Detection and Confirmation of *Salmonella* in Raw Milk Cheese with Hygiena PCR Systems

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INTRODUCTION:

Salmonella is the main cause of diarrheal diseases; 1 in 4 cases is related to *Salmonella* infection, and 33 million healthy life years are lost to Salmonella infection every year. In addition, *Salmonella* is attributed with a high lethality rate and is one of the main reasons for infection is contaminated milk, especially raw milk. With the consumption of raw milk cheese, the risk is high due to the lack of a high temperature treatment. Given this serious risk of infection, it is essential to examine the matrix using a highly sensitive detection system. To respond quickly to salmonellosis outbreaks, it is common to use alternative PCR methods and to pool samples in 375 g volumes. Even so, it must be guaranteed that the presence and absence of *Salmonella* can still be analyzed in these large sample sizes with the same sensitivity as the ISO method 6579 for 25 g samples.

Therefore, Hygiena® conducted a comparison study between real-time PCR with a sample size of 375 g versus the ISO 6579 method for 25 g samples. BAX® and foodproof® detection systems with different target sequences for the *Salmonella* genus were evaluated on a real-time cycler in accordance with ISO 16140-2 as part of an RLOD study. In addition, colony confirmation was performed by picking colonies from XLD, Brilliance and Caso agar plates, using the BAX Lysis and the corresponding Hygiena PCR systems with the respective other target sequences for confirmation. These options save biochemical confirmation with very reliable confirmation based on two different target sequences.

Hygiena assays demonstrated, in a fractional unpaired RLOD study, that the probability of detecting *Salmonella* is comparable for ISO and PCR. The RLOD study was successful for both PCR systems. Also, colony and culture confirmation using the Hygiena PCR systems confirmed the positive results successfully, meaning the presence of *Salmonella* has been proven by two different target reagents.

PURPOSE:

This study was designed to evaluate the detection of *Salmonella* genus in the challenging matrix of raw milk cheese by two Hygiena real-time PCR systems. The ISO 6579 method was used for comparison and to determine the RLOD. The results of alternative and reference method must be comparable regardless of sample size and PCR Kit.

REFERENCES:

1. ISO 6579 Standard: https://www.iso.org/standard/56712.html

2. ISO 16140 Standard: https://www.iso.org/standard/54870.html

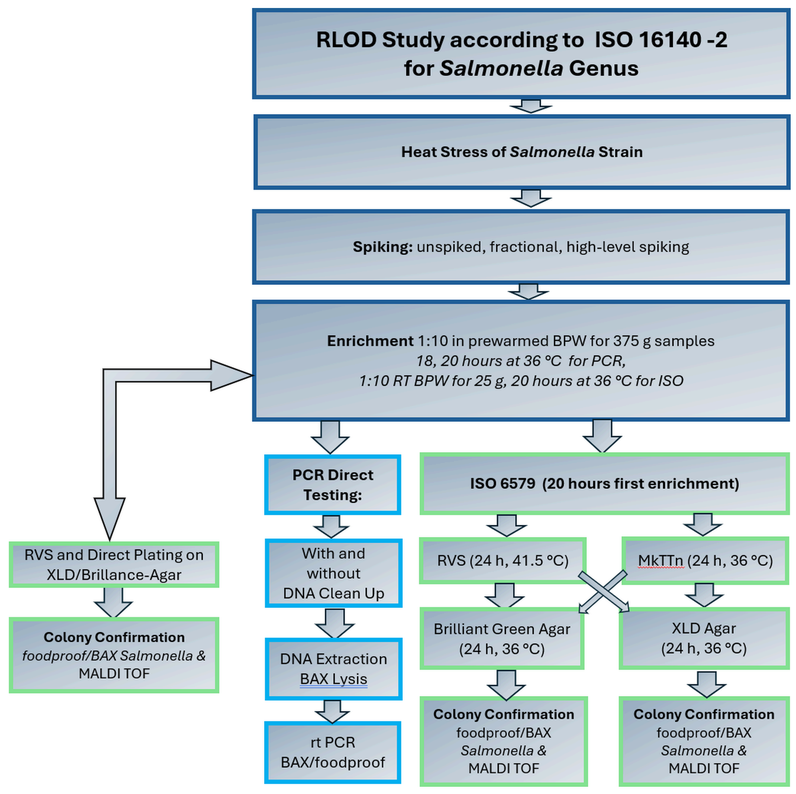
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METHOD:

For comparison of methods, the RLOD study according to ISO 16140 – 2 was conducted with two different PCR Kits, BAX System (KIT2006) and foodproof System (KIT230099) for *Salmonella* Genus, in comparison to ISO 6579. This unpaired study was tested with 375 g samples of raw milk cheese for the alternative PCR method and 25 g samples for the ISO reference method. The structure of the study included 5 uninoculated, 20 low-level (0.7 CFU/sample) inoculated and 5 high-level (1.3 CFU/sample) inoculated samples. The samples were diluted 1:10 in prewarmed buffered peptone water (BPW) for 375 g samples and in room-temperature BPW for 25 g samples, then incubated for 18 (PCR) to 20 hours (ISO and PCR) at 36 °C. The easy-to-use BAX System Prep Lysis Kit for gram-negative bacteria was used for DNA extraction, followed by real-time PCR analysis with the foodproof and BAX Kit for the detection of *Salmonella* Genus. Confirmation of PCR results was performed in accordance with the corresponding ISO reference method (ISO 6579-1). Colony confirmation was conducted by PCR using two different target sequences and by MALDI-TOF (Bruker).

WORKFLOW:



RESULTS:

Table 1. Results of the RLOD Spike Study (Comparison of ISO 6579 and Hygiena real-time PCR Methods - BAX and foodproof - for *Salmonella* Genus)

Study Design and Results: Detection of *Salmonella* in Raw Milk Cheese	
Number of Samples	20 fractional
Sample Size Reference	25 g
Sample Size Alternative	375 g
Spiking Level/Test Portion	0.7 CFU
Positives ISO Reference	9
Negatives ISO Reference	11
Presumptive Positive Alternative	11
Presumptive Negative Alternative	9
Confirmed-Positives	11
Confirmed-Negatives	9

Calculation dPOD for an Unpaired Study (AOAC Binary Workbook v5-2)					
Collaborator	POD CP	POD CC	POD C	POD R	dPOD for unpaired (C,R)
Data	0.55	0.55	0.55	0.45	**0.1**
95% LCL	0.76	0.76	0.76	0.70	**-0.46**
95% UCL	1.00	1.00	1.00	0.97	**0.61**
90% LCL	0.80	0.80	0.80	0.74	**-0.48**
90% UCL	1.00	1.00	1.00	0.97	**0.63**

* foodproof and BAX, 18 and 20 hours enrichment

The probability of detection (POD) for Salmonella in 375 g raw milk cheese samples is higher with both Hygiena *Salmonella* PCR Systems than the POD of the reference method with a sample size of 25 g, demonstrated by a dPOD of 0.1, resulting in more fractional positives with PCR than with the ISO 6579 method. All controls performed as expected. Colony confirmation by PCR was in 100% agreement with the certified MALDI-TOF detection, regardless of which PCR KIT or agar the colonies were picked from.

SIGNIFICANCE:

The detection rate of the Hygiena *Salmonella* PCR Systems in the challenging matrix of raw milk cheese with a high background flora is comparable to the ISO method but offers the advantage of being faster and easier to handle. The option of colony or culture confirmation with two different target regions for BAX and foodproof PCR systems increases the reliability of correct *Salmonella* detection. It offers the option to purchase a secure Salmonella detection workflow from a single company.