

Validation of shorter protocol for detection of *Salmonella enterica* subsp. *Enterica* in peanut butter samples followed by a rRNA detection system

Kathleen Merx¹ and Jvo Siegrist²

¹ BECIT GmbH, Edisonstr. 5, 06766 Wolfen, Germany ² Sigma-Aldrich Chemie GmbH, Industriestr. 25, 9470 Buchs Switzerland

Abstract

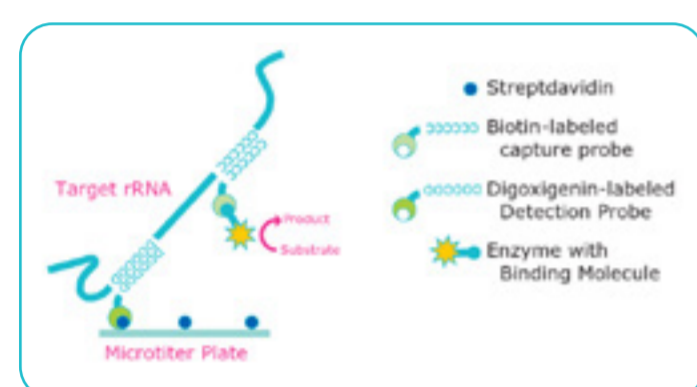
Food-borne pathogens *Salmonella* is commonly evaluated in manufacturing of peanut butter and other food products. For the HybriScan®D *Salmonella* Test (Cat. No. 55662) the ISO based enrichment method is recommended. That means sample pre-enrichment for 18 hours at 37 °C in buffered peptone water (BPW) followed by a selective enrichment step in Rappaport-Vassiliadis (RV) Broth for 24 hours at 41 °C. HybriScan®D *Salmonella* is a rRNA sandwich hybridisation detection system which needs at least 500 cfu/mL for the assay. This rapid molecular test system is desirable for the detection of *Salmonella* species like *S. Enteritidis*, *S. Typhimurium*, *S. Typhi* and *S. Paratyphi*. Results of this study on a rapid test kit demonstrate that *Salmonella enterica* subsp. *enterica* (ATTC® 13311™) can be detected and identified in a shorter time even in a difficult sample matrix like peanut butter.

Introduction

Peanut butter consist of about 20% carbohydrates, 25% proteins and 50% fat, *Salmonella* cells are just a very small component of the overall sample material and may be attached within the food matrix as single cells or clumps of cells. Normally before rapid detection methods can be used successfully, it is usually necessary to separate the target cells from the food matrix and from the background microflora. But even the HybriScan is as well a rapid molecular biological system it is practically insensitive to the sample matrix.

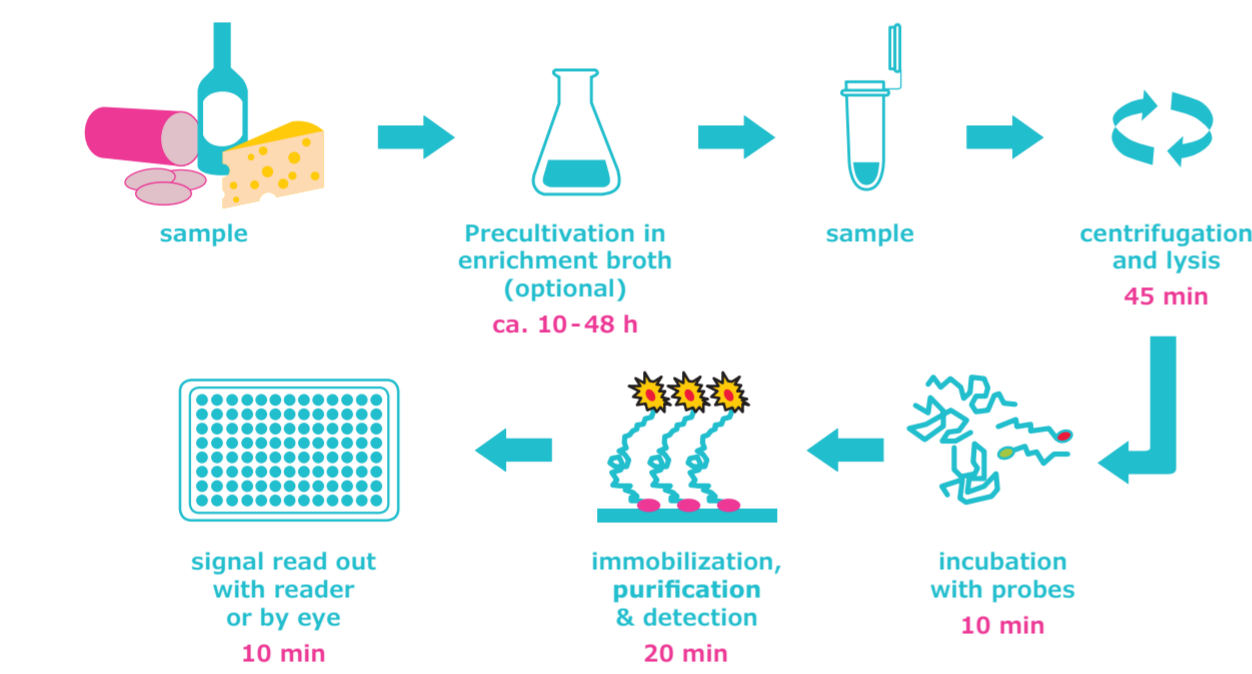
HybriScan®D *Salmonella* test is based on the detection of target molecules from the microorganisms of interest by means of specific capture and detection probes in a so-called sandwich hybridization. The hybridization reaction of the target molecules with the Biotin-labeled capture and a DIG-labeled detection probe takes place in a streptavidin coated microtiter plate (Figure 1).

Figure 1: Sandwich Hybridization between Target rRNA Capture and Detection Probe.



After coupling of the target molecule to the microtiter plate, an enzyme is attached in a subsequent incubation step. After several washing steps, reaction with a color substrate gives blue coloration that changes into yellow color after the addition of a stop solution. The yellow color enables highly sensitive photometric measurement at 450 nm (Figure 2). Comparison is made with the standard solutions contained in the test kit.

Figure 2: Work flow – HybriScan®D *Salmonella*.



Experimental

The matrix to be examined was Peanut Butter (9 different brand codes with 12 different code dates). The pre-enrichment time described in the protocol of the HybriScan®D *Salmonella* test takes 42 hours. In this experiment one target was to reduce the enrichment time to 24 hours in total. The cultivation time of the pre-enrichment peptone water culture took 18 hours; the incubation time of the selective enrichment culture in Rappaport-Vassiliades Enrichment Broth was shortened to 6 hours. Per peanut butter batch, one sample wasn't inoculated and carried as a negative control (N.C.), 9 to 10 samples were inoculated with *Salmonella enterica* subsp. *Enterica* (ATTC® 13311™). Each Peanut Butter sample was treated as follows:

- 25 g of Peanut Butter (except the negative controls) were inoculated with 1 to 5 cells of *Salmonella enterica* subsp. *Enterica*.
- 225 mL buffered peptone water were added to each sample, the mixture was homogenised for 1 minute in a Stomacher and the sample were incubated for 18 hours at 37 °C.
- After 18 hours of incubation 0.1 mL of the pre-enrichment peptone water culture were transferred to 10 mL Rappaport-Vassiliades Enrichment Broth. The selective main enrichment was conducted for 6 hours at 41°C.

Cell lysis and the HybriScan®D *Salmonella* assay were completed as described in the HybriScan®D *Salmonella* test protocol. In addition, each negative control and inoculated sample were tested for *Salmonella* according to EN ISO 6579:2002.

Results

Evaluation of the samples was performed using the following formula as described in the HybriScan®D *Salmonella* assay:

$$\text{Sample O.D.\%} = (\text{O.D.}_{\text{sample}} - \text{O.D.}_{\text{N.C.}}) / (\text{O.D.}_{\text{P.C.}} - \text{O.D.}_{\text{N.C.}}) \times 72.1\%$$

P.C. positive control (S3)

N.C. negative control (S1)

Samples with O.D.% values under 10 are considered negative.

Samples with O.D.% values from 10 to <20 are considered questionable.

Samples with O.D.% values ≥20 are considered positive.

Of the 107 inoculated with *Salmonella enterica* peanut butter samples, 106 were identified as clearly contaminated with *Salmonella* by the use of HybriScan®D *Salmonella* assay. The result of 1 sample was considered questionable. All negative controls gave negative results in the HybriScan®D *Salmonella* assay. See Table 1– 4.

Table 1

Code Date	Probe	Average O.D. 450 nm	Sample O.D.%	Cells/ 10 µL
	S1	0.046		0
	S2	0.149		10.0
	S3	0.377		30.0
	S4	1.017		90.0
1624251	1-1 N.C.	0.046	0.0	
	1-2	4.060	874.2	
	1-3	3.955	851.4	
	1-4	3.970	854.6	
	1-5	3.899	839.2	
	1-6	3.891	837.4	
	1-7	3.535	760.0	
	1-8	3.791	815.8	
	1-9	3.736	803.7	
	1-10	3.816	821.1	
1874251	2-1 N.C.	0.048	0.3	
	2-2	3.748	806.4	
	2-3	3.726	801.6	
	2-4	4.072	877.0	
	2-5	3.907	840.9	
	2-6	3.965	853.5	
	2-7	3.824	822.8	
	2-8	4.060	874.2	
	2-9	4.003	861.9	
	2-10	4.032	868.2	

Table 3

Code Date	Probe	Average O.D. 450 nm	Sample O.D.%	Cells/ 10 µL
	S1	0.058		0
	S2	0.154		10.0
	S3	0.356		30.0
	S4	0.982		90.0
93294252	6-1 N.C.	0.046	-2.8	
	6-2	3.727	886.2	
	6-3	3.994	950.8	
	6-4	3.659	869.8	
	6-5	3.512	834.4	
	6-6	3.430	814.5	
	6-7	3.588	852.8	
	6-8	1.738	405.9	
	6-9	4.067	968.5	
	6-10	3.727	886.2	
01304252	7-1 N.C.	0.046	-2.9	
	7-2	3.787	900.7	
	7-3	3.575	849.5	
	7-4	3.779	898.8	
	7-5	3.875	922.0	
	7-6	4.144	987.1	
	7-7	4.067	968.5	
	7-8	3.988	949.4	
	7-9	4.135	984.9	
	7-10	4.101	976.7	
01304252	8-1 N.C.	0.053	-1.2	
	8-2	3.507	833.2	
	8-3	3.906	929.4	
	8-4	3.708	881.7	
	8-5	3.672	873.1	
	8-6	4.059	966.4	
	8-7	3.914	931.5	
	8-8	4.101	976.7	
	8-9	3.750	891.8	
	8-10	3.852	916.5	

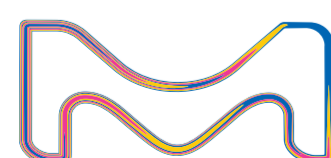
Table 2

Code Date	Probe	Average O.D. 450 nm	Sample O.D.%	Cells/ 10 µL
	S1	0.051		0
	S2	0.169		10.0
	S3	0.409		30.0
	S4	1.171		90.0
01574251	3-1 N.C.	0.057	1.2	
	3-2	0.608	112.1	
	3-3	1.665	324.6	
	3-4	0.289	47.9	
	3-5	0.120	13.9	
	3-6	1.146	220.2	
	3-7	0.701	130.8	
	3-8	0.251	40.2	
	3-9	0.981	187.0	
	3-10	0.359	62.0	
01334252	4-1 N.C.	0.051	0.0	
	4-2	3.737	741.4	
	4-3	3.682	730.3	
	4-4	3.927	779.6	
	4-5	3.700	733.9	
	4-6	3.780	750.1	
	4-7	3.813	756.6	
	4-8	3.915	777.1	
	4-9	3.682	730.4	
	4-10	4.091	812.6	
00864252	5-1 N.C.	0.053	0.4	
	5-2	0.232	36.4	
	5-3	0.267	43.4	
	5-4	0.740	138.7	
	5-5	0.880	166.7	
	5-6	0.736	137.8	
	5-7	0.561	102.7	
	5-8	1.967	385.3	
	5-9	2.093	410.7	
	5-10	3.585	710.7	

S1= Standard1 0 cells/10 µL
 S2=Standard2 10.000 cells/µL
 S3=Standard3 30.000 cells/10µL
 S4= Standard4 90.000 cells/10µL
 N.C.= Negative control: sample was not inoculated with *Salmonella* spp.

Table 4

Code Date	Probe	Average O.D. 450 nm	Sample O.D.%	Cells/ 10 µL
	S1	0.047		0
	S2	0.131		10.0
	S3	0.339		30.0
00984252	9-1 N.C.	0.060	3.1	
	9-2	2.749	668.2	
	9-3	0.267	54.4	
	9-4	1.231	292.9	
	9-5	0.166	29.3	
	9-6	1.516	363.3	
	9-7	2.969	722.7	
	9-8	1.921	463.5	
	9-9	3.646	890.1	
00894252	10-1 N.C.	0.050	0.7	
	10-2	1.372	327.7	
	10-3	3.943	963.6	
	10-4	4.063	993.3	
	10-5	3.913	956.2	
	10-6	3.543	864.7	
	10-7	3.803	929.0	
	10-8	3.869	945.3	
	10-9	4.112	1005.4	
	10-10	3.615	882.4	
01904252	11-1 N.C.	0.049	0.5	
	11-2	3.537	863.1	
	11-3	3.874	946.6	
	11-4	3.988	974.8	
	11-5	3.979	972.4	
	11-6	4.049	989.7	
	11-7	3.881	948.2	
	11-8	3.421	834.5	
	11-9	3.984	973.7	
	11-10	3.635	887.3	
00704252	12-1 N.C.	0.051	0.9	
	12-2	2.225	538.6	
	12-3	3.100	755.0	
	12-4	2.251	545.0	
	12-5	0.434	95.7	
	12-6	1.037	244.7	
	12-7	0.340	72.5	
	12-8	0.555	125.5	
	12-9	0.293	60.7	



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