NEW APPROACHES for FOOD AUTHENTICITY TESTING

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MÉRIEUX NUTRISCIENCES

National Environmental Monitoring Conference

8 August 2019

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Let's Chat About...

- Definitions
- AOACI Response
- Targeted Testing
- Non-Targeted Testing
- SMPRs and Priorities
- Some New Methods

Areas of Focus … Despite the Lack of "Internationally Agreed-upon Definition"

- Food Fraud Incidents :
  - Deliberate act
  - Aims for economic gain in an illicit manner
  - Meant to be hidden / not to be discovered
  - Misrepresents the food product to consumers

- US FDA Working definition of "Economically Motivated Adulteration" (EMA)

The fraudulent, intentional substitution or addition of a substance in a product for the purpose of increasing the apparent value of the product, or reducing the cost of its production, i.e. for economic gain.

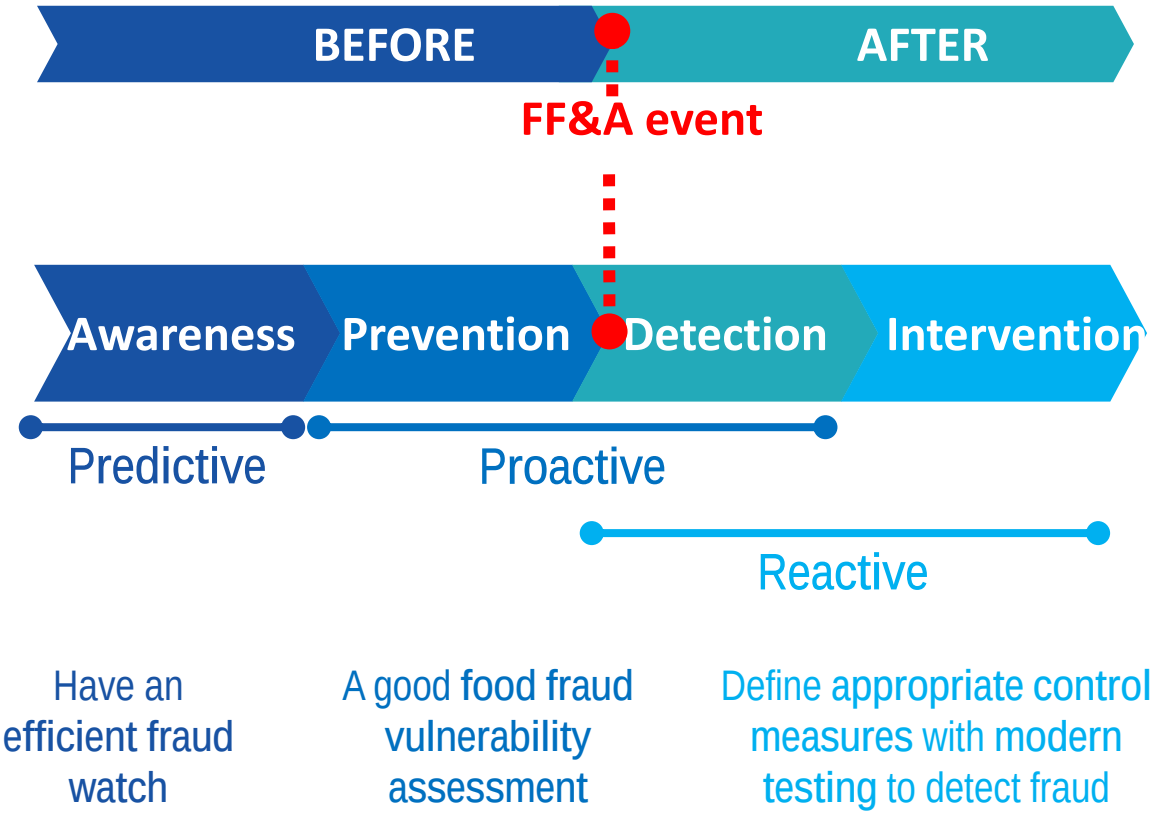
Clarifications

- *Food authentication**
  - a process to evaluate that state of being
- *Food fraud**
  - the act that creates the problem;
  - the deliberate and intentional substitution, addition, tampering, or misrepresentation of food, food ingredients, or food packaging; or false or misleading statements made about a product, for economic gain.

*John Spink, *quality Assurance & Food Safety*, 2018

Food Fraud Risk Management

- **A global approach**



AOAC Int'l Initiative

- AOAC INT'L Taskforce on Food Fraud:
  - ✓ Shape AOAC's role and future actions to address the Food Fraud
  - ✓ Leverage AOAC's leadership and stakeholder engagement to support sustained action in addressing analytical requirements for a Food Fraud Prevention
  - ✓ Framework
    - Method Availability
    - Method Standardization

AOACI 's Actions

- AOACI BOARD OF DIRECTORS created 2 working groups:
  - ✓ **Targeted Testing Working Group:** Map existing methods, their status, and ID needs for method development and standardization
    - ✓ Chaired by Dr. Joe Boison
  - ✓ **Non-Targeted-Testing Working Group :** To develop Standard Methods Performance Requirements (SMPRs) for methods used in the early detection of food fraud incidents
    - ✓ Chaired by Dr. John Szpylka

Approach for Authenticity Testing

- **Standard: Targeted Analysis**
  - ***Is it** in the food sample?*
  - Determination of **known** molecules associated with adulteration

- **Innovative: Non-Targeted Screening (NTS)**
  - ***Is Something** in the food sample?*
  - Determination of the **overall profile / fingerprinting** of the sample = **known + unknown molecules**
  - Much data collected at the same time to build an ***ad hoc* reference database for authenticity testing**. Innovative approach for **customized projects:**
    - **Geographic origin**
    - **Species varieties**
    - **Biodiversity**
    - Etc.

Targeted Testing (TT)

- Targeted Testing (TT) requires
  - **prior identification of adulterants** likely to be present
  - subject to EMA
  - employed to assure adulterants do not contain known health-risks and maintain ingredient integrity
- Targeted Testing (TT) protocols/procedures to:
  - Support authenticity assurance
  - Ensure the food supply chain integrity
  - Tells adulterers we are monitoring and will prosecut

Targeted Testing Working Group

- Current Actions:
  - Assessment of gaps of current food fraud test method and identify & validate new targeted testing methods;
  - Developing standards leading to Codex Type 1 methods;
  - Prioritizing actions of adulterants and commodities of interest.

Capabilities: Targeted approach

- Some food frauds can be detected with standard tests
  - when they are properly combined together
  - when you know what you are looking for
- Examples
  - Fish, meat and botanical species identification
  - Counterfeiting of organic products
  - Common wheat in durum wheat pasta
  - Artificially colored fruit juice
  - Adulteration of olive oil with cheaper substitutes
  - Adulteration of milk
  - Adulteration of A2 milk
  - Adulteration of Butter
  - Adulteration of spices
  - Crust % in grated parmesan
  - Aging of Grana Padano cheese
  - Arabica vs Robusta coffee
  - Dairy products produced by silage fed animals vs. grass fed
  - Fish freshness
  - Etc.

Non-Targeted Testing Methods

- New Concept
  - In The Past: Quantitative analytical methods measure amounts of known chemicals in known foods.
  - In The Past: Qualitative methods determine if a known chemical or microorganism is present at or above a known level.
  - New: Non-Target Testing models properties of the authentic material, not the properties of the adulterant.
- NTT Approach
  - Create a standardized fingerprint for an ingredient.
  - Compare new lots of the ingredient to the fingerprint.
  - Quantify "degree of difference"
    - Small difference shows something **may be** wrong
    - Large difference shows something **is** wrong

Non-Targeted Technologies

- Variety of methodologies are being used
  - LC-MS/MS
  - GC/MS
  - NMR
  - Spectroscopic
  - XRF and other ones for certain matrices
- Data analysis
  - Chemometrics
  - Principle Component Analysis
  - Customized software
- Specific method requirements don't exist
  - Some methods can give insight into adulterating substance
- Rapid Evaporative Ionization MS
- Laser Diode Thermal Desorption
- Isotope Ratio MS
- NGS-metabarcoding
- Lots of activity
  - ILSI Food Authenticity Task Force
  - AOAC Task Force (MXNS chairing NTS portion)
  - USP webinars

Capabilities: NTS-Non Targeted Screening Approach

NMR, IR
LC-HRMS

MALDI-TOF

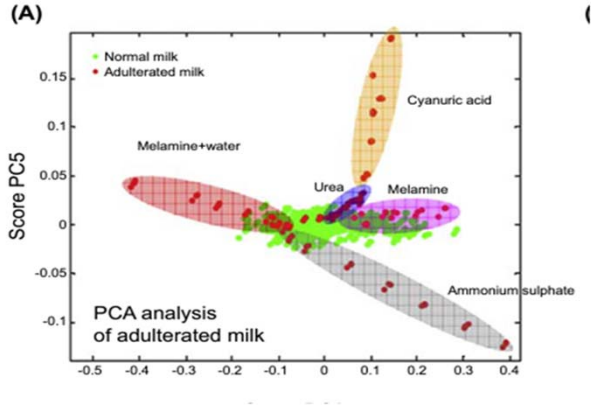
NON TARGETED ANALYSIS

CHEMOMETRICS

"MULTIVARIATE DATA ANALYSIS"

Small molecules

Big molecules



Result: 95% probability that sample is Authentic: YES/NO

Standard Method Performance Requirements (SMPRs®)

- Appendix F: Guidelines for Standard Method Performance Requirements

- SMPR
  - First define the method's minimum performance requirements
  - Is a part of a *Call For Methods*
  - Is used by an AOAC *Expert Review Panel* to judge if a submitted method can be accepted as an AOACI *First Action Official Method of Analysis*

New SMPR Components

Traditional AOAC SMPR	Non-Targeted Testing SMPR (draft)

NTT Working Group

- Created Generic SMPR
  - Demonstration of Non-Targeted Testing method effectiveness and usefulness
  - Validation/verification guidance
- Generic SMPR is being used first on prioritized commodities
  - Learnings will then be applied to other commodities
- SMPRs will sent with *Call for Methods*

Using Parts of USP Appendix XVIII

- NTT models the properties authentic material, not the properties of the adulterant
- Define what we want NTT method to do
- Define the Reference Set of authentic samples to create fingerprint
  - Incorporate natural variability
- Define Test Samples to evaluate method
  - Authentic and adulterated samples
- Method developers choose technology and mathematical assessment

Example Applicability Statements

- Example 1: “A rapid non-targeted method for detecting the adulteration of milk powder with **nitrogen-rich compounds** added at **economically motivating levels** (e.g., 0.1%) with a sensitivity rate of 99% and a specificity rate of 95%, both with a Confidence Interval of 95%.”
- Example 2: “A rapid non-targeted method for detecting the adulteration of milk powder with **any foreign material** at **economically motivating levels** (e.g., 5%) with a sensitivity rate of 90% and a specificity rate of 95%, both with a significance level of p = 0.01.”

Method Performance

- Sensitivity: ability to correctly recognize unacceptable samples/material as atypical

$$\text{Sensitivity} = \frac{(\text{correctly identified adulterated foods})}{(\text{total adulterated foods})}$$

- Specificity: ability to correctly recognize samples/materials as typical

$$\text{Specificity} = \frac{(\text{correct identified authentic foods})}{(\text{total authentic foods})}$$

NTT SMPR Two Tiered Approach

- How do reference materials and standards fit into a method looking for unknowns?

- Tier 1
  - For initial Single Lab Validation (SLV)
  - Recipes of commonly used adulterants at EMA levels
    - Number of replicates and %correctly identified as adulterated
  - Define how “authentic” reference materials representing natural variability
- Tier 2
  - For Multi-Lab Validation (MLV)
  - Third party group creates blind authentic and adulterated samples
  - Adulterants go beyond those used in SLV

Generic SMPR Components

- A non-targeted method
  - to evaluate foods and ingredients for possible EMAs.
  - Generate a fingerprint of the authentic material.
  - Compare test samples fingerprints to assess differences.
  - Binary result of either authentic or potentially adulterated.
- Single Lab Validation using prescribed adulterated materials (next slide).
- Approved SLVs proceed to 2nd level using blinded samples containing unknown adulterants.
- Method developer documents hoe authentic samples were located.

Generic NTT SMPR

Authentic Material	Adulterant	%adulterant in Validation Samples	n	#positive	%Sensitivity at 95% confidence (Correctly Identified as Adulterated)
EVOO	Sunflower Oil	0%	30	n/a	fingerprint
		15%	100 (or 35)	99 (or 35)	95%
EVOO	Safflower Oil	15%	100 (or 35)	99 (or 35)	95%
Honey	HFCS	0%	30	n/a	fingerprint
		25%			
Milk (powder)					
Milk (liquid)					

Commodities Being Examined First

- Initial Commodity List
  - Olive oil
  - Extra Virgin Olive Oil
  - Honey
  - Milk Liquid & Powder
  - Fish
  - Meat
  - Seafood
  - Grains (rice)
  - Spices

Some Additional Thoughts

- Non-Targeted and Targeted Testing Overlap
  - NTT will identify new adulterants, therefore new TT methods will be needed.
- If a major international food fraud incident happens, a rapid response will be needed.
  - AOAC will have an even bigger role.

Targeted and non-targeted approach





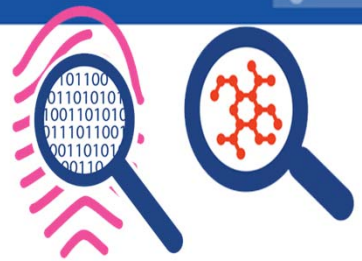


TARGETED APPROACH

- Fish, meat and botanical species identification
- Common wheat in durum wheat pasta
- Artificial colors in juice
- Adulteration of olive oil with cheaper substitutes
- Crust % in grated parmesan
- Arabica vs Robusta coffee
- ...

NTS APPROACH

- EVOO geographical origin
- Species varieties and biodiversity
- Origin of tomato products
- ...

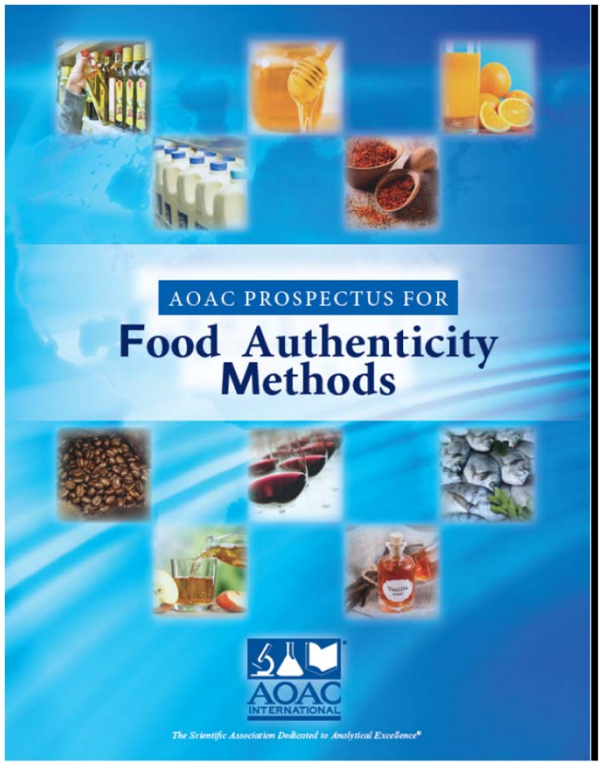


TARGETED + NTS APPROACH

- DOP Parmigiano Reggiano
- 100% italian origin (wheat)
- ...

AOAC Food Authenticity Working Group

- For information on how to join any of the AOAC Food Authenticity Working Groups below, please contact Delia Boyd, Sr. Manager at dboyd@aoac.org.
  - Non-Targeted Testing Working Group
  - Targeted Tested Working Group



DNA Microsatellites, Isotope Ratios and Metabolomics to better understand botanical and geographic origin of wheat, semolina and pasta.

E. Gritti, E. Poloni, F. Cattapan, E. De Dominicis, S. Saner - Mérieux NutriSciences Research & Science Center

G. Gambarota - De Matteis Agroalimentare

Recent MXNS Study

1. DNA Microsatellite markers

Simple Sequence Repeats (SSRs) different in length (Species - Variety - Individual)

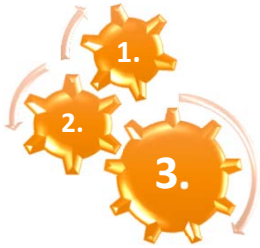
Botanical Origin

2. IRMS: C, H, O, N, S

3. Metabolomics

Non Targeted Mass Spectrometry

Geographic Origin



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REFERENCE SAMPLES for METHOD DEVELOPMENT

Variety ID

30 samples

- **6 pure samples (wheat)**
  4 Italian
  1 Kazakh
  1 Canadian
- **12 italian semolina mix**
  (prepared in-house)
- **12 non-italian semolina mix**
  (prepared in-house)

SIRA/IRMS & Metabolomics

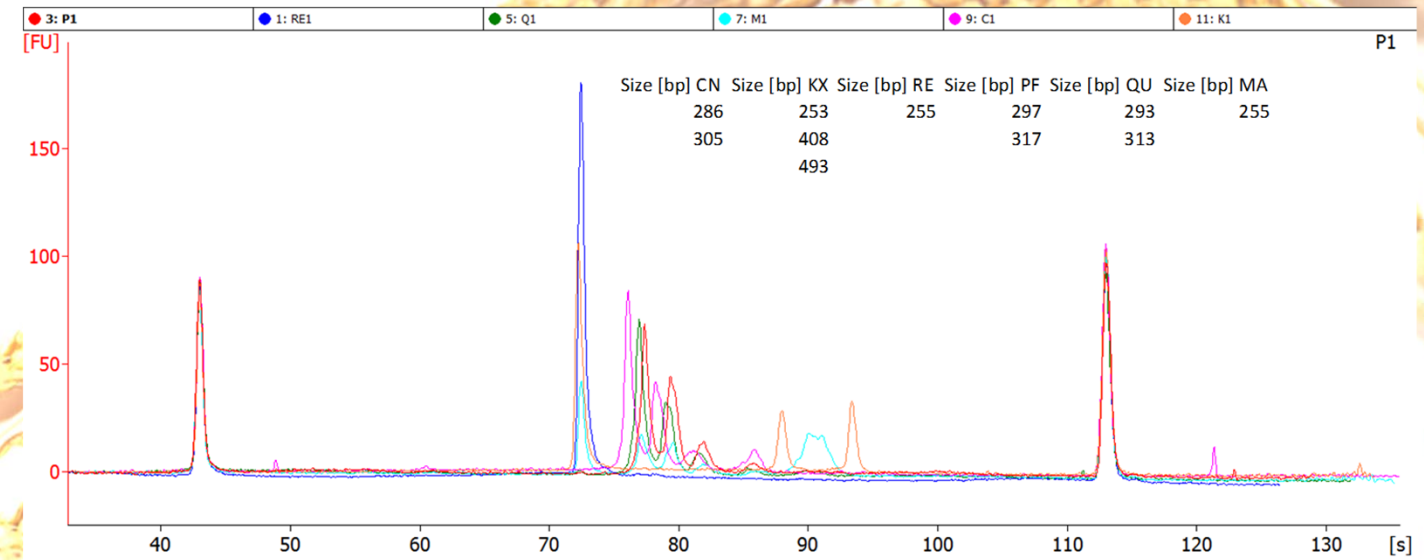
74 samples

- **44 Italian semolina**
  20 of the year 2017
  24 of the year 2018
- **20 Kazakh semolina**
  10 of the year 2017
  10 of the year 2018
- **10 Canadian semolina**
  year 2017-2018

REFERENCE SAMPLES for METHOD DEVELOPMENT

This study provided a reproducible fingerprint: Based on Italian durum wheat cultivar SSR markers

- ✓ identified the minimum number of SSRs usable for the identification of the major number of Italian cultivars
- ✓ used ABI PRISM 3100 Genetic Analyzer and GeneMapper v 3.5 genotyping software for rapid and high throughput screening.



Electropherogram showing peak sizes using DuPW 167. Wheat cultivars are distinguished according to the polymorphic fragments of the SSR.

MULTIVARIATE DATA ANALYSIS - INTERNAL VALIDATION

Soft Independent Modeling of Class Analogy (SIMCA) and Partial Least Squares Discriminant Analysis (PLS-DA) multivariate methods discriminate between samples from different geographical origins.

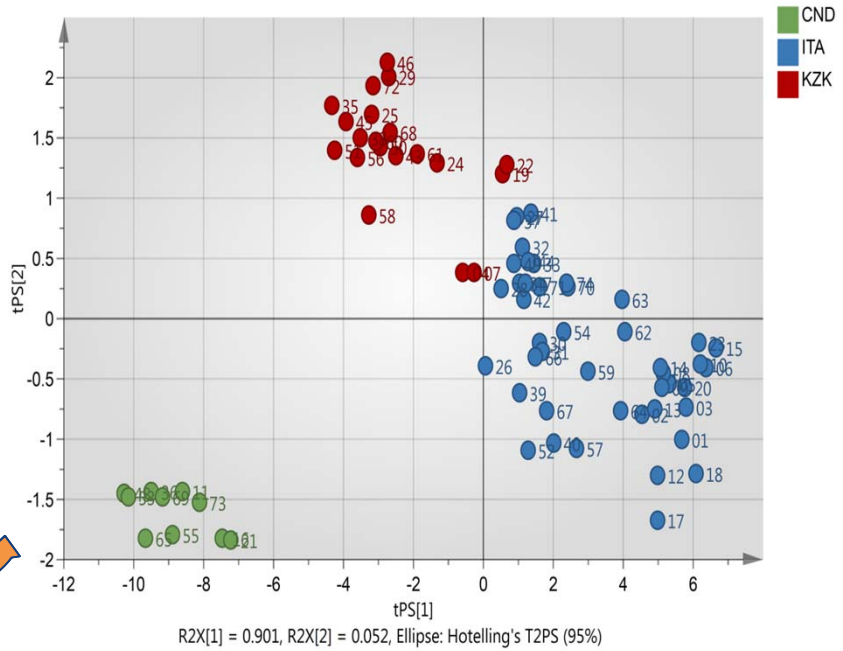
Accuracy	97,2973%
Kappa statistic	0,9433
Total Number of Instances	74

=== Detailed Accuracy By Class ===

Sensitivity	Specificity	Precision	Recall	F-Measure	MCC	MR	Class
1,000	0,933	0,957	1,000	0,978	0,945	0,00%	Italian
0,933	1,000	1,000	0,933	0,966	0,945	3,33%	Non-Italian
0,973	0,960	0,974	0,973	0,973	0,945	3,33%	Overall

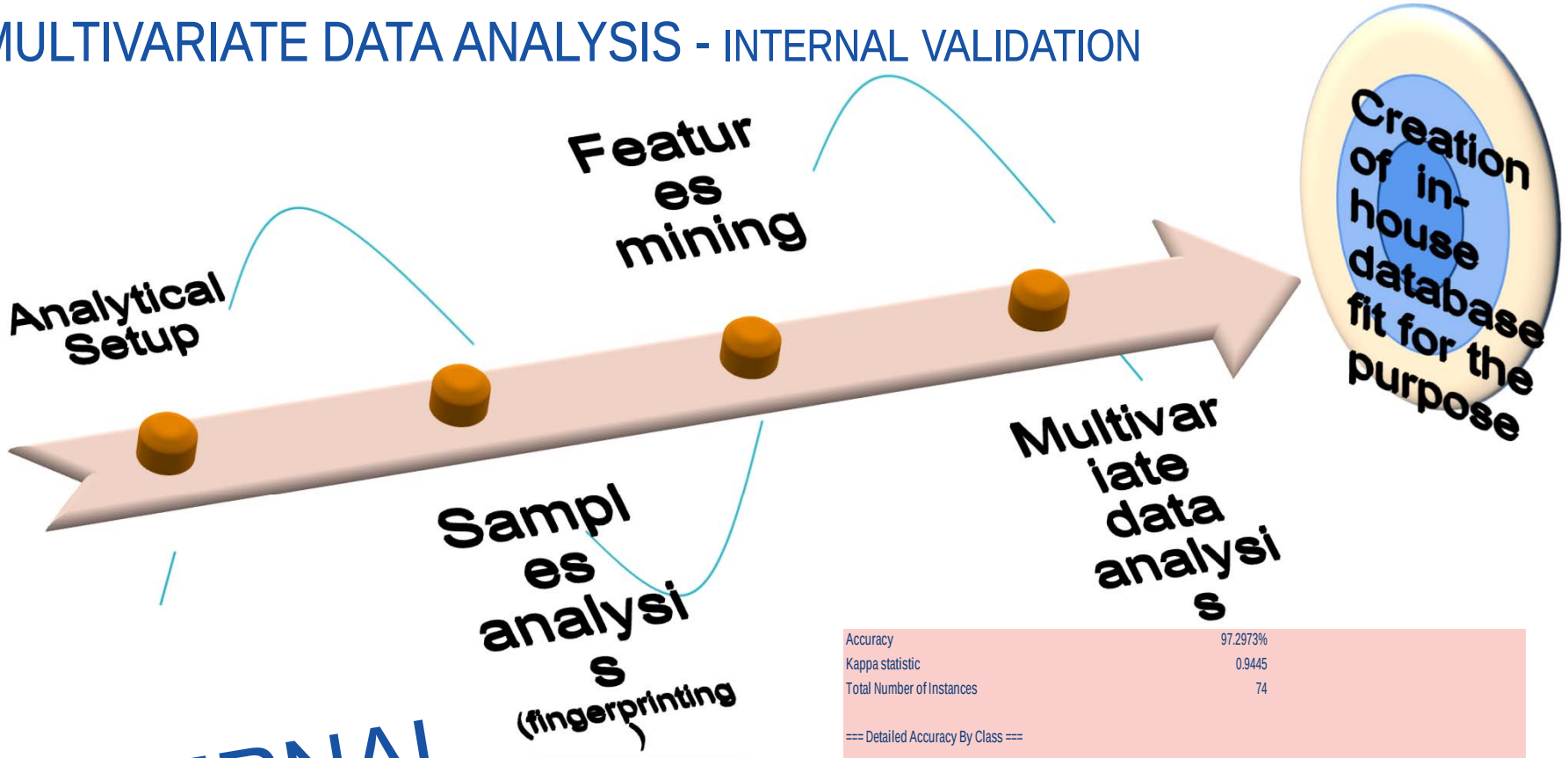
=== Confusion Matrix ===

a	b	<-- classified as
44	0	| a = Italian
2	28	| b = Not Italian



METABOLOMICS NON-TARGETED MS

MULTIVARIATE DATA ANALYSIS - INTERNAL VALIDATION



EXTERNAL VALIDATION

Accuracy	97.2973%
Kappa statistic	0.9445
Total Number of Instances	74

=== Detailed Accuracy By Class ===

Sensitivity	Specificity	Precision	Recall	F-Measure	MCC	MR	Class
0.955	1.000	1.000	0.955	0.977	0.946	2.27%	Italian
1.000	0.955	0.938	1.000	0.968	0.946	0.00%	Non-Italian
0.973	0.982	0.975	0.973	0.973	0.946	2.27%	Overall

=== Confusion Matrix ===

a	b	<-- classified as
42	2	| a = Italian
0	30	| b = Not Italian

INTERNAL VALIDATION

Closing Thoughts

- Analytical testing is a component of combating food fraud
  - as a part of an entire program
- Lots of analytical approaches are being developed
- Key factors
  - Our education is based only on events we have caught
  - Targeted Testing is for known adulterants or known authenticity factors
  - Non-Targeted Testing can be used as a screen
    - Methods being developed
    - How to Assess their reliability is being developed
  - TT and NTT should be used together