Quality Control

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Background

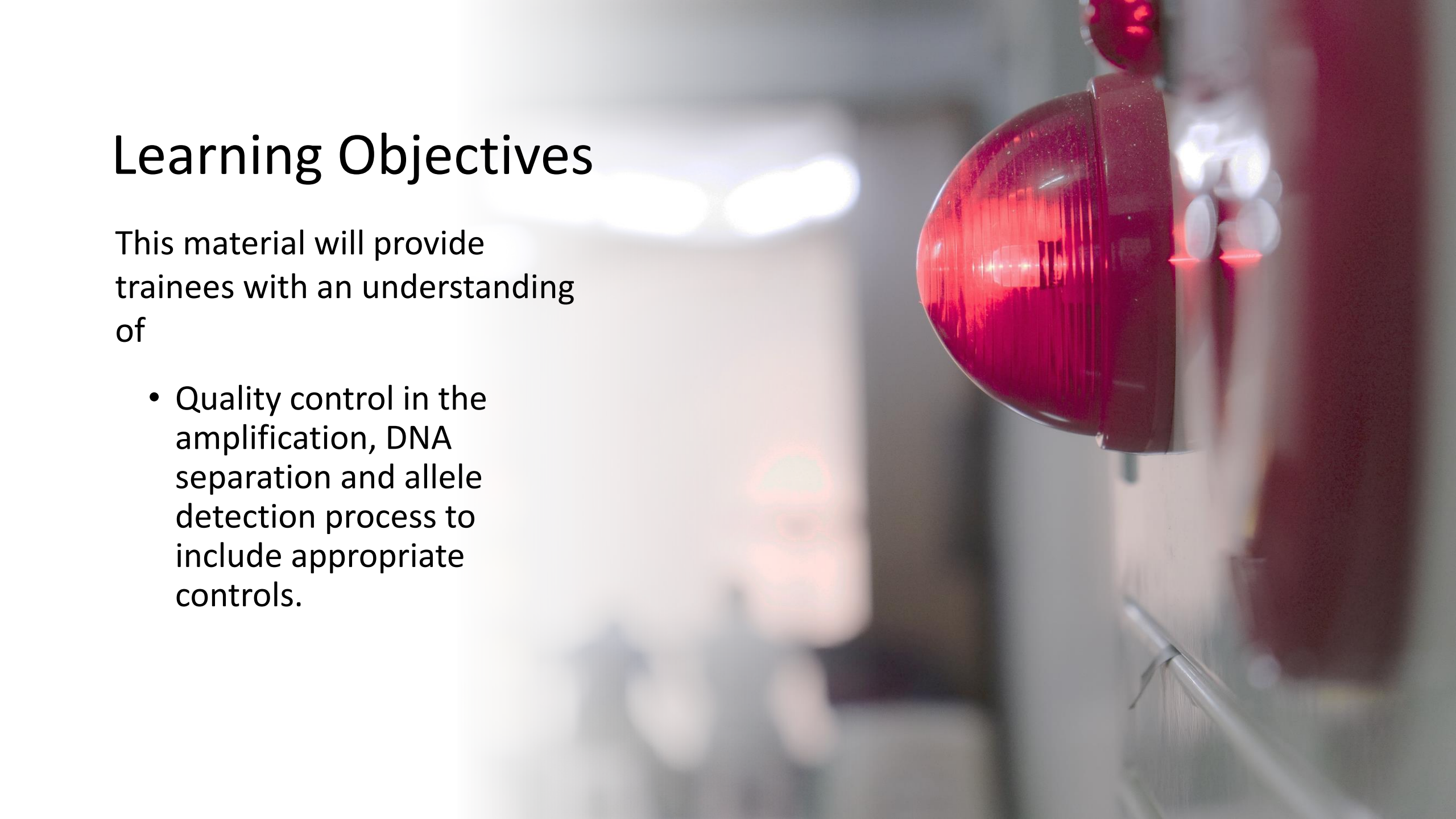
In October 2023, Towson University was awarded a cooperative agreement from NIST to develop a standardized DNA training curriculum for the United States that addresses the components in ANSI/ASB Standard 115, *Standards for Training in Forensic Short Tandem Repeat Typing Methods Using Amplification,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

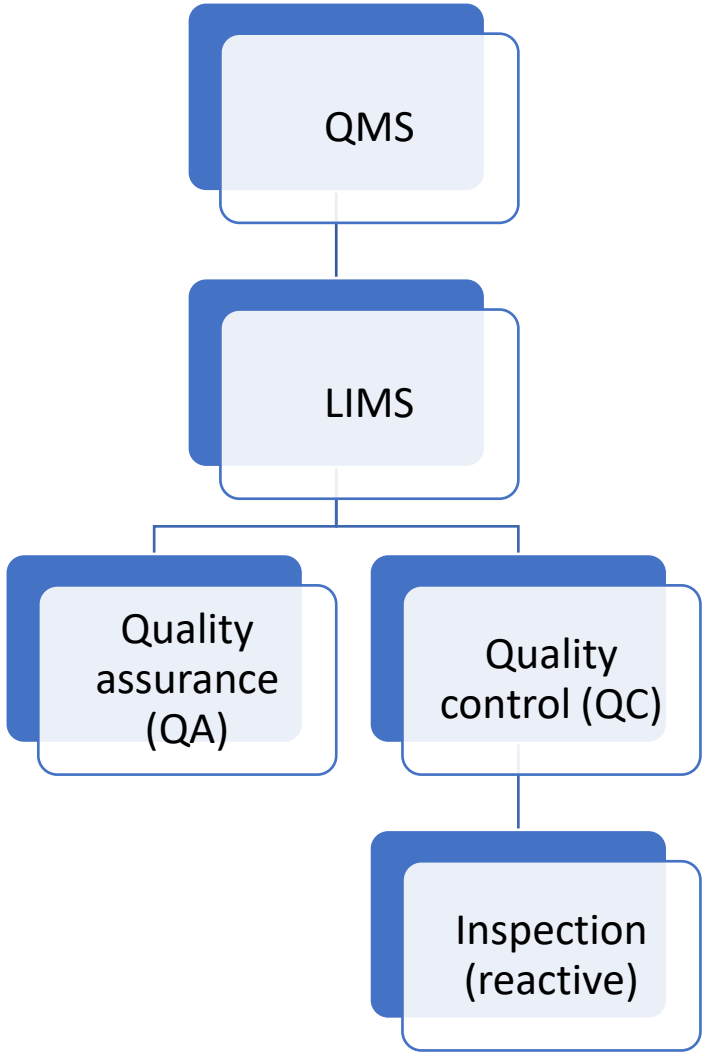
Learning Objectives

This material will provide trainees with an understanding of

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



Quality Management System (QMS)



An Overview of Quality Management Principles

- Process Approach
- Evidence-based decision making
- Leadership
- Engagement of people
- Improvement
- Relationship of management
- Customer focus

Features of a QMS

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

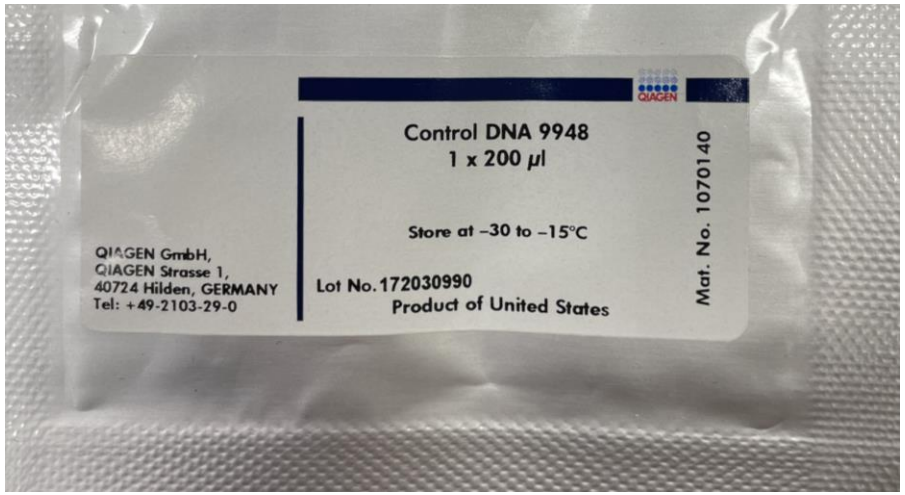
- Under the direction of the Technical Leader
- Reviewed annually
- Training is reviewed and approved
- Proficiency testing is used
- Assure data quality
- Interpretation guidelines
- Mixture interpretation guidelines

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
  - Well-characterized
  - Can be incorporated into validation processes



QA

- Ensure that all functions perform as intended
- Review of education and training to meet legal and professional requirements
- Technical Review of casework and testimony
- Coordinate and review proficiency testing, accreditation and audits
- Lead remediation efforts
- Uncover and mitigate process issues (data-driven)

Use of Controls, References and Standards

NECESSARY TO INTERPRET DATA

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

Overview of the DNA Typing Process and Controls

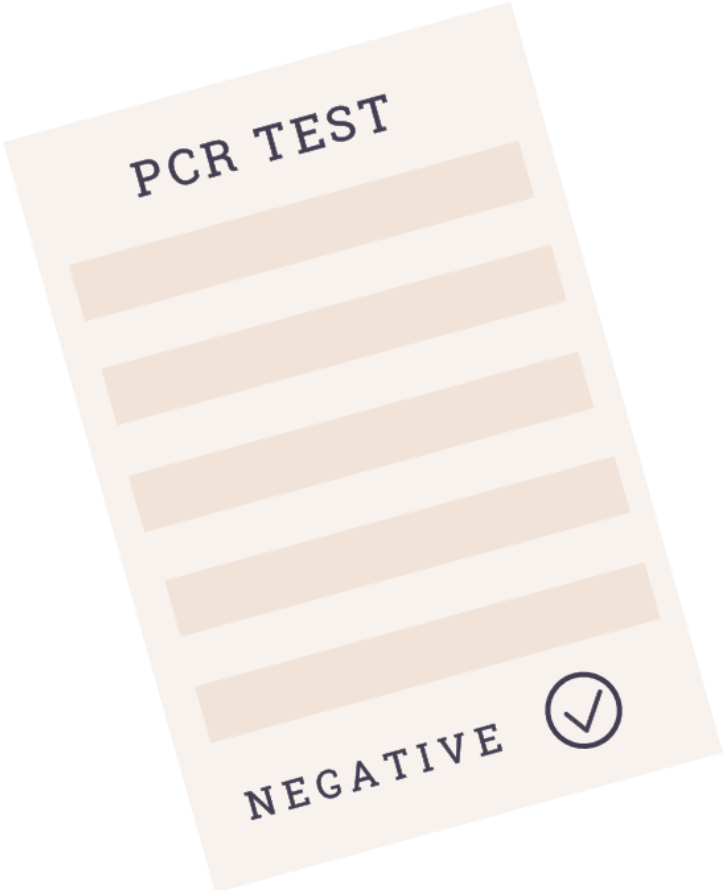
Sampling	DNA Extraction	DNA Quantitation	STR Fragment Analysis
• Evidence sample • Reference sample • Elimination sample	• Positive Control • Substrate control / NTC • RB	• Quantitation standards • Positive Control • RB	• Positive Control • NTC / RB • Elimination Sample • Allele ladder • ILSS

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

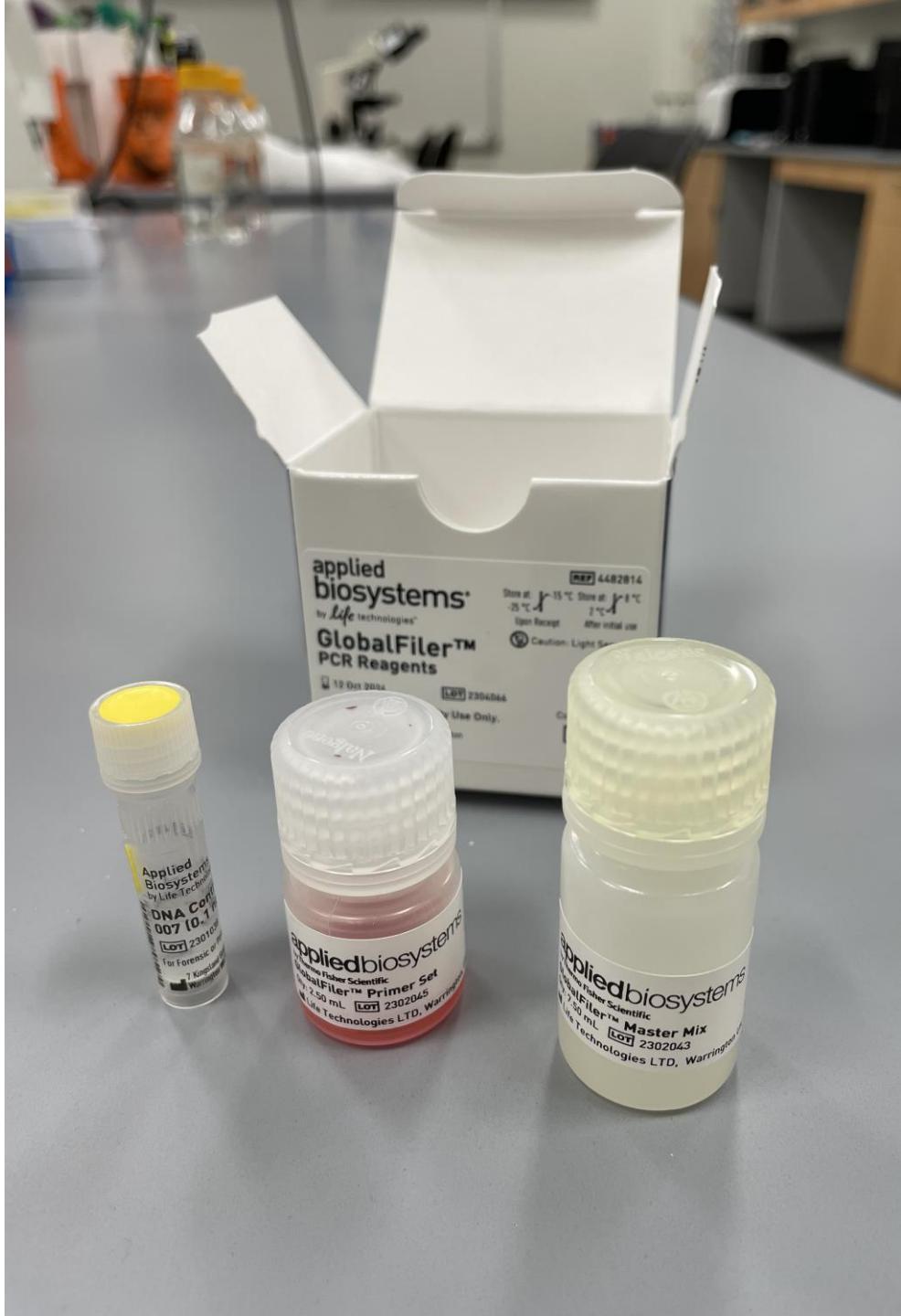


Reference Material and Known Samples and Standards

- **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.

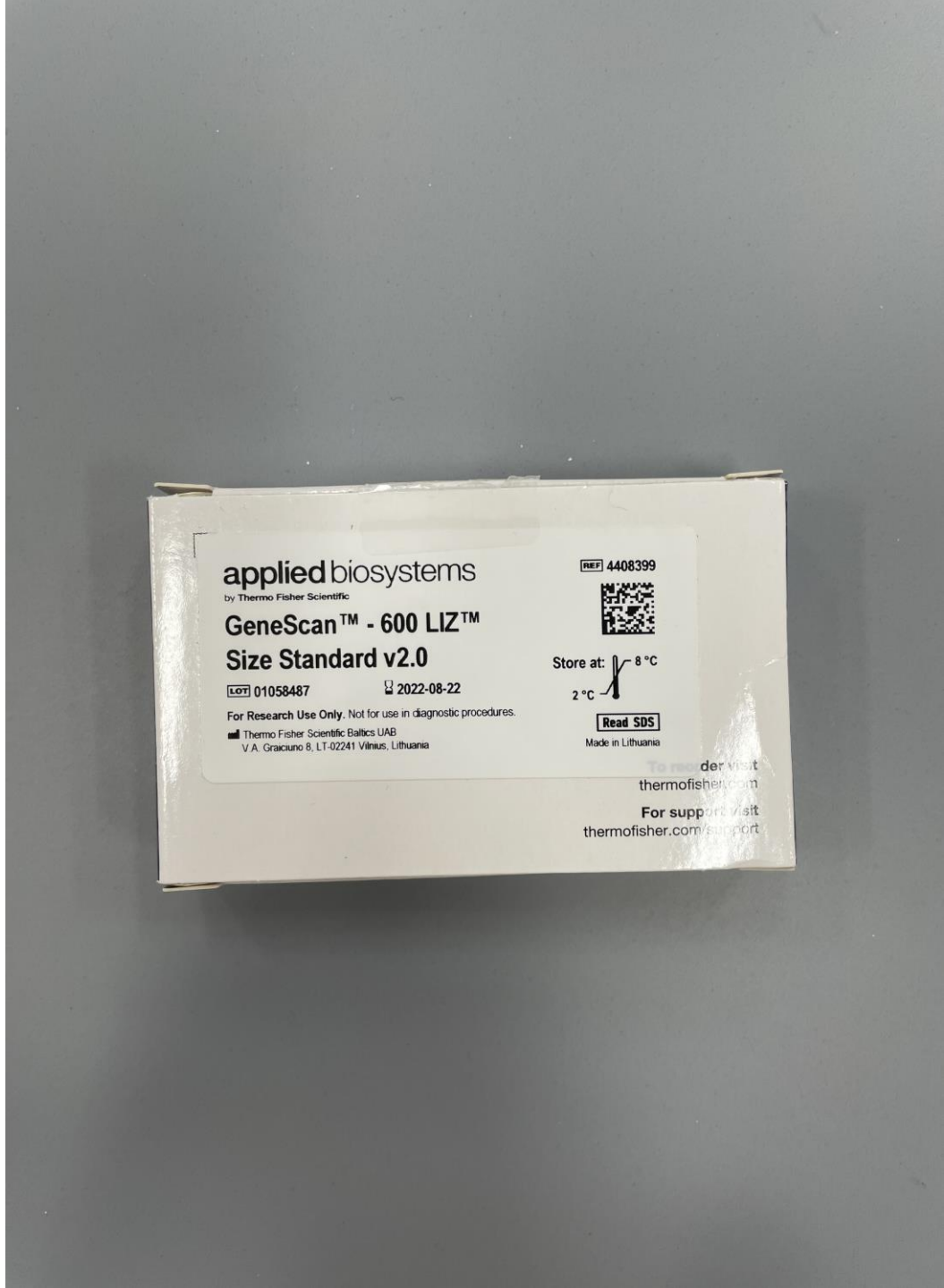
Quality Control Standards

- Use standard control DNA included in the kit or purchased separately (e.g., 007, K562, 9948, 2800M) for quantitation and amplification controls
  - Generate high quality DNA quantitation and typing results
  - Known profile
  - Reproducible
  - Process each amplification set with proper controls
  - Each batch run must include at least one correct positive control.
  - Can be used for troubleshooting if a non-conforming result is detected



Quality Control Standards

- Internal lane size standard (ILSS) or internal size standard (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.

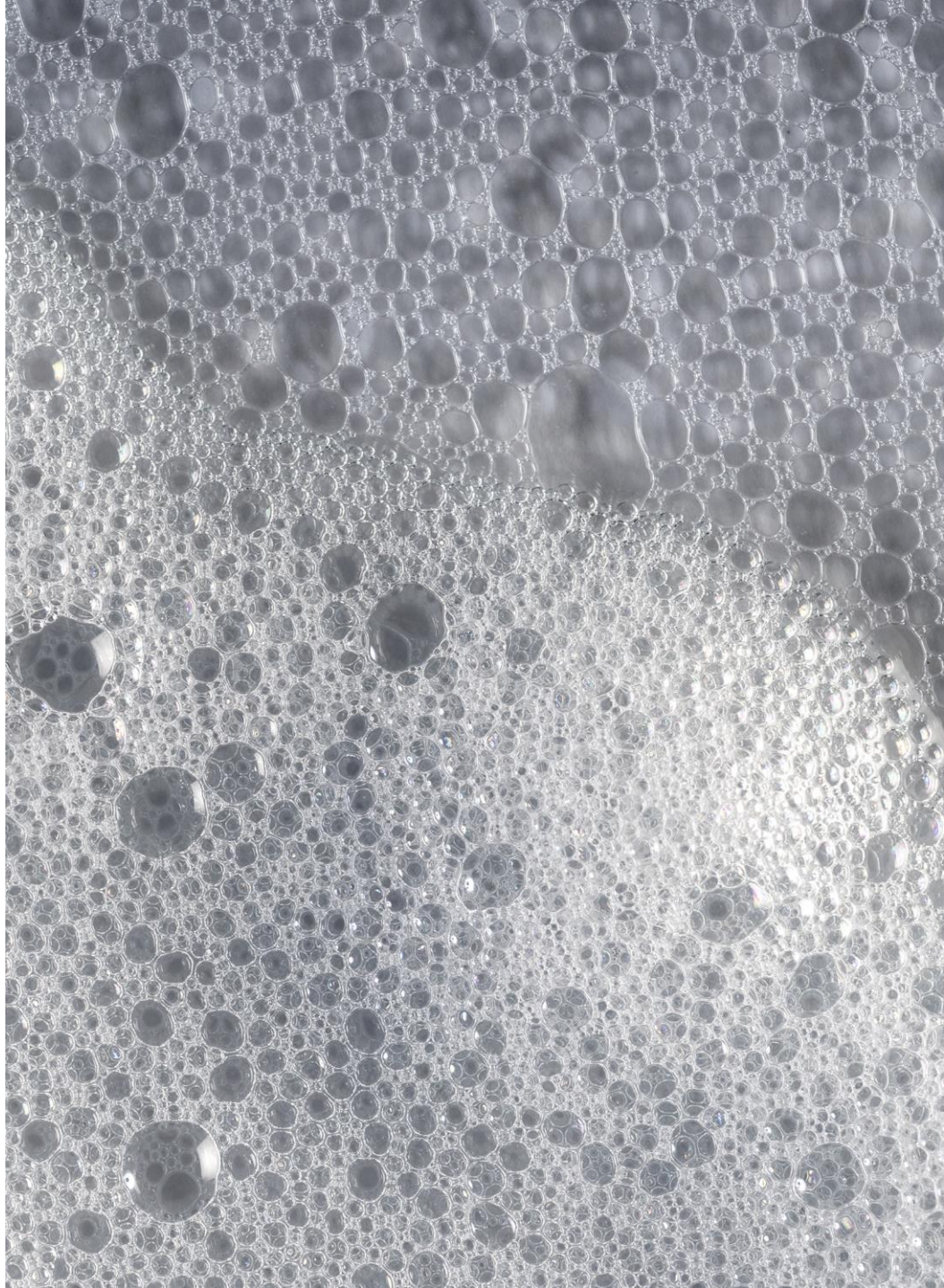


Sources of Controls and Standards

- 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
  - Ethylene oxide
  - CIDEX
  - UV irradiation



Batch Processing

- Exemplars and evidence must be handled separately (never placed on the same tray)
- Can run on the same instrument, but on separate plates (i.e., separate runs)
- Process each amplification set with controls.
- Use correct documentation and ensure set-up is witnessed.
- Controls must match or exceed injection sensitivity used for samples.
- Stagger incubation by ~1 hour if running multiple batches in a day.

Instrument and Computer Maintenance

- Handle instruments gently and keep clean.
- Monitor performance early to detect issues (e.g., leaks, bubbles, calibration).
- Defragment hard disks and delete non-essential files to improve system performance.
- Report any issues to Quality Assurance.

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
- Ability to obtain correct result with standards, competency, and proficiency test samples
- Record instrumental readings as output without rounding

Reporting Data

- Requesting officer
- Officials of the court

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

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

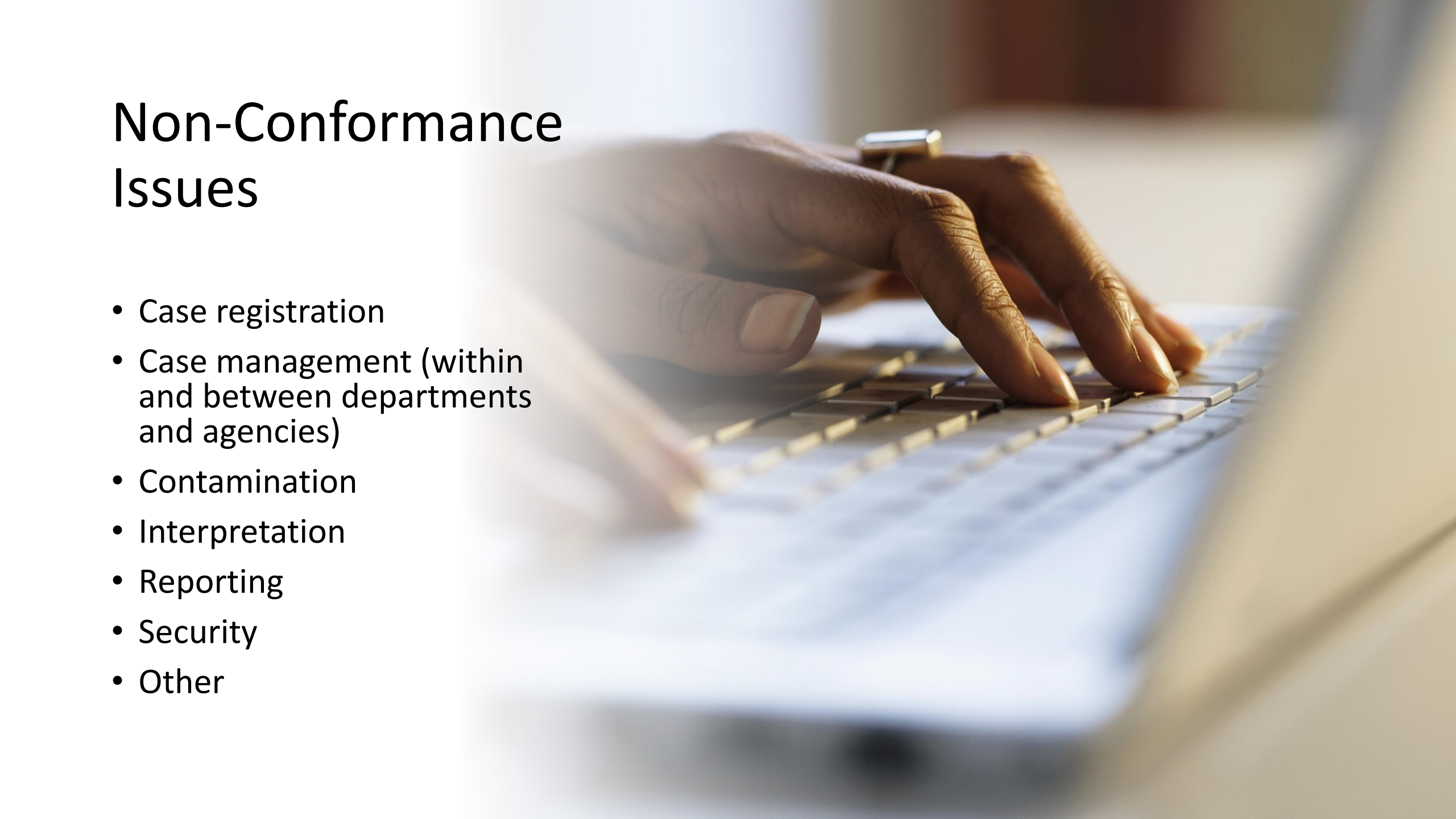
- Most forensic DNA laboratories have a quality assurance (QA) program to ensure quality testing.
- Key elements of a well-designed QA program aim to ensure samples were protected from contamination and handled by trained personnel in accordance with documented SOPs etc.
- The FBI provides guidelines 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 documented to be followed.
- Credible
  - Structure
  - Consistent
  - Valid
  - Reviewed
  - Grounded in science
- Legally defensible

Non-Conformance Issues

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

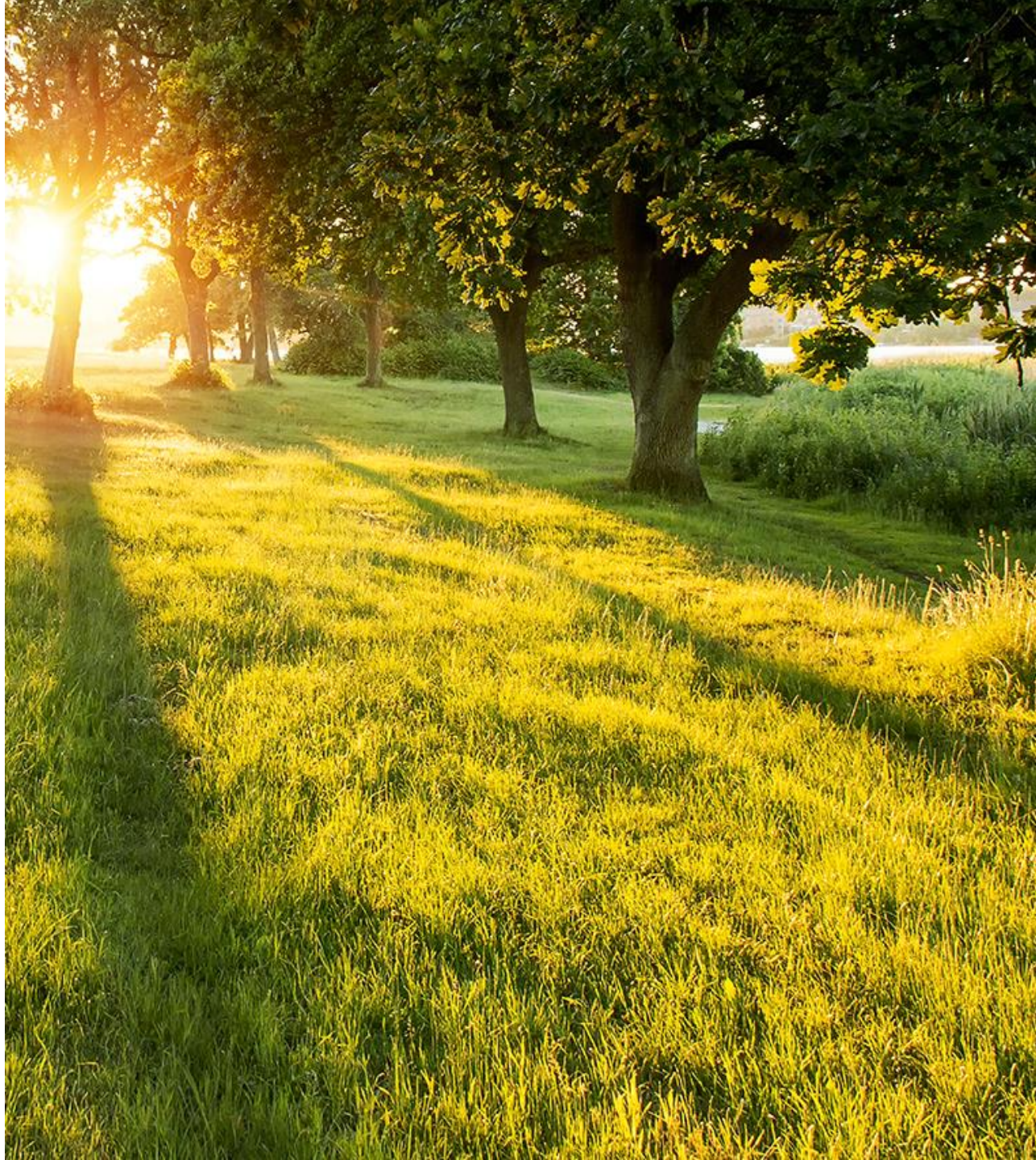


Quality Control Issue Examples

- 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 the significance of an 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 the difference between a background test, a NTC and a negative control?
- What is an elimination sample?
- Know how you should report a nonconformance issue in your lab.

Suggested Reading

- 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
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