# Testing PCR Clean™ Efficiency

## INTRODUCTION

Nucleic acid contaminations are especially problematic in the highly sensitive PCR technique. Even a single contaminant DNA or RNA molecule at PCR workstations can be detected in the amplification process leading to widespread problems throughout the testing procedure, and resulting in PCR artifacts, false positive results and inaccurate data. Removal of nucleic acid contaminations has proven to be no trivial matter, as DNA contaminations are particularly sustainable. PCR Clean™ is a ready-to-use solution for the removal of nucleic acids 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 contaminations on various surfaces, in comparison with several other cleaning agents.

## PROCEDURE

### 1. 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.

### 2. 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 ($2 \times 10^6$ genome copies) of *E. coli* genomic DNA, or 2 µl ($2 \times 10^6$ 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 $2 \times 10^6$ genome copies of *E. coli* genomic DNA, or $2 \times 10^6$ 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.

### 3. 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 were 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.

### 4. DNA extraction and qPCR amplification

DNA extraction from the swabs was carried out using our SwabUp™ Lab Monitoring kit. DNA was extracted from all collected samples and positive controls in the same manner. All DNA extracts were subsequently subjected to qPCR.

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## RESULTS

### 1. Removal of amplicon DNA from various surfaces

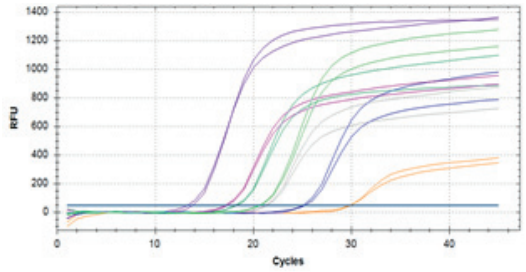
Results showed a bigger depletion of amplicon DNA after removal with PCR Clean™ from the various surfaces tested, in comparison to other cleaning agents and in reference to the positive control (Table 1). For all surfaces tested, PCR Clean™ showed the best effectiveness for the removal of amplicon DNA, 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.

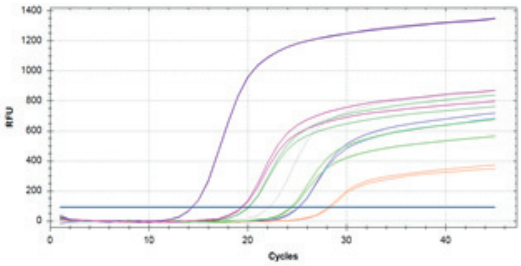
| Surface / Remover | Plastic foil | Glass | Trespa® | Plexiglas® | Aluminum |
|---|---|---|---|---|---|
| **PCR Clean™** | *23.82* | *28.82* | *24.23* | *29.78* | *28.22* |
| **Diluted dishwashing detergent** | *18.95* | *23.53* | *22.28* | *25.11* | *25.13* |
| **70 % Ethanol** | *17.74* | *22.21* | *22.96* | *18.15* | *20.06* |
| **Isopropanol** | *17.43* | *20.82* | *20.82* | *20.54* | *24.37* |
| **$H_2O$** | *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).

**A.**




**B.**




Orange = PCR Clean™
Light green = Isopropanol
Dark green = 70 % Ethanol
Blue = dishwashing detergent
Gray = $H_2O$
Pink = dry paper towel
Purple = positive control

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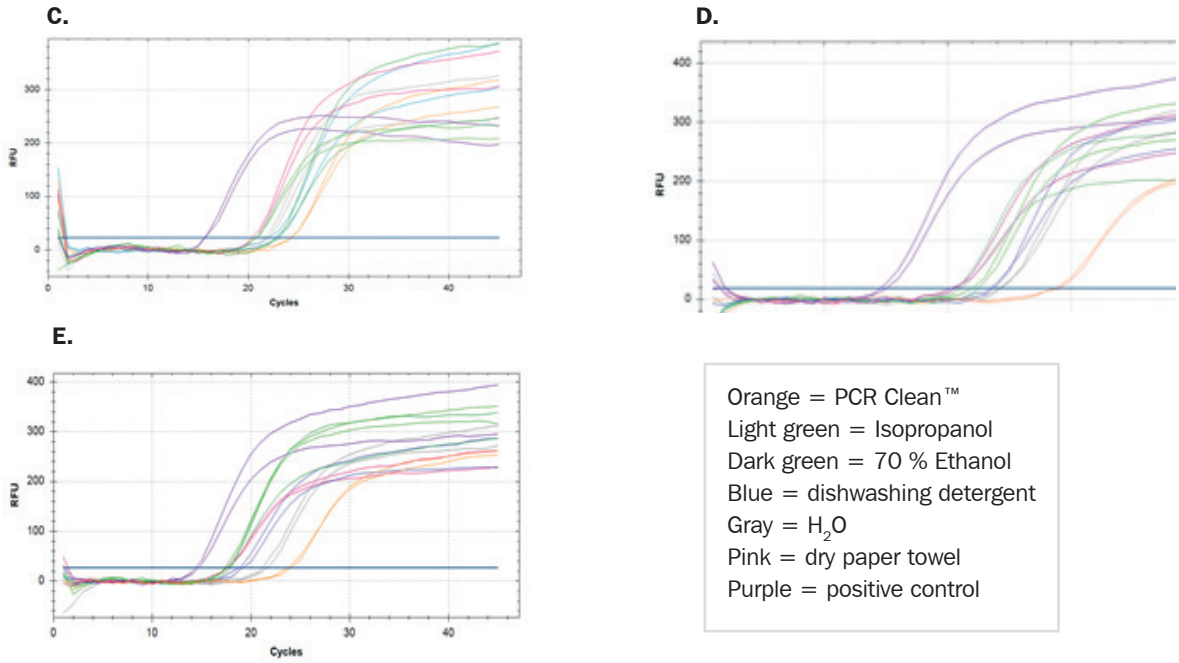


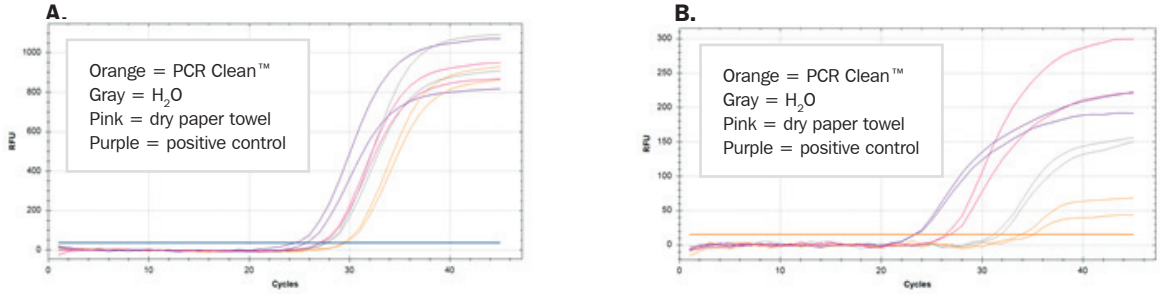

**2. Removal of genomic DNA and plasmid DNA from aluminum surfaces**

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 $H_2O$ and a dry paper towel.

| Remover \ Nucleic acid | Plastic foil | Glass |
|---|---|---|
| **PCR Clean™** | 29.45 | 34.51 |
| **$H_2O$** | 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™, $H_2O$, or a dry paper towel.



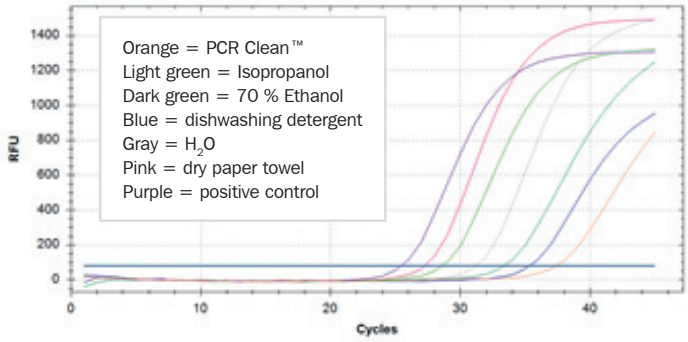
### 3. Removal of RNA from a Plexiglas® surface

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, synthetized 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, synthetized from RNA via reverse transcription, after RNA removal from a Plexiglas® surface using PCR Clean™ in comparison with different cleaning agents.

| Remover / Nucleic acid | PCR Clean™ | Diluted dishwashing detergent | 70 % Ethanol | Isopropanol | $H_2O$ | 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, synthetized from RNA via reverse transcription, after RNA removal from Plexiglas®, using different cleaning agents (s. legend below for details).




### 4. Summary of results

Depletion of nucleic acids as a result of removal using PCR Clean™ or other cleaning agents was established by calculating $\Delta C_t$ values in reference to the positive control of each qPCR assay (Table 4). Results show 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, $\Delta C_t$ values in red).

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

| Nucleic acid contamination | Amplicon DNA | | | | | RNA | Genomic DNA | Plasmid DNA |
|---|---|---|---|---|---|---|---|---|
| Surface / Remover | 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 | / | / |
| **$H_2O$** | 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 |

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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 $\Delta\Delta C_t$ values were calculated using the results of the dry paper towel ($\Delta C_t$ 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 $2^{-\Delta\Delta Ct}$, where $\Delta\Delta C_t$ values are in reference to results ($\Delta C_t$ values) of dry paper towel.

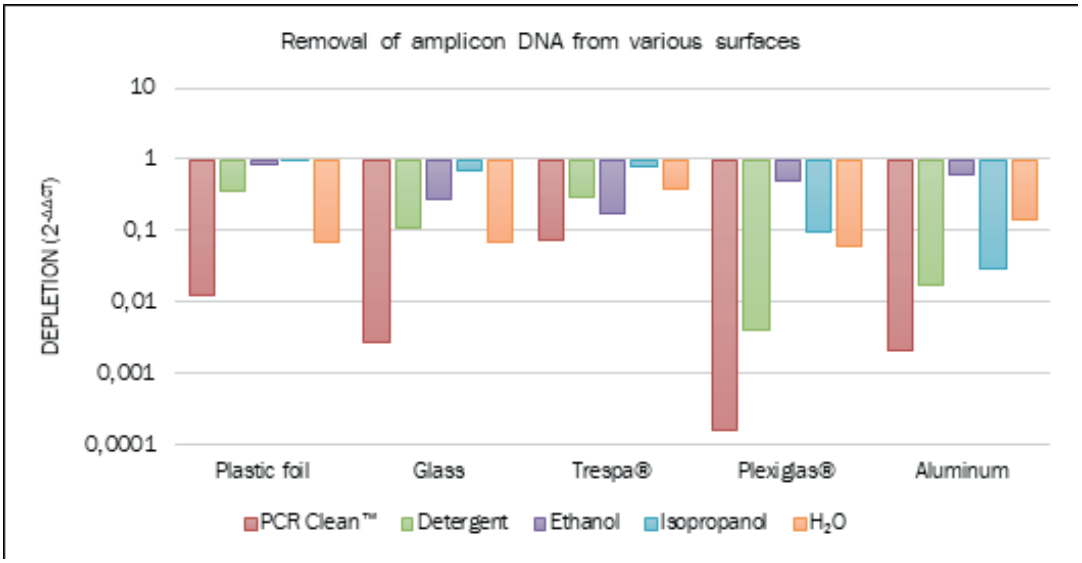



**Diagram 2.** Overview of depletion results of RNA after removal from Plexiglas® surface, using different cleaning agents. Results are shown as $2^{-\Delta\Delta Ct}$, where $\Delta\Delta C_t$ values are in reference to results ($\Delta C_t$ values) of dry paper towel.

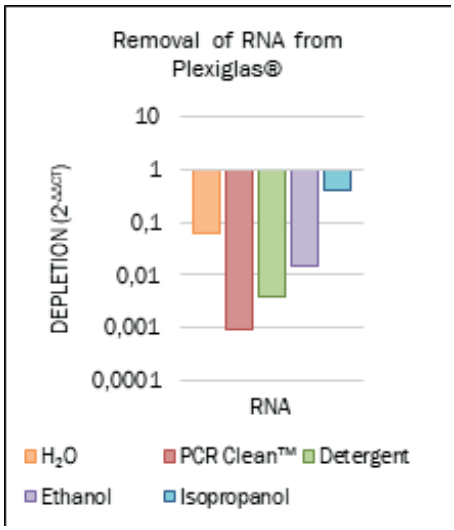
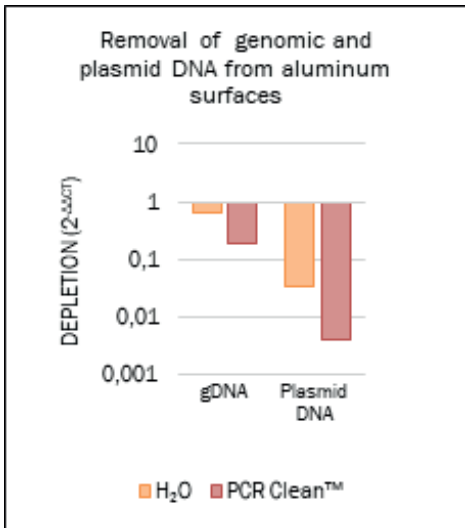



**Diagram 3.** Overview of depletion results of genomic and plasmid DNA after removal from aluminum surfaces, using PCR Clean™ or water. Results are shown as $2^{-\Delta\Delta Ct}$, where $\Delta\Delta C_t$ values are in reference to results ($\Delta C_t$ values) of dry paper towel.

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## 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. Depletion results also demonstrate superiority in effectiveness and efficiency of PCR Clean™ compared to any of the other common cleaning agents usually available at molecular biology laboratories, e.g. ethanol, isopropanol or dishwashing detergent. 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.



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