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Review

Alternative microbial methods: An overview and selection criteria

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ABSTRACT

This study provides an overview and criteria for the selection of a method, other than the reference method, for microbial analysis of foods. In a first part an overview of the general characteristics of rapid methods available, both for enumeration and detection, is given with reference to relevant bibliography. Perspectives on future development and the potential of the rapid method for routine application in food diagnostics are discussed. As various alternative "rapid" methods in different formats are available on the market, it can be very difficult for a food business operator or for a control authority to select the most appropriate method which fits its purpose. Validation of a method by a third party, according to international accepted protocol based upon ISO 16140, may increase the confidence in the performance of a method. A list of at the moment validated methods for enumeration of both utility indicators (aerobic plate count) and hygiene indicators (Enterobacteriaceae, *Escherichia coli*, coagulase positive *Staphylococcus*) as well as for detection of the four major pathogens (*Salmonella* spp., *Listeria monocytogenes*, *E. coli* O157 and *Campylobacter* spp.) is included with reference to relevant websites to check for updates. In a second part of this study, selection criteria are introduced to underpin the choice of the appropriate method(s) for a defined application. The selection criteria link the definition of the context in which the user of the method functions – and thus the prospective use of the microbial test results – with the technical information on the method and its operational requirements and sustainability. The selection criteria can help the end user of the method to obtain a systematic insight into all relevant factors to be taken into account for selection of a method for microbial analysis.

1. Introduction

Microbial analysis of foods is an integrated part of management of microbial safety in the food chain. Both control authorities and individual food business operators use microbial analysis for the purpose of monitoring of the actual situation and trend analysis in order to detect emerging risks. Also for compliance testing to defined microbiological criteria or assessment of the performance of management strategies based upon Hazard Analysis Critical Control Points (HACCP), microbial analysis is a valuable tool. Standardized methods (e.g. ISO methods) are acknowledged as the reference analytical methods for official control. These standardized methods are most of the time classical culture methods, using selective nutritious broth or agar media to grow, isolate or enumerate the target organism while suppressing the indigenous background flora of the food it resides in. They have been developed historically based upon expertise and decided upon by international consensus. Also important is that they are open access methods in a way that the composition of culture medium is freely available and/or techniques used are described in sufficient detail to reproduce and materials are to be acquired from multiple suppliers. These standardized classical culture methods are still in use by many labs, especially by regulatory agencies, because they are harmonized methods, looked at as the "gold standards" in food diagnostics and thus overall well accepted. These are important aspects in international trade and compliance testing. A serious drawback is that, although they demand no expensive infrastructure and are rather cheap in consumables, they are laborious to perform, demand large volumes usage of liquid and solid media and reagents, and encompass time-consuming procedures both in operation and data collection. During the last decades, interest has risen in the development of more rapid methods. Based on these "golden standards" a "rapid method" can be defined as any method or system that reduces the time taken to obtain a microbiological test result (Feng, 1996; Fung, 1994). Rapid may be interpreted as a shorter time to detection, but may also refer to a better flow

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through/handling of multiple samples and thus refer to convenience and automation in work in the lab. As such rapid methods may be better replaced by the terminology "alternative methods". Alternative microbiological methods may help the industry to find new ways of obtaining reliable results more efficiently to ensure food safety. At this moment it is not yet possible to take quick corrective actions and, therefore, impossible to immediately identify and control the causes of non-conformities, as still these microbial analyses take too long to give a result (Rosmini et al., 2004).

Numerous and diverse alternative methods for microbiological analysis of foods are currently brought to the market by various suppliers in a variety of formats as a result of recent developments, particularly in the field of biotechnology, microelectronics and related software development. Overall, the general rule is that regulatory bodies accept the use of alternative methods as long as they have been validated by a third party towards the standardized reference methods. The first part of this study aims to give an overview of the general characteristics of alternative methods available both for enumeration of the most common utility and hygiene parameters (aerobic plate count, Enterobacteriaceae, *Escherichia coli* and coagulase positive *Staphylococcus*) as well as for detection of the four major food safety parameters namely *Salmonella* spp., *Listeria monocytogenes*, *E. coli* O157 and *Campylobacter* spp. In a second part of the study, validation of alternative microbiological methods is highlighted while in a third part, selection criteria underpinning the choice of the appropriate method(s) for a defined application based upon a techno-managerial point of view are described.

2. Definition of methods

Methods for microbial analysis comprise methods for enumeration (quantitative methods) and methods for detection (qualitative methods). In many occasions, the classical (conventional) culture methods (based upon multiplication of target organisms in broth or agar media to numbers visual by the naked eye as turbidity, colour change or colonies) are referred to as the reference methods for microbial analysis. However, also other types of methods based upon other principles of detection (e.g. molecular based methods) may be recognized or acknowledged as reference methods if they are perceived as providing a more accurate result, e.g. such as current ISO methods for detection of non-O157 EHEC (enterohemorrhagic *E. coli*) and Noroviruses under draft. In microbiology, reference methods are commonly open access methods (not proprietary methods) and have been subjected to some kind of collaborative study or peer review by independent labs to gain a wide acceptance of proven performance.

For official analysis, most often reference methods are recommended, however, alternative methods, usually proprietary methods can be accepted as well if they have been shown to provide equivalent results by means of a validation study. In particular cases, e.g. for emerging microbial hazards in foods such as toxins of *Bacillus cereus* for which international agreement yet has to be obtained, in-house validated methods may be used for official analysis.

In the frame of assurance of food safety, often proprietary methods are the methods of choice, but still also classical reference methods are used (to some extent automated or with minor modifications) and occasionally in-house methods. Definitions of these various types of methods are depicted in Table 1.

3. Enumeration methods in food diagnostics

3.1. Classical cultural methods

Conventional methods for the enumeration of bacteria in food are colony count methods. In the colony count method the total number of bacteria in a product is determined by inoculating dilutions of suspensions of the sample onto the surface of a solid growth medium by the spread-plate method or by mixing the test portion with the liquefied agar medium in Petri dishes (pour plate method). Enumeration is performed after incubation for fixed periods at temperatures varying from 7 to 55 °C in an aerobic,

Table 1
Definition of type of methods available for microbial analysis of foods.

Type of method	Definitions according to working document of revised ISO 16140 (2009) method validation – part 1: terminology
Methods suitable for official controls (*as defined by EC 882/2004*)	Method acknowledged for use in "official control"; official control meaning any form of control that the competent authority performs for the verification of compliance with legislation. *Official control methods shall comply with internationally recognized methods such as CEN methods or methods agreed upon in national bodies. Or in absence of these (inter)national methods other methods shall be used fit for intended purpose and developed according to scientific protocols.*
Reference method	An Internationally recognized method and widely accepted. *A reference method has been thoroughly investigated and clearly and exactly describes the necessary conditions and procedures, for the measurement of one or more property values. Accuracy and precision should be commensurate with its intended use and can therefore be used to assess the accuracy of other methods for the same measurement. As a rule, the reference method is a standard or a commonly used method taken as reference.*
Alternative method	Method of analysis for detection or estimation, of the same analyte as is measured by using the corresponding reference method, for a given category of products. *The method can be proprietary or non-commercial and covers an entire analytical procedure, that is, from the preparation of samples to the test results either as such or may include references to other procedures in order to be complete.* *The alternative method exhibits attributes appropriate to the user' needs, for example:* – *speed of analysis and/or response* – *ease of execution and/or automation* – *analytical properties (precision, accuracy, limit of detection, etc.)* – *miniaturization* – *reduction of cost*
Proprietary method	Method with a registered trademark/brand name, which is owned and generally marketed by a commercial company. *Generally, some of the components of the method are undisclosed.*
Qualitative method	Method of analysis that detects either the presence or absence of the analyte, either directly or indirectly, in a certain quantity of the sample.
Quantitative method	Method of analysis whose response is the amount of the analyte measured either directly (e.g. enumeration in a mass or volume), or indirectly (e.g. colour absorbance, impedance, etc.) in a certain quantity of sample.