Fitness For Purpose Methods

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Agenda

- Describe AOAC process for Fitness for Purpose
- Review method validation principles and common protocols
- Review key elements of laboratory quality systems
- Review case study: SPIFAN (Stakeholder Panel Infant Formula Adult Nutritionals)

FSCF Food Safety Cooperation Forum
PTIN Partnership Training Institute Network

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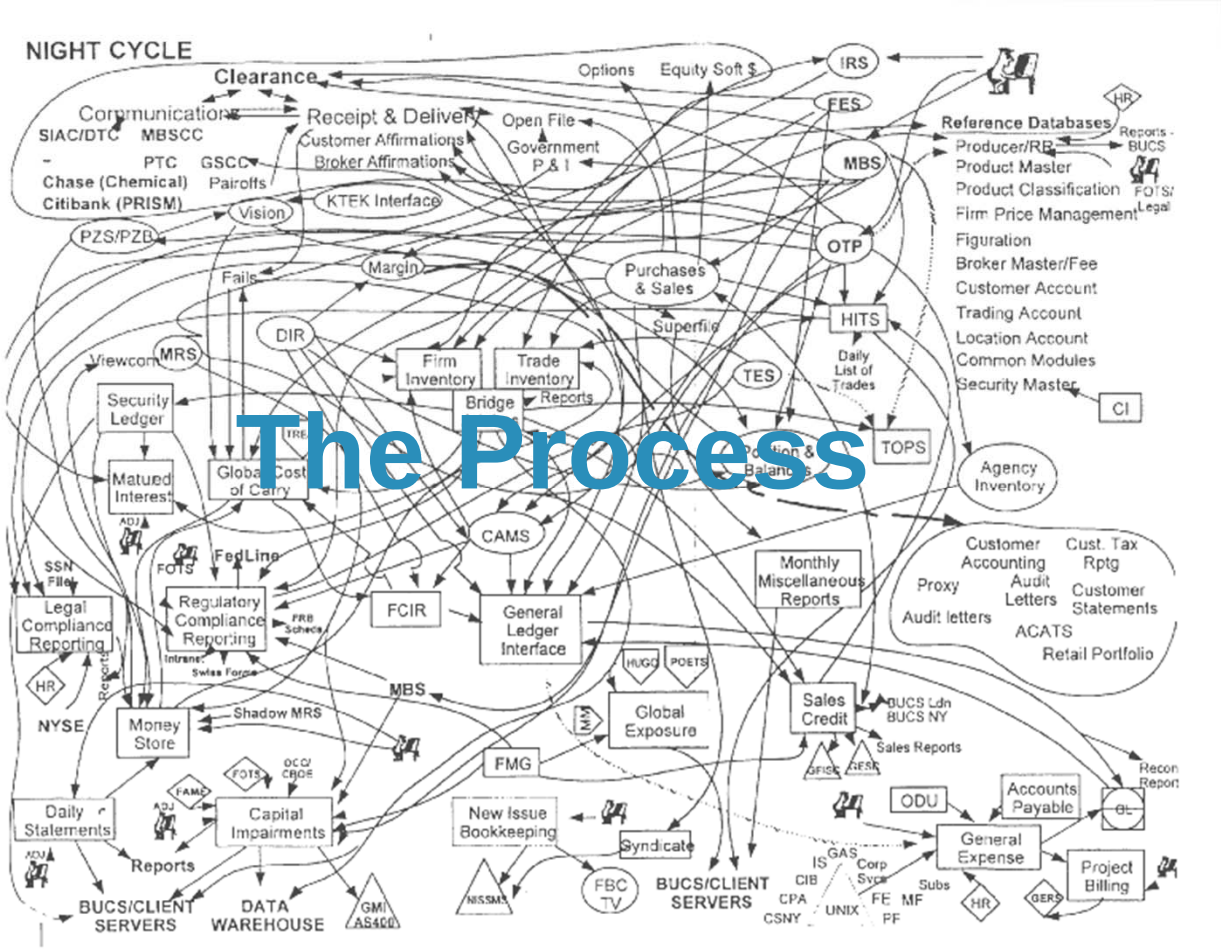
Fitness For Purpose



Catalyst

- Recognized need or gap
- Global implications to safety or trade
- Consensus required
- **Champion(s)**

AND

Key Elements

Stakeholder Community

Voting Stakeholders

Working Group

Expert Review Panel




Stakeholder Community Recruit representatives from all disciplines that share a stake in the issue and methodology Government/Manufacturers/CROs/ Academia/Trade Associations	Key Stakeholders Subset of stakeholder community (balanced composition) which makes key decisions and sets priorities.
Working Groups Subject matter experts that develop the standard method performance requirements and recommend candidate methods	Expert Review Panels Official authorizing body that decides disposition of a method. In AOAC, this would include First and Final Actions.

Fitness For Purpose Statement

- A high level summary of “what the method is intended to do”
- This would typically include:
  - applicable matrices
  - target analytes
  - intended use, e.g., field, manufacturing, surveillance, dispute

Used to identify appropriate stakeholders and subject matter experts to form a Working Group

Standard Method Performance Requirements

The working group develops the standard method performance requirements. These are shared for public comment and final revisions are presented to the Voting Stakeholders for adoption.

EXAMPLE:

AOAC SMPR Determination of Cr, Mo, and Se in Infant and Adult/Pediatric Nutritional Formula

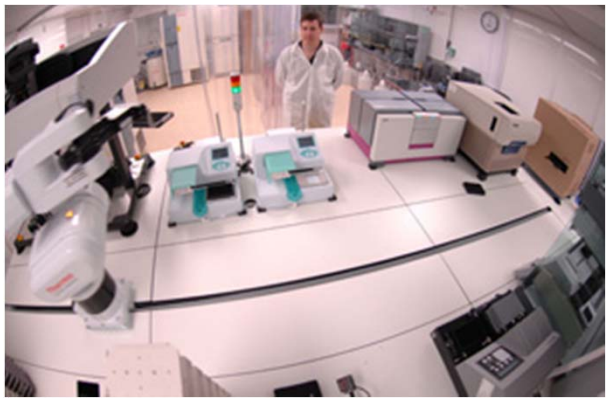
Down Selection of Candidate Methods

Based upon applicability to SMPRs, candidate methods are proposed by the working groups.

There is a broad call for methods from stakeholders. These are evaluated by the working groups. Oftentimes, the proposed candidate method may be a “hybrid” method drawing on the best parts of several submitted methods.

Study Director

- Champions a candidate method and takes it through:
  - Single laboratory validation
  - Peer collaboration
  - Collaborative Study



Digression: Test Method Validation – What is it?

ISO defines Validation as:

"Confirmation by examination and provision of objective evidence that the particular requirements for a specific intended use are fulfilled.".

-ISO 17025, 5.4.5

From Eurochem:

"…the process of defining an analytical requirement and confirming that the method under consideration has performance capabilities consistent with what the application requires. Implicit in this is that it will be necessary to evaluate the method's performance capabilities….The judgement of method suitability is important; in the past method validation has tended to concentrate on the process of evaluating the performance parameters."

-The fitness for Purpose of Analytical Methods, 3.1

Test Method Validation – What is it?

The latter definition identifies three discrete stages of method validation:

1. **Define the Analytical Requirement(s)**
2. **Determine (by collection and analysis of relevant data) the Appropriate Method Performance Metrics**
3. **Confirm the Adequacy (based on the determined performance metrics) of the Method to Meet the Established Analytical Requirement(s)**

Method validation is a separate process from development and optimization. It is an objective, systematic, evaluation that can only be undertaken AFTER development and optimization are complete. Unsatisfactory validation data may dictate the need for additional development and optimization work, after which a new validation must be executed.

Test Method Validation – What is it?

Performance Measures Typically Addressed by Validation:

1. **Linearity** (fit to calibration model, usually linear)
2. **LOD/LOQ**
3. **Specificity**
4. **Precision***
    a) **Repeatability**
    b) **Intermediate Precision** (within lab reproducibility)
    c) **Reproducibility**
5. **Accuracy** (Trueness)*
6. **Robustness** (Ruggedness)

Precision and Accuracy (Trueness) form the core of the validation process. All aspects of validation ultimately relate in some way to these two metrics.

Test Method Validation – What is it?

Validation may be either a single laboratory validation (SLV) or a collaborative study (CS) involving multiple laboratories.

SLV – Provides estimates of all performance metrics (except reproducibility) within a single laboratory

CS – Usually designed to estimate only repeatability and combined intermediate precision/reproducibility. Can be designed to give explicit estimates of intermediate precision and reproducibility .

Collaborative studies are virtually always preceded by a complete SLV, which is required to evaluate readiness for undertaking a CS.

Test Method Validation – Why is it Important?

Validation data provide the information necessary to properly interpret test results, since it is this data that is likely to be the basis for estimating the uncertainty of actual test results. Without an appropriate uncertainty estimate, it is impossible to use a test result correctly.

Recall the ISO definition of validation: *"Confirmation by examination and provision of objective evidence that the particular requirements for a specific intended use are fulfilled."* In other words, method validation allows an objective assessment of whether the method is ***fit for purpose***.

Effective method validation is good practice in all circumstances but it is mandatory for testing related to status and/or disposition of commercial products.

Test Method Validation – How is it Done?

Typical AN SLV for Nutrient Analysis

Linearity

Analyze a minimum of 5 standards, uniformly spaced across a range that exceeds the expected data range by 15% on either end. Repeat a minimum of 3 times.

For each data set, calculate the relative calibration error for each standard by back calculating concentrations and comparing to the true concentrations.

Level dependent trends in the calibration errors considered as potential factors affecting method accuracy (trueness).

Test Method Validation – How is it Done?

Typical AN SLV for Nutrient Analysis

LOD/LOQ

Usually not addressed explicitly because the ranges of interest are well above these limits. An empirical LOQ based on the method working range established during validation is typically imposed.

If necessary LOD/LOQ may be addressed by one of the following:

- Analysis of a matrix placebo spiked at low level
- Analysis of low level standard (instrument limit only)
- Uncertainty of Calibration Intercept (instrument limit only)

Test Method Validation – How is it Done?

Typical AN SLV for Nutrient Analysis

Specificity (one or more)

Analysis of a matrix placebo

- Absence of background signal demonstrates specificity
- Detectable background average ($n \geq 6$) signal considered as potential factor affecting accuracy (trueness)

Analysis of compounds known to be possible interferences in the matrix of interest

- Absence of interference demonstrates specificity

Analysis of sample that has been treated (e.g., enzymatically) to specifically remove the compound of interest

- Same as matrix placebo

Test Method Validation – How is it Done?

Typical AN SLV for Nutrient Analysis

Precision

Analyze unspiked samples in duplicate on each of six or more non-consecutive days (independent runs). Use multiple analysts (≥2), instruments, etc. to the extent feasible.

Conduct ANOVA to obtain estimates of repeatability (within-run), run-to-run, and intermediate (total) precision. The most important of these is the intermediate precision because it best reflects execution of the method over time. Knowledge of repeatability and run-to-run components allows informed use of replication to reduce uncertainty.

Test Method Validation – How is it Done?

Typical AN SLV for Nutrient Analysis

Accuracy/Trueness (one or more)

Analyze matrix placebo spiked at expected 50%, 100%, and 150% of typical level in triplicate on each of three days.

- Recoveries significantly different from 100% considered as factors affecting accuracy (trueness)

Analyze sample at 50% and 100% overspike in triplicate on each of three days.

- Same as placebo spikes

Analyze certified reference material CRM) in duplicate on each of six days.

- Difference significantly different from zero considered as factor affecting accuracy/trueness

Compare with reference method

- Same as CRM

Test Method Validation – How is it Done?

Typical AN SLV for Nutrient Analysis

Robustness (Ruggedness)

Not usually investigated by formal protocol. By using multiple analysts, instruments, etc. and by executing adequate independent replication, variation due to typical factors is incorporated into various estimates. If particular sensitivity to some experimental parameter is suspected, it will be investigated.

Test Method Validation – How is it Done?

Fit for Purpose?

Demonstration that a method is "***fit for purpose***" is the ultimate goal of the validation process. However, guidance in this critical area is generally vague and there is no consensus definition of what this means.

One widely utilized measure of acceptable method performance (precision) in a SLV is the Horwitz Ratio. Although most often, and appropriately used for evaluating data from collaborative studies, it is sometimes applied to SLVs. For repeatability:

$$\text{Horrat}_r = \frac{\text{RSD}_r}{\text{PRSD}_r}$$

Where RSD_r= *repeatability* relative standard deviation from validation data and PRSD_r =*repeatability* relative standard deviation predicted by the Horwitz equation:

$$\text{PRSD}_r = C^{-0.15}$$

Test Method Validation – How is it Done?

Fit for Purpose?

$Horrat_r$ values between 0.5 an 2.0 are generally considered to indicate acceptable precision.

Limitations of Horwitz ratios for evaluating method performance include:

Only precision is addressed and, therefore, the question of accuracy (trueness) is left to be addressed separately.

RSDs tend to be overestimated, sometimes dramatically, especially at low concentrations.

More fundamentally, it does not provide a actual basis for deciding whether method performance is *adequate for any particular purpose*. Rather, it provides a framework for evaluating whether method performance (precision only) is *consistent with expectations*, based on the empirical data that was used to formulate the Horwitz model as it is currently applied.

Test Method Validation – How is it Done?

Fit for Purpose?

A construct that AN has found useful for evaluating method performance that accounts for both precision and accuracy (trueness) is built on the concept of process capability, a metric that gives a measure of the ability of a process to operate within defined limits. Considering testing done under intermediate precision conditions, this model can be formulated in terms of the intermediate precision RSD as:

$$\frac{\left|\left(100+\mathrm{I}\right)-\mathrm{SLNT}\right|}{\frac{\mathrm{RSD_{IP}}}{\sqrt{n}}} \geq \mathrm{t}_{0.005,df}$$

Test Method Validation – How is it Done?

Fit for Purpose?

$$\frac{\left|(100+\mathrm{I})-\mathrm{SLNT}\right|}{\frac{\mathrm{RSD_{IP}}}{\sqrt{n}}} \geq t_{0.005,df}$$

I is the estimated inaccuracy (lack of trueness), expressed as a percentage.

SLNT is the specification limit nearest the target (or expected mean) for the product being tested, expressed as a percentage of the target (or expected mean) after it has been adjusted by the inaccuracy.

RSD_{IP} is the intermediate precision RSD

$t_{0.005,df}$ is the student-t value at 99% confidence for df degrees of freedom

n is the number of independent observations averaged to produce the final result

Test Method Validation – How is it Done?

Fit for Purpose?

$$\frac{\left|(100+\mathrm{I})-\mathrm{SLNT}\right|}{\frac{\mathrm{RSD_{IP}}}{\sqrt{n}}} \geq t_{0.005,df}$$

All sources of inaccuracy that remain uncorrected in the final result are considered when estimating the inaccuracy, I:

- Systematic Calibration error
- Background Interference
- Recoveries different from 100%
- Difference from CRM value or Reference Method

Test Method Validation – How is it Done?

Fit for Purpose?

$$\frac{\left|(100+\mathrm{I})-\mathrm{SLNT}\right|}{\frac{\mathrm{RSD_{IP}}}{\sqrt{n}}} \geq t_{0.005,df}$$

The model can be modified to account explicitly for repeatability and run-to-run precision components and the degree of replication associated with each. It can also be constructed to incorporate estimates of the the underlying true process variation, which results in an even more realistic assessment of method adequacy. Finally, it can be simplified somewhat by replacing $t_{0.005,df}$ with an empirical factor, typically 3 or 4.

Test Method Validation – General Resources

ISO: 5725, Parts 1-6

Eurochem Guide: The Fitness for Purpose of Analytical Methods

AOAC: Guidelines for Single Laboratory Validation

AOAC: Guidelines for Collaborative Studies

ICH: Text on Validation of Analytical Procedures

FDA: Guidance on Analytical Procedures and Methods Validation

IUPAC: Harmonized Guidelines For Single Laboratory Validation Of Methods Of Analysis

IUPAC: Protocol For The Design, Conduct and Interpretation of Method-Performance Studies

More Digression: Laboratory Quality System – Key Elements

Compliance

Document change control

Validated methods

Basic laboratory procedures

Ongoing system monitoring

Qualified laboratory equipment & instruments

Preventative maintenance

Evaluation of nonconformities

CAPA

Data integrity

Internal audit review

Training

Compliance

A well-defined laboratory quality system is the cornerstone of any laboratory operation. An integral part of the quality system is the expectation of compliance. It is considered a 'must do' and is not optional

To be most effective, the laboratory's quality system requires:

- Support from all levels of the organization, particularly senior management
- Everyone is responsible and accountable for its success
- A culture of reinforcement
- An understanding that the quality system is a living work in progress

Document Change Control

Good documentation and document change control are important to the quality system because they:

- Ensure document integrity by requiring limited edit access
- Assure that current, controlled document versions are in use
- Provide for consistent document structure, format and distribution
- Maintain an established audit trail that provides important historical perspective (traceability)

Document Change Control (cont'd)

Documentation practices to avoid include:

- Backdating to a previous date
- Postdating to a future date
- Document corrections without appropriate explanations provided
- Write-overs
- The use of pencils or erasable ink
- Obscuring original entries (e.g., the use of correction fluid, tape, etc)

Validated Methods

Test methods require validation to level appropriate based on

- intended use
- application

Validation is the systematic practice of experimentally gathering and analyzing sufficient information to assure that

- methods are reliable
- methods will yield acceptable results when performed as documented

Validated methods are typically required when used for regulatory submissions and to support marketed products

Basic Laboratory Procedures

There are minimum procedures which are fundamental to any laboratory, and include the following:

- Documentation procedures (discussed in previous slides)
- Procedures for labeling, preparation and storage of chemicals, solutions and reagents
- Environmental monitoring (measuring temperature-critical items such as lab refrigerators, freezers, incubators, etc)
- General lab housekeeping (cleaning, waste disposal)
- Calibration of instruments and equipment with measureable components (including balances, thermometers, pipettes, etc)
- Laboratory instrument and equipment maintenance procedures
- Analyst training

Ongoing System Monitoring

An effective quality system requires ongoing monitoring in order to evaluate and maintain laboratory compliance. System monitoring considers:

- Are items calibrated on a routine schedule?
- Are calibration items removed from use if an out-of-calibration tolerance level is detected?
- If calibration is not feasible (due to instrument drift), then is instrument standardization conducted at the time of use?
- Are reagents, chemicals and solutions routinely checked for expiration?
- Are control samples and blanks (if appropriate) run with test methods to assist with monitoring method suitability (i.e., control charting)?
- Are laboratory analysts re-trained in order to demonstrate ongoing competence?

Qualified Laboratory Equipment & Instruments

Laboratory instruments and equipment must be qualified for their intended purpose

Rely on instrument manufacturers' IQ/OQ, particularly for complex instrument platforms

Qualify instrument systems holistically if possible, rather than qualifying each component of the system

Re-qualify the system if:

- component parts have been changed
- the instrument will be used for a new purpose
- the instrument has not been in use for an extended period of time
- the instrument is moved from one location to another (e.g., lab X to lab Y)

Preventative Maintenance

Preventative maintenance is analogous to scheduled maintenance performed on your vehicle in order to ensure proper function (i.e., oil changes, tire rotation, etc). It is designed to prevent future instrument and equipment problems

Preventative maintenance applies to instruments or equipment that have a direct impact on laboratory analyses

Over time, instrument and component parts may become worn, non-functional or obsolete. They should be replaced as need as part of the laboratory's routine function

Laboratory instruments (in particular new and/or complex instruments) may be covered under preventative maintenance (PM) service contracts

Evaluation of Nonconformities

What is a Nonconformity?

- A nonconformity is a deviation from a controlled policy or procedure requirement. Nonconformities are atypical, unplanned and generally rare

Nonconformities should be ‘exceptions’ rather than the rule as part of a comprehensive quality system

Nonconformities must be documented. When tracked and trended, they provide important information for improvement opportunities to the laboratory’s quality system

CAPA (Corrective and Preventative Actions)

CAPA is a means to correct and prevent quality issues that are identified with materials, products, processes and/or quality systems

CAPAs may be the end result of documented nonconformities, audit observations or trends observed from process and product quality monitoring

A CAPA consists of:

- Investigation in an effort to identify a “root cause”
- Development of a resolution plan that will “correct” the problem and identify corrective and/or preventative actions
- Implementation of the resolution plan (with review & approval)
- Effectiveness check (was the resolution plan effective)?

Data Integrity

A test is only as good as the quality of the data that it produces. Data integrity assumes the quality of all data to be true and complete

Data integrity is foundational because it is the basis for both business as well as quality decision-making

Data are essentially information or facts. Data must be controlled, reliable, accurate and complete

Data must be statistically & scientifically valid and unbiased

Where data are transcribed and/or mathematical calculations are used, the accuracy must be verified

Note: Data discrepancies can be considered misleading or even falsification of documentation

Internal Audit Review

Internal audits are typically conducted annually. They offer an opportunity to:

- Comprehensively review the laboratory's quality system
- Evaluate compliance to effective policies and procedures
- Observe laboratory analysts' execution of test methods

The internal audit schedule should be communicated in advance for appropriate planning

Internal audits must be documented. Issues discovered during internal audits that impact the quality system should be followed up with appropriate actions (documented CAPAs)

Training

Training ensures that employees are qualified to perform their assigned functions based on a combination of training, education and experience

Training for laboratory analysts must be documented and should be reviewed by management at least annually

A training matrix is recommended to describe minimum analyst training requirements

A re-training frequency should be established in order to maintain analyst qualification

Back to Process……Expert Review Panel

An authoritative body (e.g., AOAC, ISO, Codex, etc.) will review validation packages and, in some cases, collaborative study results, and confer official status as warranted.

Stakeholder Panel Infant Formula Adult Nutritionals - SPIFAN

•Engaged AOAC and major manufacturers (Abbott/Danone/Fonterra/Mead Johnson/Nestle/Perrigo/Wyeth) to initiate international collaborative. Industry representation was mobilized and is coordinated through the International Formula Council (IFC).

•Recruited a large cross section of key stakeholders from government and private sectors.

- 100+ Stakeholders
- 20+ Countries
- Objective is to establish ISO/Codex dispute methods for complete nutrition label (Vitamins, Minerals, etc.)

FSCF Food Safety Cooperation Forum
PTIN Partnership Training Institute Network

How Standards are Shaping Up…

Vit A HPLC Cis + Trans

Vit D LC/MS D3/D2 + previtamin

Vit E HPLC Total Tocopherol (distinguish natural and synthetic)

Vit K HPLC all-trans

B1 HPLC Total

B2 HPLC Total

B6 HPLC Total

Niacin HPLC Total

Biotin HPLC Free

Folate LC/MS Folic acid +metafolin (including polyglutamyl)

Pantothenic Acid TBD

Vit B12 HPLC Total

Vitamin C TBD

Inositol HPLC Free + Phosphatidyl

Maj/Trace Minerals ICP

UTMs ICP/MS

Iodine Total ICP/MS

Fatty Acids GC

Choline TBD

Nucleotides TBD

Carnitine TBD

Matrices Plan

SRM 1849

Infant Formula Powder Milk-Based

Infant Formula Ready to Feed Milk-Based

Infant Formula Powder Soy-Based

Infant Formula Powder Hydrolysate Milk-Based

Infant Formula Powder Hydrolysate Soy-Based

Infant Formula Powder Elemental (amino acid-based)

Matrices Plan

Child Formula Powder

Adult Nutritional Powder

Adult Nutritional Powder Low Fat

Adult Nutritional RTF High Protein

Adult Nutritional RTF High Fat

Sustainability and Effectiveness

- Establish a laboratory certification/proficiency program
- Establish an international control sample where feasible
- Provide training on methods
- Maintain relevance

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Questions?