

Detection of *Campylobacter* spp. in Food with a New Test Kit System

Analytix Volume 9 Article 3

Jvo Siegrist, Product Manager Microbiology, Sigma-Aldrich

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 samples¹. 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 viruses⁵. 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.

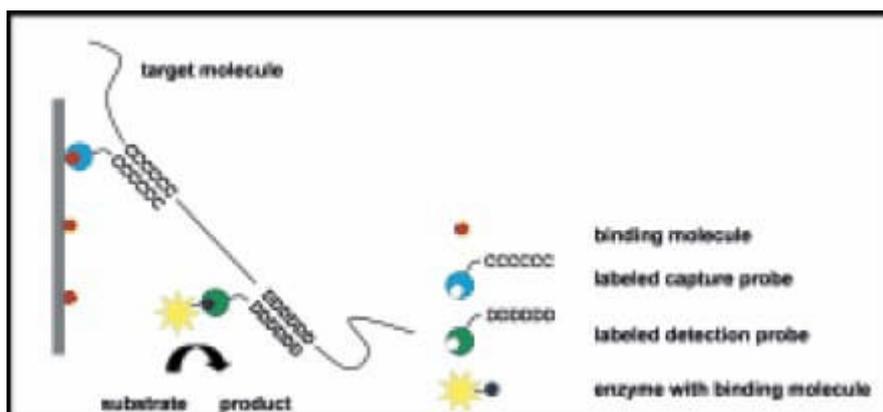


Figure 1. Principle of the HybriScan sandwich hybridisation assay

HYBRISCAN[®] CAMPYLOBACTER: DETECTION OF CAMPYLOBACTER SPP. IN FOOD

Bacteria of the species *Campylobacter* are zoonotic pathogens which annually infect 1 % of Western Europe's population. Some *Campylobacter* species are known to infect animals, especially infections of the reproductive tract.



Campylobacter are ubiquitous and often found in domestic animals. In this way, they are present frequently in the environment and on many raw foods of plant and animal origin. A very high concentration of *Campylobacter* can be found on raw poultry meat⁶.

According to authorities in Germany, human *Campylobacteriosis* is the most common reported disease caused by bacteria besides *Salmonellosis*. In 2004, around 55,000 and in 2005, 60,000 *Campylobacter* infections were observed⁷.

Therefore, a rapid and reliable detection of *Campylobacter* is required to ensure microbiological safety and quality. In contrast to HybriScan, classical cultural methods for the detection of *Campylobacter spp.* are time consuming and well-trained laboratory personnel is required for each type of bacteria.

HybriScan^D *Campylobacter* is a rapid molecular test system for the detection of bacteria of the genus *Campylobacter* in different food matrices, including the detection of the most relevant species *C. jejunii* (Figure 2), *C. coli*, *C. lari* and *C. upsaliensis*.

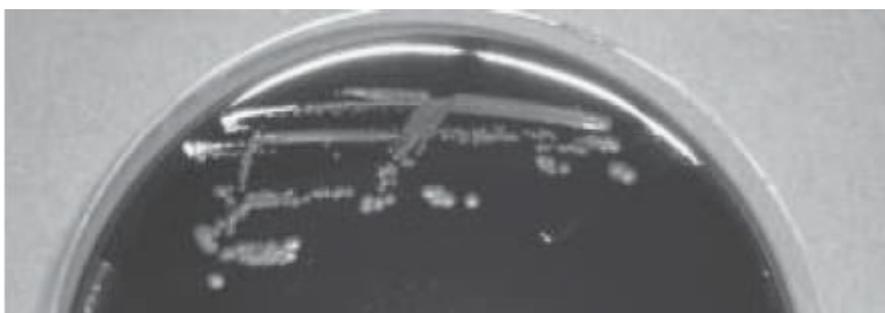


Figure 2. *Campylobacter jejunii* colonies grown on CCDA agar

HybriScan^D *Campylobacter* enables a reliable and comprehensive control of suspicious results in the context of classical microbial diagnostics but makes detection more rapid, with results available after 48 hours. An overview of the validation results of HybriScan^D *Campylobacter spp.* are presented in Figure 3. 108 food samples were analysed with HybriScan^D *Campylobacter* and compared with a cultivation-based method according to § 64 LFGB. In total, five different food categories were tested. The results of the validation lead to a relative accuracy of 95.2 %, a relative specificity of 97.5 %, and relative sensitivity of 93 %, respectively.

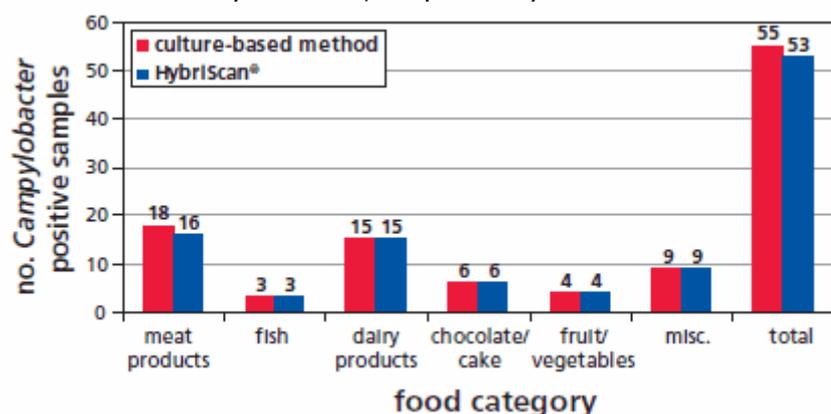


Figure 3. Overview of the validation results of HybriScan^{®D} *Campylobacter* 108 food samples were analysed with HybriScan^D *Campylobacter* and compared to a cultivation-based method according to § 64 LFGB. Numbers on the bars represent the number of analysed food samples in each food category. The validation was performed according to ISO 16140:2003 (ASU L00.00-22).



References

1. Tenhunen J, Eloranta J, Kallio A, Söderlund H. 1990. A solution hybridization method for quantification of mRNAs: Determining the amount and stability of oncogene mRNA. *Gene Analysis Techniques*. 7(8):228-233. [https://doi.org/10.1016/0735-0651\(90\)90005-z](https://doi.org/10.1016/0735-0651(90)90005-z)
2. Huhtamella S, Leinonen M, Nieminen T, Fahnert B, Myllykoski L, Breitenstein A, Neubauer P. 2007. RNA-based sandwich hybridisation method for detection of lactic acid bacteria in brewery samples. *Journal of Microbiological Methods*. 68(3):543-553. <https://doi.org/10.1016/j.mimet.2006.10.009>
3. Casadémont I, Bizet C, Chevrier D, Guesdon J. 2000. Rapid detection of *Campylobacter fetus* by polymerase chain reaction combined with non-radioactive hybridization using an oligonucleotide covalently bound to microwells. *Molecular and Cellular Probes*. 14(4):233-240. <https://doi.org/10.1006/mcpr.2000.0312>
4. Chevrier D, Popoff MY, Dion MP, Daniel H, Guesdon J. 1995. Rapid detection of *Salmonella* subspecies I by PCR combined with non-radioactive hybridisation using covalently immobilised oligonucleotide on a microplate. 10(3-4):245-252. <https://doi.org/10.1111/j.1574-695x.1995.tb00039.x>
5. Albretsen C, Kalland K, Haukanes B, Håvarstein L, Kleppe K. 1990. Applications of magnetic beads with covalently attached oligonucleotides in hybridization: Isolation and detection of specific measles virus mRNA from a crude cell lysate. *Analytical Biochemistry*. 189(1):40-50. [https://doi.org/10.1016/0003-2697\(90\)90041-7](https://doi.org/10.1016/0003-2697(90)90041-7)
6. Humphrey T, O'Brien S, Madsen M. 2007. *Campylobacter*s as zoonotic pathogens: A food production perspective. *International Journal of Food Microbiology*. 117(3):237-257. <https://doi.org/10.1016/j.ijfoodmicro.2007.01.006>
7. Wittwer M, Keller J, Wassenaar TM, Stephan R, Howald D, Regula G, Bissig-Choisat B. 2005. Genetic Diversity and Antibiotic Resistance Patterns in a *Campylobacter* Population Isolated from Poultry Farms in Switzerland. *AEM*. 71(6):2840-2847. <https://doi.org/10.1128/aem.71.6.2840-2847.2005>
8. Wittwer M, Keller J, Wassenaar TM, Stephan R, Howald D, Regula G, Bissig-Choisat B. 2005. Genetic Diversity and Antibiotic Resistance Patterns in a *Campylobacter* Population Isolated from Poultry Farms in Switzerland. *AEM*. 71(6):2840-2847. <https://doi.org/10.1128/aem.71.6.2840-2847.2005>

