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Validation of Antimicrobial Interventions for Small and Very Small Processors: A How-to Guide to Develop and Conduct Validations

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SUMMARY

It is important to assure that antimicrobial interventions applied on/into foods to control pathogenic microorganisms are functioning properly and achieving the desired goal of preventing, reducing and/or eliminating microbial hazards associated with a defined food product. This approach is necessary both to ensure that antimicrobial interventions are having the desired positive effect on food safety and to provide assurance to the processor that the investment in food safety is in fact providing the appropriate benefit for the investment. Validation is a fundamental component of the HACCP system, in that those processors currently required to have HACCP plans in place are also required to validate their HACCP plans. This manuscript provides a practical approach for developing validation protocols to evaluate the efficacy of antimicrobial interventions, especially for small and very small processors.

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INTRODUCTION

Assuring safety of food from production to consumption is a complicated process requiring an organized, deliberate approach to preventing and controling potential food safety hazards. The Hazard Analysis and Critical Control Point (HACCP) system is widely accepted as the most effective and logical approach to accomplishing this task. HACCP plans are developed on the basis of seven principles: hazard analysis, identification of CCPs, establishment of critical limits, monitoring of CCPs, defining of corrective actions, verification, and record-keeping/documentation. Of these seven principles, verification procedures may be the most misunderstood and least effectively implemented, and are often overlooked or given low priority.

The National Advisory Committee on Microbiological Criteria for Food (NACMCF) *(5)* defines verification as any activity, other than monitoring, that determines the validity of the HACCP plan and ensures that the HACCP system is operating according to the plan. Included in verification activities is validation, defined by NACMCF as the element of verification focused on collecting and evaluating scientific and technical information to determine whether the HACCP plan, when properly implemented, will effectively control the defined hazards.

Before a HACCP plan can function with assured control, it must be determined that all hazards have been identified and that specific control measures are scientifically sound and will be effective when implemented. Validation, both of individual CCPs and the entire HACCP plan, is integral to determining the plan's soundness. A HACCP plan that has not been validated may appear logical and effective; however, without thorough validation of the process, there is no assurance that factors that may compromise product safety have been evaluated. Process control and safety cannot be assured unless a HACCP plan has been validated.

The Food and Drug Administration (FDA), through implementation of the Food Safety Modernization Act (FSMA), and the United States Department of Agriculture's Food Safety and Inspection Service (FSIS) will require all food processors to provide evidence of HACCP plan validation. Proper validation of HACCP controls may be difficult to implement for all food processing operations; however, small to very small processors may find the task to be particularly burdensome. This manuscript provides a practical overview of validation, including experimental design, implementation and application, to help small, local, artisan and very small food processors to understand the concepts and protocols for validation of CCPs and HACCP plans. Basic concepts presented herein are applicable to all food processors; however, the primary focus is directed toward small to very small processors, including local and artisan manufacturers. This manuscript also discusses the importance of validation, as well as the selection of scientific justification documents to support intended process control measures. In addition, a practical approach to in-plant validation is provided, including appropriate microbiological testing, analysis and reporting.

The USDA Food Safety and Inspection Service regulations *(7)* state that "upon completion of the hazard analysis and development of the HACCP plan, the establishment shall conduct activities designed to determine that the HACCP plan is functioning as intended." Further, the Pathogen Reduction HACCP Rule states that "During this HACCP plan validation period, the establishment shall repeatedly test the adequacy of the CCPs, critical limits, monitoring and recordkeeping procedures, and corrective actions set forth in the HACCP plan (emphasis added)." In addition to other validation activities, review of the processing records themselves, routinely produced by monitoring of the HACCP system in the context of other validation activities, is a key element of HACCP plan validation.

Thus, the validation process has two aspects: (1) verifying that the antimicrobial intervention (such as a lethality process) will achieve its intended purpose of preventing, reducing and/or eliminating the hazard as implemented in the food processing operation and (2) verifying that the critical limits of the critical parameters that would impact the efficacy of the antimicrobial treatment are being met on a continual basis as implemented in the processing operation. The first aspect can be achieved only by evaluating the prevalence and/or concentrations of the organism of concern (food safety hazard). The second aspect can be achieved through review of records and by assuring that critical limits of the critical parameters are being met for the particular antimicrobial intervention in practice. These two aspects are essential components of the validation process, and assuring compliance with one aspect without the other will not assure that the antimicrobial intervention is achieving its intended purpose of preventing, reducing and/or eliminating the hazard.

Scientific and technical justification

Initial validation of the HACCP plan can be based upon various types of information, but most often utilizes scientific studies and advice of experts, regulatory guidance, industry standards or guidance, modeling programs, and university extension publications, as well as observations and data collected in the processing facility.

The most common approach to validating a process or demonstrating process control in plants is to use scientific publications that provide information on efficacy of control measures. Typically, scientific information can consist of peer-reviewed journal articles, a documented scientific study, in-house data, or data generated from published guidelines. The five primary types of scientific supporting documentation (see http://www.fsis.usda.gov/Science/HACCP_Validation/index.asp#2) are:

1. published processing guidelines (safe harbors) that achieve a stated reduction of a pathogen, such as the time-temperature guidelines in Appendix A of the final rule "Performance Standards for the Production of Certain Meat and Poultry Products";
2. a scientific article published in a peer-reviewed journal that describes the process and level of reduction of a particular food safety hazard or process stabilization; the publication or scientific article being used, however, should closely relate to the manufacturing process being validated (meet the critical parameters) with respect to species, product characteristics, processing parameters, and equipment;
3. a microbial challenge study or inoculated pack study (with non-pathogenic surrogates or indicator organisms as acceptable alternatives to the food safety hazard [pathogen]

of interest) that is designed to determine lethality or stabilization of a process; these studies are typically performed in a laboratory or pilot plant by a processing authority or expert, and it is not advocated that pathogens be introduced into the food processing plant environment;

4. data gathered in-house, which can be used to validate an antimicrobial intervention or process and which may be generated if the establishment has not implemented a process documented in the literature, and
5. regulatory performance standards as defined in the Code of Federal Regulations that outline specific prescribed procedures such as time/temperature combinations, product storage conditions, or product reconditioning procedures.

Several resources for scientific publications or pre-existing supporting documents can be found on the Internet or via county Extension agents, industry trade groups, and university libraries. Although there are several sources/access points at which information can be obtained, it is important to identify and utilize scientific information that is truly relevant to the process or the product being evaluated. In selecting scientific publications or articles relevant to the process, it is critical to look for processing parameters consistent with the specific plant's operational parameters for the product and pathogen(s) of interest. In addition to collecting pre-existing scientific and technical information, plants should obtain necessary data by repeatedly testing the adequacy of the process in preventing, reducing and/or eliminating the identified hazard and establish that the HACCP system meets the designed parameters to achieve the intended results.

In addition, the basic composition of the food, as well as the processing methods and storage conditions, should be considered in the initial analysis *(3)*. For example, processing plants often incorporate antimicrobial interventions or processes to reduce levels of certain pathogens and use published scientific support to implement that process as the first step. However, processors should demonstrate the capabilities of these new/altered interventions within specific plant environments to verify that the process step actually achieves the effect documented in the scientific study. This approach is critical because laboratory conditions often differ from conditions in the establishment, as conditions are highly controlled and on a smaller scale in the laboratory than in a processing plant; hence, specific log reductions or the ease of monitoring critical parameters achieved in the laboratory may not be readily attainable in a commercial processing operation.

Practical demonstration

Validation may be accomplished by an in-plant demonstration of achieving or meeting the critical parameters that have been identified in the scientific and technical literature. The following section discusses the main components of developing an in-plant validation process to demonstrate its effectiveness in controlling a particular food safety hazard or concern. These general considerations may be applied to a variety of processes, and may not be relevant to each distinct process.

The fundamental question in developing an in-plant demonstration is "What are you trying to validate?" While the answer to this question may seem obvious, it is in fact complex and requires considerable thought before proceeding. Initially, the answer to the question may appear to be that you are trying to validate that the product is safe. However, safety is difficult to prove, and the answer to the question should specify what the process, or a specific step in the process, is intended to accomplish.

Experimental design

The scientific and technical justification for validation provides insight regarding the expected outcome of a specific process. For example, in the scientific literature, a hot water wash is reported to have a certain impact on a specific pathogen or indicator organism for a target pathogen on a defined food product. The in-plant demonstration is intended to show that under the circumstances specific to that particular processing operation, the same result can be reproducibly/repeatedly achieved. So the answer to the question of "what is being validated" should refer to the initial justification for the use of a process. If a hot water wash is being used as an intervention, then demonstrating that the hot water wash, as described in the literature, has the same effect in your operation is the answer to the question of "what." In general, the in-plant demonstration should cover the specific interventions identified in the HACCP plan as critical control points and show that the entire process improves the microbiological safety of the product by preventing, reducing, and/or eliminating the food safety hazards identified in the hazard analysis through validation of the HACCP system.

After the initial question of "what" has been answered, a specific experiment must be designed to demonstrate both the effectiveness and the control of the process. The experiment should evaluate all of the relevant parameters previously identified in the scientific and technical justification, including but not limited to temperature, time, process speed, application pressures, and any other factors critical to a given step in the process. For both the demonstration and the reporting of the results, exact parameters and their expected ranges should be recorded. Water temperature may vary during the course of the day, so recording the target temperature as well as the variation is important in evaluating a process. It is equally important for a processor to understand and document similar variations associated with all critical parameters defined in the HACCP plan, as they may impact the effectiveness of the antimicrobial interventions being utilized. If peer-reviewed scientific research articles have been used as part of the technical justification, these articles may be useful in designing and conducting a similar in-plant demonstration.

Pathogens

If the objective is to demonstrate a reduction in the prevalence and/or levels of microorganisms, either pathogens or pathogen indicators, several additional considerations are available to evaluate the process. The choices include naturally occurring microflora, which may consist of indicator organisms, which are nonpathogenic surrogates intentionally inoculated into or onto a product for validation purposes. In general, the use of pathogens for in-plant demonstration

projects is not encouraged, unless the pathogen occurs naturally and with sufficient frequency as part of the normal microflora of the product (e.g., *Campylobacter* spp. in poultry). Artificially inoculating pathogens in a processing plant setting may compromise employee safety and product safety, and it may create sanitation and/or regulatory problems. In addition, proper disposition of contaminated product is of critical importance *(6)*, and the use of pathogens for inoculum to be used in in-plant studies would make this even more difficult.

Indicators and surrogates

In some cases, the naturally occurring microflora may serve as a useful indicator of process control. However, interpreting the results of a general aerobic population count requires knowledge of the initial microflora population. For example, if the naturally occurring microflora contains a high proportion of sporeforming bacteria, a hot water wash may appear to have little or no impact on the total aerobic population, because sporeformers are quite heat resistant. On the other hand, the presence of naturally occurring coliforms or generic *Escherichia coli* may be useful in evaluating a process, assuming that the populations are high enough to measure reliably. However, if the naturally occurring population is typically present at the lower limit of the detection method, it may not be possible to demonstrate an effect of a process.

A possible solution to this problem is the use of surrogates that can be inoculated into or onto a product at sufficient populations to demonstrate the efficacy of an antimicrobial treatment. Numerous acceptable surrogates are available, but most have been tested or designed to be used with a specific process or product *(4)*. For example, *Enterococcus faecium (1)* has been shown to be a useful surrogate for the thermal processing of almonds. Other examples are given in Table 1. It is important to match the surrogate to the intended use, as a surrogate shown to be useful for one process may not be useful for another. The production of sufficient volumes of surrogate organisms for inoculation purposes requires use of a laboratory, and therefore may be beyond the capability of some processors. In this case, the services of a research or contract laboratory may be retained to produce and supervise the use of surrogates for an in-plant demonstration. The processor must confirm with the appropriate regulatory body that the surrogate(s) being used, how they are applied, and disposition of the production units involved are acceptable prior to initiating studies.

TABLE 1. Examples of indicators and surrogates that may have application in validation studies. Specific cultures may be obtained from the American Type Culture Collection (www.ATCC.org)

Indicator or Surrogate	Strengths	Weaknesses
Mesophilic aerobic bacteria (Total Plate Count)	Easy to test for Present in every sample	Unlikely to represent pathogen population
Coliforms	Easy to test for Present in many samples	May only represent enteric pathogens
***Escherichia coli* Biotype I/II ("generic" *E. coli*)**	Easy to test for May be present in some samples	May not be present in all samples, or in populations great enough to measure
***E. coli* surrogates (ATCC 1427,1428,1429,1430,1431)**	Representative of *E. coli* O157 and salmonellae in meat products Allowed by USDA-FSIS for in-plant studies	Requires microbiologist to supervise inoculation May not represent all processes
***Enterococcus faecium* (ATCC 8459)**	Representative of thermal processes, especially with almonds and tree nuts	May have limited applicability to meat and poultry Requires microbiologist to supervise inoculation
***Pediococcus* spp.**	Readily available as starter culture Easy to inoculate	May not represent pathogen
Lactic acid bacteria	Readily available as starter culture Easy to inoculate	May not represent pathogen

Design of In-plant demonstrations

Several considerations must be addressed in the design of in-plant demonstrations. One of the most important is understanding of the expected variation that may occur under normal plant operations. An in-plant demonstration study should essentially represent a "worst case" scenario. Most operators have a general idea of the type, magnitude, and periodicity of variation that may occur within the process, based on their practical experiences with good, normal and bad days. The impact of seasonal differences should also be included in understanding this variation, which is important to answering the questions of "how much data do I need" and "how many times do I need to repeat the demonstration?" There are several statistical approaches to answering questions related to the nature and number of samples to be tested; however, some general guidelines can be applied in decision making *(8)*. There is a meaningful difference between repetitions and replications. Repetitions are multiple samples taken during the same replication; they improve the accuracy of the results, by accounting for variation within the replication. Replications are completely independent from each other, differing by lots, shifts or days; the intent of multiple replications is to accurately reflect the normal variation that occurs during the process. A more thorough discussion of variation is presented in the Data Analysis section, and those who are not familiar with this topic may want to read that section before proceeding.

The demonstration should be independently replicated at least three times. In situations where more variation is expected in the results, more data will be required to demonstrate the effectiveness of the process. Data is available on the results of the intervention (the "after" treatment in a "before" and "after" comparison), a better estimate of the number of independent replications can be determined. Table 2 provides additional suggestions regarding the number of replications required based on the expected variation. To use the table, determine the variance of the data that is expected or available from prior experience. For example, if there are 5 samples, five replicates would be required to reliably detect a statistical difference of 1.0 $\log_{10}$ CFU in a comparison of the "before" and "after" samples. As a starting point, with no available data, it would be appropriate to assume a variance of 0.5. From a realistic point of view, population reductions of < 1 $\log_{10}$ CFU/g may not have practical significance.

Other details that should be considered in the design of an in-plant demonstration include determining the location of the sampling sites within the process flow, the types of samples to be collected (e.g., sponge sample, product sample, surface excision, etc.), and the methods of analysis. This includes where and how you will collect

TABLE 2. Guidelines for the number of replications required for a given number of samples and variance, based on a 95% probability of detecting a difference of 1.0 $\log_{10}$ unit in population

Number of Samples	Variance	Difference	Number of Replicates
3	0.25	1.0	4
	0.50	1.0	9
	0.75	1.0	13
5	0.25	1.0	2
	0.50	1.0	5
	0.75	1.0	7
8	0.25	1.0	2
	0.50	1.0	4
	0.75	1.0	5
10	0.25	1.0	2
	0.50	1.0	3
	0.75	1.0	5

The basis for Table 2 can be found in van Emden *(8)*. Briefly, the Least Significant Difference can be calculated using the following formula:

$$\text{LSD } t^* \sqrt{2^* (\text{variance}/2)} =$$

where LSD is the least significant difference, t is the t statistic for a 95% with n-1 degrees of freedom, and the variance is the variance of the samples. This equation becomes:

$$\text{Number of replicates} = \frac{t^2 * 2 * \text{variance}}{\text{Difference}^2}$$

where t is the t statistic for a 95% with n-1 degrees of freedom, the variance is the variance of the samples, and the difference is the least difference which may be statistically resolved under these conditions.

TABLE 3. Two data sets with equal averages but different variances

Observation	Data Set 1	Data Set 2
1	10	25
2	20	25
3	30	30
4	40	35
5	50	35
Mean[a]	30	30
Range	40	10
Variance	250	25
Standard Deviation	15.8	5

[a]See Table 4 for formulae.

TABLE 4. Mathematical formulas for statistical calculations, for the data set

Value	Formula	Example	Excel Function[a]
Average or Mean	Sum of all data point Total number of data points	$\frac{1+2+3+4+5}{5}$	**= average ($a_1 \ldots a_x$)** Where x = the last cell in the data
Range	Maximum value = minimum value	5 - 1 = 4	**= max ($a_1 \ldots a_x$) - min ($a_1 \ldots a_x$)** Where x = the last cell in the data
Variance	Sum of each data point - average, squared, divided by the total number of data points	$\frac{\sum (\text{data point - average})^2}{5}$	**= var ($a_1 \ldots a_x$)** Where x = the last cell in the data
Standard Deviation	The square root of the sum of each data point - average, squared, divided by the total number of data points - 1	$\sqrt{\frac{\sum (x\text{-average})^2}{(N-1)}}$ Where "x" is each data point, and "n" is the number of samples in the data set	**= stdev ($a_1 \ldots a_x$)** Where x = the last cell in the data

[a] Excel, Microsoft. Mention of a specific product does not constitute an endorsement or recommendation of the product by either the authors or the International Association for Food Protection.

supporting process parameter data during the in-plant demonstration (e.g., pH, temperature, spray pressures, dwell times, etc.). Practical considerations may also affect the sampling site location, related to access to the food product. If a specific process is being evaluated, samples should be collected close to the beginning of the process and immediately after the process. For a hot water wash, the samples would be collected immediately before and immediately after the wash. The samples should be collected in a manner that neither introduces new contamination into the sample nor allows for the increased destruction of bacteria. The Food and Drug Administration, in its Bacteriological Analytical Manual (BAM) *(2)*, provides instruction on the appropriate means of collecting and handling samples, and the actual method should be documented for the report. Collecting samples using an alternate method is acceptable as long as justification for the method is provided. It is advisable to discuss sampling and analysis plans with a trained microbiologist before initiating in-plant studies.

Sample analysis

The basic properties of the method of microbial analysis need to be documented prior to the beginning of the demonstration project. The minimum level of detection, and in the case of presence/absence tests, the rate of false positive and false negatives, need to be documented. The sensitivity and specificity of the method of analysis may impact the design of the demonstration, especially if the demonstration involves the use of naturally occurring microflora. If the minimum detection limit of the analytical method is close to the typical populations encountered in the food, it may not be possible to demonstrate an effect between the initial and final populations.

Either an internal (in-house) or external laboratory may conduct the study, analyze the samples and report the results. If the samples are analyzed at an internal laboratory, it is important to clearly document that the appropriate procedures have been followed in the analysis, including not only the method used, but also the details of the method. Again, both the Microbiological Laboratory Guidebook (MLG; USDA-FSIS) and BAM provide clear, detailed methods recognized by the respective regulatory agencies. As an alternative, some methods are approved by the Association of Official Analytical Chemists (AOAC) and the methods can vary as to basic principles used for detection or the sensitivity or specificity of detection. Because of differences between methods, it is important that the appropriate method be used for the food being evaluated. If the samples are analyzed internally, it is important to document the internal quality control procedures used in the laboratory to assure that the results are reliable. If an external laboratory analyzes the samples, it is important that the external laboratory have its own procedures for ensuring quality control, whether that is ISO accreditation or another program. Prior to the start of the studies, responsible individuals within the company should understand clearly how the external laboratory will receive, store, analyze and report the needed data. Responsible individuals should ask questions and make adjustments if necessary to avoid ending up with less than optimal data and additional expense. Questions such as what the laboratory does when it receives samples on Friday and will analyze them on Monday must be considered, as this could substantially impact the results of the study.

Data analysis

Once the sample analysis is completed, the results will need to be analyzed statistically. The first step in statistical analysis is to review the actual sample results (data) as they are returned from the laboratory, an important first step in identifying any sample result that does not appear to be logical. There is always the possibility that data may be recorded incorrectly, and data analysis is only as good as the raw data being analyzed. Obvious transcription errors should be corrected (for example, pH 46.3 rather than 4.63) before the statistical analysis is conducted. Other analytical data that seem out of place or are clearly outliers should be investigated to ensure that the values in question are not errors attributable to either sample collection or analysis. The sample results cannot be excluded simply because it does not fit the expected pattern, whereas it can be excluded if there is a legitimate reason, such as a known sampling error. Any data eliminated from the final analysis must be accompanied by a written justification based on known facts. One purpose of an in-plant demonstration is to learn more about the actual process as implemented in the processing operation; therefore, deliberately excluding data from the analysis for reasons that cannot be justified not only weakens the validation process but also ignores information that is valuable in understanding the process.

Data analysis involves more than simply calculating the average. Table 3 illustrates this point by presenting two sets of data with identical averages. A measure of the variability associated with the results is necessary to put the data into context. Several measures, including the variance, standard deviation and standard error, indicate the degree of variability. These values may be calculated using the formulas shown in Table 4.

Several computer software programs are available that can assist in performing basic statistical analysis. One of the most popular spreadsheet programs has several statistical functions as part of the program, including a one-way analysis of variance (ANOVA), and the on-line tutorial will guide the user through the process. Other inexpensive statistical analysis programs are available and provide adequate means for analyzing data.

The results of the data analysis will determine if there is a statistical difference between the "before" and "after" samples, as well as providing an estimate of how significant the difference is. These results allow the processor to demonstrate that under their specific plant process and environment, a certain result can reasonably be expected to occur within some confidence limits. This result must be viewed in the context of the original question that was to be evaluated, and cannot be extended beyond that specific process. The strength of this claim is only as strong as the initial design of the demonstration, the number of samples and replications, the sample analysis methods utilized, and the statistical analysis applied.

Conclusions and reporting

An in-process demonstration provides evidence for what a process is capable of accomplishing during normal operations. Validation reflects the system's performance under the conditions and parameters defined in the study. Changes to these parameters do not necessarily mean that a new demonstration must be performed. For example,

raising the water temperature by 10°F or increasing the contact time by 5 seconds probably would not require a new in-plant demonstration, as those changes would be expected to result in greater reductions of a microbial hazard or a better control of the hazard. However, major changes that might allow for increased pathogen survival, such as lowering water temperatures, reducing contact times, changing spray nozzle types or distances, reducing pressure, or changing the supplier of a previously validated antimicrobial must be shown to produce results equivalent to previously evaluated conditions. In most cases, this will require a new in-plant demonstration.

In-plant demonstrations are process- and facility-specific. While the results may be generalized to other processes used in other processing facilities, the information developed in one facility could be part of the scientific and technical justification of a demonstration performed in another facility. However, validation of a process in one facility cannot suffice as a validation of the same process in another facility. Local or regional differences in equipment, water quality, and individual processes are such that each processing facility must conduct its own in-plant demonstration of each process.

At the conclusion of an in-plant demonstration, it is important to document the demonstration for future reference. This in-plant demonstration may be incorporated into the supporting documentation for a HACCP plan or may be used as necessary to meet regulatory requirements. From a practical standpoint, in-plant demonstrations require considerable planning, time to conduct the demonstration, and sample analysis costs. It is important to record this information in a formal report to obtain the maximum return on investment.

A report should include the following information. First, the dates, time and location of the demonstration, and lead personnel involved (including expert advisers/consultants and external laboratories) should be recorded. Any approvals requested and received (e.g., use of surrogate organisms and product disposition guidance) should be documnented, so that as personnel and processes change, the information can be viewed in the context of when it was performed. All relevant information, beginning with the initial question to be evaluated, should be clearly explained. Because the report is a means of communicating to others, both within and external to the company, what was attempted and accomplished in the demonstration, all details should be included. Although some details may seem obvious and unnecessary to include, a regulatory official who has never been in the facility may review the report, and it is thus important to clearly explain how the demonstration was conducted and how the results were evaluated. The report should also include the actual sample analysis data and all of the calculations used in the analysis. If a computer program for statistical analysis is used, the name of the program as well as the specific procedures used should be described. A printout of the results should be included as part of the results. An example of a report format is shown in Table 5.

Other considerations

If a product is inoculated with a surrogate organism during the demonstration, consideration needs to be given to the disposition of the product. A raw product that will be cooked by a further processor, may present no additional concerns; however, if the product would not normally be cooked sufficiently by the consumer, it may be necessary to divert the product to an alternate process or end user where use of the product will be under more control than it would be if used by the general consumer.

Where to start

An in-plant demonstration project is important to understanding specific process capabilities as well as for meeting regulatory requirements. Although it may appear to be complicated, as with any process, it can be broken down into specific tasks, a general outline of which is shown in Table 6.

When preparing to conduct an in-plant demonstration, all necessary resources must be assembled prior to beginning the validation. Basic questions such as who will do which step in the demonstration, from collecting samples to analyzing the samples, must be addressed. Accurate written procedures for each type of sampling and appropriate training of personnel involved with the study are absolutely crucial. Having the necessary sampling materials pre-labeled is an important detail that makes the process of sample collection easier. If the samples are being sent to an external laboratory, having the necessary shipping items on hand (boxes, coolers, cold packs, shipping temperature recorders, etc.) is important prior to beginning the project. As previously mentioned, pre-study communication and agreement on expectations of both entities (processor and laboratory) is imperative.

TABLE 5. Generalized outline of a report format

1. **Initial Details:**
   - Who did the study (key personnel)?
   - When was it done?
   - Where was it done?
   - What process was evaluated?
2. **What was the question to be evaluated?**
   - What was the overall design?
   - What samples were collected?
   - How many samples were collected?
   - How were the samples collected to assure independent replication?
   - How were the samples analyzed?
   - What laboratory quality assurance programs were in place?
   - Where were the samples analyzed?
3. **What were the results?**
   - Overview of raw data
   - Overview of data analysis
   - Results of data analysis
4. **Conclusions**
5. **Other considerations**
   - Disposition of product
6. **Appendices**
   - Table of sample data
   - Table of data analysis results (may include graphs)
   - Printout of data analysis

TABLE 6. List of steps (check sheet) for validation protocols

	Task	Comments
☐	**1. Identify the process to be validated**	
☐	**2. Determine the appropriate test to conduct**	What is the question to be answered?
☐	**3. Determine if natural microflora or inoculated surrogates will be used**	Use of surrogates may require an external laboratory
☐	**4. Design the in-plant demonstration**	Keep in mind the number of samples and the number of independent replications
☐	**5. Determine the appropriate methods of analysis**	Determine the minimum detection limit, sensitivity and specificity of the analytical method
☐	**6. Schedule the in-plant demonstration**	Consider production schedules and product disposition issues
☐	**7. Begin the in-plant demonstration**	Be sure that the samples are collected in accordance with recognized procedures and transported to the laboratory in a timely fashion
☐	**8. Analyze the samples**	Assure that laboratory quality assurance programs are in place
☐	**9. Overview of the data**	Look for obvious errors in the data
☐	**10. Analyze the data in-plant demonstration**	Use appropriate statistical tools to evaluate the results of the
☐	**11. Draw the appropriate conclusions**	Do not generalize the results beyond the limits of the demonstration (process and facility specific)
☐	**12. Prepare a detailed report in a timely fashion**	The demonstration is not complete until the report is written

CONCLUSION

Validation is a critical aspect of HACCP and should be conducted to assure the safety of the product being produced in a particular food processing operation. Food safety regulations require that processing operations implementing HACCP systems should validate their critical control points and the overall HACCP system. The goal of a food safety management system such as HACCP is to ensure the safety of the food products being produced under that system. Validation includes ensuring that the CCPs within the process are achieving their intended purpose.

These validation activities should be properly designed and executed in the processing operation to evaluate the effectiveness of the CCPs as implemented in preventing, reducing and/or eliminating the food safety hazard and that the products produced under the HACCP plan are safe.

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