

Molecular work flow & Best Practice (MBP)

Laboratory Environment and PCR Workflow

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Objectives

Upon completing this section, the trainee should be able to:

- Understand the influence of the laboratory environment on PCR quality
- Understand the important aspects of PCR laboratory environment and workflow

Introduction

Classification of Contamination in PCR

Development of the Roche PCR workflow

The Uracil-N-glycolysase

Contamination transmission

Contamination prevention

Introduction

- Routine PCR is a very reliable and sensitive method in diagnostics.
- Multiple factors will influence the quality and accuracy of PCR results
 - Target
 - Performance of the assay, in particular primer and probe design
 - Reliability of instruments and software
 - Quality of reagents
 - Purity of nucleic acids.
- These factors are optimized in our assay and are RMD's key competence.
- **What is the support organization's responsibility?**
 - **We need to ensure optimal instrument and operator performance.**
 - **We need to optimize the PCR laboratory beyond our systems.**

Definition of PCR Workflow *In Molecular Diagnostics*

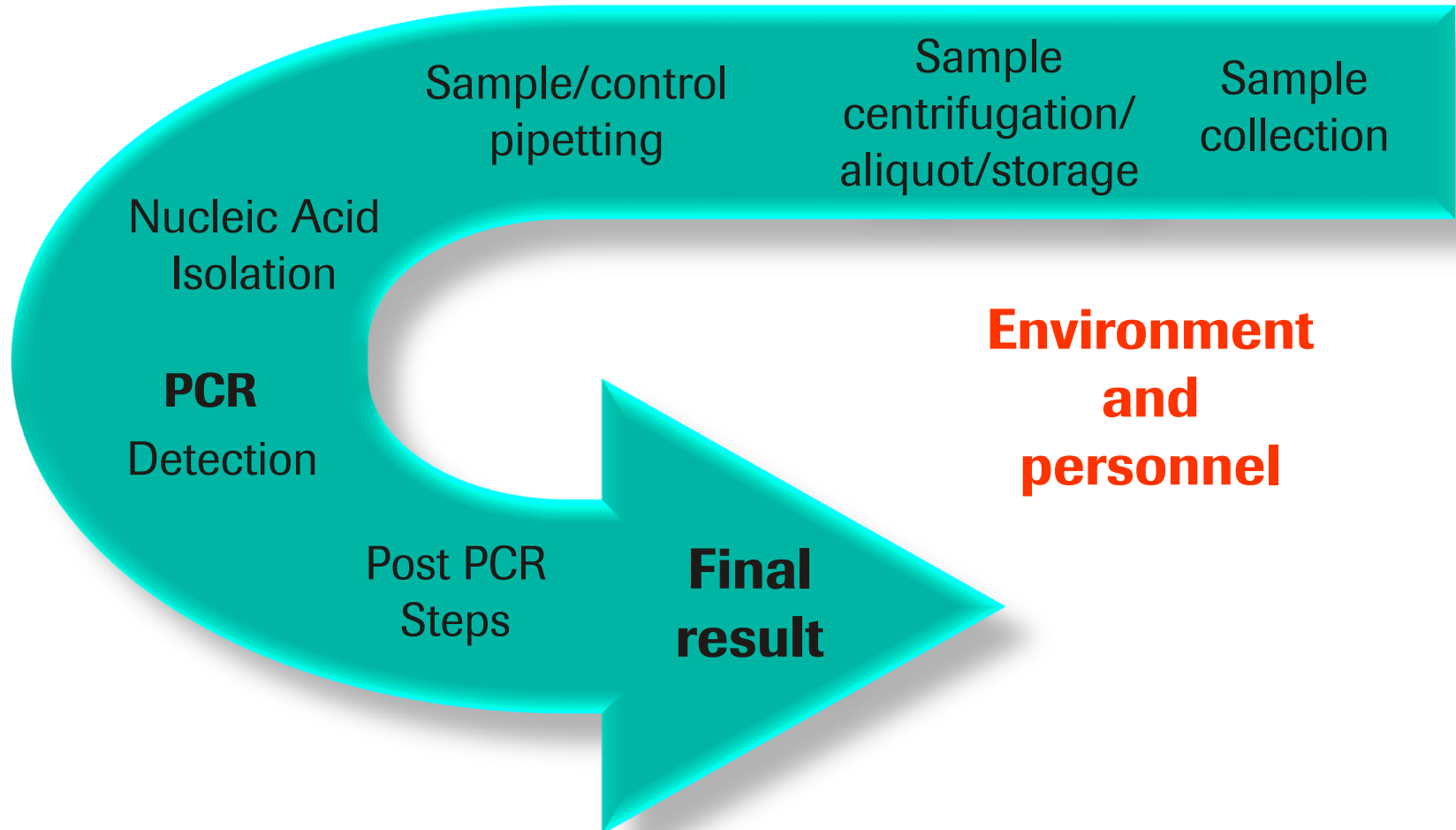


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Best Performance



Influences on PCR performance

- Influences on PCR performance can be grouped in 3 categories:
 1. System-related
 2. Operator-related
 3. Environment-related

- Environmental influences usually equates to ***contamination***

- What is contamination in the context of PCR?

- And what can we do to prevent it?

Classification of Contamination in PCR

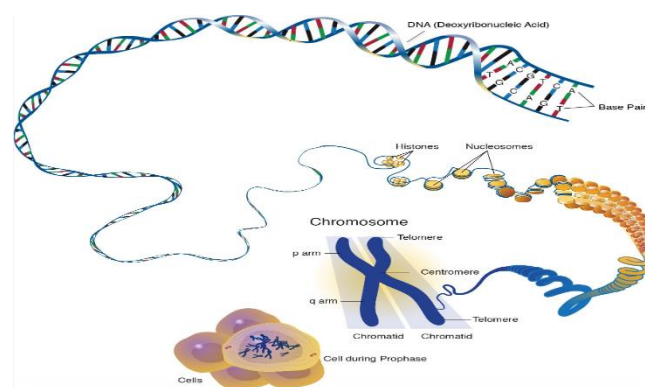
- Contamination leading to ***false positive results*** (specific nucleic acids)
 - Crossover contamination = via native sample/control
 - Carry-over contamination = via PCR products (amplicon)

- Contamination leading to ***Inhibition***
 - Endogenous
 - Exogenous

Inhibition of PCR by endogenous substances

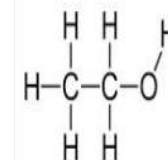
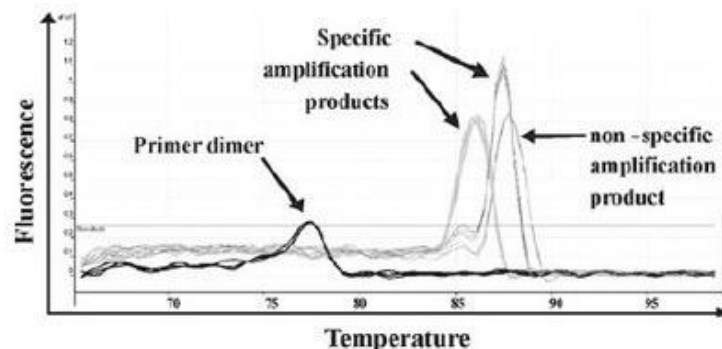
Native in the patient sample, depending on the material:

- Viral DNA, RNA (multiple infections)
- bacterial/fungal DNA
- Human DNA; RNA
- Lipids
- Medication
- Heparin
- Urea
- DNAses/RNAses/Proteinases
- Polysaccharides
- Heme / hemoglobin / myoglobin / immunoglobulin G
- ...



Inhibition of PCR by exogenous substances

- **Nucleic acids**
 - primer dimers
 - primary extension products
 - PCR products/side reaction products (HMWP)
 - genomic DNA
- bacterial/fungal metabolites
- dust/soot/pollen, etc.
- alcohols (Ethanol, Isopropanol, etc.)
- DNAses, RNAses, proteinases, etc.
- collagen/melanin from hair or skin
- silicon based grease
- ...



Ethanol



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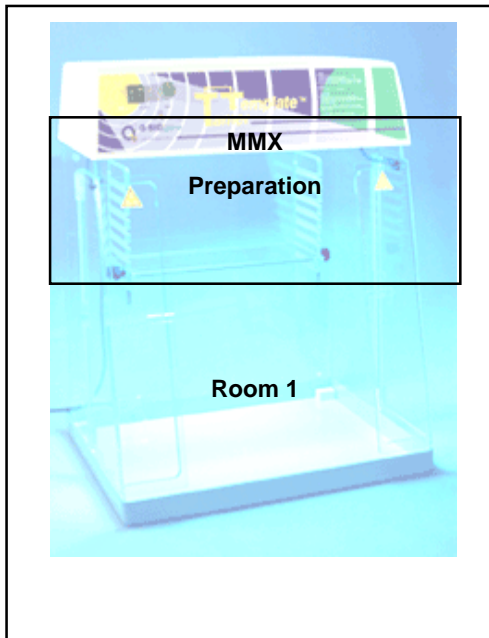
Contamination prevention

How did we deal with contamination in the past?

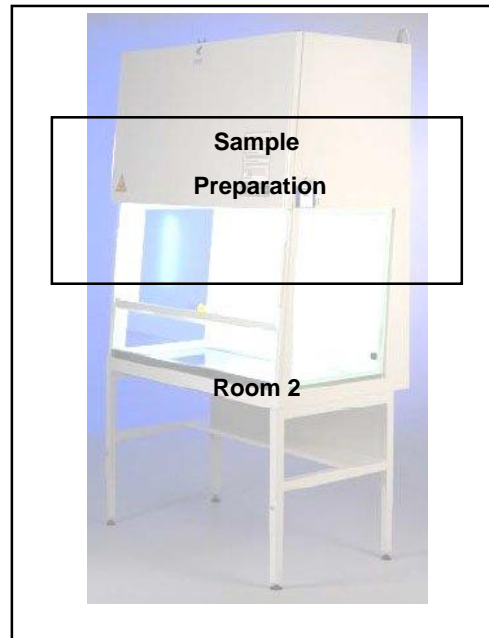
In the AMPLICOR® world

Preventive measures against contamination

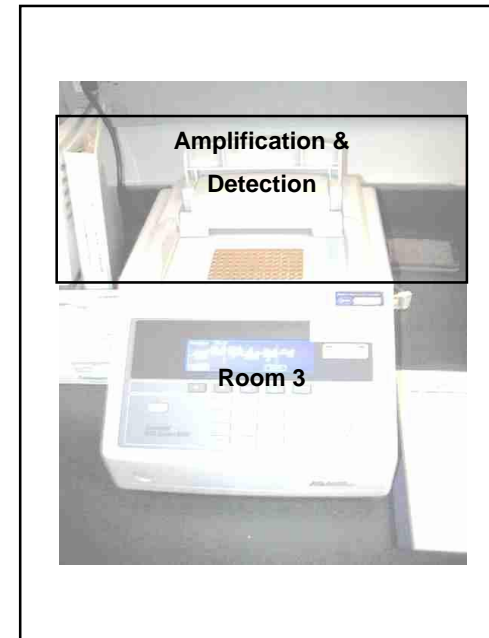
- 3 room concept and unidirectional workflow
- Clean everything before and after with fresh bleach
- UNG / Uracil-containing amplicons



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Global Customer Support

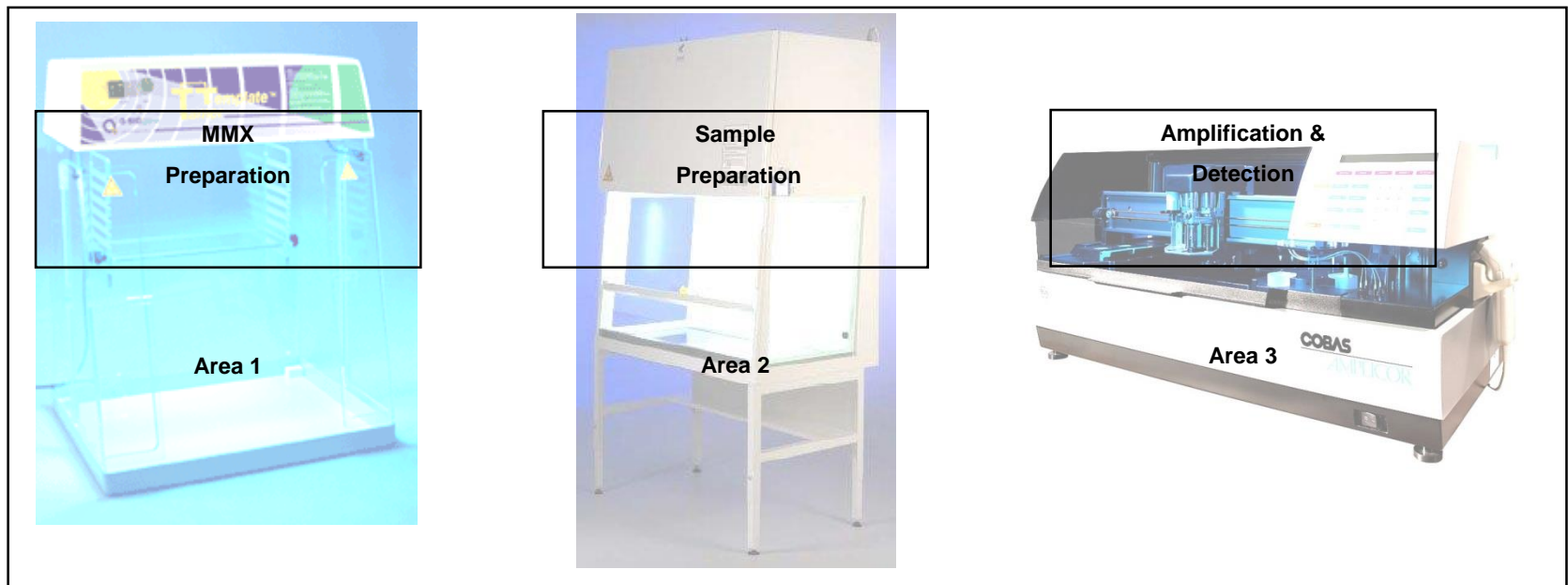


Laboratory Environment and PCR Workflow

In the COBAS® AMPLICOR® world

Preventive measures against contamination

- 3 area concept and unidirectional workflow
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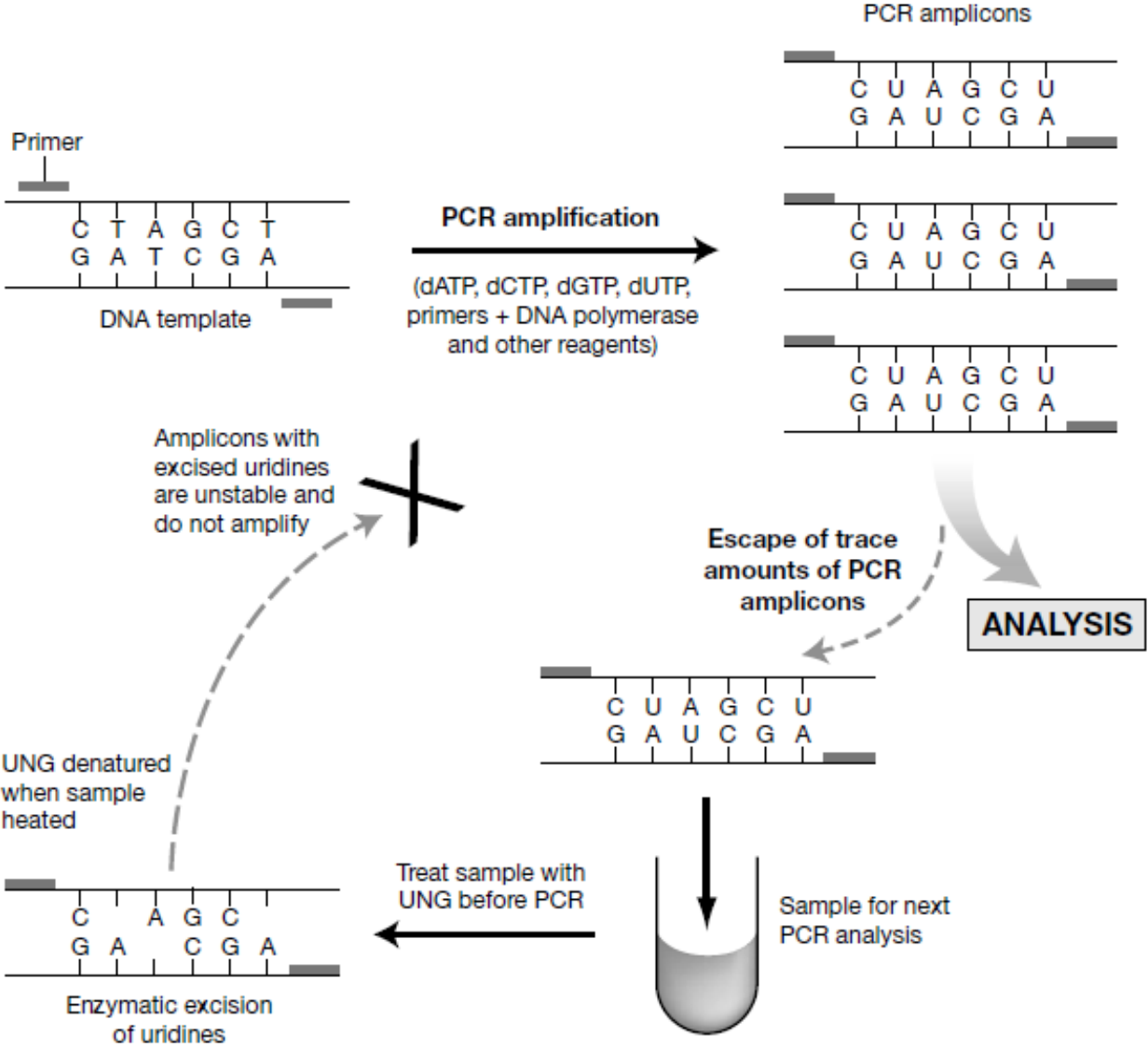
Contamination transmission

Contamination prevention

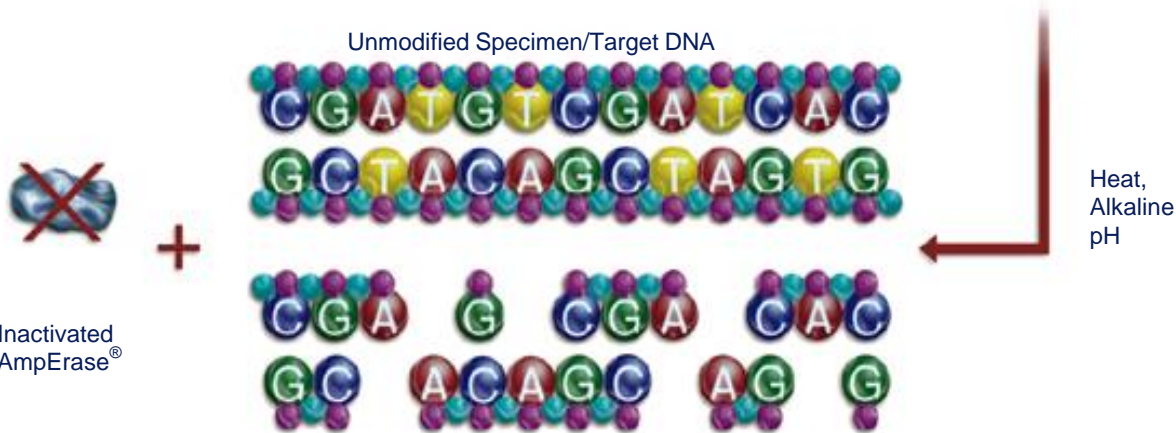
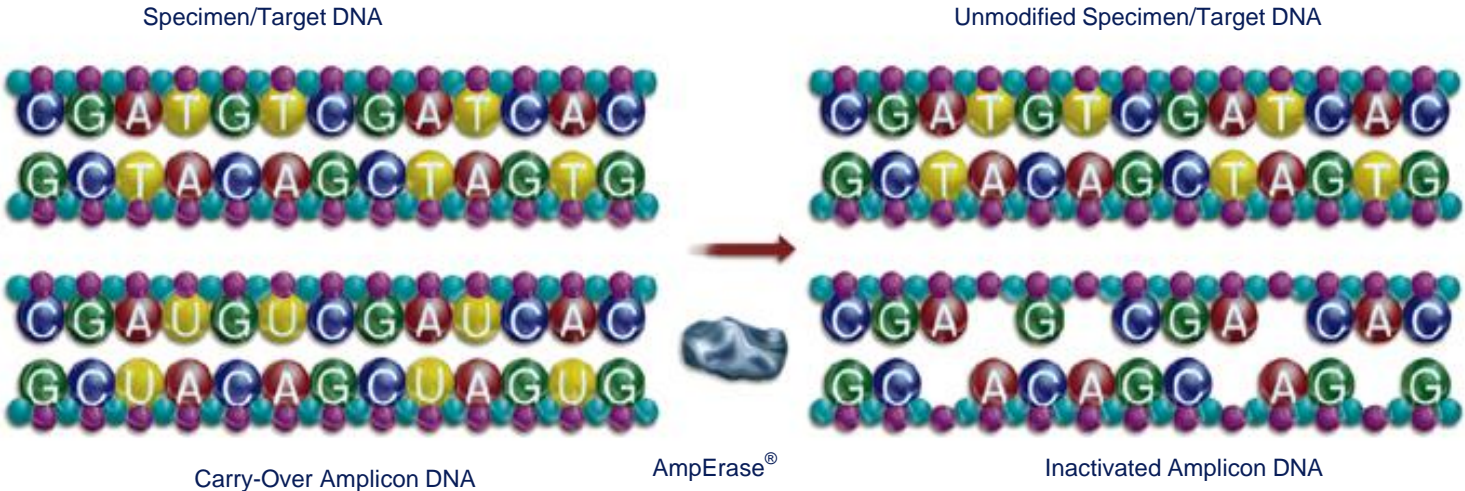
UNG / AmpErase

- Uracil-N-glycosylase is an enzyme capable of recognition and removal of uracil-residues from DNA
- The DNA strand containing abasic sites is subsequently destroyed by breaking the sugar-phosphate backbone
- The efficiency of AmpErase is assay and target dependent:
 - TaqMan HIV-amplicons in the 10^4 range
 - TaqMan HCV amplicon in the 10^5 range
 - Certain unspecific/side products from PCR-reactions might be cleaved very inefficiently (e.g. high GC-content, high secondary structure)

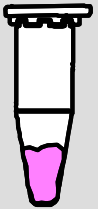
Functionality of AmpErase (UNG)



Functionality of AmpErase (UNG)

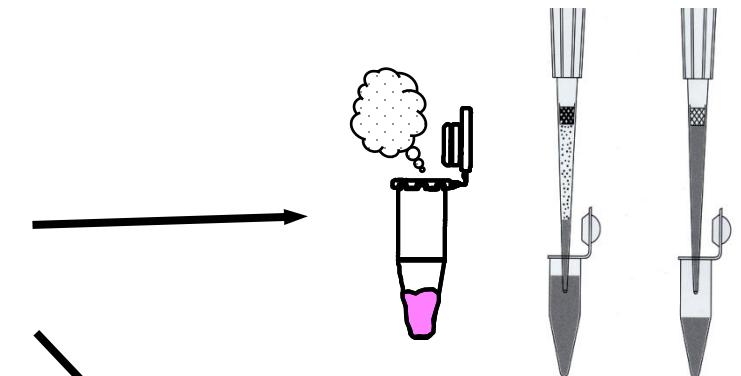


Carry-over contamination by amplicon



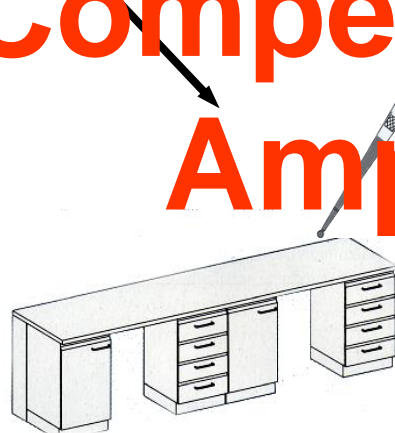
**100 μ L PCR
contains 1×10^9
Amplicons
(1,000,000,000)**

**Compensated by
AmpErase**



If / when reaction tube is opened aerosols are created.

These Aerosols can contain up to **1×10^3 Amplicons**



1 μ L PCR-reaction (10^7 Amplicons)
Is equally distributed on a surface of 100 m².

1 cm²

On the area of 1 cm² are still **10 Amplicons**

The Transfer of **10 Amplicons** is more than sufficient for a **false-positives** result !

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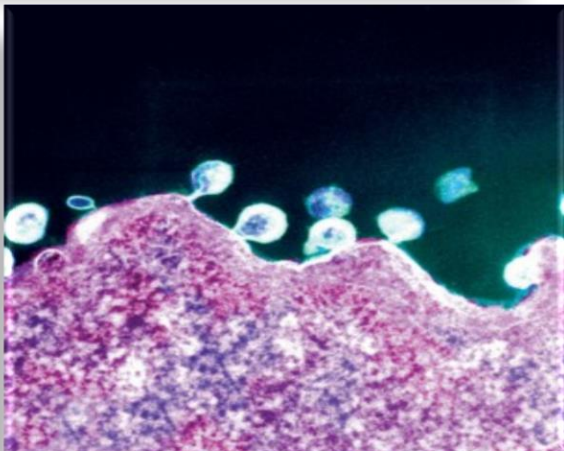
Contamination prevention

Contamination is the most likely way of introducing unwanted material

2 major pathways exist



Via physical contact (most likely human touch)

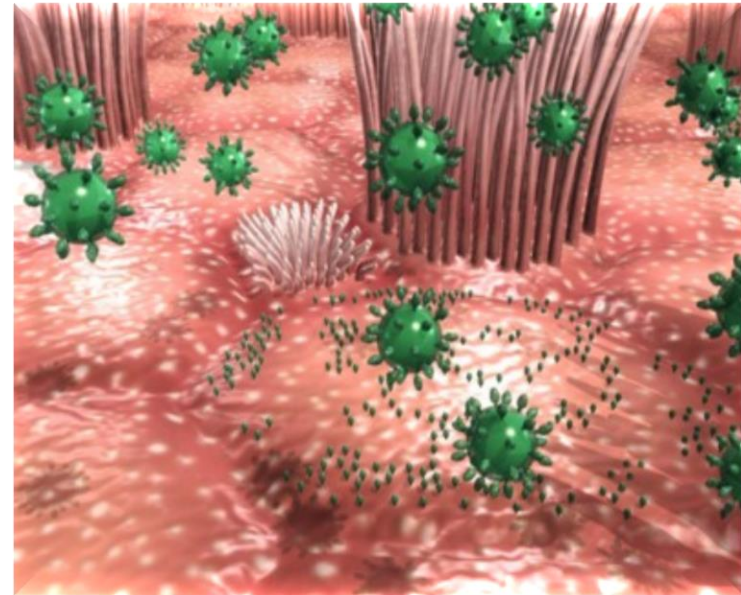


As aerosols or piggyback on other particles

Possible transmission pathways

By human interaction/contact

- Pipettors
- Primary tubes
- Waste containers
- Centrifuges
- Vortex
- Surfaces in safety benches
- Workspace
-



Possible transmission pathways II

By human interaction/contact

- Gloves
- Door handles (also for refrigerators)
- Keyboard and Computer mouse
- Sink, water tap, faucet
- Benches
- Floor
- Insufficient personal protection (skin, hair, etc)
- Maintenance/other tools

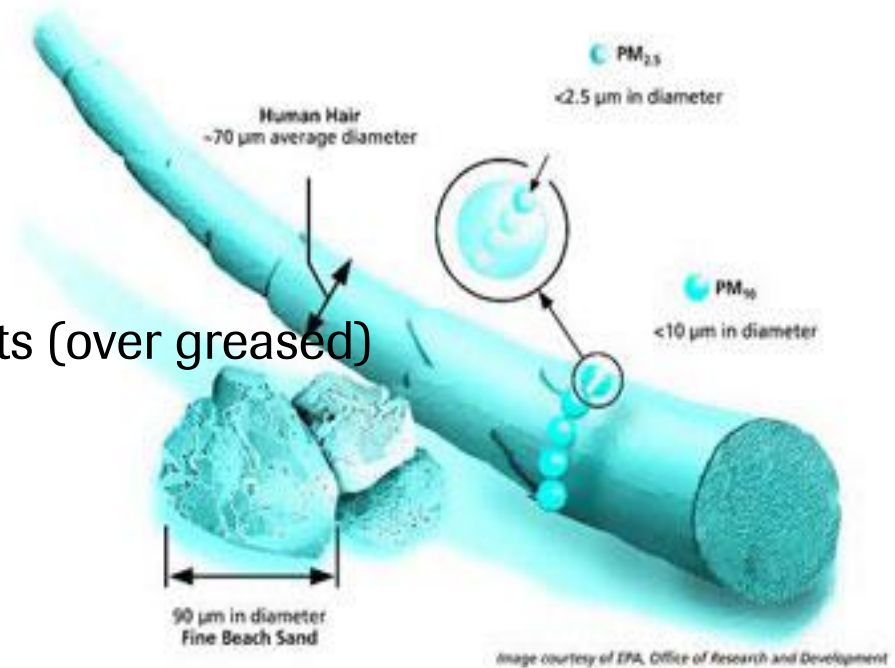


Possible transmission pathways

Without human involvement

Aerosols and particles

- By stirring the air
- Source of particles (dust):
 - Ventilation / A/C / Fans
 - Centrifuges
 - Moving parts in instruments (over greased)
 - Other instruments
 - Sweeping the floor
 - Spraying
 -



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What can we do?

**Eliminate all
(or at least the most prominent)
sources of contamination**

How do we do this?

1. Clean all the touching-hotspots
2. Clean surfaces where dust collects
3. Minimize the entry of particles
4. Minimize the creation of aerosols
5. Consult your customer in planning the PCR lab

Recommendations on cleaning

- REGULARLY clean all workspace areas (benches, equipment)
- REGULARLY clean **the floor**
- **Use a chemical that destroys nucleic acids (e.g. bleach) in addition to acting as a disinfectant (e.g. 70% ethanol)**
- REGULARLY clean the equipment (racks and carriers) and inside the instruments
- Be aware that gloves are a contamination protection only to the carrier, not to the environment
- **Use common sense, more cleaning is not always the answer!**
- Adapt certain instrument maintenance intervals (fan filters) to specific customer situation

Lab Design

Consult your customer in planning the PCR lab

- Follow local safety regulations
- Design cleaning and drying areas
- Design waste areas and choose the right size of waste bins
- Design storage area for disposables, reagents and equipment (e.g. racks)
- Plan for a separate sink to discard liquid waste
- Plan for robust door handles, surfaces, floor
- Plan room specific A/C with sufficient distance to the instrumentation

Doing now what patients need next