Contamination

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Slides prepared by Kelly M. Elkins, Ph.D., Towson University, 2024

Background

In October 2023, Towson University was awarded a cooperative agreement from NIST to develop a standardized DNA training curriculum for the United States that address the components in ANSI/ASB Standard 115, *Standards for Training in Forensic Short Tandem Repeat Typing Methods Using Amplification, DNA Separation, and Allele Detection*. 2020. 1st Ed.

This presentation addresses the knowledge-based portion of the training program and covers the topic outlined in 4.2.3f in ANSI/ASB Standard 115.

Learning Objectives

This material will provide trainees with an understanding of:

1. sources (environmental, procedural) of contamination;
2. sample handling strategies and preventative methods;
3. decontamination procedures;
4. detection limitations;
5. root cause analysis, corrective action when contamination occurs.

Terms & Definitions
Contamination
(ASB 115 3.7)

- The unintentional introduction of **exogenous** DNA or other biological material in a DNA sample, PCR reaction, or item of evidence; the exogenous DNA or biological material could be present before the sample is collected or **introduced** during collection or testing of the sample.
- Not native to the evidence
  - Accidental or unintentional

Sources of Contamination

- **Exogenous DNA:** DNA contamination from outside of the organism or case of study
  - **Environmental**
    - Genomic DNA from air, surfaces, soil, and water rather than the human (or wildlife) suspect or victim sample
  - **Procedural**
    - DNA contamination from lab analysts such as sneezing
    - Reusing dirty tips or not changing gloves
    - Mixing or comingling samples

Sources of Contamination

- First responders
- Laboratory personnel
- Crime scene technicians
- Law enforcement
- Medical personnel
- Objects or surface transfer

It can never be known with certainty that a casework or database sample is contamination-free.

The **sensitivity** of testing instrumentation and methods in human forensic DNA laboratories has steadily **increased** and has resulted in a **greater chance of detecting** low-level contamination events.

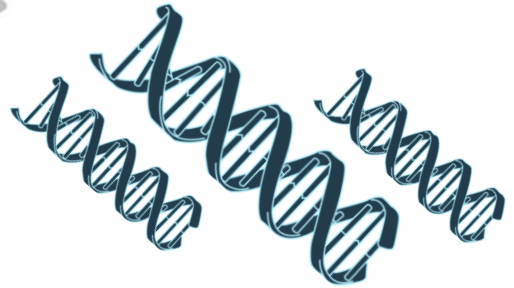
Labs should have **documented procedures** designed to minimize contamination and loss

DNA Typing Process

Evidence collection at crime scene

Sampling evidence

Extraction of DNA from sample



Amplification of DNA for quantitation and typing

Tozzo, P.; Mazzobel, E.; Marcante, B.; Delicati, A.; Caenazzo, L. Touch DNA Sampling Methods: Efficacy Evaluation and Systematic Review. *Int. J. Mol. Sci.* **2022**, *23*, 15541. https://doi.org/10.3390/ijms232415541

Contamination can occur at various stages

Sample collection	Sampling	DNA extraction[#]	PCR
• Sample contamination with genomic DNA, body fluids, tissue or exogenous DNA already present in the environment • Sample contamination with genomic DNA traces on crime scene equipment	• Sample contamination from DNA present on lab surfaces • Sample contamination from DNA present on scissors, lab paper, swabs, etc. • DNA contamination between samples during preparation	• Sample contamination from DNA present on scissors, lab paper, swabs, etc. • DNA contamination in extraction reagents*	• DNA contamination of a sample with amplified DNA from a previous PCR reaction • DNA contamination in PCR reagents*

[#]Most susceptible step

*Obtain reagents and consumables (e.g., tubes, tips) from an ISO 18385 compliant manufacturer or PCR grade products, and document lot numbers in documentation

Contamination at Evidence Collection

Sample collection

- Sample contamination with genomic DNA, body fluids, tissue or exogenous DNA already present in the environment
- Sample contamination with genomic DNA traces on crime scene equipment

Contamination in Sampling

Sampling

- Sample contamination from DNA present on lab surfaces
- Sample contamination from DNA present on scissors, lab paper, swabs, etc.
- DNA contamination between samples during preparation

Contamination in DNA Extraction

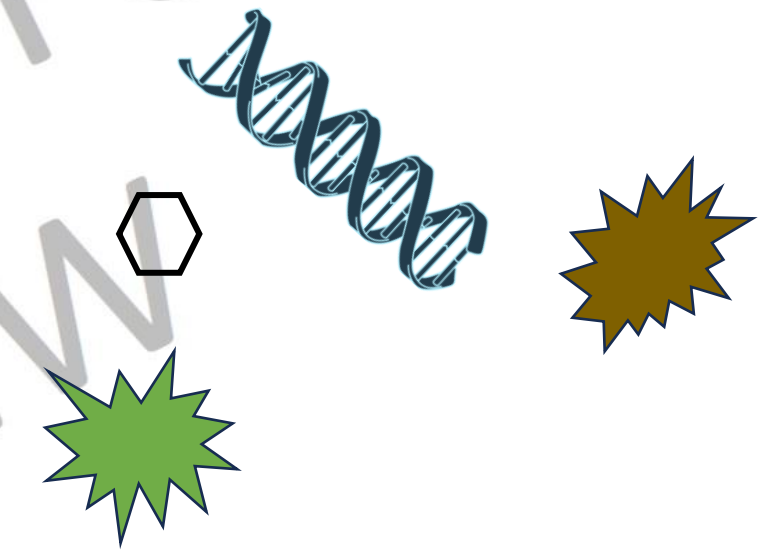
DNA Extraction

- Sample contamination from DNA present on scissors, lab paper, swabs, etc.
- DNA contamination in extraction reagents
- DNA contamination in consumables such as pipette tips or tubes

Contamination in PCR Reaction

PCR

- DNA contamination of a sample with amplified DNA from a previous PCR reaction
- DNA contamination in PCR reagents or consumables



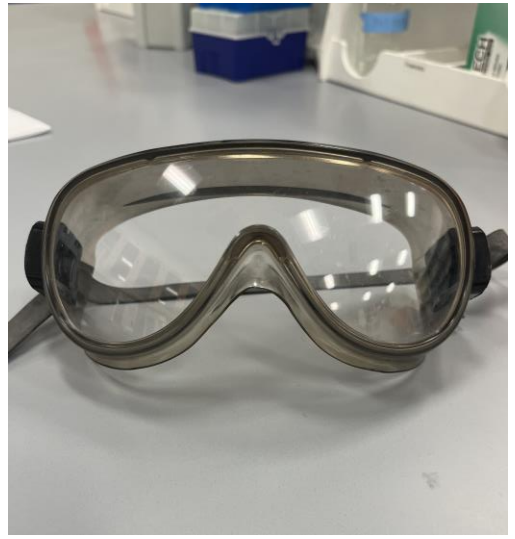
The working **environment** and **procedures** should mitigate contamination.

Preventative Methods: Avoiding Contamination

Access	Limit access to necessary personnel
PPE	Wear and change personal protective equipment (PPE) including gowns/lab coats, booties, gloves, hair covering, face mask and beware of static cling
Awareness	Avoid excessive speaking over samples/ bench surfaces Avoid opening more than one sample at a time
Decontamination procedures	Utilize decontamination procedures frequently
Separate processing	Process evidence (crime scene) samples separately from reference (exemplar) samples
Separate handling	Open tubes with tube openers Open one tube at a time
Separate spaces	Set up PCR amplification samples in PCR prep hoods in designated area Do not move post-PCR amplification samples or waste to the pre-PCR amplification areas; store in each space Equip pre and post amplification areas so that equipment can remain in place
Use of controls	Implement appropriate controls (e.g., reagent blanks, extraction controls, amplification controls) Implement tube labelling conventions
Elimination database	Establish a DNA elimination database for laboratory staff and visitors and rules for searches and notification policies for privacy purposes

Preventative Methods: Personal Protective Equipment & Safety

- Personal protective equipment (PPE) includes googles, gloves, lab coats, booties, hair coverings, masks, heat protection gloves, Tyvek or Kleengard suit, and a respirator
- In the event that a foreign material enters the eye, an eyewash located in the lab should be used immediately



Preventing Contamination: Personal Protective Equipment (PPE)

- Goggles are PPE that work to reduce the risk of samples and reagents entering the eyes
- Wearing PPE reduces the risk of DNA transfer to surfaces, equipment, other evidence and reference samples and extracts as well as microorganisms from samples to staff

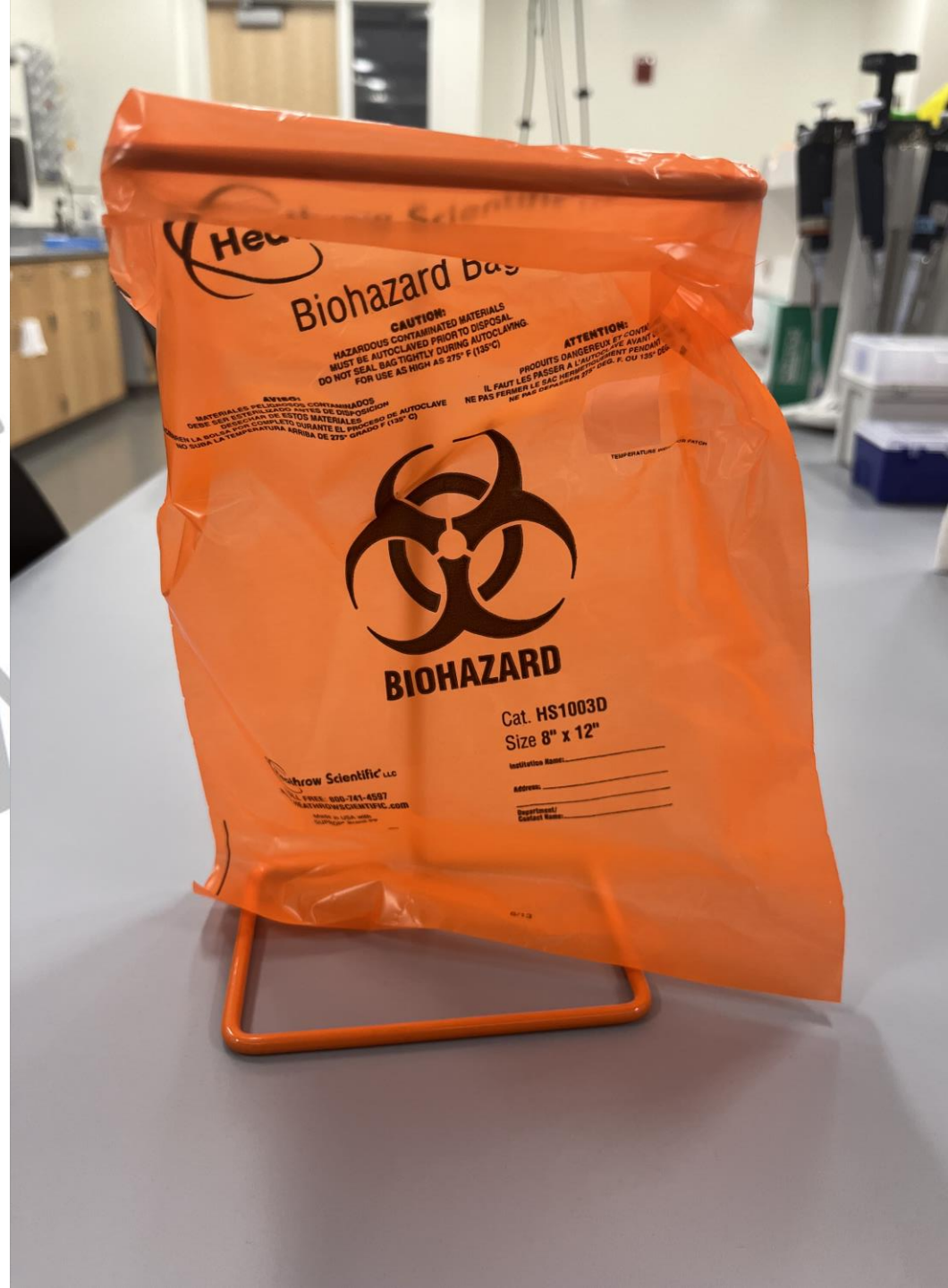
Safety, Quality and Avoiding Contamination: Activities Not Allowed in Lab*

- Smoking
- Eating
- Drinking
- Makeup application
- Hanging jewelry

*Items may fall into evidence, cause a chemical reaction or fire, or become contaminated by evidence or chemicals that could cause injury or harm.

Preventative Methods: Biohazard Disposal

- Use biohazard labels to signify biohazard waste
- Dispose of contaminated gloves, masks, suits and consumables in red biohazard bags



Preventative Methods for Avoiding Contamination: PCR Preparation Hood

- UV light is used to destroy surface DNA that could be introduced to samples
- Change paper surface to prevent carry over and contamination of samples
- Dedicated filtered pipette tips, pipettes, and equipment in pre- and post-PCR amplification areas eliminate DNA carryover
- Biosafety laminar flow hoods should be used when working with potentially pathogenic materials as the airflow carries the airborne pathogens away from the analyst

Decontamination Procedures*

*Use prior to opening evidence or samples and reagents and consumables to destroy cellular and DNA material on surfaces.

Do not spray bleach, water, ethanol, etc. directly on pipettes/instruments when cleaning


10% bleach


70% ethanol


DNA off


Soap and water


UV light


Autoclaving


Ethylene oxide

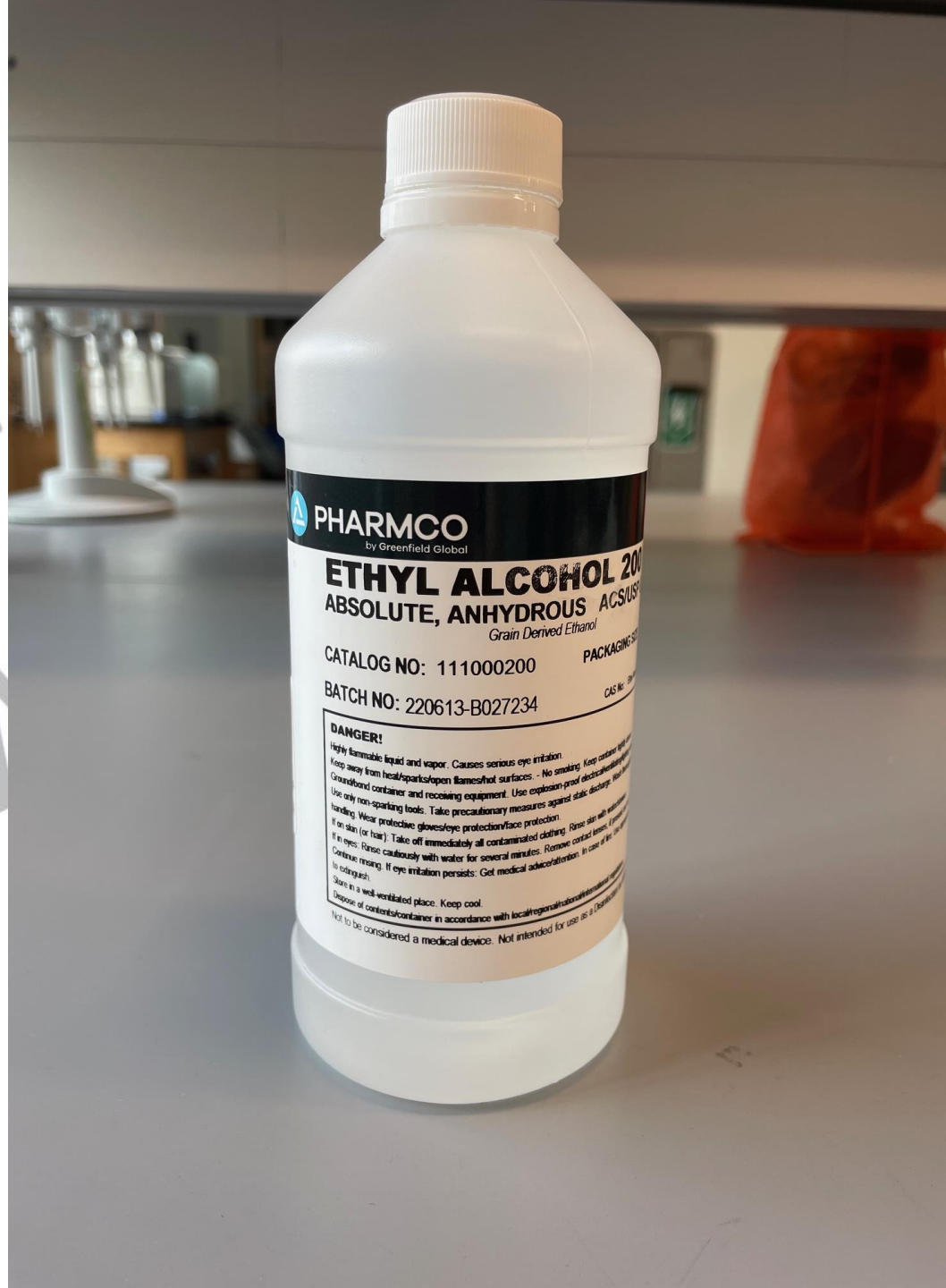
1. 10% Bleach (NaOCl)

- Oxidizes DNA and cleaves the strands into smaller pieces
- Wear PPE and avoid contact, corrosive and discolors clothing
- Prepare by diluting 1 part bleach with 9 parts water
  - Bleach degrades over time in the presence of light and at room temperatures
  - Prepare fresh daily or weekly per SOP
- Apply to surface by spraying
- Apply and let stand on surfaces for at least 10 minutes or according to SOP
  - Bleach damages metal surfaces and sensitive equipment so an alternative method may be indicated
- Wipe with a paper towel or cloth
  - Rinse with water or ethanol
- Soak bone in at least 3% bleach for at least 15 minutes

Kemp BM, Smith DG. Use of bleach to eliminate contaminating DNA from the surface of bones and teeth. Forensic Sci Int. 2005 Nov 10;154(1):53-61. doi: 10.1016/j.forsciint.2004.11.017.
Goodyear N. Effectiveness of five-day-old 10% bleach in a student microbiology laboratory setting. Clin Lab Sci. 2012 Fall;25(4):219-23.
Nilsson M, De Maeyer H, Allen M. Evaluation of Different Cleaning Strategies for Removal of Contaminating DNA Molecules. Genes (Basel). 2022 Jan 17;13(1):162. doi: 10.3390/genes13010162.

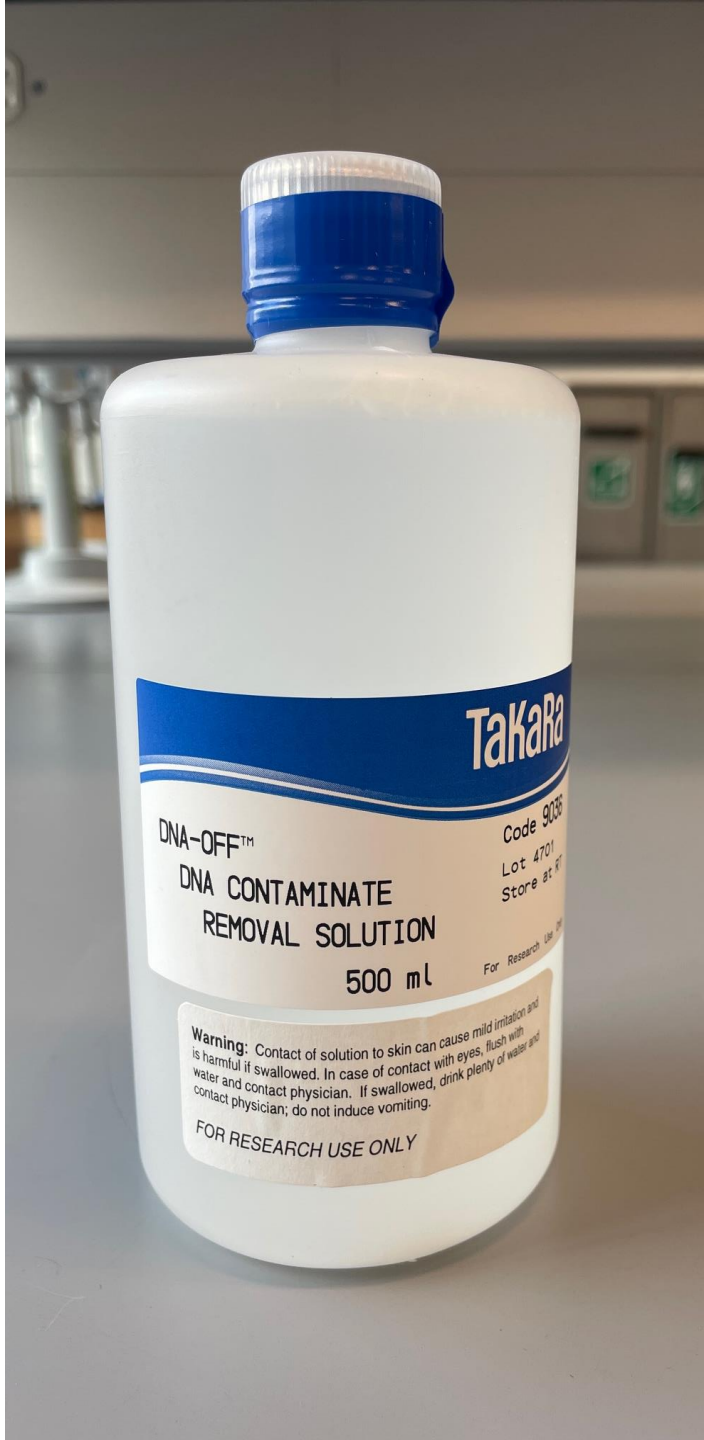
2. 70% Ethanol (CH_3CH_2OH)

- Flammable, eye irritant and toxic
- Evaporates quickly so 10-minute application is not achievable
- Apply following bleach to wipe down surfaces and equipment
- Small tools (e.g., tweezers, scissors) may be soaked in the solution for at least 10 minutes or follow SOP



3. DNA OFF™

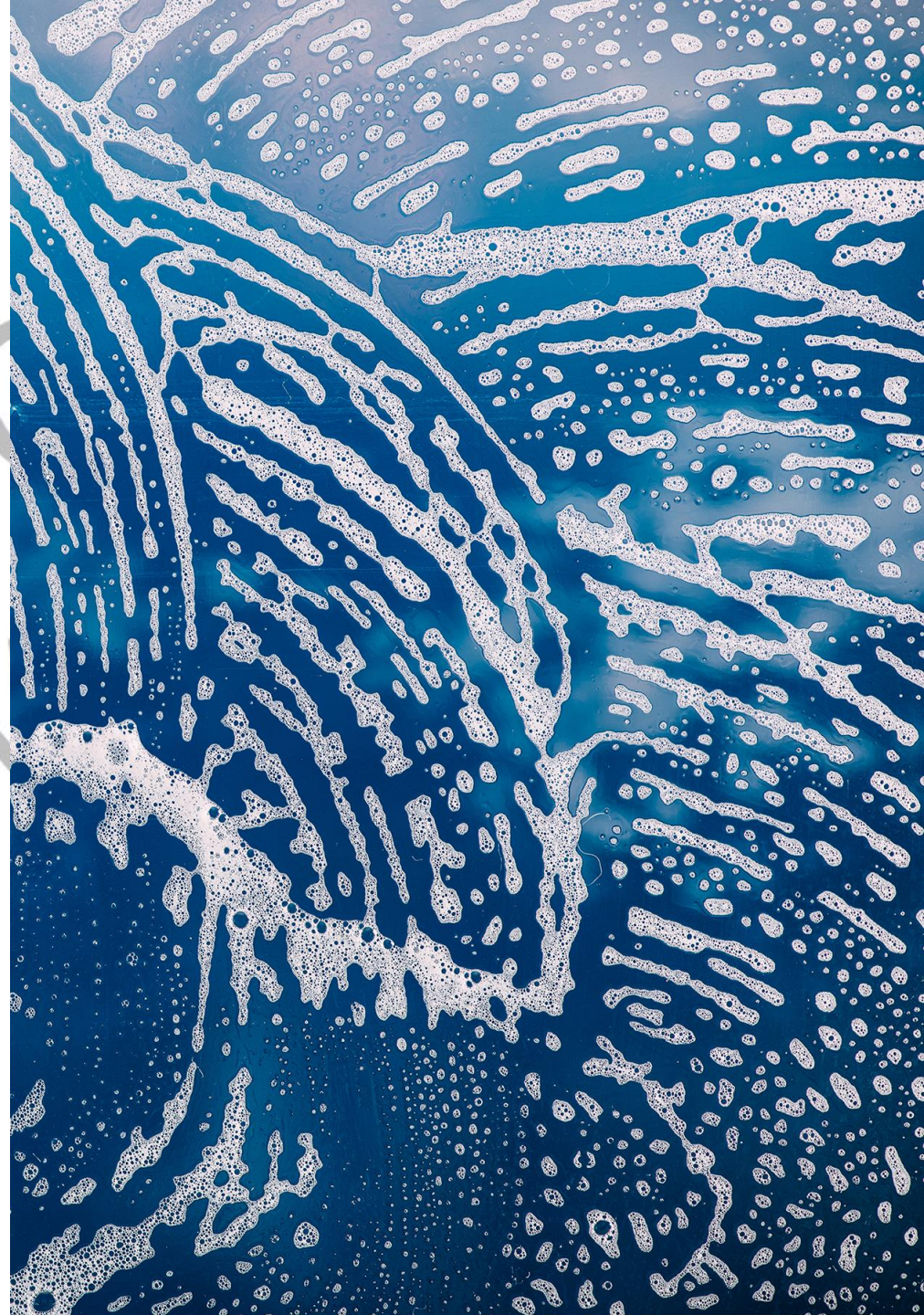
- According to the manufacturer DNA-OFF is a "non-alkaline, non-corrosive, and non-carcinogenic cleaning solution"
  - Store at room temperature
  - Heat at 37 °C to redissolve if precipitates form
  - Apply directly to surfaces such as PCR prep hoods for a few minutes, wipe with a paper towel, rinse with water and wipe again with a paper towel
  - Wear PPE as the product "may cause eye or skin irritation; may be harmful if inhaled"
- Cationic surfactants (e.g., octyl-trimethyl-ammonium bromide (OTAB), dodecyl-trimethyl-ammonium bromide (DTAB) and cetyl-trimethyl-ammonium bromide (CTAB)) induce structural changes in DNA



Bhattacharya S, Mandal SS. Interaction of surfactants with DNA. Role of hydrophobicity and surface charge on intercalation and DNA melting. Biochim Biophys Acta. 1997 Jan 14;1323(1):29-44. doi: 10.1016/s0005-2736(96)00171-x.

4. Soap and Water

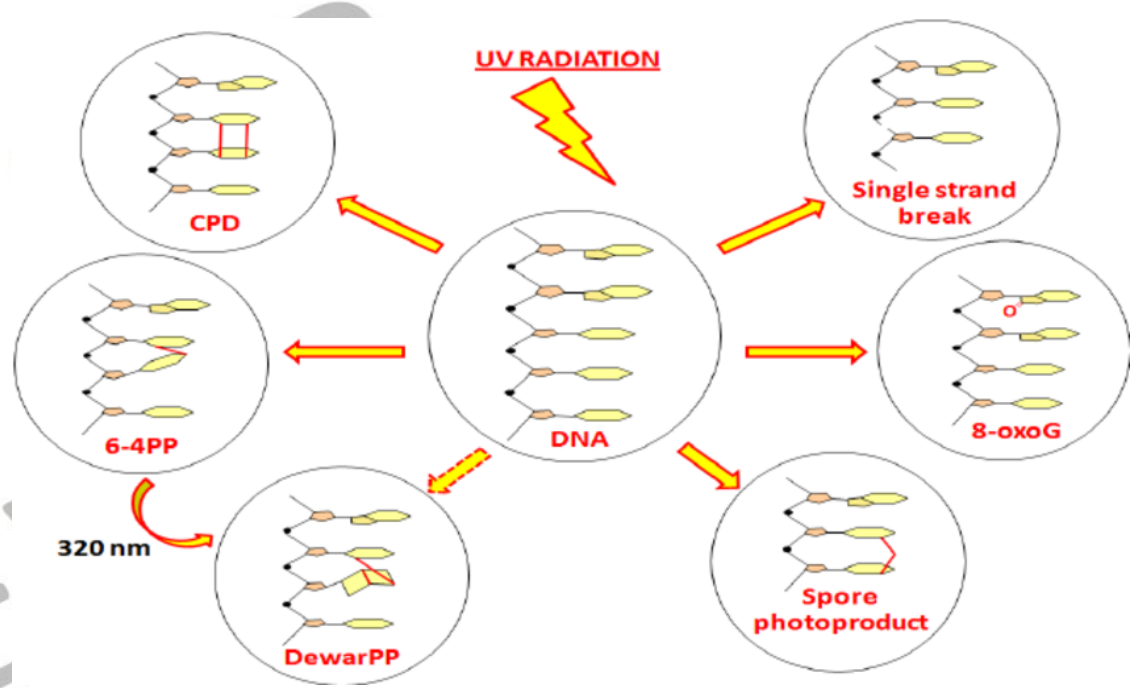
- Soap disrupts and dissolves cell lipid membranes
- Released DNA is water soluble and dissolves and can be wiped away
- Water, especially at high temperatures, hydrolyzes DNA into fragments
- Wash surface with hot soapy water and wipe away and rinse with water
- Cells are persistent and items soaked in water at neutral pH can still yield DNA profiles



Helmus J, Zorell S, Bajanowski T, Poetsch M. Persistence of DNA on clothes after exposure to water for different time periods-a study on bathtub, pond, and river. Int J Legal Med. 2018 Jan;132(1):99-106. doi: 10.1007/s00414-017-1695-2.
Harris KA, Thacker CR, Ballard D, Syndercombe Court D. The effect of cleaning agents on the DNA analysis of blood stains deposited on different substrates. International Congress Series 2006; 1288: 589-591.

5. UV-Light

- Short wave UV-B (290-320 nm) or UV-C (254 nm) light is typically employed
- UV-B causes pyrimidine (e.g., thymine, cytosine) cyclobutane formation dimerization
- UV-C causes strand breaks and generates reactive oxygen species including singlet oxygen, hydrogen peroxide, and hydroxyl radicals that oxidize DNA bases
- Decontamination procedures should be written in SOP and scheduled routinely
- 15 to 30 minutes depending upon distance to the surface and intensity of the source



Yagura T, Makita K, Yamamoto H, Menck CF, Schuch AP. Biological sensors for solar ultraviolet radiation. Sensors (Basel). 2011;11(4):4277-94. doi: 10.3390/s110404277.

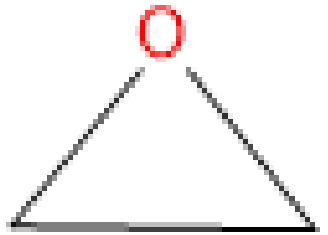
6. Autoclave

- Destroys DNA template using high heat, steam, and pressure
- Heat induced hydrolysis of DNA strand into small fragments
- Autoclave 80 minutes at 121 °C to remove intact DNA that may serve as a template in amplification
- Consumables such as tips and tubes (packs) are typically autoclaves at 121 °C for 20 minutes

Fattorini P, Marrubini G, Bonin S, Bertoglio B, Grignani P, Recchia E, Pitacco P, Procopio F, Cantoni C, Pajnič IZ, Sorçaburu-Cigliero S, Previdere C. Prolonged DNA hydrolysis in water: A study on DNA stability. Data Brief. 2018 Aug 30;20:1237-1243. doi: 10.1016/j.dib.2018.08.120.
Suyama T, Kawaharasaki M. Decomposition of waste DNA with extended autoclaving under unsaturated steam. Biotechniques. 2013 Dec;55(6):296-9. doi: 10.2144/000114113.

7. Ethylene Oxide (C_2H_4O)

- Wear PPE, probable carcinogen
- Ethylene oxide is an SN_2 alkylating agent in which the molecule is reacted via backside attack of the bond
- Destroys DNA via hydrolytic deamination via hydroxy group on alkyl side chain



Li F, Segal A, Solomon JJ. In vitro reaction of ethylene oxide with DNA and characterization of DNA adducts. Chem Biol Interact. 1992 Jun 15;83(1):35-54. doi: 10.1016/0009-2797(92)90090-8.
Gates KS. An overview of chemical processes that damage cellular DNA: spontaneous hydrolysis, alkylation, and reactions with radicals. Chem Res Toxicol. 2009 Nov;22(11):1747-60. doi: 10.1021/tx900242k.

Example of Decontamination of a Thermal Cycler

- Soak a cotton swab in isopropanol or 1:10 v/v dilution of 5.25% bleach or apply with an atomizer
  - Clean the sample wells by swabbing
  - Let the isopropanol evaporate
  - Rinse the block with deionized water following bleach treatment
- Avoid excessive use of bleach as the sample blocks can corrode

Applied Biosystems. Veriti Thermal Cycler User Manual, 2020. https://assets.thermofisher.com/TFS-Assets/LSG/manuals/MAN0018441_VeritiThermalCycler_UG.pdf

Detection and Control of Contamination

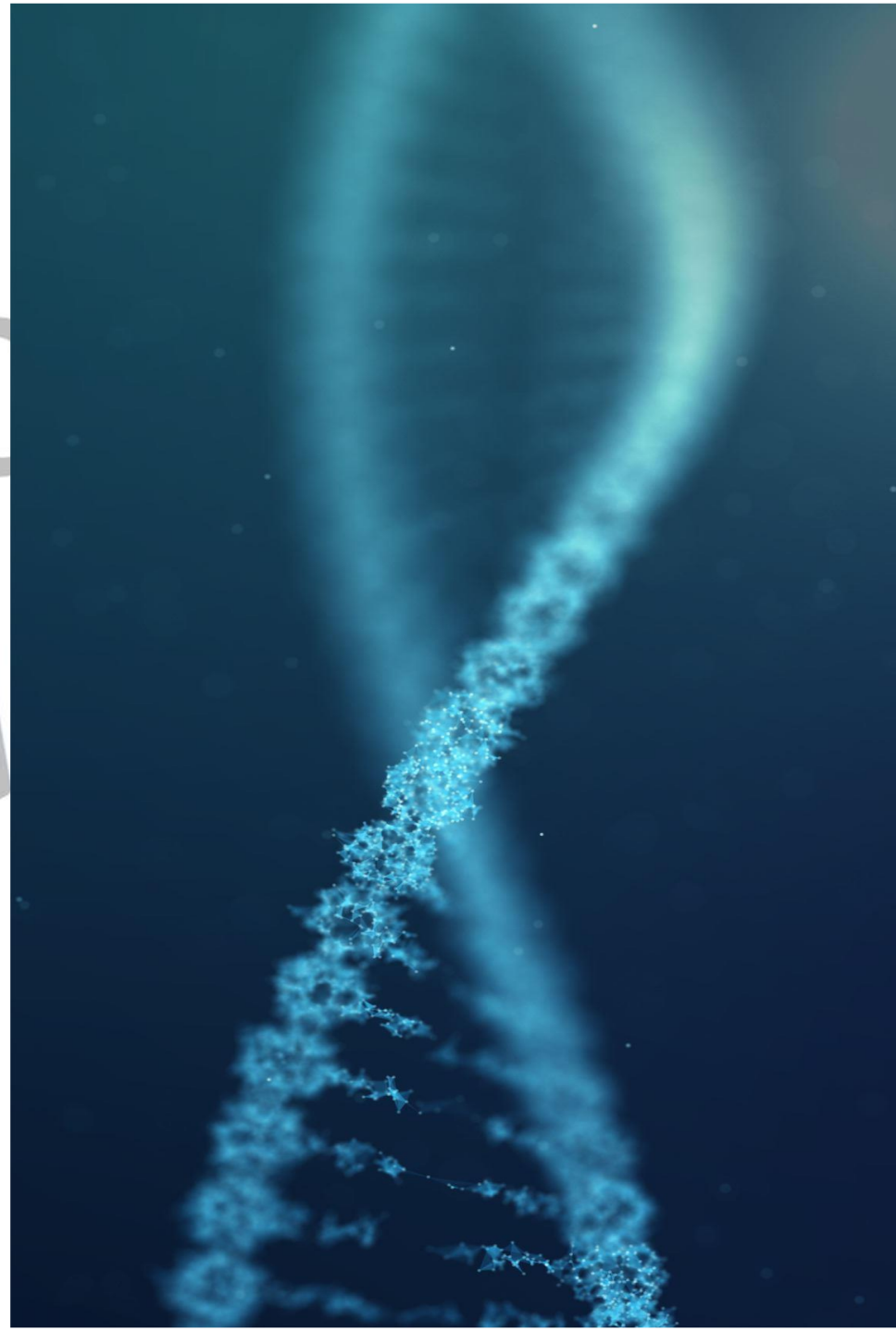
- Documenting
  - Log events and when likely occurred
- Monitoring and restricting access
- Wearing and changing PPE
- Evaluation of environmental DNA on surfaces and consumables
- Creating an elimination database for comparison
- Perform intra-batch comparisons and cross-contamination checks
- Use probabilistic genotyping software to aid in detection

Contamination Monitoring

- Lab visitors
- Cleaning staff
- Ventilation
- Surfaces
- Medical personnel
- Production staff
- Crime scene technicians
- Forensic staff
- Interns

Contamination Monitoring

- Regular evaluation of lab surfaces, instruments, and equipment for detectable and amplifiable DNA



Elimination Database

- Voluntary samples collected from lab and law enforcement staff including technicians, analysts, technical leads, interns, investigators, and administrators
- Not associated with the crime scene samples
- People who had access to the crime scene or samples
  - People working with samples can shed DNA on the samples
- Cannot detect all forms of contamination

Detection Limitations

PCR is very sensitive, and caution must be taken to avoid contamination

Can detect amplifiable DNA that amplifies in the assay using fluorescent-tagged primers or intercalating dyes

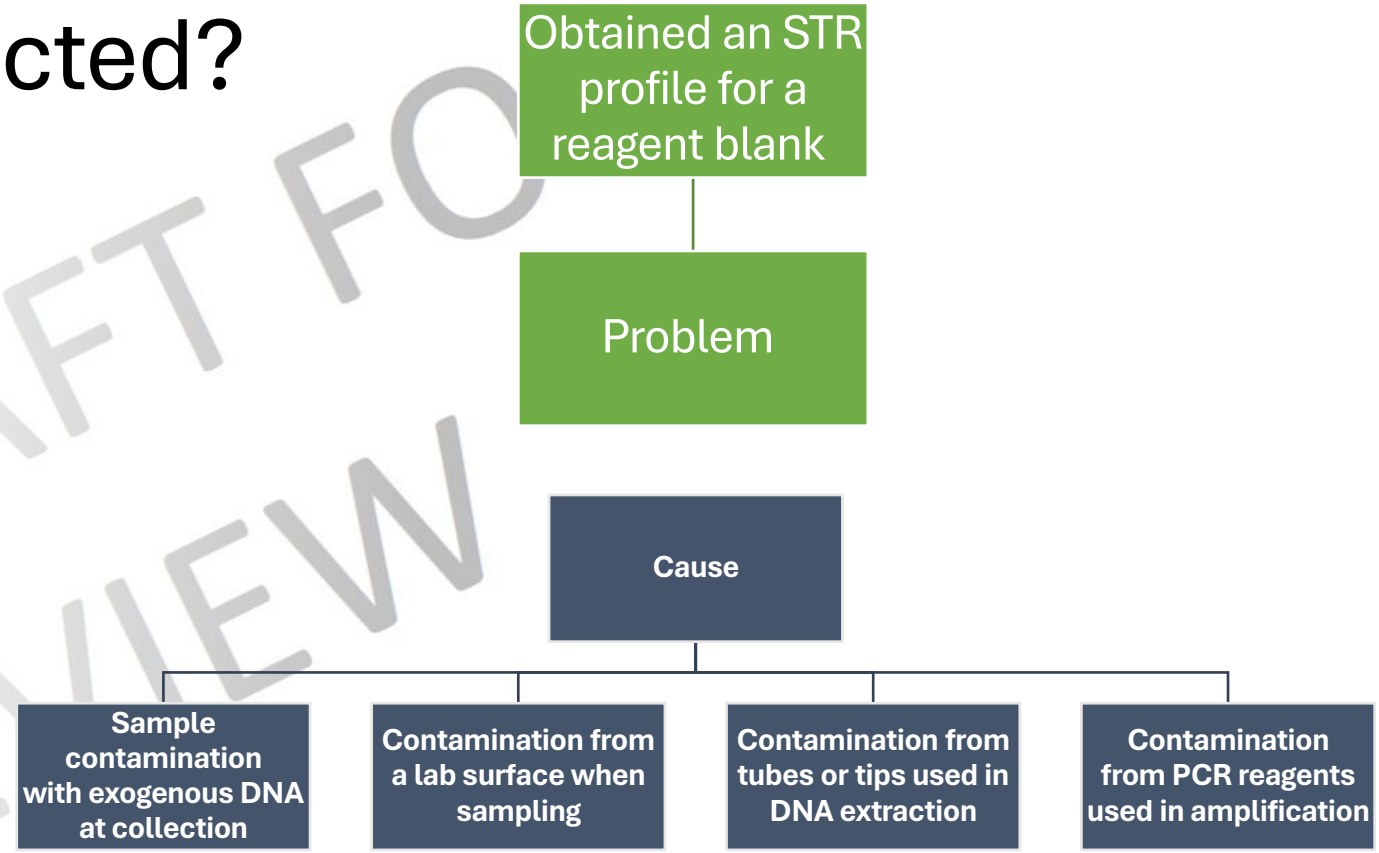
Need sufficient amplicons to exceed fluorescence detection threshold

Can detect one cell (6 pg) if the DNA is intact and amplifiable with the primers; to avoid stochastic effects, 100 pg to 1 ng is recommended for most reactions*

*depending on the PCR cycle number used

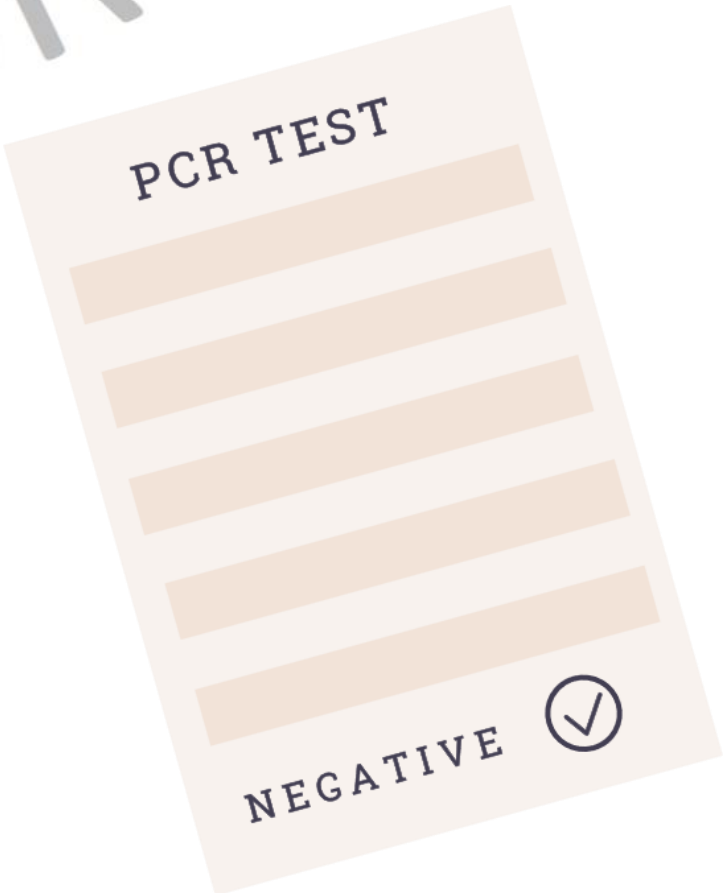
What do you do if contamination is detected?

- Root cause analysis
- Possible observations, symptoms or issues indicating contamination
  - Detectable quantifiable DNA in negative amplification control or reagent blank/extraction control
  - Obtaining an STR profile or partial profile for a negative or substrate control



Types of Controls

- **Negative control** - an amplification reaction in which no DNA template is added to the primer and reaction mix to ensure the method produces no detectable or a negative response
  - Substrate negative control – control to test if environmental DNA is present on the surface or swab the biological material is sampled from
  - Reagent blank (extraction negative) control – control to test if the extraction reagents are contaminated
  - Quantitation negative control – control to test if the quantitation reagents are DNA free
  - Reagent blank (amplification negative) control- control to test if amplification reagents are contaminant free
  - No template control (NTC) – an amplification reaction in which DNA molecules are not added to the reaction and primer mix



Reference Samples

- Samples of known origin to compare to evidence sample
  - Buccal swabs from victim / suspects
  - Hair sample from victim / suspects
- Tested after the evidence sample(s)

Tolerance Level

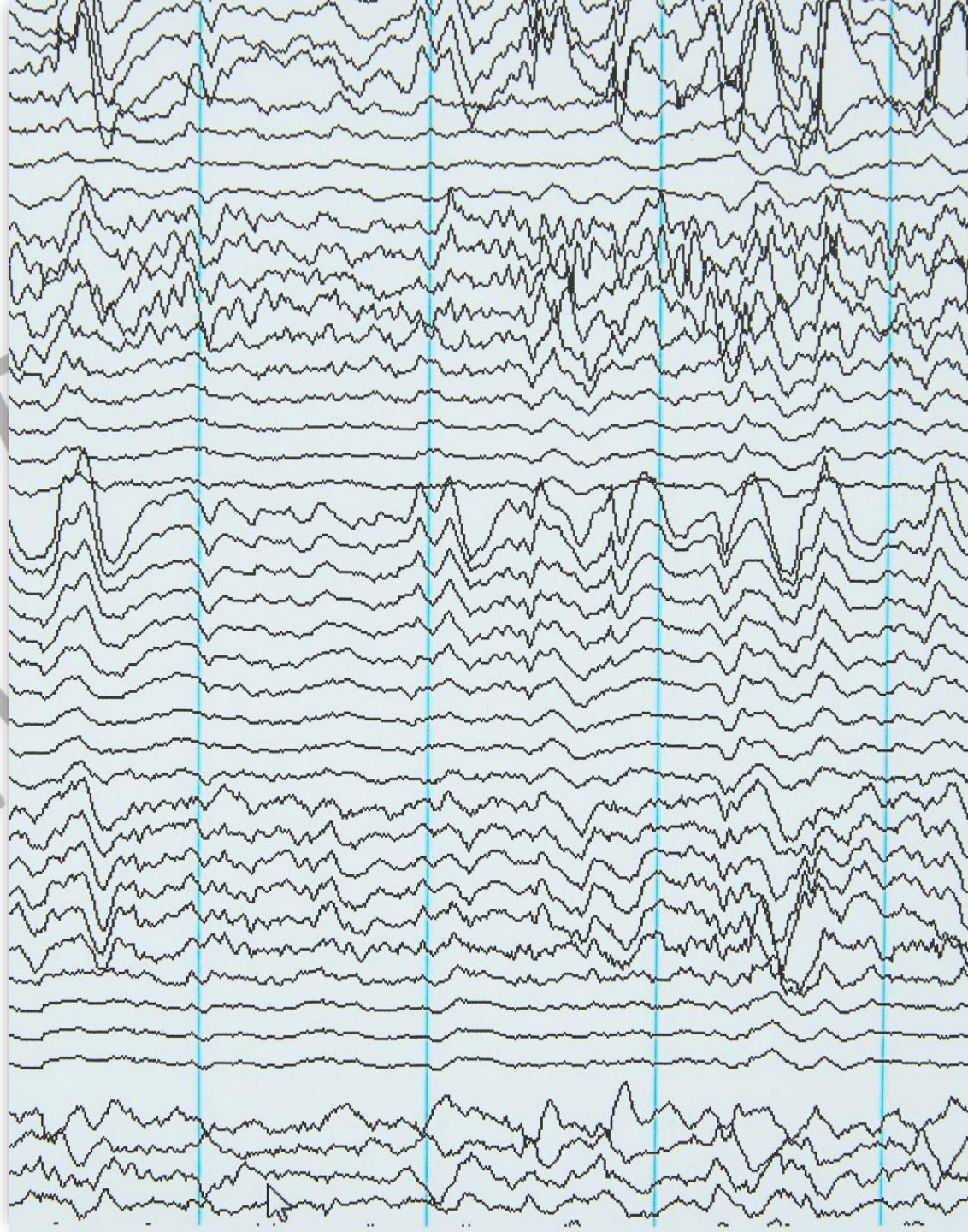
- A contamination tolerance is the level of detection at or below which contamination does not interfere with a confident interpretation of the data based on validation
- In DNA typing the tolerance threshold is the interpretation threshold

Gloria Jansen, Daniel Gebert, Tharini Ravindra Kumar, Emily Simmons, Sarah Murphy, Felipe Karam Teixeira, Tolerance thresholds underlie responses to DNA damage during germline development. bioRxiv 2024.01.07.574510; doi: https://doi.org/10.1101/2024.01.07.574510
Now published in *Genes & Development* doi: 10.1101/gad.351701.124

Analytical Threshold

1) The minimum height requirement at and above which detected peaks on a STR DNA profile electropherogram can be reliably distinguished from background noise; peaks above this threshold are generally not considered noise and are either artifacts or true alleles.

2) A “Relative Fluorescence Units” (RFU) level determined to be appropriate for use in the PCR/STR DNA typing process; a minimum threshold for data comparison is identified by the specific forensic laboratory through independent validation studies.



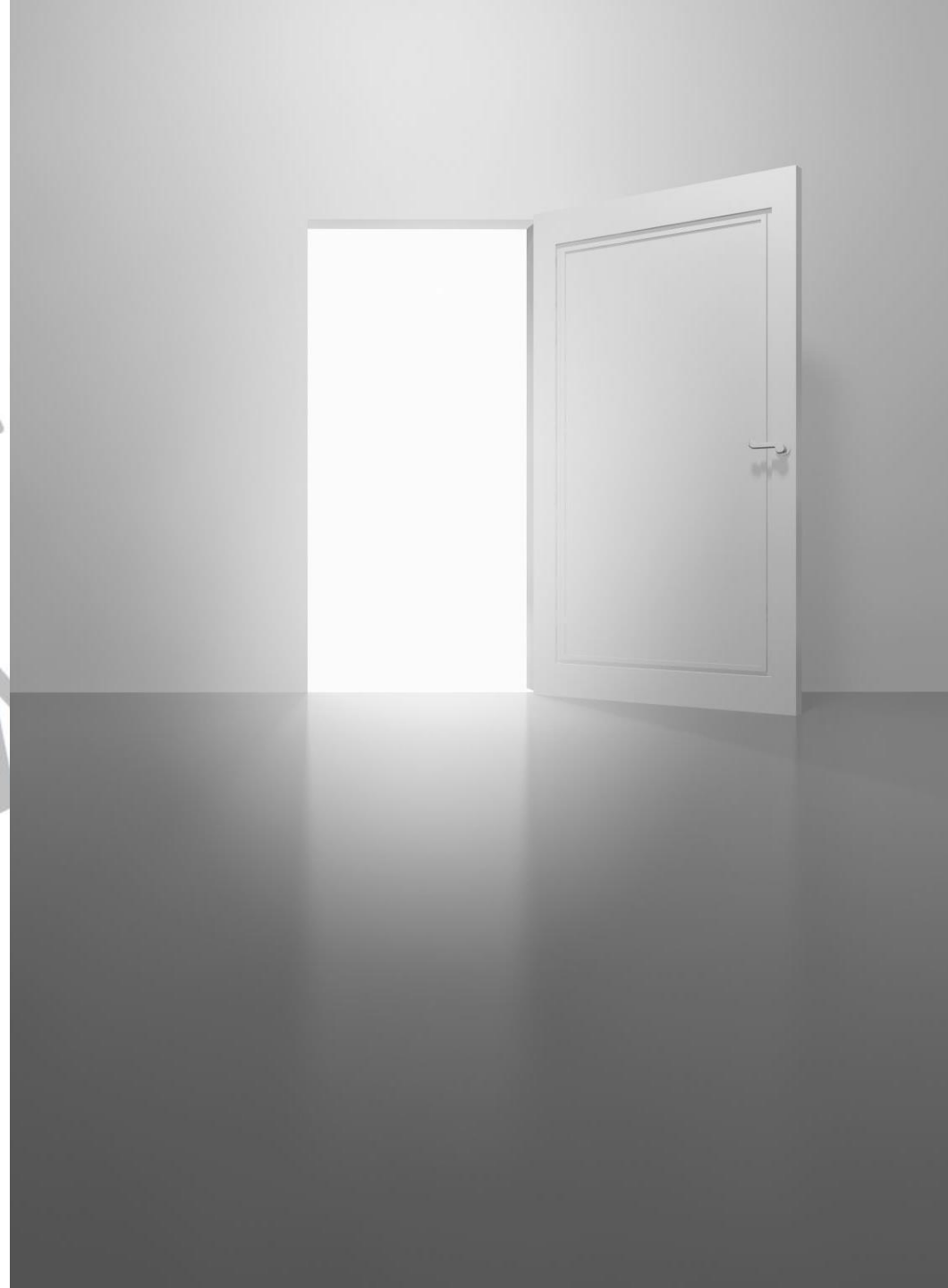
Approaches to Determining the Analytical Threshold

- The peak is real if it is above the stochastic or interpretation threshold setting determined in validation studies (e.g., 50 or 200 RFU)
- The peak is real if it reliably amplifies in concordance studies and is above the analytical threshold (e.g., 30 RFU)
- The peak is real if the ratio of heterozygote loci peaks less than 60%

Butler JM. Forensic DNA Typing, 2nd. ed. Elsevier, 2005.

Root Cause Analysis Steps

- Define the problem
- Determine the "Why" or cause of the problem
- Collect data about the steps preceding the problem
- Locate the root cause of the contamination and eliminate that cause using a well-developed and effective corrective action
- Types of contamination
  - *Material*: Contaminated consumable tips, plastics and/or gloves
  - *Material*: Contaminated reagents and solutions
  - *Environmental*: Contaminated bench or surface or air flow
  - *Machine*: Contaminated instrument(s)
  - *Method*: Altered procedural steps
  - *Staff*: Skills need remediation



Defining the "Problem"

"Problems" may be categorized into three main types:

- **Type 1:** Deviation, non-conformity or inconsistency where the actual and expected results differ. For example, an erroneous result obtained during proficiency testing
- **Type 2:** An undesirable situation or event. For example, accidents such as the loss of a sample, contamination events, loss of electronic data
- **Type 3:** Undesirable performance. For example, failure to follow established validated and documented protocols

The level of difficulty to detect and analyze the problem is dependent upon the type/category it falls within.

Identifying a Failed Control

- Positive or negative control that produces an unexpected result

Types of Contamination Events

- Contamination in a negative control or reagent blank
- Contamination in a positive control
- Contamination in a forensic or reference sample

Why controls may fail

Handling error

Contamination with exogenous DNA

Faulty lot of reagents

- Allelic ladder contamination in PCR reagents in 2007 (ABI)
- Manufacturing change instituted to prevent this in the future

Identifying the Type of Contamination Issue

- Single contamination event
  - Pipette tip
  - Tube
- Low level contamination in all samples but no impact on interpretation
  - DNA profile from a high-quality single source or two-person mixed DNA profile with a very low-level minor component consistent with the profile in the negative control and possibly other samples below interpretation threshold
- Cross contamination or event could not be determined
- …Need to change gloves frequently

Goray M, Pirie E, van Oorschot RAH. DNA transfer: DNA acquired by gloves during casework examinations. Forensic Sci Int Genet. 2019 Jan;38:167-174. doi: 10.1016/j.fsigen.2018.10.018.

Interpreting Failed Controls and Contamination Events

Risk assessment

Impact

Cause

Not suitable for interpretation

DNA test results are determined to be compromised and no retesting

Contaminated control and sample contained mixture of more individuals that validated interpretation permits

Suitable for interpretation

Must be within constraints of lab's internal validation studies and documented interpretation protocols

The source may be identified by name, employment position or other descriptor as permitted by law and agency policies (i.e. analyst whose DNA was detected in negative control)

Use applicable statistical analysis to assess the similarity or difference of the two DNA profiles

Corrective Action

Create

Create actionable strategy

- Purchase new kit(s) or consumables
- Clean/decontaminate instrument(s)/surface(s)
- Retest step prior to error or issue
- Provide retraining or additional training

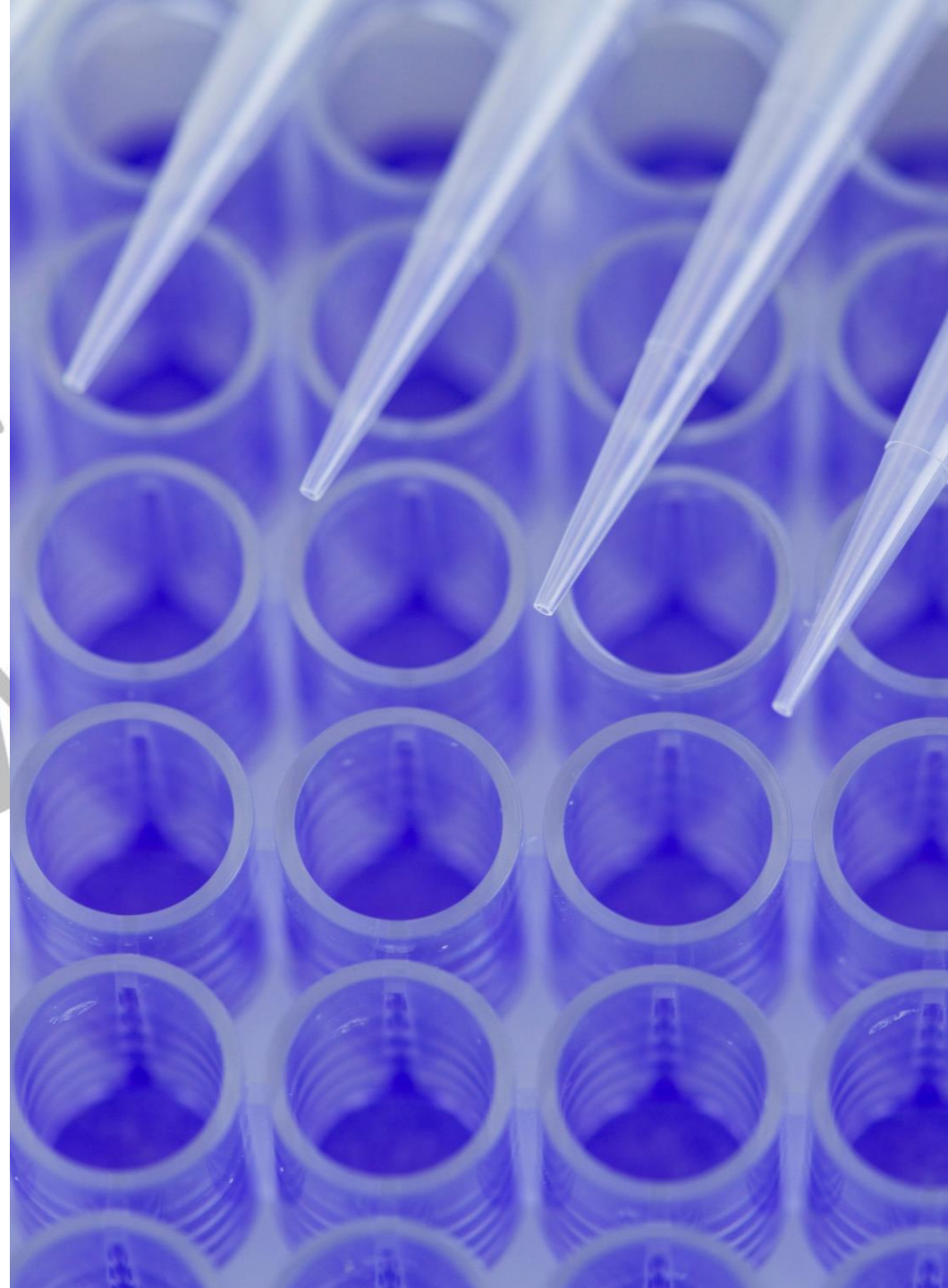
Confirm

Confirm the solution worked

- Evaluate skill with proficiency test samples
- Run QC samples with new products or cleaned instruments/surfaces

Retesting Do's and Don'ts

- **When to retest**
  - Sufficient evidence material to resample or retest
    - Analyze new extract or material prior to comparing to previous results
  - Issue can be determined
  - Associated profiles needed for comparisons
- **When not to retest**
  - Sample was consumed during the initial analysis
  - Additional testing would exhaust the remaining portion of the sample or DNA extract eliminating the possibility of future testing
  - Associated profile(s) would not be suitable for comparison even if the controls produced the expected results



Reporting Contamination

- Document
  - Tracking (e.g., lot numbers, case numbers, dates)
  - Remediation
  - Improvement
- Report
  - Transparency
  - Original and a retest data
- Communicate
  - Legal discovery

A Case Study Example

- Observation
  - Contamination by a crime scene technician was identified in multiple samples in a year using the QC elimination database and laboratory management system
- Corrective action
  - Observation
    - Technician was not changing gloves as frequently as required in the SOP
- Confirmation
  - Observation
  - Sample tracking
- Maintaining a quality control elimination database of employee profiles is key

Pickrahn I, Kreindl G, Müller E., Dunkelmann B, Zahrer W, Cemper-Kiesslich J, Neuhuber F. Contamination when collecting trace evidence—An issue more relevant than ever? Forensic Science International: Genetics Supplement Series, Volume 5, e603 – e604.

Study Questions

- Define contamination in DNA analysis.
- List some causes of contamination.
- List some processes and procedures to avoid contamination.
- List some decontamination methods.
- Explain how root cause analysis can be used to trace contamination events.
- Explain which failed controls can be interpreted and why.

Suggested Readings

- OSAC 2020-S-0004, Standard for Interpreting, Comparing and Reporting DNA Test Results Associated with Failed Controls and Contamination Events (on the OSAC Registry).
- ANSI/ASB Standard 115, Standard for Training in Forensic Short Tandem Repeat Typing Methods using Amplification, DNA Separation, and Allele Detection. 2020. 1st Ed. https://www.aafs.org/sites/default/files/media/documents/115_Std_e1.pdf
- ANSI/ASB Standard 136, Forensic Laboratory Standard for Prevention, Monitoring, and Mitigation of Human DNA Contamination. 2022. 1st ed. https://www.aafs.org/sites/default/files/media/documents/136_Std_Balllot03.pdf