Ribo Technologies
MICROBIAL DIAGNOSTICS

TocScan™

Real–time PCR identification and quantification of Bacterial total count

Total count Bacteria

Reliability

Speed

Easy

Molecular bacterial diagnostics

Identification of micro-organisms was until recently limited to determination of the morphological and physiological traits of isolated individual cells using cultivation dependent methods. Determination of many of these phenotypic traits is laborious, but most of all very unreliable. Moreover, studies have shown that only 0.1 to 10% of all bacteria can be cultivated. Under equal conditions, some bacterial groups are unable to compete with others. Consequently, enumeration of bacteria by cultivation can lead to enormous underestimation of actual values, and the micro-organisms found do not represent the true population structure.

At the end of last century DNA based methods were developed which can be applied to the detection of micro-organisms. In DNA, information is stored for all cellular processes. These processes are performed by proteins. Special regions on the DNA (genes) code for these proteins. The proteins are synthesised by ribosomes coded for by RNA coding genes. Due to their essential role in cellular processes the structure of RNA coding genes is similar in all organisms. Most of the phylogenetic analysis performed nowadays makes use of the information stored in the 16S RNA molecule. This molecule is approximately 1600 nucleotides long and comparison of more than 80.000 sequences presently found in the freely accessible databases has lead to the identification of group- or even species-specific sequences (signature sequences). Conserved regions are found across different taxa or even across the eubacterial kingdom. Using various molecular analytic techniques small oligonucleotide probes or primers (16-20 nucleotides long) are able to detect the presence of complementary specific regions on the rRNA target molecule. With the modern high-end techniques used by Microscreen it is now possible to identify all micro-organisms present in a population, regardless of their phenotypic capabilities.

TocScan

The TocScan, which is developed by Microscreen, is a very fast quantitative Real-time PCR tool for the determination of the total count of bacteria. The bacterial cells are isolated using a rapid procedure, after which the DNA is extracted. The target DNA is amplified using two universal eubacterial primers which amplify the 16S rRNA of all bacteria. SYBR green binds to the amplified target, which causes an increase in the fluorescent signal. This is visualized as a growth curve in a graph, when the number of PCR cycli is plotted against the fluorescence (see Fig. 1). Assay results are obtained by measuring the increase of fluorescence that occurs during the amplification After completion of the PCR a meltcurve is performed on the amplicons, which results in Melt peaks. The TM of these Melt peaks is used to determine if the amplicon is the expected product or a result of primer dimmer formation.

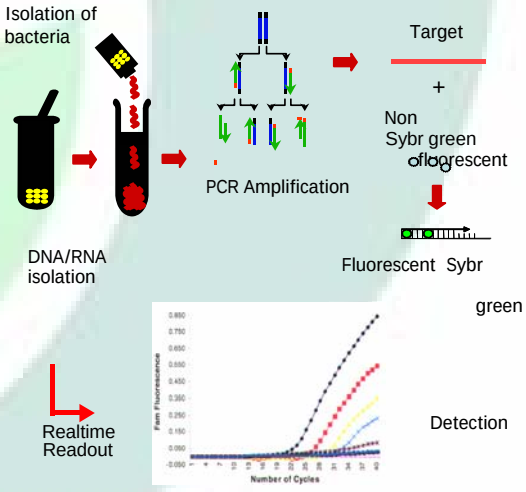

Fig. 1: Workflow TocScan

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Features

Specifications

Real time PCR or Q-PCR offers the ability to analyse data during the log-linear phase of the PCR reaction. A plot of the fluorescence *vs* cycle number produces a sigmoidal shaped curve called a growth curve. To analyse Q-PCR assays, the log-linear phase of the reaction is used to determine the cycle threshold (Ct) for each sample. To obtain a reference line the Ct values of a number of samples with known numbers of bacteria are determined. The calculated linear fit equation derived from the reference line can be used to quantify a sample with an unknown number of bacteria.

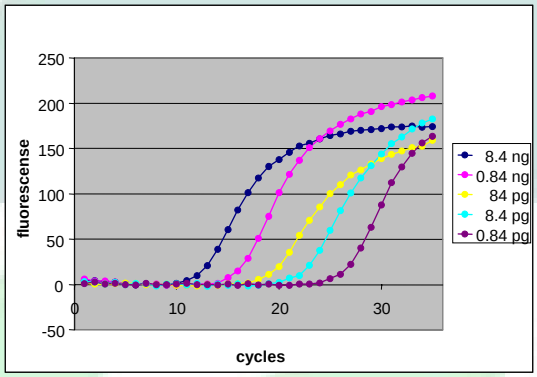

Fig. 2: Dilution series PCR total count

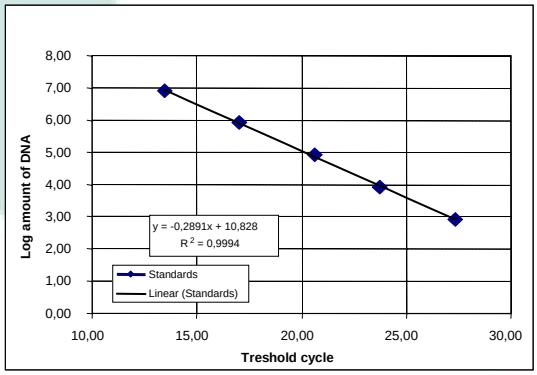

Fig. 2. Referenceline total count Q-PCR test

Conclusion

The lower limit of the test is 1000 bacteria. Below this level, the bacterial contamination of the reagents will negatively influence the outcome of the test. For most applications this is not a problem

The TOCSCAN can detect and ennumerate the presence of bacteria within 4 hours. This presents a major advantage compared to the laborious and time consuming traditional methods. The ease with which the test can be performed allows high through-put screening. Microscreen can assist in laboratory design and implementation of Q-PCR applications and can supply training of personnel

Testkit components

Comprehensive and easy to follow protocol

Oligo's
Forward primer
Reverse primer

Fluorescent probe
Sybrgreen

Positive control
Lyophilized DNA of *Escherichia coli*

Ordering information

Product	Contents	Cat. No.
TOCScan	on request	20-TS-001

Disclaimer:
The Polymerase Chain Reaction (PCR) process is covered by patents owned by Hoffmann-La-Roche, Inc. Use of the PCR process requires a license.

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