RAPID AND SENSITIVE DETECTION OF *BACILLUS ANTHRACIS* BY REAL-TIME PCR

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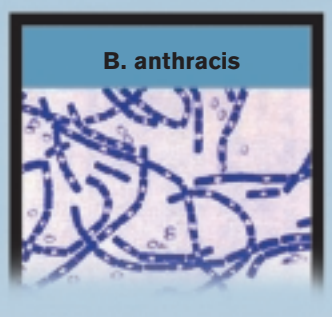
RealArt™ B. anthracis PCR

for use with the LightCycler® Instrument (Roche Diagnostics)[1]



B. anthracis is the causative agent of Anthrax, mainly a disease of herbivorous animals, particularly cattle and sheep. It is a gram-positive endospore-forming bacterium capable of producing fatal infections both in livestock and humans. At present, artificially engineered, highly virulent spore powder designed for Biological Warfare is a dangerous threat in the hands of "Bio-terrorists". Usually, humans can contract the disease by handling infected animals, products made thereof, or after contact with soil-borne spores.

In humans the disease manifests in three forms of different severity, defined on the basis of their transmission mode. Cutaneous, intestinal, or pulmonary anthrax is contracted when spores get into open wounds, are ingested or inhaled, respectively. Regardless of the transmission mode, Anthrax begins as a localized infection of the affected tissue and, if untreated, can develop into septicemia.

Virulent strains of *B. anthracis* are encapsulated and cause death by producing various toxins, including the lethal factor. Both, toxins and the capsule are encoded by genes present on two large plasmids, designated pX01 and pX02, respectively. Attenuated ("vaccine") strains lack either one or both plasmids.

The *RealArt™ B. anthracis LC PCR Kit* allows a rapid molecular detection of one representative target gene in each of the two virulence plasmids (***lef*** gene in pX01 and ***cap*A** gene in pX02).

Internal Control and Purification Control

Internal Control: The ***lef*** master includes an internal control that monitors possible inhibition of DNA amplification and does not influence the analytical PCR (Fig. 2A).

Purification Control: For ***cap*A** PCR a purification control is supplied that monitors possible mistakes during both the purification and the DNA amplification. It does not influence the analytical PCR (Fig. 2B).

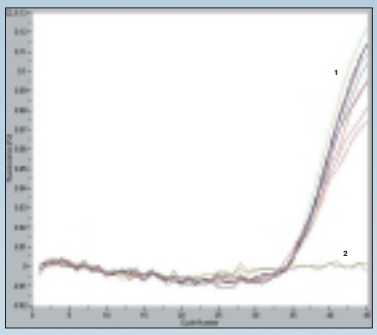


Fig. 2A: **IC** signals in F2 of the samples shown in Fig. 1A. (1) The positive signal of the negative samples (in F1) excludes the possibility of PCR Inhibition. (2) The **IC** is competed out by the strong amplification of QS 1 in F1.

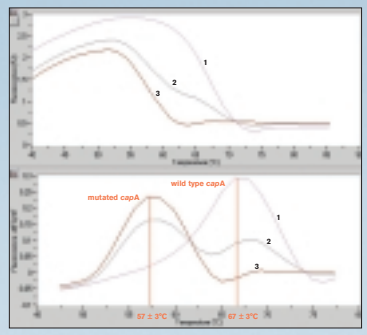


Fig. 2B: Melt Curve analysis of *B. anthracis* PCR (***cap*A** gene) in F2 of the samples (Fig. 1B). All samples contain mutated ***cap*A** DNA (**EC/IC**), (1) competed out by high copy number of ***cap*A** wild type DNA (peak at 67 ± 3°C)[2] but (2) coamplified in the low copy number ***cap*A** wild type DNA (peak at 57 ± 3°C). (3) Negative Control (PCR water).

Specific analytical PCR

Reliable interpretation of results: Positive samples can be unequivocally distinguished from negative ones. The test reliable discriminates highly virulent *B. anthracis* (***lef*:** signal in F1; ***cap*A:** signal in F2) from harmless *B. anthracis* strains and other bacteria (no signal). Sensitivity is beyond the range of 50 spores per PCR.

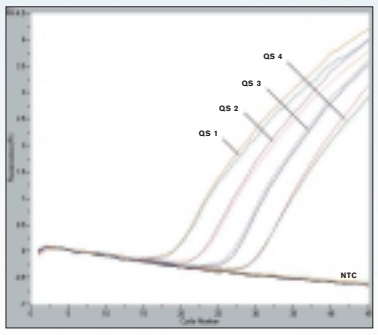


Fig. 1A: Specific detection of *B. anthracis* PCR (***lef*** gene) in F1: QS 1-4, (QS 1) 40000 copies/µl, (QS 2) 4000 copies/µl, (QS 3) 400 copies/µl, (QS 4) 40 copies/µl, Negative Control (PCR water).

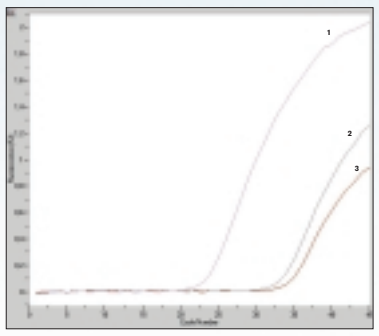


Fig. 1B: Specific detection of *B. anthracis* PCR (***cap*A** gene) in F2: (1) High positive, (2) low positive, (3) Negative Control (water), incl. EC/IC mutated ***cap*A**-DNA.[2]

Basics for Practice

- **Recommended sample material** — Spores and vegetative cells from diverse origin, even from soil (using the Qiagen Stool Kit for purification).
- **DNA extraction** — **spores or vegetative cells:** *DNeasy Tissue Kit* or *QIAamp DNA Blood Kit*[3], **soil:** *QIAamp DNA Stool Kit*[4]
- **Kit features** — **Master Mix:** includes primers, probes, enzymes, buffers, and *Internal Control* in one tube. **Positive Control:** A dilution series of four positive controls (***lef*** PCR only) is included which allows quantification of pathogens. **Sensitivity:** Below 50 *B. anthracis* cells or spores per PCR.

Sensitivity was measured by Probit analysis

Highest Sensitivity: Each *RealArt™ PCR Kit* is adapted to one real-time cycler in order to provide the most effective pathogen detection: Using the LightCycler® limits are ***lef*** = 46 copies/µl and ***cap*A** = 18 copies/µl.

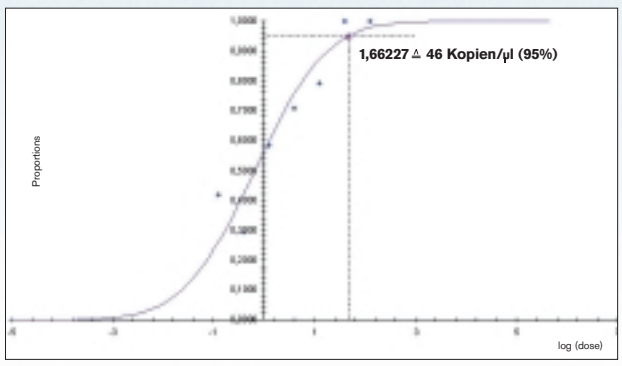


Fig. 3A: Probit analysis of the ***lef*** PCR.

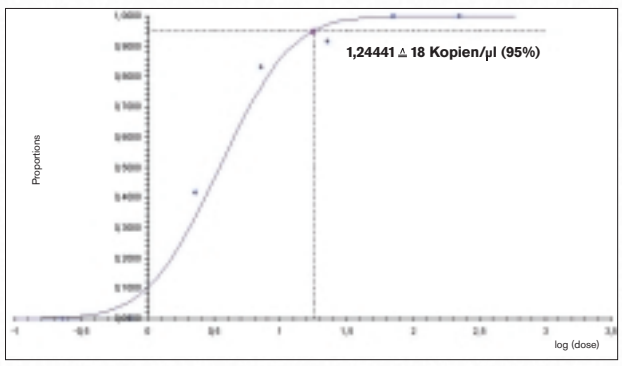


Fig. 3B: Probit analysis of the ***cap*A** PCR.

[1] Also available for the Rotor-Gene (Corbett Research), coming soon for ABI Prism Detection System 7000/7700/7900 (Applied Biosystems).
[2] No positive controls of ***cap*A**-DNA are provided with the Kit. In order to generate a strong ***cap*A** (mutated) signal similar to curve 1 (Fig. 2A), use 5 µl of **EC/IC** (instead of a sample) for a ***cap*A** PCR. The melting curve will be at 57°C (as the **EC/IC** in Fig. 2B), but the crossing point will be earlier than in curve 3 in Fig. 1B.
[3] Special protocol adaption required: see Qiagen Protocol B, p. 26 (Tissue Kit Cat. # 69504) or Protocol D, p. 43 (Blood Kit Cat. # 51104).
[4] Special protocol adaption required (supplied separately on request).

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the PCR reference