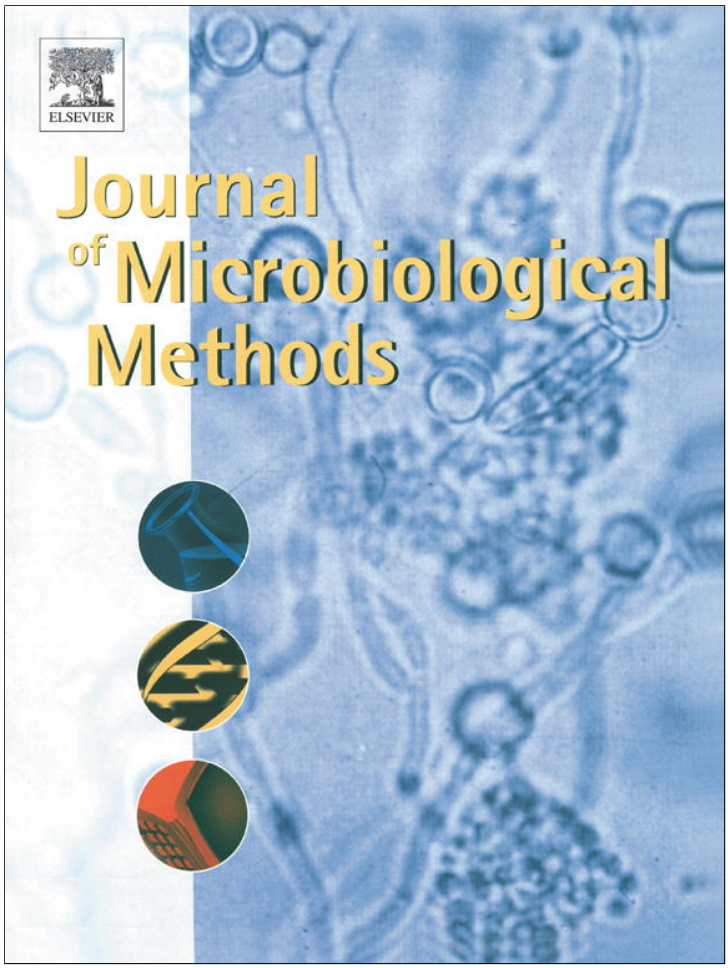

(This is a sample cover image for this issue. The actual cover is not yet available at this time.)

Journal of Microbiological Methods 92 (2013) 358–365

Contents lists available at SciVerse ScienceDirect

Journal of Microbiological Methods

journal homepage: www.elsevier.com/locate/jmicmeth

Detecting laboratory DNA contamination using polyester-rayon wipes: A method validation study

E. Marielle Remillard, Laura K. Taylor, Julie Layshock, Sheila Van Cuyk, Kristin M. Omberg *

Los Alamos National Laboratory, P.O. Box 1663, Los Alamos, NM 87545, USA

ARTICLE INFO

Article history:
Received 31 October 2012
Received in revised form 3 January 2013
Accepted 3 January 2013
Available online 11 January 2013

Keywords:
Contamination
Detection limit
DNA
PCR
Robustness
Wipe

ABSTRACT

Due to the high sensitivity of many PCR assays, extraneous target DNA in a laboratory setting can lead to false positive results. To assess the presence of extraneous DNA, many laboratories use gauze wipes to sample laboratory surfaces. The accuracy, precision, limits of detection, linearity, and robustness of a wipe test method and each associated wipe processing step were evaluated using *E. coli* genomic DNA. The method demonstrated a limit of detection of 108 copies of DNA, which equates to detectable surface concentration of 4.5×10^5 copies of DNA per area sampled. Recovery efficiency or accuracy is $22\pm10\%$ resulting from a $>58\%$ loss of DNA occurring at the wipe wash step. The method is robust, performing consistently despite deliberate modifications of the protocol.

1. Introduction

Polymerase Chain Reaction (PCR) amplification is a powerful tool for measuring DNA at low concentrations. However, the high sensitivity of PCR assays means that small amounts of extraneous target DNA, either primary genomic or amplicon, can lead to false positive results. Lo *et al.* were among the first to publish false positive results as a consequence of interfering DNA (Lo et al., 1988); between 1990 and 2002, about 2% of published papers on PCR reported DNA contamination (Borst et al., 2004). The consequences of false positives range from loss of data or time in research settings to incorrect diagnoses and fatalities in clinical settings (Patel et al., 2000).

This problem is typically addressed by implementing stringent protocols to minimize DNA contamination. Standard procedure includes performing different stages of the PCR process in separate areas or rooms, with work conducted in a unilateral direction from "clean" areas (those containing only low levels of DNA) to "dirty," where researchers handle large quantities of amplicons (Mifflin, 2007; USEPA, 2004). A number of methods have been adapted to decontaminate work areas including UV irradiation, uracil–DNA glycosylase, exonuclease III, hydroxylamine hydrochloride, and sodium hypochlorite (Borst et al., 2004). However, one study reports that these methods may be less effective than presumed (Dwyer and Saksena, 1992), while others suggest that decontamination may reduce the sensitivity of PCR through reagent interference (Niederhauser et al., 1994).

Surface wipe samples are commonly collected to assess residual DNA contamination, identify possible areas of contamination, and ensure that residual decontaminant does not inhibit PCR. Early studies reported success in detecting DNA contamination using wipes (Cone et al., 1990), and regulatory agencies and professional associations, such as the American Society of Histocompatibility and Immunogenetics (ASHI), stipulate regular wipe tests in accredited PCR laboratories. However, the literature on the efficacy of the wipe method for detecting laboratory contamination is sparse. Moreover, wipe tests have been shown to produce widely varying results depending on the type of surface sampled, the type of solvents and wipes used, the analyte in question, and the sampling technique (Billets, 2007).

To address these gaps, a quantitative assessment of a surface wipe method for detecting laboratory DNA contamination was performed. The wipe test protocol consisted of four distinct stages: removal of DNA from surfaces using absorbent wipes, extraction of DNA from wipes into buffer solution, purification of DNA, and analysis of the extract using a real-time quantitative PCR (qPCR) assay. A known quantity of DNA was introduced to separate sets of samples at each stage, allowing calculations of method accuracy, precision, detection limits, linearity, robustness, repeatability, and inter-technician variability to be made. Recovery and error are defined for each individual process component as well as for the wipe test as a whole.

* Corresponding author at: Los Alamos National Laboratory, P.O. Box 1663, MS F606, Los Alamos, NM 87545, USA. Tel.: +1 505 667 9628; fax: +1 505 665 5204.
E-mail address: komberg@lanl.gov (K.M. Omberg).

0167-7012/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.mimet.2013.01.003

2. Materials and methods

2.1. Experimental conditions

Experimental details, including DNA concentrations and replicate numbers, are given in the supplemental information. All activities associated with the determination of accuracy, precision, detection limit, linearity and specificity were performed by one technician. Robustness tests included evaluation of the effect of residual bleach and of dust in the sample matrix, wipe pattern, wipe processing technique, the length of time a sample was stored before being analyzed, and the effect of using multiple technicians to complete the process. Repeatability tests were performed for each process step and involved a large number of replicate tests performed by the original technician. Inter-technician variability tests were performed by multiple technicians to evaluate the contribution of technician variability to the method error.

2.1.1. Sample preparation

Dry *Escherichia coli* DNA (Affymetrix, Santa Clara, CA) was suspended in DNA Suspension Buffer (10 mM Tris-HCl, 0.1 mM EDTA, pH 8.0, Teknova, Hollister, CA) at an initial concentration of 2×10^7 copies μL^{-1}. The concentration of DNA was verified before each trial using the Quant-iT™ PicoGreen® dsDNA Assay Kit (Life Technologies™, Grand Island, NY) with a microplate fluorescence reader (model FLx800T, BioTek, Winooski, VT). DNA was diluted to the desired concentration via serial dilution in QIAGEN elution buffer (QIAGEN, Valencia, CA), PBST (1× PBS with 0.03% Tween 20, prepared from PBS and PBS with 1% Tween 20, Teknova, Hollister, CA), or PCR-grade water (Teknova, Hollister, CA). Calibrated pipettes (20 to 1000 μL, Rainin, Oakland, CA) were verified daily using an analytical balance (Sartorius, Goettingen, Germany). Temperature, pressure, and evaporative losses were taken into account. Arizona Test Dust (ATD, 5 to 10 micron particles, Powder Technology Inc., Burnsville, MN) was used to assess matrix effects. A defined amount of ATD was suspended in PBST, then the initial suspension of *E. coli* genomic DNA was diluted to the desired concentration using the ATD suspension.

2.1.2. Surface sample collection

Sterile 2×2 in. polyester-rayon non-woven wipes (Dukal, Ronkonkoma, NY) were placed into sterile 50 mL conical tubes (VWR, Radnor, PA). Four milliliters of PBST were transferred to each conical tube using a sterile serological pipette (VWR, Radnor, PA). In trials where the wipe was directly inoculated with DNA, 100 μL of DNA in PBST was applied to the wipe within the conical tube using a micropipette.

Polypropylene sheet protectors (8.5×11 in., Avery, Brea, CA) were cut to produce an inert, clean surface of 0.12 m^2 in area. Twenty 5-μL droplets of sterile PCR-grade water or *E. coli* genomic DNA in PCR-grade water were applied to the surface of the polypropylene sheets and allowed to dry overnight at room temperature. Pre-moistened wipes were used to sample the surface of the plastic sheet using an S-stroke collection pattern (Brown et al., 2007) covering the entire surface once. After the sample was collected, the wipe was returned to the 50 mL conical tube. Gloves were changed between each sample collected.

Several surface parameters were varied to evaluate method robustness. To evaluate the impacts of the area sampled, multiple sheets were used to produce areas of 0.12, 0.48 and 0.96 m^2. To evaluate the impacts of decontamination protocols, the sheets were pre-cleaned with 0.5% sodium hypochlorite wipes (equivalent to 10% household bleach, Medtrol, Niles, IL) then wiped with a moist paper towel prior to spiking with samples or controls. To evaluate the impacts of different wipe patterns, two patterns were evaluated: a unidirectional S-stroke covering the entire sampling area once, and an S-stroke in three directions (vertical, horizontal, and diagonal), which sampled the entire area three times (NIOSH, 1996).

2.1.3. Wipe extraction

Ten milliliters of PBST were added to each wipe sample with a sterile pipette and then the tubes were vortexed at the maximum speed for 20 minutes using a Vortex Genie 2 (Scientific Industries, Bohemia, NY). A 200 μL aliquot of the supernatant was processed using a nucleic acid purification kit (QIAamp DNA Blood Mini Kit, QIAGEN, Inc, Valencia, CA; Dauphin et al., 2009), using a QIAcube with the "Blood and body fluid spin protocol V3" (QIAGEN, 2010). The QIAcube lyses the sample, stabilizes and binds DNA to a selective membrane, washes impurities with a series of buffers, and elutes the pure DNA sample.

Robustness tests included evaluation of four PBST wash volumes (5, 10, 15, and 20 mL). Additionally, a subset of samples was processed with a slightly altered protocol to optimize DNA recovery. In this protocol, spiked wipes were vortexed for 20 minutes and a 200 μL aliquot of the supernatant was processed; these followed the regular protocol and served as control samples. Then, from the 50 mL conical containing the samples, the wipes were compacted to remove additional sample by using a sterile serological pipette to press the wipe against the side of the conical tube. The wipe was removed and discarded, and a second set of 200 μL samples was then collected. Finally, the samples were centrifuged for 10 minutes at 2700 rpm. All but 2 mL of the PBST was removed, the samples were vortexed briefly to re-suspend any pelleted material, and a third set of 200 μL aliquots was collected.

To evaluate storage stability, a subset of samples was stored at two points in the extraction process: after the sample had been vortexed (with and without the gauze wipe remaining in the 50 mL conical tube), and after the samples had been purified by the QIAcube. Vortexed samples were stored at 4 °C for eight days. Subsequent extraction and analysis were performed at 1, 2, and 8 days after the initial reading. Two concentrations of purified DNA samples (150 and 90 copies μL^{-1}) were stored for two days at 4 °C, then for four days at -20 °C. Measurements were taken at 1, 2, and 7 days after the initial reading.

2.1.4. PCR procedure

DNA extracts from the QIAcube were analyzed using a TaqMan® qPCR assay targeting highly conserved regions of the 16S rRNA gene (Yang et al., 2002). Seven rRNA operons appear in each copy of *E. coli* genomic DNA (Stevenson and Schmidt, 2004). Each 25 μL reaction contained 5 μL of purified DNA suspension, 12.5 μL Path-ID™ qPCR master mix (Applied Biosystems, Grand Island, NY), 5 μL of primer/probe mix (4.5 μM of each primer, 0.5 μM probe; custom oligonucleotides ordered from Integrated DNA Technologies, Coralville, IA), and PCR-grade water. All trials were run on an Applied Biosystems 7500 Fast Dx Real-Time PCR Instrument (ABI) in MicroAmp® Fast Optical 96-Well Reaction Plates (Applied Biosystems, Carlsbad, CA) using the following parameters: Mode: Standard; Detector: FAM (reporter)/TAMRA (quencher); Passive reference: ROX; Cycling conditions: 50 °C for 2 minutes, 95 °C for 10 minutes, 45 cycles at 95 °C for 15 seconds and 60 °C for 1 minute each.

2.2. Quality control

Each batch of samples processed included positive and negative controls (see Table 1). All controls were processed through each analytical step alongside the samples, and three to eight control replicates were used in each batch. All sample results were blank-corrected according to the appropriate control for that test. Preliminary studies (data not shown) established the initial spiking concentrations for the entire method and the individual process stages. Depending on the process stage, these concentrations ranged from 10^3 to 10^6 copies of *E. coli* DNA and were chosen based on expectant recoveries. Samples were

Table 1
Experimental controls.

Control type		Process stage	Description	DNA detected (copies)	Number of samples (n)
Negative:	Method Blank	Surface	Polypropylene sheet spiked with PCR grade water	48 ± 20	22
	Matrix Blank	Wipe	Wipe moistened with PBST	39 ± 21	21
	Reagent Blank	QIAcube	200 μL of PBST	30 ± 20	18
	PCR Blank	PCR	5 μL QIAGEN elution buffer	1 ± 1	43
Positive:	Matrix Spike	Wipe	Wipe moistened with PBST, spiked with 10^6 copies *E. coli* DNA	406 ± 56	20
	PCR Spike	PCR	5 μL QIAGEN elution buffer, spiked with 10^3 copies *E. coli* DNA	926 ± 117	27

extracted, purified, and analyzed by PCR on the same day that they were collected, excepting the samples collected to assess storage times and conditions as part of robustness tests.

2.3. Data analysis

Each PCR plate contained a six-point calibration curve used to translate Ct values from the ABI into *E. coli* DNA concentration values. The calibration curve, prepared as a dilution series of *E. coli* genomic DNA in QIAGEN elution buffer, ranged from 10 to 10^6 copies *E. coli* DNA per PCR reaction and had an average $R^2 > 0.999$. Method validation calculations and statistical evaluations were performed using Microsoft® Excel. *P*-values were determined using the Student's *t*-test or analysis of variance (ANOVA).

Accuracy was calculated as the relative sample bias (Eq. (1)) for the whole method and isolated process steps (wipe extraction, QIAcube and PCR).

$$\text{accuracy} = \frac{\overline{x} - x_{\text{blank}}}{x_{\text{true}}} \times 100\% \quad (1)$$

Where $\overline{x}$ is the average DNA measured in the samples, x_{blank} is the level of background DNA in the blank sample, and x_{true} is the expected value. Eight samples collected on the same day under identical conditions were used for this determination. Precision was calculated as the relative sample standard deviation (Eq. (2)):

$$\text{precision} = \frac{\sqrt{\frac{1}{n-1}\sum_{i=1}^{n}(x_i - \overline{x})^2}}{x_{\text{true}}} \times 100\% \quad (2)$$

Where $\{x_1, x_2, \ldots, x_n\}$ are the observed values of the sample items, $\overline{x}$ is the mean value of these observations, n is the number of samples, and x_{true} is the expected value. The limit of detection (LOD) was calculated as three standard deviations from the established sample blank using negative controls (Willetts and Wood, 2000). Linearity was determined using a least-squares regression for multiple replicates at either seven concentrations (surface and wipe samples) or nine concentrations (spiked solutions).

Specificity, the ability of the wipe test to detect the target DNA from a complex matrix (e.g., ATD) compared to the ability to detect pure DNA, was calculated as a ratio (Eq. (3)).

$$\text{Specificity} = \frac{\text{amount of DNA recovered from a pure sample}}{\text{amount of DNA recovered from a complex matrix}} \quad (3)$$

3. Results

3.1. Accuracy, recovery, precision, and detection limits

Accounting for sample diminution through all process steps, the complete wipe test method detected 22% of maximum available *E. coli* DNA from a clean, smooth surface (a polypropylene sheet) initially spiked with 10^6 DNA copies. Individual evaluation of the accuracy at each process step revealed that 41% of available DNA was detected for spiked wipes and 67% was detected for spiked solutions processed with the QIAcube. The method displayed precision within 10% standard deviation at each process step. Table 2 lists the calculated accuracy and precision at each step along with all constituent data.

Some DNA was lost at each process step, influencing the process accuracy. Table 3 shows average DNA recovery efficiencies at each stage. To determine recovery efficiency, the maximum recovery was calculated by assuming a theoretical 100% recovery for all process steps and taking into account all dilutions. Then, the recovery efficiency was determined for each individual stage beginning with the PCR step and back-calculating the recovery efficiency for the QIAcube purification, the wipe wash, and the surface removal step. Each individual step had at least a 59% recovery.

While step-specific recoveries were quite high, overall recovery appears low. This is due to the volume of sample carrying through the QIAcube and PCR steps, where 99.9% of the initial sample is discarded before analysis. As evident in Tables 2 and 3, the DNA detected at the PCR step reached over 20% of the maximum possible value.

The limits of detection (LOD) ranged from one copy of DNA for the PCR step to 108 copies of DNA for the entire method, starting with the spiked surfaces (Table 4). A more practical detection limit was calculated from the LOD by taking into account dilution factors and removal efficiencies (see supplemental information for stepwise calculations). Overall, the wipe method can detect DNA at a minimum of 4.5×10^5 copies of DNA on a surface, 2.5×10^5 copies on a wipe, 17 copies/μL in a spiked solution, or 1 copy of DNA at the PCR step. Note that while LODs were calculated from background levels as described in Section 2.3, detection of one copy at the PCR step remains a tractable limit due to the presence of multiple rRNA operons per genomic copy.

3.2. Linearity

The DNA measured by PCR demonstrated an inverse logarithmic trend between measured Ct values and the amount of DNA initially added to the sample (Fig. 1). Spiked solutions could be modeled over six orders of magnitude, from 50 to 5×10^6 initial copies of DNA per sample ($R^2 = 0.99$). Spiked wipes followed a similar trend between 10^2 and 10^5 initial copies of DNA per wipe ($R^2 = 0.99$). Samples collected from spiked surfaces trended logarithmically over a

Table 2
Summary of accuracy and precision for the detection of *E. coli* DNA from polypropylene surfaces using polyester–rayon gauze wipes, $n = 8$.

	PCR	QIAcube	Wipe Extraction	Overall Process
DNA spike (copies)	1000	20,000	1,000,000	1,000,000
Recoverable DNA (copies)	1000	1000	1000	1000
DNA detected (copies)	887	694	386	373
	890	675	391	145
	911	623	410	246
	950	733	430	166
	974	740	432	307
	918	618	409	308
	1009	621	437	103
	879	643	408	105
Accuracy (% bias)	93%	67%	41%	22%
Precision (Standard Deviation)	±5%	±5%	±2%	±10%

Table 3
Summary of DNA recovery for each process step compared to the theoretical maximum recovery and initial input. For isolated process steps, the following replicates apply: PCR $n=27$, QIAcube $n=26$, Wash $n=17$, Overall Process $n=25$.

	Maximum possible recovery, DNA copies (maximum overall recovery as % of initial)	DNA copies measured for isolated process step (step recovery as % of step maximum)	Total DNA copies remaining through continuous process (overall recovery as % of initial)
Wipe	1,000,000 (100%)	590,000 (59%)	590,000 (59%)
Wash	1,000,000 (100%)	740,000 (74%)	440,000 (44%)
QIAcube	20,000 (2%)	12,000 (59%)	5200 (0.5%)
PCR	1000 (0.1%)	930 (93%)	240 (0.02%)

smaller range, from 10^2 to 5×10^4 initial copies of DNA deposited on the surface ($R^2=0.99$). For DNA quantities above 5×10^4 copies per surface spike, the sensitivity began to decline.

3.3. Robustness

Robustness tests aided in determining how well the method performed under normal work conditions by evaluating the effects of minor, deliberate protocol variations on the final results.

3.3.1. Laboratory variations

Several commonly encountered factors (i.e., bleach, dust, sampled area, storage and technician variability) were assessed as part of the robustness tests. One of the first tests involved cleaning polypropylene surfaces using bleach prior to spiking with 10^6 copies of *E. coli* DNA. The results showed that DNA recovery was not significantly diminished compared to non-bleached surfaces ($n=4$, $p=0.47$). Bleached surfaces exhibited an average DNA recovery of $23\pm5\%$, compared to non-bleached surfaces, which had $26\pm6\%$ recovery (Table 5).

ATD was added to three types of samples to evaluate the ability of the method to detect *E. coli* from a complex matrix (Fig. 2). Calculated specificity values (Eq. (3)) were equal to 1 for the solution and wipe, while specificity was equal to 2.7 for the spiked surface. ATD had no statistical effect ($p>0.05$) on the DNA recovery for spiked solutions and spiked wipes, but impaired DNA recovery for spiked surfaces ($p<0.05$). In fact, average DNA recovery from spiked surfaces declined from 22% (no ATD) to 6% (ATD).

Statistical differences were observed when different areas were sampled. DNA recovery was compared for three different areas (0.12 m^2, 0.48 m^2, and 0.96 m^2) containing the same initial DNA loading, 10^6 copies of *E. coli* DNA in water. Recovery was equivalent from surfaces 0.12 m^2 ($25\pm5\%$) and 0.48 m^2 ($26\pm7\%$); however, a lower recovery value ($p=0.06$) was observed for the 0.96 m^2 surface ($11\pm5\%$) (Fig. 3).

Multiple wipe samples are typically collected from laboratory spaces, and larger sample numbers may require multiple days for full analysis. Consequently, collected samples may be stored at some point during processing. To determine the effect of sample storage on data quality, samples were stored at two points in the wipe process: after the sample had been vortexed (with and without the gauze wipe remaining in the conical tube); and after the samples had been purified by the QIAcube. There was no change in DNA recovered from the wipes stored in conical vials at 4 °C for up to eight days; values fluctuated between 96% and 111% of the initially measured DNA concentration. Furthermore, these samples showed no decline over eight days, whether or not the wipe was left in the conical tube; $n=5$, $p=0.23$. There was, however, a trend of decreasing concentration observed for the eluted DNA samples over seven days of storage. The higher concentration of purified DNA (150 copies μL^{-1}) declined 21% over the weeklong trial, while the lower concentration of DNA (90 copies μL^{-1}) declined 18%. However, the change in the lower concentration sample was not statistically significant ($p=0.08$, Fig. 4).

Since multiple technicians may assist in performing one wipe method, wipes were spiked with 10^6 copies of *E. coli* DNA and processed by up to four different technicians to produce a single result. Results were compared to data collected by a single laboratory technician. There was no difference ($p=0.41$, $n=18$) in DNA recovery between spiked wipes that were processed by one technician ($42\pm5\%$) compared to wipes that were processed by multiple technicians performing various steps of the wipe process ($39\pm10\%$) (Fig. 5).

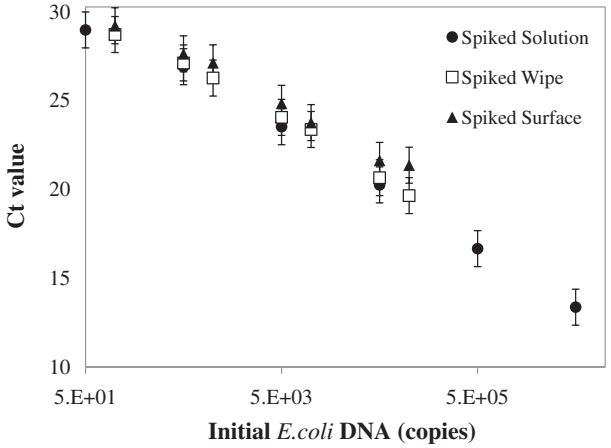

Fig. 1. Linearity of instrumental response for dilution corrected samples: solution ($n=4$), wipe ($n=4$) and surface ($n=4$). Error bars represent one standard deviation.

3.3.2. Method techniques

Two different wiping patterns were used to sample polypropylene sheets spiked with 10^6 copies of *E. coli* DNA. The recovery of DNA from polypropylene sheets sampled using unidirectional S-strokes and covering the surface once ($26\pm6\%$) was comparable to DNA recovered from surfaces sampled using S-strokes in three directions ($37\pm9\%$). Although higher recoveries were recorded using three stroke directions, there was no statistical difference at the 95% confidence interval ($n=4$, $p=0.06$).

Table 4
Summary of limits of detection of *E. coli* DNA from polypropylene surfaces using polyester-rayon gauze wipes. PCR $n=43$, QIAcube $n=18$, Wipe Extraction $n=21$, Overall Process $n=22$.

	PCR	QIAcube	Wipe Extraction	Overall Process
Limit of detection	1 copy DNA	94 copies DNA	103 copies DNA	108 copies DNA
Practical detection Limit[a]	1 copy DNA	17 copies DNA/μL	250,000 copies DNA/wipe	450,000 copies DNA/surface

[a] Practical detection limit used data from the limit of detection to back-calculate the minimum concentration of DNA required at the onset of processing in order to detect DNA via PCR. It takes into account process dilutions and efficiencies.

Table 5
Comparison of DNA recovery for bleach-cleaned vs. non-bleached surfaces, $n=4$, $p=0.47$.

	Bleach-cleaned surface	Non-bleached surface
DNA Spike (copies)	1,000,000	1,000,000
Recoverable DNA (copies)	1000	1000
DNA Recovered (copies)	168	218
	213	209
	294	268
	237	341
Recovery (%)	23 ± 5	26 ± 6

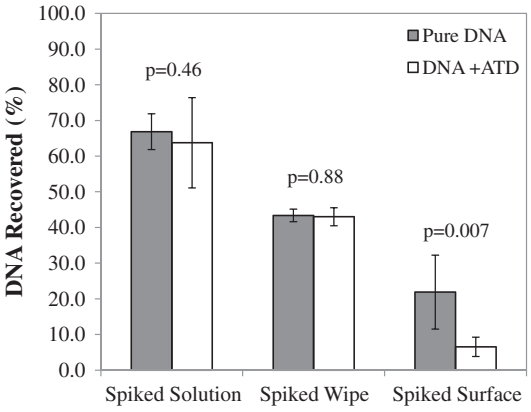

Fig. 2. Specificity samples spiked with *E. coli* DNA are represented by shaded bars, and samples containing 50 μg/mL dust and *E. coli* DNA are represented by white bars. Error bars represent one standard deviation, $n=8$ for all samples.

Spiked wipes were washed with various volumes of buffer to determine the optimal wash volume. Overall, the recovered DNA for the larger wash volumes produced similar results with percent recovery ranging from $50\pm9\%$ to $53\pm4\%$ ($p>0.3$), but recovery decreased with the lowest wash volume of 5 mL ($39\pm6\%$), Fig. 6A. Since the wipe method is a screening method, it is more useful to analyze the data produced by PCR analysis without accounting for volume dilutions. In this case, the amount of DNA detected was greatest for wipes washed with 5 mL of PBST and decreased with increasing wash volume: 780 ± 120 copies using 5 mL, 520 ± 50 copies using 10 mL, 360 ± 30 copies using 15 mL, and 260 ± 50 copies using 20 mL (Fig. 6B).

Tests were also conducted to determine whether certain techniques affected method performance. The data shows no significant difference ($p>0.05$) in the DNA recovered from sample aliquots collected prior to removing the wipes from the conical tube ($n=12$); after the wipe was wrung out and removed ($n=9$); or after the sample was centrifuged ($n=5$). The analysis was repeated with the addition of matrix effects (i.e., ATD). This did not affect DNA recovery, regardless of processing method.

3.4. Repeatability

Data from all trials, collected over three months, was compiled to determine the consistency in results over time. From 27 samples spiked at the PCR stage, collected over six different days, $93\pm12\%$ of the expected DNA was detected, with values ranging from 80% to 110%. Less DNA was recovered from solutions spiked prior to QIAcube extraction; the average DNA recovered from 26 samples over five days was $59\pm10\%$, with a range from 39% to 77%. Recovery from spiked wipes averaged $41\pm6\%$, ranging from 26% to 48% in 20 samples collected over 7 days. Finally, from spiked surfaces, recovery averaged $24\pm7\%$ and ranged from 10% to 37% for 25 samples collected over five days.

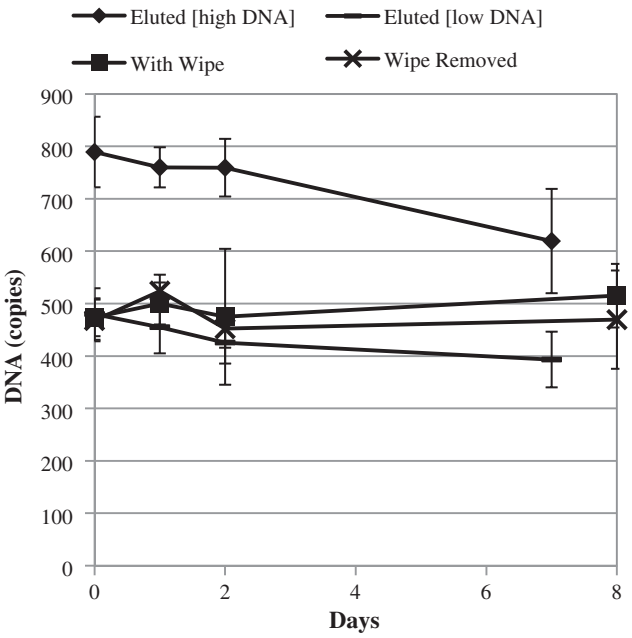

Fig. 4. Storage stability tests performed after the sample was eluted with higher (Eluted [high DNA]) and lower (Eluted [low DNA]) DNA concentration and before elution in the extraction vial with the wipe (With wipe) and with the wipe removed (Wipe removed) for up to eight days. Error bars represent one standard deviation, $n=5$.

3.5. Inter-Technician Variability

Small differences in technique between laboratory technicians introduced variability in the results. In this test, four technicians sampled 0.12 m^2 polypropylene surfaces that were spiked with 10^6 copies of *E. coli* DNA in water and five replicate samples were collected per technician According to an analysis of variance test (ANOVA), there is a significant difference in DNA recovery depending on which technician

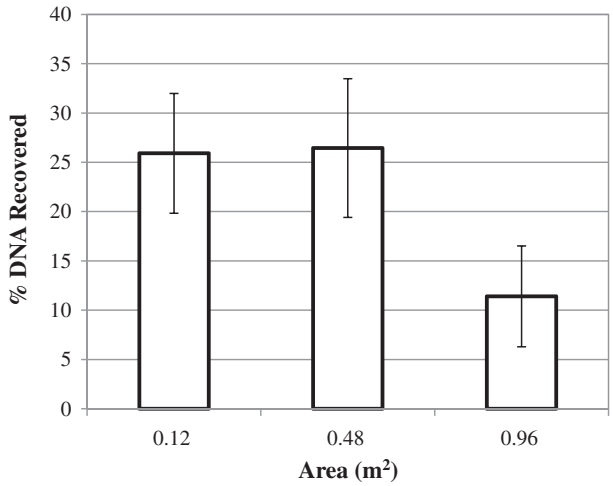

Fig. 3. DNA recovery based on sampled surface areas of 0.12 m^2, 0.48 m^2 and 0.96 m^2. Error bars represent one standard deviation, $n=4$ for all samples.

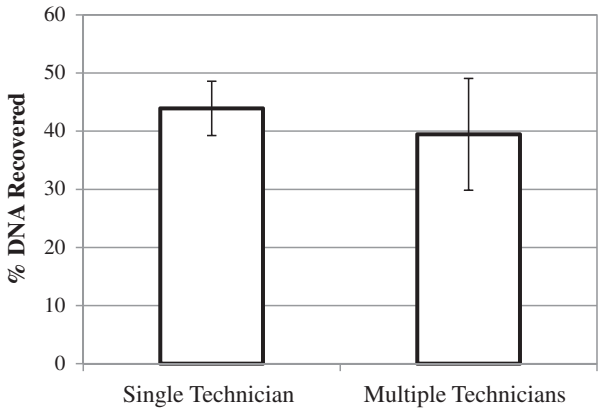

Fig. 5. DNA recovery from the wipe process was comparable whether the wipes were processed by a single technician or processed by different technicians during each step. Error bars represent one standard deviation, $n=18$.

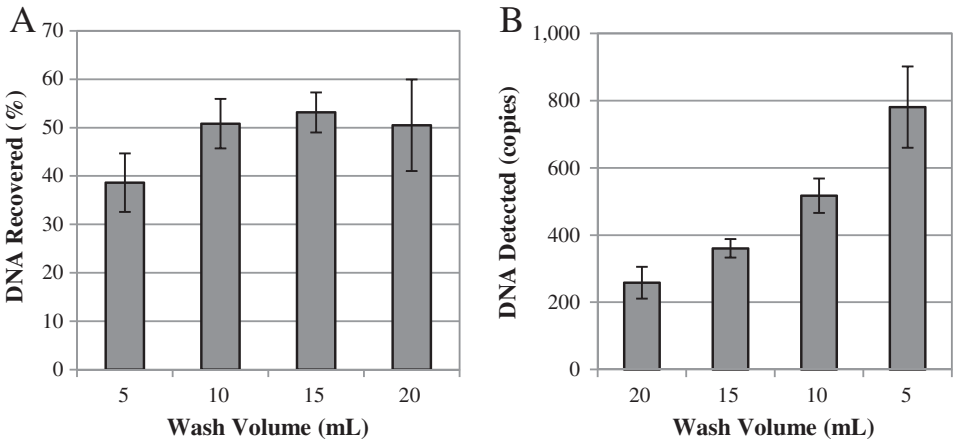

Fig. 6. Effect of PBST wash volume on the DNA recovery of wipes spiked with 10^6 copies of *E. coli*. Fig. 6A shows the dilution-corrected recovery for each volume of buffer as a percentage of the DNA spiked, and Fig. 6B shows the amount of DNA detected by PCR for each sample. Error bars represent one standard deviation, $n=4$.

collected and processed the sample ($p<0.05$, $F>F_{crit}$, correlation = 0.85). Recoveries for four technicians ranged from $16\pm1\%$ to $45\pm3\%$, with an average of $26\pm13\%$ (Fig. 7). The average standard deviation for any individual technician was 4%, indicating consistent precision in technique, although accuracy varied.

4. Discussion

Environmental wipe tests play a key role in detecting DNA on surfaces. Simplicity and low cost make these tests ideal for rapid detection; however, the number of studies detailing the efficacy of wipe tests varies widely across applications. For instance, many papers have compared the effect of wipe materials, surface types, protocols, or spore species on detection efficacy (Brown et al., 2007; Buttner et al., 2004; Edmonds, 2009; Estill et al., 2009; Frawley et al., 2008; Krauter et al., 2012; Sanderson et al., 2002). While these papers contribute significantly to pathogen detection methodology, the basic, quantitative parameters of a wipe method (e.g., LOD) for detecting laboratory contamination have remained largely unexplored, even with explicit endorsement of the wipe method to identify laboratory contamination (Cone et al., 1990) and the availability of several commercial DNA wipe kits (Invitrogen UniTray DNA Wipe Test, Biofortuna HLA Wipe Test kit).

In this study, the wipe test was characterized by a method validation process using *E. coli* genomic DNA. Based on the results of the current study, the wipe method was capable of detecting laboratory DNA contamination above 4.5×10^5 copies (~2 ng) with recovery efficiency from polypropylene surfaces around 22%. Similar detection limits were published for the Biofortuna HLA Wipe test, where genomic DNA was detected above 0.1 ng/μL. It is important to note that the size of the target DNA will affect the sensitivity of the test, and smaller DNA fragments (amplicon DNA) are expected to have a lower limit of detection based on amplicon studies using the Biofortuna HLA Wipe test (Biofortuna, 2011). Although the sensitivity of the wipe test may appear low, this range is relevant to laboratory settings; an aerosolized droplet of amplified PCR product of 10^{-6} μL can contain as many as 10^5 copies of DNA (Persing, 1991).

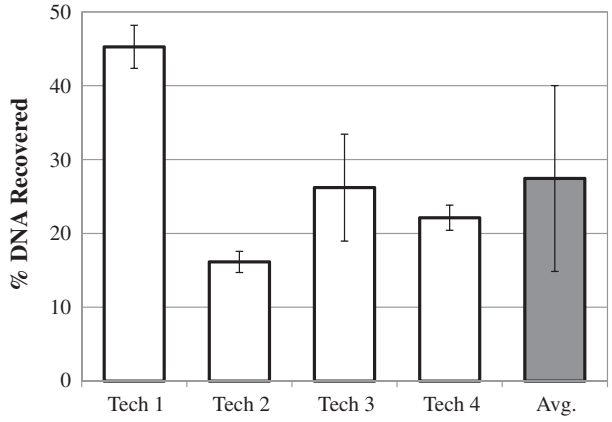

Fig. 7. Comparison of the precision and accuracy of individual technicians on the recovery of the wipe method using *E. coli* DNA spiked on polypropylene sheets. Error bars represent one standard deviation, $n=5$ for each technician (Tech).

Significant losses occur at various process steps. The method validation process revealed that only 0.02% of the DNA present on a surface was detected by PCR, the equivalent of 240 copies detected with an initial surface loading of 10^6 copies of DNA. Sampling efficiencies were determined at each step of the process based on all samples collected during the testing period and were ≥59% for each stage of the wipe process. Major DNA losses occurred due to surface sampling inefficiencies, the retention of DNA in the wipe, and DNA discarded in excess wash solution.

The test response was proportional to the initial DNA loading, with Ct values trending logarithmically over 3.5 orders of magnitude. At concentrations higher than 5×10^7 copies of DNA contamination on the surface, the logarithmic nature of the test declined, likely due to the inability of the wipe to efficiently remove DNA from the polypropylene surface. However, there could be other factors to consider: in one study, higher concentrations of wipe extract decreased PCR sensitivity, sometimes to the point of complete inhibition (McCormack et al., 1997). Since most laboratories use wipe tests to identify the presence of contamination rather than quantify contamination present, the decline in sensitivity above 5×10^7 copies should not reduce the utility of the wipe test.

Deliberate modifications to the wipe sampling protocol were made to determine if recovery could be improved. As a result, a number of inefficiencies in the wipe washing stage were identified and removed. Initially, the wipe test mimicked sampling protocols used to detect spores from impermeable surfaces (CDC, 2002). It included steps to increase yield such as wringing out the gauze wipe after it was washed and centrifuging the sample extract to remove spores. The data indicates that these steps are irrelevant when isolating DNA from wipes. Consequently, by eliminating these steps, a laboratory could save time, money and materials.

Other alterations had varied effects on DNA recovery. One important result is that increasing the sampled area may not increase the collection efficiency of the wipe. A current gauze wipe protocol utilized for surface collection recommends a sampling area greater

than 100 cm^2 with no upper bound limit (CDC, 2002). In the present study, the results demonstrated that increasing the sampled area above 4000 cm^2 decreases recovery efficiency. These results were counter-intuitive based on a previous study by Buttner et al. which found that larger sampling areas (10,000 cm^2) yielded more sensitive results compared to smaller areas sampled using a commercial biological sampling kit (BiSKit™) (Buttner et al., 2004). The lower yields might be attributed to the wipe drying out while sampling the larger area. The foam used in the BiSKit worked best when used dry, and consequently, would be unaffected by changes in moisture when sampling larger areas. Furthermore, there was no significant difference between sampling an area one time versus three times, even though many sources recommend sampling the area more than once (Brown et al., 2007).

The volume of wash played an important role in providing adequate volume for maximum extraction efficiency and in concentrating the sample. The highest extraction efficiency was achieved using wash volumes between 5 and 10 mL, indicating no added benefit of larger wash volumes. However, since wipes for lab contamination typically would not account for wash volume, concentrating the sample with a 5-mL wash volume compared to 10 mL wash volume would increase the chances of detection by nearly two-fold according the present study. Typical wash volumes for wipes range from 1 mL (Frawley et al., 2008) to 50 mL (Estill et al., 2009).

Another important finding is that certain storage conditions proved to be more ideal. Samples stored just after vortexing had more consistent DNA concentration over the course of a week compared to samples that were purified. The microcentrifuge tubes used to store the eluted samples may have contributed to the loss of DNA over time. In one study, Gaillard and Strauss report DNA retention as high as 5 ng mm^{-2} for polypropylene tubes, with large variation between tube lots, and recommend using polyallomer tubes as an alternative (Gaillard and Strauss, 1998). Additionally, eluted samples were stored at 4 °C to avoid multiple freeze-thaw cycles; however, storing the DNA at −20 °C or −70 °C, as QIAGEN recommends, may improve long term stability (QIAGEN, 2010).

Manipulations to the surface conditions for robustness tests, including background levels of dust or potential bleach residue, had negligible effects on the wipe test results. Arizona Test Dust or ATD is commonly used to validate spore extraction efficiencies from environmental samples using concentrations of dust from 2 mg per wipe (Rose et al., 2011) to 250 mg per wipe (Kane et al., 2009; Letant et al., 2011). In this study, a lower concentration of ATD was selected in order to represent conditions of a freshly cleaned lab. The results suggest that dust does not affect the specificity of the wipe test and agree with similar spore studies (Hodges et al., 2010; Rose et al., 2011). However, the significantly lower DNA recovery from dusty surfaces, 6% compared to 22% recovered from clean surfaces, deviated from both Rose and Hodges who reported recoveries between 15% and 55% for both clean and dusty samples. A comparison between these results, however, is inconclusive due to the intrinsic differences in detection methods between spores (detected with CFUs) and DNA (using PCR) and the different materials used for sampling the surface.

Although the method demonstrated excellent repeatability when one technician performed multiple repeat tests over several months, the inter-technician variability tests revealed a high degree of variability. Recorded recovery efficiencies differed by almost 30% when comparing certain technicians. Inter-technician variability showed that the highest source of error when comparing one wipe test to another may be the process technician. Given this variability and the sources of DNA loss, the wipe tests were successfully implemented to identify DNA contamination hotspots on office keyboards and mice, the bench tops where the wipe samples are prepared, and equipment used in post-amplification processes including PCR workstations, a centrifuge, a fridge, PCR instrument tray, and instrument keyboards.

5. Conclusion

Even with stringent measures to avoid contamination and false positive readings, DNA contamination remains a concern; identifying sources of DNA contamination in a PCR laboratory can reduce the prevalence of false positive results that lead to erroneous results and possibly expensive and life-threatening errors. The method validation process reported herein defines the boundaries of a robust wipe method for detecting DNA contamination. This data demonstrates that the method is capable of reliably detecting surface contamination above 4.5×10^5 copies (~2 ng), with few noticeable effects on recovery efficiency upon various alterations of the method. Through these tests, method improvements were identified which made the test more sensitive and the process more time and cost efficient. Although the validation was performed using *E. coli*, similar results are expected with other types of DNA.

Role of the funding source

The Department of Homeland Security (DHS) Office of Health Affairs funded this work in its entirety. However, no stipulations or input were given by DHS regarding this study, including the design, analysis, documentation or submission for publication.

Acknowledgements

The authors gratefully acknowledge the Department of Homeland Security for funding this project. Additionally, the authors would like to thank Eric N. Generous, Emily A. Lyon, and Zoe F. Mark for assistance in the laboratory preparing materials and participation in the inter-technician variability testing.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.mimet.2013.01.003.

References

Billets, S., 2007. A literature review of wipe sampling methods for chemical warfare agents and toxic industrial chemicals. US EPA.
Biofortuna, 2011. HLA Wipe Test Kit Product Information Document, 2nd ed.
Borst, A., Box, A.T., Fluit, A.C., 2004. False-positive results and contamination in nucleic acid amplification assays: suggestions for a prevent and destroy strategy. Eur. J. Clin. Microbiol. Infect. Dis. 23, 289–299.
Brown, G.S., Betty, R.G., Brockmann, J.E., Lucero, D.A., Souza, C.A., Walsh, K.S., Boucher, R.M., Tezak, M., Wilson, M.C., Rudolph, T., 2007. Evaluation of a wipe surface sample method for collection of Bacillus spores from nonporous surfaces. Appl. Environ. Microbiol. 73, 706–710.
Buttner, M.P., Cruz, P., Stetzenbach, L.D., Klima-Comba, A.K., Stevens, V.L., Emanuel, P.A., 2004. Evaluation of the biological sampling kit (BiSKit) for large-area surface sampling. Appl. Environ. Microbiol. 70, 7040–7045.
CDC, 2002. Procedures for collecting surface environmental samples for culturing *B.anthracis*. Center for Disease Control and Prevention.
Cone, R.W., Hobson, A.C., Huang, M.L., Fairfax, M.R., 1990. Polymerase chain reaction decontamination: the wipe test. Lancet 336, 686–687.
Dauphin, L.A., Moser, B.D., Bowen, M.D., 2009. Evaluation of five commercial nucleic acid extraction kits for their ability to inactivate *Bacillus anthracis* spores and comparison of DNA yields from spores and spiked environmental samples. J. Microbiol. Meth. 76, 30–37.
Dwyer, D.E., Saksena, N., 1992. Failure of ultra-violet irradiation and autoclaving to eliminate PCR contamination. Mol. Cell. Probes 6, 87–88.
Edmonds, J.M., 2009. Efficient methods for large-area surface sampling of sites contaminated with pathogenic microorganisms and other hazardous agents: current state, needs, and perspectives. Appl. Microbiol. Biotechnol. 84, 811–816.
Estill, C.F., Baron, P.A., Beard, J.K., Hein, M.J., Larsen, L.D., Rose, L., Schaefer, F.W., Noble-Wang, J., Hodges, L., Lindquist, H.D.A., Deye, G.J., Arduino, M.J., 2009. Recovery efficiency and limit of detection of aerosolized *Bacillus anthracis* Sterne from environmental surface samples. Appl. Environ. Microbiol. 75, 4297–4306.
Frawley, D.A., Samaan, M.N., Bull, R.L., Robertson, J.M., Mateczun, A.J., Turnbull, P.C.B., 2008. Recovery efficiencies of anthrax spores and ricin from nonporous or nonabsorbent and porous or absorbent surfaces by a variety of sampling methods. J. Forensic Sci. 53, 1102–1107.

Gaillard, C., Strauss, F., 1998. Avoiding adsorption of DNA to polypropylene tubes and denaturation of short DNA fragments. Elsevier Trend Journal Technical Tips. Elsevier.

Hodges, L.R., Rose, L.J., O'Connell, H., Arduino, M.J., 2010. National validation study of a swab protocol for the recovery of *Bacillus anthracis* spores from surfaces. J. Microbiol. Methods 81, 141–146.

Kane, S.R., Letant, S.E., Murphy, G.A., Alfaro, T.M., Krauter, P.W., Mahnke, R., Legler, T.C., Raber, E., 2009. Rapid, high-throughput, culture-based PCR methods to analyze samples for viable spores of *Bacillus anthracis* and its surrogates. J. Microbiol. Methods 76, 278–284.

Krauter, P.A., Piepel, G.F., Boucher, R., Tezak, M., Amidan, B.G., Einfeld, W., 2012. False-negative rate and recovery efficiency performance of a validated sponge wipe sampling method. Appl. Environ. Microbiol. 78, 846–854.

Letant, S.E., Murphy, G.A., Alfaro, T.M., Avila, J.R., Kane, S.R., Raber, E., Bunt, T.M., Shah, S.R., 2011. Rapid-viability PCR method for detection of live, virulent *Bacillus anthracis* in environmental samples. Appl. Environ. Microbiol. 77, 6570–6578.

Lo, Y.M.D., Mehal, W.Z., Fleming, K.A., 1988. False-positive results and the polymerase chain-reaction. Lancet 2, 679.

McCormack, J.M., Sherman, M.L., Maurer, D.H., 1997. Quality control for DNA contamination in laboratories using PCR-based class II HLA typing methods. Hum. Immunol. 54, 82–88.

Mifflin, T.E., 2007. Setting up a PCR laboratory. CSH Protoc 2007, pdb top14.

Niederhauser, C., Hofelein, C., Wegmuller, B., Luthy, J., Candrian, U., 1994. Reliability of PCR decontamination systems. PCR Methods Appl. 4, 117–123.

NIOSH, 1996. LEAD in Surface Wipe Samples, NIOSH Manual of Analytical Methods (NMAM), 4th ed.

Patel, R., Grogg, K.L., Edwards, W.D., Wright, A.J., Schwenk, N.C., 2000. Death from inappropriate therapy for Lyme disease. Clin. Infect. Dis. 31, 1107–1109.

Persing, D.H., 1991. Polymerase chain reaction: trenches to benches. J. Clin. Microbiol. 29, 1281–1285.

QIAGEN, 2010. QIAamp DNA Mini and Blood Mini Handbook, 3rd ed. , pp. 1–72.

Rose, L.J., Hodges, L., O'Connell, H., Noble-Wang, J., 2011. National validation study of a cellulose sponge wipe-processing method for use after sampling *Bacillus anthracis* spores from surfaces. Appl. Environ. Microbiol. 77, 8355–8359.

Sanderson, W.T., Hein, M.J., Taylor, L., Curwin, B.D., Kinnes, G.M., Seitz, T.A., Popovic, T., Holmes, H.T., Kellum, M.E., McAllister, S.K., Whaley, D.N., Tupin, E.A., Walker, T., Freed, J.A., Small, D.S., Klusaritz, B., Bridges, J.H., 2002. Surface sampling methods for *Bacillus anthracis* spore contamination. Emerg. Infect. Dis. 8, 1145–1151.

Stevenson, B.S., Schmidt, T.M., 2004. Life history implications of rRNA gene copy number in *Escherichia coli*. Appl. Environ. Microbiol. 70, 6670–6677.

USEPA, 2004. Quality Assurance/Quality Control Guidance for Laboratories Performing PCR Analyses on Environmental Samples. In: Water, O.o (Eds.) .

Willetts, P., Wood, R., 2000. Principles and Practices of Method Validation. Royal Society of Chemistry, Cambridge.

Yang, S., Lin, S., Kelen, G.D., Quinn, T.C., Dick, J.D., Gaydos, C.A., Rothman, R.E., 2002. Quantitative multiprobe PCR assay for simultaneous detection and identification to species level of bacterial pathogens. J. Clin. Microbiol. 40, 3449–3454.