

Quality Control

Knowledge Based Training 4.2.3g

*Standard for Training in Forensic Short Tandem Repeat Typing Methods
using Amplification, DNA Separation, and Allele Detection*

ANSI/ASB Standard 115, First Edition, 2020

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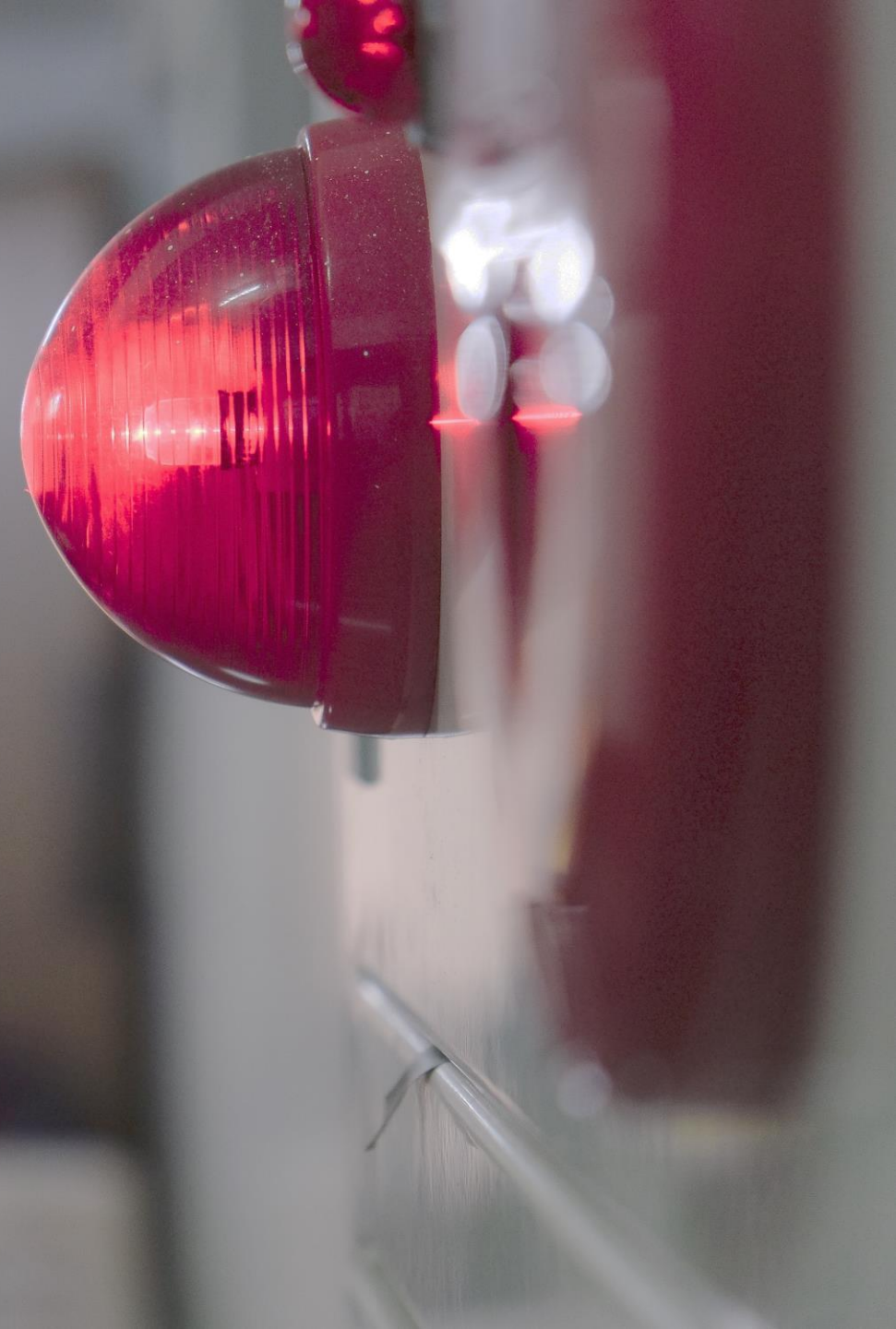
Background

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 4.2.3g in ANSI/ASB Standard 115.

Learning Objectives

- Quality control in the amplification, DNA separation and allele detection process to include appropriate controls.



Features of a QMS

- Goals and objectives
- Organization and management
- Personnel
- Facilities

- Reviewed annually
- Under the direction of the Technical Leader
- Training is reviewed and approved
- Proficiency testing is used
- Assure data quality
- Interpretation guidelines
- Mixture interpretation guidelines

Quality Control (QC)

- A system of maintaining standards by testing the reagents, platforms, and products to ascertain that a specification or threshold is met
 - Used to detect **non-conforming** results
- Fulfills QMS requirements
- **Routine** quality control is part of the *process*
 - Each part of the process is inspected, tested and measured
 - QC using premade and purchased chemicals and products including kits and reagents as well as in-house prepared reagents
 - With each set of samples, daily, or for a lot number



QC

- Standards
 - Control DNA isolated from a known, documented, and reproducible source
 - Scientifically sound / proven to work
 - Can be incorporated into validation processes

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Use of Controls, References and Standards



NECESSARY TO INTERPRET DATA



ASSESS IF INSTRUMENTS AND
PRODUCTS ARE WORKING PROPERLY
AND AVOID INSTRUMENT BIAS

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Overview of the DNA Typing Process and Controls

Sampling

- Reference sample
- Elimination sample
- Evidence sample

DNA Extraction

- Positive Control
- Substrate control / NTC
- RB

DNA Quantitation

- Quantitation standards
- Positive Control
- RB

STR Fragment Analysis

- Positive Control
- NTC / RB
- Elimination Sample
- Allele ladder
- ILSS

Types of Controls in DNA Analysis

- **Reagent blank control** is an analytical control sample that contains no template DNA and is used to monitor contamination from extraction to final fragment or sequence analysis. This control is treated the same as, and parallel to, the forensic and or casework reference samples being analyzed.
 - No DNA template, control or standard DNA is added
- **Positive amplification control** is an analytical control sample that is used to determine if the PCR performed properly. This control consists of the amplification reagents and a known DNA sample.
 - Known is frequently a standard
- **Negative amplification control** is used to detect DNA contamination of the amplification reagents. This control consists of only amplification reagents without the addition of template DNA.
- **Background controls** are used to test that the background is negative or as expected for the test run without a sample. These are used in fluorescence detection.

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 Material and Known Samples and Standards

- **Reference material** (certified or standard) is a material for which values are certified by a technically valid procedure and accompanied by, or traceable to, a certificate or other documentation which is issued by a certifying body.
- **Known samples** are biological material whose identity or type is established.

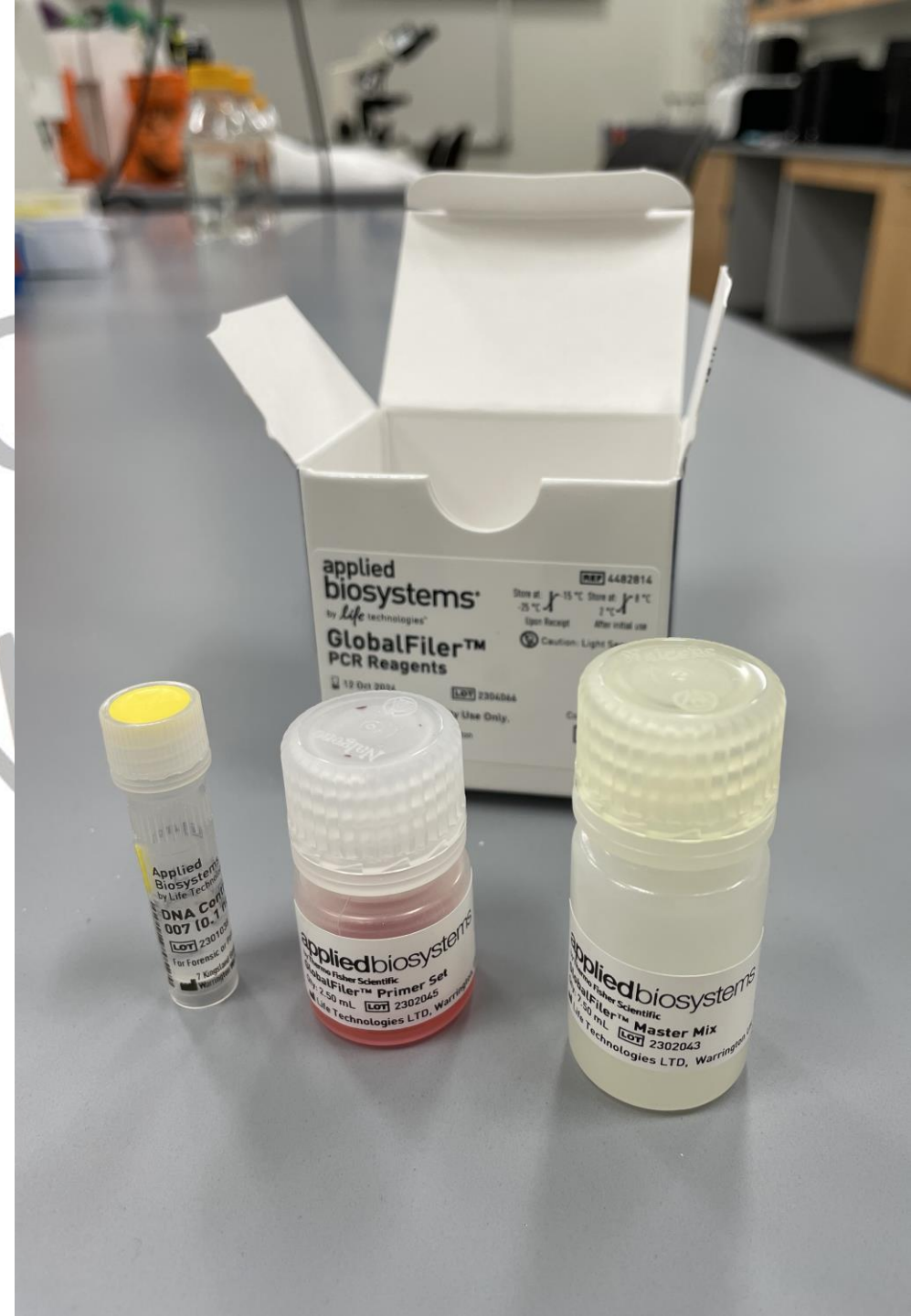


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Quality Control Standards

- Use standard control DNA included in the kit or purchased separately (e.g., 007, K562, 9948, 2800 M) for quantitation and amplification controls
 - Generate high quality DNA quantitation and typing results
 - Known profile
 - Reproducible
 - Can use for troubleshooting if a non-conforming result is detected
- Internal lane size standard (ILSS or ISS) for electrophoresis sizing
 - Used to determine a correlation coefficient to size sample fragments
 - For troubleshooting, if all of the peaks for the sizing standard are not present, it suggests a temperature, run time, or injection problem.



Sources of Controls and Standards



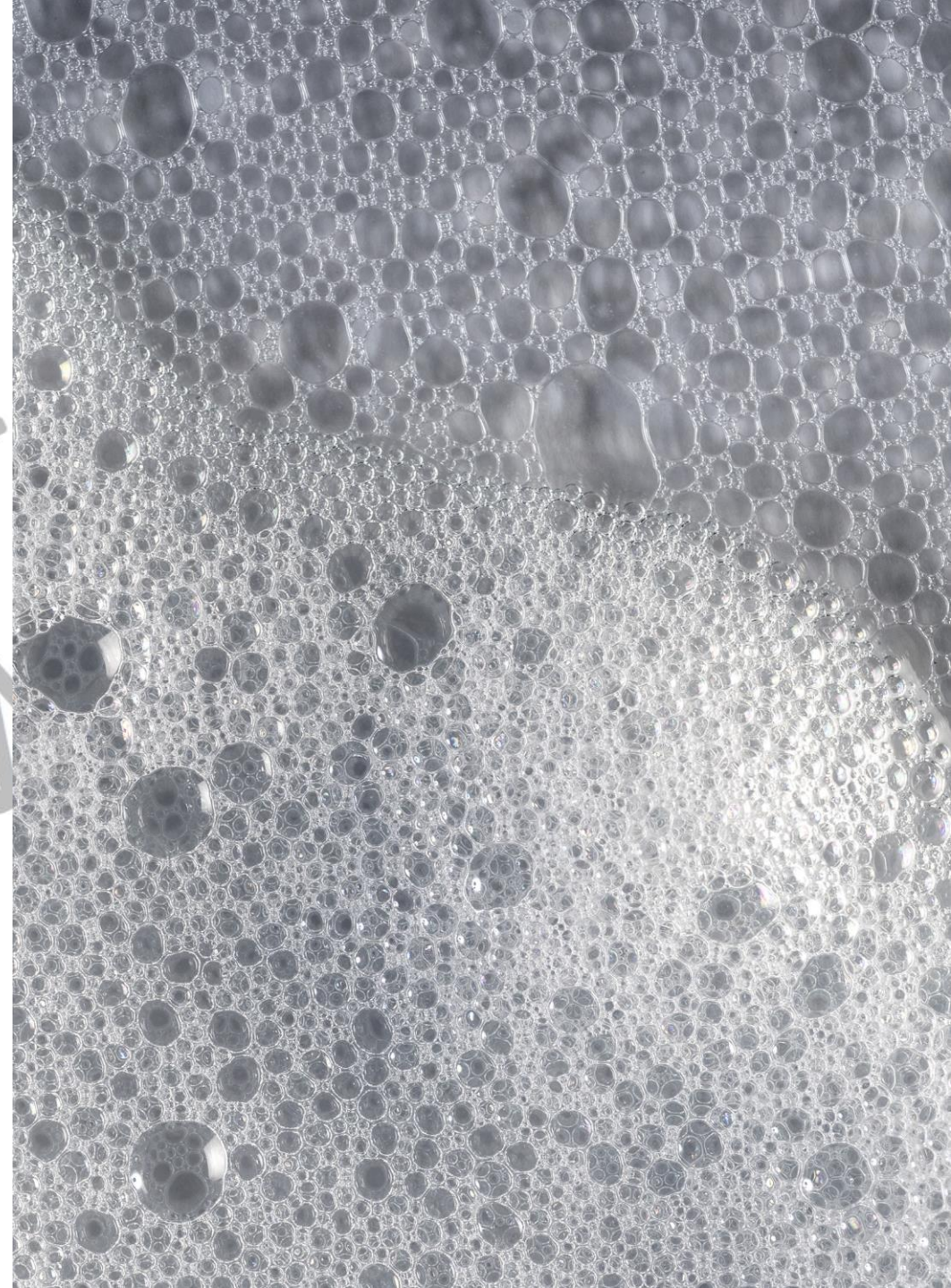
- Independent Institutes
 - NIST Standard Reference Materials (SRMs)
 - Coriell Cell Lines
- Manufacturers' Included Standards (e.g., 007, 2800M, 9947A, 9948, K562, ISS, etc.)
- Molecular Biology Suppliers
 - OriGene



Preparing a surface for use

- Surfaces can be cleaned, disinfected and sterilized for use
 - Soap and water
 - 70% ethanol
 - 10% bleach
 - DNAZap
 - DNA OFF
 - DNA OUT
 - UV irradiation
 - Ethylene oxide
 - CIDEX

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Safety Data Sheets (SDS)

- Describe the handling safety, storage, transport, disposal, and chemical and physical properties of a product or chemical
- Can be downloaded from the manufacturer's website



Safety Data Sheets (SDS)



Information on the identity of the substance or mixture

Composition/ingredients

Personal protection equipment for handling

Handling and storage

First aid measures in case of exposure

Precautions in case of accidental release

Firefighting measures

Supplier details and contact

Physical and chemical properties

Stability and reactivity

Toxicological information

Ecological information

Disposal considerations

Hazards information including classification of the substance or mixture

Transport information and precautions

Regulator information

Accuracy

- **Accuracy** is the degree of conformity of a measured quantity to its actual (true) value.
 - Instruments and tools are calibrated using standards
 - Pipette performance is assessed using balances
- Record instrumental readings as output without rounding
- Ability to obtain correct result with standards, competency, and proficiency test samples



Reporting

- Requesting officer
- Officials of the court

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Documentation

- Track reagents lot and product used
- Case report results of analysis
- Contemporaneous by the person who performed the testing
- Should be complete
- Supporting information
- Retained and provided as required by law



Administrative Review

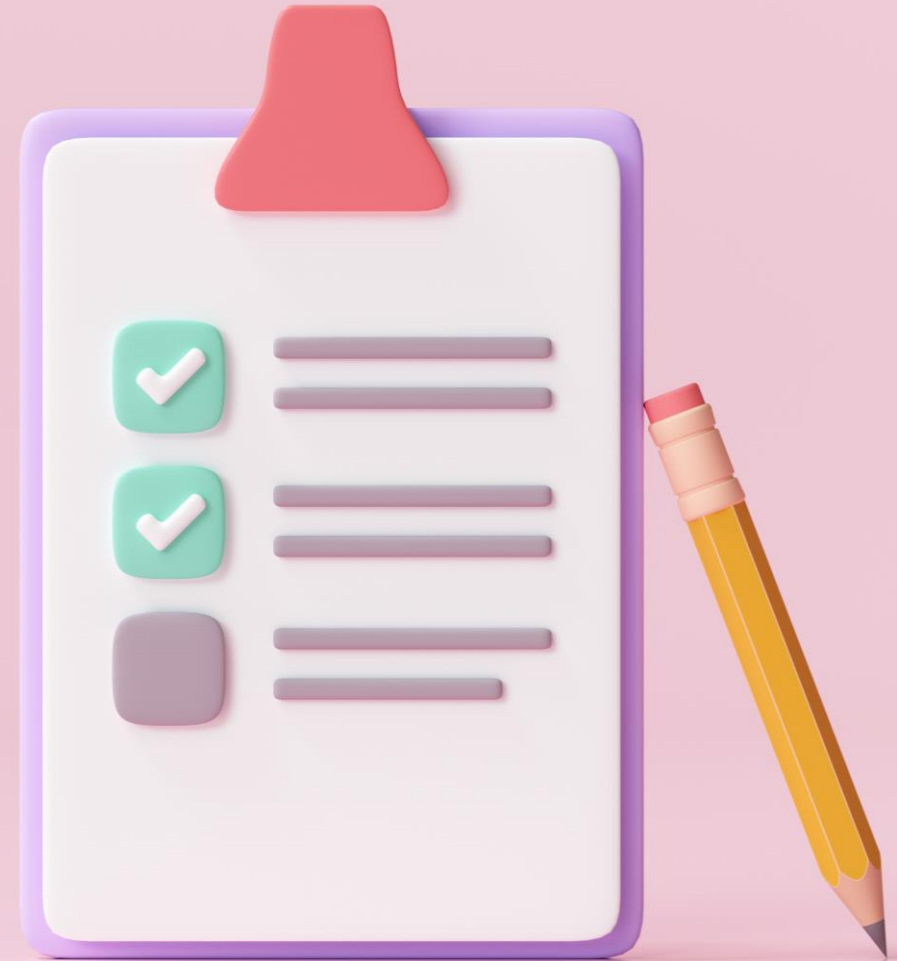
- **Review** is an evaluation of documentation to check for consistency, accuracy, and completeness.
- Evaluation of lab data, documentation, and reports for accuracy and consistency with lab SOPs
- Checks for editorial correctness



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Technical Review

- Evaluation of reports, notes, data, and other documents to ensure there is an appropriate and sufficient basis for the scientific conclusions.
- Ensures that conclusions are concordant with data/results obtained.



QMS

- Most forensic DNA laboratories have a quality assurance (QA) program to ensure quality testing.
- Key elements of a well-designed QA program aim to ensure samples were protected from contamination and handled by trained personnel in accordance with documented SOPs etc.
- The FBI provides guidelines for laboratories conducting forensic DNA testing and convicted offender databasing.



Accreditation

- Signifies the quality of the lab and work product and that specific standards are followed.
- Credible
 - Structure
 - Consistent
 - Valid
 - Reviewed
 - Grounded in science
- Legally defensible

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Non-Conformance Issues



- Case registration
- Case management (within and between departments and agencies)
- Contamination
- Interpretation
- Reporting
- Security
- Other

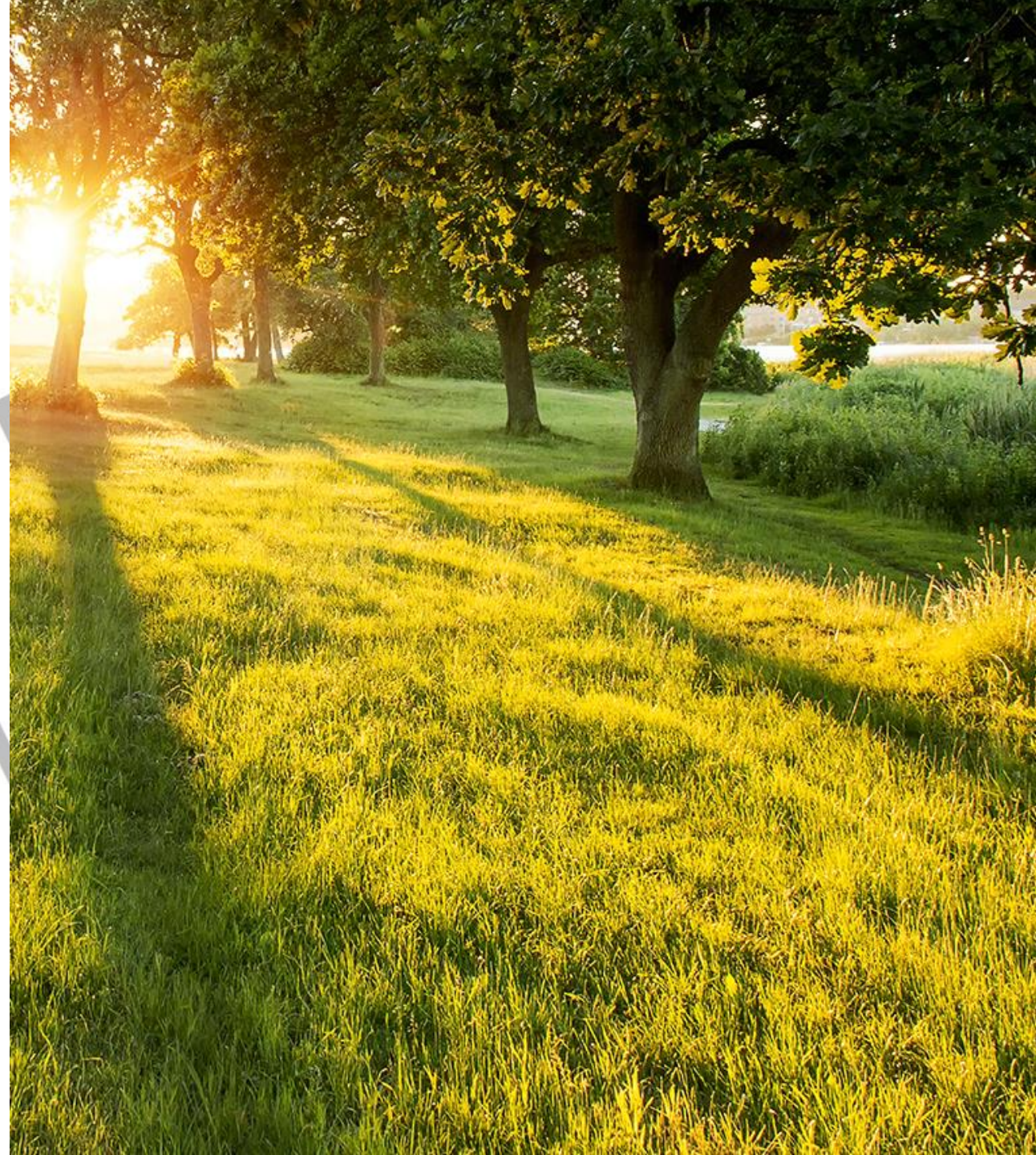
Quality Control Issue Examples

A large group of sheep, mostly white, with one black sheep standing out in the center. The sheep are in a field, and the background is slightly blurred.

- Extraction negative is contaminated and DNA is detected in quantitation or peaks in CE post-amplification
- DNA extract quantities are low for buccal swabs or reference samples
- Suspect sample profile is a mixture

Non-Conformance Reporting & Correction

- Non-conformance is any aspect of testing, result or analysis that does not conform to SOPs or agreements with the customer.
 - Training, consumables, reagents, instruments
- May be escalated to a corrective action
- A corrective action deals with errors as they occur and to prevent reoccurrences
- Improve practice
- Solve and remediate issue
- Create and institute new policy to avoid issues in the future
- Accreditation requirement



Non-Conformance Reporting & Correction

- Evaluate risk and significance of error or issue
- Determine how the error or issue occurred
- Log errors and issues
- Communicate and discuss



Maintaining Quality

- Curiosity to solve problems encountered
- Professional development and continued learning
- Ask questions
- Provide input to supervisors and managers



Study Questions

- Define quality control.
- What is the purpose of a positive control?
- What is the purpose of a negative control. Give some examples of negative controls.
- What is an elimination sample?
- Know how you should report a nonconformance issue in your lab.

Suggested Reading

- FBI, *Quality Assurance Standards for DNA Databasing Laboratories*, effective July 1, 2020.
- FBI, *Quality Assurance Standards for Forensic DNA Testing Laboratories*, effective July 1, 2020.
- 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 171, Best Practice Recommendation for the Management and Use of Quality Assurance DNA Elimination Databases in Forensic DNA Analysis. 2024. 1st Ed.
<https://www.aafs.org/asb-standard/best-practice-recommendations-management-and-use-quality-assurance-dna-elimination>