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Preparation, Separation,
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Application Note

Rapid Detection and Identification of Bacteria and Yeasts in Food, Beverages and Water

Abstract

HybriScan® system is a new technology based on the detection of microbe-specific rRNA using sandwich hybridization. It allows comprehensive and reliable routine control of microbial contamination during food production, from raw materials to finished goods. The system is sensitive and specific since the method is based on molecular genetic identification, and it allows detection of a group of microorganisms as well as specific species. The signal read-out is triggered optically by an enzymatically generated color change. No PCR is required because the method is quantitative without cell counting (using standards) and uses standard laboratory equipment.

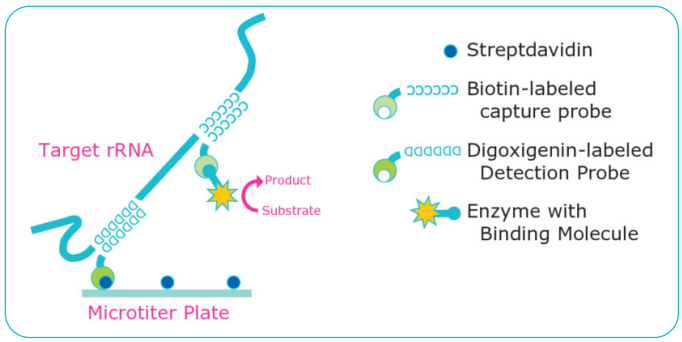
The HybriScan® method is an economical, high throughput, 96-well microplate format system. The test is performed in less than 3 hours (in addition to the prep time) and offers a time saving of up to 10 days compared to cultivation-based assays. It is ideal for safety and quality control of alcoholic and nonalcoholic beverages, water and food. Specific examples include the detection of food-borne pathogens like *Salmonella*, *Campylobacter*, *Listeria* and *Cronobacter* spp., and counting of *Legionella* in water, including the most relevant species, *L. pneumophila*. Organisms can be detected at any level of classification, from species or genus to higher ranks.

Introduction

Methods for a rapid, sensitive and reliable detection and quantification of microorganisms and pathogens in food, beverages and water are receiving increasing attention. The sandwich hybridization method used in the HybriScan® test system is a suitable alternative for such analysis. This test method is independent of the influence 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 rRNA via hybridization events and specific capture and detection probes **(Figure 1)**. Specificity is achieved by targeting conserved or unique rRNA sequences. A biotin-labeled capture probe is used to immobilize the target sequence on a solid support plate (streptavidin-coated microtiter plate). A digoxigenin-labeled detection probe provides an enzyme-linked optical signal read out. Detection results from application of anti- DIG-horseradish peroxidase Fab fragments. The bound complex is visualized by horseradish peroxidase substrate TMB (3,3′,5,5′-tetramethylbenzidine). Photometric data are measured at 450 nm and compared with standard solutions. The HybriScan® software enables easy measurement and data analysis.

Figure 1: Principle of the HybriScan® Sandwich Hybridization Assay



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Discussion

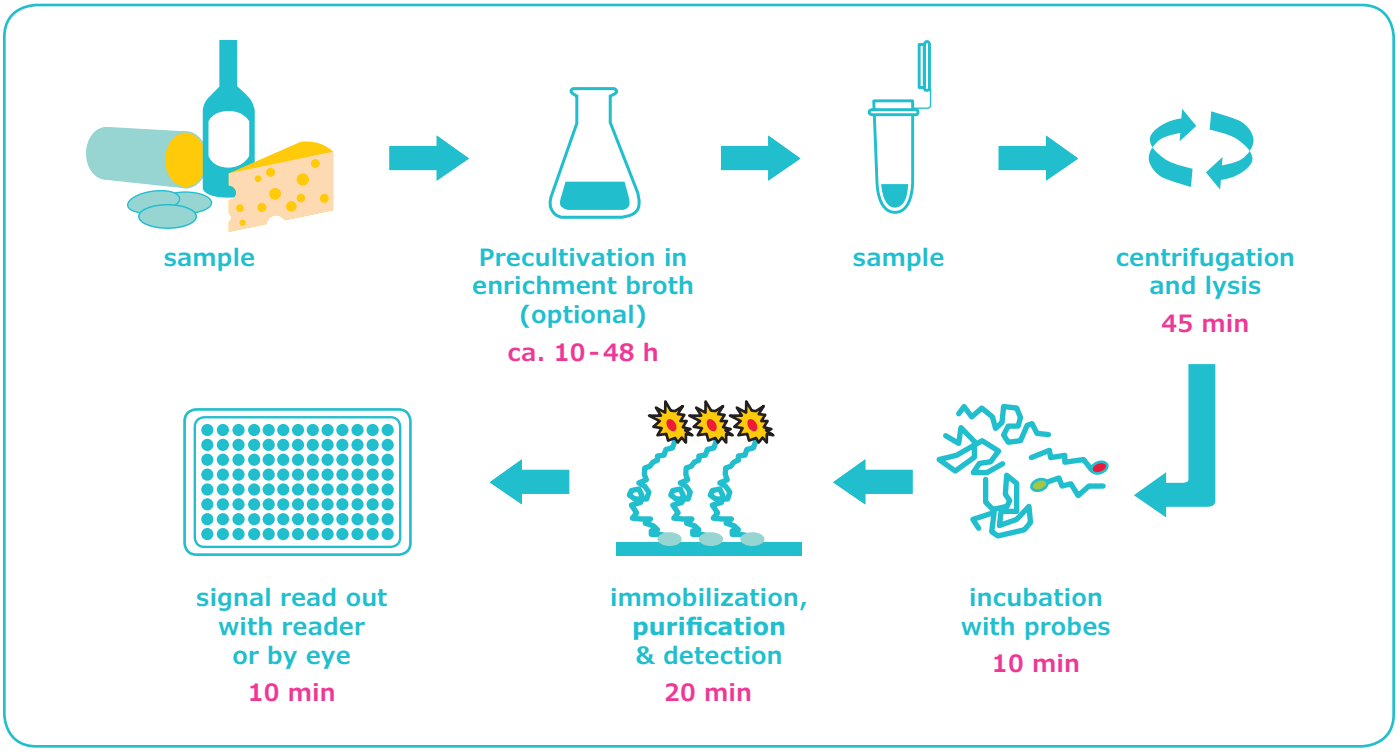
Sensitivity, specificity, flexibility and applicability of HybriScan® technology

Sandwich hybridization is very sensitive, detecting attomoles of the respective target rRNA molecules [1]. The ideal hybridization target for bacteria and yeast is rRNA. These cells contain a large number of rRNA-containing ribosomes; a single cell therefore contains several thousand copies of rRNA but only one DNA. Sandwich hybridization also provides sensitivity in crude biological samples because it is not susceptible to matrix interference.

By using specific probes, the HybriScan® technology allows flexible group- and species-specific detection. It is applicable to many analytical fields, including monitoring the microbial content of beer, wine, non-alcoholic beverages, drinking water, a wide variety of foods and wastewater. The HybriScan® system rapidly and accurately identifies, detects and quantifies many important pathogenic species, including *Salmonella, Campylobacter, Listeria* and *Legionella* including the most relevant species *L. pneumophila*[2,3,4]. The HybriScan® test system is ideal for the comprehensive and reliable routine control of raw materials and concentrates in all production steps up to the quality check of finished goods.

Figure 2: Workflow of HybriScan® test method

The HybriScan® system is a simple, time-saving assay that can be performed with standard laboratory equipment.



Benefits over conventional detection methods and PCR

The HybriScan® system has significant time- and labor-saving benefits over traditional methods. It also has benefits over PCR and real time PCR, which, although highly sensitive, are susceptible to experimental interferences, like template inhibition from insufficient purification, and lack quantification accuracy due to biases associated with PCR and reverse transcription reactions[5]. In contrast, the HybriScan® method is nearly independent of the influences of sample matrix and detects only living cells. It also permits the detection of non-culturable microbes. **Table 1** compares the benefits and disadvantages of the various methods.

HybriScan® *Listeria monocytogenes*: An example of the rapid and innovative test system

One of the most important foodborne pathogens is *Listeria monocytogenes* **(Figure 3)**, which poses a health threat in foods that have long, refrigerated shelf lives[6]. Listeriosis, caused by ingestion of foods contaminated with *Listeria monocytogenes,* has increased dramatically in recent years, causing a great deal of distress and even death. Milk, cheese, ice cream and meat contaminated with this pathogen have led to recent outbreaks of listeriosis[7].

L. monocytogenes proliferates at refrigeration temperatures and is able to grow over a wide pH range from 4.4 to 9.4.

Table 1: Advantages of HybriScan® system over other detection techniques

Detection technology	Advantage	Disadvantage
HybriScan® system	- detects only living cells - minimal interference by sample matrix - high specificity - low cross-reactivity - easy handling - cost-efficient read-out devices - quantitative and qualitative - high sample throughput (96-microwell plates) - detects non-culturable microbes	- no differentiation of serotypes or subspecies - limited probe design (rRNA target)
PCR	- high sample throughput - sensitive - quantitative	- no live/dead cell differentiation sensitive to matrix interference (high extraction effort) - susceptible to polymerase inhibition
ELISA	- differentiation of serotypes or subspecies - high sample throughput (96-microwell plates) - quantitative and qualitative	- low sensitivity - low specificity, higher cross- reactivity - slow and expensive assay development
Conventional cultivation-based methods	- relatively inexpensive - simple - specific - widely accepted method	- time-consuming (up to 10 days) - no detection of non-culturable Microbes - low sample throughput - laborious

Figure 3. *Listeria monocytogenes* colonies grown on PALCAM Agar

Conventional culture-based methods to detect *L. monocytogenes* generally involve selective enrichment followed by culturing on selective medium, isolation and biochemical identification[8]. This laborious and time-consuming approach often takes several days to show results. Also, compared to molecular biological and immunological methods, culture-based methods often give false negatives.

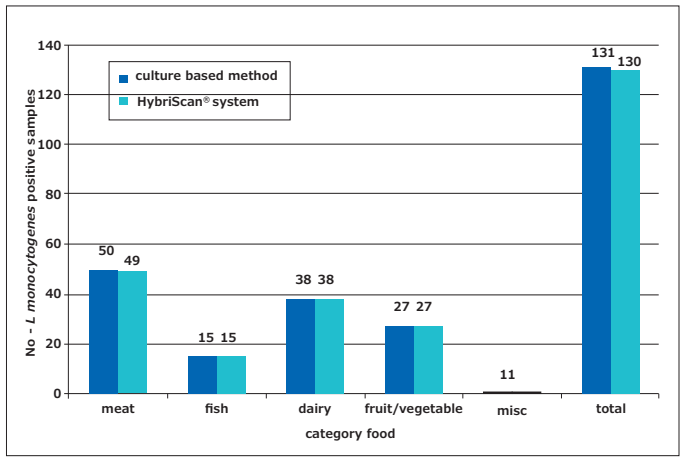
HybriScan® *Listeria monocytogenes* is an excellent alternative to lengthy culture- based methods. It is as reliable and comprehensive as classical methods but permits rapid detection and quantification with results available within 48 hours.

The species-specific probe permits direct detection of *L. monocytogenes,* thereby eliminating false positives caused by other *Listeria* species. Even more compelling, suspected single colonies can be identified within one hour using the HybriScan® identification kit without need for further cultivation.

Figure 4 shows the validation results of HybriScan® *Listeria monocytogenes*. Food samples were analyzed with the HybriScan® method and compared to the culture- based method according to 64-LFGB. Five different food categories were tested. 355 food samples were analyzed and compared to culture-based method according to 64-LFGB. The blue values are the number of analyzed food samples in each category. Validation was according to ISO 16140:2003 (ASU L00.00-22). Results of validation showed a relative accuracy of 99.2%, relative specificity of 98.5% and relative sensitivity of 99.6%.

Two versions are available. HybriScan® *Listeria monoytogenes* is used for the extremely rapid, sensitive and economical identification of suspect colonies of *L. monocytogenes*. HybriScan® *Listeria monocytogenes* is used for the detection, identification and quantification of *L. monocytogenes* in different food matrixes.

Figure 4: Validation of HybriScan® *Listeria monocytogenes*



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