hogeschool Leiden

# PCR contamination issues

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TopLab

# What is PCR contamination?

- Contamination: target NA in PCR is derived from sample but….
- PCR amplifies all DNA targets to which primers do anneal, no matter the origin
- PCR is an extremely sensitive technique: every level of contamination of DNA target will be amplified
- Result: false positivity

# What can be contaminated in PCR and what are the consequences?

- Important issue: PCR mix contamination. What can be the cause?
  - 1)
  - 2)
  - 3)
  - 4)
  - 5)

# The contaminants

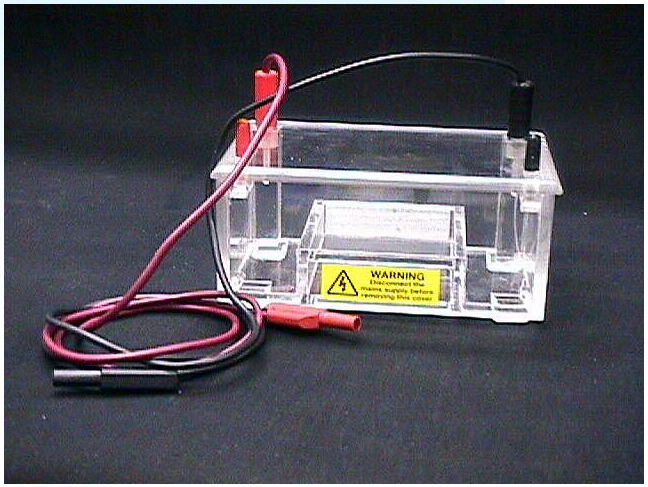
- Pathogens at the receptionroom for clinical samples
  - Bacteria, virusses, fungi, parasites
- Colonized personnel
- Vectors with inserts
- Cultured bacteria, virusses
- Amplicons from analysis lab
- Commensals (no pathogens)

# How arises PCR contamination?

- Transportation by airflow (aerosols)
- Stick onto material and equipment
- Contamination of solutions
- Transport by lab personnel (labcoat!!)

# Stick onto material and equipment

- pipets,
- homogenizers,
- Preparation needles, scalpels
- centrifuges,
- exsiccator,
- elektroforesis equipment,
- Gel-doc system
- (Dry)ice machine/incubator,
- waterbath,
- glassware



# Measurements to avoid contamination

- 3 categories
  - 1)
  - 2)
  - 3)

# Strategies to avoid contamination

- Systematic working according to GLP standards
- Keep everything clean (cloride, UV).
- Airconditioning (under-/overpressure)
- Close all bottles, reaction vessel
- Autoclave
- gloves
- Labcoats (special colored labels per room)

# Strategies to avoid contamination

- **Sterilitity (RNase/Dnase-free) of solutions**

- Autoclave (all relevant solutions (not from the kit))
- Water quality
  - UV or gamma-”irradiated” water
  - autoclaved
- Use as little as possible solutions
  - Eliquot and freeze

# Strategies to avoid contamination

- **”Ready to use" reagents/-solutions**
  - each PCR: pipet schedule
  - 10X stocks(bijv. 10X dNTP; 10X PCR buffer)
  - Combine: master-mix
  - Mark reaction vessels
  - Use filtertips (expensive, but a must!!

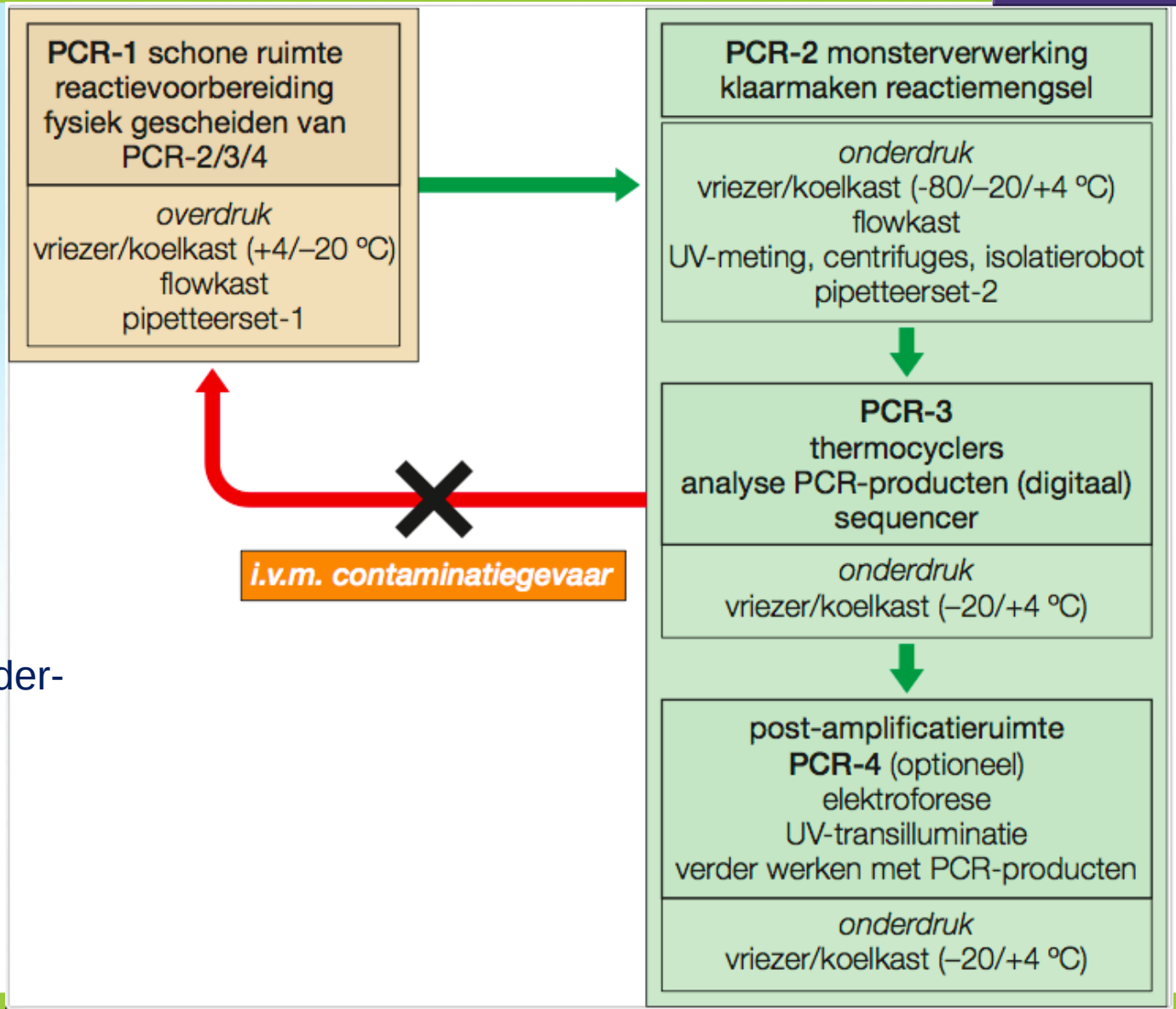
# Strategies to avoid contamination

- **Decontamination**
  - Lab tables, pipets, ice boxes, small lab equipment
    - Clean with 1N HCl (NA depurinization and hydrolysis); dry with ethanol
    - UV- or gamma radiation
- **Swipe tests**
  - Pre-determined frequency and -spots
  - All targets used for diagnostics

# Strategies to avoid contamination

- Work in physically separated labs
  - Allowable and not-admitted routes of materials, equipment and solutions (transport boxes)
  - Each lab has its own facilities

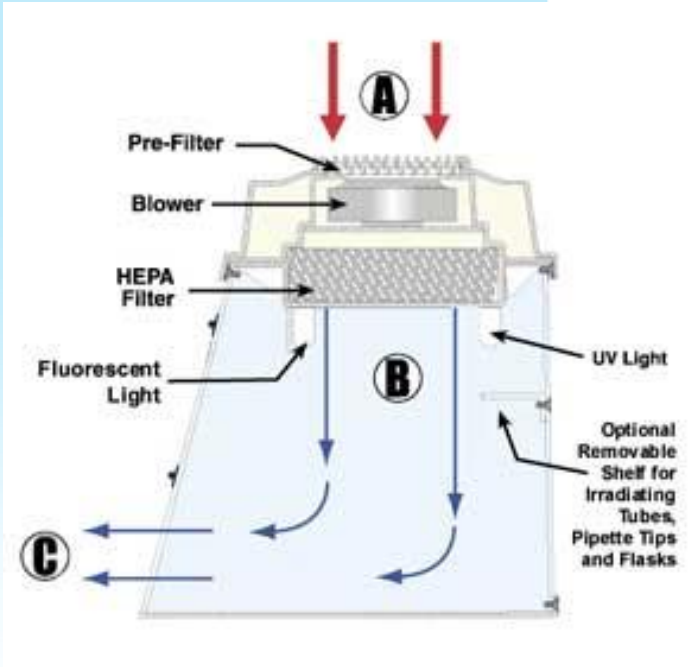
# Contamination-free working; physical separated labs



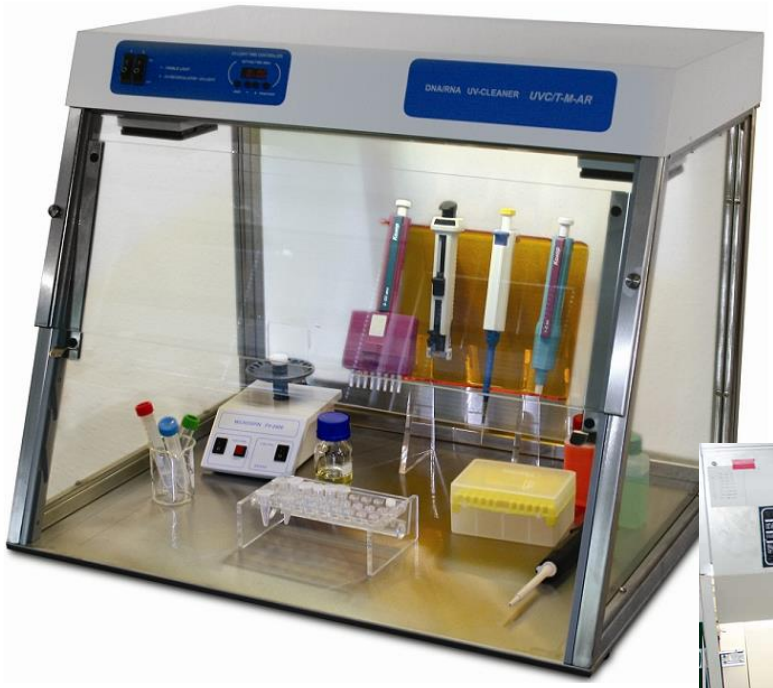

N.B. discuss the under- and overpressure

# PCR-1 “clean room”

- Storage of PCR reagents
  - 1)
  - 2)
  - 3)
- Preparation of master mixes
  - 1)
  - 2)
- Work in UV cabinet
- Keep everything clean (by yourself)
- Decontaminate routinely (UV, cloride)
- Gloves



# Do’s and dont’s; UV cabinet

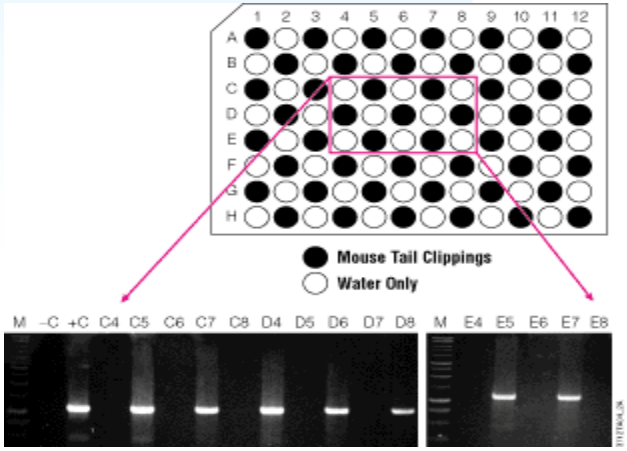




# Sample preparation

## PCR-2 (bio-hazard/flow cabinet)

- Acceptance and preparation of samples
- Work in cabinet and use an
  - NA extraction robot
- Minimalize sample-to-sample contamination risk
  - Closed tubes
  - No material outside lab
  - No amplification in this lab




contaminatietest

# PCR analysis (PCR-3 lab)

- Transport (closed) of PCR reagent/sample combi from PCR 2 to this lab
- Only diluted POS controles

- N.B. real- time PCR reduces the contamination-risk
- Reasons:.....

# PCR analysis (PCR-4)

- Only for gel- or capillary elektroforesis, analysis of amplicons **outside** the PCR reaction vessel
- Gel-doc system
- Conventional PCR machine
- Peripheral computer equipment
- Over- or underpressure?

# Monitoring contamination

- Using:
  - 1)
  - 2)
- How do you handle PCR contamination in your lab?
- What to do?
  - Source detection
  - clean
  - New reagents (also kits)
  - Check