NA Dok 62
RT-PCR testing (food, feed & water)
Guidance for technical assessors

Document Category: Guide
Technical area: Technical Assessors (Microbiology)

Objectives and changes in this version:

Endret fra NA Doc til NA Dok for bedre søkbarhet etter dokumentet på nettsiden

ISO 17025	Requirements	Reference to specific ISO-standards
5.3.3	DNA from test materials and amplified DNA generated from PCR shall be physical separated. Minimum 4 dedicated, separated 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. If performed in area c – tubes with amplified PCR-products shall not be opened in this area!	ISO 22174 6.1 ISO 22174 6.3.2
	Change of laboratory coat in prePCR step and postPCR step. Within each working area - routines for change of clothing at described frequencies.	ISO 22174 6.2
	Disposable gloves (applicable quality!) should be used in working area a, b and c. Change of gloves between each of the working areas.	ISO 22174 6.2
	Cleaning of work benches etc. with DNA-destroying agents. ✓ 3 % Na-hypochlorite ✓ 0,5-1% chlorine ✓ Commercial available “DNA-removers”	ISO 22174 6.3.2
5.4	**Sample preparation** Standard enrichment method is preferred. Deviation from standard method shall be validated Special protocols can be needed to avoid inhibition ✓ 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	 ISO 20837 6.1 6.2.1.2
	Confirmation of PCR-product Appropriate method other than size determination: ✓ DNA-sequencing of PCR-product ✓ Hybridization of PCR-product with specific DNA-probes ✓ Restriction analysis of PCR-product	 ISO 20838 7.2

<table>
<tr><th>ISO 17025</th><th>Requirements</th><th>Reference to specific ISO-standards</th></tr>
<tr><td></td><td>Alternatively can standard cultural method be used</td><td></td></tr>
<tr><td>5.5</td><td>Regents:
✓ Analytical purity appropriate for molecular biology
✓ Shall be used according to manufacturers' instruction
✓ Ultra pure water free of DNase og RNase</td><td>ISO 20838
5</td></tr>
<tr><td>5.5
Cont.</td><td>Thermal cycler:
✓ Maintenance program shall be established base on documented working hours or number of runs
✓ Instrument performance (temperature) shall be tested indirectly by biochemical method or directly by physical method (methods described in annex)
<u>Physical test method:</u>
▪ 96-well equipment – Plate is divided in six, two temperature-probes are used pr section
▪ Temperature probes have to be traceable to accredited, calibrated temperature device.
▪ Temperature shall not deviate more than 0,5ºC from the programmed temperature
Pipettes:
Three different sets:
a) Sample preparation & enrichment
b) Preparation of mastermix/reactionmix
c) Post-amplification step

For a) and b) use tips of "PCR-quality" with filters</td><td>ISO 20836
5
ISO 20836
6.2/6.3

ISO 20838
6.2</td></tr>
<tr><td>5.6</td><td>Controls needed:

<table>
<tr><th></th><th>Neg process ctr [a]</th><th>Pos process ctr [a]</th><th>Neg extraction ctr [b]</th><th>Pos extraction ctr [b]</th><th>Internal/ external amplif. ctr[c]</th><th>Pos PCR ctr [d]</th><th>Neg PCR ctr [d]</th></tr>
<tr><td>Sample prep. Enrichment</td><td>↓</td><td>↓</td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>Isolation & purification, DNA</td><td>↓</td><td>↓</td><td>↓</td><td>↓</td><td></td><td></td><td></td></tr>
<tr><td>Amplification</td><td>↓</td><td>↓</td><td>↓</td><td>↓</td><td>↓</td><td>↓</td><td>↓</td></tr>
<tr><td>Detection</td><td>↓</td><td>↓</td><td>↓</td><td>↓</td><td>↓</td><td>↓</td><td>↓</td></tr>
</table>
a) Frequency – shall be given in the QA-program
b) Not needed when negative and positive process control is included. To document the lysis step, the positive extraction control must have equal properties to the target organism.
c) Included in each PCR-reaction
d) Included in each cycler-run

Check that the controls have different placing from run to run

The extraction control shall be monitored in each run. The control ensures:
• Proper quality of reagents
• Reagents are added in the order specified in the procedure
• Proper pipetting
The process is normally controlled by the positive process control if pre-preparation and enrichment is included

Commercial kit internal PCR-controls are normally pure DNA and should be</td><td>ISO 22174
9.3

&

Harmonized practice agreed (NA - TAs)</td></tr>
</table>

<table>
<tr><th>ISO 17025</th><th>Requirements</th><th>Reference to specific ISO-standards</th></tr>
<tr><td></td><td>avoided as positive extraction control (process control) for e.g bacteria

Evaluation:

<table>
<tr><th>Sample</th><th>Neg process ctr</th><th>Pos PCR ctr</th><th>Neg process ctr
Neg extraction ctr
Neg PCR ctr</th><th>Pos process ctr
Pos extraction ctr
Pos PCR ctr</th><th>Internal amplif. ctr</th><th>External amplif. ctr</th><th>Interpretation results</th></tr>
<tr><td>+</td><td>+</td><td>+</td><td>-</td><td>+</td><td>+/-</td><td>+</td><td>+</td></tr>
<tr><td>-</td><td>+</td><td>+</td><td>-</td><td>+</td><td>+</td><td>+</td><td>-</td></tr>
<tr><td>+</td><td>+</td><td>+</td><td>+</td><td>+</td><td>+/-</td><td>+/-</td><td>?? [a]</td></tr>
<tr><td>-</td><td>-</td><td>+</td><td>-</td><td>+</td><td>-</td><td>-</td><td>?? [b]</td></tr>
</table>

a) Possible contamination
b) Possible inhibition</td><td></td></tr>
</table>

Use of CT-values for qualitative analysis in test reports

Some laboratories want to include the CT-value when reporting positive PCR results from qualitative analysis. The CT-values are semi-quantitative and is an expression of the DNA/RNA content (high or low) in the sample. When CT-values are included in the test report shall they be regarded as an interpretation of the test result, and this shall be expressed as such in the report. Laboratories not accredited for opinions and interpretations shall label the CT-values with “not accredited” in the test report.