

Guidelines for Single Laboratory Validation of Botanical Identification Methods (BIMs)

James Harnly¹ and Robert LaBudde²

**¹Food Composition and Methods Development Lab
Beltsville Human Nutrition Research Center,
Agricultural Research Service,
U.S. Department of Agriculture,
Beltsville, MD, 20705 USA**

**²Least Cost Formulations, Ltd., 824 Timberlake Drive,
Virginia Beach, VA 23464
Department of Mathematics and Statistics,
Old Dominion University, Norfolk, VA 23529**

***AOAC Working Group on Qualitative Chemistry Guidelines
for Alternative Methods
29June2011***

ERP: Guidelines for Validation of Botanical Identification Methods

James Harnly, Chair

Wendy Appleyquist

Paula Brown

Steven Caspar

Peter Harrington

Danica Harbaugh-Reynaud

Norma Hill

Robert LaBudde

James Neal-Kababick

Mark Roman

Shauna Roman

Darryl Sullivan

Barry Titlow

Paul Wehling

USDA, ARS

Missouri Botanical Garden

British Columbia Institute of Technology

US FDA, CFSAN

Ohio University

AuthenTechnologies, LLC

Alcohol & Tobacco Tax & Trade Compliance lab

Least Cost Formulations & Old Dominion University

Flora Research Laboratories

Tampa Bay Analytical Laboratories

4Life Research

Covance Labs

Compound Solutions

General Mills

Background

In January 2011 an ERP was established to develop guidelines for validation of botanical identification methods (BIMs).

In June 2011 a draft of “**Guidelines for Validation of Botanical Identification Methods**” was placed on the AOAC website for comment.

These guidelines are based on the **Probability of Detection (POD)** statistical model developed for identification of microbiological and bio-threat organisms.

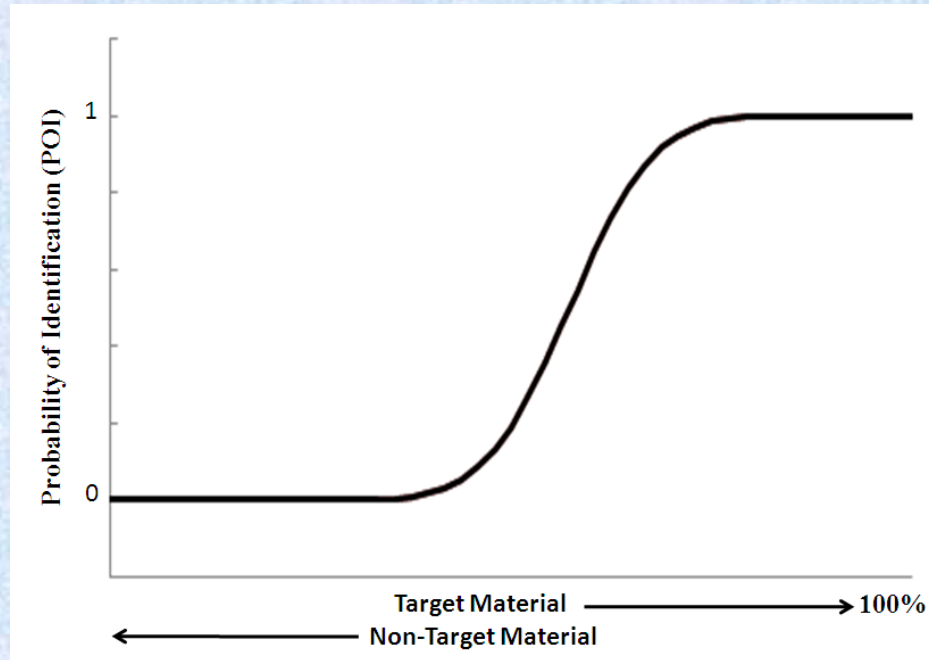
For botanical materials, the model was named **Probability of Identification (POI)** and the language and model were modified appropriately.

Concepts Specific to BIMs

- **An identification method is for materials (ingredients) not individual components, analytes, or markers**
- **For validation purposes, the method can be regarded as a black box that returns a YES or NO answer (Top down view)**
- **Identification is based on the comparison of an unknown botanical material to the target material.**
- **Target material: the botanical of interest as described in the identity specifications.**
- **Identity Specification: Morphological (macro and micro), genetic (DNA sequencing, barcoding), and chemical (chromatographic [CE, GC, LC, TLC] a/o spectral [IR, NIR, NMR, MS, UV/VIS]) characteristics.**
- **Non-target material – Any material that does not meet the identity specification.**

General Guidelines for Validation of BIMs

Based on Probability of Identification model



Validation requires:

Inclusivity/Exclusivity test

Superior/Inferior materials test

Characterization of POI (optional?)

Target Material

What is the target material?

Botanical, ingredient, or complex organic mixture that meets the identity specs.

Material that you want to give a YES result

Not an individual component, analyte, or marker.

What is the variability of the target material?

Is there seasonal, location, processing variability?

How many examples of the target material:

Can you think of: Inclusivity list

Can you get: Inclusivity sampling frame

Will you use: Inclusivity panel

Target Material Example

Target material: American ginseng grown in Wisconsin

Inclusivity list:

Samples from every farm in Wisconsin for the last twenty years.

Inclusivity sampling frame:

**43 samples – from 19 farms over last 4 years
(but not all 19 farms for all 4 years)**

Inclusivity panel

Used all 43 samples

Non-Target Material

What is a non-target material?

Botanical materials that don't meet the identity specs

Material that you want to give a NO result

Modified target material:

Adulterated, contaminated, or impure?

Is there an acceptable level of modification?

Material that is similar to the target material:

Taxonomically, morphologically, chemically?

What is the variability of the non-target material?

How many examples of non-target material:

Can you think of: Exclusivity list

Can you get: Exclusivity sampling frame

Will you use: Exclusivity panel

Non-Target Material Example

Non-target material: Chinese ginseng

Exclusivity list:

**Any non-American ginseng botanical ever grown
Chinese ginseng from multiple provinces for the
last twenty years.**

Exclusivity sampling frame:

**8 samples: imported from China, purchased
on the internet, and purchased from AHP**

Exclusivity panel

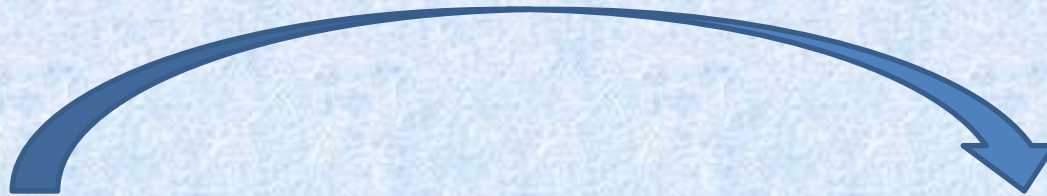
Used all 8 samples

Analytical Measurement

- **Comparison of a test material to the target material requires analytical measurement(s).**
- **Measurement(s) must be appropriate for the materials.**
- **Measurements may be based on morphological features, genetic sequences, chromatographic patterns, spectral patterns, or any other metric appropriate for the target material.**
- **Analytical measurements must be reduced to a single value that is used to make the YES/NO decision.**
- **Summary: You must have yardstick and a value (threshold) on the yardstick that separates the target (YES) and non-target (NO) materials.**

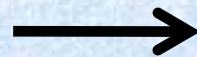
Analytical Measurements

Integrate Results



Measure characteristics:

Morphological features
Base pair sequences
TLC features
Chromatographic fingerprint
Spectral fingerprint
Organoleptic features



Analytical parameter:

% Match
% Match
% Match
Statistical match
Statistical match
Aroma match?

Analytical Measurement Example

Methodology:

MS fingerprints - direct injection (with no separation), negative ionization mode

Data:

43 American ginseng and 8 Chinese ginseng samples

950 ions per sample from m/z 50 to m/z 1000

Final data matrix 51 samples x 950 ions

Data processing:

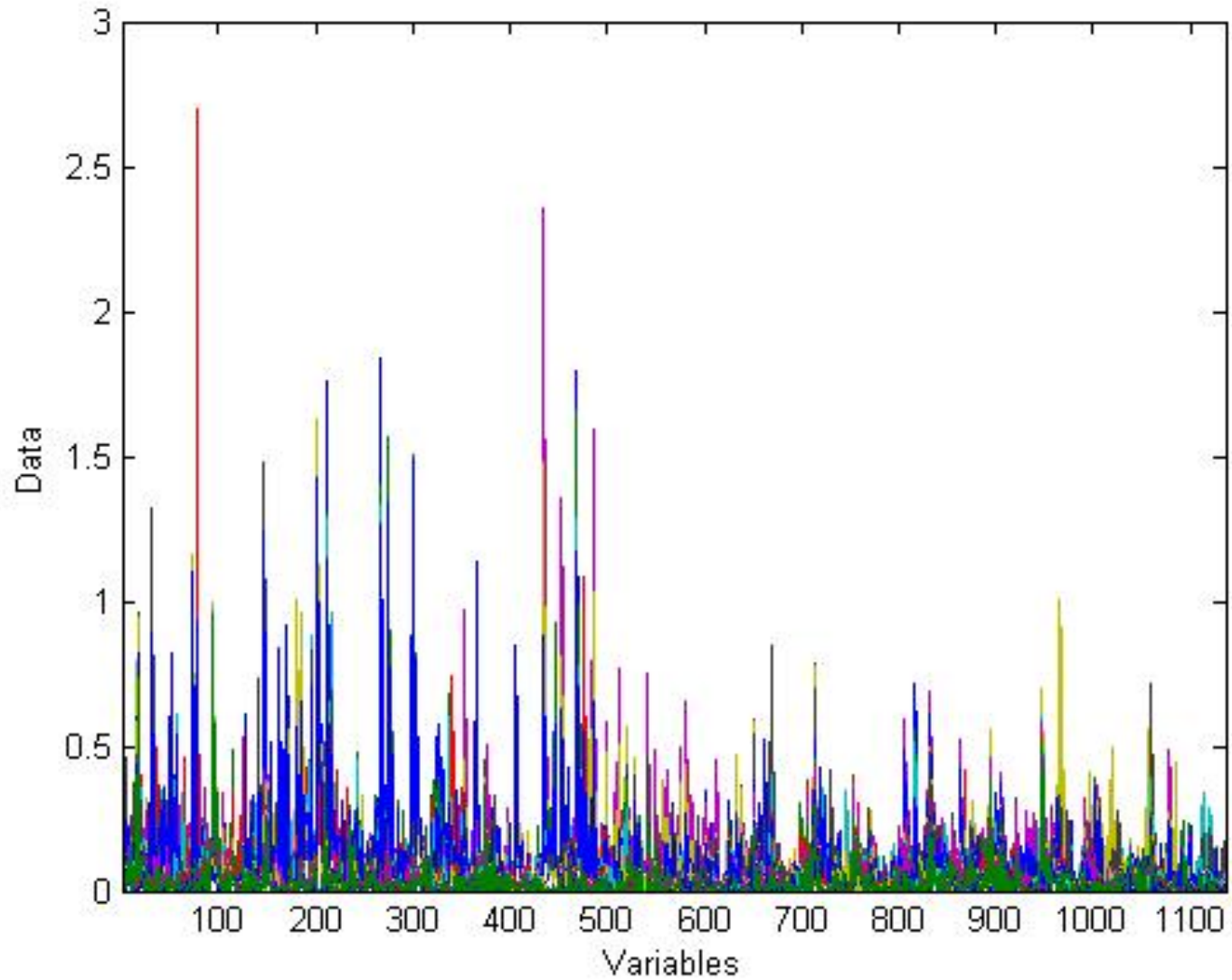
SIMCA (soft independent modeling of class analogy)

PCA (principal component analysis) model of American ginseng (inclusivity panel)

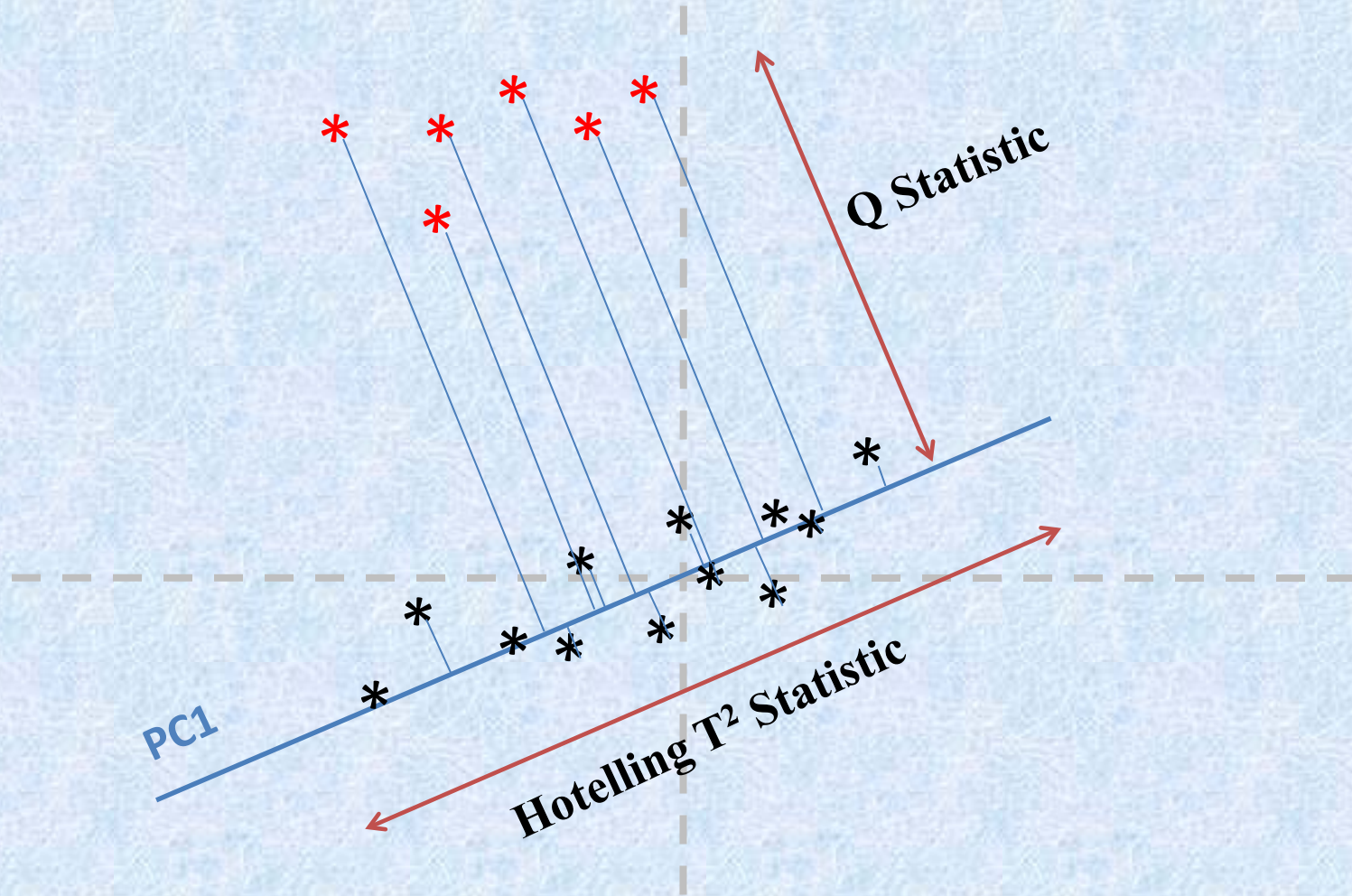
Apply model to all data (exclusivity panel)

Calculate Q residual (how well does data fit the PCA model)

Analytical Measurement Example



Example: PCA Model

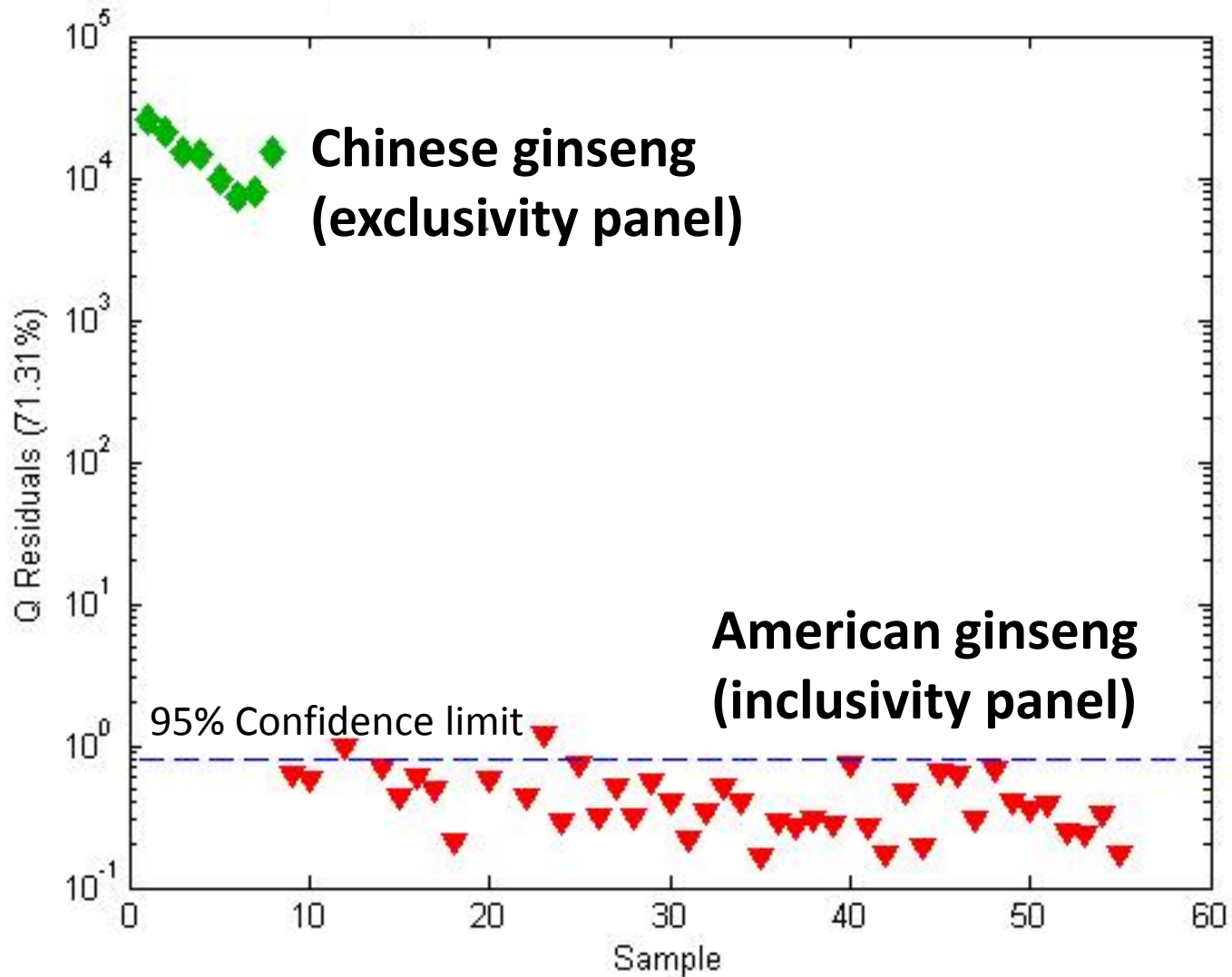


*** Modeled target sample data**

*** Test sample data**

Example

Q residuals for American and Chinese ginseng samples



Acceptable Target Material

Superior test material (STM): a mixture that contains the **lowest acceptable** concentration of target material.

Specified by Standard Method Performance Requirements (SMPRs).

Probability of Identification (POI) = 1.0

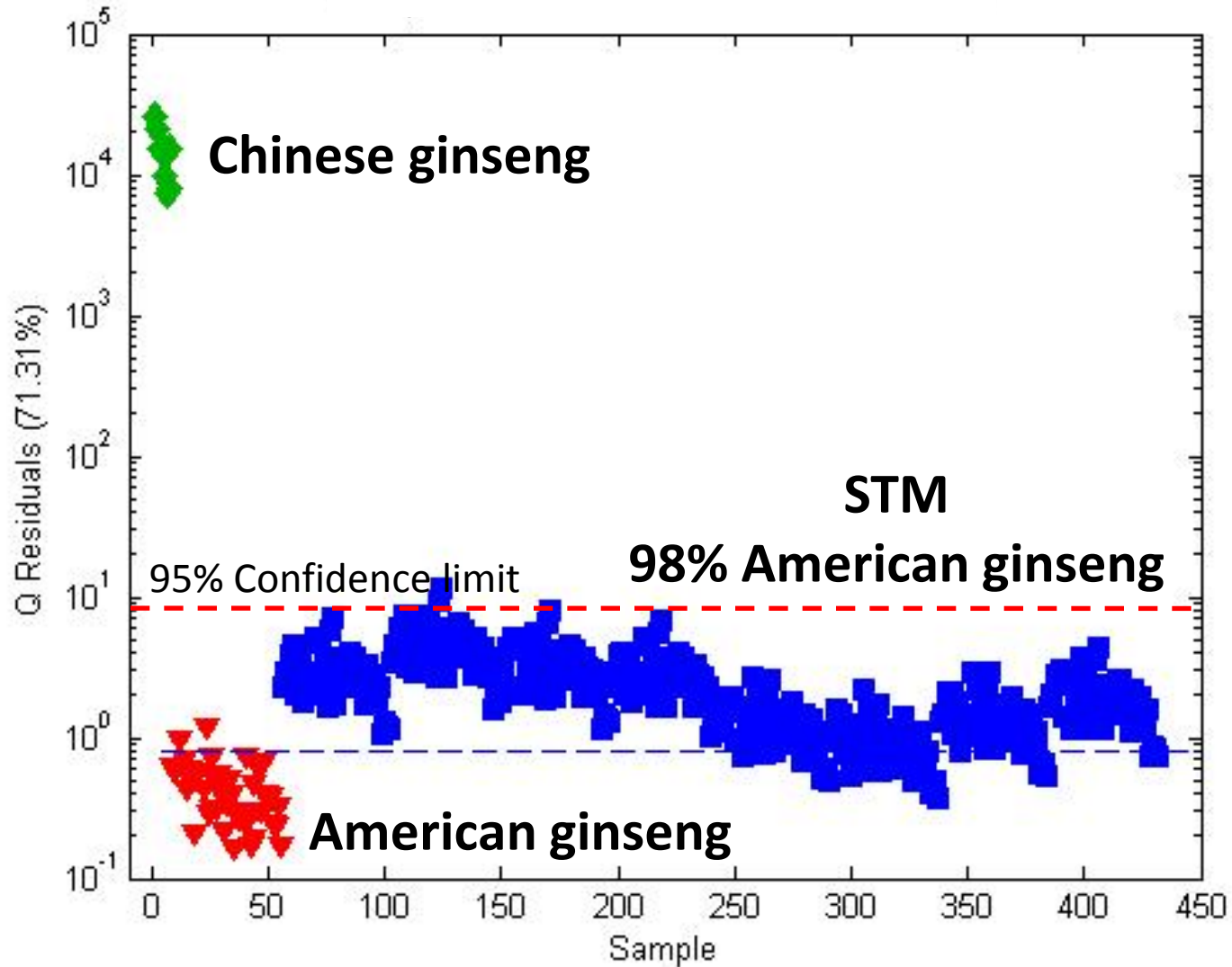
Inferior test material (ITM): a mixture that contains the **highest un-acceptable** concentration of target material.

Specified by SMPRs.

Probability of Identification (POI) = 0.0

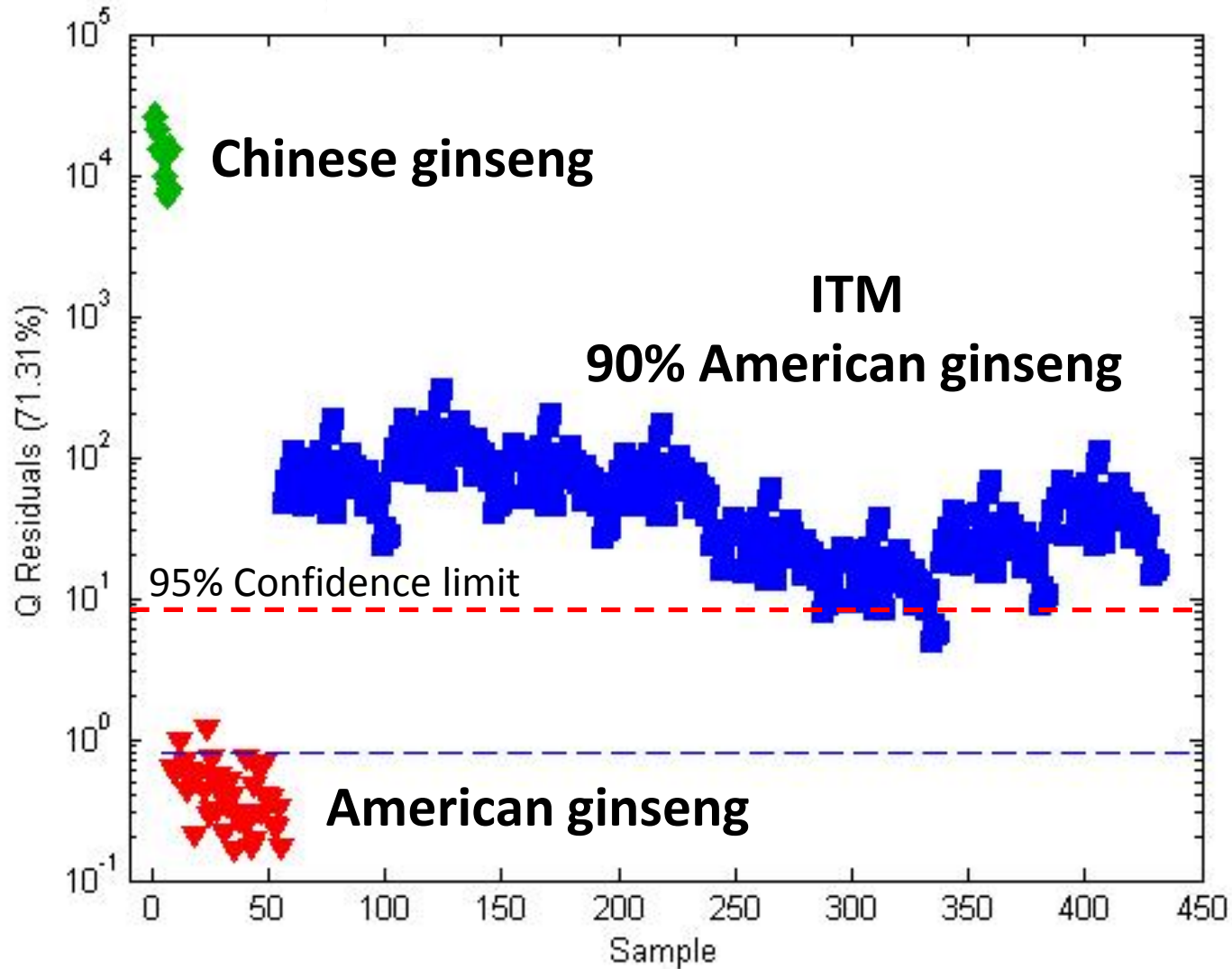
STM Example

98% American ginseng: **lowest acceptable** concentration



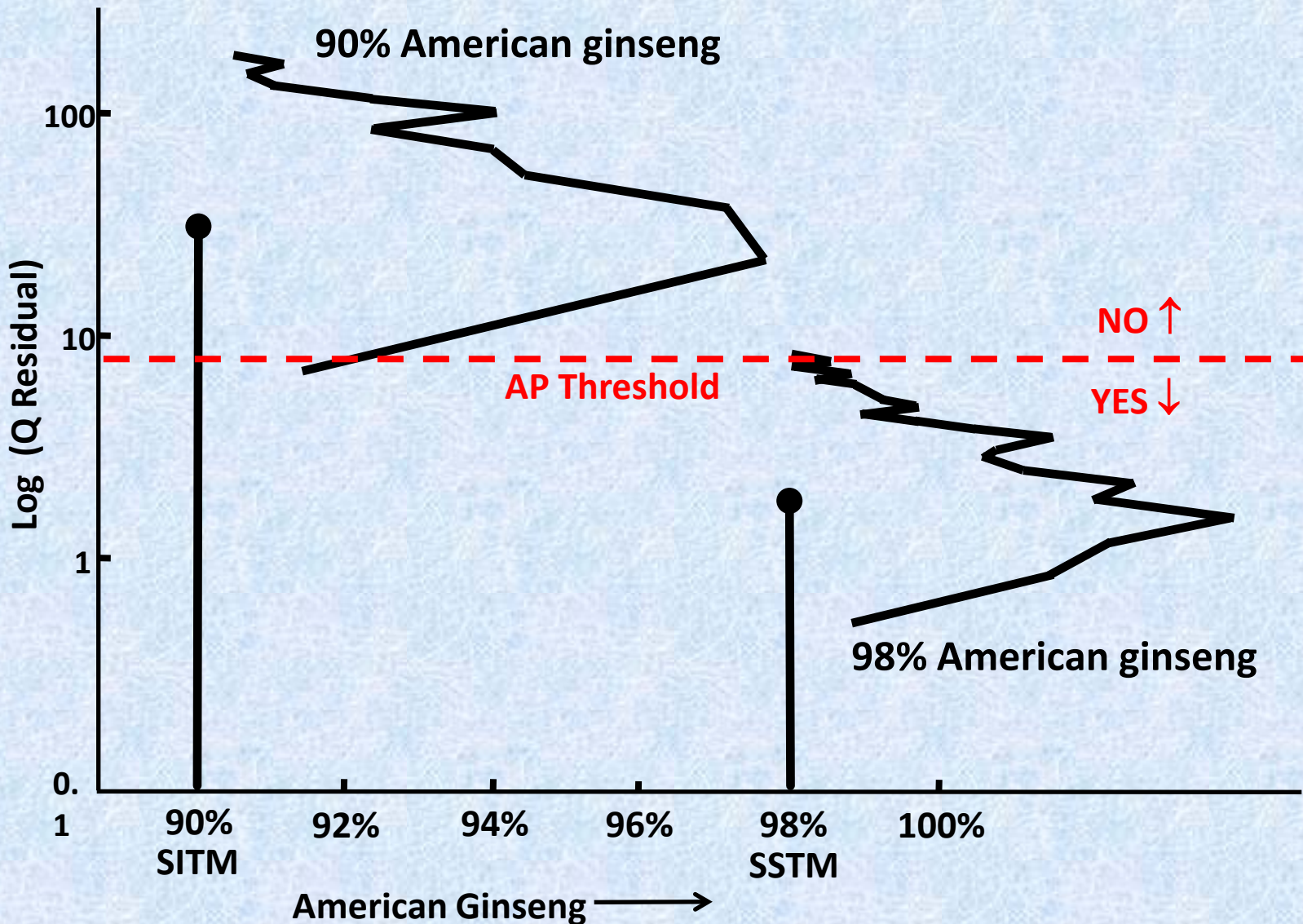
ITM Example

90% American ginseng: **highest un-acceptable** concentration



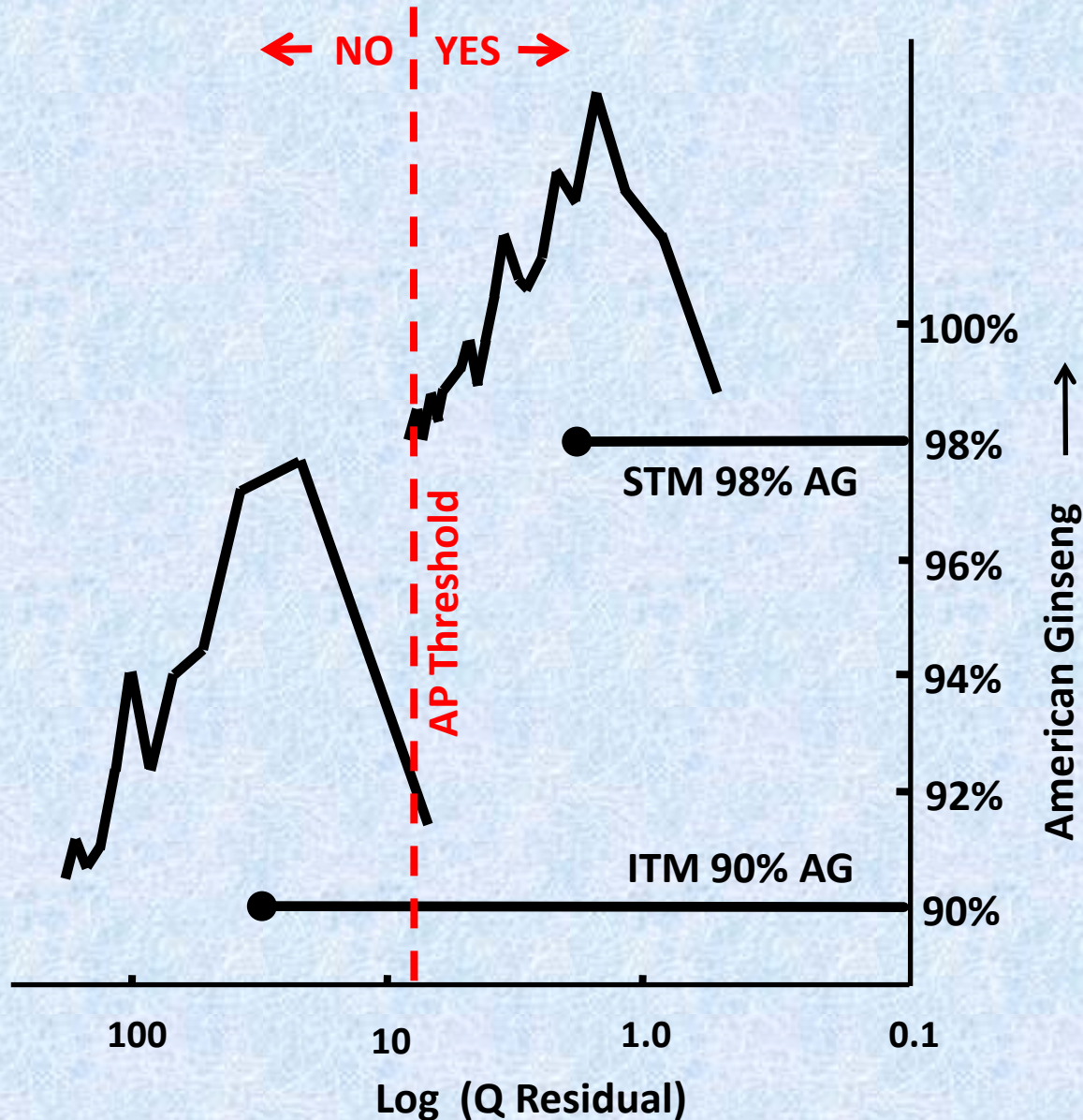
STM/ITM Example

Q residuals for American and Chinese ginseng samples



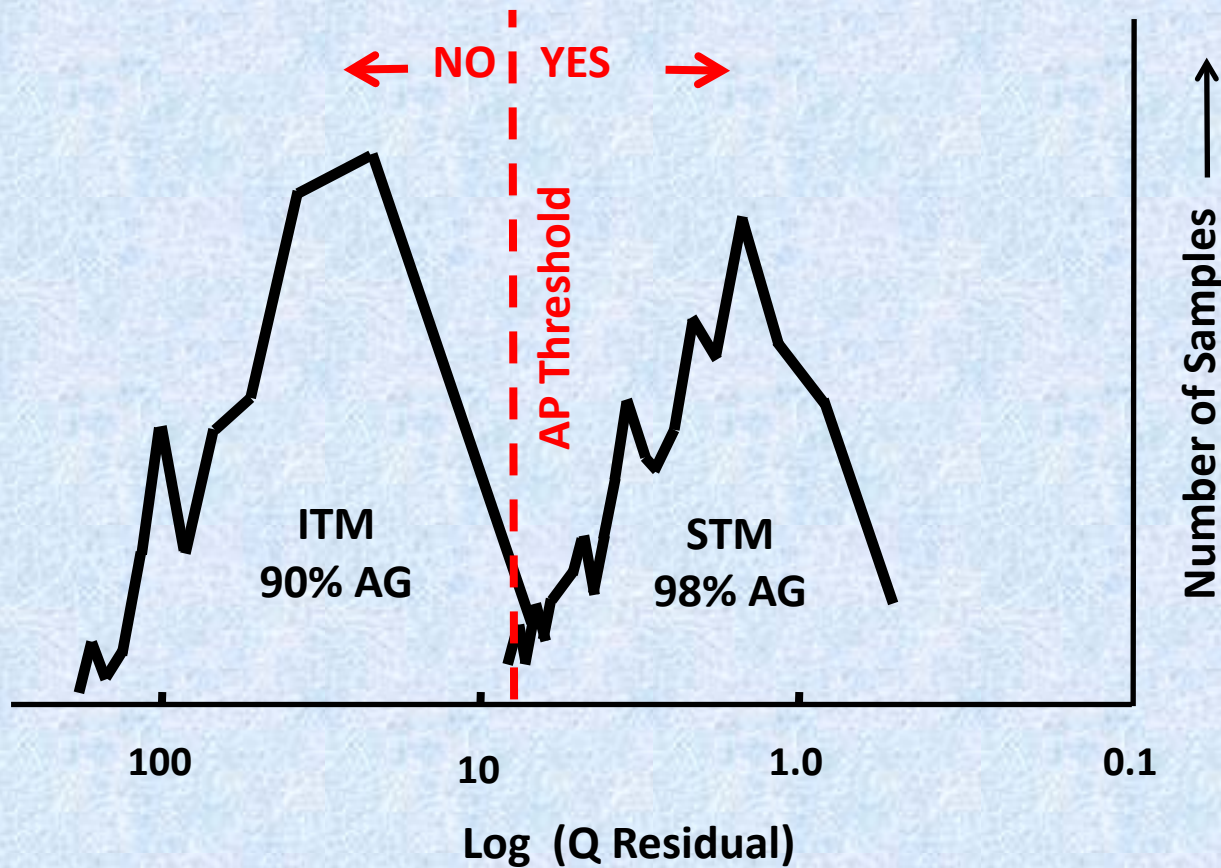
STM/ITM Example

Q residuals for American and Chinese ginseng samples



STM/ITM Example

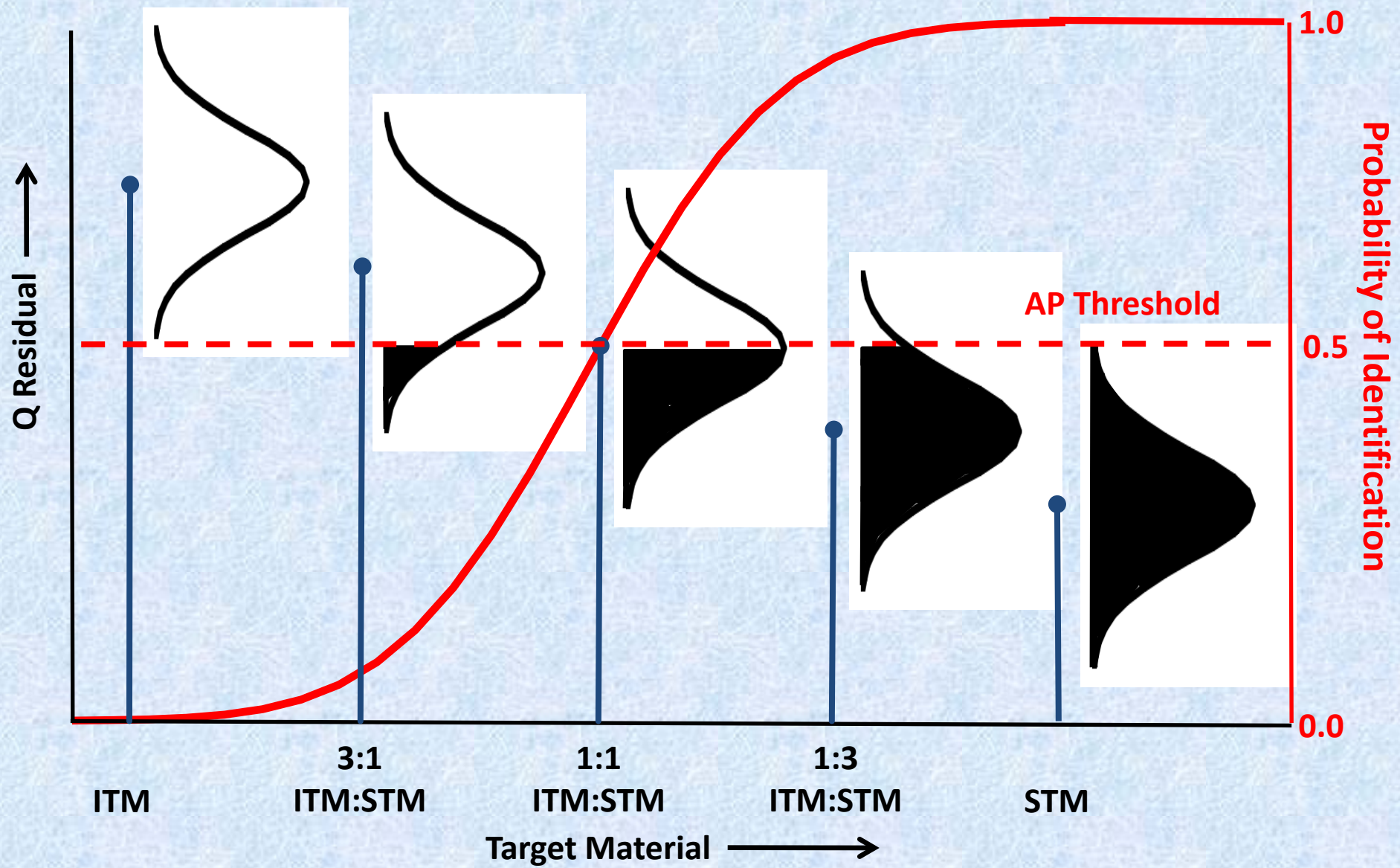
Q residuals for American and Chinese ginseng samples



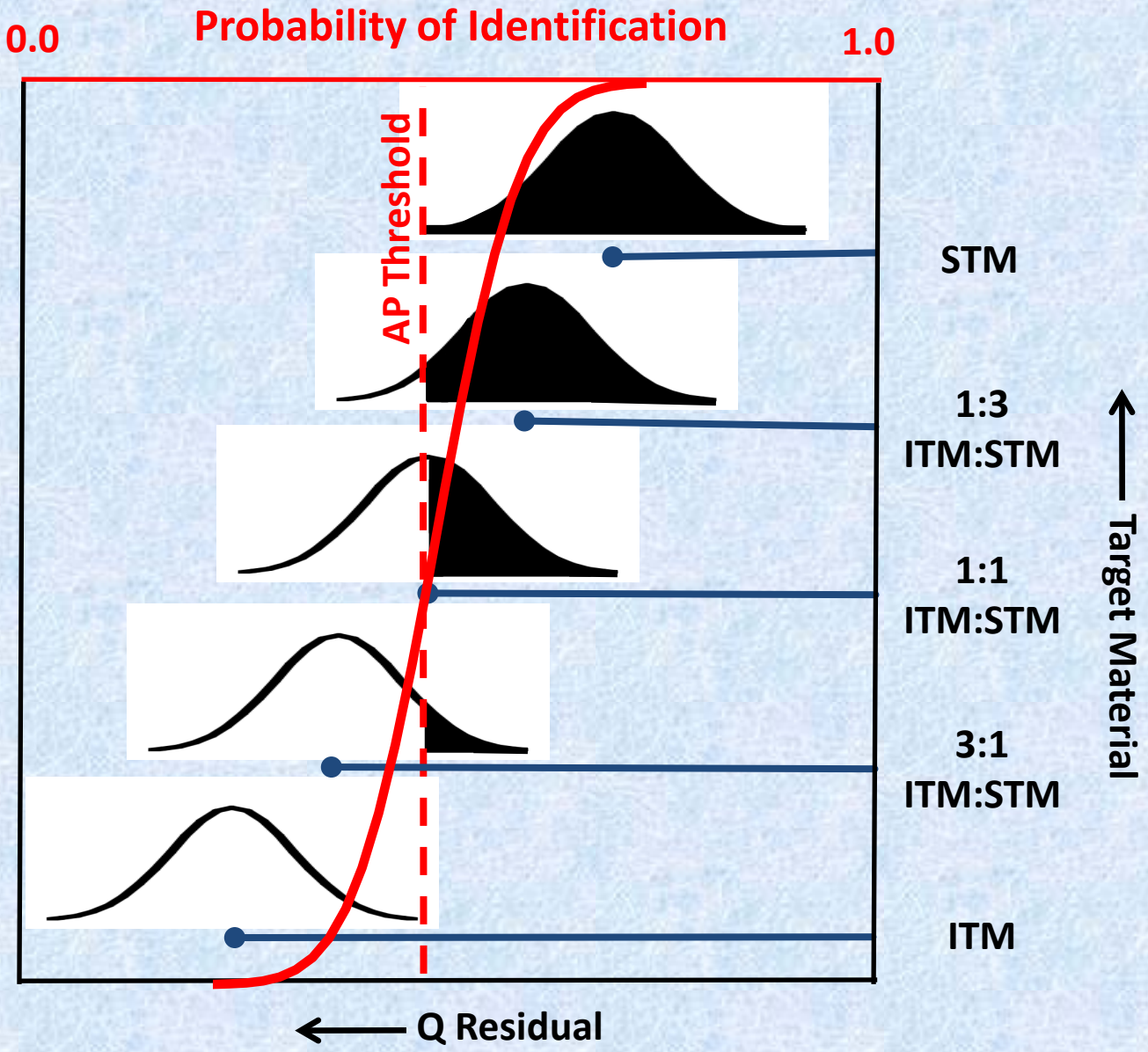
Characterization of POI

Curve describing the transition from 1.0 (YES this is the target material) to 0.0 (NO this is not the target material).

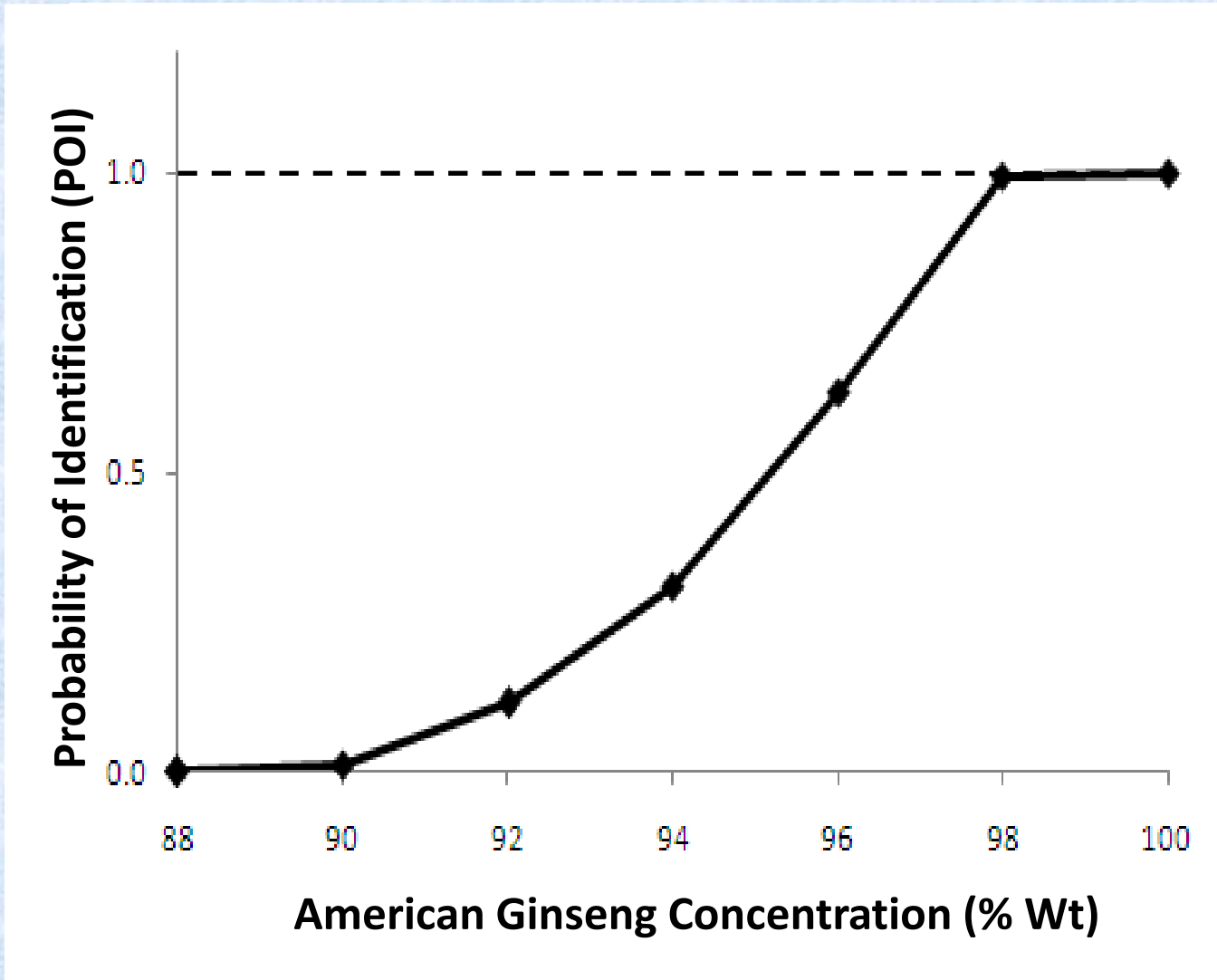
POI Curve Example



POI Curve Example



POI Curve for American Ginseng



If the Non-Target Material is Unknown? (not covered by the validation guidelines)

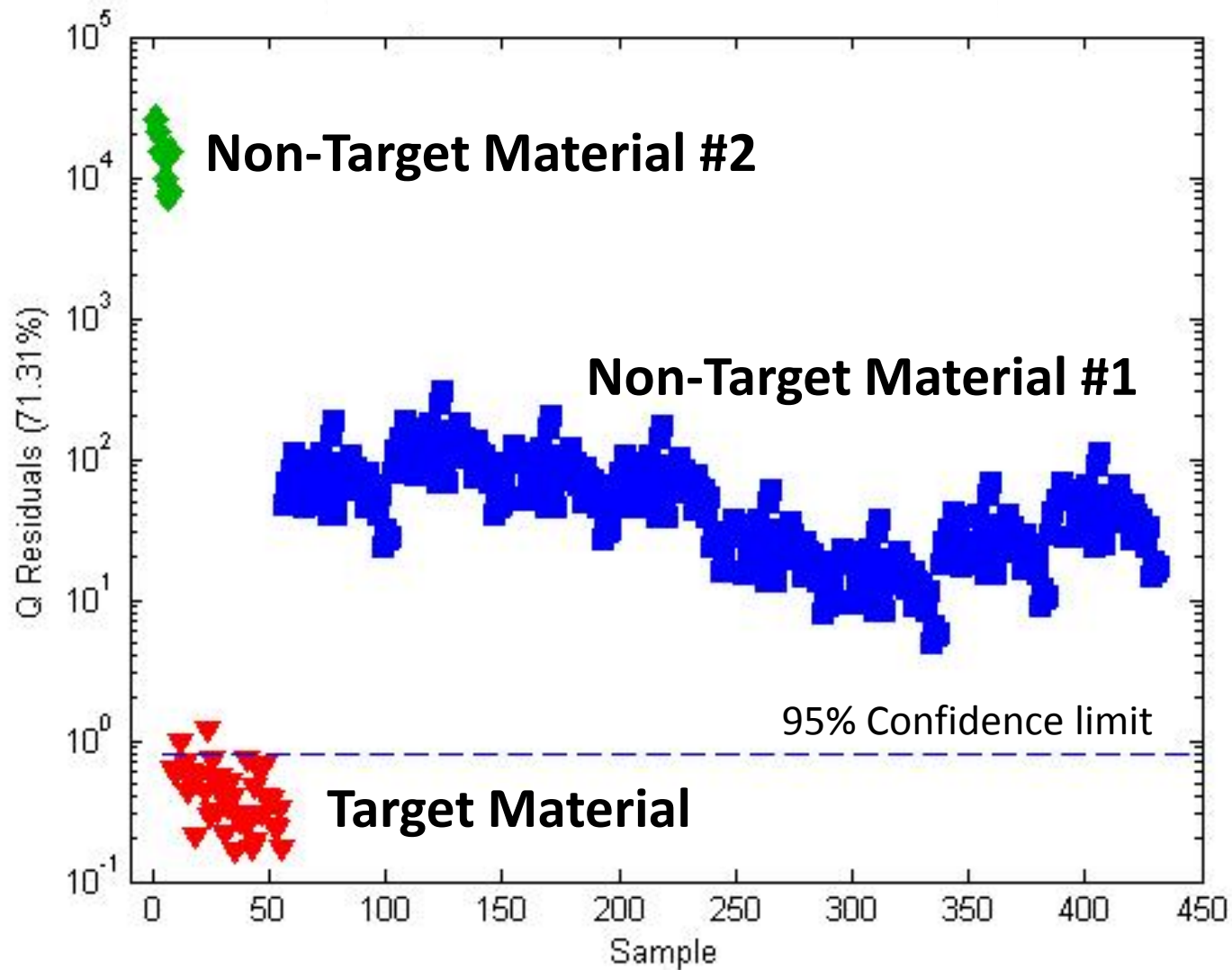
**Target material established by the inclusivity panel.
(average spectrum and expected variance)**

**Non-target material is any material that falls outside
the confidence limit established by the inclusivity
panel.**

**Sensitivity of non-target material is critical where
sensitivity is defined as:**

**Deviation from the average spectra caused by
difference in chemical composition.**

Unknown Non-Target Material Example



Summary

POI statistical method has been established for the identification of botanical materials.

General method involves establishing:

- Inclusivity/Exclusivity panels (target/non-target materials)**
- Superior/Inferior materials (acceptable/un-acceptable limits)**
- Characterizing the POI curve (optional)**

If non-target material is unknown:

- Inclusivity panel determines mean spectra and variance**
- Non-target materials fall outside the confidence limit determined from the variance**