Using Ultraviolet Light to Eliminate DNA Cross-Contamination in Forensic Laboratories

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Introduction

Criminal cases are built on forensic science, but can be destroyed by contamination from outside sources during processing, transporting, or storage. Cross-contamination of DNA evidence by outside sources can take place by a variety of transfers. One of the most common sources is improperly sanitized field instruments used for data collection and crime scene processing. These tools include fingerprint brushes, forceps, shears/scissors, evidence collection jars, and other equipment used to process crime scenes and evidence in the lab. Effective non-destructive decontamination of instruments and tools is the best way to ensure that DNA evidence for every case is legally and scientifically viable.

DNA Decontamination in Forensic Science

Forensic scientists have long known that even minimal contamination from outside DNA sources can lead to erroneous results from highly sensitive polymerase chain reaction (PCR) experiments. Several main methods of DNA decontamination have been developed over the years, including bleach decontamination, autoclave procedures, complete disposal/destruction, and ultraviolet (UV) light decontamination.

Bleach Decontamination

In 1992, scientists documented that bleach was more effective than hydrochloric acid at decontaminating surfaces with rogue DNA. The efficacy of bleach in DNA decontamination depends on the amount of free and available chlorine. A concentration of 0.05 – 0.5% of free and available chlorine is considered an intermediate level disinfectant. A 10-100x dilution from a commercial bleach stock, containing 5.84% available chlorine, is normally used to decontaminate surfaces in laboratories.

Bleach is an effective, simple, and relatively inexpensive decontaminate. No special equipment is needed, and as long as proper laboratory procedures are followed, this method will sufficiently decontaminate surfaces. Unfortunately, bleach can be corrosive to metal surfaces and must be allowed to sit on the surface for up to 30 minutes before being wiped off for full effectiveness. Additionally, bleach mixtures break down over time and become less effective at decontaminating surfaces due to a lower concentration of available chlorine.

Autoclaves

Autoclaves decontaminate surfaces with high pressure steam, essentially killing DNA by subjecting objects to extremely high temperatures. Autoclaves are often used in laboratory and medical settings where metal and polypropylene instruments can be sanitized and reused.

Autoclaves are an effective decontamination option, however they can be expensive to incorporate into a laboratory and use a considerable amount of resources which may not be practical for some forensic laboratories.

Disposal

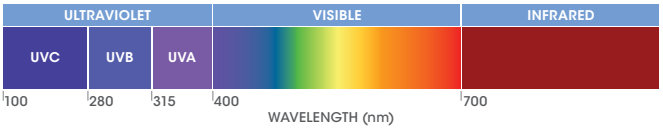
Another method to prevent contamination by DNA is to dispose of all instruments and tools used during the collection and analysis process. While effective, this method is exceptionally expensive and wastes valuable resources.

UV Irradiation

Scientists identified as early as 1989 that treating laboratory surfaces with doses of UV light could break down DNA and help minimize cross-contamination in sensitive polymerase chain reaction experiments. An article in the journal *Nature*[1] claimed that UV irradiation was a way to "quickly damage any DNA left on exposed surfaces." Most damage from UV irradiation occurs via the formation of cyclobutane rings between neighboring pyrimidine bases, thymidine or cytidine. The cyclobutane rings form intrastrand pyrimidine dimers that inhibit polymerase-mediated chain elongation.

There are three main subtypes of ultraviolet light, UVA, UVB, and UVC, the names of which correspond to different wavelengths on the light spectrum. Not all types of UV are effective at decontaminating surfaces of erroneous DNA.

The Spectrum of Light:



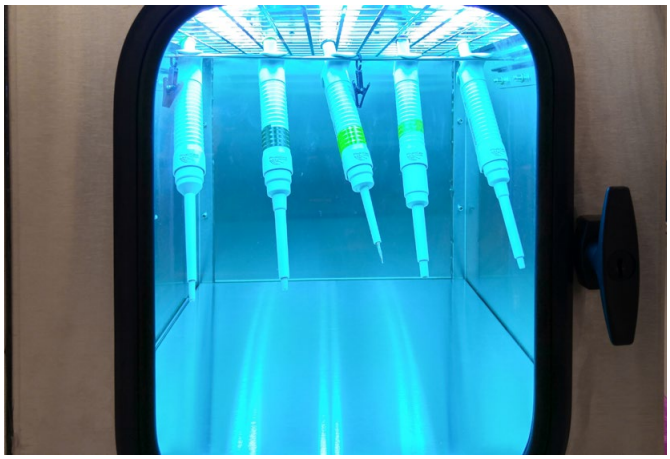
- **UVA light** has a wavelength of 315 to 400 nanometers and is considered "long wave" UV light. This light is not absorbed by the ozone layer and does not decontaminate surfaces of DNA.
- **UVB light** has a wavelength of 280 to 315 nanometers and is called "medium wave" UV light. This light is mostly absorbed by the ozone layer and has a minimal effect on DNA with direct exposure.
- **UVC light** has a wavelength of 100 to 280 nanometers and is known as "short wave" UV light. This light is considered germicidal and can damage living cells, but cannot effectively penetrate skin layers. UVC light is completely absorbed by the atmosphere.

Decontamination practices typically call for UV light in the wavelength of 254 nanometers and direct exposure of the surfaces for a period of 90 minutes to ensure all DNA and microbes have been eradicated. The maximum distance from the contaminated surface is recommended to be no more than 10 cm and must be directly above the item to be effective. Simple light fixtures fitted with UV lights are not sufficient to ensure DNA decontamination. The light emitted often does not directly impact all surfaces of every piece of equipment being treated and the intensity of light exposure is not enough to ensure full decontamination.

1 Brill, Steven J., and Bruce Stillman. "Yeast Replication Factor-A Functions in the Unwinding of the SV40 Origin of DNA Replication." *Nature* vol. 342 (1989): 92-95. Nature Publishing Group, doi: 10.1038/342092a0

The Solution: The Air Science UV-Box

The Air Science UV-Box™ is a desktop unit that provides easy, effective UV radiation decontamination of forensic instruments, tools, and materials. High intensity UV lamps positioned within the cabinet produce short wave ultraviolet light at 254 nm to destroy exposed surface DNA and bacteria, leaving evidence free of contamination prior to other forensic tests, analyses, or procedures.



Features

UV lamps are optimally placed throughout the UV-Box to eliminate blind spots and ensure that all surfaces of equipment being decontaminated come into direct contact with UV light. The corners and walls of the box are smooth to make cleaning easy. Stainless steel surfaces naturally reflect UV radiation to ensure contents are fully irradiated from all directions. Additional design and construction features offer convenience and protect personnel during use, cleaning, and maintenance of the cabinets.

The operator is protected from UV radiation by the UV absorbing window and safety controls which ensure lamps cannot be activated until the cabinet door is securely closed. Other features include UV timer, hanging rod, and shelf to support or suspend items for decontamination.

The UV-Box has been independently tested and validated based on strict regulatory requirements to meet the needs of forensic laboratories and ensure laboratory methods can legally stand in court cases.

Standards and Regulatory Compliance

Forensic laboratories are held to stringent regulatory compliance standards for validation of decontamination methods to ensure that the level of DNA decontamination is repeatable and low enough not to affect results. The American Society of Crime Lab Directors (ASCLD) requires validation based on the principles outlined in ISO 17025. The ISO 17025 General Requirements document under section 5.4, subsection 5.4.5 "Validation of Methods," states that "validation is the confirmation by examination and the provision of objective evidence that the particular requirements for a specific intended use are fulfilled."[2]

In that same subsection, the techniques used to determine performance of a method are outlined as:

1. Calibration using reference standards or reference materials
2. Comparison of results achieved with other methods
3. Interlaboratory comparisons
4. Systematic assessment of the factors influencing the result
5. Assessment of the uncertainty of the results based on scientific understanding of the theoretical principles of the method and practical experience

Additionally, the 2011 ISO 17025 Supplemental Requirements for Forensic Laboratories notes that:

> *"Validation studies can be conducted by the scientific community (as in the case of standard or published methods) or by the forensic laboratory itself (as in the case of methods developed in-house or where significant modifications are made to previously validated methods)."*[3]

Thus, if published, data from a validated study exists for a particular method or product. Under ASCLD standards, that method or product is able to be used in the forensic laboratory and will maintain legal validity in court proceedings.

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2 "ISO/IEC 17025:2005 General Requirements for the Competence of Testing and Calibration Laboratories." *International Organization for Standardization (ISO)*. N.p., 15 May 2005. www.iso.org/iso/catalogue_detail.htm?csnumber=39883.

3 5.4.5.2, "Supplemental Requirements for the Accreditation of Forensic Science Testing Laboratories." *American Society of Crime Laboratory Directors / Laboratory Accreditation Board (ASCLD/LAB)*. N.p., 2011, Corresponds to ISO/IEC 10725:2005. des.wa.gov/SiteCollectionDocuments/About/1063/RFP/Add7_Item4ASCLD.pdf.

Validation Testing

The UV-Box was recently evaluated and validated by independent testing at the National Forensic Science Technology Center in Largo, Florida. The purpose of the study was to test the UV decontamination capabilities of the UV-Box in a typical laboratory setting. The UV-Box is designed mainly for forensic labs, which require equipment validation as part of ASCLD credentials. By having published data, the labs will not have to rely on expensive and time consuming in-house validation studies to use the equipment.

Hypothesis

The working hypothesis was that the design of the UV-Box allowed effective eradication of human DNA on common forensic instruments, which could be supported and validated if technicians observed at least a 2-log (99%) reduction in DNA present following treatment within the UV-Box.

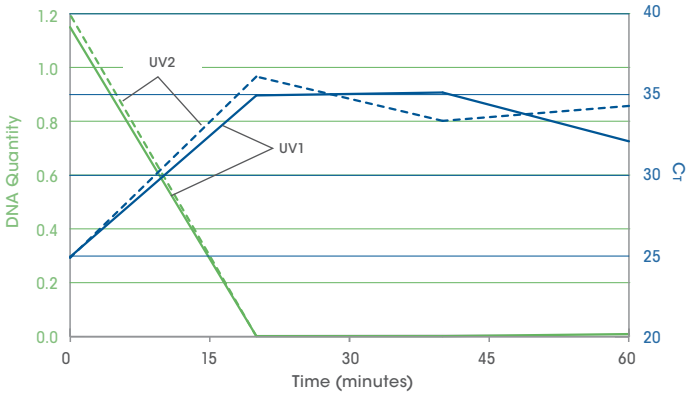
Personnel

Robert O'Brien, a well respected scientist in the Forensic community who worked with the Florida Department of Law Enforcement for five years prior to beginning his nine-year tenure with the National Forensic Science Technology Center, conducted the testing. O'Brien is a DNA and forensic testing expert and developed the testing protocol to mimic conditions found in real-world forensic analysis scenarios.

Methods

Methods were developed to approximate forensic equipment and tools that have been contaminated with human DNA from sources other than a crime scene. Glass slides were used as a controlled surface on which a thin layer of human blood was spread to replicate contaminated equipment. The slides were placed inside the UV-Box and certain sets were treated for 30 minutes, 45 minutes, and 60 minutes, with a set of slides going untreated to serve as a control. The procedure was repeated twice with different sets of slides for greater statistical scope. Once treatment was completed, swabs from the surface of each slide were taken and processed using an Applied BioSystems 7500 PCR Instrument. The results were quantified against an HID Standard curve and compared over time.

Results



	Quantity	C_T
Control 1, 0 minutes	1.1496	24.9459
Control 2, 0 minutes	1.1964	24.8871
UV1, 30 minutes	0.0013	34.9496
UV2, 30 minutes	0.0006	36.1153
UV1, 45 minutes	0.0012	35.1293
UV2, 45 minutes	0.0038	33.374
UV1, 60 minutes	0.009	32.1064
UV2, 60 minutes	0.002	34.2985

The graphs and resulting quantification tables show that the control slides contained a mean fluorescence quantity of 1.15 and 1.20 for large autosomes respectively. Slides exposed to 30 minutes of UV light had 0.00 fluorescence for large autosomes, as did the 45-minute exposure, and the 60-minute exposure times. This represents greater than a 2-log (99%) reduction in quantifiable DNA present on the slide following treatment in the UV-Box.

Discussion

The results indicate that treatment within the UV-Box is particularly effective at decontaminating surfaces that contain trace DNA and achieving a reduction at zero or near zero.

The treatment by the UV-Box was effective at achieving a greater than 2-log (99%) reduction of quantifiable DNA when slides were exposed for 30 minutes, 45 minutes, and 60 minutes. Large autosomal fluorescence was directly impacted by treatment in the UV-Box and represents a decontamination level appropriate for use in any forensic laboratory. This becomes especially true when it is considered that the media used was a thin layer of blood, which would in nearly all laboratory settings be wiped off or pre-cleaned prior to decontamination treatment in the UV-Box.

Conclusions

UV light is effective at decontaminating surfaces for trace DNA, if the proper procedures are followed. The UV-Box is a product that provides effective UV decontamination in a convenient, desktop application. The UV-Box also has a host of features to assist achieving repeatable results that have been validated through independent laboratory testing. The results achieved by the National Forensic Science Technology Center are sufficient to meet the burden of proof for ISO and the ASCLD requirements, which will allow laboratories to use the UV-Box based on the published results of this test. Future studies will look at the effectiveness of shorter UV exposure times and different substrates.

About the Author: Andre Chambre

Andy Chambre is the founder and CEO of Air Science, LLC and has been associated with the ductless fume hood industry for more than 25 years. He was formerly the US Vice President for Captair Labx and President of Astec Microflow US. He was named President of Filtco Corporation in 2003 and currently also serves as a Director of Air Science Technologies Ltd. in the UK. Mr. Chambre has written numerous articles on fume hood safety and assisted in the development of safety standards by serving on various committees such as the Canadian Standards Association subcommittee on fume hoods and the SEFA 9 Ductless Enclosures Committee.

Sources

- "ISO/IEC 17025:2005 General Requirements for the Competence of Testing and Calibration Laboratories." *International Organization for Standardization (ISO).* N.p., 15 May 2005. www.iso.org/iso/catalogue_detail.htm?csnumber=39883.
- 5.4.5.2, "Supplemental Requirements for the Accreditation of Forensic Science Testing Laboratories." *American Society of Crime Laboratory Directors / Laboratory Accreditation Board (ASCLD/LAB).* N.p., 2011, Corresponds to ISO/IEC 10725:2005. des.wa.gov/SiteCollectionDocuments/About/1063/RFP/Add7_Item4ASCLD.pdf.

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