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# Microbial contaminants in food: a big issue for a working group of the MoniQA NoE project

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## Abstract

*Introduction* The MoniQA Network of Excellence is an EC funded project working towards the harmonization of analytical methods for monitoring food quality and safety along the food supply chain. This paper summarises both the structure and tasks of the working group on microbial contaminants within the MoniQA NoE and specifically focuses on harmonisation strategies important in the microbiological analysis of food. *Objectives* There is a need for rapid microbiological methods in order to quickly and efficiently identify harmful pathogens in food sources. However, one of the major problems encountered with many new methods is their market acceptance, as they have to pass extensive validation/standardisation studies before they can be declared as official standard methods. *Methods* The working group on microbiological contaminants aims to contribute towards speeding up these prerequisites by collecting information on food law, quality assurance, quality control, sampling, economic impact, measurement uncertainty, validation protocols, official standard methods and alternative methods. *Results* The present report provides an overview of currently existing methodologies and regulations and addresses issues concerning harmonisation needs. One of the deliverables of the working group is the development of extended fact sheets and reviews based on relevant 'hot' topics and methods. The selection of food borne analytes for these fact sheets have been selected based on global, local and individual parameters. The working group has identified 5 groups of stakeholders (governmental bodies, standardisation/validation organisations, test kit/equipment manufacturers, food industry and consumers). *Conclusion* Current challenges of food microbiology are driven by new analytical methods, changes in the food market and altered consumer desires. The MoniQA NoE is contributing in overcoming these risks and challenges by providing a profound platform on microbiological rapid methods in food analysis to all stakeholders and it is expected that strong interaction within the network and beyond will foster harmonization.

## Activities and working plan

The MoniQA Network of Excellence (NoE) is structured into 9 'Work Packages', which follow a diversity of interrelated aims. In addition, but independent of the work packages, specialized working groups have been established to deal with the following areas in a more focused way: microbiological contaminants; phycotoxins and mycotoxins, chemical contaminants, food allergens, food authenticity, food additives, qualitative method validation, and socioeconomics.

The field of microbiological contaminants is multifactorial and encompasses a wide variety of microorganisms together with their role in the food supply chain. Extensive surveys of the literature and databases of surveillance

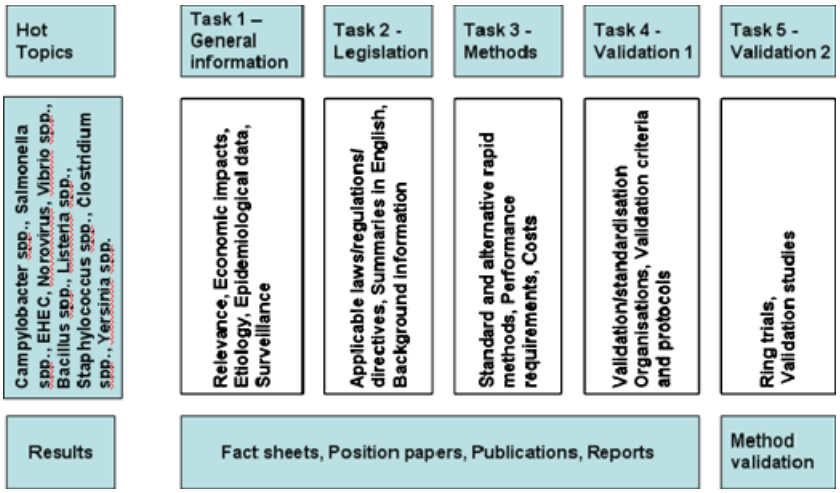


**Figure 1** Strategic concept of the MoniQA working group on microbiological contaminants.

programmes including RASFF, FoodNet or the EFSA Report on Zoonoses, have led to the risk ranking of the most important microorganisms responsible for foodborne diseases in the Asian–Pacific region (Australia, China and New Zealand), the European Union and the United States. For each of the three regions a short-list displaying the most relevant disease-causing microorganisms has been extracted, taking into account that not all microbiological hazards are of the same importance for each region or country due to a number of reasons including different nutritional practices, technological aspects and local hygiene standards. Based on this information, the strategic focus of the microbiological contaminants working group is outlined in Figure 1.

**TASK 1**: Searches in national and international surveillance reports will aim to address questions such as 'what is under surveillance?', 'which region is controlled?', 'who are the responsible organisations?' and 'how is the information collected?'. Based on this information a pool of reliable resources, which will help the working group to select possible future topics, will be created.

**TASK 2**: Laws/Regulations/Directives will be collected and summarized. This will include a short description of the legislation; how food law, both generally and specifically, is integrated into it; the area of application; who is in charge of controlling law abidance and how penalties, in the case of contraventions, are implemented.

**TASK 3**: Collecting information on traditional standard methods as well as modern/rapid methods will provide answers to the following questions: (a) Which methods are available and which are approved by supervisory/governmental bodies? (b) Performance descriptions; (c) Requirements; (d) Precision indicators; (e) Costs.

**TASK 4**: In the next phase, guidelines for the validation of (rapid) methods will consider the specific prerequisites for validated methods according to different regions and corresponding guidelines.

**TASK 5**: Depending on the available budget, practical studies (e.g., ring-trials, validation studies, proof of applicability of methods in different types of food, etc.) may be performed.

A survey on microbiological risks, based on epidemiological data (see Fact finding based on epidemiology) and the concept of the working group, has already been presented to the project partners in February 2008. Discussion on related topics at work group meetings has led to information on partner-specific microbiological expertise. As a result, three review papers on *Salmonella*, *Campylobacter* and *Listeria* have been recently completed and submitted to the Board of the working group. These three review articles will be published in the new MoniQA-supported journal 'Quality Assurance and Safety of Crops and Foods'. This journal also provides a potential opportunity to disseminate other current issues related to the 'top ten' list of microbiological contaminants along with future activities and outputs from the working group.

All information acquired within the microbiological contaminants working group will be collected in a central online database, established and administered by CSL (Central Science Laboratories, York, UK). The database will be connected to the password-protected area of the MoniQA homepage in order to enable the user to access both areas with one password. In order to promote the WP 6 database as a product for sustainability access could potentially be given to associated partners and registered external

**Table 1** Epidemiological data on foodborne illness (compiled from different sources; a–g) (details about microorganisms according to capital letters; A–M).

| | *Campylobacter* spp. | *Salmonella* spp. | EHEC | Norovirus | *Vibrio* spp. | *Bacillus* spp. | *Listeria* spp. | *Staphylococcus* spp. | *Clostridium* spp. | *Yersinia* spp. |
|---|---|---|---|---|---|---|---|---|---|---|
| USA | | | | | | | | | | |
| Estimated annual foodborne diseases[a] | 1 963 141 | 1 342 532[A] | 93 714 | 9 200 000 | 5 218[B] | | 2 493[C] | | 248 520[D] | 86 731[E] |
| Estimated annual deaths due to foodborne diseases[a] | 99 | 553[A] | 78 | 124 | 31[B] | | 499[C] | | 7[D] | 2[E] |
| EU | | | | | | | | | | |
| Reported Human Infections in the EU in 2006[b] | 175 561 | 165 023 | 4 916 | | | | 1 583 | | | 8 979 |
| Cases/100.000 in the EU; 2006[b] | 46.1 | 34.6 | 1.1 | | | | 0.3 | | | 2.1 |
| Total foodborne Qutbreaks in the EU; 2006[b] | 400 | 3 131 | | | | 78 | 9 | 236 | 81 | 26 |
| % of Total foodborne outbreaks in the EU; 2006[b] | 6.9 | 53.9 | | | | 1.3 | 0.2 | 4.1 | 1.4 | 0.4 |
| Estimated foodborne diseases in GB, Wales from 1996 to 2000[c] | 337 655 | 73 370[F] | 1 140 | 61 584 | 485[H] | 10 717 | 221[C] | 9 196[G] | 168 436[D] | 129 338 |
| Estimated deaths in GB, Wales due to foodborne diseases from 1996 to 2000[c] | 80 | 209[F] | 26 | 10 | 2[H] | 0 | 78[C] | 0[G] | 177[D] | 3 |
| Australia | | | | | | | | | | |
| Notified infections by OzFoodNet in 2005[d] | 16 479 | 8 376 | 78 | | | | 56 | | | |
| Cases/100.000 in 2005[d] | 121.6 | 41.2 | 0.4 | | | | 0.3 | | | |
| Foodborne disease Outbreaks in 2005[d] | 9 | 26[L] | | 4 | | | 1[C] | 2[G] | 4[D] | |
| China | | | | | | | | | | |
| Reported foodborne disease outbreaks from 1994 to 2005[e] | | 181 | | | 211[J] | 145[I] | | 84[G] | | |
| Individual cases affected by the outbreaks from 1994 to 2005[e] | | 12 769 | | | 10 790[J] | 5 744[I] | | 3 055[G] | | |
| New Zealand | | | | | | | | | | |
| Notified infections in 2006[f] | 15 873 | 1 335 | 87 | 3 945 | | | | | | 487 |
| Rate/100.000 in 2006[f] | 383.5 | 32.3 | 2.1 | | | | | | | 11.8 |
| Foodborne Transmission[a] | 80 | 95[M] | 85 | 40 | 90[K] | 100[I] | 99[C] | | 100[D] | 90[E] |

a, Mead et al. (1999); b, Anonymous 1 (2006); c, Adak et al. (2005); d, Anonymous 2 (2005); e, Wang et al. (2007); f, Anonymous 3 (2006).

A, ***Salmonella*** nontyphoidal and Typhi; B, ***Vibrio cholerae***, ***Vibrio vulnificus*** and other ***Vibrio*** species; C, ***Listeria monocytogenes***; D, ***Clostridium perfringens***; E, ***Yersinia enterocolitica***; F, ***Salmonella*** nontyphoidal, Typhi and Paratyphi; G, ***Staphylococcus aureus***; H, ***Vibrio cholerae*** and other ***Vibrio*** species; I, ***Bacillus cereus***; J, ***Vibrio parahaemolyticus***; K, ***Vibrio cholerae***; L, ***Salmonella typhimurium***; M, ***Salmonella nontyphoidal***.

stakeholders with the provision that a disclaimer is in place and that free access may be later closed or changed to access against a fee. In order to guarantee the high quality of this database a usability questionnaire will be distributed among database users (including stakeholders).

## Fact finding based on epidemiology

A summary of data relating to foodborne pathogens and confirmed human cases is presented in Table 1. Unfortunately the reporting, as well as the surveillance strategies, are very different worldwide and therefore need to be harmonized. For example, data available from the United States only represent 10 member states and data obtained from Australia does not cover the entire country. The working group on microbiological contaminants has already established a database and compiled information relating to global outbreak and incidence of foodborne pathogens and corresponding disease. However, there is a need to keep this information updated if we are to successfully evaluate the potential need for validation of methodological protocols.

Based on the information available and the expertise within the group, three microorganisms have been selected for further investigation where attention will be focused on the evaluation of traditional methods versus rapid detection methods. Exemplarily, preliminary work has been initiated based on high impact foodborne pathogens such as *Salmonella*, *Campylobacter* and *Listeria*. Comprehensive information about the nature of the organism and the detection methods available for these microorganisms will be subject of forthcoming work among the working group and will form the basis for further publications. In addition, this task will help to identify the needs and the gaps of different techniques and will constitute the platform for further validation.

## Towards a harmonization guideline

### Gaps and needs in microbiological methodology

In general, microbiological methods have to meet two different requirements. First they should be able to detect a certain microorganism (or group of microorganisms) depending on their state of viability or dormancy. Second, some microbiological methods should also be able to enumerate a defined microorganism (or group of microorganisms) in different kinds of matrices as precisely as possible. Ideally, for examining food samples, the chosen methodology should facilitate the detection of low numbers of target microorganisms or contaminants (e.g. microbial toxin).

### Features and disadvantages of rapid methods

Among other reasons, new duties for producers resulting from new developments in food legislation (e.g. Regulations EC No. 178/2002, No. 852/2004) have stimulated the demand for new and improved analysing strategies.

Unfortunately, there is still no official definition of the term 'Rapid Method'. When compared with more labour–intensive traditional methods, rapid methods, using new techniques, should aim to reduce the workload, leading to a result in a shorter time period. However, there can also be derivatives from techniques using the same principles as the corresponding reference method, but in an automated, partially automated or miniaturized way.

In this context, quality parameters of analytical methods need to be met. According to Fung (1995), *accuracy* describes the minimum extent of false positive or false negative results where the limit of detection should be as low as possible. Regarding the *costs*, it is generally expected that despite higher costs in the initial phases (introduction of a rapid method), the costs should decline over time. Of course, any new and rapid method has to undergo some *validation* approved by the scientific community. It is essential that the validation step includes a robust examination of the assay's sensitivity and specificity taking into consideration current knowledge of the taxonomy and diversity of the target organism. Failure to consider this aspect can lead to false positive and negative results on a large scale.

Rapid tests also need to be *user friendly*, all reagents and supplementary material must be *easily available* and the preparation of reagents should be fast and easy.

One of the major limitations of rapid methods can be found in the problem of the diversity of food sample matrices, which often affect the quality of the result. In addition to water, carbohydrates, fats, oils and proteins, food may contain other substances which can inhibit the growth of bacteria (Feng, 1996). Moreover, some of the so-called rapid microbiological methods still include labour-intensive and time consuming pre-enrichment techniques. This can be regarded as one of the most important burdens and is a particular disadvantage in pathogen detection, where due to the very small concentration of analyte in a sample, pre-enrichment steps are inevitable (Scanlan, 1995).

However, rapid methods without incubation or pre-enrichment may also be disadvantageous. For example, bioluminescence-based methods suffer from their very limited application mainly when considering the determination of total viable count and automated flow cytometry, unfortunately, only allows some insufficient differentiation between living and dead cells (van der Zee & in't Veld, 1997).

On the other hand, direct detection of bacteria in food by the use of PCR methods is very prone to false-positive results due to contamination. Moreover, these methods are related to very complex sample preparations (Rijpens & Herman, 2002).

The largest group of rapid methods is constituted by antibody-based assays. Major benefits of these assays are their ease of handling and their specificity based on the antibody-antigen interaction. These benefits lead to a great variety of formats and assays. For example the latex agglutination assays (LA) are quick and easy to perform, but only applicable for the identification of pure cultures due to their lack of sensitivity (Feng, 2007)

Immunomagnetic separation is a useful tool to reduce the previously described negative effects of the food matrix as the analyte can be specifically selected or concentrated before examination. Major benefits of this technique, in comparison with traditional enrichment procedures include a higher specificity, less cell damage and less time consuming. In addition immonomagnetic separation can be used in combination with most types of assays. But one should consider that this technique only reduces the number of non-target bacteria and does not produce a pure culture. Furthermore, the applicability and effectiveness of this method depends on the food (Feng, 2007).

Another interesting application is the matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) which is a fast and relatively easy method to use. It enables analysts to identify mixed cultures and it is applicable for rapid detection of biomarkers when only very little genetic data are available (Mandrell *et al*., 2005). Several studies indicate the applicability of MALDI-TOF MS for the determination of foodborne microorganisms. The aforementioned authors for example have shown that species (and also some subspecies) of *Campylobacter* isolated from different food and animal samples display high discriminative power using MALDI-TOF MS compared with other methods. Different experimental conditions like culture medium, the growth time, the bacterial concentration, the sample preparation and MALDI matrices can affect reproducibility and accuracy of the result and thus a standardized analytical protocol is inevitable (Mazzeo *et al*., 2006).

One disadvantage which all rapid methods have in common is their 'black box effect'. Modern methods often display only the final result and all the information on how the result was achieved is not visible or poorly accessible (e.g., only by using special software).

Nevertheless, rapid methods need to become widely accepted on the market, where they stand in direct competition with well-established and proven traditional methods. Therefore, validation certificates awarded by an accredited organization such as AOAC, AFNOR or ISO are important for successfully introducing a new method to the market.

## Quality criteria of rapid methods

The performance of a method can be assessed based on various indicators. According to the AOAC, indicators for qualitative methods are sensitivity, specificity, false negative and false positive rates. Performance of quantitative methods can be examined by criteria such as repeatability, reproducibility, reproducibility value and the relative standard deviation (Feldsine *et al*., 2002).

Comparing the validation protocols and validation criteria originating from different standardization organizations (for example, ISO, IDF, AOAC) will provide the consortium with an overview of the most important indicators for method performance. Moreover, it will also highlight any possible differences between techniques.

Depending on the region and the food products, the quality assurance and control criteria as well as the corresponding legislations may differ considerably. However, the recently emerged food crisis of adulteration of milk products in China has shown that there are still considerable gaps in global surveillance and control systems.

In terms of a globalized food market, it may be of potential interest to build on an international consortium and to establish a catalogue with the most important regulations concerning food quality assurance and control. Regulations, however, need to be executed based on suitable analytical methodologies, ranging from sampling to detection and verification. Today, various organisations, such as ISO, IDF and ICMSF among others, provide clear sampling guidelines. For example, the European Commission also considers sampling within Regulation (EC) No 882/2004.

## Economic impact

Foodborne diseases impact substantially on national and international political economies. This has been demonstrated in several reports. In 2000, the total costs in New Zealand resulting from foodborne diseases amounted to $NZ 55.1 million (direct medical costs: $NZ 2.1 million, direct non-medical costs: $NZ 0.2 million, indirect cost of lost productivity: $NZ 48.1 million, and intangible cost of loss of life: $NZ 4.7 million) (Scott *et al*., 2000). In the United States, seven foodborne pathogens (six bacteria and one parasite) resulted in annual economic costs of USD 5.6–9.4 billion (Buzby *et al*., 1996).

## The role of stakeholders

Given five groups of stakeholders relevant to this field (Governmental bodies, Standardization/validation organizations, Test kit manufacturers, Food industry, Consumers) it is planned to establish a short, but tailor-made questionnaire for each of the five groups. The purpose of this questionnaire will be to define possible individual needs in order to facilitate efficient work, which is in accordance with the stakeholders' interest. Furthermore stakeholders will be involved in evaluating final results. For example a validation protocol developed within the microbiological contaminants working group could be distributed among stakeholders to assure the applicability for each of the different groups.

## Conclusion

Global control of foodborne hazards undoubtedly requires suitable methodologies of high specificity and precision. Increasing consumer awareness and demand for quality and safe products will increase pressure on industry and regulators to deliver the desired results. This task becomes complicated by deviating results (when methods are applied in different geographical regions and in the various stages of food production), different specifications and subsequently by different conclusions. These challenges drive the need for standardization and harmonization of new technologies to provide solid and robust data for a global food market.

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