e-CAM: Classification of microbiological analysis methods

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During the past decades, a lot of rapid and/or automated methods dedicated to the detection or enumeration of microorganisms in food, have been developed all around the world. However, the choice of a method, even for a given purpose, is somewhat difficult and, currently, very little is known about how laboratories choose methods or how selected methods are validated.

In that context, and in order to help microbiologists from different horizons in their choices, a project called "e-CAM" (Electronic Compilation of Analytical Methods) was developed by AOAC INTERNATIONAL, with the help of federal funding (Food safety and Inspection Services 'FSIS of USDA', and US/FDA).
It seems interesting, as an introduction to a course on "rapid methods and automation in microbiology", to be aware of that project in order to classify not only the methods but also our ideas on the possible and adequate use of such or such methods in a given context.

The background of that project will be shortly described in a first part; in a second part, the basis of classification will be indicated, then discussed; finally, we shall try to point out the usefulness of the classification in the context of the present course.

1- Background

As everybody knows, foodborne illness is a significant public health problem all around the world: as an example, in the USA, the Center for Disease Control and Prevention estimates that 325000 hospitalizations and 5000 deaths per year are caused by foodborne pathogens (it must be mentioned that the comparison with data obtained in different countries is quite difficult, as the collection of data is performed according to different ways). In addition to the usual problem of foodborne illness, it must also be added that bioterrorism seems to have been one important factor leading to take into account rapid methods in microbiology.

In fact, regulatory agencies have a current primary goal to reduce the prevalence of foodborne hazards, throughout the farm to table continuum, and including all stages. In that context, it is of main importance to be aware of the "status" of such method leading to such result: that is the reason why it was early recognised that the development of a database trying to classify methods should be a key component for a better understanding of data obtained in different geographical areas. In the century we are living, the computerization of data appeared as the best tool to be used.

In 2001, a cooperative agreement was established between the USDA/FSIS and AOAC; and advisory group was formed, including 4 subcommittees (chemistry, microbiology, database design planning and

implementation, and statistics). These groups have further defined the categories of methods validation, criteria format and other requirements for a successful system.

The challenge was to create an international database including all types of methods which may be used for a given purpose; in that context, the method should be described as well as the system of validation to which it had been submitted.

2- The e-CAM system and the classification of methods

I think we have at first to remember the variety of methods which have been developed during the last decades for detection/enumeration of microorganisms in food and feedingstuffs, but also the number of validation systems being used in different countries all around the world. The question of an internet based system giving to the users the opportunity of knowing on line the methods as well as the degree of validation was raised many years ago (and I have in mind a question I had to answer after I presented a paper in K. State in 2000, i.e.: which may e the usefulness of internet in that field? isn't it for a very next future?

The classification which will be described has been proposed by AOAC and an Advisory group including scientist of different countries; from now, it must be mentioned that the initiative originates from USA, i.e. USDA and AOAC INTERNATIONAL which is an independent, not for profit, internationally recognised organisation, showing 120 years of experience in validating and approving methods for food and agriculture.

The purpose of e-CAM is to permit federal state, international organisations and industry conducting food safety work to have electronic access on a subscription basis, to the latest methods of analysis. The use of validated methods will provide uniformity and constancy throughout the scientific community which will allow to share analytical data nationally and internationally. Such exchange of data will enhance rapid, appropriate, and effective public health and regulatory responses to food safety threats. The methods would be validated according to international criteria and would be easily accessible and affordable to users globally.

The system consists of five separate categories of methods: regulatory methods and four other categories differentiated by the degree of validation.

Regulatory (REG) Methods

According to e-CAM, Regulatory Methods are methods which are specified by national and international regulatory agencies for enforcement purposes. These include currently recommended methods and methods specified n regulations.
The word "reference" must not be confused with "regulatory" as " reference" methods become "regulatory" only when they have been included in regulations. However, it may be logical that "reference" methods become "regulatory".

In fact, some confusion may remain as the definition of the "reference" has sometimes to be clarified and also the necessary steps to be fulfilled before proposing a standard.
Concerning the definition, it seems interesting to remind that "reference" does not mean "static". In that context, it seems interesting to remind the content of resolution n°74 taken during the meeting of CEN/TC275/WG6 held in Parma (It), 23rd April 2004-3 WG6 members agreed the following points:

- Each time a standard reference method is established or revised, the possible use of new technologies including PCR will be reviewed alongside the existing culture method to determine the most suitable method for the reference method.
- In addition to the existing reference standard for a given microorganism, the need to develop a standard method with a different objective (such as pathogenicity…)
- When new technologies including PCR are used as an alternative method to the standard reference method, they shall be validated against the reference method.

Concerning the different steps and requirements necessary to set up a standard, a group of ISO/TC34/SC9 is working on that topic.

- The fact that reference methods are not identical all around the world; for example, USDA and USFDA take into account AOAC methods or their own methods and not ISO ones; however, some progress has been set during the last years for *Salmonella* detection: during an European project, a collaborative study was performed by European and American laboratories as well in order to compare the results obtained for three matrices (mince poultry meat, cheese curd and egg powder) artificially or naturally contaminated by *Salmonella*.

There was finally an agreement for a mutual recognition of ISO and AOAC methods and the results were recently published in the Journal of AOAC.

- The fact that the approaches of validations systems have also been very different during a very long period and the harmonisation is not yet perfect; however, it must be mentioned that a lot of progress has been made: the international EN/ISO protocol for validation of alternative methods, which was published in May 2003 is very close to the AOAC protocol previously published for the same purpose. As was published a few years ago "HANDS TOUCH ACROSS THE SEA". We shall see that e-CAM refers to both systems.

Harmonised collaborative Validation (HCV) Methods

"Methods validated through a full collaborative study that meet the standards set forth in the ISO 16140 or AOAC Methods Committee Guidelines for Validation of Qualitative and Quantitative Microbiological Methods for full collaborative study. The collaborative study must report valid data from at least eight laboratories for quantitative methods and ten laboratories for qualitative methods. A methods comparison study conducted in a single laboratory on other foods has to be included"

The proposal is that validation and approval will be administered by AOAC INTERNATIONAL and other reciprocally recognised validation bodies in accordance with the category criteria in e-CAM. Methods submitted in this category must follow program requirements of the *Official methods* program.

Multiple-Laboratory Validated (MLV) Methods

"MLV methods are methods that do not meet the requirements of the HCV category but have undergone validation studies following a third party approved protocol. The study manuscript must include valid data from two or more laboratories analysing at a minimum the same five different test materials as blind replicates under the same test conditions.
Submission must comply with ISO 16140 or "AOAC Microbiology Guidelines for Validation of Qualitative and Quantitative Microbiological Methods except that the collaborative study requirement is removed. The alternative test method results are compared with those from a reference method using common samples of a minimum of 2 foods with three contamination levels. For quantitative methods, 5 test portions are run for each of three levels of inoculation or three lots of contaminated food. A methods comparison study conducted in a single laboratory must be included.
Validation and approval will enter a reciprocal relationship with third party validation bodies that follow specified criteria for methods validation and who agree to administer validation in accordance with e. Cam criteria. Specific arrangements with each organization will be made through a Memorandum of Understanding or an equivalent mechanism.

Single –laboratory Validation (SLV) Methods

Those are "Methods validated through single laboratories studies meeting the standards set forth in the EN/ISO 1640 or AOAC Methods Committee Guidelines for validation of Quantitative and Qualitative Microbial Methods, Microbiology Methods must include a methods comparison study conducted in a single laboratory. Submission must comply with EN/ISO 16140 or "AOAC Microbiological Methods Committee guidelines for Validation of Qualitative and Quantitative Microbiological Methods" except that the collaborative study requirement is removed, the methods comparison study is conducted in a single laboratory. The conditions of submission are identical to those described in the previous category.

Developmental Non Validated (DNV) Methods

"DNV methods may be of interest to the analytical community, but have not been validated to the SIV criteria. They may include evolving methods which may not yet be optimised or fully characterized with respect to performance characteristics. A factual disclaimer will be incorporated stating that this method has not been peer-reviewed by technical experts, that it has not been validated through a third party approval scheme and therefore it is provided on an informational basis only"

The conditions of submission and validation are identical to those required for the previous category. Methods submitted into HCV, MLV, SLV and DNV must include the following, as applicable :

- Submission date;
- Title;
- Appropriate identification (AOAC will assign reference number);

- LOING assign unique identifier (if applicable);
- Regulatory reference (if applicable);
- Abstract;
- Safety warnings;
- Scope which includes method applicability; i.e. food matrices, analyse, range and interference;
- Definitions;
- Method principle;
- Reagents/Materials/Media;
- Apparatus and Equipment (with any technical requirements);
- Preparation of analytical samples;
- Test Procedure;
- Test Result Acceptance Criteria;
- Calculation;
- Precision (if applicable); recovery; HORRAT.
- Quality control requirements.

3- General considerations and prospective

"When methods are reclassified to another category, they will be removed from the existing category in which they are currently listed, as for example, when a Single Laboratory (SLV) method is later validated as a Multiple-Laboratory Validated (MLV method or a Harmonised Collaboratively Validated (HCV) Method". The possibility of an "on line validation" has been considered.

However, the question is raised to know who will "filter" the acceptability of a method…
That is an important topic as it seems absolutely necessary to have a consensus; the possibility of using an "on line" system of validation seems very attractive as a principle but maybe difficult to apply…
So, different questions need to be solved.

Presently, e-CAM has been proposed but is not yet used, especially for financial reasons. Anyway, should we use the system as such, or keep the idea and try to find an international consensus from the basis which is available actually ????. What has been proposed must surely be kept in mind…

And, far from financial problems, a good exercise could be to classify the methods which will be described and used during this week according to the e-CAM system…Why not?