

Testing PCR Clean™ Efficiency

INTRODUCTION

DNA, RNA, but also DNase and RNase contaminations represent constant although diverse threats for molecular biology laboratories specialized in PCR. Due to their high sensitivity, PCR-based methods may wrongfully amplify and detect even a single DNA or RNA molecule contaminating e.g. PCR workstations. In most cases, this leads to widespread problems throughout the testing procedure, resulting in PCR artifacts, inaccurate data, and often false positive results. Additionally, contamination with nucleases - which degrade DNA or RNA - are as common as harmful for molecular biology experiments. Especially RNases, which are ubiquitous (e.g. human skin, microbes etc.) and robust, are known to dramatically affect downstream applications by degrading RNA molecules.

Removal of nucleic acids and nucleases contaminations has proven to be no trivial matter, as especially DNA or RNases contaminations are particularly resistant to treatment.

PCR Clean™ is a ready-to-use solution for the removal of nucleic acids and nucleases from any surface at PCR workstations and/or lab devices and equipment. The solution contains a surfactant and a non-alkaline and non-carcinogenic agent. In this study we examined the efficiency and effectiveness of PCR Clean™ against different nucleic acid and nucleases contaminations on various surfaces, in comparison with various other cleaning agents.

PROCEDURE

1. Surface decontamination of DNA

1a. Removal of amplicon DNA from various surfaces

The efficiency of PCR Clean™ was tested for the removal of bacterial amplicon DNA from *E. coli* 0104 and *E. coli* 0157 and compared to several other cleaning materials. Bacterial amplicon DNA was applied by pipetting 2 µl (0.05 ng) *E. coli* 0104 DNA on a plastic foil, glass surface, and lab work surface area (Trespa®), or 2 µl (0.2 ng) *E. coli* 0157 DNA on Plexiglas® and aluminum-surfaces. After DNA was completely air-dried, it was removed by using a paper towel moisturized with PCR Clean™, a diluted dishwashing detergent, 70 % Ethanol, Isopropanol, water, or a dry paper towel, by wiping off the spot where DNA was pipetted with one stroke. Samples were then collected by using a moisturized swab and wiping off (swabbing) the same area. As positive control, either 0.05 ng *E. coli* 0104 DNA (for plastic foil, glass or Trespa® assays), or 0.2 ng *E. coli* 0157 DNA (for Plexiglas® and aluminum assays), were directly pipetted into 250 µl PCR grade water and extracted in the same manner as the other samples.

1b. Removal of genomic DNA and plasmid DNA from aluminum surfaces

The efficiency of PCR Clean™ was also tested for genomic and plasmid DNA removal from aluminum surfaces by pipetting 2 µl (2×10^6 genome copies) of *E. coli* genomic DNA, or 2 µl (2×10^6 genome copies) *E. coli* plasmid DNA on an aluminum surface. DNA was air-dried before the spot was wiped off using either a paper towel moisturized with PCR Clean™, water, or a dry paper towel, with one stroke. Samples were then collected by using a moisturized swab and wiping off (swabbing) the same area. As positive control, either 2×10^6 genome copies of *E. coli* genomic DNA, or 2×10^6 genome copies of *E. coli* plasmid DNA were directly pipetted into 250 µl PCR grade water and extracted in the same manner as the other samples.

2. Surface decontamination of RNA

2. Removal of RNA from a Plexiglas® surface

RNA was extracted from human cells using our ExtractNow™ RNA extraction kit. RNA concentration was measured and 5 µg (11 µl) were pipetted on each of 6 different spots of a Plexiglas® surface. RNA was completely air-dried, then removed using a paper towel moisturized with PCR Clean™, a diluted dishwashing detergent, 70 % Ethanol, Isopropanol, water, or a dry paper towel, by wiping off the spots where RNA was pipetted, with one stroke. Samples were then collected by swabbing the same area, using a moisturized swab. Swabs carrying the collected RNA samples were transferred into 400 µl of lysis buffer and RNA was extracted using ExtractNow™ RNA extraction kit. As positive control, 5 µg RNA extract from human cells were directly transferred into 400 µl lysis buffer and processed in the same manner as the rest of the samples. Reverse transcription of all RNA samples, including the positive control, was carried out and synthesized cDNA samples were subsequently subjected to qPCR.

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3. Surface decontamination of DNases

3a. Removal of DNase from glass surfaces

The efficiency of PCR Clean™ and PCR Clean™ Wipes was also tested concerning their ability to remove DNases from glass surfaces. Briefly, DNase I (1 µl, 3 U/µl, AppliChem, Cat. No. A3778.0010) was pipetted onto a glass surface and let air-dry. Next, the pre-coated spot was wiped off or not with a dry paper towel, a 70 % isopropanol-soaked, a PCR Clean™-soaked or a PCR Clean™ Wipe, with one homogenous, reproducible stroke.

3b. DNase activity assay

Persisting DNase contaminations were identified by measuring the enzymatic activity of DNases, indirectly by qPCR, using a genomic DNA (gDNA) standard as substrate/indicator. Briefly, after the wiping step, 20 µl of gDNA (*M. gallisepticum*, 10³ genome copies/µl, 10mM MgCl₂ in PCR grade Water) were added to the DNase-pre-coated spot. The mixture of contaminating DNase and its substrate gDNA were then collected by pipetting up and down 10 times and incubated 15 minutes at 37 °C to allow for the enzymatic reaction (digestion) to take place.

Relevant DNA degradation due to contaminating DNases was then inferred by assessing the performance of the qPCR amplification with Microsart® ATMP Mycoplasma (Sartorius STEDIM Biotech). As a reference of a fully impaired qPCR reaction, positive controls were included and obtained by incubating DNase I (1 µl, 3 U/µl) with 20 µl of gDNA (10³ genome copies/µl) for 15 minutes at 37 °C without prior surface spotting. The same procedure was used to generate the negative controls, omitting the DNase I.

4. Surface decontamination of RNases

4a. Removal of RNase from glass surfaces

The efficiency of PCR Clean™ (spray and pre-wetted wipes) was also tested concerning its ability to remove RNases from glass surfaces. Briefly, RNase A (5 µl, 0.03 U/ml in glycerol, Thermo Fisher Scientific, Cat. No. AM1964) was pipetted onto a previously decontaminated glass surface and incubated for 30 min at RT. Next, the RNase-coated spot was cleaned or not with a dry paper towel, a PCR Clean™ Wipe, or a towel soaked with 70 % isopropanol, PCR Clean™ or the commercially available RNase decontaminant RNaseZap® (Thermo Fisher Scientific, Cat. No. AM1964), with reproducible one homogenous, reproducible stroke after 15 seconds contact time.

4b. RNase activity assay

After RNase contamination was performed in controlled conditions, 50 µl nucleases-free H₂O (Thermo Fisher Scientific, Cat. No. AM1964) were added to the pre-contaminated spot and pipetted up and down 10 times to collect any present RNase. 45 µl of such samples were then used for the commercially available, fluorescence-based assay RNaseAlert® (Thermo Fisher Scientific, Cat. No. AM1964). The kit was used as recommended by the manufacturer. Subsequently, raw fluorescences (ex 490/em 520) were measured during incubation at 37 °C, every 5 minutes for max. 2 hours with CFX96 Touch™ (Bio-Rad).

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RESULTS

1. Surface decontamination of DNA

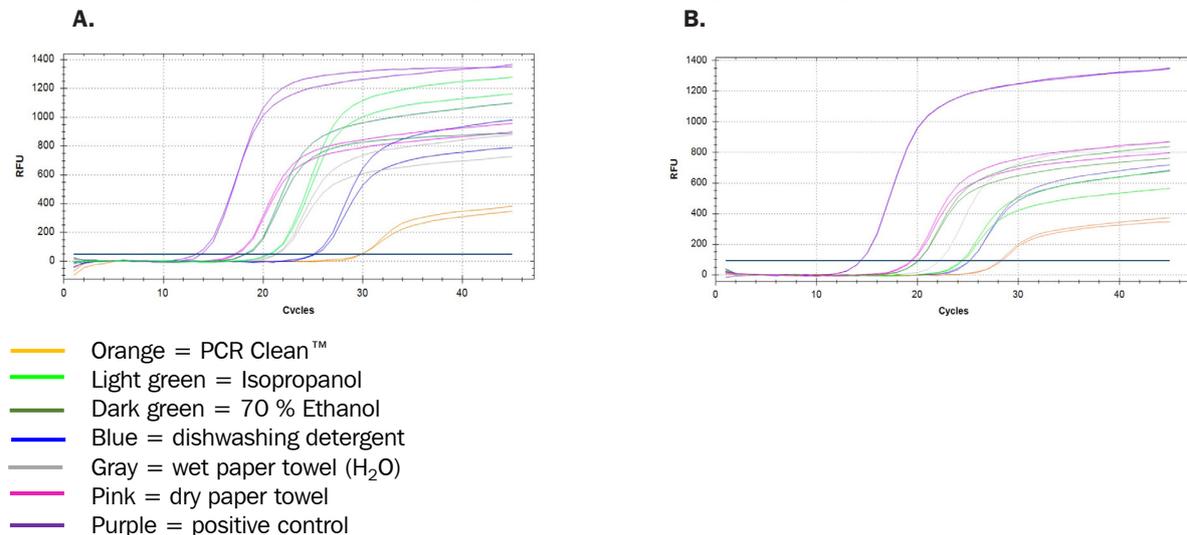
1a. Removal of amplicon DNA from various surfaces

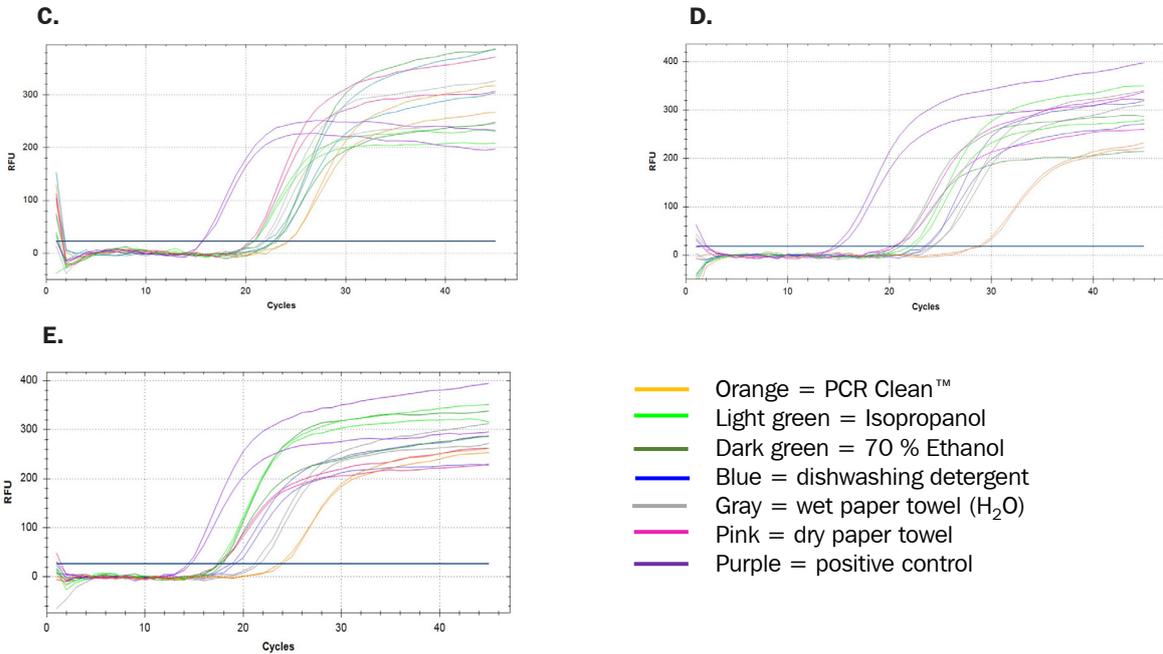
Results showed a bigger depletion of amplicon DNA after removal with PCR Clean™ from the various surfaces tested, in comparison to other cleaning agents and in reference to the positive control (Table 1). For all surfaces tested, PCR Clean™ showed the best effectiveness for the removal of amplicon DNA, which could be observed in Graph 1, where C_t-values for PCR Clean™ were higher in comparison to other cleaning agents and in reference to the positive control, regardless of the surface material (Graph 1, A-E), indicating a higher decrease in DNA amount.

Table 1. C_t-values measured in qPCR amplification of bacterial amplicon DNA after removal from various surfaces using PCR Clean™ in comparison with other cleaning agents.

Surface Remover	Plastic foil	Glass	Trespa®	Plexiglas®	Aluminum
PCR Clean™	23.82	28.82	24.23	29.78	28.22
Diluted dishwashing detergent	18.95	23.53	22.28	25.11	25.13
70 % Ethanol	17.74	22.21	22.96	18.15	20.06
Isopropanol	17.43	20.82	20.82	20.54	24.37
H ₂ O	21.37	24.21	21.83	21.23	22.13
Dry paper towel	17.52	20.35	20.52	17.18	19.36
Positive control	14.49	14.77	15.64	13.47	14.34

Graph 1. qPCR amplification curves of E. coli amplicon DNA after removal from A. Plexiglas®, B. aluminum, C. Trespa®, D. Glass, and E. Plastic foil, using different cleaning agents (s. legend below for details).





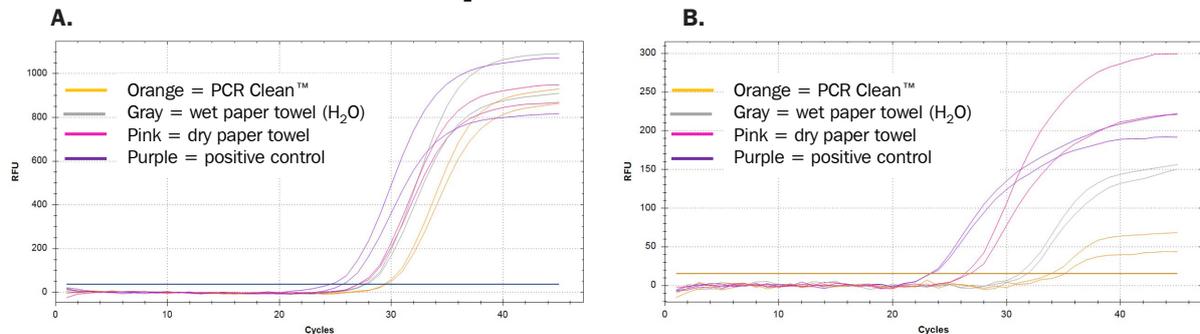
1b. Removal of genomic DNA and plasmid DNA from aluminum surfaces

Results showed a bigger depletion of genomic DNA and plasmid DNA after removal with PCR Clean™ from an aluminum surface, in comparison to water and a dry paper towel, and in reference to the positive control (Table 2). This can also be shown in Graph 2, where C_t-values for genomic DNA amplification (Graph 2, A) and C_t-values for plasmid DNA amplification (Graph 2, B) after removal with PCR Clean™ were higher in comparison to C_t-values after removal with the other cleaning agents and in reference to the positive control, indicating a higher decrease in DNA amount.

Table 2. C_t-values measured in qPCR amplification of genomic DNA or plasmid DNA after removal from an aluminum surface using PCR Clean™ in comparison with H₂O and a dry paper towel.

Remover	Surface	Plastic foil	Glass
PCR Clean™		29.45	34.51
H ₂ O		27.69	31.49
Dry paper towel		27.06	26.56
Positive control		25.11	23.33

Graph 2. qPCR amplification curves of A. E. coli genomic DNA or B. E. coli plasmid DNA, after removal from aluminum surfaces using PCR Clean™, H₂O, or a dry paper towel.



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2. Surface decontamination of RNA

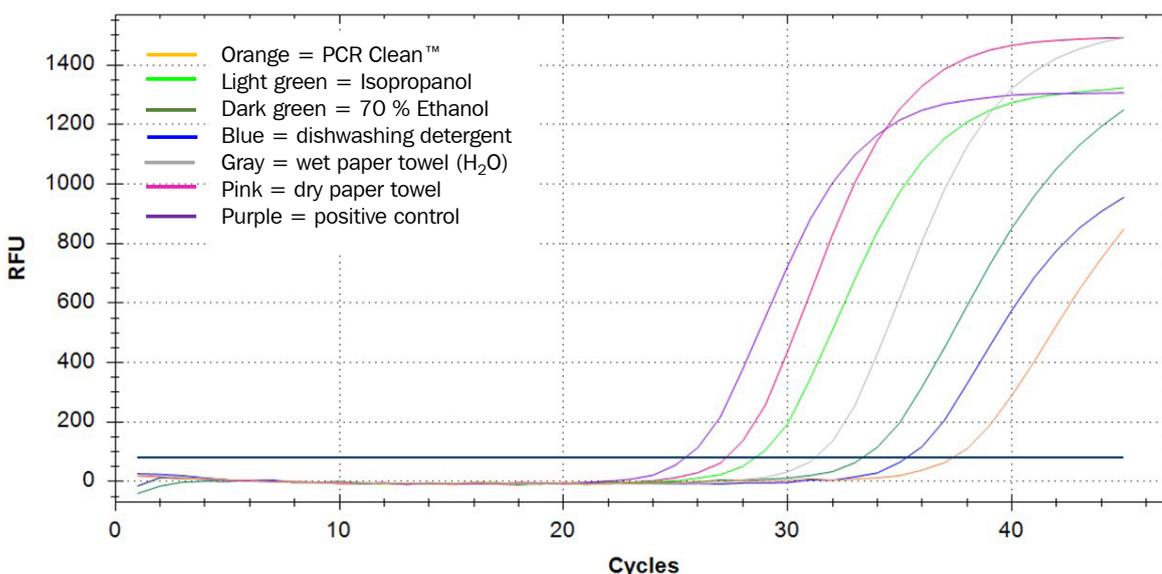
2a. Removal of RNA from a Plexiglas® surface

Results showed a bigger depletion of RNA after removal with PCR Clean™ from a Plexiglas® surface, in comparison to other cleaning agents, and in reference to the positive control (Table 3). This can also be shown in Graph 3, where C_t-values for cDNA amplification, synthesized from RNA via reverse transcription, after RNA removal with PCR Clean™ were higher in comparison to C_t-values after RNA removal with the other cleaning agents and in reference to the positive control, indicating a higher decrease in RNA amount.

Table 3. C_t-values measured in qPCR amplification of cDNA, synthesized from RNA via reverse transcription, after RNA removal from a Plexiglas® surface using PCR Clean™ in comparison with different cleaning agents.

Nucleic acid \ Remover	PCR Clean™	Diluted dishwashing detergent	70 % Ethanol	Isopropanol	H ₂ O	Dry paper towel	Positive control
RNA	37.33	35.27	33.3	28.52	31.19	27.23	24.08

Graph 3. qPCR amplification curves of cDNA, synthesized from RNA via reverse transcription, after RNA removal from Plexiglas®, using different cleaning agents (s. legend below for details).



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5. Summary of results on nucleic acids

Depletion of nucleic acids

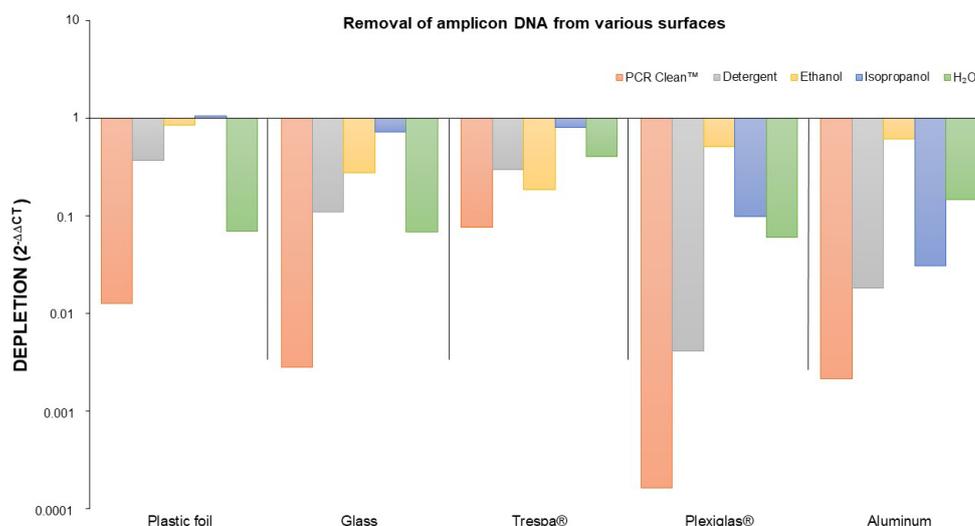
Depletion of nucleic acids as a result of removal using PCR Clean™ or other cleaning agents was established by calculating ΔC_t values in reference to the positive control of each qPCR assay (Table 4). Results showed that depletion of amplicon, genomic and plasmid DNA, as well as depletion of RNA after removal with PCR Clean™ is higher than any other cleaning agent, regardless of the surface material used for the testing (Table 4, ΔC_t values in red).

Table 4 Overview of all nucleic acid depletion results after removal from various surfaces using different cleaning agents (ΔC_t value, decrease in reference to positive control).

Nucleic acid contamination	Amplicon DNA					RNA	Genomic DNA	Plasmid DNA
	Plastic foil	Glass	Trespa®	Plexiglas®	Aluminum	Plexiglas®	Aluminum	
PCR Clean™	9.33	14.05	8.59	16.31	13.88	13.25	4.34	11.18
Diluted dishwashing detergent	4.46	8.76	6.64	11.64	10.80	11.19	/	/
70 % Ethanol	3.25	7.44	7.32	4.68	5.72	9.22	/	/
Isopropanol	2.94	6.05	5.18	7.07	10.03	4.44	/	/
H ₂ O	6.88	9.44	6.19	7.76	7.79	7.11	2.58	8.16
Dry paper towel	3.03	5.58	4.88	3.71	5.02	3.15	1.95	3.23

Depletion of nucleic acids after removal with PCR Clean™ or other cleaning agents was visualized in diagram 1 (for amplicon DNA), diagram 2 (for RNA), and diagram 3 (for genomic and plasmid DNA), as $2^{-\Delta\Delta C_t}$ values were calculated using the results of the dry paper towel (ΔC_t values), where no cleaning agent was used, as reference. By doing so, the sole removal effect of the dry paper towel was subtracted.

Diagram 1. Overview of amplicon DNA depletion results after removal from various surfaces using different cleaning agents. Results are shown as $2^{-\Delta\Delta C_t}$, where $\Delta\Delta C_t$ values are in reference to results (ΔC_t values) of dry paper towel.



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Diagram 2. Overview of depletion results of RNA after removal from Plexiglas® surface, using different cleaning agents. Results are shown as $2^{-\Delta\Delta C_t}$, where $\Delta\Delta C_t$ values are in reference to results (ΔC_t values) of dry paper towel.

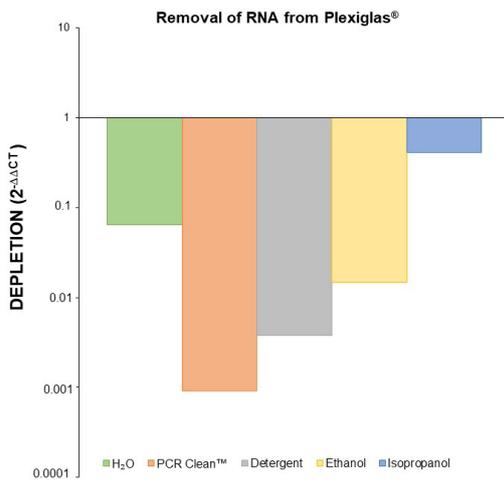
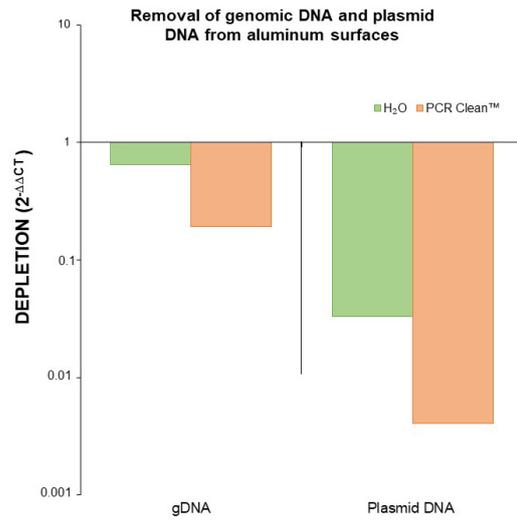


Diagram 3. Overview of depletion results of genomic and plasmid DNA after removal from aluminum surfaces, using PCR Clean™ or water. Results are shown as $2^{-\Delta\Delta C_t}$, where $\Delta\Delta C_t$ values are in reference to results (ΔC_t values) of dry paper towel.



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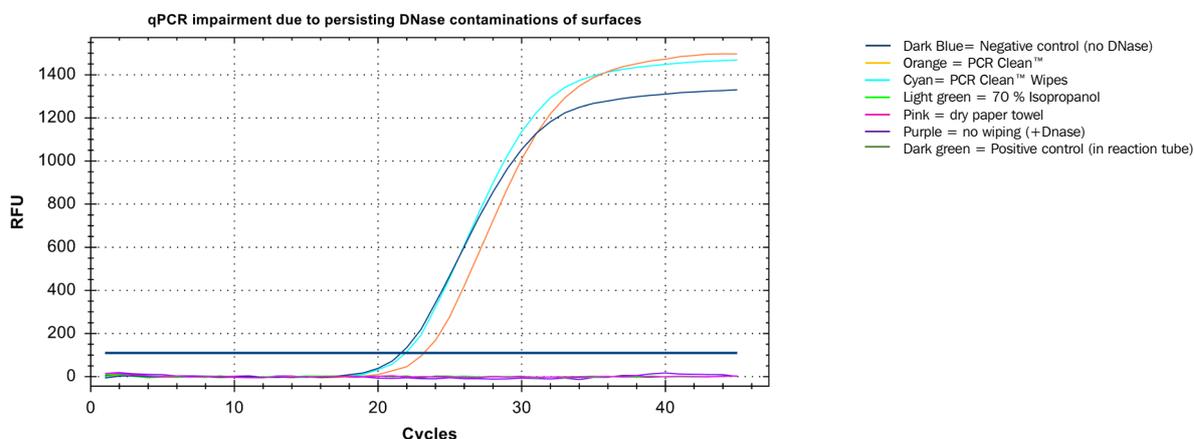
3. Surface decontamination of DNases

Effective removal of DNases (3 U) was assessed by qPCR of gDNA (*Mycoplasma gallisepticum*, 10³ C/μl) spiked on the DNase-contaminated spot, after decontamination was carried out with different methods. Prior to qPCR, the mixture of gDNA and DNase was collected from the contaminated glass surface and incubated in optimal conditions for the enzymatic reaction. Decontamination was considered to be complete when no impairment of the qPCR reaction was observed. This was achieved by wiping with PCR Clean™ wipes or PCR Clean™-moistened paper towels, as demonstrated by successful amplification of the spiked gDNA. The Ct values obtained for spiked gDNA from samples of decontaminated surfaces with PCR Clean™ wipes or PCR Clean™-moistened paper towels were comparable to those of the negative control, where no DNase was pipetted prior to the gDNA spike (Table 5). Omitting the wiping step, using dry paper towels, or towels moistened with 70 % isopropanol induced substantial gDNA degradation by the previously pipetted DNases (no Ct, Table 5). A similar, complete degradation was observed in the positive control group, where DNases-induced DNA degradation was allowed to occur in optimal reaction conditions. Graph 5 shows clear amplification curves above threshold, only for PCR Clean™- treated surfaces and negative control, thereby confirming the differential qPCR performance depending on the removal agent employed.

Table 5. Residual DNase activity after surface cleaning using subsequent DNA recovery as an index of decontamination efficiency. The table shows the qPCR C_t-values for gDNA spiked on a DNase-precoated glass surface, later wiped with several methods, in comparison with controls. Lack of amplification (no Ct), as obtained with optimal enzymatic digestion (positive control), indicated significant DNase-dependent DNA degradation, hindering qPCR. Similarly, inefficient decontamination methods also led to qPCR impairment. Successful amplification of the spotted target (within 2 Ct values from negative control) demonstrated significant removal of previously contaminating DNases.

Remover	Results	Ct	Persisting DNase contamination:
Negative control (no DNase)		21.60	no (DNA recovery)
PCR Clean™		23.19	no (DNA recovery)
PCR Clean™ Wipes		21.92	no (DNA recovery)
70 % Isopropanol		no Ct (> 45)	yes (DNA degradation)
Dry paper towel		no Ct (> 45)	yes (DNA degradation)
No wiping		no Ct (> 45)	yes (DNA degradation)
Positive control (DNase)		no Ct (> 45)	yes (DNA degradation)

Graph 5. qPCR amplification curves of gDNA spiked on DNase-contaminated glass surfaces after several cleaning methods were applied in comparison with control conditions (s. legend below for details).

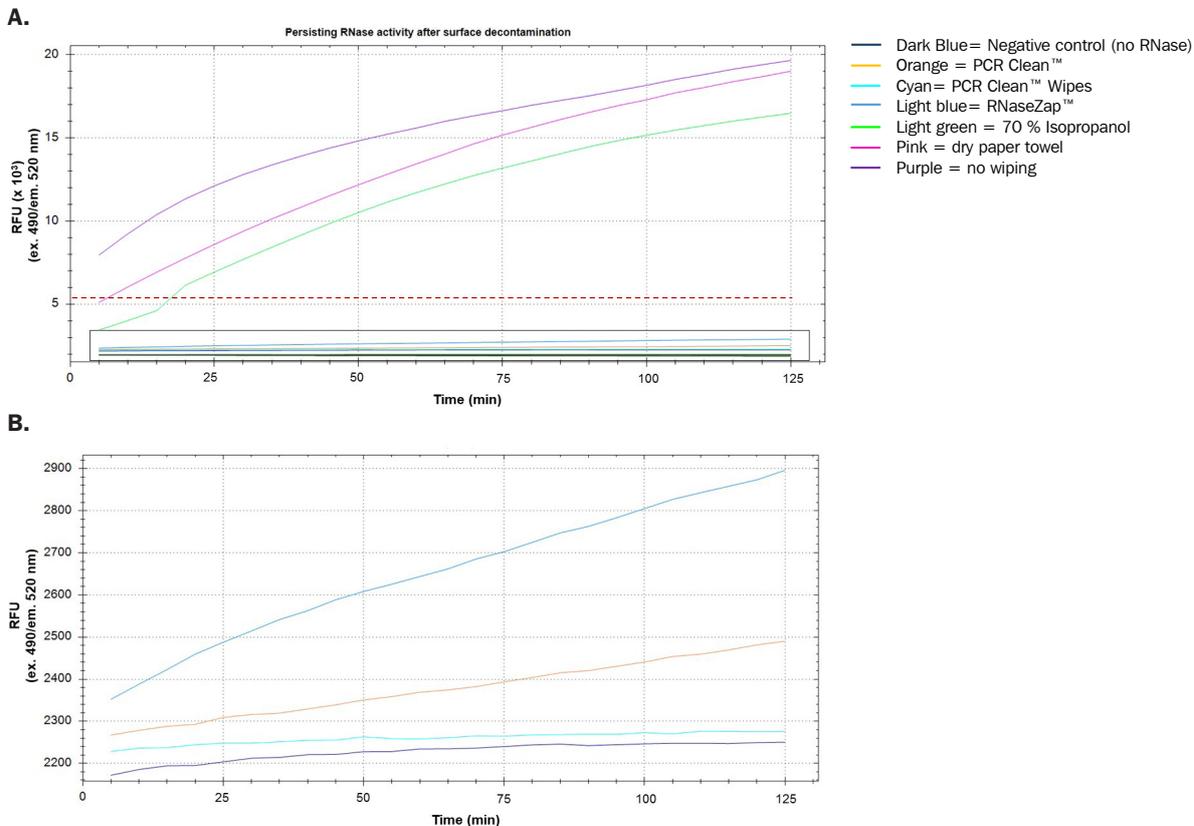


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4. Surface decontamination of RNases

Effective removal of RNases was assessed with a commercially available, fluorescence-based RNase activity assay after surface cleaning or not. Prior to the assay, surfaces, including those where RNase (negative control) or wiping (positive control) were omitted, were then sampled by thorough spot-pipetting and assayed with fluorescence-based method RNaseAlert® (Thermo Fisher Scientific). RNase activity attributable to a significant degree of contamination was consistently observed in samples where no wiping was carried out (Graph 6A, purple curve, representative results). Decontamination was considered to be complete when no residual activity of the RNase was measured, as in the negative controls. In terms of fluorescent signals, a cut-off was defined following the kit's manufacturer recommendations, as 2.5-fold the fluorescence of the negative controls (Graph 6A, red dotted line). Based on this parameter, omitting the wiping step, using dry paper towels, or towels moistened with 70 % isopropanol did not prevent RNase activation in at least 2 or 3 replicate experiments (Table 6 and Graph 6A). To better highlight the differences between negative controls and other cleaning methods, the corresponding zoomed area of Graph 6A (black rectangle) is shown in Graph 6B. Significant and reproducible decontamination was only achieved after wiping with PCR Clean™ (both prewetted wipes and soaked paper towels) or with RNaseZap®-moistened paper towels, as demonstrated by low fluorescence levels even after 2 hours incubation at 37 °C (Graph 6B and Table 6).

Graph 6. Representative fluorescence curves for the analysis of RNase contamination with RNaseAlert™ (Thermo Fisher Scientific). A, RNase activity was evaluated after wiping the contaminated glass surface with several methods in comparison with control conditions (no wiping: positive control; no RNase spotted: negative control). Quick and stable fluorescence signals were observed in significantly contaminated samples, whereas samples with negligible RNase amounts showed a trend similar to the negative controls. A cut-off of 2.5-fold the RFU of the negative controls (red-dotted line) was applied to discriminate between contaminated and non-contaminated samples. For this purpose, RFU values for all the experimental groups were averaged through 10 measurements at plateau (after recommended incubation time). In B, the zoomed portion of Graph A (black rectangle) is shown.



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Table 6. Persisting RNase contaminations on glass surfaces after decontamination with several methods.

RNase-contaminated glass surfaces were successfully (–, non-contaminated) or not (+, contaminated) cleaned by wiping with dry towels, isopropanol-soaked, PCR Clean™-soaked, RNaseZap® (Thermo Fisher Scientific)-soaked paper towels, or PCR Clean™ Wipes. Persisting RNase contamination of the surfaces (+) was identified by mean fluorescence values (over 10 measurements) which were at least 2.5-times higher than the negative controls. Samples showing RFU below such cut-off were considered as non-contaminated (–). The experiment was repeated 3 times in controlled conditions.

Remover	Persisting RNase Contamination (n=3):
Negative control (no RNase)	– – –
RNaseZap®	– – –
PCR Clean™ Wipes	– – –
PCR Clean™	– – –
Isopropanol	+ – +
Dry paper towel	– + +
No wiping (Positive Control)	+ + +

CONCLUSIONS

The results of our study showed that PCR Clean™ is highly effective against amplicon, plasmid, and genomic DNA, and RNA contaminations from all surfaces tested, even within seconds after use. Experiments to evaluate the removal of nucleases showed also efficient DNases and RNases decontamination of glass surfaces by PCR Clean™ (spray and wipes). Depletion results also demonstrate superiority in effectiveness and efficiency of PCR Clean™ compared to most common cleaning agents usually available in molecular biology laboratories such as ethanol, isopropanol or dishwashing detergent. Furthermore, here we found comparable RNase decontamination efficiencies for PCR Clean™ (both spray and wipes) and for a commonly used RNase cleaning product, RNaseZap®.

Therefore, the regular use of PCR Clean™, both before and after PCR analysis is fast, easy and ideal to maintain a clean work area, which can be critical in molecular biology laboratories and PCR workstations, and thereby saves time and expenses. Remarkably, PCR Clean™ is an excellent cleaning agent for molecular biology laboratories, as it can be successfully used against several known contaminants at once.

Trademarks

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