Development and Application of a Tool To Assess Laboratory Hygiene in Contained-Use Facilities[▽]

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To gain information on laboratory hygiene in contained-use laboratories, a method was developed to study the presence of microorganisms on laboratory equipment. Focusing detection on genetically modified organisms (GMOs) containing the universal M13 primer binding sites enabled the detection of a broad range of GMOs using a single PCR. Swabbing surfaces in three different contained-use laboratories led to detection of M13-containing PCR products in 26 out of 34 swabs. Most sequences (up to five per sample) were detected in swabs from the centrifuge and sink, followed by swabs taken from the bin and incubator (up to four sequences per sample). The obtained sequences varied in length from 171 nucleotides (nt) to 878 nt. In most cases, sequences were only partially similar to sequences published in GenBank. The lengths of the regions with high similarity varied from 94 nt to 795 nt, and these similarities ranged from 81% to 100%. Similarities with more than one sequence were commonly found, complicating the identification of detected sequences. Nonetheless, 84% of the detected sequences were actually handled in the laboratory at the time of sampling. This demonstrates that the method may be used as a quality control tool to assess the efficacy of decontamination and cleaning of commonly used surfaces, such as laboratory benches, freezer doors, and centrifuge rotors, without prior knowledge of the identity or characteristics of the GMOs.

In the last decades, accidental releases of human or veterinary pathogenic microorganisms, such as *Bacillus anthracis* spores, foot-and-mouth disease virus, and hemorrhagic fever viruses, have been reported (9, 13–15). Both national and international legislation on the management of pathogens, contained-use laboratories, the use of genetically modified material, transportation of these agents, and decontamination and cleaning is in place to diminish the probability of such accidental escapes from laboratory facilities. Laboratory incidents may be due to improper inactivation and decontamination procedures (13). The two most common methods of decontamination are sterilization and chemical disinfection, of which the latter has to be used for the decontamination of surfaces present in the laboratory, such as door handles, benches, and laboratory equipment. A wide variety of chemicals, such as hypochlorite, alcohols, acids, and peroxygens, have been used to disinfect hard, nonporous surfaces in industrial, laboratory, and health care settings (16). The ideal disinfectant should have a fast and broad-range activity against bacteria, viruses, fungi, spores, and prions, while being easy to use and not harmful to the surfaces to be disinfected. Traditionally, alcohols such as ethyl alcohol and isopropyl alcohol are the disinfectants most commonly used. They have been shown to be active against most bacteria and lipid-containing viruses but are inactive against nonenveloped viruses, spores, and prions

(3). The most optimal disinfection concentrations are between 60 and 90% (vol/vol) (18). Alcohols are not suitable to decontaminate protein-rich matrices, as they may precipitate proteins resulting in protection of the microorganism. Hypochlorites have a more broad-range activity and are also effective against most nonenveloped viruses such as enteric viruses. They are active in the presence of organic materials, although the concentration of chlorine may need to be increased up to 3% sodium hypochlorite (5, 7, 8). The use of hypochlorite is limited due to its corrosive characteristics and instability (18). To be able to gain information on the efficacy of decontamination and cleaning in contained-use laboratories and laboratory hygiene in general, the presence of microorganisms on several laboratory surfaces was studied.

In the present study, it was decided to focus on the detection of genetically modified organisms (GMOs) because of the presence of universal DNA sequences, such as M13 forward and reverse primer binding sites. In this way, a broad range of microorganisms can be detected using a single PCR. The M13 primer binding sites are present on extrachromosomal plasmid DNA molecules, which may be present in high copy numbers, increasing the sensitivity of detection (6). Oligonucleotides complementary to these sequences are commercially available (12) and can be used to identify the inserted DNA by PCR and sequencing analysis. To study whether this method may be used as a quality control tool for determining the efficacy of decontamination and cleaning of laboratory equipment, commonly used surfaces, such as laboratory benches, freezer doors, and centrifuge rotors, in contained-use laboratories were swabbed. Subsequent amplification of the swabbed material by M13 PCR was carried out to demonstrate whether and which M13 sequence-containing plasmid DNAs

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were detectable, thereby gaining information on laboratory hygiene.

MATERIALS AND METHODS

Sampling. Swab samples were taken from flat surfaces of 100 cm² or other surfaces such as centrifuge rotor holes or pipette swabs using the premoistened Enviroswab (Tecra [bio Trading], Mijdrecht, Netherlands), which enables access to a variety of surfaces because of its tip shape and flexible stem. The premoistened Enviroswab samples were transported to the laboratory in the supplied sealing tube at room temperature and stored at 4°C until further treatment for a maximum period of 72 h.

DNA extraction. Thirteen milliliters of ultrapure water was added to the swabs, and samples were vortexed for 1 min. After extensive wringing of the swab against the wall of the tube, the suspension was collected in a 15-ml tube and centrifuged for 15 min at room temperature at 4,500 × *g*. The supernatant was discarded and the pellet resuspended in 300 µl ATL buffer, which is present in the DNeasy tissue kit (Qiagen, Venlo, Netherlands). DNA was extracted from 100 µl sample using the DNeasy tissue kit according to the instructions of the manufacturer. The remaining sample (200 µl) was stored at -20°C for possible further analysis. All operations concerning sample treatment and PCR were performed in such a way that carryover contamination is minimized, i.e., by working in a class II biological safety cabinet (11).

Amplification of isolated DNA. Isolated DNA was amplified using primers M13 forward (5'-GTT TTC CCA GTC ACG AC-3') and M13 reverse (5'-CAG GAA ACA GCT ATG AC-3') and the Expand long-template PCR system (Roche, Almere, Netherlands). Briefly, 10 µl of the extracted sample, a volume which was shown to be efficiently amplified without inhibition, was added to a PCR master mix consisting of 1× Expand long-template buffer 2 containing 2.75 mM MgCl₂, 1.2 mM deoxynucleoside triphosphates (dNTPs), 1 µM primers M13 forward and M13 reverse, and 2.5 U Expand enzyme mix composed of thermostable *Taq* DNA polymerase and *Tgo* DNA polymerase, a thermostable DNA polymerase with proofreading activity. Samples were denatured for 4 min at 94°C and subjected to 40 cycles (94°C for 15 s, 50°C for 30 s, and 72°C for 3 min), followed by an 8-min extension at 72°C. PCR products were analyzed by agarose gel electrophoresis.

IAC. To obtain controlled PCR performance conditions, an internal amplification control (IAC) DNA was included in the PCR. The IAC was synthesized by cloning an M13 PCR amplification product of *Arabidopsis thaliana* of approximately 1,500 bp in the pBAD-TOPO vector. To exclude false-negative results in case of a negative M13 PCR result, the M13 PCR was repeated using a master mix containing 1 µl of the IAC at a concentration that did not interfere with amplification of the target DNA.

Spiking experiments on bench surfaces. To be able to optimize the method and determine several performance characteristics, spiking experiments on bench surfaces were performed. To be able to perform spiking experiments with plasmids that contain M13 amplification products of different sizes, M13 amplification products of *Echinostoma* fragments of 550 bp and 1,150 bp were cloned into pBAD-TOPO, resulting in M13 PCR amplification products of 800 bp and 1,400 bp, respectively. As a control, a plasmid without an insert was generated, resulting in M13 PCR amplification products of 250 bp. Plasmids were transformed in *E. coli* strain JM109, resulting in GMOs generating M13 amplification products of 250 bp (*E. coli* M13-250), 800 bp (*E. coli* M13-800), or 1,400 bp (*E. coli* M13-1400). Overnight cultures of these GMOs in 500 µl Luria-Bertani (LB) medium (4) were spread on surfaces of 100 cm² and were incubated for 3 h. Dilutions of the overnight cultures were plated onto LB-ampicillin plates to be able to determine the concentration of the culture used. When surfaces were dry, swab samples were taken, processed to isolate the DNA, and analyzed for the presence of plasmid DNA containing M13 primer binding sites by PCR.

Sampling of contained-use laboratories. Three different contained-use laboratories at different research institutes in the Netherlands were sampled as described above. At lab 1, 8 samples were taken from different objects present in the laboratory; at lab 2 and lab 3, 15 and 11 samples were taken, respectively (see Table 2). Of the samples obtained in every laboratory, one sample was a swab of a deliberate spill. Table 1 summarizes the sampled objects. At lab 3, one sample was obtained from outside the contained-use laboratory. To include a negative control, two samples were obtained from a laboratory where no GMO work was performed, one from a centrifuge and one from the display of a DNA extraction machine.

Cloning and sequencing of PCR products. Because several different GMOs may be present in a swab sample, all PCR products that were present as distinct bands of >250 bp on a 2% agarose gel were excised from the gel, purified by using a QIAquick gel extraction kit (Qiagen, Venlo, Netherlands), and treated

TABLE 1. Overview of sampled objects and the number of detected sequences

Sampled object	No. sampled	No. (%) positive	No. of sequences/ sampled object
Centrifuge	6	6 (100)	1-5
Sink	3	3 (100)	1-5
Bin	2	2 (100)	1-4
Incubator	1	1 (100)	4
Biosafety cabinet	1	1 (100)	3
Balance	1	1 (100)	3
Telephone	1	1 (100)	2
Spectrophotometer	1	1 (100)	1
Outside containment	1	1 (100)	1
Refrigerator	3	2 (67)	1-3
Pipette	4	2 (50)	1
Bench	5	2 (40)	1-3
Vortex	2	0 (0)	
Deliberate spill	3	3 (100)	1

with dNTPs and *Taq* polymerase (AmpliTaq) to add a deoxyadenosine to the 3' end in a template-independent way. Products were cloned into vector pBAD-TOPO or pCRII-TOPO (Invitrogen, Breda, Netherlands), which are supplied with single overhanging 3' deoxythymidine residues, allowing *Taq*-amplified PCR inserts to ligate efficiently with the vector. The enzyme topoisomerase is attached to the vector, enabling efficient ligation of vector and insert. Vectors were cloned into competent *E. coli* JM109 cells, and the resulting colonies were analyzed for the presence of an insert by PCR using M13 forward and reverse primers/T7 (TAA TAC GAC TCA CTA TAG GG) and SP6 primers (ATT TAG GTG ACA CTA TAG). If possible, eight clones of each PCR product were sequenced by using the BigDye Terminator cycle sequencing ready reaction kit (Applied Biosystems, Nieuwerkerk a/d IJssel, Netherlands).

Data analysis. Nucleotide sequences without primer sequences were compared with sequences present in GenBank, using the basic BLAST program at the website of the National Center for Biotechnology Information (NCBI) (<http://www.ncbi.nlm.nih.gov/BLAST/Blast.cgi>) to identify individual clones. The sequence analysis tools were set as follows: nucleotide query BLASTn, search set "others" and "nucleotide collection (nr/nt)," and program selection for somewhat similar sequences "blastn."

RESULTS

Detection of genetically modified microorganisms containing M13 primer binding sites. To determine whether GMO spills in contained-use facilities can be detected by taking swab samples from surfaces and amplifying swabbed microorganisms/DNA by an M13 PCR, several performance characteristics of the method were determined, such as detection efficiency, recoveries, storage conditions, and effect of interfering flora present on the surface.

To determine the detection efficiency of the method, 500 µl of 10-fold serial dilutions of overnight cultures of *E. coli* M13-250, *E. coli* M13-800, and *E. coli* M13-1400 were spiked on surfaces of 100 cm² on the bench to be swabbed and further processed (Fig. 1), as well as plated directly onto LB-ampicillin agar plates to count the number of CFU in the dilution used for spiking the surfaces. Upon processing and PCR detection, the numbers of PCR-detectable units (PDU) were estimated as most probable numbers by endpoint dilution (19). Mean concentrations from several experiments were used to estimate the difference between the numbers of CFU and PDUs and thereby the detection efficiency. The detection efficiency was estimated for the three different pBAD-TOPO-containing *E. coli* strains and varied from 0.01 CFU for *E. coli* M13-1400 to

FIG. 1. Schematic representation of the consecutive steps taken in the detection of M13-containing DNA sequences on surfaces.

0.004 CFU for *E. coli* M13-800, with a mean detection efficiency of 0.007 CFU.

To estimate overall recoveries, 500- μ l portions of overnight cultures of *E. coli* M13-250, *E. coli* M13-800, and *E. coli* M13-1400 were either spiked on surfaces of 100 cm² on the bench to be swabbed and further processed (Fig. 1) or transferred to 13 ml of ultrapure water directly. The detection was three times more efficient when the culture was analyzed directly, indicating that the recovery by this method for the isolation and detection of plasmid DNA from swabbed surfaces was around 30%.

Similar spiking experiments were performed to study whether cooled transportation from the field laboratory to the laboratory analyzing the samples is required by incubating swabbed samples at 4°C or at room temperature for a period of 12 h. The incubation temperatures did not detectably influence the results (data not shown). Furthermore, it was demonstrated that a storage time of 72 h at 4°C did not have detectable adverse effects.

To study whether the detection of *E. coli* DNA decreased rapidly after spiking surfaces with live bacteria, surfaces were swabbed after different incubation periods of up to 5 weeks. The efficiency of detection of *E. coli* DNA decreased by less than 1 log₁₀ unit after an incubation period of 5 weeks on surfaces (data not shown).

By swabbing, not only DNA of the GMO of interest will be collected but also DNA from any organism present, whether genetically modified or not. Therefore, the effect of *E. coli* M13-250 on the detection of plasmid DNA of either *E. coli* M13-800 or *E. coli* M13-1400 was studied at different dilutions. A 250- μ l portion of a 10⁻⁶ dilution of an overnight culture of *E. coli* M13-800 or *E. coli* M13-1400 was added to 250 μ l of 10-fold serial dilutions (10⁻¹ to 10⁻⁹) of an overnight culture of *E. coli* M13-250. Mixtures were spiked on a surface of 100 cm² and processed as described above. As shown in Fig. 2, both *E. coli* M13-800 and *E. coli* M13-1400 were detected in the presence of a 100-fold excess of *E. coli* M13-250. Repeating this experiment with an *E. coli* strain without an M13-containing plasmid revealed that *E. coli* M13-800 and *E. coli* M13-1400 were detected in a >10⁷ times excess of this *E. coli* strain, indicating that adverse effects of the presence of non-M13-carrying plasmids were negligible.

Survival of *E. coli* on surfaces. As the aim of this study was to develop a method to gain information on laboratory hygiene in contained-use laboratories, it was preferable that viable microorganisms should be detectable instead of plasmid DNA. To study whether not only DNA but also viable GMOs were isolated by this method, swab samples were taken from spiked surfaces as described above. However, instead of incubating the swab in 13 ml of ultrapure water, LB medium was added to the swab. After centrifugation to concentrate bacteria into a smaller volume, samples were incubated overnight at 37°C. No viable bacteria were detected after drying of surfaces for not more than 3 h (data not shown). Thus, survival of *E. coli*, here the host strain JM109, on surfaces in general may be very limited, and detection of viable GMOs may be hampered.

FIG. 2. Effect of *E. coli* M13-250 on the detection of plasmid DNA of either *E. coli* M13-800 or *E. coli* M13-1400. A 250- μ l portion of a 10⁻⁶ dilution of an overnight culture of *E. coli* M13-800 or *E. coli* M13-1400 was added to 250 μ l of 10-fold serial dilutions (10⁻¹ to 10⁻⁹) of an overnight culture of *E. coli* M13-250. Mixtures were spiked on a surface of 100 cm² and processed as outlined in Fig. 1.

TABLE 2. Sequences detected in each sampled contained-use laboratory

Lab	Swabs	No. of:										
		Sequences detected				Sequences detected in the following no. of swabs:						
		Total	Coding	Vector	Unknown ^a	1	2	3	4	5	6	7
1	8	4	2	1	1	4	NO ^b	NO	NO	NO	NO	NO
2	15	13	9	3	1	6	1	2	2	1	NO	1
3	11	11	7	3	1	7	4	NO	NO	NO	NO	NO
Total	34	28	18	7	3	17	5	2	2	1	NO	1

^a No similar sequences detected in published databases.^b NO, not observed.

Analyses of field samples obtained from three different contained-use laboratories. In total, 34 swab samples obtained from three different contained-use laboratories were analyzed by the method described in Fig. 1. In addition, two swab samples that were obtained from a laboratory where no GMO work was performed were analyzed to serve as negative controls. Following DNA extraction, M13 PCRs were performed. When no PCR fragments were detectable upon agarose gel electrophoresis, M13 PCRs were repeated in the presence of an IAC to check for inhibition and possible false-negative results. No inhibitory effects on PCR performance could be demonstrated, as IAC signals were observed in all reactions where no target DNA was detected. PCR products were obtained from 26 of 34 samples (Table 1), including 3 samples taken from surfaces with deliberate spills obtained from the three different laboratories. PCR products varied in size from 280 bp to 3,000 bp. M13 PCR products smaller than 250 bp were not taken into account, because these products were shown to contain mainly multiple-cloning sites, which are commonly present between the M13 forward and reverse primers in cloning vectors (data not shown).

Samples taken at lab 1 were used to determine the reproducibility of the method. The analysis of duplicate DNA extractions per sample, followed by five replicates of the M13 PCR per extracted DNA, revealed that the 10 replicates gave identical PCR products in two of the four PCR-positive swabs. These swabs were obtained from a centrifuge rotor and from the deliberate spill on the laboratory bench. In the remaining two PCR-positive samples, only 1 or 2 of the 10 replicates gave a PCR product (the samples were obtained from the laboratory bench close to the deliberate spill and from the freezer door, respectively), suggesting that the number of positive replicates may be indicative of the level of contamination.

PCR products obtained from the swabs taken at the three contained-use laboratories were cloned separately in either a pBAD-TOPO or pCR-II vector. A maximum of eight clones of each cloned PCR product were amplified by M13 primers or T7/SP6 primers, depending on the vector used. Depending on the cloning efficiency, at least one of the clones of every PCR product was sequenced and analyzed by a BLAST search for the presence of similar sequences in GenBank. Twenty-eight DNA sequences were obtained from 26 PCR-positive samples. The number of different sequences generated per swab varied from one to five. Most sequences (up to five per sample) were detected in swabs from the centrifuge and the sink, followed by swabs taken from the bin and incubator (up to four sequences

per sample) (Table 1). One sample was taken outside the containment area, and one DNA sequence was detected in this sample. Therefore, negative controls were taken at an institute where no GMO activities were ever performed.

The obtained sequences varied in length from 171 nucleotides (nt) to 878 nt. In most cases, sequences were only partially similar to sequences published in GenBank. The lengths of the regions with high similarity varied from 94 nt to 795 nt, and these similarities ranged from 81% to 100%. After the identification of the relatively large fragment with the highest similarity, which was usually located at the central part of the cloned sequence, a BLAST search on the 3' and 5' flanking sequences was done separately. Eighteen sequences were shown to be coding sequences, and seven sequences were highly similar to vector sequences that may be used for expression in host cells (Table 2). From three sequences of 280 nt, 565 nt, and 581 nt, no similarities were found in the published databases. Of the total of 28 identified sequences, 17 were detected once in a swab sample and 5 were present in two different swabs (Table 2). One sequence was detected in seven different swabs and one in five different swabs. Both sequences were obtained from lab 2, indicating that the two plasmids carrying those sequences were abundantly present in this laboratory. The remaining four sequences were detected in three or four different swabs. Many small fragments of the identified sequences, which varied in length from approximately 50 to 100 nt, were found to be highly similar to sequences in GenBank but did not give specific information on their possible identity. These noninformative sequences comprised general vector sequences, such as multiple-cloning sites, but regulatory subunits, 3' untranslated regions (UTRs) of partial genes, and rRNA sequences also were detected. These sequences were not included in the 28 detected sequences. A schematic overview of how to determine similarities of the obtained sequences with GenBank sequences is given in Fig. 3.

Comparison of detected sequences with contained-use activities in field laboratories. Twenty-one (lab 1, three; lab 2, nine; lab 3, nine) of the 25 sequences (84%) that showed a high similarity with sequences in GenBank were known to have been used in the sampled contained-use laboratories. In all three swabbed deliberate spills, DNA sequences were detected. In two of them, the spilled DNA sequences were detected. In one of the swabs a sequence which was used in the sampled laboratory was detected, but the sequence of the deliberate spill was not detected. By doing a BLAST search, fragments of 2 of 25 (8%) sequences showed similarities with

FIG. 3. Schematic representation of the steps needed for the interpretation of sequencing results, from the BLAST search in public databases (GenBank) to determination of the meaning of the presence of certain sequences.

GenBank sequences that were not known by the workers in the sampled laboratory. However, comparing the detected sequences with a lab-specific database revealed that fragments of the detected DNA were identical to DNA sequences used in the studied laboratory. In the three sampled contained-use laboratories, no cloning of uncharacterized sequences was performed, so the origin of the three sequences without similarities in the public databases remained unclear.

DISCUSSION

Swabbing of commonly used surfaces, such as laboratory benches, doors of refrigerators, and rotors or lids of centrifuges, and subsequent detection and identification of DNA present was shown to be an adequate tool to gain information on laboratory hygiene and the efficacy of decontamination and cleaning procedures. Swabbing in contained-use laboratories and subsequent amplification and sequencing of the swabbed material by M13 PCR demonstrated that DNA sequences were detected in 28 of 34 swabs. Identification of DNA sequences was shown to be feasible without prior knowledge of the identity or characteristics of the GMOs that were handled in the laboratory.

To study the feasibility of the method, four different labs were sampled, i.e., three contained-use laboratories and one laboratory where no GMO activities were performed. In the samples taken at the last lab, no PCR products and thus no sequences were obtained, demonstrating the specificity of the method. In the 34 swabs that were sampled in the contained-use laboratories, 28 different sequences were detected, which may be informative on the activities performed in the studied

laboratories. Of these sequences, 84% were actually used in these laboratories, whereas 8% were initially identified as coding sequences that were not known by the studied laboratory. However, reanalysis of these sequences by comparing them with a lab-specific database revealed that fragments of the detected DNA were identical to DNA sequences used in the studied lab. In these 8% of the samples, a more detailed BLAST search could have improved the success rate of the method to 92%. An inventory of the activities performed in the labs revealed that at the time of sampling, activities were indeed performed with the detected sequences, indicating that decontamination and cleaning after the laboratory activities were finished were insufficient to remove all traces of GMOs. Sequences that were isolated from two or more different swabs were in all cases DNA sequences which were used by the laboratories. The sequences that were not known by these labs were in all cases detected once.

No correlation between the most commonly used DNA sequences and the detected sequences was present. This may indicate that spoilage during lab activities does not occur with every lab worker to the same extent or that cleaning efficiencies vary between lab workers. Most DNA sequences were acquired by sampling different components of the centrifuge, such as the lid, rotor, or display, and the sink. Contamination of the centrifuge was not unexpected, because it is a frequently used apparatus in which small accidents occasionally occur, such as the opening of the lid of a centrifuge tube. The frequent detection of up to five different sequences revealed that decontamination and/or cleaning after such incidents may be improved. Contamination of the sink was less expected, because discarding biological materials in the sink is not allowed. The sink was sampled in two of the three labs, and in both labs DNA sequences were detected. In the two samples of the vortex, no DNA was detected, suggesting that this contamination-prone apparatus is more regularly cleaned than the centrifuge. Whether this is indeed the case could not be confirmed by the labs.

A comparison of different disinfection and cleaning products revealed that the most efficient removal of plasmid DNA with $>4 \log_{10}$ units was obtained with 70% ethanol and hypochlorite concentrations of at least 3% (data not shown), as has been shown by others as well (10), indicating that depending on the level of contamination, DNA may still be present after cleaning. Due to the increase in spread of nosocomial pathogens, interest in decontaminants for surfaces is increasing, resulting in a surplus of products claiming microbicidal activities (16). Beekes et al. (3) described a broad-range disinfection formulation of 0.2% SDS and 0.3% NaOH in 20% *n*-propanol (pH 13.0 ± 0.05) that exerted a strong disinfecting activity against bacteria, fungal spores, nonenveloped viruses, and prions. This formulation was also effective in the presence of protein-rich matrices such as coagulated blood. However, whereas wiping is often an integral part of surface decontamination, products meant for this purpose are rarely assessed with the physical effect of wiping incorporated. Moreover, ideally the microorganism is not only inactivated by the decontamination procedure but also removed, because remaining DNA may be of concern during PCR and cloning activities (17).

PCR products may be obtained either from plasmid DNA present in bacteria, either viable or not, or from naked DNA

on surfaces. In a study of viability of the detected microorganisms, experiments performed on artificially contaminated surfaces revealed that the weakened *E. coli* strain JM109 was not detectable 3 h after spiking of the surfaces. This indicates that these GMOs were rapidly inactivated during the suboptimal conditions on surfaces, hampering the detection of viable GMOs (20). With this knowledge in mind, no attempt was made to isolate cultivable GMOs from the field samples obtained from the contained-use laboratories studied. However, the method aims to select for relatively large particles by spinning down biological material at 4,500 rpm by centrifugation (Fig. 1). Plasmid DNA will remain in the supernatant, and because the pellet is used for further analysis, naked plasmid DNA will not be detected, suggesting that detected plasmid DNA may be obtained from an (inactivated) actual GMO.

Identification of the detected sequences was done by BLAST search, using the "blastn" program selection, indicating that besides homologous sequences, sequences with fewer similarities also could be detected. Indeed, sequences with maximum similarities varying from 81% to 100% were detected. Only in the case of 100% homology are DNA sequences identified. If similarities are lower, search results may give an indication of the identity but certainly no decisive answer. Which minimal similarity is required to give an indication of laboratory activities is difficult to state in general. This will depend mainly on the function of a coding sequence; if a sequence encodes a functional protein which is highly conserved, a high similarity is needed for true identification. Alternatively, if the sequence encodes, for example, variable domains in the heavy chain of an antibody, much lower similarities may give information on the identity of the detected DNA. Furthermore, the longer the fragment that gives homologous results, the more informative the sequence will be. However, this does not mean that small homologous sequences are never informative. To make the interpretation more complicated, several fragments of one sequence appeared to show similarities with different sequences in the public databases. In lab 2, a sequence of 800 nt was detected that had similarities with different sequences in the public databases in five different regions of the sequence, which were partially overlapping. Moreover, there is no restriction on what is submitted to GenBank, so all information from these databases should be interpreted with care. This demonstrates that experience in sequencing analysis and BLAST searching is required for the correct interpretation of detected sequences.

In Europe, a license is required to produce and work with genetically modified organisms (GMOs), as is prescribed by EU directives 98/81/EC and 2001/18/EC (1, 2). In the Netherlands, this license has to be obtained by the organization where the operations with GMOs will be performed from the Dutch Ministry of Housing, Spatial Planning and the Environment. The regulations for producing and working with such GMOs aim to protect both human health and the environment from negative effects by use of control measures such as containment. The license holder has to follow the level of physical containment as imposed in the license, which is based on a risk assessment that takes into account the impact of an accidental

release on the environment and public health. The current method enables the detection of sequences that were present in the contained-use laboratory. Thus, besides information on laboratory hygiene, the method may also give information on which GMO activities may have been performed in the laboratory, which may be informative for the contained-use license holders and authorities.

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REFERENCES

1. Anonymous. 1998. Council directive 98/81/EC. Offic. J. Eur. Commun. L **330**:13–31. http://www.biosafety.be/PDF/98_81.pdf. Accessed 22 July 2010.
2. Anonymous. 2001. Council directive 2001/18/EC. Offic. J. Eur. Commun. L **106**:1–38. http://www.biosafety.be/PDF/2001_18.pdf. Accessed 22 July 2010.
3. Beekes, M., et al. 2010. Fast, broad-range disinfection of bacteria, fungi, viruses and prions. *J. Gen. Virol.* **91**:580–589.
4. Bertani, G. 1951. Studies on lysogenesis. I. The mode of phage liberation by lysogenic *Escherichia coli*. *J. Bacteriol.* **62**:293–300.
5. Duizer, E., et al. 2004. Inactivation of caliciviruses. *Appl. Environ. Microbiol.* **70**:4538–4543.
6. Elbing, K., and R. Brent. 2002. *E. coli*, plasmids and bacteriophages, p. 1.14.1–1.14.5. In F. M. Ausubel, R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith, and K. Struhl (ed.), *Current protocols in molecular biology*. John Wiley and Sons, Inc., New York, NY.
7. Eterpi, M., G. McDonnell, and V. Thomas. 2009. Disinfection efficacy against parvoviruses compared with reference viruses. *J. Hosp. Infect.* **73**:64–70.
8. Girard, M., S. Ngazoa, K. Mattison, and J. Jean. 2010. Attachment of norovirus to stainless steel and their inactivation, using household disinfectants. *J. Food Prot.* **73**:400–404.
9. Jeggo, M. 2007. The changing world of biocontainment and biosecurity. *Dev. Biol. (Basel)* **128**:47–51.
10. Kemp, B., and D. G. Smith. 2005. Use of bleach to eliminate contaminating DNA from surface of bones and teeth. *Forensic Sci. Int.* **154**:53–61.
11. Kwok, S. H. R. 1989. Avoiding false positives with PCR. *Nature* **339**:237–238.
12. Messing, J. 1983. New M13 vectors for cloning. *Methods Enzymol.* **101**:20–78.
13. ProMED-mail. 2004. Laboratory safety & disease dissemination (02). ProMED-mail 20040606.1527. <http://www.promedmail.org>. Accessed 19 July 2010.
14. ProMED-mail. 2004. Laboratory safety & disease dissemination: request for information. ProMED-mail 20040601.1487. <http://www.promedmail.org>. Accessed 19 July 2010.
15. ProMED-mail. 2007. Foot & mouth disease—UK (England): possible accidental release. ProMED-mail 20071126.3829. <http://www.promedmail.org>. Accessed 19 July 2010.
16. Sattar, A. A. 2010. Promises and pitfalls of recent advances in chemical means of preventing the spread of nosocomial infections by environmental surfaces. *Am. J. Infect. Control* **38**:S34–S40.
17. Stals, A., et al. 2009. Laboratory efforts to eliminate contamination problems in the real-time RT-PCR detection of noroviruses. *J. Microbiol. Methods* **77**:72–76.
18. Werkgroep Infectiepreventie. 2004. Beleid reiniging, desinfectie en sterilisatie. http://www.wip.nl/free_content/richtlijnen/11beleid%20reiniging%20desinfectie%20en%20sterilisatie.pdf. Accessed 7 December 2010.
19. Westrell, T., et al. 2006. Short- and long-term variations of norovirus concentrations in the Meuse river during a 2-year study period. *Water Res.* **40**:2613–2620.
20. Williams-Smith, H. 1978. Is it safe to use *Escherichia coli* in recombinant DNA experiments? *J. Infect. Dis.* **137**:655–660.