Application Note:
DNA Amplicon Contamination in the PCR Laboratory

Prevention and control of DNA amplicon contamination in the PCR laboratory is critical for the integrity of experiments and equipment, which can affect the accuracy of PCR test results. Polymerase Chain Reaction (PCR) amplifies specific DNA sequences for various downstream applications, including gene expression analysis, genetic testing, disease diagnosis, and pathogen detection. DNA amplicon can exponentially grow through the PCR reaction to incredibly high copy numbers and carries the risk of spreading across a laboratory. Amplicon release can be a result of numerous unplanned events, including autoclaving PCR tubes. During autoclaving, the extreme heat and pressure can pop open tubes which cause the DNA amplicon to aerosolize quickly, causing widespread contamination in the immediate area. Amplicon from the environment can then spread to the user's gloves and be carried throughout the laboratory and to all equipment the user interacts with. DNA amplicon can also spread when PCR tubes become compromised inside of the PCR instrument, which will also quickly aerosolize the DNA amplicon due to the thermal cycling of the instrument. Once DNA amplicon contamination occurs, it may be difficult to manage without a well-structured plan.

Eliminating DNA Amplicon Contamination

In many laboratories, 70% isopropyl alcohol (IPA) is commonly used as a laboratory disinfectant. While it is effective for inactivation of bacteria, it is ineffective against DNA. To eliminate DNA, a strong oxidizing agent such as bleach, also known as sodium hypochlorite, is needed. It works by producing reactive oxygen species, such as hydroxyl radicals and chlorine radicals, which can cause oxidative damage to DNA molecules. These free radicals induce breaks in the sugar-phosphate backbone of DNA, and promote cross-linking between DNA strands, leading to DNA fragmentation and degradation. Another recommended DNA degrading product is DNAZap™, which degrades high levels of contaminating DNA and RNA from surfaces. It works on contact, and is effective on PCR instruments, general lab equipment, and lab benches. While either a freshly made 10-20% bleach solution or DNAZap™ is an effective means to degrade DNA, it must be used cautiously, ensuring that after use it is wiped away with a distilled water rinse as it may inhibit results of future experiments and damage laboratory equipment, instruments, and metal surfaces.

Environmental Monitoring

When DNA amplicon contamination begins to affect the accuracy and integrity of PCR test results, a swift response must be taken by the laboratory team. When experiments generate data that is unrealistic or unexpected, such as negative controls testing positive via PCR or repeat false positive results, an investigation should be conducted to determine if amplicon release has occurred in the laboratory. Depending on the severity and circumstances which should be determined via risk assessment, two strategies can be implemented. These strategies both utilize environmental monitoring via surface swabs, using sterile cotton swabs dampened with sterile water to swab specific surfaces, equipment, and instruments in the laboratory.

The first strategy tests three swabs per area. This is the more expensive approach, but allows for data trending over time. The second strategy tests 1 swab per area which is a cost-effective alternative. The swab is snapped off into a microcentrifuge tube with 500 µL of sterile water, followed by vortexing, and then PCR to detect contamination. Once regions have been screened via PCR, PCR-positive areas are targeted with a freshly prepared 10-20% bleach solution and/or DNAZap™, then rinsed twice with distilled water to remove any inhibitory residue. The environmental monitoring swab test is repeated until all regions test negative. Environmental monitoring for amplicon contamination may be considered as part of a risk mitigation strategy. This protocol should include both instructions for routine cleaning, and instructions for when and how to conduct regular environmental monitoring for DNA amplicon.

Case Study

In a laboratory routinely utilizing a multiplex PCR assay with three targets (A, B, and C), the laboratory staff noticed that target A was testing positive in the no-template control (NTC) and in samples that were not able to be confirmed with culture techniques. An environmental swab test determined that the laboratory environment was contaminated with DNA amplicon. It was not one single event that caused DNA amplicon contamination in the laboratory, but rather a steady buildup of amplicon contamination over time with no protocol to eliminate the contamination. Once the laboratory staff located the most contaminated regions in the laboratory, the regions were plotted on a laboratory schematic of the lab to show the distribution of amplicon. It was noted that the area of highest contamination surrounded the PCR bench and radiated outward with decreasing positivity further away from the PCR bench. The laboratory surfaces, equipment, and instruments were thoroughly wiped down with a freshly prepared 10% bleach solution, followed by a distilled water rinse, then wiped down with DNAZap™, followed twice by a distilled water rinse to eliminate the DNA amplicon and inhibitory residues from the environment.

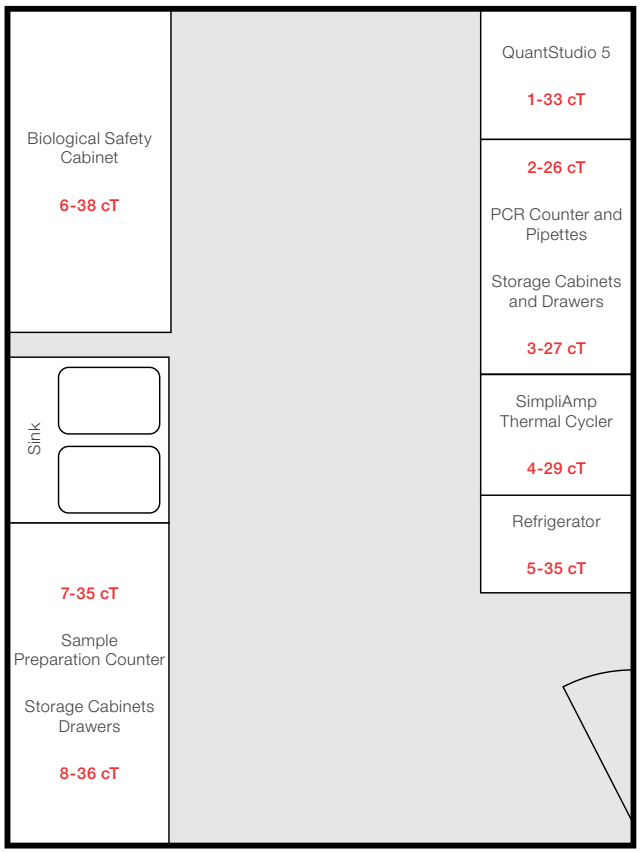

Figure 1. Heat map of Ct values prior to cleaning

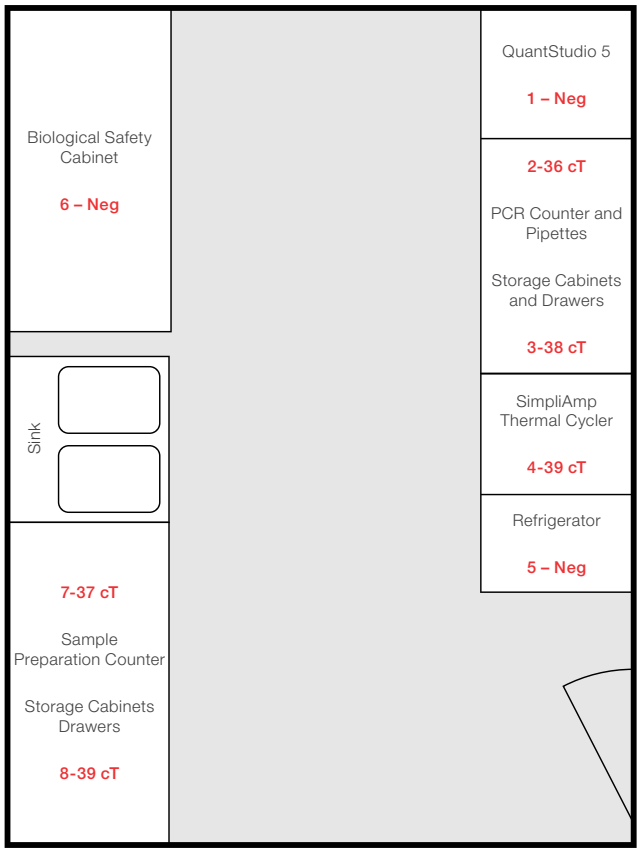

Figure 2. Heat map of Ct values after cleaning

Table 1. Summary of contamination findings

Location	PCR Target Ct Value	Relative Contamination Level* [High / Medium / Low]
1 Swabbed inside, outside, and on the counter in front of the QuantStudio 5	33	Low
2 PCR Counter - Left side of counter, left drawer, left cabinet, and pipettes	26	High
3 PCR Counter - Right side of counter, right drawer, right cabinet, vortex and plate spinner	27	High
4 SimpliAmp Thermal Cycler	29	Medium
5 Refrigerator	35	Low
6 Biological Safety Cabinet	38	Low
7 Sample Preparation - Right counter, right drawers, right cabinet, vortex	35	Low
8 Sample Preparation - Left counter, left drawers, left cabinet, and pipettes	36	Low

*The designations High, Medium, and Low were based on the data generated, high = < 28, medium = 28-32, low = > 32

Once the laboratory was cleaned with one pass, another environmental monitoring swab test occurred to ensure the laboratory was free of DNA amplicon. Some swabs still tested positive, but this time were markedly weaker (higher Ct values) and only affected 5 of the 8 regions, indicating that the level of contamination had decreased significantly following the first round of DNA amplicon removal. The laboratory staff then repeated the decontamination and environmental testing procedure and all swabbed regions tested negative.

Potential root cause: Low Ct values indicate higher contamination is present. In Table 1, the PCR counter contains the lowest Ct value; therefore, it is the likely source of the contamination. The PCR counter is where benchwork occurs - involving lysate transfer occurs, the thermal cycling step, and the PCR run on the Applied BioSystems™ QuantStudio 5™ Food Safety Real-Time PCR System.

Next steps:

- Decontaminate the entire laboratory including the inside and outside of pipettes (special care should be taken when decontaminating pipettes, such as ensuring all cleaning steps are followed by multiple distilled water rinse steps to ensure inhibitory residues are eliminated), laboratory equipment, and instruments per their cleaning procedure, followed by swab testing. Repeat this until all areas in the laboratory are negative.
- Integrate a DNA elimination step into the existing cleaning procedure, along with an environmental monitoring program to monitor for amplicon contamination as needed.
- All processes conducted in the most likely area of amplicon release should be investigated for potential repeat occurrences, e.g. processes related to capping of tubes.

Table 2. Risk assessment and mitigations

Root Cause	Impact	Likelihood	Mitigation
Tubes crushed in thermal cycler	Medium	Low	Ensure tubes are properly placed in the instrument. If alignment issues are apparent, ensure that racking is not damaged and PCR tubes are not warped.
Loose caps in PCR tubes	High	Low	Use tools and consumables specified by the PCR kit manufacturer to ensure a tight seal.
Lysate leaking from lysis tube onto PCR counter and onto gloved hands	Low	Moderate	Ensure that care is taken to prevent spills, and that spills are handled accordingly. Use all recommended racks and tools and replace soiled gloves immediately. Clean PCR and sample preparation counter at regular intervals with a DNA degrading solution, e.g. DNAZap™.
Improperly seated PCR tubes may crush and leak during a run	High	Moderate	Ensure that the proper racking is used and both tubes and plates are properly seated prior to performing a PCR run.

Conclusion

In this case study example, the laboratory determined the root cause of the DNA amplicon contamination and developed a cleaning protocol to eliminate the DNA amplicon from the laboratory surfaces, instruments, and equipment.

DNA contamination is a continuous challenge that laboratory staff must be ready to face; therefore, performing risk assessments and implementing a robust cleaning schedule is essential. Regular environmental monitoring and cleaning of all surfaces, equipment, and instruments using a freshly prepared 10-20% bleach solution and/or DNAZap™ aids in preventing future DNA amplicon contamination.

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