CERTIFICATION

AOAC Research Institute
Performance Tested Methods℠

Certificate No.

061901

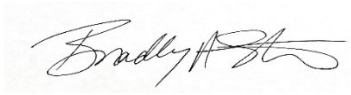
The AOAC Research Institute hereby certifies the method known as

Foodproof® *Vibrio* Detection LyoKit, with **food**proof® StarPrep Three Kit

manufactured by

Hygiena Diagnostics GmbH
Hermannswerder 17
14473 Potsdam, Germany

This method has been evaluated and certified according to the policies and procedures of the AOAC *Performance Tested Methods*℠ Program. This certificate indicates an AOAC Research Institute Certification Mark License Agreement has been executed which authorizes the manufacturer to display the AOAC Research Institute *Performance Tested Methods*℠ certification mark on the above-mentioned method for the period below. Renewal may be granted by the Expiration Date under the rules stated in the licensing agreement.



Bradley A. Stawick, AOAC Research Institute Senior Director

Issue Date December 22, 2025

Expiration Date December 31, 2026

2275 Research Blvd., Ste. 300, Rockville, Maryland, USA Telephone: +1-301-924-7077 www.aoac.org

AUTHORS
Hanna Hartenstein, Benjamin Junge, Cordt Grönewald, Kornelia Berghof-Jäger

SUBMITTING COMPANY
BIOTECON Diagnostics GmbH
Hermannswerder 17
14473 Potsdam, Germany

CURRENT SPONSOR
Hygiena Diagnostics GmbH
Hermannswerder 17
14473 Potsdam, Germany

METHOD NAME
foodproof® *Vibrio* Detection LyoKit, with **food**proof® StarPrep Three Kit
Formerly **food**proof® *Vibrio* Detection LyoKit, 5'Nucelease with **food**proof® StarPrep Three Kit

CATALOG NUMBERS
KIT 2301 17; KIT 2301 18; KIT 2301 87

INDEPENDENT LABORATORY
Q Laboratories, Inc.
1400 Harrison Avenue
Cincinnati, Ohio 45214-1606

APPLICABILITY OF METHOD
Analytes – *Vibrio* species: *Vibrio parahaemolyticus*, *Vibrio vulnificus* and *Vibrio cholerae.*

Matrixes
quantitative: raw oysters (12 animals pooled), cooked octopus (50 g), raw scallops (12 animals pooled), raw fish (50g)

qualitative: raw oysters (25 g), cooked octopus (25 g), raw shrimp (central portion, 25 g), raw scallops (25 g), raw fish (25 g)

Performance claims – Performance was found to be equivalent to that of the U.S. Food and Drug Administration *Bacteriological Analytical Manual* (BAM) chapter 9, *Vibrio* (2) reference culture methods for all matrixes (raw oysters, cooked octopus, raw shrimp, raw scallops and raw fish) in the internal and the external method comparison.

ORIGINAL CERTIFICATION DATE
June 03, 2019

CERTIFICATION RENEWAL RECORD
Renewed through December 2026.

METHOD MODIFICATION RECORD
1. **February 2022 Level 1**
2. **November 2024 Level 1**

SUMMARY OF MODIFICATION
1. **Rebranding to include Hygiena, editing, and formatting changes to inserts and labeling.**
2. **Editorial changes and removal of 5'Nuclease from method title.**

Under this AOAC *Performance Tested Methods℠* License Number, 061901 this method is distributed by:
NONE

Under this AOAC *Performance Tested Methods℠* License Number, 061901 this method is distributed as:
NONE

PRINCIPLE OF THE METHOD (1)
The method describes the detection of *Vibrio cholerae* with the toxin gene *ctx*, *Vibrio parahaemolyticus* with the toxin genes *tdh1, trh1* and *trh2* and *Vibrio vulnificus* with the **food**proof® *Vibrio* Detection LyoKit in food. The **food**proof StarPrep Three Kit in combination with reagent D is used for DNA isolation from enriched food samples. The **food**proof *Vibrio* Detection LyoKit detects *Vibrio* specific DNA by means of real-time PCR using 5´Nuclease- (Taqman-) based instruments.

DISCUSSION OF THE VALIDATION STUDY (1)
The data demonstrate no statistical difference for the detection of *Vibrio* species in with the **food**proof *Vibrio* detection LyoKit in combination with **food**proof StarPrep Three and Reagent D in the method comparison part. The specificity of the **food**proof *Vibrio* detection LyoKit could be shown with the Inclusivity and Exclusivity studies. The DNA isolates have shown equivalent results on different cyclers (LightCycler 480, ARIA MX qPCR System, LightCycler 96, CFX 96 and ABI 7500 fast). For *V. vulnificus* and *V. parahaemolyticus*, an enrichment of 25 g in APW 1:10 was made additionally to the requirements of an MPN study according to BAM Chapter 9. For raw shrimp it was shown that *V. cholerae* could be also detected after an enrichment of 6-8 h with the **food**proof *Vibrio* detection LyoKit. With the microbiological reference method *V. cholerae* could not be detected after 6-8 h in all PCR positive samples, but after an enrichment of 18-24 h, all samples that gave positive PCR results could be confirmed as positive. This indicates a higher sensitivity for the detection with the **food**proof *Vibrio* detection LyoKit. The DNA extraction in single reaction tube and in 8-strip gave comparable results as shown in the qualitative method comparison part. Cooked octopus spiked with *V. parahaemolyticus* was analyzed in the method developer and the independent study with the qualitative method and the MPN method. Both studies gave comparable results.

Table 3. Inclusivity tests were performed on the LightCycler 480, ARIA MX qPCR System, LightCycler 96, CFX 96 and ABI 7500 fast with equivalent results. All species and toxin genes were be detected correct with the foodproof *Vibrio* Detection LyoKit. (1)

Inclusivity List - *Vibrio parahaemolyticus*									
Strain description			**PCR results**						
Source/Strain Number	Origin	tox genes	Vp	Vv	Vc	*tdh*	*trh 1*	*trh 2*	*ctx*
V06/016	Clinical Strain, Vietnam, CEFAS	tdh +	+	-	-	+	-	-	-
V05/064	Clinical Strain, CEFAS	tdh +	+	-	-	+	-	-	-
CCUG 43362	Human diarrhea, India	tdh +	+	-	-	+	-	-	-
CCUG 43363	unknown	tdh +	+	-	-	+	-	-	-
V05/012	Clinical Strain, CEFAS	tdh +	+	-	-	+	-	-	-
V05/062	Clinical Strain, CEFAS	tdh +	+	-	-	+	-	-	-
V05/063	Clinical Strain, CEFAS	tdh +	+	-	-	+	-	-	-
BCD 16558	clinical strain	tdh +, trh +	+	-	-	+	+	-	-
V05/014	Clinical Strain, CEFAS	tdh +, trh +	+	-	-	+	+	-	-
CCUG 43364	unknown	trh1 +	+	-	-	-	+	-	-
V05/070	*Mytilus edulis*, CEFAS	trh +	+	-	-	-	+	-	-
ATCC 17802	Shirasu food poisoning, Japan	trh2 +	+	-	-	-	-	+	-
CCUG 43365	unknown	trh2 +	+	-	-	-	-	+	-
DSM 10027	invertebrate	trh2 +	+	-	-	-	-	+	-
i 712	unknown	trh +	+	-	-	-	-	+	-
i.809, IRTA	unknown	trh +	+	-	-	-	-	+	-
ISS 521, IRTA	mussels	trh2 +	+	-	-	-	-	+	-
ISS 542	water	trh2 +	+	-	-	-	-	+	-
ISS 915	mussels	trh2 +	+	-	-	-	-	+	-
V05/018	Clinical Strain, CEFAS	trh +	+	-	-	-	-	+	-
FU697	water Baltic Sea	trh2 +	+	-	-	-	-	+	-
FU759	water Baltic Sea	trh2 +	+	-	-	-	-	+	-
BCD 16557	unknown	/	+	-	-	-	-	-	-
CECT 612	unknown	/	+	-	-	-	-	-	-
FU1121	blue mussels	/	+	-	-	-	-	-	-
FU1519	King Prawns	/	+	-	-	-	-	-	-
FU506	blue mussels	/	+	-	-	-	-	-	-
FU544	blue mussels	/	+	-	-	-	-	-	-
FU545	shrimps	/	+	-	-	-	-	-	-
FU546	shrimps	/	+	-	-	-	-	-	-
FU549	shrimps	/	+	-	-	-	-	-	-
FU551	shrimps	/	+	-	-	-	-	-	-
FU552	shrimps	/	+	-	-	-	-	-	-
FU553	shrimps	/	+	-	-	-	-	-	-
FU554	shrimps	/	+	-	-	-	-	-	-
FU555	shrimps	/	+	-	-	-	-	-	-
FU558	shrimps	/	+	-	-	-	-	-	-
FU560	shrimps	/	+	-	-	-	-	-	-
FU562	shrimps	/	+	-	-	-	-	-	-
FU563	shrimps	/	+	-	-	-	-	-	-
FU564	shrimps	/	+	-	-	-	-	-	-
FU571	shrimps	/	+	-	-	-	-	-	-
FU574	shrimps	/	+	-	-	-	-	-	-
FU575	shrimps	/	+	-	-	-	-	-	-
FU606	shrimps	/	+	-	-	-	-	-	-
FU608	shrimps	/	+	-	-	-	-	-	-
FU609	shrimps	/	+	-	-	-	-	-	-
FU678	shrimps	/	+	-	-	-	-	-	-
FU694	water Baltic Sea	/	+	-	-	-	-	-	-
FU695	water Baltic Sea	/	+	-	-	-	-	-	-
FU696	water Baltic Sea	/	+	-	-	-	-	-	-
FU698	water Baltic Sea	/	+	-	-	-	-	-	-
FU699	water Baltic Sea	/	+	-	-	-	-	-	-

FU700	water Baltic Sea	/	+	-	-	-	-	-	-
FU701	water Baltic Sea	/	+	-	-	-	-	-	-
FU702	shrimps	/	+	-	-	-	-	-	-
FU703	shrimps	/	+	-	-	-	-	-	-
FU704	shrimps	/	+	-	-	-	-	-	-
FU705	shrimps	/	+	-	-	-	-	-	-
FU712	shrimps	/	+	-	-	-	-	-	-
FU760	water Baltic Sea	/	+	-	-	-	-	-	-
FU761	water Baltic Sea	/	+	-	-	-	-	-	-
FU764	water Baltic Sea	/	+	-	-	-	-	-	-
FU765	water Baltic Sea	/	+	-	-	-	-	-	-
FU766	water Baltic Sea	/	+	-	-	-	-	-	-
FU767	water Baltic Sea	/	+	-	-	-	-	-	-
ISS 1005	mussels	/	+	-	-	-	-	-	-
ISS 967	mussels	/	+	-	-	-	-	-	-
CEFAS V05/022	Environmental strain	/	+	-	-	-	-	-	-
CEFAS V05/023	Environmental strain	/	+	-	-	-	-	-	-
CEFAS V05/024	Environmental strain	/	+	-	-	-	-	-	-
CEFAS V05/024	Environmental strain	/	+	-	-	-	-	-	-
CEFAS V12/033	Environmental strain	/	+	-	-	-	-	-	-

Inclusivity List - *Vibrio cholerae*									
Strain description	**PCR results**								
Source/Strain Number	Origin	tox genes	Vp	Vv	Vc	*tdh*	*trh 1*	*trh 2*	*ctx*
ATCC 14034 (*V. cholerae O1)*	from cholera epidemic in Egypt	ctx +	-	-	+	-	-	-	+
ATCC 14103 (V. cholerae O1)	Clinical specimen - human	ctx AB+	-	-	+	-	-	-	+
CCUG 9118 (V. cholerae O1)	unknown	ctx +	-	-	+	-	-	-	+
CCUG 34707 (V. cholerae O139)	unknown	ctx +	-	-	+	-	-	-	+
ISS 14 (V. cholerae O1/139)	human isolate	ctx AB +	-	-	+	-	-	-	+
ISS 561 (V. cholerae O1/139)	water	ctx AB +	-	-	+	-	-	-	+
FU1599	unknown	/	-	-	+	-	-	-	-
FU371	unknown	/	-	-	+	-	-	-	-
FU373	unknown	/	-	-	+	-	-	-	-
FU407	unknown	/	-	-	+	-	-	-	-
FU572	unknown	/	-	-	+	-	-	-	-
FU573	lake water	/	-	-	+	-	-	-	-
FU579	shrimps	/	-	-	+	-	-	-	-
FU659	shrimps	/	-	-	+	-	-	-	-
FU711	shrimps	/	-	-	+	-	-	-	-
FU733	shrimps	/	-	-	+	-	-	-	-
FU735	Baltic Sea	/	-	-	+	-	-	-	-
FU736	Baltic Sea	/	-	-	+	-	-	-	-
FU738	Baltic Sea	/	-	-	+	-	-	-	-
FU739	Baltic Sea	/	-	-	+	-	-	-	-
FU740	Baltic Sea	/	-	-	+	-	-	-	-
FU748	Baltic Sea	/	-	-	+	-	-	-	-
FU749	Baltic Sea	/	-	-	+	-	-	-	-
FU751	Baltic Sea	/	-	-	+	-	-	-	-
FU874	Baltic Sea	/	-	-	+	-	-	-	-
FU875	Baltic Sea	/	-	-	+	-	-	-	-
FU876	Baltic Sea	/	-	-	+	-	-	-	-
FU877	blue mussels	/	-	-	+	-	-	-	-
FU880	blue mussels	/	-	-	+	-	-	-	-
FU881	blue mussels	/	-	-	+	-	-	-	-
FU882	blue mussels	/	-	-	+	-	-	-	-
FU883	blue mussels	/	-	-	+	-	-	-	-
FU884	blue mussels	/	-	-	+	-	-	-	-
FU885	blue mussels	/	-	-	+	-	-	-	-

FU886	blue mussels	/	-	-	+	-	-	-	-
FU887	blue mussels	/	-	-	+	-	-	-	-
FU888	blue mussels	/	-	-	+	-	-	-	-
FU889	blue mussels	/	-	-	+	-	-	-	-
FU890	blue mussels	/	-	-	+	-	-	-	-
FU891	blue mussels	/	-	-	+	-	-	-	-
FU892	blue mussels	/	-	-	+	-	-	-	-
FU893	blue mussels	/	-	-	+	-	-	-	-
FU894	blue mussels	/	-	-	+	-	-	-	-
FU895	blue mussels	/	-	-	+	-	-	-	-
FU896	blue mussels	/	-	-	+	-	-	-	-
FU897	shrimps	/	-	-	+	-	-	-	-
FU898	blue mussels	/	-	-	+	-	-	-	-
FU912	blue mussels	/	-	-	+	-	-	-	-
ISS 932	blue mussels	/	-	-	+	-	-	-	-

Inclusivity List - *Vibrio vulnificus*

Strain description			**PCR results**						
Source/Strain Number	Origin	tox genes	Vp	Vv	Vc	tdh	trh 1	trh 2	ctx
ATCC 27562	Human blood, Florida, United States	/	-	+	-	-	-	-	-
ATCC 33149	Diseased eels, *Anguilla japonica*, Japan	/	-	+	-	-	-	-	-
ATCC 33815	Leg ulcer, Wisconsin	/	-	+	-	-	-	-	-
BCD 16559	unknown	/	-	+	-	-	-	-	-
BCD 16560	unknown	/	-	+	-	-	-	-	-
CCM 2838	Corneal ulcer	/	-	+	-	-	-	-	-
CCM 2839	Blood.	/	-	+	-	-	-	-	-
CECT 4863	Leg wound, Rhode Island; Biotype 2	/	-	+	-	-	-	-	-
CECT 4865	Vibriosis in shrimps, Taiwan; Serovar E	/	-	+	-	-	-	-	-
CECT 4866	Human blood, Australia; Serovar E	/	-	+	-	-	-	-	-
CECT 4867	Diseased eel; Serovar E	/	-	+	-	-	-	-	-
CECT 4868	Diseased eel, Norway; Serovar E	/	-	+	-	-	-	-	-
CECT 4869	Diseased eel, Belgium	/	-	+	-	-	-	-	-
CECT 5164	Human septicemia, US	/	-	+	-	-	-	-	-
CECT 5165	Sea water, US	/	-	+	-	-	-	-	-
CECT 5167	Human blood, US	/	-	+	-	-	-	-	-
CECT 5763	Tank water, Spain	/	-	+	-	-	-	-	-
ISS 946	mussels	/	-	+	-	-	-	-	-
CEFAS V06/014	Clinical Strain, Year unknown, Japan	/	-	+	-	-	-	-	-
CEFAS V06/015	Clinical Strain, Bt3, Israel	/	-	+	-	-	-	-	-
CEFAS V11/016	Environmental strain, Gulf of Mexico, USA	/	-	+	-	-	-	-	-
CEFAS V11/017	Environmental strain, Gulf of Mexico, USA	/	-	+	-	-	-	-	-
CEFAS V11/018	Clinical Strain, Gulf of Mexico, USA	/	-	+	-	-	-	-	-
CEFAS V12/001	Environmental strain, 2012, France (English Channel)	/	-	+	-	-	-	-	-
CEFAS V12/002	Environmental strain, 2012, France (Atlantic Coast)/ Mussels	/	-	+	-	-	-	-	-
GCSL K4567	Human Clinical; source unknown	/	-	+	-	-	-	-	-
GCSL K4572	Human Clinical; Blood	/	-	+	-	-	-	-	-

GCSL K4574	Human Clinical; Blood	/	-	+	-	-	-	-	-
GCSL K4633	Human Clinical; Blood	/	-	+	-	-	-	-	-
GCSL K4767	Human Clinical; Blood	/	-	+	-	-	-	-	-
GCSL K4776	Human Clinical; source unknown	/	-	+	-	-	-	-	-
GCSL K4778	Human Clinical; source unknown	/	-	+	-	-	-	-	-
GCSL K5008	Human Clinical; Blood	/	-	+	-	-	-	-	-
GCSL K5041	Human Clinical; Blood	/	-	+	-	-	-	-	-
GCSL VVR42-D10	Oyster, Louisiana, USA	/	-	+	-	-	-	-	-
GCSL VVR51-A12	Oyster, Louisiana, USA	/	-	+	-	-	-	-	-
GCSL VVR51-E9	Oyster, Louisiana, USA	/	-	+	-	-	-	-	-
GCSL VVR27-C9	Oyster, Florida, USA	/	-	+	-	-	-	-	-
GCSL VVR30-C10	Oyster, Texas, USA	/	-	+	-	-	-	-	-
GCSL VVR57-B10	Oyster, Louisiana, USA	/	-	+	-	-	-	-	-
GCSL VVR59-B3	Oyster, Louisiana, USA	/	-	+	-	-	-	-	-
GCSL VVR80-G3	Oyster, Alabama, USA	/	-	+	-	-	-	-	-
GCSL VVR80-G5	Oyster, Louisiana, USA	/	-	+	-	-	-	-	-
GCSL VVR80-H6	Oyster, Louisiana, USA	/	-	+	-	-	-	-	-
GCSL VVR81-C6	Oyster, Louisiana, USA	/	-	+	-	-	-	-	-
GCSL VVR84-F1	Oyster, Virginia, USA	/	-	+	-	-	-	-	-
GCSL VVR84-F4	Oyster, Virginia, USA	/	-	+	-	-	-	-	-
GCSL VVR73-C11	Oyster, Delaware, USA	/	-	+	-	-	-	-	-
GCSL VVR60-F9	Oyster, New Jersey, USA	/	-	+	-	-	-	-	-
GCSL VVR98-C11	Oyster, Louisiana, USA	/	-	+	-	-	-	-	-

Vp: *Vibrio parahaemolyticus*, Vv: *Vibrio vulnificus*, Vc: *Vibrio cholera*, *tdh*: *thermostable direct hemolysin*, *trh1* and *trh*2: *tdh-related hemolysin*, *ctx*: *cholera toxin /:* tox genes are not present, +: positive PCR result for a target, -:,negative PCR result for a target

Strain collections:

ATCC: American Type Culture Collection, BCD: BIOTECON Diagnostics Strain Collection

CCM: Czech Collection of Microorganisms, CCUG: Culture Collection, University of Göteborg, Sweden, CECT: Spanish Type Culture Collection, FU: Free University of Berlin Collection, DSM: Deutsche Sammlung von Mikroorganismen (German Collection of Microorganisms), IRTA: Institute of Agrifood Research and Technology, ISS: Instituto Superiore di Sanità, CEFAS: Centre for Environment Fisheries and Aquaculture Science, UK, GCSL:'s Gulf Coast Seafood Laboratory, Alabama

Table 4 Exclusivity tests were performed on the LightCycler 480, ARIA MX qPCR System, LightCycler 96, CFX 96 and ABI 7500 fast with equivalent results. All strains tested for Exclusivity gave negative result for all targets with the foodproof *Vibrio* Detection LyoKit. (1)

Exclusivity List										
Strain description				**PCR results**						
Organism	Serotype	Source/ Strain Number	Origin	Vp	Vv	Vc	*tdh*	*trh 1*	*trh 2*	*ctx*
Vibrio	*aestuarianus*	FU878	Blue mussel	-	-	-	-	-	-	-
Vibrio	*alginolyticus*	DSM 2171	spoiled horse mackerel, causing food poisoning	-	-	-	-	-	-	-
Vibrio	*alginolyticus*	DSM 2172	Mussel	-	-	-	-	-	-	-
Vibrio	*alginolyticus*	FU366	unknown	-	-	-	-	-	-	-
Vibrio	*alginolyticus*	FU368	unknown	-	-	-	-	-	-	-
Vibrio	*alginolyticus*	FU716	shrimps	-	-	-	-	-	-	-
Vibrio	*alginolyticus*	FU374	unknown	-	-	-	-	-	-	-
Vibrio	*alginolyticus*	FU393	unknown	-	-	-	-	-	-	-
Vibrio	*alginolyticus*	FU394	unknown	-	-	-	-	-	-	-
Vibrio	*alginolyticus*	FU398	unknown	-	-	-	-	-	-	-
Vibrio	*alginolyticus*	FU399	unknown	-	-	-	-	-	-	-
Vibrio	*alginolyticus*	FU403	unknown	-	-	-	-	-	-	-
Vibrio	*alginolyticus*	FU418	unknown	-	-	-	-	-	-	-
Vibrio	*anguillarum*	DSM 11323	unknown	-	-	-	-	-	-	-
Vibrio	*campbelli*	ISS 983	Mussel, Spain	-	-	-	-	-	-	-
Vibrio	*campbelli*	CCM 2582	unknown	-	-	-	-	-	-	-
Vibrio	*fluvialis*	ATCC 33809	Human feces, Dacca, Bangladesh	-	-	-	-	-	-	-
Vibrio	*furnissii*	CCM 3696	unknown	-	-	-	-	-	-	-
Vibrio	*harveyi*	DSM 6904	seawater	-	-	-	-	-	-	-
Vibrio	*harveyi*	FU1496	shrimps	-	-	-	-	-	-	-
Vibrio	*mediterranei*	CCM 3961	unknown	-	-	-	-	-	-	-
Vibrio	*metschnikovii*	FU782	shrimps	-	-	-	-	-	-	-
Vibrio	*metschnikovii*	BCD 5889	unknown	-	-	-	-	-	-	-
Vibrio	*metschnikovii*	CCM 7098	unknown	-	-	-	-	-	-	-
Vibrio	*mimicus*	ATCC 33653	Ear, North Carolina, USA	-	-	-	-	-	-	-
Vibrio	*mimicus*	FU409	unknown	-	-	-	-	-	-	-
Vibrio	*mimicus*	FU410	unknown	-	-	-	-	-	-	-
Vibrio	*mimicus*	FU412	unknown	-	-	-	-	-	-	-
Vibrio	*mimicus*	FU413	unknown	-	-	-	-	-	-	-
Vibrio	*natriegens*	CCM 2575	unknown	-	-	-	-	-	-	-
Vibrio	*ordalii*	CCM 3553	unknown	-	-	-	-	-	-	-
Vibrio	*proteolyticus*	DSM 30189	intestine of *Limnoria tripunctata*	-	-	-	-	-	-	-
Vibrio	*sp.*	V05/011, CEFAS	Clinic isolate, Norway	-	-	-	-	-	-	-
Acinetobacter	*lwoffii*	BCD 15660	Isolate BCD	-	-	-	-	-	-	-
Alteromonas	*macleodii*	DSM 6062	seawater	-	-	-	-	-	-	-
Arthrobacter	*crystallopoietes*	CCM 2386	unknown	-	-	-	-	-	-	-
Bacillus	*badius*	B-2412, BCD 2929	unknown	-	-	-	-	-	-	-
Bacillus	*subtilis*	DSM 10	unknown	-	-	-	-	-	-	-
Brochothrix	*thermosphacta*	DSM 20171	fresh pork sausage	-	-	-	-	-	-	-
Carnobacterium	*piscicola*	DSM 20730	diseased rainbow trout	-	-	-	-	-	-	-
Citrobacter	*freundii*	DSM 30040	feces	-	-	-	-	-	-	-
Clostridium	*sporogenes*	ATCC 19404	Gas gangrene	-	-	-	-	-	-	-
Cronobacter	*sakazakii*	DSM 4485	child's throat	-	-	-	-	-	-	-
Edwardsiella	*tarda*	DSM 30052	human feces	-	-	-	-	-	-	-
Escherichia	*coli*	DSM 30083	urine	-	-	-	-	-	-	-
Klebsiella	*pneumoniae*	ATCC 13883	unknown	-	-	-	-	-	-	-
Kurthia	*zopfii*	DSM 20580	unknown	-	-	-	-	-	-	-
Lactobacillus	*delbrueckii sub.*	DSM 20074	sour grain mash	-	-	-	-	-	-	-
Lactobacillus	*hilgardii*	DSM 20051	wine	-	-	-	-	-	-	-
Lactococcus	*garvieae*	DSM 20385	raw milk	-	-	-	-	-	-	-

Microbacterium	*paraoxydans*	BCD 16120	unknown	-	-	-	-	-	-	-
Micrococcus	*luteus*	DSM 20030	unknown	-	-	-	-	-	-	-
Paracoccus	*denitrificans*	DSM 413	unknown	-	-	-	-	-	-	-
Pediococcus	*parvulus*	CECT 4795	unknown	-	-	-	-	-	-	-
Plesiomonas	*shigelloides*	DSM 8224	dog feces	-	-	-	-	-	-	-
Proteus	*sp.*	FU734	Baltic Sea	-	-	-	-	-	-	-
Proteus	*vulgaris*	DSM 2140	inner ear infection	-	-	-	-	-	-	-
Rhodococcus	*erythropolis*	DSM 312	soil	-	-	-	-	-	-	-
Salmonella	*Senftenberg*	ATCC 43845	unknown	-	-	-	-	-	-	-
Salmonella	*enteritidis*	BgVV 2627/00	Chicken isolate	-	-	-	-	-	-	-
Serratia	*marcescens*	DSM 1636	unknown	-	-	-	-	-	-	-
Shigella	*sonnei*	BCD 7889	unknown	-	-	-	-	-	-	-
Sporosarcina	*ureae*	DSM 2281	unknown	-	-	-	-	-	-	-
Staphylococcus	*aureus*	ATCC 6538	Human lesion	-	-	-	-	-	-	-
Stenotrophomona	*sp.*	V05/039-2, CEFAS	Environmental strain; France	-	-	-	-	-	-	-
Yersinia	*enterocolitica*	DSM 4780	glanders-like infection of face	-	-	-	-	-	-	-
Yersinia	*ruckeri*	ATCC 29473	Rainbow trout; USA	-	-	-	-	-	-	-

ATCC: American Type Culture Collection, BCD: BIOTECON Diagnostics Strain Collection, BgVV: Bundesinstitut für gesundheitlichen Verbraucherschutz und Veterinärmedizin (existed until 2002)
CCM: Czech Collection of Microorganisms, CCUG: Culture Collection, University of Göteborg, Sweden, CECT: Spanish Type Culture Collection, FU: Free University of Berlin Collection, DSM: Deutsche Sammlung von Mikroorganismen (German Collection of Microorganisms)

Table 6. Results of the Method Comparison Study for the qualitative detection of *Vibrio* species with the candidate method (DNA-extraction in Eppendorf tube and 8-strips) and the reference method. The PCRs were done on the LightCycler 480, ARIA MX qPCR System, LightCycler 96, CFX 96 and ABI 7500 fast with equivalent results. (1)

				Candidate method StarPrep Three tubes/ 8-strips					Reference method	
samples				presumptive		confirmed				
Matrix 25 g	**Serotype**	**Inoculation**	**N[1]**	**X[2]**	**POD_{CP}[3]**	**X**	**POD_{CC}[4]**	**$dPOD_{CP}$[5]**	**X**	**POD_R[6]**
Raw shrimp (6 - 8 h)	*Vibrio cholerae*, *ctx* positive ATCC 14034	uncontaminated	5	0	0.00	0	0.00	0.00	0	0.00
		low	20	15	0.75	15	0.75	0.00	9	0.45
		high	5	5	1.00	5	1.00	0.00	5	1.00
Raw shrimp (16 -18 h)		uncontaminated	5	0	0.00	0	0.00	0.00	0	0.00
		low	20	15	0.75	15	0.75	0.00	15	0.75
		high	5	5	1.00	5	1.00	0.00	5	1.00
Raw oysters (16 -18h)	*Vibrio parahaemo-lyticus tdh* positive CCUG 43362	uncontaminated	5	0	0.00	0	0.00	0.00	0	0.00
		low	20	10	0.50	10	0.50	0.00	10	0.50
		high	5	5	1.00	5	1.00	0.00	5	1.00
Cooked octopus	*Vibrio parahaemo-lyticus trh* 1 positive CCUG 43364	uncontaminated	5	0	0.00	0	0.00	0.00	0	0.00
		low	20	15	0.75	15	0.75	0.00	15	0.75
		high	5	5	1.00	5	1.00	0.00	5	1.00
Raw scallops	*Vibrio parahaemo-lyticus trh* 2 positive DSM 10027	uncontaminated	5	0	0.00	0	0.00	0.00	0	0.00
		low	20	12	0.60	12	0.60	0.00	12	0.60
		high	5	5	1.00	5	1.00	0.00	5	1.00
Raw fish	*Vibrio vulnificus* CECT 4865	uncontaminated	5	0	0.00	0	0.00	0.00	0	0.00
		low	20	15	0.75	15	0.75	0.00	15	0.75
		high	5	5	1.00	5	1.00	0.00	5	1.00
Cooked octopus*	*Vibrio parahaemo-lyticus trh 1* positive CCUG 43364	uncontaminated	5	0	0.00	0	0.00	0.00	0	0.00
		low	20	9	0.50	9	0.50	0.00	9	0.50
		high	5	5	1.00	5	1.00	0.00	5	1.00

* results of the independent laboratory
[1]N = Number of test potions
[2]x = Number of positive test portions
[3]POD_{CP} = Candidate method presumptive positive outcomes
[4]POD_{CC} = Candidate method confirmed positive outcomes
[5]$dPOD_{CP}$ = Difference between the candidate method presumptive result and candidate method confirmed result POD values
[6]POD_R = Reference method

Table 7. Average MPN values obtained for the different matrixes in the quantitative study. (1)

	average MPN/level PCR			average MPN/level BAM MPN			*Microbio-logical load CFU/ml
Matrixes	**low level**	**medium level**	**high level**	**low level**	**medium level**	**high level**	
Raw scallops	0.18	0.48	37.2	0.18	0.48	37.2	4.7×10^5
Raw oysters	0.74	9.1	61	0.74	9.1	61	2×10^2
Cooked octopus	0.14	2.24	14.24	0.14	2.24	14.24	1.3×10^3
Raw fish	4.1	25.56	110	4.1	25.56	110	1×10^5
Cooked octopus*	0.7	3.1	13.72	0.7	3.1	13.72	< 10

*result from the independent laboratory

REFERENCES CITED

1. Hartenstein, H., Junge, B, Grönewald, C., Berghof-Jäger, K., BIOTECON Diagnostics foodproof® *Vibrio* Detection LyoKit, in combination with **food**proof® StarPrep Three, AOAC *Performance Tested Methods*℠ certification number 061901. Approved June 2019
2. U.S. Food and Drug Administration (2004) *Bacteriological Analytical Manual.* Chapter 9. *Vibrio*. https://www.fda.gov/food/foodscienceresearch/laboratorymethods/ucm070830.htm (accessed May 9, 2019)
3. *Official Methods of Analysis* (2019). 21st Ed. Appendix J. AOAC INTERNATIONAL. Gaithersburg. MD. http://www.eoma.aoac.org/app_j.pdf (accessed May 9, 2019)
4. Probability of Detection (POD) as a Statistical Model for the Validation of qualitative Methods, Wehling P., LaBudde RA, Brunelle SL, Nelson MT.: Journal of AOAC International Vol. 94, No 1 (2011)
5. VibrioNet Europe. http://www.vibrionet.de (accessed May 9, 2019)
6. U.S. Food and Drug Administration. (2015) *Bacteriological Analytical Manual*. Appendix 2: Most Probable Number from Serial Dilutions https://www.fda.gov/food/foodscienceresearch/laboratorymethods/ucm109656.htm (accessed May 9, 2019)