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, 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 septicaemia. 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. Attenuated (“vaccine”) strains lack either one or both plasmids.

Specific analytical PCR
Reliable interpretation of results: Positive samples can be unequivocally distinguished from negative ones. The test reliably discriminates highly virulent B. anthracis (lef gene) from harmless B. anthracis strains and other bacteria (no signal). Sensitivity is beyond the range of 50 spores per PCR.

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).

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® Instrument (Applied Biosystems) or the Rotor-Gene (Corbett Research), coming soon for ABI Prism Detection System 7000/7700/7900.

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: QIAamp DNA Blood Kit, QIAamp DNA Stool Kit or QIAamp DNA Soil Kit
  - soil: QIAamp DNA Soil Kit
- 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.

Fig. 1A: Specific detection of B. anthracis PCR (lef gene) in F1, 1-4. (3) Negative Control (PCR water).
Fig. 1B: Specific detection of B. anthracis PCR (capA gene) in F2. (1) High positive, (2) low positive, (3) Negative Control (water), incl. EC/IC mutated capA DNA.
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 (capA gene) in F2 of the samples (Fig. 1B). All samples contain mutated capA DNA. EC/IC, (1) competed out by high copy number of capA wild type DNA (peak at 67 ± 3°C) but (2) coamplified in the low copy number capA wild type DNA (peak at 57 ± 3°C), (3) Negative Control (PCR water).
Fig. 3A: Probit analysis of the lef PCR.
Fig. 3B: Probit analysis of the capA 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 capA DNA are provided with the Kit. In order to generate a strong capA (mutated) signal similar to curve 1 (Fig. 2A), use 3 µl of EC/IC (instead of a sample) for a capA 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.
² Special protocol adaption required: see Qiagen Protocol B, p. 26 (Tissue Kit Cat. # 69504) or Protocol D, p. 43 (Blood Kit Cat. # 69505) or Protocol D, p. 43 (Blood Kit Cat. # 69505).
² Special protocol adaption required (supplied separately on request).