Food testing

Quantification of Salmonella in primary production and in-production samples

Thermo Scientific™ SureCount™ Salmonella species, Typhimurium and Enteritidis PCR Kit method

Quantification of *Salmonella* throughout the poultry production process, with a focus on serotypes that are commonly associated with human illness, allows for a more detailed picture of the impact of control measures than detection in finished product alone.

Samples taken during production and from the live production environment are likely to have highly variable levels of *Salmonella*, other microflora, and interferants from the process and environment[1,2,3]. These factors may impact the performance of rapid quantification methods that often utilise predictive models to estimate the contamination level of *Salmonella* from a PCR result, following a short enrichment step. Enrichment is required to bring low levels of contamination up to a level which can be detected and quantified. These methods rely on consistent growth of *Salmonella* during enrichment to obtain reliable results.

To minimize the variation in result caused by numerous extrinsic factors, it is necessary for testing laboratories to ensure their rapid solution is verified for use with their specific in-production and primary production samples. The Thermo Scientific™ SureCount™ Salmonella species, Typhimurium and Enteritidis Multiplex PCR Kit method (SureCount Salmonella Multiplex PCR Kit method) enables users to rapidly quantify *Salmonella* species, *S.* Enteritidis, and *S.* Typhimurium in meat products (including poultry), production environment and primary production samples using customised software without further analysis (Figure 1).

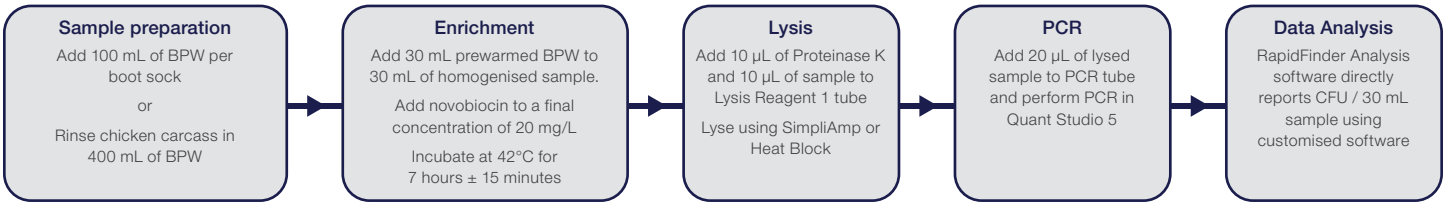

Figure 1: SureCount™ Salmonella Multiplex PCR workflow

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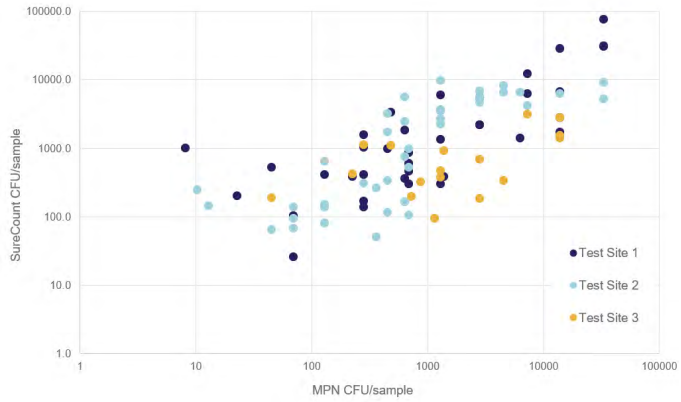

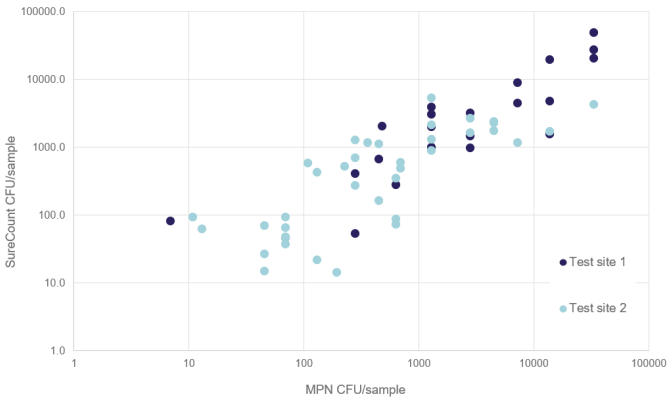

Figures 2 & 3: Correlation between MPN and SureCount Salmonella Multiplex PCR Kit methods estimated *Salmonella* species (Left) and *Salmonella* Enteritidis (Right) CFU/Sample in boot sock samples.

Primary production - Boot socks

The SureCount Salmonella Multiplex PCR Kit method uses a predictive model to estimate the number of *Salmonella* in a sample utilising PCR after a 7-hour incubation period. For highly variable samples such as rehang rinses and primary production boot socks this model is built using data generated from naturally contaminated samples from each producer.

An interlaboratory study was conducted across three laboratories using naturally contaminated boot sock samples from a single poultry producer (Figures 2 & 3). Results from the MPN and SureCount Salmonella Multiplex PCR Kit workflows, conducted simultaneously, were used to build a predictive model which was then applied to the data to assess accuracy. Quantitative estimates of *Salmonella* species and *S.* Enteritidis from the two workflows were compared. *Salmonella* Typhimurium was not analysed due to a lack of positivity in the naturally contaminated samples. Greater than 90% of SureCount Salmonella Multiplex PCR Kit method estimates were found to be within 1 $\log_{10}$ of the MPN (Table 1). Results were consistent between testing sites, providing evidence for the reproducibility of the PCR workflow.

A study was conducted to demonstrate the impact of using artificial contamination to build a predictive model for quantifying *Salmonella* species in complex samples.

Boot sock samples screened negative for *Salmonella*, from the same producer used in the aforementioned study were artificially contaminated with *Salmonella* at a variety of contamination levels. A new predictive model was built from the data gathered (from simultaneous MPN and SureCount Salmonella Multiplex PCR Kit workflows) and applied to both sets of data (Figure 4).

Table 1: Accuracy of SureCount quantitative estimates compared to MPN in boot socks samples tested at multiple locations.

Target	Number of Samples	Accuracy (within 1 $\log_{10}$ of MPN)
Salmonella Species	95	92.63%
Salmonella Enteritidis	60	96.67%

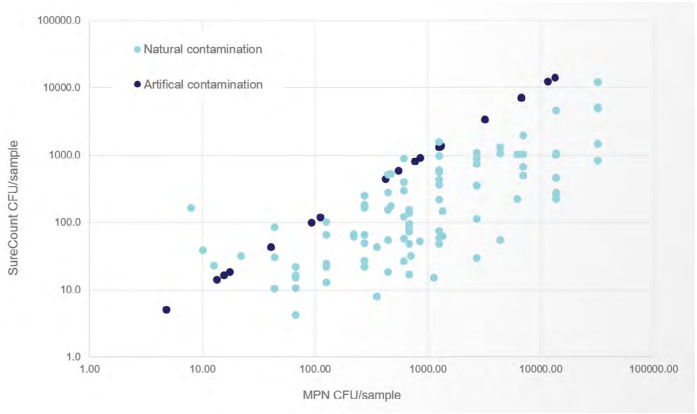

Figure 4: Correlation between MPN and SureCount Salmonella Multiplex PCR Kit estimates for *Salmonella* species CFU/ 30 mL sample in boot socks, using a predictive model built from artificially contaminated sample data.

Whilst this model gave accurate quantification estimates for the artificially contaminated samples (95.00% within 1 $\log_{10}$ of the MPN), when applied to the data gathered from naturally occurring contamination it showed reduced accuracy (65.26% within 1 $\log_{10}$ of the MPN) (Table 2). The predictive model built from natural contamination data showed greater accuracy of 92.36% for these samples (Table 1).

Naturally occurring *Salmonella* was consistently underestimated when applying the artificial contamination model. There was an estimated 9-minute increase in the average replication rate of the *Salmonella* when it was naturally occurring compared to when it was artificially introduced. Lab strains of *Salmonella* are likely to replicate at a faster rate than *Salmonella* that may have been sub-lethally injured in the production environment[4]. In complex samples this effect is further amplified by the carry-over of these extrinsic pressures within the sample enrichment.

When evaluating a rapid method against a traditional MPN method for use with complex samples, it is important that samples with natural contamination are used, as predictive models built upon artificial contamination may not provide accurate and representative results.

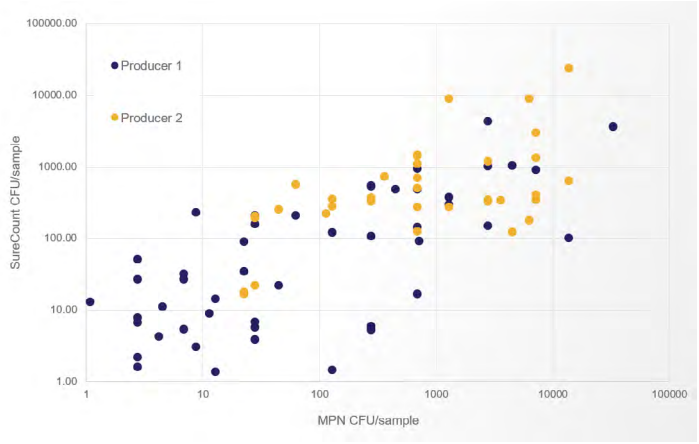

Figure 5: Correlation between MPN and SureCount Salmonella Multiplex PCR Kit method estimated *Salmonella* species CFU/Sample in rehang rinse samples when custom predictive models are used.

Table 2: Accuracy of SureCount Salmonella Multiplex PCR Kit method quantitative estimates for *Salmonella* species compared to MPN in boot sock samples, when using a model built from artificially contaminated sample data.

Contamination type	Number of samples	Accuracy (within 1 $\log_{10}$ of MPN)
Artificial	20	95.00%
Natural	95	65.26%

Table 3: Accuracy of SureCount quantitative estimates for Salmonella species compared to MPN in rehang rinse samples when using a custom predictive model vs. one designed using data from another producer.

Predictive model design	Producer	Number of samples	Accuracy (within 1 $\log_{10}$ of MPN)
Designed for Producer 1	Producer 1	51	80.39%
Designed for Producer 2	Producer 2	36	83.33%
Designed for Producer 1	Producer 2	36	69.44%

In-production – Rehang rinses

Naturally contaminated rehang samples from two different poultry producers were analyzed in a comparative study between the MPN method and SureCount Salmonella Multiplex PCR Kit method. PCR results were used to build predictive quantitative models of *Salmonella* species for each producer (Figure 5).

The accuracy of the model and software was producer-dependant. Greater than 80% of SureCount Salmonella Multiplex PCR Kit method estimates were within 1 $\log_{10}$ of the MPN when a custom model was used. However, when the predictive model designed for Producer 1 was applied to a different producer the accuracy of the model was reduced (Table 3). This was due to the differences in background microflora and microbial control practices at each of the producers and their impact on the growth of *Salmonella* within the sample enrichment.

When developing a predictive model for *Salmonella* quantification in highly variable sample types, it is important that growth-impacting factors that vary between producers are accounted for.

Conclusions

- Evaluation and creation of rapid quantification methods for complex sample types should include **naturally contaminated samples** to ensure they provide accurate and representative results.
- Rapid quantification methods may require **producer specific customisation** when analysing highly variable sample types.
- The SureCount Salmonella Multiplex PCR Kit method enables users to rapidly quantify *Salmonella*, *S.* Enteritidis and *S.* Typhimurium in highly complex sample types using custom software **without the need for further analysis**.

References

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