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Journal of Microbiological Methods 53 (2003) 165–174
Journal of Microbiological Methods
www.elsevier.com/locate/jmicmeth

Review article

The use of NASBA for the detection of microbial pathogens in food and environmental samples

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Abstract

The isothermal amplification method nucleic acid sequence-based amplification (NASBA), which amplifies RNA, has been reported as useful for the detection of microbial pathogens in food and environmental samples. Methods have been published for *Campylobacter* spp., *Listeria monocytogenes* and *Salmonella enterica* ser. *Enteritidis* in various foods and for *Cryptosporidium parvum* in water. Both 16S rRNA and various mRNAs have been used as target molecules for detection; the latter may have advantages in allowing specific detection of viable cells. Most of the methods to detect pathogens in foods have employed enrichment in nutrient medium prior to NASBA, as this can ensure sensitivity of detection and encourage the detection of only viable target cells. Although a relatively recent method, NASBA has the potential for adoption as a diagnostic tool for environmental pathogens.

Keywords: NASBA; Microbial pathogens; Food; Water

1. Introduction

For many microbial pathogens, the environmental route of transmission is important, with water and food being particularly significant. Often, the numbers of pathogens are not high, which can complicate their detection in samples taken from these environments. The polymerase chain reaction, with its potential for exquisite sensitivity, can mediate detection of low numbers of microorganisms rapidly and with a high degree of specificity, and its use for this has been propounded in various published studies. So far, there have been few published methods for detection of foodborne or waterborne pathogens using the alternative technique, nucleic acid sequence-based amplification (NASBA) (Chan and Fox, 1999) and those which are available concern very few pathogenic types. This review will detail the publications that have appeared to date. The advantages and limitations of NASBA will be overviewed, with regard to its use in analysis of food and environmental samples. In addition, the issue of detection of viable target will be discussed, as will other relevant issues such as sample pretreatment. Finally, a view of the future of NASBA-based methods, in relation to the current position of PCR-based method for the detection of food and environmental pathogens, will be provided.

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0167-7012/03/$ - see front matter.
doi:10.1016/S0167-7012(03)00022-8

2. NASBA

NASBA is specifically designed to detect RNA and employs three enzymes: a reverse transcriptase, RNaseH and T7 RNA polymerase, which act in concert to amplify sequences from an original single-stranded RNA template. Oligonucleotide primers, complementary to sequences in the target RNA, are incorporated in the reaction. One primer also contains a recognition sequence for T7 RNA polymerase. The reaction contains both dNTPs and NTPs. The first primer binds to the RNA, allowing the reverse transcriptase to form a complementary DNA strand. Then the RNase digests away the RNA and the second primer binds to the cDNA, allowing the reverse transcriptase to form a double-stranded cDNA copy of the original sequence. This double-stranded DNA then acts as a kind of mini "gene", which is transcribed by the T7 RNA polymerase to produce thousands of RNA transcripts which then cycle through the reaction. The reaction is performed at a single temperature, normally 41 °C. At this temperature, the genomic DNA from the target microorganism remains double-stranded and does not become a substrate for amplification. This eliminates the necessity for DNase treatment, which is required when using RT-PCR for RNA detection (Klein and Kuneja, 1997; Szabo and Mackey, 1999), and it also offers the possibility of specific detection of viable cells, as will be discussed below. The product of a NASBA reaction is mainly single-stranded RNA. This may be detected on by gel electrophoresis followed by ethidium bromide staining, but to ensure product specificity, a confirmatory step, generally involving probe hybridization, is usually employed.

3. NASBA-based detection methods for pathogens in food and water

The following sections will focus on the particular microorganisms for which NASBA-based methods have been reported, describing salient aspects within each study. The studies are summarised in Table 1.

3.1. Campylobacter spp.

The identification of the pathogens *Campylobacter coli*, *Campylobacter jejuni* and *Campylobacter lari* is conventionally based on biochemical tests, resistance patterns and growth temperature, and the complexity of this analysis makes precise identification time-consuming and sometimes uncertain. NASBA-based detection of specific 16S rRNA sequences can provide a more rapid means of identifying these pathogens (Uyttendaele et al., 1994). In a series of published studies, Uyttendaele et al. have demonstrated the applicability of NASBA to the detection of *Campylobacter* spp. in foods.

In the first of the series, Uyttendaele et al. (1995a) added *C. jejuni* to 10-g samples of chicken skin and ground beef which had been homogenised in selective (Preston) medium. These homogenates had previously been heated to reduce the numbers of background microflora, or had been left untreated. Half of the homogenates were processed immediately, and the rest incubated at 42 °C for 24 h in a CO_2-enriched atmosphere to increase the numbers of *C. jejuni* cells. Food debris was removed from samples of the homogenates by slow-speed centrifugation (1000 × *g*) for 2 min, then 1-ml aliquots were taken for nucleic acid extraction according to the commonly used Boom et al. (1990) method. After NASBA, amplicons were detected by enzyme-linked gel electrophoresis (ELGA), where they were hybridized to a horseradish peroxidase-labeled species-specific probe, run on an acrylamide gel, and stained in the gel by immersion in a substrate solution. Where homogenates were neither heat-treated nor incubated, no NASBA signal could be obtained, whereas incubation of samples resulted in a positive NASBA signal regardless of whether heat treatment had been employed or not. A range of inoculated food types were then tested by the selective enrichment/NASBA method, and *C. jejuni* could be detected to less than 10 cfu/10 g (except for raw milk where 30 cfu/10 ml was the limit). This method could be completed in less than 48 h.

Enrichment, or incubation of samples in nutrient medium, which can be selective for a particular bacterial type, is commonly employed in several microbial detection techniques associated with foods, for several reasons. As well as ensuring the presence of target cells in numbers above the detection limit of the technique, it can remove food-derived inhibitory substances either through dilution or allowing their digestion through bacterial growth (Szabo and Mackey, 1999). It may also be useful when employing

Table 1
NASBA-based methods reported for detection of pathogenic microorganisms in food and environmental samples

Target	Matrix	Isolation procedure	Amplicon detection	Detection limit	Reference
C. jejuni, *C. coli*, *C. lari* (16S rRNA)	poultry products	SE	ELGA	<10 cfu/10 g	Uyttendaele et al. (1995a)
C. jejuni (16S rRNA)	poultry products	SE	ELGA	naturally contaminated (10 g)	Uyttendaele et al. (1995b)
L. monocytogenes (16S rRNA)	poultry products, meat products, seafood, vegetables, dairy products	SE	ELGA	<10 cfu/25 g	Uyttendaele et al. (1995c)
C. jejuni (16S rRNA)	poultry products	SE	ELGA	naturally contaminated (10 g)	Uyttendaele et al. (1996)
L. monocytogenes (hlyA mRNA)	egg products, dairy products	SE	colorimetric probe hybridization	<10 cfu/60 g	Blais et al. (1997)
C. jejuni (16S rRNA)	poultry products	direct detection	ELGA	$<10^5$ cfu/10 g	Uyttendaele et al. (1997)
C. jejuni (16S rRNA)	poultry products, meat products, dairy products	buoyant density separation	ELGA	$5 \times 10^2 - 5 \times 10^4$ cfu/20 g	Uyttendaele et al. (1999)
C. parvum (hsp70 mRNA)	water	filtration/IMS	ECL	50 oocysts/100 l	Baeumner et al. (2001)
Hepatitis A virus (VP2 gene)	blueberies, lettuce	elution	probe hybridization/ dot-blot		Jean et al. (2001)
	wastewater	direct detection			
E. coli (clpB mRNA)	water	–	ECL	40 cfu/ml	Min and Baeumner (2002)
Rotavirus (Gene 9)	sewage	direct detection	ELISA		Jean et al. (2002b)
S. enterica ser. *Enteritidis* (dnaK mRNA)	liquid egg	enrichment	ECL	<10 cfu/25 g	Cook et al. (2002)

SE: selective enrichment. ELGA: enzyme-linked gel assay. IMS: immunomagnetic separation. ECL: electrochemiluminescence.

NASBA to detect the presence of viable bacteria in a sample, as will be discussed in a later section.

Uyttendaele et al. (1995b, 1996) compared the selective enrichment/NASBA method with a traditional isolation procedure based on agar plating (modified *Campylobacter* charcoal desoxycholate agar (mCCDA)) and biochemical identification. They examined poultry products by each method for the presence of naturally contaminating *C. coli*, *C. jejuni* and *C. lari*. In total, 160 samples were tested, and the methods gave similar results to each other in 93% of cases. No false negative results were obtained for the NASBA-based method, i.e. the agar-based method did not detect campylobacters where NASBA did not. The traditional method failed to detect campylobacters in nine samples where a positive NASBA signal was obtained. On retesting, two of these samples gave a positive result by mCCDA; therefore, the first test results were recorded as falsely negative for the agar-based method. The other seven samples could, therefore, be reported as falsely positive for the NASBA-based method; however, there were indications that in some cases, the mCCDA method failed due to overgrowth by noncampylobacters, and it was considered possible that all the NASBA results were actually true positives.

To demonstrate that the speed of analysis could be improved by simplifying the nucleic acid extraction step, Uyttendaele et al. (1999) heat-lysed *C. jejuni* cells and used the extract in the NASBA reaction directly. To overcome inhibition by food-derived substances, buoyant density centrifugation was employed to separate the cells from food components prior to heating. A food homogenate was layered on

top of a density gradient medium, and centrifugation resulted in sedimentation of the cells. The food material was discarded along with the rest of the medium. This sample pretreatment was applied to various foodstuffs, including poultry, meat and dairy products. Food homogenates were inoculated with *C. jejuni* to 500–5000 cfu/ml, the cell concentration which could be expected after an enrichment of samples containing low numbers (0.3–3000 cfu) of cells. Positive results were obtained from each food sample, except soft cheese, where removal of fat was not fully accomplished.

3.2. Cryptosporidium parvum

The protozoan parasite *C. parvum* has been responsible for many outbreaks of waterborne gastrointestinal disease (Smith and Rose, 1998). Baeumner et al. (2001) devised a method to detect viable oocysts by NASBA amplification of heat shock protein mRNA (hsp70). This transcript could be produced more abundantly by incubating oocysts at 42 °C for 20 min, potentially facilitating the detection efficiency of the amplification method. Nucleic acids were extracted from oocysts by incubation at 60 °C for 10 min with intermittent vortexing, followed by lysis and purification by a commercial extraction kit. Detection of the NASBA signal was by hybridization to labeled probe followed by electrochemiluminescence (ECL). This mediated the detection of five oocysts per reaction in six tests out of seven.

To demonstrate the application of NASBA detection to *C. parvum* oocysts in environmental water, Baeumner et al. (2001) had 10-ml samples of deionised and turbid water (which had been previously concentrated by vortex flow filtration) seeded with various amounts of oocysts. They then applied an immunomagnetic separation (IMS) procedure to concentrate the oocysts out of the samples and deliver them to the extraction system. They were able to detect 50 oocysts in all the deionised water samples, and in 83% of the turbid water samples. Lower numbers of oocysts could be detected, but less efficiently, possibly due to losses during the IMS procedure. Current standard detection methods rely upon microscopic identification of the transmissive stage, or oocysts, in water samples; although reliable, they are labor-intensive and may not effectively determine viability. With development, the sensitive and specific NASBA-based methodology should become a useful and complementary addition to the armory of techniques available for detection of *C. parvum* in the environment (Smith, 1998).

3.3. Escherichia coli

E. coli is widely used as an indicator organism of fecal contamination in food and water. In conventional water analysis, culture-based detection methods are the rule (Bordner et al., 1978). They allow detection of low numbers of *E. coli* in complex matrices, but can require several days to complete. To show the potential of an alternative gene-based method, Min and Baeumner (2002) developed a NASBA assay for the detection of viable *E. coli*. The target for amplification was another heat shock protein mRNA, clpB. A minimum of 5 min at 41 °C was sufficient to induce detectable clpB mRNA. Nucleic acids were extracted by mild sonication followed by purification by a commercial extraction kit. Signal detection was achieved by probe hybridization and electrochemiluminescence.

The authors reported that this assay was used to detect *E. coli* cells seeded into drinking water, although no details of sample size or any method used to extract and concentrate the cells from the water were provided. Positive signals were consistently obtained down to 40 cells/ml water. No signals could be obtained from cells that had been killed through boiling, sonication or chlorination.

It appeared that the assay could permit quantitation of viable cells, as there was a linear relationship between the magnitude of the ECL signal and colony counts after incubation of sample aliquots on solid medium.

3.4. Hepatitis A virus

Enteric viruses such as hepatitis A virus (HAV) can contaminate various foodstuffs, leading to outbreaks of disease (Cook, 2001). In many instances, molecular methods offer the best means of detecting foodborne viruses, other methods such as ELISA or cell culture being too inefficient or slow (Cook and Myint, 1995). A NASBA assay for HAV was developed by Jean et al. (2002a,b), targeting the capsid protein gene VP2.

Amplicon detection was via hybridization with a digoxigenin-labeled oligonucleotide probe in dot-blot format. This assay was capable of detecting two plaque-forming units (PFU) when tested with pure virus suspensions. The assay was capable of detecting approximately 10^6 PFU of HAV artificially inoculated onto the surfaces of samples of lettuce and blueberries. The samples were washed with 100-μl high-protein buffers or water to elute the virus particles from the surface of the fruit, then a 5-μl aliquot of the eluate was introduced directly (after heating to release viral RNA from the capsids) into the NASBA. Only very small samples of foodstuff appear to have been used (3 cm^2 lettuce, sample size not given for blueberries), and consequently the concentration of foodstuff-derived inhibitory substances in the final extract may have been low. To be of practical value, the method should be able to process realistic food sample portions (Kurdziel et al., 2001), which may require more complex extraction procedures to remove inhibitors.

Jean et al. (2001) also reported that the HAV NASBA assay could directly detect viruses artificially inoculated into wastewater samples (10^6/ml). But the volume used directly (5 μl) would be too low to detect viruses in naturally contaminated waters, for which considerable preconcentration is required to deliver target viruses, present if at all in low numbers, to a detection method (Cook and Myint, 1995). The authors stated that work was under way to improve the pre-NASBA extraction techniques for detection of viruses in complex sample matrices.

3.5. Listeria monocytogenes

Uyttendaele et al. (1995c) devised a NASBA to detect *L. monocytogenes* using 16S rRNA sequences as the target. The detection limit was determined at 10^6 cfu in 1 ml of cell suspension used for extraction of nucleic acids prior to amplification. They evaluated the method against a modified FDA culture method for detection of *L. monocytogenes* in foods, using 25-g food samples of various kinds, spiked with approximately 1, 10 and 100 cfu of *L. monocytogenes*. Selective enrichment was performed for 48 h on each sample prior to NASBA detection. Identical results could be obtained with the two methods for chicken breast, meat, minced meat, raw milk, soft cheese, mushrooms and radish, and all spiking levels could be detected. The NASBA-based method, however, could generate results within 3 days, whereas the culture-based method took 6–7 days to perform. In addition, with shrimp and dry sausage samples, the modified FDA method could not detect low spiking levels, whereas the NASBA-based method could. The NASBA-based method was also evaluated against an ELISA method for detection of *Listeria*: both methods performed equally well, but NASBA could give a rapid confirmation to species level through the specificity of the oligonucleotide probe used in the ELGA.

Blais et al. (1997) devised a NASBA assay to detect *L. monocytogenes* using the inducible hlyA mRNA as target for amplification. Amplicons, which incorporated biotin-labeled UTP, were hybridized to an oligonucleotide probe immobilized on a nylon membrane, and detected by colorimetry. The detection limit which could be consistently achieved by this was 500 cfu per reaction. The authors concluded that this limit was not sensitive enough for direct detection of *L. monocytogenes* in foods, so when the method was applied to various dairy products and egg powder inoculated with *L. monocytogenes* at levels down to 0.2 cfu/g, a 48-h enrichment in a selective broth was used to increase target cell numbers before application of NASBA. The assay correctly identified inoculated samples in 92.6% of cases. Some of the samples inoculated with very low levels (e.g. 0.5 cfu/g) did not produce a NASBA signal, although this may have been through the statistical possibility that these samples have actually received no *L. monocytogenes* cells. Some apparently false positive results were obtained from uninoculated samples of egg powder and mozzarella cheese. These samples had previously been analysed by a traditional culture technique which confirmed them as negative. However, the possibility remains that they were in fact contaminated with low numbers of *L. monocytogenes*; Uyttendaele et al. (1997) found that a culture-based method occasionally gave negative results from low inoculated food samples which were NASBA-positive.

3.6. Rotavirus

Rotaviruses are the major etiologic agents of acute gastroenteritis in infants and in the young of many

animals (Kapikian, 1996). Jean et al. (2002a) developed a NASBA assay for rotavirus. The target of amplification was sequences of the gene encoding the antigenic VP7 rotavirus coat protein, and DNA–RNA hybrid amplicons were detected by an ELISA system. The limit of detection of rotavirus in pure suspension was 40 PFU.

Rotaviruses are excreted in large numbers by those infected, and can be found in high concentrations in sewage (Hejkal et al., 1984). Rapid gene-based detection of rotaviruses in sewage and wastewater would facilitate epidemiological studies, and PCR-based methods for this purpose have been reported (Dubois et al., 1997). To demonstrate the potential of an alternative NASBA-based method, the assay of Jean et al. (2002a) was applied to the direct detection of rotavirus artificially inoculated into sewage treatment effluent (Jean et al., 2002b). A limit of 3×10^3 PFU/ml virus could be detected. Rotaviruses can be present in sewage in such numbers (Hejkal et al., 1984); therefore, with further sample concentration development to allow detection of lower numbers of viruses, the NASBA-based method could be usefully applied to natural samples.

3.7. *Salmonella enterica*

Whole liquid egg is used in the manufacture of various food products, the egg generally being pasteurized prior to use. A low level of contamination of raw egg with *S. enterica* ser. *Enteritidis* may exist (Humphrey et al., 1991), and the current test to determine whether pasteurization has been correctly performed can give false positive and negative results (Pether et al., 1991). Cook et al. (2002) devised a method to detect *S. enterica* ser. *Enteritidis* in liquid whole egg. Having previously shown (Simpkins et al., 2000) that NASBA was capable of identifying viable *S. enterica* cells, the use of the technique as a basis for screening of liquid whole egg for this pathogen was considered possible. Enrichment of 25-g samples of liquid egg in buffered peptone water was employed prior to extraction of nucleic acids because it was found that otherwise, positive results could not be obtained: concentration of bacterial cells form the food matrix by a variety of methods before nucleic acid extraction apparently also concentrated inhibitors of the NASBA. The final method could detect at least three *Salmonella* cells per sample.

4. The issue of viability

The detection of messenger RNA has been proposed as an indicator of cell viability (Bej et al., 1991, 1996; Klein and Kuneja, 1997; del Mar Lleò et al., 2000), as defined by capability of cell division, metabolism or gene transcription. Messenger RNA can have a short half-life within viable cells, and is rapidly degraded by specific enzymes (RNases) which are themselves very stable even in environments outside the cell itself (Sela et al., 1957). Simpkins et al. (2000) showed that NASBA can selectively amplify mRNA sequences in a background of genomic DNA, which indicated that NASBA amplification of mRNA can be used to specifically detect viable cells. It was noted that when nucleic acids were immediately extracted from pasteurized *Salmonella* cultures, a signal was sometimes obtained. Subsequently, it was found that leaving the cultures for 15 min after pasteurization, before extracting nucleic acids, abolished this residual signal, probably by allowing degradation of mRNA by cellular RNase enzymes (Simpkins and Cook, unpublished). Birch et al. (2001) reported, however, that mRNA sequences in *E. coli* could be detected by NASBA for up to 30 h after cell death. Twenty-four-hour post-heat killing was necessary to eliminate a NASBA signal from *C. parvum* oocysts (Baeumner et al., 2001) and *E. coli* cells (Min and Baeumner, 2002). It has been demonstrated that mRNA degradation can be dependent on the susceptibility of the transcript (Alifano et al., 1994), or regions thereof (Norton and Batt, 1999), and this may help to explain the differences in the above observations. Accordingly, NASBA primers for a viability assay should be complementary to regions of the mRNA target most susceptible to enzymatic degradation, where these are known or can be predicted.

Detection of mRNA in viable cells may depend on their physiological state (Barer and Harewood, 1999): cells which are stressed, or viable but non-culturable, may contain mRNA in quantities too low for detection. To avoid false negative results which may arise in such instances, NASBA primers could be chosen to

target mRNA transcripts of genes which may be constitutively expressed in all physiological states (Deiman et al., 2002).

Plasmid-encoded gene transcripts may not be suitable for a NASBA viability assay. Voisset et al. (2000) found that undenatured plasmid DNA could be amplified by NASBA, and hypothesized that this may have been due to amplification of single-stranded intermediates of plasmid replication. Such an occurrence may result in false positives in a NASBA viability assay.

4.1. Enrichment NASBA

The use of an enrichment step prior to NASBA detection may also be a safeguard against false positive results in a viability assay caused by residual RNA in dead cells. Uyttendaele et al. (1997) inoculated 10-g pieces of chicken skin with 10^5 cfu *C. jejuni*, then heated them for 10 min at 100 °C in a water bath. Samples were taken at various times and either enriched or subjected to direct extraction, comprising homogenisation and centrifugation prior to extraction of nucleic acids. When direct extraction was employed, NASBA signals could be obtained from samples taken for up to 24 h after heating, but no NASBA signal was obtained from any of the enriched samples. It was concluded that the RNA was being completely degraded, through increasing the activity of RNase enzymes, both cellular and derived from the food matrix, at the elevated enrichment temperature. Interestingly, in the study by Uyttendaele et al. (1995a) described previously, NASBA signals were obtained from some uninoculated food samples which were analysed prior to enrichment, but not from similar samples which had been enriched; a possibility that these were naturally contaminated with *C. jejuni* but the cells were dead may be considered. In the studies of Uyttendaele et al. (1995a, 1997), 16S rRNA sequences were amplified; mRNA is less stable than rRNA (King and Schlessinger, 1987) and, thus, enrichment may be even more efficacious when amplification of mRNA sequences is used to detect viable cells in a food or environmental matrix.

Enrichment prior to PCR may also be considered capable of avoiding detection of dead cells (Candrian, 1995). But the accuracy of this approach will depend on the number of dead cells in a sample being below a threshold detection level. As an illustration, one may make the following hypothetical example. A food sample is enriched in 250-ml broth, then 1 ml is taken and nucleic acid is extracted, producing a final extract of 50 μl, 5 μl of which is incorporated in a PCR. If one assumes that the DNA from all cells in the 1-ml sample is found in the final extract, then the PCR is set up to detect one-tenth of these cells. If the PCR has a theoretical detection limit of one cell per reaction, then the detection limit of the complete method will be $1 \times 10 \times 250$ cells per original food sample. Consequently, if there are more than 2.5×10^3 dead cells present in the original sample, a PCR signal will be obtained. Increasing the volume of enrichment culture, or decreasing the volume taken for nucleic acid extraction, will increase the threshold number of dead cells which will give a signal, but there will always be a level of uncertainty as the number of dead cells in the sample will not be known. With a NASBA assay, this would not be a problem.

The use of enrichment prior to NASBA may have other advantages. As seen in many studies, it may be the best or only way to avoid inhibition of the reaction by food components. In addition, it would confer a degree of familiarity to end users, mainly routine high-throughput laboratories and general technical staff, which could encourage them to consider eventually replacing conventional methodology with a NASBA-based method.

5. Other issues

5.1. Sample pretreatment

Generally, NASBA's requirements for sample pretreatment are similar to those of PCR, in that nucleic acids must be extracted from the target organism in the food or environmental matrix and delivered to the reaction in a reasonable pure solution, i.e. one that does not contain any inhibitors of the reaction. The reaction itself is more complex than PCR in that it requires three enzymes and the effect of potential inhibitors has not been studied in detail, as for PCR (Rossen et al., 1992), nor have suitable facilitators (Al Soud and Rådström, 2000), substances which can combat the presence of matrix-derived inhibitors, been identified. This would be a useful line of

research if direct NASBA detection of microorganisms, i.e. without a prior enrichment step, is to be considered for a food or environmental sample type. Incorporation of enrichment culture directly into the NASBA reaction, as is possible with some PCR-based assays (Knutsson et al., 2001), cannot be done with NASBA because the reaction temperature is not high enough to lyse the target cells and release nucleic acid. Further, the NASBA enzymes are not thermostable and would not withstand an initial heat-lysing step; moreover, such a step may result in denaturation of genomic DNA, which would nullify a viability assay.

5.2. Internal amplification controls

The use of an internal amplification control (IAC) is essential if an amplification assay is to be effectively and reliably employed in analysis, especially of samples which may contain inhibitors which can cause false negative results (Jones et al., 2000; Abdulmawjood et al., 2002). In a NASBA assay, IACs can be based on RNA sequences which are identical to the target nucleic acid sequence except for internal modifications which allow binding of a different detector probe. This type of control has been used as a calibrator to facilitate quantification of target RNA (van Gemen et al., 1994; Deiman et al., 2002), but should also be appropriate for identification of inhibited reactions.

6. Future directions

Nucleic acid-based diagnostics for pathogenic microorganisms, in its main current incarnation as PCR, is very young compared to traditional detection methods. However, it is considered that PCR can be established as a routine reference method, alongside traditional techniques, within the next decade (Hoorfar and Cook, 2002). Currently, there are international collaborative efforts to produce PCR-based methods for foodborne pathogens which are suitable for standardization, and thereafter adoption as routine diagnostic procedures (Hoorfar and Cook, 2002; http://www.pcr.dk). The framework of these efforts is based on three principal steps: demonstration of primer specificity; evaluation of the method through collaborative trial; and finally, proposal to an international standardization body such as CEN. NASBA for detection of pathogens in food and the environment is at around the same stage as PCR was a decade or so ago, with a few methods being published sporadically in the scientific press. Considerable further development is, therefore, required before NASBA can follow in PCR's footsteps to realize its potential in routine use. However, with its potential of equalling the rapidity and accuracy of PCR and of matching the surety of culture-based methods in detection of viable pathogens, NASBA, as a diagnostic tool for food and environmental samples, is surely worthy of committed developmental effort.

Acknowledgements

The unpublished work from the author's laboratory was funded by the United Kingdom Food Standards Agency.

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