Rapid microbiological methods for food safety testing

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With recent food poisoning outbreaks such as the E. coli outbreak in Germany, public awareness of food safety issues is increasing. In addition, standardisation of regulations for food safety and the evolution of rapid microbial tests are important drivers for the market for food safety testing.

In Europe, regulations for food safety testing are more stringent than in other parts of the world. Therefore, the European market share for microbial food testing is globally the major one. EU countries performed an estimated 275 million food microbial tests in 2011. In comparison, 213 million of such tests were conducted in the US in 2010.

More and more, alternative rapid microbial tests replace the reference methods in food safety testing.

Rapid tests are important to food producers since the analysis time for food and environmental samples is much shorter when using rapid test than using traditional reference methods. And time is money.

It can take at least three to five days to obtain a result using traditional methods of detection for some pathogens. Many of these alternative tests are currently available commercially and have been successfully validated by validation and certification organisations.

MicroVal is a not-for-profit European platform organisation for the validation and approval of alternative methods for the microbiological analysis of food and beverages. MicroVal validates and certifies alternative methods in order to show that such, primarily proprietary, methods perform equally well as internationally standardised reference methods.

MicroVal certification of alternative methods will facilitate the use by Food Business Operators in the framework of EU regulation No. 2073/2005 and ensure their acceptance by the competent authorities throughout Europe and thus improving acceptability of results and facilitating commerce within the European Union.

MicroVal issued eight new certificates in 2011. In total 24 certificates were granted by MicroVal to kit manufacturers. Four validation studies were carried out as a joint validation study with AOAC with both MicroVal and AOAC certificates as a result.

One of these studies was also performed in cooperation with NordVal. Nine validation studies are currently performed by the MicroVal independent Expert Laboratories.

The certified methods comprise of chromogenic culture media, PCR tests and immunological tests for the major pathogens, yeast and moulds or for the total viable count.

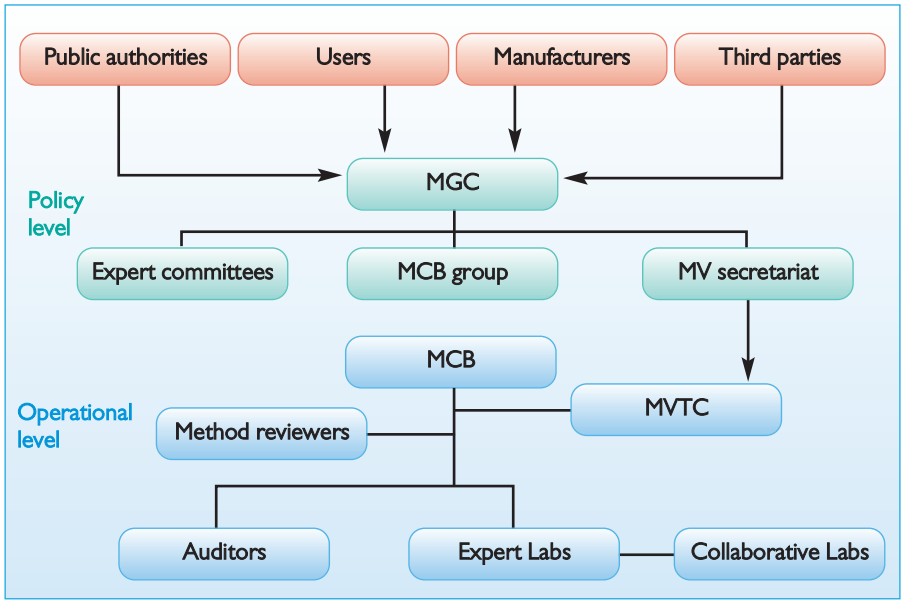

Fig. 1. Organisational scheme of MicroVal.

The history of MicroVal

MicroVal started as a Eureka project in the nineties. In its final stages the project consisted of 21 full partners from seven European countries (Denmark, Germany, UK, Netherlands, France, Spain and Portugal) and delivered:

- Technical rules for validation – at a later stage brought into European standardisation channels and finally published in 2003 as: EN-ISO 16140 'Microbiology of food and animal feeding stuffs – Protocol for the validation of the alternative methods'.
- The MicroVal Rules and Certification Scheme for the quality control of validation.

In 2006 the organisation became operational. The first certificate was handed out in 2007 and in 2008 another four certificates were approved.

MicroVal organisation

MicroVal is a European validation and certification organisation for the validation of alternative microbiological methods.

The aim is to provide a single validation system within Europe. Stakeholders in the validation process are represented in the organisation.

The MicroVal organisation is based on an impartial European structure (Fig. 1) which consists of:

- An impartial European Board of Experts, the MicroVal General Committee (MGC), in which the interests of stakeholders, consisting of public authorities, manufacturers, users and third parties, are evenly covered. The most important focus of the Board is to develop and maintain the MicroVal validation and certification system.
- Expert Committees for the selection of Expert Laboratories, Method Reviewers,

interpretation of ISO 16140, and complaints.
- The MicroVal Certification Body (MCB) Group, which is open to participation by all European Certification Bodies.
- A neutral MicroVal secretariat, for which NEN (Dutch standardisation body) is responsible.
- The European network consisting of Expert Laboratories, Collaborative Laboratories, reviewers and auditors.
- The MicroVal Technical Committee (MVTC), in which all stakeholders are represented, for the evaluation of validation studies.

MicroVal validation

The EU Legislation: Commission Regulation (EC) 2073/2005 on microbiological criteria for foodstuffs of 15th November 2005, published in the Official Journal of the European Union on 22nd December 2005, states in Article 5 'Specific rules for testing and sampling':
- The use of alternative analytical methods is acceptable when the methods are validated against the reference method in Annex I and if a proprietary method, certified by a third party in accordance with the protocol set out in EN-ISO standard 16140 or other internationally accepted similar protocols, is used.

MicroVal Certification conforms to these requirements as follows:
- Validation is performed against a CEN method or ISO method, or, if a CEN or ISO method is not available, against a European official method.
- Validation is granted by third party MicroVal certification bodies.
- Validation is in accordance with the protocol set out in EN-ISO standard 16140 'Microbiology of food and animal feeding stuffs – Protocol for the validation of the alternative methods', which also forms the basis of MicroVal.

Therefore, alternative methods certified with the MicroVal organisation fulfill the requirements as stated in the European Commission regulation.

Certification principles

The MicroVal certification procedure is based on three principles:
- To perform a method comparison study and collaborative (inter-laboratory) study according to EN/ISO 16140.
- The manufacturer where the materials are produced shall have a quality management and control system in place in accordance with ISO 9001/ISO 13485.
- A regular verification of the quality of the certified methods, which is made after the certification, is granted. The validity of the certificate is four years. Thereafter a renewal has to be carried out.

Validation for qualitative and quantitative methods is as follows:

- **Qualitative alternative methods**

The method comparison study is performed by the MicroVal Expert Laboratory. If the alternative method is to be validated for all foods, five categories of food must be involved. For each food category, 60 test samples should be analysed. Inclusivity and exclusivity studies, with target and non-target organisms, and determination of the minimum level of detection are also necessary.

The samples used should be naturally contaminated but, if that is not possible, a limited number of artificially contaminated samples may be used with cells in a similar state of stress and a similar type of background microflora. The availability of naturally contaminated samples depends strongly on the food categories selected as relevant for the target organism and for the scope of the method.

The inter-laboratory performance study is organised by the Expert Laboratory. At least 24 samples, with a minimum of three contamination levels and at least eight samples per level, are analysed by 10 Collaborative Laboratories from at least three different countries. Samples are analysed by the alternative and the reference method, with results returned to the Expert Laboratory for analysis. On completion, the Expert Laboratory submits a final report to the method reviewers and the MicroVal Technical Committee for assessment.

- **Quantitative alternative methods**

Again, comparison and inter-laboratory studies are organised by the Expert Laboratory. The comparison study determines linearity, relative accuracy, detection and quantification limit. The additional validation procedure is the same as for qualitative methods. If methods have already been validated and/or certified by another organisation, specific rules apply in order to consider such results.

Expert Laboratories

The independent Expert Laboratory must be accredited to EN-ISO 17025 for the reference method involved in the validation study. It is also important that the Expert Laboratory has experience in the development of new techniques, is involved in organising inter-laboratory studies, and has statistical knowledge.

It is selected by the manufacturer (or the MCB) from the database of MicroVal Expert Laboratories established by the MGC. The qualification 'Expert Laboratory' is only valid for the MicroVal certification of the alternative method it was selected for.

Collaborative Laboratories

Collaborative Laboratories participating in the inter-laboratory study are selected by the MicroVal Expert Laboratory on the basis of their capability and geographic distribution (at least three different European countries). The Expert Laboratory coordinates the inter-laboratory study in which the Collaborative Laboratories are involved.

Harmonisation with others

MicroVal works together with AOAC RI and NordVal in joint validation studies. One validation study protocol meeting each organisation's requirements is advantageous for the manufacturer, since it is less expensive and more efficient than two individual validation studies.

MicroVal and AOAC RI decided to take a pragmatic approach to accommodate the manufacturers by combining the requirements for a MicroVal certificate (basis EN ISO 16140) and an AOAC RI (Performance Tested Methods) review.

It is important for each organisation to maintain and fulfill its own requirements with respect to the reviews, policies and procedures and fees, but also to design a validation study protocol that meets both AOAC and MicroVal requirements.

Combined validation studies will benefit from further harmonising validation standards and procedures.

Conclusion

Validation systems are developed to provide fully validated methods that can be used with confidence by public authorities, food industry, laboratories, and end users.

A certified method provides a level of confidence to all parties because of its reliability.

MicroVal offers the opportunity for test kit manufacturers to undertake a third party evaluation programme according to EU legislation to gain acceptance for the product across Europe.

A MicroVal certificate will show, to the method user, that the alternative method has been thoroughly tested using an approved and standardised procedure.

It means that a method can be used confidently and with the knowledge that the results of that method will be accepted without question throughout Europe.

MicroVal celebrates five years of validations with a symposium in 2012 in Rotterdam, the Netherlands. The symposium is of interest to all parties involved with food safety testing.

At the international level MicroVal is actively working with AOAC harmonising validation requirements and allow results of certifications to pass between these international markets. With NordVal and AOAC validations are being performed as joint studies. This benefits the manufacturers at least with respect to the validation costs.

Furthermore, MicroVal validations are being performed by Expert Laboratories in Europe.

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