Observed	+	258 TP FN	6 TN	264
	-	7	306	313
Total		265	312	577

Positive (+) and negative (-) results for 577 individual samples  
 Diagnostic Sensitivity,  $TP/TP+FN = 97.4\%$ ; Diagnostic Specificity,  $TN/TN+FP = 98.1\%$ .

## Real-time Results:

- 28 panels were analyzed and returned for scoring
- Failures resulted from one or more of the following reasons:
  - FAM portion
    - 1 failure due to out-of-range Ct for a positive sample
    - 6 failures due to contamination of water samples
      - 1 in dilution
      - 4 in undiluted
      - 1 carryover
  - TxRed portion
    - 1 Control failure - contaminated plant DNA

# Real-time Results

Summary statistics: Target samples (*Phytophthora ramorum*) included in the real-time panel for the 2006 PT program.

Statistic	Sample 7	Sample 8	Sample 9	Sample 10	Sample 11	Sample 12	Sample 18	Sample 19	Sample 21	Sample 24
N	27	27	27	26	27	27	14	20	5	2
Mean	17.99	25.06	32.56	26.94	22.19	22.23	27.33	31.37	29.46	26.95
Std. Dev	1.27	1.42	2.57	1.60	1.25	1.23	1.21	2.45	0.82	0.92
CV(%)	7.06%	5.67%	7.91%	5.94%	5.63%	5.54%	4.44%	7.82%	2.77%	3.41%
Expected	17.58	24.57	31.46	25.79	22.07	21.99	26.34	29.92	26.32	25.79

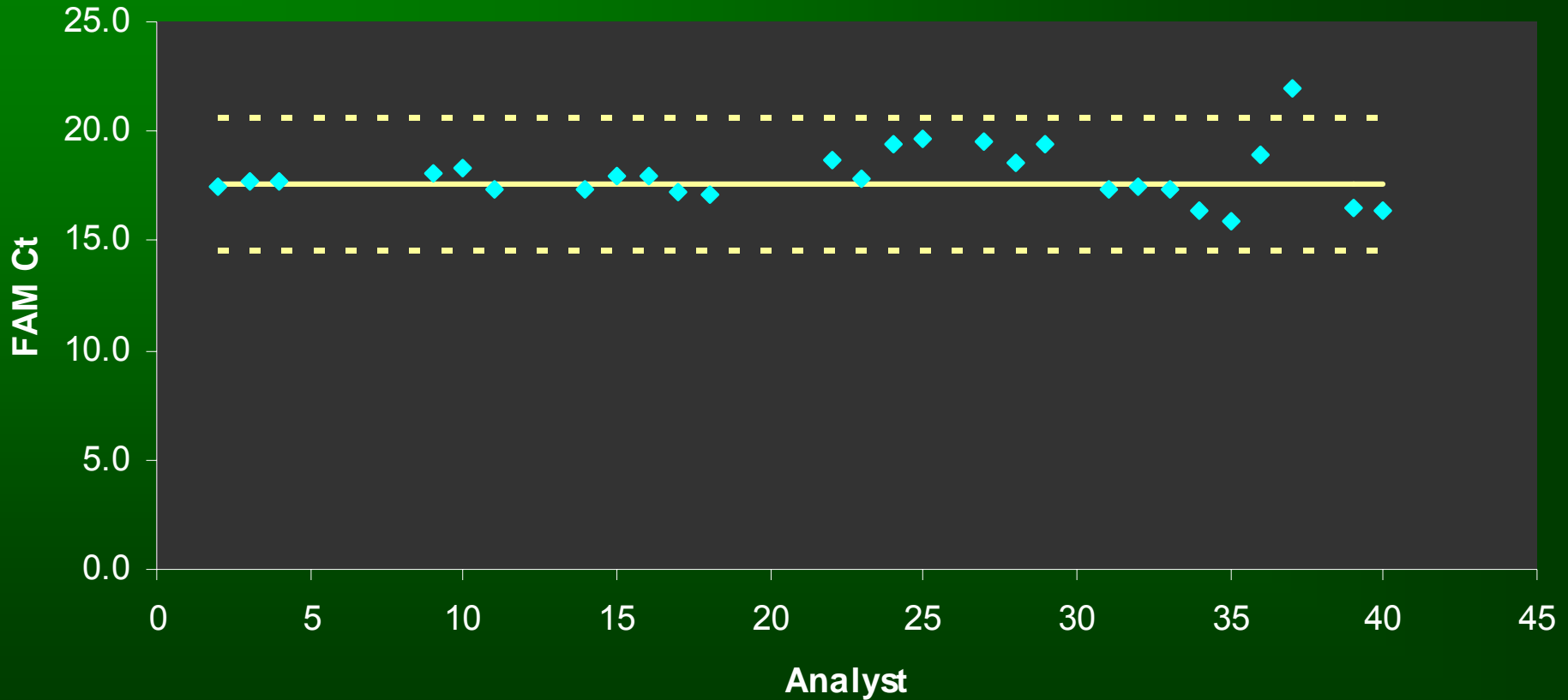
# Real-time Results

Summary statistics: Target samples (*Phytophthora ramorum*) included in the real-time panel for the 2006 PT program.

Statistic	Sample 7	Sample 8	Sample 9
N	27	27	27
Mean	17.99	25.06	32.56
Std. Dev	1.27	1.42	2.57
CV(%)	7.06%	5.67%	7.91%
Expected	17.58	24.57	31.46

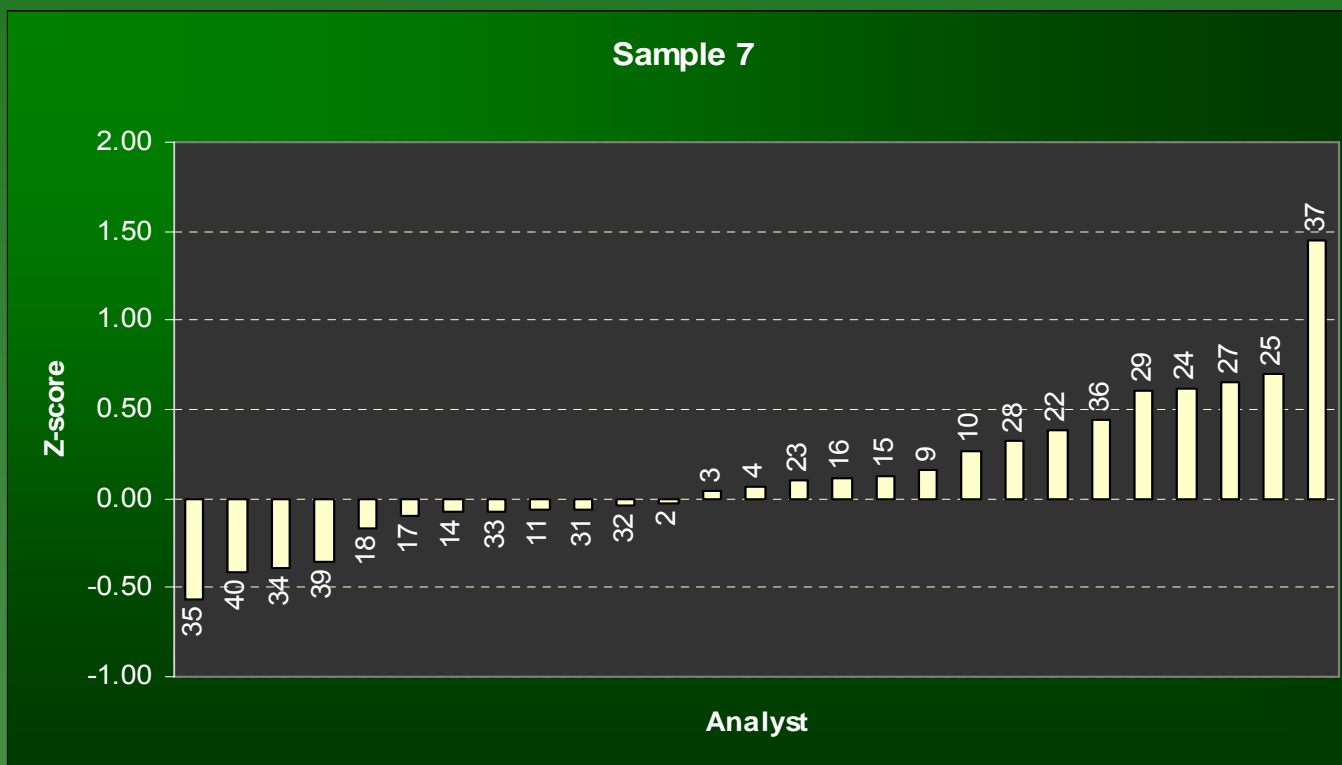
# Real-time Results

## Sample 7



Solid line is the expected value; dashed lines =  $\pm 3$  Ct's

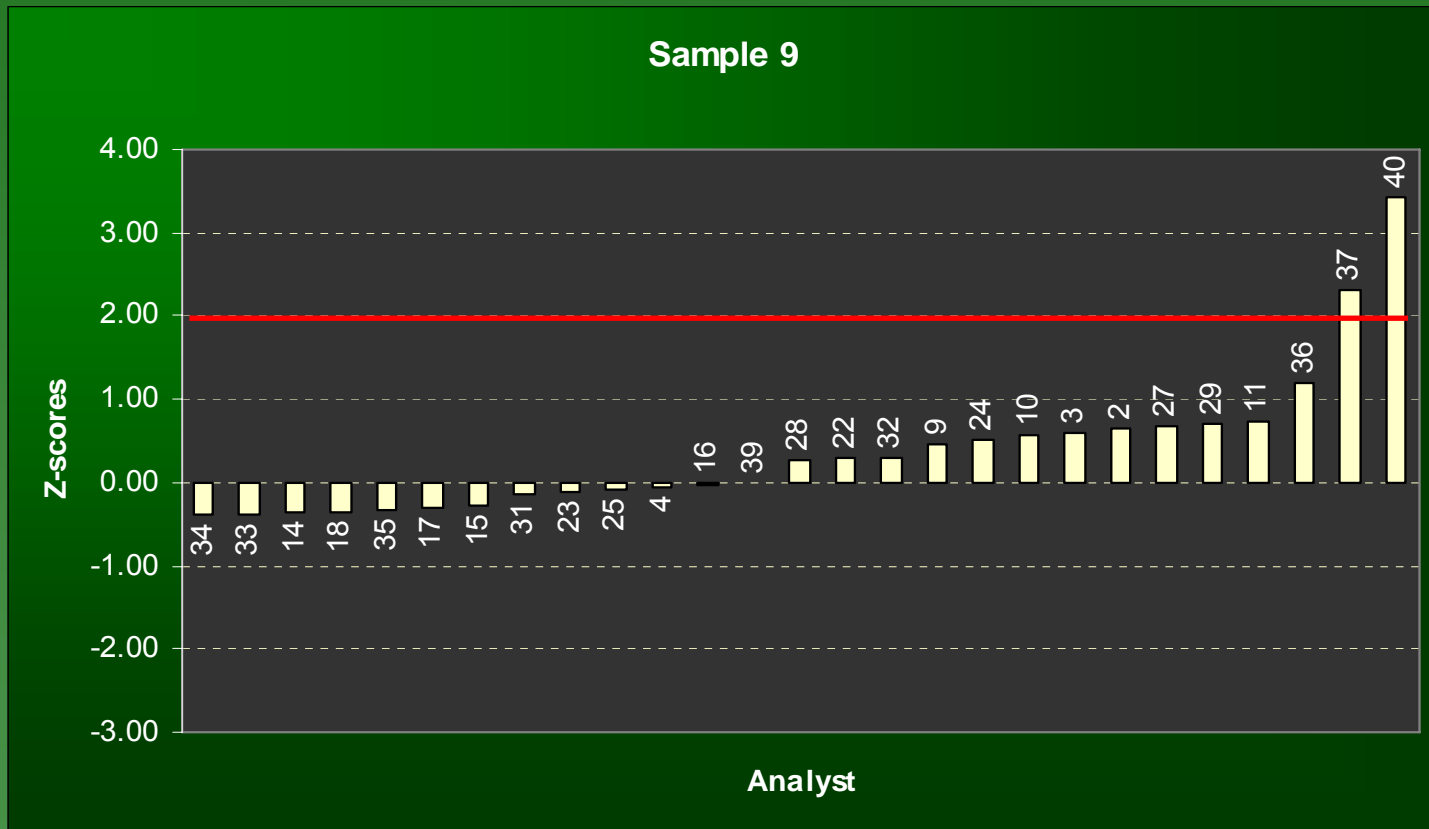
# Real-time Results



Z-scores derived as:  $z = \frac{x - X}{s}$ , where  $x$  = reported,  $X$  = expected,  $s$  = target standard deviation

Z-scores of  $\leq 2$  are considered satisfactory. Scores between 2 and 3 are of concern and scores  $> 3$  are unsatisfactory.

# Real-time Results



Z-scores of  $\leq 2$  are considered satisfactory. Scores between 2 and 3 are of concern and scores  $> 3$  are unsatisfactory.

# Real-time Results

Expected

		Expected		Total
		+	-	
Observed	+	198 TP	0 FP	198
	-	7 FN	232 TN	239
Total		205	232	437

Positive (+) and negative (-) results for 437 individual samples  
 Diagnostic Sensitivity,  $TP/TP+FN = 96.6\%$ ; Diagnostic Specificity,  $TN/TN+FP = 100\%$ .

# Proficiency Testing – Does it Work?

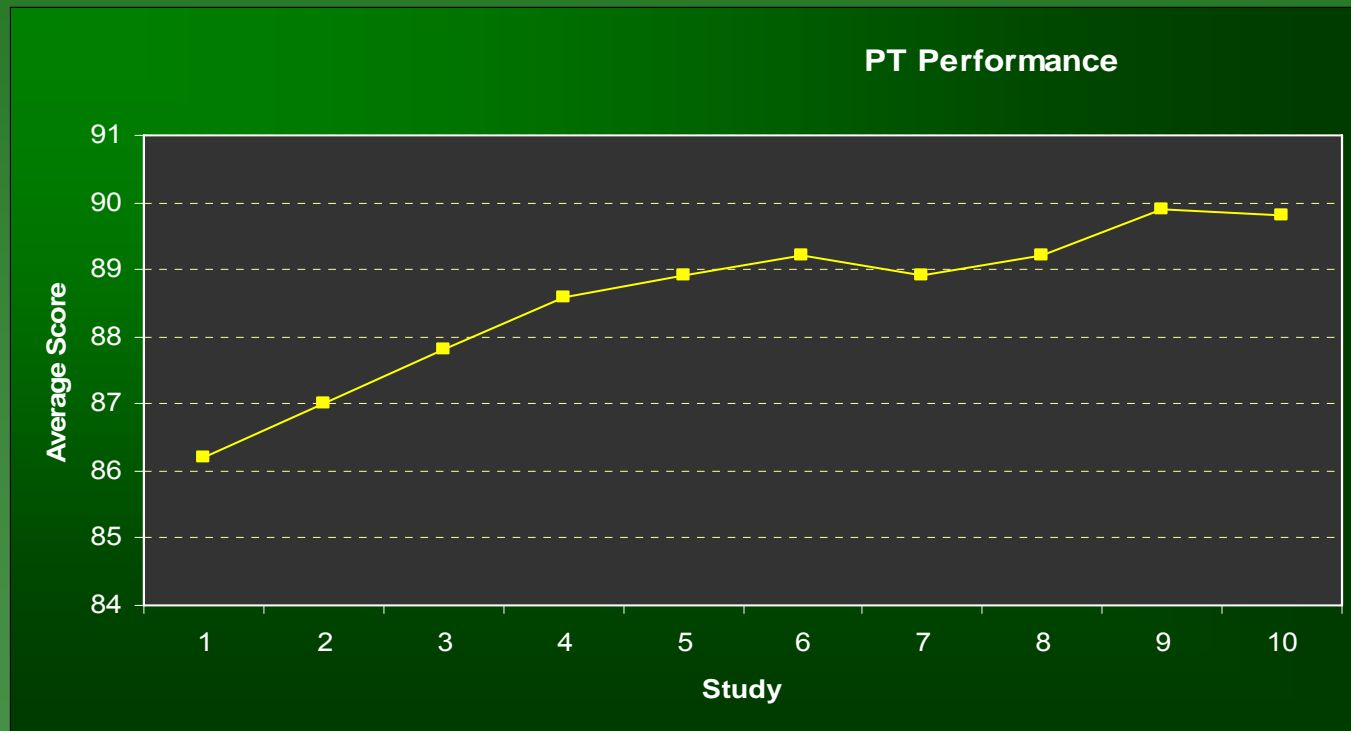


Figure 3. Comparison of average PT score for all parameters combined.

# Proficiency Testing – Does it Work

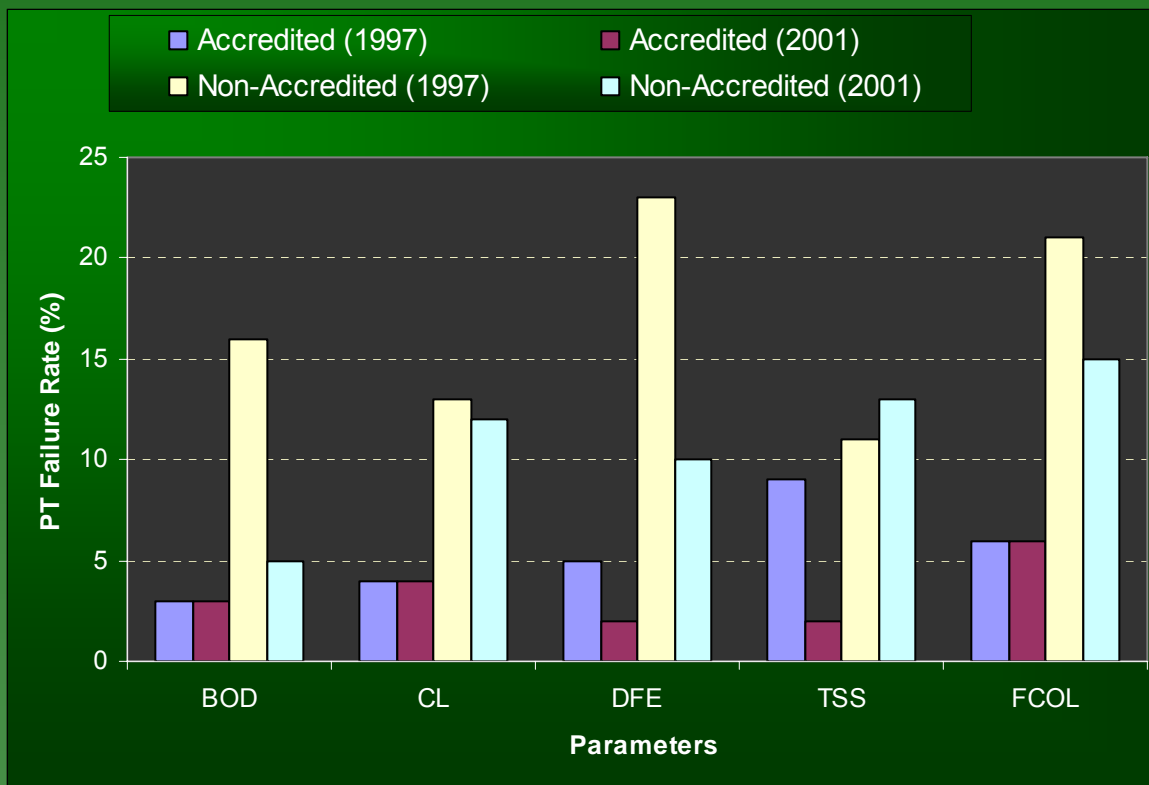


Figure 1. A comparison of proficiency testing failure rates between accredited and non-accredited laboratories.

# Proficiency Testing Summary

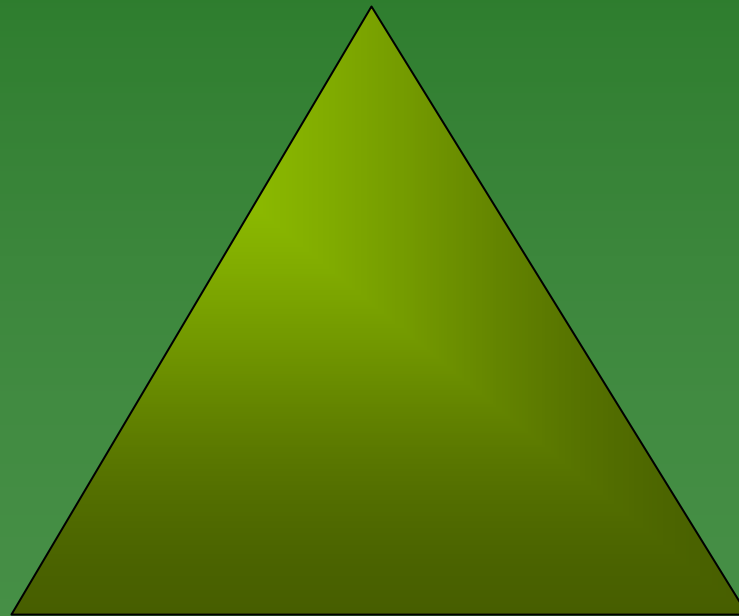
- Inter-lab variability is very low (Robust)
- Panel showed excellent stability over the distribution and analysis period (6 months)
- Next generation of PT in development
  - Lower sample #'s, Tissue based
  - **Enhanced reports to participating labs**
- Panels in development
  - PCN, HLB, Citrus Canker, PPV
- Panels waiting for development
  - CVC
  - Next *P. ramorum*
    - Triplex involving, elicitin,  $\beta$ -tubulin, and *P. kernoviae*

# Conclusions

- We're all in this together, and NPPLAP is one of many ways to ensure continuous improvement
- NPPLAP is still in the formative stage
  - still evolving
    - PPQ evaluating internally
  - learn from experience
  - improve our processes
  - assist our partners in improving their processes
- Comments, suggestions, etc.: Contact John Payne or Phil Berger

# The Laboratory Reliability Triangle

Accreditation



Proficiency Testing

Validated Methods