Quantitative detection of pathogens and of mRNA/gene mutants

Internal and external reference RNAs and DNAs for the development of real-time PCR assays and for high level quality & performance controls

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Customized DNA and RNA reference controls are available for any chosen target sequence and with a wide size range (www.amp-tec.com).

NEW: Synthetic High Efficiency mRNAs for transformation & reprogramming of human somatic cells. Terminal and internal modifications increase desired efficiency & reduce innate antiviral response.

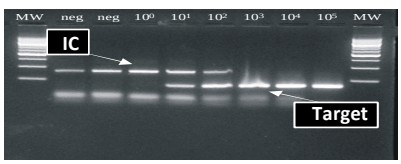
One important aspect of using synthetic internal control RNAs: false negative results are easily discovered. Availability of customized RNAs allow monitoring of both steps in RT-qPCR tests: the crucial cDNA synthesis and the subsequent PCR amplification. High quality dilution series of positive controls allow routine implementation of performance control of the tests and guarantee continuous high quality standards. The development of mutant screening tests is straightforward and any desired sequence variant is available in homogeneous form, independent from the limited availability of (uni-variant) biological samples.

An important aspect of pathogen detection is the evaluation of test specificity: only the intended target sequence(s) give positive results, closely related species are not detected and give negative results. Availability of the corresponding sequence panels can be challenging for new test developers without resources that have been accumulated over many years. The availability of customized reference sequences offers unlimited possibilities for any facility and for new fields.

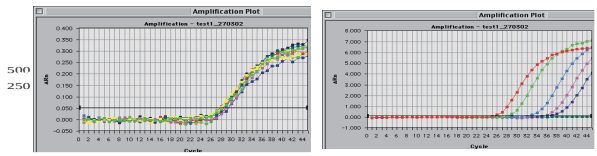
Applications of these reference nucleic acids are demonstrated with instrument platforms from the manufacturers ABI, Qiagen and Roche. Examples are shown for quality & performance control and for the detection of the closely related viral sequences HSV-1 and HSV-2 in one single test.

Monitoring of both steps in RT-qPCR tests: avoid false Negatives

RNA with unrelated sequence and size is included before cDNA synthesis



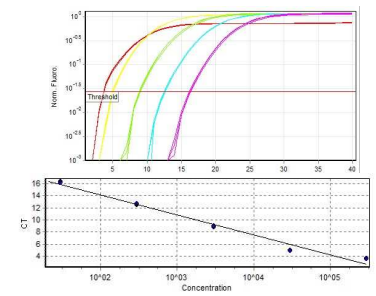
Agarose gel electrophoresis of RT-PCR products
An unrelated **internal control RNA** (IC) is spiked into all samples, containg logarithmically increasing amounts of target RNA: 10^0 to 10^5 copies.
Negative controls: only IC is amplified; high target amounts outcompete IC amplificatio (lanes 10^3 to 10^5). The fuzzy primer dimer band at the bottom decreases with increasing specific amplifications.



Real-time IC and Target detection with two different reporter dyes
ABI instrument SDS 7700 and TaqMan probes
Left panel: IC in JOE channel to detect potential inhibition in the RT or in the qPCR step.
No inhibition: All samples show strong signals for IC.
Right panel: Target amplicons in FAM channel are positive (red, green, blue, purple, dark blue curves) or negative (dark green base line)

Target Quantification

Dilution series of Positive Control

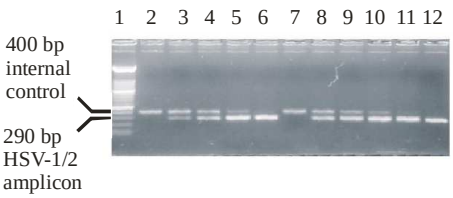


Dilution series of Target
RotorGene 6000 instrument and TaqMan probes
Top panel: Amplifcation curves, all reactions were run in triplicates.
Bottom panel: Graphic quantification plot

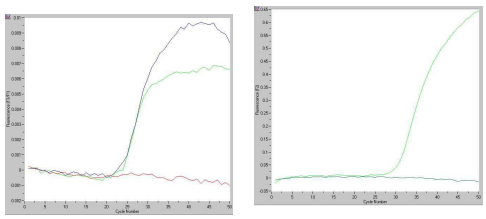
Differentiation of two closely related viral sequences HSV-1 and HSV-2

Hybridisation probes in Roche LightCycler instrument

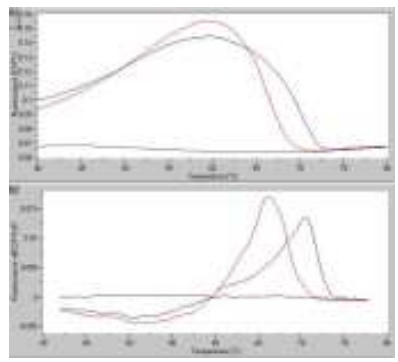
Further application: Specificity of Target Sequence detection can be checked with an extensive panel that includes all relevant, closely related sequences



Agarose gel electrophoresis of PCR products
Lanes 2+7: Negative controls. Lanes 3-6: Increasing amounts of **HSV-1 DNA** (10 to 10^4 copies), IC amplicon is outcompeted in lane 6.
Lanes 8-12: Increasing amounts of **HSV-2 DNA**, IC amplicon is outcompeted in lanes 11 and 12.



HSV detection with LightCycler and hybridization probes
Left panel: IC in channel F3 to detect potential inhibition in the qPCR step.
No inhibition: Negative result for HSV-DNA (right panel, channel F2) corresponds to strong signal for IC in F3 (blue curves).
High HSV amount outcompetes IC (red curve), the corresponding curve is not shown in right panel.
Right panel: HSV amplification in channel F2 is positive (green curve) or negative (blue baseline).



Melting curve analysis for differentiation of HSV 1 and HSV 2.
Detection of fluorescence signal is in channel F2.
Top panel: raw data, **bottom panel**: first derivative of curves in top panel.
Melting temperatures are easily recognized as maxima in curves, at 67°C for HSV-2 (red) and at 71°C for HSV-1 (blue).