Free detection and identification of bacteria in food

Science, Biology

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Rapid detection and identification of bacteria infoodand clinical laboratories

Abstract

Moderntechnological progresshas affected how microbiology is practiced. There is emphasis on the minimalisation of laboratory costs, cost-efficiency and reliability of tests for efficient bacterial identification from food cultures. Before using anytechnology, it is recommended that the products' performance characteristics be first tested, particularly as theses characteristics, are often not determined by the manufacturers. Consequently, the sensitivity and specificity, amongst other factors, associated with the use of these tests will also not have been determined. Additional factors would benefit from the use of controls, such as in the form of large scale and controlled clinical trials, in order to study the products'

performance. It is to be borne in mind that the involvement of ‘ rapid’ tests, including an enzyme-linked immunosorbent assay, in bacterial detection may serve bests as methods for expeditious detection and screening than for the purposes of confirmation.

1. Introduction

In order to help diagnose infectious diseases, such as the bacteria Salmonella, a leading cause of food poisoning, the need for specialised microbial tests has arisen. Testing food products using rapid methods is a complicated process requiring the balance of sensitivity and specificity for the achievement of a reliable result. The following sections will discuss the use of five different detection methods, flow cytometry, the enterotube II system, chromogenic media, the Enzyme linked immunoassay and polymerase chain reaction and the necessity to balance the specificity and sensitivity of each technique, for the most accurate means of bacterial detection.

2. 0 Flow Cytometry

Flow cytometry (FCM) is based on the principles of excitation of light, light scattering and fluorochrome molecular emission for the purposes of generating data covering a number of different parametric readings. FCM focuses on cells that measure 0. 5um to 40? m in diameter. The technique of FCM relies on the provision of a light source, which, are usually lasers, and the cells must first be covered in a layer of phosphate buffered saline before being able to intercept the focused source of light. In this technique, a

sample, containing the cells being tested, are injected into the centre of a sheath flow. Flow cytometry provides an analysis of cellular interactions at the macromolecular level. FCM is a technique that is considered to be a critical component of research in the biomedical field (Nolan & Sklar, 1998).

2. 1 Milk testing

FCM is one technique which may be useful when testing the safety and quality of milk. Testing milk requires analysis of somatic cell count and microbial analysis. Tests have shown (Gunasekera, et al., 2003) that the analysis of milk, where a known number of cells have been inoculated, upon clearing can be performed by FCM. FCM is able to give a good indication of the somatic cell count in raw milk and when coupled with other methods such as techniques involving fluorescence staining, can be used in testing biological milk quality. This therefore has an important application in the dairy industry, particularly in quality testing.

2. 2 Analysis of Water Quality

The use of flow cytometry has to date also occurred in tandem with heterotrophic plate count (HPC) for the rapid detection of the bacterial count of potable as well as raw water (Hoefel, et al., 2005). The results showed that FCM was much quicker than HCP, in detecting viable bacteria in samples that were classed as viable but not amenable toculture. The FCM method detected bacteria within an hour as opposed to several days, for the HCP technique.

Studies have tested the sensitivity of FC-based assays in comparison to the plaque assay method, to measure levels of an infection virus in a sample (Cantera, et al., 2010). Poliovirus infection (PV1) was tested and the FCM method applied to a water sample infected with PV1-infected cells. The study revealed that a combination of flow cytometry, used with fluorescence resonance energy transfer technology, is able to sensitively and quickly detect the presence of infectious virus in a sample of environmental water.

2. 3 Specificity of FCM

FCM has also been used to investigate whether T4 phage infected cells with E. coli ATCC 111303 can be differentiated from uninfected cells, based on phage DNA fluorescent detection. The technique, involving the lysis of bacterial cells by phage, allowed for the detection for infected cells 35 minutes post infection. Thus, FCM is able to be specific, when used combined with phages of predetermined host specificity. Overall, FCM is able to quantitatively measure and sensitively detect molecular level interactions and as such it may be considered to be a robust and adaptable technology (Nolan & Sklar, 1998).

3. 0 The enterotube ll system

The Enterotube II was described for the first time in 1969 (Painter & Isenberg, 1973). This technology is an example of a rapid system of multi-test nature, functioning as a biochemical and enzymatic test method. The test system, functions by identifying unclassified gram-negative, rod shaped and oxidase-negative bacteria, belonging to thefamilyEnterobacteriaceae. The test is often conducted within clinical laboratories. The machine

comprises a flat-sided tube within which are 12 compartments, developed to allow different biochemical tests to be conducted. The system does consistently produce accurate results, and hence is liable to produce occasional false results.

3. 1 Sensitivity and specificity

Reports such as the one by Dalton et al., (1993) in the detection of bacteriuria, have found that upon screening, only 55% specificity and 93% sensitivity have been obtained. O'Hara (2005) reports that it may be valuable for thediagnosticlaboratory running tests, using equipment such as the Enterotube II system, to first stipulate what levels of ' accuracy' and ' discrimination' they consider are acceptable from their systems of identification. Accuracy of identification may be maximised by using the skills of a qualified microbiologist to confirm the bacterial classification (O'Hara, 2005). An additional way to potentially maximise sensitivity and specificity is to send an isolate to a reference laboratory in order to confirm identity. Use of enterotube II system will be for the testing of oxidase-negative bacteria and hence it should first be established that the oxidase test is not positive. To achieve this, and improve the specificity, an oxidase test may be performed on the relevant cultures.

In order to improve interpretation of results from use of the Enterotube II system, a suitable incubation time should be used, such as 16 hours (in the analysis of carbohydrate reactions (Woolfrey, et al., 1981). Furthermore, tests resulting in ambiguous classifications should be reevaluated (Woolfrey,

et al., 1981) in order to improve specificity, without hampering the tests' sensitivity.

4. 0 Chromogenic Media

Chromogenic media (or fluorogenic media) are a microbial growth media of microbial nature. The media contains enzymes that are linked to either fluorogen (involved in light reaction) or chromogen (involved in colour reaction) or a combination of both. The method works by detecting activities that are enzymatic in nature, that are produced by the target microorganisms. Enzymatic activities are detected by the use of either organic compounds or dyes, as microorganisms, which grow in the proximity of these compounds are liable to make a distinctive pattern of colouring or alternatively fluoresce, which can be detected under UV light. Chromogenic media were first designed for application in clinical settings, but have proven to be useful in food testing.

4. 1 Sensitivity and specificity of chromogenic media

Chromogenic media are considered to be a sensitive method of media analysis, when compared to more conventional types of media analysis (Downes, 2001). This is because the chromogenic media method allows for a faster analysis, with a turnover time of 24 hours, and it is also considered to have a higher sensitivity. In the identification of E. coli or Listeria monocytogenes, for example, specially designed chromogenic media are available for the purposes of improving test sensitivity.

When considering Salmonella detection, a number of specialised chromogenic media that are able to improve the specificity of detection are available. A study by Perez et al., (2003) showed that both broth enrichment and increasing the incubation time by a factor of two (from 24 hours to 48 hours) effectively increases the sensitivity of all of the media being used. Furthermore, due to the specificity of the chromogenic media, (determined to be greater than 84% following a two-day incubation period), a reduction in the need to undergo confirmatory tests improved the overall sensitivity of the specialized chromogenic media. A second study by Monneri et al., (1994), for the comparison of two new types of agar, media of chromogenic nature, Salmonella Detection and Identification Medium (SMID) and Rambach agar, against two conventional types of media for the detection of Salmonella. The results revealed that the newer chromogenic agar media were notably more specific than the more conventional media. Rambach agar was furthermore slightly more specific than SMID, being able to detect all Salmonella serotypes following a complementary C8 esterase test. Hence, sensitivity and specificity can be maximised by increasing culture time to 2 days fully, and using Rambach agar where appropriate, such as in the detection of Salmonella serotypes.

5. 0Enzyme Linked Immunoassay

The Enzyme linked immunoassay (ELISA) is a common antibody based technique designed for microorganism, or pathogenic, detection. The method is noted to have a high standard of specificity and sensitivity (Evans et al., 1989). A quantitative, or qualitative method may be used for the

purposes of interpreting the results, which are, respectively, via the use of an instrumental read-out or through visual means. Specialised test kits to aid in the detection of Listeria, Salmonella and other microorganisms are commercially available.

5. 1 Sensitivity and specificity

A study by Evans et al., (1989) utilised ELISA in the detection of Campylobacter pylori. The specificity and sensitivity of the test allowed for the detection of serum immunoglobulin G (IgG) antibodies targeted against the cell-associated proteins of C. pylori. Values of specificity and positive predictive value were revealed to be 100% for the high molecular weight cell-associated proteins. Furthermore, the assay sensitivity was measured at 98. 7%, with the negative predictive value recorded as 98. 6%. This indicates that specialised ELISA tests are likely to be valuable in such instances as in the detection of H. pylori. Furthermore, the costs of using the ELISA, as noted by Evans et al., (1989) are that it is cost effective and readily usable, with a lower likelihood of obtaining false negatives than with other tests, such as the use of a ‘ urea breath test’ which is also amenable to be useful for the same purpose.

Svennerholm & Holmgren, (1978) report that E. Coli can be sensitively detected using a ganglioside ELISA. The method was deemed to be reliable and allow a high level of reproducibility. In general, it has been reported that the specificity and, or, sensitivity of assays that are commercially available, such as the ELISA may be maximised by having set cut-off values decreed by the manufacturers, according to the target disease (Cuzzubbo, et al., 1999).

Furthermore, the IgG test, due to having 100% specificity, is highly likely to be reliable, as a method for bacterial testing.

6. 0Polymerase Chain Reaction

Similar to the ELISA test, the ‘ PCR’ or the polymerase chain reaction (PCR) is one of the most readily recognised and used diagnostic tool currently in use. PCR works by identifying a highly specific sequence of DNA from a microorganism that is under target. Subsequent to this, the sequence much be amplified in order to allow for detection of the microorganism. PCR is considered to be reliable and specific, as a detection method, being able to detect bacteria of pathogenic nature within a time frame of a day. As a form of DNA-based assay, PCR has been developed to detect foodbourne pathogens. For the purposes of DNA hybridization, PCR is able to amplify one single DNA copy in fewer than 2 hours by one million times. However, in situations where amplification is not completely efficient, such as when inhibitors are present in food, the normally extremely high levels of sensitivity of PCR become reduced. In order to improve sensitivity therefore, a form of cultural enrichment is likely to achieve this (Rose & Stringer, 1989).

As a rapid method to screen food samples for bacteria, PCR tests that are run and found to yield positive results are regarded as being ‘ presumptive’ and require methods that are more conventional to confirm this (Feng, 1996). For direct testing, due to a lack of adequate specificity and sensitivity, pre-analsysis culture enrichment is frequently called for, which serves to increase specificity (Feng, 1997).

6. 1 Sensitivity and specificity of PCR

To maximise the sensitivity of certain types of PCR, such as NK-1R PCR, a form of ‘ nested’ PCR, and for this an increased number of cycles of the primary PCR may be helpful. For example, 35 secondary PCR cycles and 45 primary PCR cycles, were performed by O’Connell (2002) as opposed to a more standard number of between 25 and 30 cycles for both to increase sensitivity. In order to identify and detect bacteria furthermore, qcRT-PCR is likely to be less sensitive overall than more conventional PCR and hence, single-target PCR is advisable for a higher level of sensitivity.

It has also been noted that PCR conditions and parameters of cycling should ideally be optimised for every, and each primer in order to allow the achievement of a maximum yield of specific product and miminise monotarget sequence amplification. Knowles (1992) suggests that nested PCR may be helpful in improving both sensitivity and specificity. It is noted that increasing the speed of amplification of PCR has not effect upon test sensitivity, and hence this alteration it is unlikely to be worth the additional costs or time-saving advantage associated with increasing the cycling protocol.

7. Conclusion

Rapid tests such as PCR, the Enterotube II system, ELISA, flow cytometry and chromogenic methods have both benefits and limitations. The relative availability of these techniques and the speed of detection of bacterial pathogens, amongst other factors, suggest advantages but the sensitivity

and specificity of the tests must be such that a reliable test result is ensured. In conclusion, a balance of sensitivity and specificity is required, but, by using the techniques mentioned, the reliability of the results obtained by the microbiologist is most likely to be improved.

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