Objectives and changes in this version:
[Reference to 17025:2005 and to specific ISO standards, e.g. ISO 22174, is removed]

Innhold

1 Working areas

DNA from test materials and amplified DNA generated from PCR should be physical separated.

Accredited laboratories should separate working areas (preferably different rooms):
a) Isolation & purification of DNA from testing material.
b) Preparation of mastermix/reactionmix.
c) Addition of nucleic acid from the test material.
d) Detection and confirmation of PCR-product.

Amplification can be performed in area c or d.
The laboratory should have a risk-based approach when deciding the need for separate working areas.

2 Coat, gloves, and cleaning

The laboratory should implement a routine with regards to:
- Change of laboratory coat in prePCR step and postPCR step.
- Change of gloves between each of the working areas.
- Routines to clean work benches etc. with DNA-destroying agents.

3 Sample preparation

Standard enrichment method is preferred.

Deviation from standard method shall be validated.

Special protocols can be needed to avoid inhibition, e.g.
✓ Products with high fat content.
✓ Products with high Calcium content (Dairy products).
✓ Products with high protein content.

Quality & yield of DNA should be repeatable and reproducible.

4 Confirmation of PCR-product

Appropriate method can be:

- ✓ Size determination (fragment).
- ✓ DNA-sequencing of PCR-product.
- ✓ Hybridization of PCR-product with specific DNA-probes.
- ✓ Restriction analysis of PCR-product.
- ✓ Standard cultural method

5 Reagents

The laboratory should ensure that reagents:

- ✓ Have analytical purity appropriate for molecular biology.
- ✓ Are used according to manufacturers' instruction, including consideration to expiry date.
- ✓ Are mixed with ultra-pure water free of DNase og RNase.

6 Thermal cycler

- ✓ Maintenance program shall be established. The program should be based on documented working hours or number of runs.
- ✓ The temperature in the instrument must be traceable to an accredited, calibrated temperature device.
- ✓ If the temperature deviate from the programmed temperature, this should be evaluated by the laboratory. Actions should be taken if temperatures are outside the accept values.

7 Pipettes

The laboratory should evaluate the need for different pipettes for

a) Sample preparation & enrichment.
b) Preparation of mastermix/reactionmix.
c) Post-amplification step.

Appropriate considerations with regards to type and quality of pipettes should be taken.

8 Controls

The laboratory should evaluate the need for
Negative process control
Positive process control
Negative extraction control
Positive extraction control
Internal/external amplification control
Positive PCR control
Negative PCR control

The evaluation should take each step of the process into consideration when selecting appropriate controls. The rationale for the chosen frequency for using the different types of controls should be documented. The laboratory should ensure that the controls have different placing from run to run.

Commercial kit internal PCR-controls are normally pure DNA and should be avoided as positive extraction control (process control) for e.g bacteria.

The laboratory should describe how controls are monitored, including how possible contamination in negative controls and lack of results in positive controls are handled.