Lab decontaminants

Use of DNA *AWAY* and RNase *AWAY* Surface Decontaminants in everyday laboratory workflows

Introduction: Choose your decontamination reagents carefully

There are overwhelming reasons for the use of strong decontaminants in laboratories, including to prevent cross-contamination of laboratory instruments, tools, benches, biological safety cabinets (BSCs), centrifuges, cold-storage equipment, and most notably, samples themselves. Despite all precautions, cross-contamination remains a significant issue, particularly in laboratories that rely on PCR-based assays. Bleach and other commercial reagents are commonly used to help prevent cross-contamination of samples. However, careful consideration should be given to the choice of decontamination reagent because of potentially undesirable effects or inadequate performance. Other methods, like UV radiation or physically separating the PCR preparation from downstream sample manipulation, do not always prevent contamination from nucleic acids in molecular diagnostic laboratories [1-4].

In this white paper, the utility of **Thermo Scientific™ DNA *AWAY*™** and **RNase *AWAY*™ Surface Decontaminants** was compared to that of bleach and other commercially available decontaminants used in molecular biology laboratories. We explain the advantages that DNA *AWAY* and RNase *AWAY* reagents have over bleach and other products on the market used for laboratory decontamination.

Don't use bleach for large laboratory equipment—it is corrosive and leaves detrimental residue

Large laboratory equipment like automated liquid handlers, BSCs, lab benches, and other surfaces need to be decontaminated regularly. However, it is particularly important to choose a decontaminant that will not impede the laboratory workflow or damage expensive equipment.

The Centers for Disease Control and Prevention (CDC), in their "Guidelines for safe work practices in human and animal medical diagnostic laboratories" [5], states that hypochlorite solutions (bleach) are classified as irritants and corrosives, and that undiluted bleach solution is corrosive to stainless steel. Thorough rinsing must follow use in BSCs and stainless-steel sinks to remove the remaining residue.

It has been reported [6,7] that bleach can cause metal corrosion and damage valuable equipment such as BSCs and instruments. Moayed and Golestanipour [6] demonstrated that bleach can cause pitting on metal surfaces. Also, Postlewaite and Hollands [7], studying the corrosive activities of bleach, observed that corrosion appears on metal after only 4 days of contact with bleach. Nestor et al. [8], in their review of sterilization equipment, recommended the use of bleach only for benchtops and glassware sterilization. However, because bleach leaves a residue, they recommended wiping and cleaning the surfaces with water after the decontamination with bleach. In addition, the authors recommended not using bleach for metal surface decontamination, due to its corrosive character.

Illumina, a manufacturer of microarray genotyping platforms, uses standard fluorescent dyes [9] in their Infinium™ assays. However, the company has a compelling reason to warn its customers not to use bleach for decontamination in laboratories where Infinium BeadChip assays are processed [10]. The company claims that bleach fumes can degrade the fluorescent dyes used in their BeadChip assays, rendering the products useless. Therefore, Illumina has a warning label that advises customers not to perform bleach-cleaning protocols in laboratories that process BeadChip assays. In addition, their website [11] recommends not using bleach for decontamination of the Hamilton Microlab™ STAR™ automated liquid handler system.

They are not alone—Thermo Fisher Scientific strongly discourages the use of bleach solutions for the decontamination of Thermo Scientific™ centrifuges [12]. In addition, the use of bleach for decontamination of Thermo Scientific™ BSCs is not recommended, due to the irreparable damage bleach may cause to the metal material [13].

Finally, Kruse et al. [14], in their comprehensive review of BSCs, do not recommend bleach for decontamination.

Not all decontaminants are equal—why shouldn't you use bleach?

Bleach can inhibit PCR. Several methods have been developed to better understand the effects of inhibition, degradation, and low copy number in the recovery of information in forensic DNA casework. In situations where a DNA sample is of low quality, containing degraded DNA, PCR inhibitors, or both, the sample can have an incomplete genotype exhibiting problems such as allele loss, low signal intensity, or inefficient amplification. McCord et al. [15] showed that forensic blood samples contaminated with bleach, PCR-amplified with the PowerPlex™ 16 kit (Promega), and genotyped on the Applied Biosystems™ 310 Genetic Analyzer have partial profiles (loss of loci and alleles) due to partial degradation of genomic DNA (Figure 1). As expected, larger amplicons are most affected. Such results can necessitate repeated genotyping or even cause loss of samples.

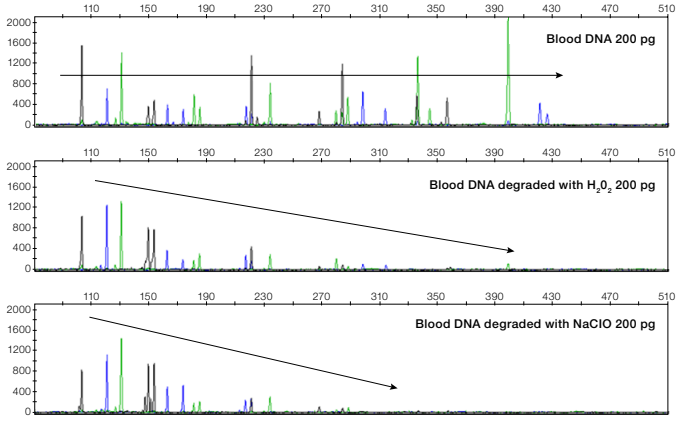

Figure 1. Partial DNA profile resulting from bleach contamination. Electropherograms are shown of PCR-amplified DNA from blood samples that were untreated (top), treated with hydrogen peroxide (middle), or treated with bleach (bottom). Figure used with permission from McCord et al. [15].

Bleach can also cause alterations of DNA sequences [16]. In studies of ancient DNA, decontamination protocols have been developed in which samples are soaked in household bleach solutions. However, samples containing human mitochondrial DNA (mtDNA), after treatment with bleach, showed a higher frequency of cytosine to thymine conversion than untreated samples (Figure 2). Conversion was assessed by the frequency and relative read position of cytosine to thymine substitutions in aligned sequence reads. The results revealed a tendency for increased cytosine to thymine substitutions in bleach-treated samples compared to untreated samples. These results suggest that the use of bleach in ancient DNA studies must be limited to avoid DNA sequence alteration of the samples.

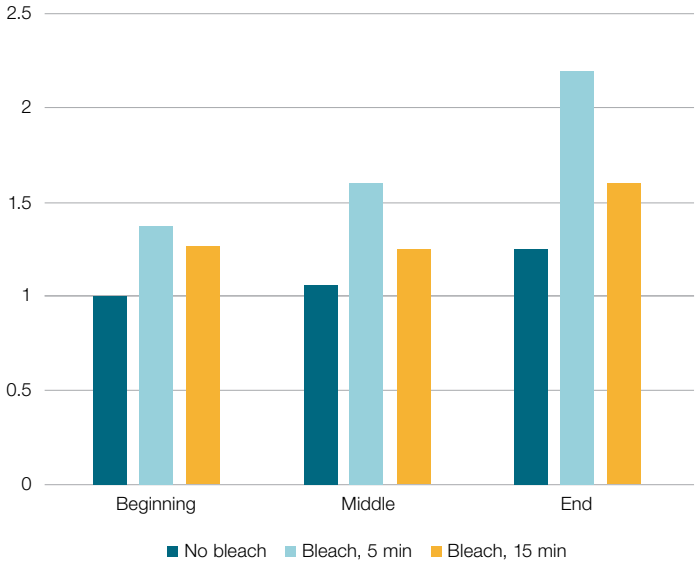

Figure 2. Conversion frequency of cytosine to thymine at the beginning, middle, and end of the DNA fragment reads. Dark blue: untreated specimens containing human mtDNA; light blue: specimens submerged in bleach for 5 minutes; yellow: specimens submerged in bleach for 15 minutes. Figure based on data from Riley et al. [16].

Bleach can inhibit PCR—use DNA *AWAY* and RNase *AWAY* reagents to decontaminate laboratory gloves instead

Molecular biology laboratory practice requires frequent decontamination of laboratory gloves throughout protocols. To clear any exogenous nucleic acid coming from microorganisms or humans, laboratory gloves are commonly decontaminated with either bleach or commercial decontaminants. However, the decontaminants may leave dry particles on the gloves that may inhibit PCR if shed into the reaction tubes. To test this hypothesis, we looked at the inhibitory effects of residue from RNase *AWAY* reagent and bleach on RT-qPCR. Although DNA *AWAY* and RNase *AWAY* reagents are based on sodium hydroxide and are chemically similar, RNase *AWAY* reagent is formulated at a higher strength. Therefore, only RNase *AWAY* reagent was tested.

One pair of laboratory gloves was sprayed with RNase *AWAY* reagent and another pair with 8.25% bleach. After drying overnight in the BSC, gloves sprayed with RNase *AWAY* reagent had no visible residue on their surface, but gloves sprayed with bleach displayed visible powder on the surface (Figure 3). Shedding of the residue particles from the gloves with the dried decontaminants was simulated by rubbing the gloves over plastic dishes. Shed residue particles were only visible from the gloves originally sprayed with bleach (Figure 4).

Samples were taken for RT-qPCR analysis by swabbing the surface of the gloves or dishes twice with buccal swabs. Swabs were placed into separate tubes containing Applied Biosystems™ TaqCheck™ SARS-CoV-2 Control RNA diluted in PCR buffer. After incubation, aliquots of the diluted control RNA from each tube were analyzed by RT-qPCR. Reactions were run in duplicate, including positive and negative controls. The RT-qPCR reaction was prepared by using 2.5 µL of Applied Biosystems™ TaqPath™ 1-Step RT-qPCR Master Mix, 0.5 µL of Applied Biosystems™ TaqCheck™ SARS-CoV-2 Fast PCR Assay, 2 µL of PCR-grade water, and 5 µL of a sample (for the positive control: 2 µL of TaqCheck SARS-CoV-2 Control RNA plus 3 µL of sterile water).

Figure 3. Laboratory gloves dried after decontamination. Gloves were sprayed with **(A)** RNase *AWAY* reagent and **(B)** bleach.

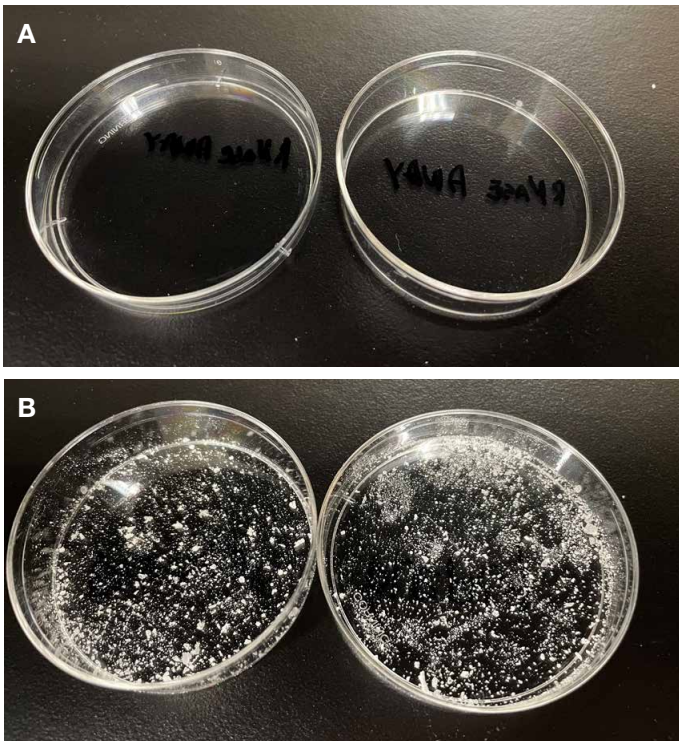

Figure 4. Plastic dishes with particles shed from gloves. Gloves were originally sprayed with **(A)** RNase *AWAY* reagent and **(B)** bleach.

The Applied Biosystems™ QuantStudio™ 5 Real-Time PCR System was used with the following cycling protocol: one cycle of reverse transcription at 50°C for 4 minutes, one cycle of activation at 95°C for 2 minutes, and 40 cycles of denaturation at 95°C for 1 second and annealing/extension at 60°C for 20 seconds. Swab samples from gloves sprayed with RNase *AWAY* reagent had no inhibitory effects on the RT-qPCR, but swab samples from gloves sprayed with bleach had inhibitory effects (Figure 5). Similarly, inhibition was only seen with swab samples from dishes that contained particles shed from gloves sprayed with bleach (Figure 6).

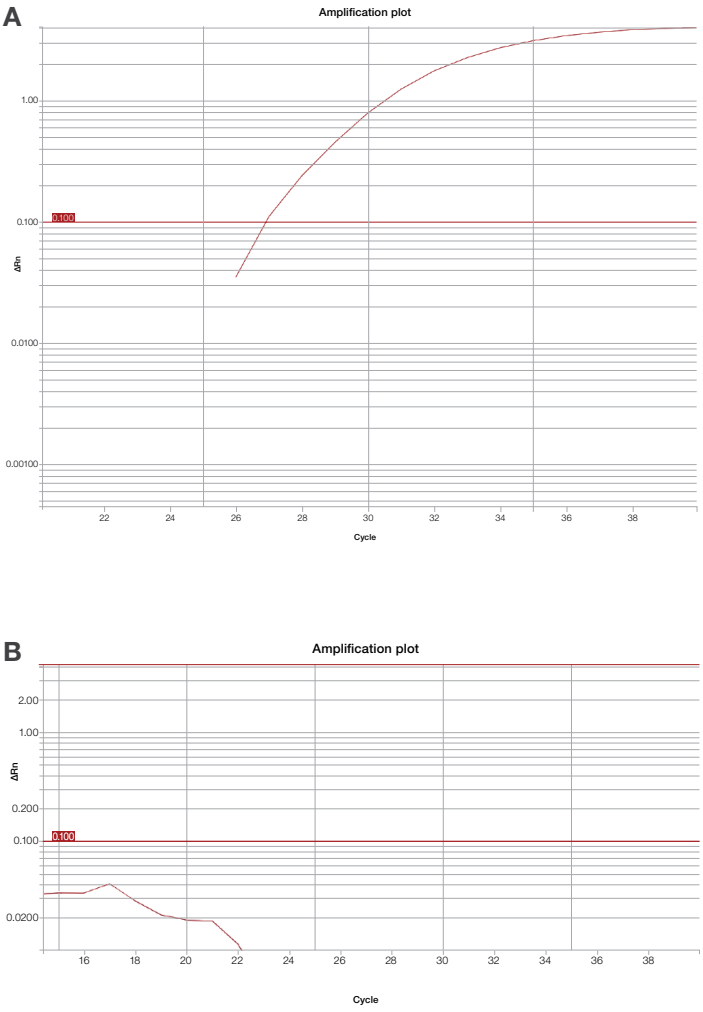

Figure 5. RT-qPCR amplification curves from swab samples taken from gloves. (A) Samples from gloves sprayed with the RNase *AWAY* reagent showed amplification with no inhibition. **(B)** Samples from gloves sprayed with bleach showed no amplification.

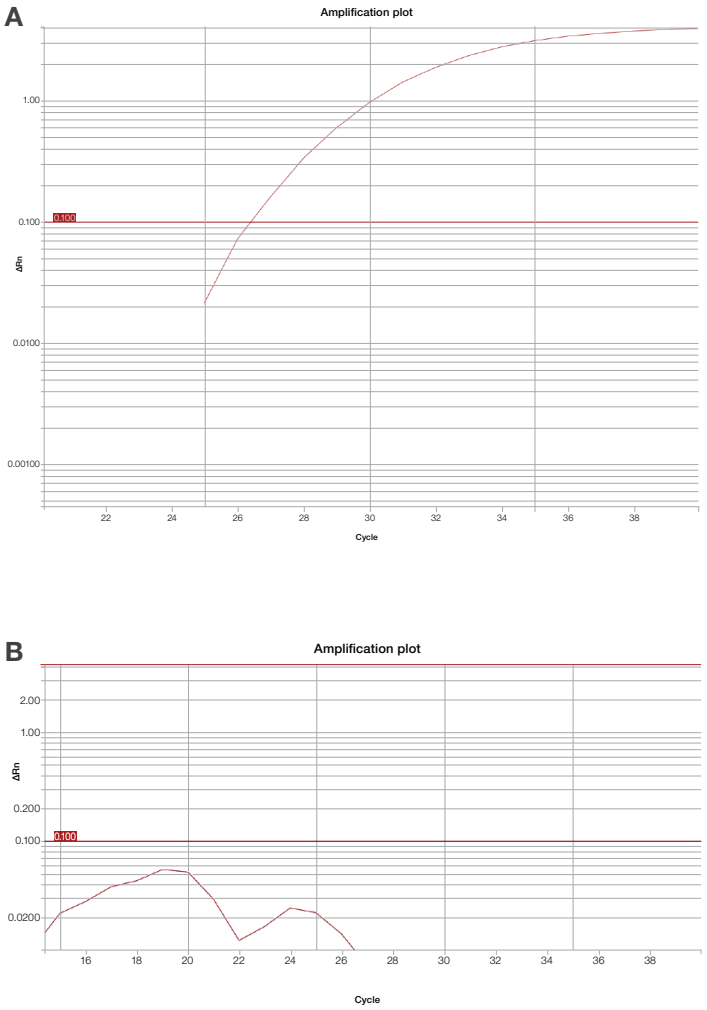

Figure 6. RT-qPCR amplification curves from swab samples taken from plastic dishes. (A) Swab samples from plastic dishes containing any leftover particles from the RNase *AWAY* reagent (not visible) showed amplification with no inhibition. **(B)** Swab samples from plastic dishes containing dry residue particles from bleach showed no amplification.

This experiment suggests that the use of bleach for decontamination of laboratory gloves can leave particles on the gloves that, if shed into the PCR tubes, can inhibit the PCR reaction. Leftover particles on bleach-decontaminated surfaces are the reason that many standard operating procedures (SOPs) recommend follow-up cleaning with water. As safe alternatives to bleach, DNA *AWAY* and RNase *AWAY* reagents do not leave particles on laboratory gloves after decontamination. Therefore, PCR inhibition will not occur when the gloves are decontaminated with either reagent, and there is no need for follow-up cleaning with water.

Some decontaminants are better than others—DNA *AWAY* and RNase *AWAY* reagents show superior performance over other commercial decontaminants

Fischer et al. [17] studied the performance of DNA *AWAY*, DNA Remover (Minerva Biolabs), and DNA-ExitusPlus™ IF (AppliChem) reagents, comparing their efficiency in removing DNA and RNA from a surface or in solution. For surface decontamination efficiency, surface areas inside plastic wells were intentionally contaminated with a known quantity of DNA amplicon or *in vitro* transcribed RNA and then allowed to dry overnight. The dry surfaces were then decontaminated for either 2 or 10 minutes using each of the commercial decontaminants. For solutions, a known quantity of the DNA amplicon and *in vitro* transcribed RNA was added to diluted solutions of the commercial decontaminants and allowed to incubate for 2 and 10 minutes. The nucleic acids were extracted, then amplified and detected by real-time PCR. The results indicated that less DNA or RNA remained on the surface after decontamination with the DNA *AWAY* reagent, compared to the amount remaining after decontamination with the other two reagents (Figure 7). Furthermore, less DNA and RNA remained in the solution of the DNA *AWAY* reagent than did in the other two commercial reagents (Figure 8). These experiments suggest that the DNA *AWAY* reagent is very efficient at removing both DNA and RNA from surfaces and solutions.

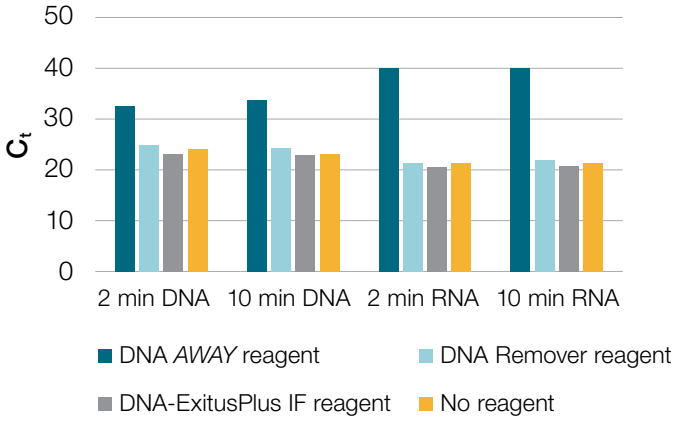

Figure 7. Comparison of remaining DNA and RNA on surfaces. Mean C_t values of target nucleic acids remaining on a surface were determined by real-time PCR after applying 1:4 diluted DNA *AWAY*, DNA Remover, or DNA-ExitusPlus IF reagent. Figure based on data from Fischer et al. [17].

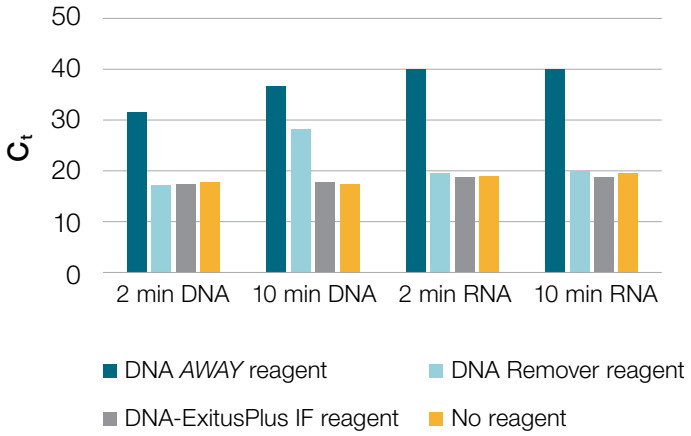

Figure 8. Comparison of remaining DNA and RNA in solutions. Mean C_t values of target nucleic acids remaining in a solution were determined by real-time PCR after incubation with 1:4 diluted DNA *AWAY*, DNA Remover, or DNA-ExitusPlus IF reagent. Figure based on data from Fischer et al. [17].

Why use DNA *AWAY* and RNase *AWAY* reagents?

Your chosen decontamination reagent must effectively eliminate exogenous DNA or RNA molecules without damaging laboratory equipment, reagents, and consumables. In diagnostic and research laboratories, contamination by exogenous DNA or RNA must be controlled to minimize the risk of faulty results. However, an inappropriate decontaminant, like bleach, can damage expensive laboratory equipment by causing corrosion, or adversely affect laboratory reagents, such as by degrading fluorescent dyes, inhibiting PCR, and destroying or altering sample genetic material. Champlot et al. [18] recommended a decontamination procedure for corrosion avoidance in molecular biology laboratories that incorporates DNA *AWAY* and RNase *AWAY* reagents. These reagents are also recommended in protocols and SOPs of many laboratories and institutions such as the Centers for Disease Control and Prevention [19], the Houston Forensic Science Center [20], and the Environmental Toxicology Laboratory at the University of Saskatchewan [21]. Considering the findings of this paper, the choice of decontaminant for molecular biology laboratories should be based on the following conclusions:

- Bleach can cause metal corrosion and leave residues that can inhibit PCR, but DNA *AWAY* and RNase *AWAY* reagents do not.
- DNA *AWAY* and RNase *AWAY* reagents do not require follow-up cleaning with water and provide a safe, fast, and proven alternative to arduous and time-consuming procedures.
- DNA *AWAY* and RNase *AWAY* reagents are more effective as decontaminants than other commercial products.
- DNA *AWAY* and RNase *AWAY* reagents are recommended as decontaminants by nationally recognized organizations.

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