Detection of Cronobacter in Food with a New Test Kit System

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BACKGROUND

Development of novel methods for a rapid, sensitive and reliable detection and quantification of microorganisms and pathogens in food, beverages and water is receiving increasing attention. The sandwich hybridisation method used in the HybriScan® Test System is a suitable alternative for a sensitive and reliable detection and identification of microorganisms.

The HybriScan method is nearly independent of influences of sample matrices, and is able to distinguish between live and dead cells. Furthermore, the detection of non-culturable microbes is possible.

The HybriScan method is based on the detection of hybridisation events between two specific oligonucleotide probes and target nucleic acids. The capture probe is used to immobilise the target sequence on a solid support and the detection probe is labeled with a detectable marker (**Figure 1**). Sandwich hybridisation is relatively sensitive and can be performed with crude biological samples[1]. Sandwich hybridisation assays from crude cell samples or in connection to PCR have been extensively used in clinical diagnostics for detection of nucleic acids from bacteria[2, 3, 4] and viruses[5]. The sandwich hybridisation method is ideal for identification of specific rRNAs in bacterial cells and yeasts. The sensitivity of this RNA-based assay benefits from the typically high number of ribosomes in each cell. Compared to only a few copies of genomic DNA a single cell contains several thousand copies of rRNA. Although a direct detection of the ribosomal RNA does not match the sensitivity of a PCRbased DNA assay, it offers advantages like quantification, live/ dead-discrimination, no additional amplification steps and simple assay protocols with standard laboratory equipment.

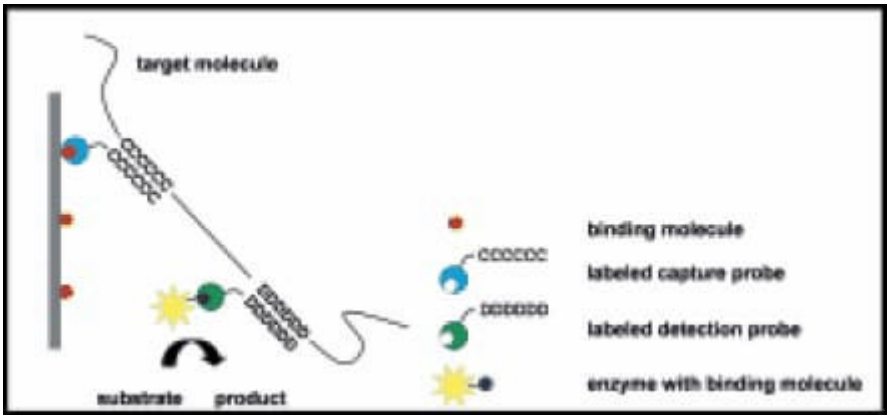

Figure 1. Principle of the HybriScan sandwich hybridisation assay

HYBRISCAN***D*** CRONOBACTER: DETECTION OF CRONOBACTER IN FOOD

Cronobacter is ubiquitous and frequently found on vegetables, meat and dairy products, and especially in baby food. Consumption of contaminated powdered infant formula milk (IFM) can result in life-threatening neonatal infections caused by the pathogen. Taxonomic studies have determined

that *Cronobacter* comprises a high genetic heterogeneity and should be reclassified as a novel genus, "Cronobacter"[8] (**Figure 4**).

Figure 4. *Cronobacter* colonies grown on M1 agar

Accurate methods are required for the rapid detection and identification of *Cronobacter*, since even low cell numbers have been reported to cause a disease. HybriScan***D*** Cronobacter is a rapid molecular test system for detection of bacteria of the genus Cronobacter in food, especially in dried infant formula milk and its production environment. **Figure 5** shows the specificity of HybriScan***D*** Cronobacter. Different cell amounts and related *Enterobacteriaceae* were tested within a validation study. No signals were observed using 2.3 x 10^8 *Enterobacter* cloacae cells or 7 x 10^8 *Citrobacter freundii* cells per assay, whereas clear specific signals were detectable using 2.6 x 10^3, 1.3 x 10^4, and 2.6 x 10^4 cells of Cronobacter, respectively. These results demonstrate that the HybriScan system is highly specific for *Cronobacter*.

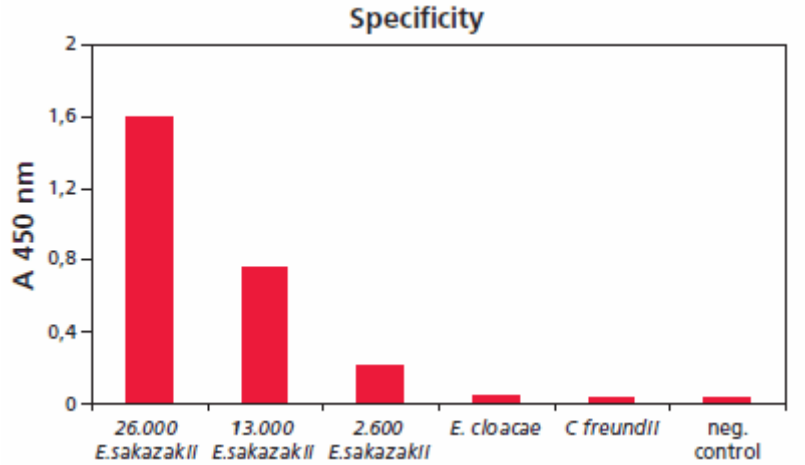

Figure 5. Specificity of HybriScan***D*** Cronobacter. Different cell numbers of *Cronobacter* and related Enterobacteriaceae like *E. cloacae* and *Citrobacter freundii* were tested. Measurement data for HybriScan analyses represent absorption at 450 nm.
A validation study of HybriScan***D*** Cronobacter was performed using two different enrichment procedures: (1) single-step enrichment for 24 –26 hours at 37 °C in ESSB broth (AES Chemunex) and (2) twostep enrichment starting with a pre-enrichment for 18 –20 hours at 37 °C in buffered peptone water and followed by a selective enrichment for 24 –26 hours at 45 °C in mLST selective broth. The results of the above-mentioned validation study are presented in **Table 2**.

Table 2. Results of a validation study of HybriScan***D*** Cronobacter

	Total Number of Samples	Number of Positive Samples	
	n	ISO/TS 22964	HybriScan
Single-Step enrichment	31	26	25
Two-Step enrichment	99	71	70

130 samples of powdered infant formula milk were analysed with HybriScan***D*** Cronobacter and compared to two cultivation-based methods according to ISO/PFR TS 22964. In total, six products from different manufacturers were tested. Results of the validation study lead to a relative accuracy of 95 %, a relative specificity of 92.9 % and relative sensitivity of 95.7 %, respectively.

HybriScan***D*** Cronobacter enables a reliable and comprehensive control of suspicious products in the context of classical diagnostic but makes detection more rapid with results available after 48 hours.

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