BioChek

BIOCHEK, SMART VETERINARY DIAGNOSTICS

DNA

Validation of Real-Time PCR Reagents to Identify Salmonella spp. DNA in Enriched Cultures

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Introduction

The National Poultry Improvement Plan (NPIP) provides a cooperative program through which new rapid diagnostic methods for **Salmonella** can be effectively applied to the continuous improvement of poultry and poultry products throughout the country. Additionally, the Association Française de Normalisation (AFNOR) Group develops international standardization, information provision, certification and training through a network of key partners throughout Europe for the improvement of **Salmonella** testing in food, environmental and primary production samples. Current NPIP and AFNOR bacteriological reference methods [1,3] for detecting **Salmonella** species in environmental samples, cloacal swabs and hatchery samples are accurate and sensitive, but can be time-consuming and laborious.

Our aim is to provide NPIP-authorized microbiology laboratories with an approved and cost-efficient **Salmonella** species rapid alternative testing method, allowing these laboratories to:

- Effectively help feed mills, broiler breeder farms, layer breeders, commercial layers/broilers, hatcheries and processing plants to closely monitor, control and react to emerging Salmonella in poultry and poultry products.
- Meet regulatory requirements.
- Support streamlining operational processes.

Following an ISO 16140-2 culture method comparison study, consecutive AFNOR interlaboratory and NPIP field trial studies were conducted to determine the performance of the BioChek Salmonella qPCR Reagents against two reference methods for **Salmonella** testing.

Materials and Methods

NPIP Field Trial Study

Poultry house environmental samples (boot swabs and dust, n = 368) were tested at four NPIP-authorized laboratories against the NPIP culture methods [2] for **Salmonella** using the Salmonella qPCR Reagents (BioChek). Each laboratory collected ≥25 positive and ≥50 negative samples.

AFNOR Interlaboratory Study (ISO 16140-2:2016) [3]

Samples were provided by ADRIA expert laboratory (Quimper, France) and tested by 10 laboratories (collaborators) comparing Salmonella spp qPCR Reagents against ISO 6579-1 [4]: Each laboratory tested 24 identical samples (contaminated ground beef, **Salmonella Typhimurium** A00C060) at the same time, comprising three inoculation levels: 0 CFU/25 g (L0), ~ 2 CFU/25 g (RLOD level) (L1), and ~ 8 CFU/25 g (L2).

DNA Extraction

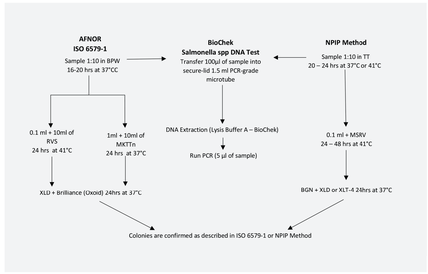
DNA was extracted from 100 µl of enriched sample using a rapid lysis method (Lysis Buffer A - BioChek BP900 or BP910).

Real-Time PCR Method

For NPIP - Samples were tested using BioChek Salmonella qPCR Reagents (BioChek BP300, MP304 and MP404) on Bio-Rad CFX-96® and Applied Biosystems® 7500. For AFNOR ISO 16140-2 - Samples were tested using the BioChek Salmonella species DNA test kit ((BioChek MP104) – contains the BioChek Salmonella qPCR reagents) on either Bio-Rad CFX-96®, Applied Biosystems® 7500, Roche Lightcycler® 480 or Agilent Mx3005P®.

Study design

Samples were pre-enriched in selected media according to each method in a 1:10 dilution (BPW – Buffer Peptone Water/ TT – Tetrathionate Broth). For temperatures and timings please refer to diagram below.



**RVS – Rappaport-Vassiliadis-Soya Broth, MKTTn – Muller-Kauffmann Tetrathionate-Novobiocin Broth, MSRV – Modified Semi-Solid Rappaport Vassiliadis Medium Base, XLD – Xylose Lysine Deoxycholate agar, Brilliance (Oxoid) – Brilliance Salmonella Agar Base and BGN – Brilliant Green Agar*

Results

NPIP Field Trial Study

There was one confirmed discrepancy against culture after repeat: BioChek Salmonella qPCR Reagents generated a weak positive result (Cq 38/39). The NPIP approved culture method and an alternative rapid test used for confirmation both showed negative.

Table 1. Comparison of NPIP culture method and BioChek Salmonella qPCR Reagents

	NPIP Culture Method (+)	NPIP Culture Method (-)	Total
qPCR Reagents (+)	PA = 128	PD = 1	129
qPCR Reagents (-)	ND = 0	NA = 239	239
Total	128	240	368

**PA – Positive Agreement, PD – Positive Deviation, NA – Negative Agreement & ND – Negative Deviation*

Statistical evaluation indicates excellent assay performance for BioChek Salmonella qPCR Reagents against the NPIP method:

- Diagnostic sensitivity 100%
- Diagnostic specificity 99.6%
- Positive predictive value 99.2%
- Negative predictive value 100%

AFNOR Interlaboratory Study (ISO 16140-2:2016)

Table 2. Comparison of ISO 6579-1 and BioChek Salmonella qPCR Reagents (n = 240).

Collaborators	Contamination Levels								
	Negative (0)			Low Level (Low)			High Level (High)		
	PCR result	Culture result	Final result	PCR result	Culture result	Final result	PCR result	Culture result	Final result
A1	1	0	0	7	7	7	8	8	8
A2	1	0	0	8	7	7	8	8	8
C1	0	0	0	8	8	8	8	8	8
C2	0	0	0	8	8	8	8	8	8
C3	0	0	0	8	8	8	8	8	8
D1	0	3	0	8	8	8	8	8	8
D2	0	2	0	6	6	6	8	8	8
E1	1	0	0	8	8	8	8	8	8
F1	0	0	0	7	7	7	8	8	8
G	0	0	0	8	8	8	8	8	8
Total	$P_{(0)} = 3$	$C_{(0)} = 5$	$CP_{(0)} = 0$	$P_{LOW} = 76$	$C_{LOW} = 75$	$CP_{LOW} = 75$	$P_{HIGH} = 80$	$C_{HIGH} = 80$	$CP_{HIGH} = 80$

**P = Number of positive PCR results , C = Number of positive confirmation results & CP = Final Call*

For sample sets D1 and D2, contamination was observed on negative samples during enrichment of MKTTn broth after enrichment of BPW. Accompanying selective RVS enrichment (reference method) and BioChek Salmonella qPCR Reagents results were in accordance with predicted negative results. Hence, MKTTn results were excluded by AFNOR Technical Committee. A further breakdown of the results is explained below.

Table 3. Comparison of ISO 6579-1 culture method (final result) and BioChek Salmonella qPCR Reagents (0–8 CFU/25 g, n=240).

	ISO 6579 Culture Method (+)	ISO 6579 Culture Method (-)	Total
qPCR Reagents (+)	PA = 155	PD = 3	158
qPCR Reagents (-)	ND = 0	NA = 82	82
Total	155	85	240

**PA – Positive Agreement , PD – Positive Deviation , NA – Negative Agreement & ND – Negative Deviation*

NA= 82 includes 1 PPNA (1 presumed positive by alternative method and tested negative after confirmation (by reference method)). Statistical evaluation indicates excellent assay performance for BioChek Salmonella qPCR Reagents against ISO 6579-1 reference method:

- Diagnostic sensitivity 100%
- Diagnostic specificity 96.5%
- Positive predictive value 98.1%
- Negative predictive value 100%

Conclusions

BioChek Salmonella qPCR Reagents offer:

- Excellent specificity, sensitivity and interlaboratory robustness against NPIP approved and ISO 6579-1 culture methods.
- Rapid screening: high-negative predictive value, facilitating quick hygiene monitoring and early release of food animals and food products without any further testing.
- An NPIP validated Real-Time PCR method [5] for accurately detecting and monitoring the presence of DNA from Salmonella species in environmental samples, cloacal swabs and hatchery samples.
- An AFNOR validated rapid alternative method [6] for the presence of Salmonella in ready-to-eat and ready-to-reheat foods, meat products, ingredients and specific foods, feed products, production environmental samples and primary production samples.
- A cost-efficient workflow: easy and efficient genomic DNA extraction using a straightforward lysis method, and rapid high-throughput screening with few handling steps.
- Broad validated instrument compatibility.

References

1. *National Poultry Plan Program Standards, USDA APHIS Veterinary Services, January 2017.*
2. *NPIP 9-CFR Subpart B §147.10 - Bacteriological Examination Procedures.*
3. *ISO 16140-2: 2016. Microbiology of the food chain -- Method validation -- Part 2: Protocol for the validation of alternative (proprietary) methods against a reference method.*
4. *ISO 6579-1: 2017. Microbiology of the food chain - Horizontal method for the detection, enumeration and serotyping of Salmonella - Part 1: Detection of Salmonella spp.*
5. *National Poultry Improvement Plan, Interim Approved Salmonella Assays from the 2019 General Conference Committee Meeting, June 13, 2019, www.poultryimprovement.org.*
6. *AFNOR Certificate Reference BCK 40/01-07/19.*