RESEARCH ARTICLE

DNA decontamination methods for internal quality management in clinical PCR laboratories

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Background: The polymerase chain reaction (PCR) technique, one of the most commonly applied methods in diagnostic and molecular biology, has a frustrating downside: the occurrence of false-positive signals due to contamination. In previous research, various DNA decontamination methods have been developed to overcome this limitation. Unfortunately, the use of random or poorly focused sampling methods for monitoring air and/or object surfaces leads to the incomplete elimination during decontamination procedures. We herein attempted to develop a novel DNA decontamination method (environmental surveillance, including surface and air sampling) and quality management program for clinical molecular diagnostic laboratories (or clinical PCR laboratories).

Methods: Here, we performed a step-by-step evaluation of current DNA decontamination methods and developed an effective procedure for assessing the presence of decontaminating DNA via PCR analysis. Performing targeted environmental surveillance by sampling, which reached optimal performance over 2 weeks, and the decontamination process had been verified as reliable. Additionally, the process was validated to not affect PCR amplification efficiency based on a comparative study.

Results: In this study, effective guidelines for DNA decontamination were developed. The method employed ensured that surface DNA contamination could be effectively identified and eliminated. Furthermore, our study highlighted the importance of overall quality assurance and good clinical laboratory practices for preventing contamination, which are key factors for compliance with regulatory or accreditation requirements.

Conclusions: Taken together, we provided the evidence that the presented scheme ranged from troubleshooting to the elimination of surface contamination, could serve as critical foundation for developing regular environmental surveillance guidelines for PCR laboratories.

KEYWORDS

DNA contamination, DNA decontamination, environmental surveillance, internal quality management

1 | INTRODUCTION

Polymerase chain reaction (PCR) has been developed to identify multiple pathogens in clinical specimens, which has revolutionized diagnostic approaches in a variety of fields. However, the high sensitivity of

PCR has a corresponding limitation: the possibility of amplifying non-specific products, creating so-called DNA contamination, leading to false-positive results.¹ When a pathogen's nucleic acids are falsely recorded as present in a clinical molecular biology laboratory, therapeutic decisions could be affected. DNA contamination can originate from

several different sources, and it is problematic when high copy numbers of DNA exist in the air or on the surfaces of objects in a laboratory.

Different contamination sources require different methods of treatment. DNA contamination of laboratory surfaces and instruments from various sources can occur at any stage of an operation and can be avoided by ensuring that rooms stay clean and by using decontamination methods, such as UV-irradiation² and application of disinfectants containing alcohol and chlorine. Amplicons from the most common sources of contamination are produced at very high copy numbers during PCR and can seriously impact diagnostic analysis because they can be homologous to target molecules and amplified at high efficiency, resulting in carry-over contamination.³ Contamination of PCR reagents⁴ and DNA extraction kits is another major problem when broad-range primers, such as those used for bacterial 16s rDNA, are used for detection in clinical specimens.

In recent years, various DNA decontamination methods have been developed to try to solve this problem, including the use of UV⁵ or γ -irradiation, hydroxylamine hydrochloride or ethidium monoazide treatment, exonuclease III or endonucleases⁶ treatment (such as DNase I^{7,8} or restriction enzymes), and, autoclaving or the use of radical-based gas generator decontamination.⁹ Currently available decontamination methods often utilize oxidative reagents and UV irradiation in clinical PCR laboratories. Unfortunately, these methods do not completely eliminate DNA contamination and show inconsistencies in their decontamination results.¹⁰ Most importantly, these treatments do not effectively eliminate very low-molecular-weight DNA fragments.¹¹ Thus, there is a need for reliable and efficient decontamination methods that can be easily integrated into the SOPs of clinical PCR laboratories.

To ameliorate the risks associated with false-positive PCR results in clinical PCR laboratories, we have developed a successful decontamination method that is based on regular monitoring of DNA contamination by sampling the environment and air. Internal quality control (IQC) is key to the effectiveness of a clinical PCR laboratory and should be an indispensable component of the SOPs for the laboratory. IQC is also very important for maintaining the reliability of reports produced from a laboratory and, in turn, the reputation of the laboratory. A reliable program for contamination surveillance is essential for the development of new molecular detection techniques and should also serve as the important basis for the development of molecular diagnostic techniques.

Here, we describe an innovative method capable of effectively eliminating contamination. In addition, we describe the importance of instituting a clinical PCR laboratory quality management program for monitoring air and surface contamination. Finally, we provide suggestions for improvements to such programs for clinical molecular diagnostic laboratories.

2 | MATERIALS AND METHODS

2.1 | Sampling locations

The present study was performed in the clinical PCR laboratory of the Fourth Affiliated Hospital Zhejiang University School of Medicine in

Zhejiang Province of China, from May 24th to June 7th of 2015. The clinical PCR laboratory has four transfer windows, four buffer wards and four rooms, which are divided according to function into rooms for reagent preparation (first room), specimen handling (second room), gene amplification (third room), and product analysis (fourth room). Air sampling was performed at four locations in the first and second rooms, and the samples collected from the rooms were individually stored in a biosafety cabinet. Samples from the third and fourth rooms, the transfer windows and the four buffer wards were collected at two sites. Each area was sampled over 3 days to determine the aerosol contaminant levels. Therefore, a total of 72 samples were analyzed for airborne contaminant levels, in addition to six negative controls. Additionally, to understand the patterns of how potential airborne contaminants settled on the surfaces of the laboratory, 129 surface samples were collected from 27 predetermined sampling locations among the four operation rooms at four different time points.

2.2 | Environmental sampling

To ensure the elimination of carry-over contamination, the air and surfaces of the PCR laboratory were sampled to measure the degree of contamination. As the four rooms in the PCR laboratory have different functions, different numbers of air samples were collected in different rooms. To sample the air, a plate with a 9-cm diameter that contained 2 mL of 0.9% sodium chloride solution was left open for 30 minutes. To sample object surfaces, sterile swabs moistened with saline were used to swab an approximate 10 cm×10 cm area. After this, the swabs were individually placed into sterile tubes containing 2 mL of 0.9% sodium chloride solution. The above procedure was developed in reference to current Chinese hospital disinfection hygiene standards (GB 15982-2012). 200 μ L of each sample was used. Environmental surveillance was performed every 3 days.

2.3 | Fluorescent PCR assessment

PCR experiments were performed in a manner consistent with that used in our laboratory (namely, quantitative PCR was used to detect hepatitis B DNA (HBV DNA)) and using the same reagent kits (DaAnGene, Guangzhou, China). PCR reactions were conducted using an ABI StepOne Plus Instrument (Applied Biosystems, Life Technology, ThermoFisher Scientific, Waltham, MA, USA). C_t (cycle threshold) values and amplification efficiency values were calculated using StepOne Plus Software version v2.2.2, (Applied Biosystems, Life Technology, ThermoFisher Scientific) as supplied by the manufacturer. The collected environmental samples were used as templates for HBV DNA amplification via quantitative PCR according to the kit manufacturer's instructions.

2.4 | Decontamination of surfaces and equipment

DNA amplicons present in the environment can cause widespread contamination of air, working surfaces, equipment, and personnel.

Referring to current Chinese standards for disinfection techniques in healthcare settings (WS/T 367-2012, China), we used a hypochlorite solution to achieve high-level disinfection, alcohol to achieve medium-level disinfection and UV irradiation to achieve decontamination. The following procedures for DNA decontamination were employed: (1) spraying a 75% ethyl alcohol solution into the air before cleaning the rooms; (2) irradiating the rooms with UV-light for 1 hour; (3) wiping objects and equipment in the rooms with a hypochlorite solution to remove settled particles; (4) wiping equipment, such as disassembled centrifuge rotors or PCR instruments, with absolute ethyl alcohol; and (5) disinfecting laboratory coats before starting a new experiment. Note that separate sets of cleaning tools were used for each room and were not mixed. These steps were carried out twice per day for approximately 2 weeks.

2.5 | Assessment of the effectiveness of the DNA decontamination procedure

Comparison with a reference laboratory was performed after using the above-described DNA decontamination for 2 weeks. The comparison was based on the quantitative detection of HBV DNA. The procedure used was the same as that used for the PCR experiments described above. We selected 20 samples containing HBV DNA levels ranging from less than 100 IU/mL (international units/per milliliter; the lower detection limit of the reagent kit used; considered a negative result) to 10^8 IU/mL (the upper detection limit of the reagent kit used; considered a positive result) for the comparison. Statistical comparisons of the collected results were performed. A coincidence rate (%) of >90% was used for comparisons to the External Quality Assessment (EQA) evaluation criteria from the National Health and Family Planning Commission of the People's Republic of China. Log-transformed values of the data were used, and data within the range of ± 0.4 ($\pm$ standard deviation value) from the standard were linear. An R^2 value >.9 following correlation analysis was considered to indicate a relatively high correlation.

2.6 | Statistical analysis

Statistical analysis was performed using Microsoft Excel (Microsoft, Redmond, WA, USA) and SPSS 19.0 (IBM, Armonk, NY, USA). Data were analyzed using *t* tests, and similarity analysis was performed using R^2 values and Pearson correlation coefficients. A *P* value <.05 was considered statistically significant, and all tests used for statistical analysis were 2-sided.

3 | RESULTS

3.1 | Determining contamination sources

The background for this study was a DNA contamination incident that occurred in May 2015. The DNA contamination in this incident resulted from a non-standard process performed by cleaning staff. We later determined that optimized management procedures

TABLE 1 PCR assessment of aerosol samples

Locations		PCR results (C_t values)		
		1st	2nd	3rd
First room (n=6) ^a	Room (n=2)	30 ^b	30 ^b	30 ^b
	Biosafety cabinet (n=2)			
	Transfer window I (n=2)			
Second room (n=10) ^a	Room (n=2)			
	Biosafety cabinet (n=2)			
	Transfer window I (n=2)			
	Transfer window II (n=2)			
Third room (n=4) ^a	Room (n=2)			
	Buffer ward (n=2)			
Fourth room (n=4) ^a	Room (n=2)			
	Buffer ward (n=2)			
Negative control (n=2) ^a	0.9% sodium chloride broth (n=1)			
	Outside the room (n=1)			

^aNumbers of tests.

^b30 of C_t values means a non-amplified result.

are not a solution for preventing contamination of the clinical PCR laboratory. Due to this incident, we initiated emergency plans and transferred the affected experiment to another laboratory. DNA contamination in clinical PCR laboratories can result from the sources of reagents used as well as air and surface contamination (a problem that is especially prevalent in clinical PCR laboratories). In addition, automation of nucleic acid extraction is unpopular in clinical PCR laboratories. In the above-described incident, it was necessary to identify the contamination and determine its location. To accomplish this, hundreds of samples, including 72 air samples and 129 surface samples as well as negative controls, were collected to identify the contamination source. Additionally, the air quality (Table 1) and characteristics of object surfaces (Table 2) were monitored based on guidelines for nosocomial infection surveillance procedures.

3.2 | Identification of PCR amplicon contamination

First, we discarded any reagents that had been opened. Second, we identified the rooms that had been, contaminated by subjecting samples from the first and second rooms and non-template controls to PCR to measure HBV DNA amplification. To accomplish this, we added PCR buffer into two ABI MicroAmp Optical 8-tube strips in the first room. In the second room, one strip was left open and the other strip was capped with an 8-cap strip. HBV DNA amplification occurred in both sets of tubes, which suggested that both rooms were contaminated with HBV DNA. As the contamination could have occurred either through in the air or as a result of surface contamination, we used special methods to assess the remaining DNA.

TABLE 2 Locations of surface sampling

Locations	First room (n=9) ^a	Second room (n=18) ^a	Third room (n=9) ^a	Fourth room (n=5) ^a	Lobby wards (n=3) ^a
1	Doorknob	Biosafety cabinet (outside)	Doorknob	Doorknob	Doorknob (front)
2	Operation desk	Biosafety cabinet (inner)	Operation desk	Operation desk	Doorknob (back)
3	Floor	Removable UV car	Floor	Floor	Switches
4	Biosafety cabinet(outer)	Eppendorf guns	PCR instrument (outer)	Instruments	Negative contron 1
5	Biosafety cabinet (inner)	Guns rack	PCR instrument (inner)	Removable UV car	Negative contron 2
6	Removable UV car	Tip boxes	Rack	Negative contron 1	—
7	Eppendorf guns	EP tube boxes	Removable UV car	Negative contron 2	—
8	Transfer window I	Metal bath	Calulator	—	—
9	Refrigerator	Centrifuge(outer)	Office supplies	—	—
10	Negative contron 1	Centrifuge(inner)	Negative contron 1	—	—
11	Negative contron 2	Mini-centrifuge	Negative contron 2	—	—
12	—	Mixer	—	—	—
13	—	Transfer window I	—	—	—
14	—	Transfer window II	—	—	—
15	—	4°C refrigerator(inner)	—	—	—
16	—	−20°C refrigerator(inner)	—	—	—
17	—	Refrigerator(outer)	—	—	—
18	—	Timer	—	—	—
19	—	Negative contron 1	—	—	—
20	—	Negative contron 2	—	—	—

^aNumbers of tests.

PCR of air samples produced no amplification (Table 1); however, environmental DNA contamination on object surfaces was found (Figure 1).

3.3 | Surface decontamination procedure

In the following decontamination procedure, we focused on environmental decontamination, and target surveillance was performed after decontamination. Established methods were used to detect pathogens in the environment in each of the four rooms. A greater number of sampling locations was used in the first and second rooms compared to the remaining rooms. Enrichment procedures were used to improve detection sensitivity, and surface contamination was detected using plates filled with medium or sterile swabs, which proved to be effective detection methods (Figure 1). The procedures described above were used to detect contaminating DNA, and the procedures described below were used to decontaminate the rooms. First, the air in the rooms was sprayed with a 75% ethyl alcohol solution before the rooms were subjected to UV irradiation. This approach increased the humidity in the rooms and maximized the DNA damage caused by the UV irradiation. Next, the objects and equipment in the rooms were wiped with a hypochlorite solution to eliminate surface contamination caused by settled particles. Some surfaces could not be wiped directly with the chemical solution, and other methods had to be used. For example, the hypochlorite solution would have corroded the disassembled centrifuge rotor and PCR instruments; therefore, absolute ethyl

alcohol was used to treat these objects, according to the manufacturers' recommendations. In addition, some supplies (e.g., tips and tubes) and tools were replaced, and some supplies were discarded, such as the Eppendorf automated pipettors used in the second room. These decontamination procedures were repeated two times per day for 2 weeks. Over this period, 129 samples were collected at four specified time points. C_t value was used as an index of DNA contamination and showed that the contamination decreased each day over the 2-week period. C_t value is inversely proportional to the concentration of target DNA. A C_t value of 30 indicates non-amplification of the target DNA. Although a few of the locations that were tested showed barely detectable levels of HBV DNA and produced C_t values of 30, most of the sampling locations showed obvious improvement following the decontamination. Generally, the mean HBV DNA levels in the sampled locations were lower after contamination than the mean levels before decontamination (Figure 1).

3.4 | Reliability of the surface decontamination method

Currently available laboratory decontamination methods, including UV irradiation and the use of chemical solutions, can damage equipment and other materials, which can in turn influence PCR amplification efficiency. We therefore required needed a reliable method for assessing the influence of our decontamination methods on PCR amplification.

FIGURE 1 Efficiency analysis of the surface decontamination in each location at four sampling times. The C_t values were used as an index and showed that DNA contamination decreased each day over the 2 week period. C_t values are inversely proportional to the concentration of the target DNA, and the greater the C_t value is, the lower the DNA concentration is. A C_t value of 30 indicates non-amplification of the target DNA. (A) Surveillance of surface decontamination during the reagent preparation (first room). (B) Surveillance of surface decontamination in the gene amplification (third room). (C) Surveillance of surface decontamination in the specimen handling room (second room). (D) Surveillance of surface decontamination during product analysis (fourth room). (E) Surveillance of surface decontamination in the lobby ward (Buffer ward)

FIGURE 2 Comparison of clinical samples between two laboratories. Statistical analysis of 15 positive samples was performed using *t* test. A *P*-value of .80 (significance threshold of $P > .05$) was considered to be a non-statistically significant difference. Similarity analysis results gave an R^2 value of .98, which implies high similarity. Pearson correlation analysis showed a high similarity between two groups, a correlation coefficient of .99 ($P < .01$)

To accomplish this, we analyzed 20 clinical specimens and compared the results with those produced by a reference PCR laboratory. Fifteen of the samples were considered positive (Figure 2), while the other five samples were considered negative because the range of amplified HBV DNA was below 100 IU/mL. Statistical comparison of the HBV amplification test results using similarity analysis produced an R^2 value of .98, which implies high similarity. Pearson correlation analysis also showed a high similarity between the two groups, with a correlation coefficient of .99 ($P < .01$). These data were analyzed in consideration of the EQA evaluation criteria for Zhejiang Province, and a coincidence rate (%) of $>90\%$ showed and that the two laboratories produced highly similar results. The obtained *P* value of .80 (significance threshold of $P > .05$) was considered statistically non-significant (Figure 2). These results imply that surface decontamination methods can be successfully applied to a PCR laboratory. In addition, we have written such procedures into SOPs for quarterly maintenance.

4 | DISCUSSION

In the present study, we described a method in which an episode of amplicon contamination in a clinical PCR laboratory was successfully resolved in 2 weeks. Air and surface samples were collected and analyzed four times over this period, and each sampling session was followed by routine cleaning and disinfection procedures on the same day. Finally, data showed that this method was effective. In addition, to ensure that the decontamination procedure did not alter PCR amplification efficiency, HBV DNA was amplified from the same set of samples in two different laboratories. The amplification results were not significantly different, indicating that the procedure did not cause damage that would affect subsequent PCR amplification. To the best of our knowledge, this is the first systematic study to report the

occurrence of amplicon contamination from environmental surfaces in a clinical PCR laboratory. Overall, we developed a surveillance and disinfecting protocol that was more effective than conventional DNA decontamination procedures.

Microbial surveillance of the environment has been used to monitor the presence of specific nosocomial pathogens and to evaluate the efficacy of routine cleaning and disinfection operations.¹² Assessments of hospital hygiene show that routine disinfection procedures might not be performed efficiently and that nosocomial pathogens may not be sufficiently reduced.¹³ Compared to bacterial culturing methods, there are differences in detecting viral DNA due to the difficulties associated with culturing viruses in vitro. In most clinical PCR laboratories, diagnostic detection of DNA/RNA for viruses such as HBV, HPV (human papillomavirus), influenza virus (H7N9, H1N1 or H3N2), human cytomegalovirus (CMV), and Epstein-Barr virus (EBV) is popular. Clinical PCR laboratories typically have viral DNA present, enabling the development of molecular methods to detect environmental contamination. PCR was primarily developed for in vitro diagnostic use for the identification of pathogenic microorganisms.¹⁴ Our group uses PCR to amplify HBV DNA as an indicator of contamination under such conditions.

Environmental surveillance, including sampling air and object surfaces, is an important component in preventing contamination in clinical PCR laboratories. Surveillance of airborne and surface viral DNA/RNA, especially HBV DNA, HPV DNA, and influenza H7N9 RNA, in our laboratory provides important information about aerosol or surface contamination, and is used periodically as a measure of quality control.¹⁵ Although many types of pathogenic microorganisms are common in hospitals, *Escherichia coli*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, and *Pseudomonas aeruginosa* are the most common pathogens associated with nosocomial infections. These organisms are genetically diverse, and novel sampling methods could be used to identify their presence. Surface swabbing has been used in numerous studies to sample the environment,¹⁶ and plates provide an effective method for sampling of air. In contrast with other methods for sampling airborne pathogenic microorganisms, our current method avoided the need to culture and incubate samples on agar for 48 hours. Results from the surface samples indicated an obvious decrease in DNA decontamination over the study period (Figure 1).

Most previous reports focused on eliminating contamination are based on a DNA degradation strategy that includes the use of DNase I, UV irradiation, exonuclease III, restriction endonucleases, and radical gas-based decontamination to eliminate the contaminating DNA.¹⁷ It should be noted that most of these approaches do not achieve complete or efficient surface decontamination. For instance, degradation of DNA synthesized in the presence of dUTP, which is an efficient method for eliminating carry-over contamination,¹⁸ but not suitable for genetic testing. Although UV irradiation at 254 nm is one of the most common methods of decontamination and is simple to perform, it is only suitable for surfaces,² and not for the inner regions of complex instruments.¹⁹ Additionally, UV irradiation does not completely eliminate amplifiable DNA fragments that are >200 bp (base pairs),²⁰

and also potentially reduce PCR amplification efficiency if the reaction components, including Taq DNA polymerase and primers, are exposed.^{21,22} Another method typically used to maintain sterile working conditions involves spraying with chemical solutions, such as ethyl alcohol and hypochlorite solutions. However, these solutions can cause corrosion, and therefore some equipment cannot be cleaned with them, although ethyl alcohol solutions are typically safe in this context. Alcohol solutions are widely used in nucleic acid precipitation, whereas hypochlorite solutions may reduce DNA/RNA stability. The above reagents are easily acquired and show excellent decontamination efficacy in our study.

Data showed that reliable methods for assessing how surface decontamination efforts affect PCR results are required (Figure 1). Comparative analysis with a reference PCR laboratory showed that any differences in HBV DNA amplification caused by the decontamination methods employed were insignificant. Most importantly, the surface decontamination proposed here could be useful for other clinical PCR laboratories. It is worthwhile that environmental surveillance have been integrated into SOPs as a monthly quality check. IQC is a long-standing topic of importance for clinical laboratories, although IQC efforts are often not adequate in the management of molecular biology laboratories, especially in clinical next-generation sequencing laboratories.²³ A PCR laboratory could also designate separate rooms for different functions or perform certain functions in pre-rooms (see the reference document entitled "Administrative measures for the laboratory management of clinical gene amplification in medical institutions of China"), which should also eliminate false-positive signals generated by contaminant PCR amplicons. The use of negative controls in this study decreased the risk of obtaining false-positive results. At present, the necessary accompanying IQC procedures have not been widely implemented. By producing false-positive results, PCR contamination often wastes time and increases the costs associated with research studies. As the field of molecular biology has rapidly advanced, the application of PCR for clinical diagnostics has become increasingly widespread according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI).²⁴ Initial molecular detection via PCR is mainly used for qualitative or quantitative detection of bacteria or viral specimens and other microorganisms, although it is also useful for detecting damage caused by tumor cells, gene mutations associated with disease, and disease or therapeutic response genes associated with transcriptional regulation. However, quality control remains a bottleneck for the above-described applications. Thus, there is an urgent need for the development of standardized guidelines for PCR laboratories, especially with respect to manual extraction of nucleic acids.

In contrast with previous reports focusing on methods of eliminating only contaminating amplicons, a key advantage of this study is the optimized sampling method, which targets different environmental locations to systematically resolve all sources of contamination. Follow-up surveillance was also performed. We must note that some limitations existed in this study, including the sampling numbers, monitoring protocol, and comparisons of chemical solutions used for decontamination, which all require further optimization.

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REFERENCES

1. Champlot S, Berthelot C, Pruvost M, et al. An efficient multistrategy DNA decontamination procedure of PCR reagents for hypersensitive PCR applications. *PLoS ONE*. 2010;5:e13042.
2. Gefrides LA, Powell MC, Donley MA, et al. UV irradiation and autoclave treatment for elimination of contaminating DNA from laboratory consumables. *Forensic Sci Int Genet*. 2010;4:89-94.
3. Banasik M, Stanisławska-Sachadyn A, Sachadyn P. A simple modification of PCR thermal profile applied to evade persisting contamination. *J Appl Genet*. 2016;57:409-415.
4. Czurda S, Smelik S, Preuner-Stix S, et al. Occurrence of fungal DNA contamination in PCR reagents: approaches to control and decontamination. *J Clin Microbiol*. 2016;54:148-152.
5. Chang SS, Hsu HL, Cheng JC, et al. An efficient strategy for broad-range detection of low abundance bacteria without DNA decontamination of PCR reagents. *PLoS ONE*. 2011;6:e20303.
6. Silkie SS, Tolcher MP, Nelson KL. Reagent decontamination to eliminate false-positives in Escherichia coli qPCR. *J Microbiol Methods*. 2008;72:275-282.
7. Mennerat A, Sheldon BC. How to deal with PCR contamination in molecular microbial ecology. *Microb Ecol*. 2014;68:834-841.
8. Glushkov SA, Bragin AG, Dymshits GM. Decontamination of polymerase chain reaction reagents using DEAE-cellulose. *Anal Biochem*. 2009;393:135-137.
9. Morono Y, Yamamoto K, Inagaki F. Radical gas-based DNA decontamination for ultra-sensitive molecular experiments. *Microbes Environ*. 2012;27:512-514.
10. Philipp S, Huemer HP, Irschick EU, et al. Obstacles of multiplex real-time PCR for bacterial 16S rDNA primer specificity and DNA decontamination of Taq polymerase. *Transfus Med Hemother*. 2010;37:21-28.
11. Espy MJ, Smith TF, Persing DH. Dependence of polymerase chain reaction product inactivation protocols on amplicon length and sequence composition. *J Clin Microbiol*. 1993;31:2361-2365.
12. Galvin S, Dolan A, Cahill O, et al. Microbial surveillance of the hospital environment: why and how? *J Hosp Infect*. 2012;82:143-151.
13. Davies A, Pottage T, Bennett A, et al. Gaseous and air decontamination technologies for *Clostridium difficile* in the healthcare environment. *J Hosp Infect*. 2011;77:199-203.
14. Peng Y, Ou QT, Lin DX, et al. Metro system in Guangzhou as a hazardous reservoir of methicillin-resistant Staphylococci: findings from a point-prevalence molecular epidemiologic study. *Sci Rep*. 2015;29:16087.
15. Stocks GW, Self SD, Thompson B, et al. Predicting bacterial populations based on airborne particulates: a study performed in nonlaminar flow operating rooms during joint arthroplasty surgery. *Am J Infect Control*. 2010;38:199-204.
16. Saegeman V, Flamaing J, Muller J, et al. Clinical evaluation of the Copan ESwab for methicillin-resistant Staphylococcus aureus detection and culture of wounds. *Eur J Clin Microbiol Infect Dis*. 2011;30:943-949.
17. Muhl H, Kochem AJ, Disque C, et al. Activity and DNA contamination of commercial polymerase chain reaction reagents for the universal 16S rDNA real-time polymerase chain reaction detection of bacterial pathogens in blood. *Diagn Microbiol Infect Dis*. 2010;66:41-49.
18. Chowdhury S, Kitamura K, Simadu M, et al. Concerted action of activation-induced cytidine deaminase and uracil-DNA glycosylase

- reduces covalently closed circular DNA of duck hepatitis B virus. *FEBS Lett.* 2013;587:3148-3152.
19. Tamariz J, Voynarovska K, Prinz M, et al. The application of ultra violet irradiation to exogenous sources of DNA in plastic ware and water for the amplification of low copy number DNA. *J Forensic Sci.* 2006;51:790-794.
 20. Setlow B, Parish S, Zhang P, et al. Mechanism of killing of spores of *Bacillus anthracis* in a high-temperature gas environment, and analysis of DNA damage generated by various decontamination treatments of spores of *Bacillus anthracis*, *Bacillus subtilis* and *Bacillus thuringiensis*. *J Appl Microbiol.* 2014;116:805-814.
 21. Nilsen IW, Øverbø K, Havdalen LJ, et al. The Enzyme and the cDNA Sequence of a thermolabile and double-strand specific dnase from northern shrimps (*Pandalus borealis*). *PLoS ONE.* 2010;5:e10295.
 22. Padua RA, Parrado A, Larghero J, et al. UV and clean air result in contamination-free PCR. *Leukemia.* 1999;13:1898-1899.
 23. de Abreu FB, Peterson JD, Amos CI, et al. Effective quality management practices in routine clinical next-generation sequencing. *Clin Chem Lab Med.* 2016;54:761-771.
 24. Clinical and Laboratory Standards Institute. Molecular Methods for Clinical Genetics and Oncology Testing; Approved Guideline-Third Edition (MM01-A3). 2011.

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