Are you asking the right questions about quantification?

The Hygiena Technical Team is pleased to cover the topic of quantification in this application note.

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"I've been on board for quantification and commercially launched the first quantification method (or kit) being SalQuant, and have been along for the whole ride," April told International Food & Meat Topics.

"I wanted to share some experiences and attributes as a customer, and what you should be looking for to ask your vendor about quantification. As someone with hands-on experience developing quantification for the industry, I wanted to share the critical components we consider when we start the development of any type of quantification, as well as how it works for the industry to ultimately fulfill the goal of reducing consumer risk."

Flexibility of data

When considering what data to collect, it is important to think about what will be understood from it.

For example, how is a result of 5.7 CFU per/g used as a decision making tool? Part of this is some of the consultative discovery that Hygiena goes through with their customers to get to the root of how to utilise the additional information.

Knowing the answer to these questions helps to decide which of the two types of quantification is needed based on the flexibility and purpose of the data.

- True quantification, like SalQuant, utilises an identified timepoint of incubation coupled with software to determine the number of bacteria in the original sample.
- Limits testing simply identifies when a positive occurs at a specified timepoint, indicating that the number of bacteria is greater than the threshold that has been set. Sometimes this style of testing is also known as semi-quantitative.

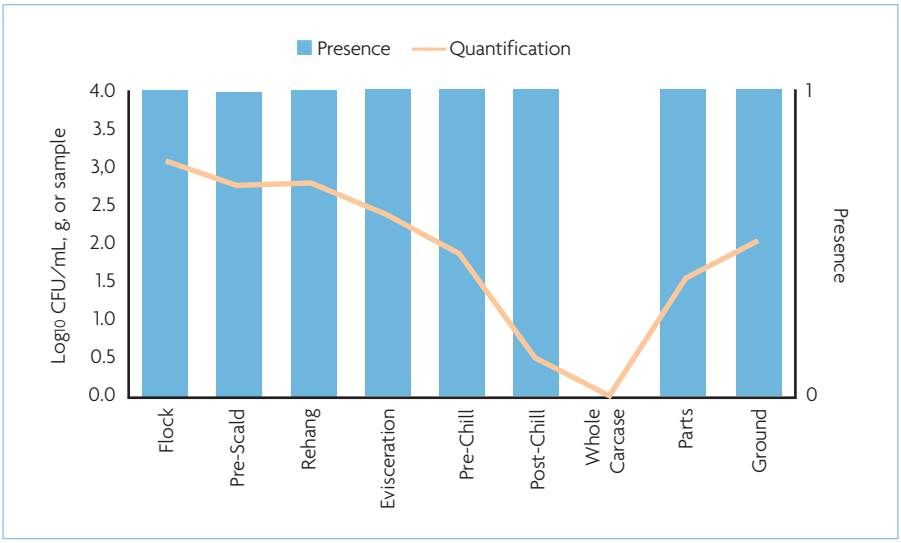

Fig 1. Prevalence vs. quantification testing in a poultry processing facility.

Understanding what type of data to look for and how to make that decision using the data is essential. Ultimately, ask: is the method being used flexible enough to collect both quantification and limits testing data from a single sample?

Dynamic matrix options

"Food safety is not just for final products; although the majority of times those samples do get the biggest focus," April said. Processing and pre-harvest sampling should also be a concern when managing the whole realm of food safety.

When this journey first started three years ago, Hygiena first developed quantification for final products and collected excellent data, but still needed to determine why the bacteria were still surviving after all of the interventions applied throughout the process.

If microbial load at the front end of animal and food production can be reduced, there is a greater chance of removing salmonella throughout the process and reducing that risk. That is why industries and influencers not only gathered data from final product testing, but added data testing locations throughout their processes to truly evaluate if the interventions were working.

Hygiena also continued to work backward to data from farm or pre-harvest levels to identify where the load of salmonella coming in can be impacted and reduce final product levels.

It is vital for processors to find robust testing methods that work across a wide variety of matrices and can detect contamination in even 'dirty' samples such as rehang versus post-chill rinsates. Any method chosen must work across the full range of 'dirtiness'.

Bottom line: not all samples are created equal even if they appear to be similar. Therefore, it is critical to have verification testing for all sample types taking during processing.

Wider enumerable range

"Methods must be able to get to span both the lower end (1 CFU) as well as the higher end (10,000 CFU) of the range of quantification, because we need flexibility based on the sample type as well as when there is a critical failure that happens with bacteria flourishing out of control," April said.

Within food safety programmes, sometimes there will be an outlier (low or high CFUs) and the method needs to be able

to quantify versus reporting a negative or greater than enumerable range, which is not a helpful data point.

"I like to use the example of the rinsate, as mentioned in dynamic matrix options, for process control to look from dirty to clean," April added.

"Being able to quantify the impact of a pre-intervention step where there is a high number of bacteria, and then showing that post-intervention there is still a positive but at a very low level is critical; you know that the level is still detectable and quantifiable. This is important to truly measure the effectiveness of the process and the interventions taken."

It is important to understand that the expectation for a final product is cleanness and the majority of the time, pathogen-free. Results should not be at high levels (100 CFU/g or mL).

Even 10 CFU/g can be too high depending on the industry. Methods that have a lower end of detection for quantifying 100 CFU/g are not helpful in a final product because the user will have a false sense of 'cleanliness' when quantification is seen as negative.

Easy to use process

From a regulatory perspective, prevalence will always be a critical component to measure consumer risk. Quantification is not meant to fully replace prevalence. It is about adding value. When thinking about the process of needing to fulfill regulatory needs but also gather more information, utilising the same sample throughout the process is helpful to minimise time and labour resources.

"Utilising a single sample is primarily why we designed our protocols at Hygiena to have a streamlined workflow where the sample can be prepared once to get both quantification results, telling me if my sample is positive with a high risk, or alternatively, that it is negative and I need to continue to prevalence testing to manage the regulatory risk," April said.

"The value of quantification is not meant to be disruptive of normal processes. If you are adding more resources and more cost or labour, does that add value at the end of the day? No, but the data is still very valuable, but if it is so hard to gather because of the process, it becomes prohibitive. We need to have dynamic, numerical data versus just ones and zeros signifying only positive and negative results."

If it is not easy to do in the lab, industries and companies may fall back to just doing the minimum requirement of prevalence testing, which is not needed when improving food safety.

See the data

For any method, demonstrating success with data from validation or verification, data is important to ensure that both vendors and method developers have done their due diligence.

The beauty of using PCR for quantification is that the assays have already been validated for detecting the pathogen at a 1 CFU/sample level for prevalence, which is more difficult than quantification.

Since all the work has already been completed for inclusivity and exclusivity, what happens next is adding quantification testing using already established microbiology tools.

Not only should the data be seen, but it is important to understand the amount of data and how it was collected and compared. Developing or validating a method using only 10 samples is not enough; at minimum 30 samples creates statistical relevance and trust in the comparisons to reference methods, but that is an absolute minimum.

Conclusion

The way to think about quantification and the reasons behind these types of questions and comments that may be heard in the industry and in government is to ensure that everyone has the correct information and awareness when it comes to pathogen testing.

"Utilising a single sample is essential; our testing ensures you have a streamlined workflow where the sample can be prepared once to generate both quantification and prevalence results; knowing the level of contamination determines if there is a high risk or low risk for regulatory purposes," April concluded.

"The value of quantification is not meant to be disruptive of normal processes. If you add more resources and more cost or labour, that is not adding value to your organisation. The data is still valuable, but if it is so hard to gather because of the process, it becomes prohibitive. We need dynamic, numerical data showing us the risk level versus just ones and zeros for positive or negative results."

Industries and companies must have easy to use methods or they will continue to do the minimum amount of testing (prevalence), which is not helping advance food safety. ■

SalQuant is registered with the US Patent & Trademark Office