

Performance of the Assurance GDS® Assay for the Detection of *L. monocytogenes* in Pure Cultures and Spiked Food Samples

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Abstract

Listeria monocytogenes is a foodborne pathogen with significant impacts on public health and economy worldwide. Reliable and fast detection of *L. monocytogenes* is of major importance for both diagnostic laboratories and the food industry. The current study evaluated the performance of the Assurance GDS® assay for the detection of *L. monocytogenes* in pure cultures and spiked food samples. In the pure culture experiments, the Assurance GDS® assay for *Listeria monocytogenes* accurately detected the target strains of different serotypes and was correctly negative for a variety of other *Listeria* species. For reliable detection of *L. monocytogenes* in pure culture experiments, colony counts $>10^5$ cfu/ml were required, which emphasizes the need for an adequate enrichment step. The challenge test experiments (steak tartare, bologna type sausage, Gorgonzola cheese) using a one-broth enrichment strategy showed that the Assurance GDS® assay reliably detected *L. monocytogenes* after 16 h of enrichment in Half-Fraser broth, provided that spiking levels of the different matrices were $\geq 10^2$ cfu/g. Depending of the food matrix, longer incubation times of 24 h or 48 h were required when the initial spiking level was $<10^2$ cfu/g, as to be expected in a proportion of naturally contaminated food products. Thus, the Assurance GDS® *Listeria monocytogenes* assay has proven to be a reliable and easy to handle, rapid test system for the specific detection of *L. monocytogenes*. This system is a suitable tool for generating microbiological results used for a "positive batch release", especially for RTE foods with short shelf lives. However, longer enrichment times (24 h or 48 h) are required in a one-broth enrichment strategy, when the contamination level of the food matrix is low ($<10^2$ cfu/g).

Keywords: Assurance GDS® detection system; *Listeria monocytogenes*; Performance; Pure cultures; Spiked food samples; Enrichment times

Introduction

Listeria monocytogenes is an important foodborne pathogen that has significant impacts on public health and economy worldwide. *L. monocytogenes* belongs to the genus *Listeria*, which actually includes 18 further species: *L. aquatica*, *L. booriae*, *L. cornellensis*, *L. denitrificans*, *L. fleischmannii*, *L. floridensis*, *L. grandensis*, *L. grayi*, *L. innocua*, *L. ivanovii*, *L. marthii*, *L. murrayi*, *L. newyorkensis*, *L. riparia*, *L. rocourtiae*, *L. seeligeri*, *L. weihenstephanensis*, and *L. welshimeri* (<http://www.bacterio.net>). *L. monocytogenes* has the potential to cause serious and life-threatening conditions (including septicemia, meningitis, meningoencephalitis, and abortion) in persons with reduced immunity [1]. In the European Union, a total of 2.161 confirmed human cases of listeriosis (notification rate of 0.52 cases per 100.000 population) were reported in 2014 [2]. Ready-to-eat (RTE) foods seem to cause the majority of human *L. monocytogenes* infections and RTE products have also been implicated in large-scale outbreaks [3-6]. *Listeria* spp. are widely distributed in the environment and certain strains may become established and persist in the processing environment [7-9].

Detection of *L. monocytogenes* traditionally involves culture methods including selective enrichment and plating, followed by the biochemical identification of presumptive *L. monocytogenes* colonies. Using culture-based techniques, as e.g. ISO/EN 11290-1 [10], it takes up to one week until the identification is completed. However, there is

a growing need for rapid tests generating results comparable to standard methods. Such rapid tests are of special importance for products with short shelf lives and (real-time) batch releases by food processing companies. Hence, several immunological and molecular biological methods have been developed [11]. Commercially available rapid molecular detection systems for *L. monocytogenes* or *Listeria* spp. include e.g. the Assurance GDS® assays, the GeneQuence® assay, the iQ-Check® kit, the Qualicon BAX® system, or the TaqMan® detection kit. The Assurance GDS® assay thereby combines the PCR approach with a preceding immunomagnetic separation (IMS) step [12,13]. The aim of the present study was (i) to determine the diagnostic specificity and sensitivity of the Assurance GDS® assay for *L. monocytogenes* and (ii) to evaluate the performance of this system for detection of *L. monocytogenes* in selected ready-to-eat food products using a one-broth enrichment strategy.

Materials and Methods

Specificity of the assurance GDS® *L. monocytogenes* assay

To determine the specificity of the Assurance GDS® *L. monocytogenes* kit (Bio Control Systems, Bellevue, WA, USA), pure culture experiments were performed using a collection of seven *L. monocytogenes* strains (serotypes 1/2a, 1/2b, 1/2c, 3a, 3c, 4b, O-group 4 non-motile) and 13 strains of various other *Listeria* species (Table 1). Strains originated from the collection of the Institute for Food Safety and Hygiene (University of Zurich, Switzerland) or were obtained from the Leibniz Institute DSMZ (German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany). Moreover, the serotype 3a

L. monocytogenes strain was obtained from the BC Centre for Disease Control (BCCDC, Vancouver, Canada). After growth of the 20 strains on sheep blood agar (overnight at 37°C; Oxoid, Pratteln, Switzerland), single colonies were inoculated into 10 ml of brain heart infusion broth (BHI; Oxoid) and incubated overnight (16 h) at 37°C. Colony counting (plate count agar, 24 h at 37°C; Oxoid) confirmed that this procedure yielded stationary phase cultures containing about 10⁹ cfu/ml. Subsets of the incubated BHI broth cultures were tested using the Assurance GDS® *L. monocytogenes* (Bio Control Systems) according to the manufacturers' instructions.

Species	Sero-type	Strain designation	Assurance GDS® <i>L. monocytogenes</i> test results ^a
<i>L. monocytogenes</i>	1/2a	N14-2420	+
<i>L. monocytogenes</i>	1/2b	N14-2234	+
<i>L. monocytogenes</i>	1/2c	N14-2232	+
<i>L. monocytogenes</i>	3a	C23 FE8-1	+
<i>L. monocytogenes</i>	3c	N14-0326	+
<i>L. monocytogenes</i>	4b	N14-2079	+
<i>L. monocytogenes</i>	4-nm ^b	N14-0600	+
<i>L. aquatica</i>		DSM 26686	-
<i>L. cornellensis</i>		DSM 26689	-
<i>L. fleischmannii</i>		DSM 24998	-
<i>L. floridensis</i>		DSM 26687	-
<i>L. grandensis</i>		DSM 26688	-
<i>L. grayi</i>		DSM 20601	-
<i>L. innocua</i>		DSM 20649	-
<i>L. ivanovii</i>		DSM 20750	-
<i>L. riparia</i>		DSM 26685	-
<i>L. rocourtia</i>		DSM 22097	-
<i>L. seeligeri</i>		DSM 20751	-
<i>L. weihenstephanensis</i>		DSM 24698	-
<i>L. welshimeri</i>		ATCC 35897	-

^aEach isolate was incubated for 16 h in brain heart infusion (BHI) broth at 37°C; + or - indicates a positive or negative test result; ^bO-group 4 non-motile.

Table 1: Specificity of the Assurance GDS assays for *Listeria monocytogenes* and *Listeria* species.

Detection limit of the Assurance GDS® *L. monocytogenes* assay

For these experiments, stationary phase BHI broth cultures of the seven *L. monocytogenes* strains (prepared as outlined above; about 10⁹ cfu/ml) were 10-fold serially diluted in saline solution (0.85%) to obtain 10 ml broth cultures containing approximately 10³, 10⁴, 10⁵, 10⁶, 10⁷, and 10⁸ cfu/ml. Subsets of each concentration and strain were then tested in triplicate using the Assurance GDS® *L. monocytogenes*

assay (Bio Control Systems) according to the manufacturers' instructions.

Performance of the assurance GDS® *L. monocytogenes* assay in spiked food samples using a one-broth enrichment strategy

In the challenge test experiments, three ready-to-eat (RTE) food products were tested: steak tartare (dish from finely chopped or minced raw beef), bologna type sausage (traditional Swiss cooked sausage), and Gorgonzola cheese (veined Italian blue cheese). Tested food products were obtained from commercial retailers in Switzerland. In the laboratory, food samples were spiked with strain N14-2420 (*L. monocytogenes* serotype 1/2a). This strain was selected due to the frequent occurrence of serotype 1/2a strains in foods and their increasing proportion among human infections [14-18]. For each product type, three different spiking levels were used (10⁴, 10³, and <10² cfu/g). Spiked food samples (two for each spiking level and product type) were then enriched (25 g in 225 ml of Half-Fraser broth; Oxoid) for up to 48 h at 30C. After 16 h of incubation in Half-Fraser broth (Oxoid), the Assurance GDS® *L. monocytogenes* assay (Bio Control Systems) was performed according to the manufacturers' instructions. In the case of a negative result, the Assurance GDS® test was repeated after 24 h and 48 h of incubation. *L. monocytogenes* colony counts of the spiked food samples and the enrichment broths (after 16 h, after 24 h, and if necessary after 48 h) were determined using Chromogenic *Listeria* agar (48 h at 37C) supplemented with *Listeria* selective and differential supplement (Oxoid).

Results and Discussion

The Assurance GDS® *L. monocytogenes* assay reliably detected all target strains in pure culture experiments, whereas isolates of other *Listeria* species yielded negative results (Table 1). Bosilevac et al. [12] reported for the first time specificity results for the Assurance GDS® test kit. However, in the present study, additional *L. monocytogenes* serotypes (3a and 3c) and a variety of recently described *Listeria* species (e.g. *L. aquatica*, *L. cornellensis*, *L. fleischmannii*, *L. floridensis*, *L. grandensis*, *L. riparia*, *L. rocourtia*, *L. weihenstephanensis*) were also included.

To determine the detection limit of the Assurance GDS® *L. monocytogenes* assay, 10-fold serial dilutions of the seven *L. monocytogenes* strains (pure cultures; concentrations: 10³-10⁹ cfu/ml) were tested in triplicate. Detailed evaluation results are shown in Table 2. With the exception of one run of the serotype 3c strain, the Assurance GDS® assay constantly detected the *L. monocytogenes* strains at concentrations ≥ 10⁶ cfu/ml. A more heterogeneous picture was evident at 10⁵ cfu/ml and 10⁴ cfu/ml. Negative Assurance GDS® results in at least one run were observed at 10⁵ cfu/ml for four strains and at 10⁴ cfu/ml for six strains (four of them negative in two or three runs). At 10³ cfu/ml, the Assurance GDS® assay did not detect any of the *L. monocytogenes* strains. Hence, concentrations >10⁵ cfu/ml were required for reliable detection of *L. monocytogenes* using the Assurance GDS® system. This emphasizes the need for an adequate enrichment step (as specified by the manufacturer) when examining food products using this system.

For the challenge test experiments (steak tartare, bologna type sausage, Gorgonzola cheese; two different products of each type), RTE food samples were first spiked with the serotype 1/2a *L.*

monocytogenes strain (three different initial spiking levels) and then enriched in Half-Fraser broth (for up to 48 h).

	Serotype	Assurance GDS® <i>L. monocytogenes</i> test results at different concentrations (cfu/ml)						
		10 ⁹	10 ⁸	10 ⁷	10 ⁶	10 ⁵	10 ⁴	10 ³
N14-2420	1/2a	+/+/+	+/+/+	+/+/+	+/+/+	+/-/-	+/-/-	-/-/-
N14-2234	1/2b	+/+/+	+/+/+	+/+/+	+/+/+	+/-/-	+/-/-	-/-/-
N14-2232	1/2c	+/+/+	+/+/+	+/+/+	+/+/+	+/-/-	-/-/-	-/-/-
C23 FE8-1	3a	+/+/+	+/+/+	+/+/+	+/+/+	+/+/+	+/+/+	-/-/-
N14-0326	3c	+/+/+	+/+/+	+/+/+	+/+/-	+/-/-	-/-/-	-/-/-
N14-2079	4b	+/+/+	+/+/+	+/+/+	+/+/+	+/+/+	+/-/-	-/-/-
N14-0600	4-nmb	+/+/+	+/+/+	+/+/+	+/+/+	+/+/+	+/-/-	-/-/-

^aEach *L. monocytogenes* isolate was incubated for 16 h in brain heart infusion (BHI) broth at 37°C. After decimal serial dilution (10⁹ to 10³ cfu/ml), subsets of each concentration were tested in triplicate in the Assurance GDS® *L. monocytogenes* assay; each + or - indicates a positive or negative test result; ^bO-group 4 non-motile.

Table 2: Detection of *L. monocytogenes* by the Assurance GDS® *L. monocytogenes* assay at different concentrations (cfu/ml).

Product/spiking level	Assurance GDS® results and colony counts (cfu/ml) after enrichment ^a					
	After 16 h		After 24 h		After 48 h	
	GD S	cfu/ml	GD S	cfu/ml	GD S	cfu/ml
Tartare ^b						
<10 ² cfu/g	-	1.9 x 10 ²	+	2.7 x 10 ⁴	nd	nd
	-	1.9 x 10 ²	-	1.5 x 10 ⁴	+	3.0 x 10 ⁵
10 ³ cfu/g	+	2.2 x 10 ⁷	nd	nd	nd	nd
	+	1.4 x 10 ⁷	nd	nd	nd	nd
10 ⁴ cfu/g	+	8.8 x 10 ⁷	nd	nd	nd	nd
	+	9.8 x 10 ⁷	nd	nd	nd	nd
Bologna type sausage ^b						
<10 ² cfu/g	-	2.0 x 10 ²	+	3.0 x 10 ³	nd	nd
	-	9.0 x 10 ¹	+	2.0 x 10 ³	nd	nd
10 ³ cfu/g	+	3.0 x 10 ⁶	nd	nd	nd	nd
	+	7.4 x 10 ⁵	nd	nd	nd	nd
10 ⁴ cfu/g	+	4.2 x 10 ⁷	nd	nd	nd	nd
	+	1.7 x 10 ⁷	nd	nd	nd	nd
Gorgonzola ^b						
<10 ² cfu/g	-	<10 ²	na	2.0 x 10 ²	+	4.2 x 10 ⁴
	-	9.0 x 10 ²	+	1.5 x 10 ³	nd	nd
10 ³ cfu/g	+	1.0 x 10 ⁶	nd	nd	nd	nd
	+	5.4 x 10 ⁵	nd	nd	nd	nd

10 ⁴ cfu/g	+	4.0 x 10 ⁶	nd	nd	nd	nd
	+	2.0 x 10 ⁶	nd	nd	nd	nd

^aEnrichment in Half-Fraser broth for up to 48 h at 30C; + or - indicates a positive or negative test result; nd: not determined; na: no amplification; ^bTwo different products were tested at each inoculation level.

Table 3: Results of the Assurance GDS® assay and colony counts after enrichment of food samples inoculated with *L. monocytogenes* (strain N14-2420, serotype 1/2a) in Half-Fraser broth.

Other studies addressing the performance of the Assurance GDS® test system for detection of *L. monocytogenes* in food products are so far widely lacking. Kerr and Bright [19] evaluated the Assurance GDS® assays for detection of *L. monocytogenes* and *Listeria* spp. in fish and seafood products by testing spiked and non-spiked samples after enrichment (for 18-22 h). These authors showed that the performance of the Assurance GDS® test systems were equivalent to the reference culture method, while being a much faster option.

In the present study, the Assurance GDS® assay reliably detected *L. monocytogenes* after 16 h of enrichment when the initial spiking level of the RTE food samples (steak tartare, bologna type sausage, Gorgonzola cheese) was between 10² and 10⁴ cfu/g. The corresponding *L. monocytogenes* colony counts after 16 h of enrichment ranged from 5.4 x 10⁵ to 9.8 x 10⁷ cfu/ml in the enrichment broth (Table 3). On the other hand, the Assurance GDS® assay for *L. monocytogenes* yielded consistently negative results after 16 h of enrichment when the initial spiking levels were <10² cfu/g and the corresponding colony counts in the enrichment broth after 16 h <10³ cfu/ml (Table 3). In these cases, incubation times of 24 h or even 48 h were required to obtain a positive Assurance GDS® test result. The corresponding *L. monocytogenes* colony counts (24 h or 48 h of enrichment and a positive Assurance GDS® test result) ranged from 1.5 x 10³ to 3.0 x 10⁵ cfu/ml. Thereby it must be considered that the *L. monocytogenes* counts on naturally contaminated (ready-to-eat) food products might vary widely, but frequently low contamination levels (<10² cfu/g) are expected [2,20-23].

Interestingly, one steak tartare sample showing 1.5×10^4 cfu/ml after 24 h of enrichment was still negative in the GDS® Assurance *L. monocytogenes* assay, whereas enriched bologna type sausage and Gorgonzola cheese samples yielded positive test results when colony counts in the enrichment broth were in the range of 1.5×10^3 to 4.2×10^4 cfu/ml (Table 3). A special challenge was thereby the examination of Gorgonzola cheese samples with initial spiking levels $<10^2$ cfu/g. The first two examined Gorgonzola samples yielded negative Assurance GDS® test results for *L. monocytogenes* even after 48 h of enrichment. Determination of colony counts showed the presence of a dominant *Listeria* spp. background flora, which hampered the growth of the spiked *L. monocytogenes*. Examinations were therefore repeated with two additional Gorgonzola samples that gave the results shown in Table 3.

Conclusions

In summary, the Assurance GDS® *Listeria monocytogenes* assay has proven to be a reliable and easy to handle, rapid test system for the specific detection of *L. monocytogenes*. This system is suited as a tool for generating microbiological results, which can be used for a “positive batch release” especially also for RTE foods with short shelf lives. However, in a one-broth enrichment strategy, depending of the food matrix, enrichment times of 24 h or 48 h are required when the initial contamination level of the food matrix is $<10^2$ cfu/g.

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