Response to Questions Posed by the Food Safety and Inspection Service: Enhancing *Salmonella* Control in Poultry Products

National Advisory Committee on Microbiological Criteria for Foods
To be presented at NACMCF Plenary Session, November 15, 2022

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**Executive Summary**

Chicken and turkey are major sources of animal protein in the United States. Per capita chicken consumption is increasing from 77.4 lb. in 2000 to a projected 99.6 lb. in 2023, with processed and cut-up chicken parts comprising the majority of purchases, whereas beef has declined from 67.5 lb. to an estimated 55.8 lb. during the same period (103). Of the estimated over 1 million cases of foodborne salmonellosis acquired annually in the US, over 24% are attributed to consumption of chicken and turkey products (Figure 1) (76, 115). Although *Salmonella* is killed during cooking, undercooking (e.g., in raw breaded or stuffed chicken products), and cross contamination with other ready-to-eat foods in the refrigerator or during preparation are contributing factors to transmission of this pathogen. Therefore, FSIS instituted *Salmonella* performance standards for raw poultry carcasses, chicken parts and raw comminuted poultry products. Data revealed a steady decline in *Salmonella* prevalence. For example, prevalence on chicken parts (legs, breast and wings) decreased 75% over an 8-year period from 2012 to 2019.

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1 Participating agencies include the U. S. Department of Agriculture, Food Safety and Inspection Service; U.S. Department of Health and Human Services, Food and Drug Administration, and Centers for Disease Control and Prevention; U.S. Department of Commerce, National Marine Fisheries Service; and U.S. Department of Defense, Veterinary Service Activity.

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2020 (177). Furthermore, *Salmonella* Typhimurium and Heidelberg infections associated with poultry have declined in the past two decades, likely due to the use of group B *Salmonella* poultry vaccines on broiler farms and other interventions. However, in spite of this reduction of *Salmonella* prevalence in poultry products, there has not been a concomitant reduction of overall *Salmonella* illnesses (Figure 2; (24)) nor in the proportion attributed to poultry (Figure 1) (76), and the proportion of illnesses associated with serotypes Enteritidis and Infantis have increased (177).

In an attempt to resolve this discrepancy, NACMCF reviewed the scientific evidence on *Salmonella* control in the US and abroad, foodborne illness surveillance data, quantitative microbial risk assessments, and microbiological testing of indicator organisms vs. *Salmonella* on poultry throughout the farm to fork continuum. Based on this information, this document seeks to provide guidance to FSIS and the poultry industry on what types of microbiological criteria might be used to identify effective intervention strategies pre- and postharvest to reduce in *Salmonella* in poultry products and thereby prevent human *Salmonella* infections associated with these products.

The infectious dose of *Salmonella* vary widely between serotypes (144). Recent data suggest that most poultry-associated salmonellosis in the US is associated with Enteritidis, Typhimurium, 4,5,12:i:-, Infantis and Heidelberg, and even fewer serogroups: groups O:4 (formerly group B), O:7 (group C1), and O:9 (group D1). In live poultry, serovars Infantis and Typhimurium are predominant in the Atlantic region, whereas a higher proportion of serovar Schwarzengrund are found in the Southeast, with the exception of the state of Georgia, which has higher proportion of Kentucky. Vaccination against specific serotypes, such as S. Typhimurium, are common among US broiler breeders, a strategy which has reduced the incidence of contamination of that serotype (98). However, vaccine development takes years and lags behind the shifting of predominant serotypes found in flocks. Other US preharvest management practices include competitive exclusion, controlling quality of feed, biosecurity, moisture control in the barns, and clean transport coops. Microbial testing (qualitative testing) for total *Salmonella* at breeder or broiler farms can be focused on environmental samples (boot or drag swabs) and cecal testing (12); however sufficiently sensitive tests and specific serotype testing is needed to determine if any changes are required for overall *Salmonella* control that are most often associated with human illness. One suggestion is that based on results from microbial testing at farms, highly contaminated birds can be targeted for logistic slaughter (scheduling) or other interventions. However, value of this approach for scheduling purposes has not yet been realized (108, 119).

As noted above, the relative number of infections in the US caused by Typhimurium and Heidelberg have declined during the past 20 years (24). This decrease corresponds to the use of a commercial poultry vaccine against S. Typhimurium, which may also deliver cross protection against Heidelberg (100), targeted environmental testing to develop strategic management programs, as well as implementing other intervention strategies. While progress has been made against these two serotypes, overall cases of salmonellosis attributed to poultry remain unchanged with Enteritidis, Typhimurium, Infantis, and 4,5,12:i:- accounting for 83% of the chicken associated illnesses. These data suggest that alternate methods for control of *Salmonella* or detection that do not rely solely on serotype may be needed. While vaccines are effective serotype-specific intervention strategies, other methods need development to reduce carriage of *Salmonella* in the bird. Furthermore, attribution data does not
specifically identify whether sources of Salmonella were whole carcasses, parts, comminuted product, or breaded raw poultry products. More granular data will help determine if all poultry products pose the same risk and allow a targeted management program.

Indicator organisms such as Enterobacteriaceae (Eb) or aerobic plate counts (APC) have been used by the industry as gauges of process control and to measure the microbial reduction from carcasses at slaughter to post chill (175). However, some studies have shown that populations of these indicators are not directly correlated to populations of Salmonella (14, 44, 127). Because of the conflicting and apparent weak correlation between indicators and either the presence or level of Salmonella post-carcass wash (175, 176), one approach is to base microbiological criteria on Salmonella enumeration. Microbial risk assessments suggest that diverting ground turkey product above a set threshold of Salmonella colony forming units (CFU) per gram, rather than based on Salmonella presence alone, is expected to remove product from the market that has higher chances of causing infection and therefore reduce illness (85). This concept is currently used by industry whereby poultry used in breaded and stuffed raw chicken product are enumerated for Salmonella by quantitative PCR (qPCR) not targeted for specific serotypes. qPCR presents logistical advantages over MPN (most probable number) methods due to its relatively rapid time to detection and therefore, more actionable (such as diverting product to high pressure pasteurization or cooking) during a production day. However, qPCR methods must have high sensitivity to consistently detect Salmonella loads at the ranges most often seen in poultry products.

In October 2022, USDA announced that they will be proposing a revised regulatory framework to reduce Salmonella infections linked to poultry products (168). The key components include requiring that incoming flocks be tested for Salmonella before entering an establishment, that establishments enhance process control monitoring, and the agency implementing an enforceable final product standard. Quantitative risk assessments paired with additional data, perhaps including historical data collected by industry, are needed to better establish if quantification specifically of highly pathogenic serotypes, rather than including all Salmonella serotypes, as a performance standard will be more effective in diverting more infectious products compared with the current approach, and thus reduce salmonellosis in the US.

The Committee’s responses to the charge questions are based on the information available at the time of writing. The Committee identified a multitude of data gaps that could affect findings and recommendations to FSIS, including the need for completion of the two quantitative risk assessments for chicken and turkey that are in progress (164). These risk assessments will evaluate the public health impact (change in illnesses, hospitalizations, and deaths) achieved by eliminating a proportion of poultry or poultry products that are contaminated with specific levels of Salmonella and/or specific Salmonella subtypes. The risk assessments will also explore the public health impacts of various monitoring and enforcing process control methods, from the re-hang to post-chill stages. Finally, the risk assessments will address the public health impact of implementing combinations of the aforementioned risk management strategies.
96 Recommendations:
97 1. Collect appropriate data to refine food attribution models to determine which form(s) of raw
98 poultry (further processed vs. parts vs. whole carcasses) and food handler practices that contribute
99 most to salmonellosis associated with chicken and turkey.
100 2. Expand systematic FSIS sampling for *Salmonella* levels, prevalence, and subtypes on poultry pre-
101 harvest (hatcheries, feed, broiler houses) and post-harvest (slaughter through processing).
102 Prioritize comminuted poultry products, mechanically recovered poultry meat, tenders, and
103 breaded stuffed raw chicken products, to identify *Salmonella* levels and evolution of predominant
104 serotypes.
105 3. Incentivize industry to deposit data (anonymized, non-punitive) on levels of indicator organisms
106 and *Salmonella* prevalence, concentration and serotypes, found at various stages of processing
107 (pre-harvest through final product) along with practices that may mitigate contamination.
108 a) Analyze data to identify alternate process control indicators and to use in risk assessments
109 to update performance standards
110 b) Analyze data to determine how non-*Salmonella* quality indicator sampling (perhaps other
111 than APC) could be established that would provide guidance to companies on how to
112 identify houses that have a higher probability of *Salmonella* contamination and modify
113 mitigation strategies
114 4. Frequently (e.g., every 2-3 years) compare the serotypes that are isolated from patients with those
115 isolated from poultry products to determine if intervention strategies used by the industry are
116 effective against all *Salmonella* equally or are selecting for specific serotypes.
117 5. Develop and validate quantitative testing methods to determine if and how testing and processing
118 scheduling can reduce the likelihood that carcasses and parts with higher levels of *Salmonella* that
119 are most capable of causing illness are released into commerce
120 6. Consider changes to performance standards based on enumeration of *Salmonella* in the product
121 rather than prevalence of *Salmonella* only or on serotype found.
122 7. Complete risk assessments for chicken and poultry to assesses public health impacts of different
123 risk-based *Salmonella* control strategies
124 8. Incentivize industry to universally implement robust *Salmonella* mitigation programs and
125 qualitative *Salmonella* testing at the breeder, hatchery, grow out, and transport levels. Eliminate
126 conditions in houses that harbor and transmit *Salmonella* by implementation of known and
127 validated mitigation strategies.
128 9. Due to extensive data gaps identified by the Committee, the agency should reevaluate this
129 document in three to five years after appropriate data has been collected and risk assessments are
130 complete.
According to the Centers for Disease Control and Prevention (CDC), *Salmonella* is responsible for approximately 1.35 million cases of foodborne illness each year in the United States. The U.S. Department of Agriculture (USDA) Food Safety and Inspection Service (FSIS) has established qualitative performance standards to limit the occurrence of *Salmonella* in poultry products (i.e., carcasses, parts, and comminuted products). The goal of these performance standards is to allow FSIS to verify each regulated establishment’s control for this pathogen in raw products throughout the slaughter process, and, by meeting these standards, achieve national food safety goals. Over the past 25 years, there have been significant reductions in the proportion of poultry contaminated with *Salmonella*, but no meaningful reduction in human *Salmonella* infections attributed to poultry products (178). Therefore, FSIS is seeking guidance on how to improve the current system for reducing *Salmonella* in poultry to better protect public health.

In 2019, the National Advisory Committee on Microbiological Criteria for Food (NACMCF) recommended that FSIS move toward risk-based disposition of finished raw poultry product, informed by *Salmonella* amount and serotype (101). Since then, several studies suggest that setting microbiological criteria (e.g., performance standards) to limit the amount of *Salmonella* in products and/or to address serotypes more frequently associated with foodborne illness would better protect public health than the current approach. In addition, several studies have demonstrated the feasibility of developing quantitative microbiological criteria based on a change in the concentration of indicator organisms correlated to *Salmonella* occurrence. These findings, along with new technologies and advancements in rapid quantification of pathogens in products, present opportunities for FSIS to enhance the microbiological criteria it establishes to measure industry control of *Salmonella* in poultry products. FSIS seeks input from the NACMCF on the best options for using quantification and/or particular pathogen characteristics, along with a relevant pathogen indicator, to enhance its microbiological criteria and reduce *Salmonella* illnesses attributed to poultry products consumed in the U.S.

**BACKGROUND**

*Salmonella* bacteria are a leading cause of foodborne illness. According to CDC estimates, *Salmonella* is responsible for approximately 1.35 million illnesses, 26,500 hospitalizations, and 420 deaths every year in the United States. Using weighted outbreak data from 1998–2018, the Interagency Food Safety Analytics Collaboration, a joint effort between CDC, the U.S. Food and Drug Administration, and USDA, estimates that over 20 percent of foodborne salmonellosis is attributed to poultry products (76).

FSIS established limits, referred to as performance standards, on the occurrence of *Salmonella* in poultry products as part of the Pathogen Reduction, Hazard Analysis and Critical Control Point (PR/HACCP) Systems Final Rule. These standards were designed to improve food safety, reducing the risk of foodborne illness, and enable FSIS to verify process control. Process control is a defined procedure or set of procedures designed by an establishment to provide control of those operating conditions that are necessary for the production of safe, wholesome food. The procedures typically include some means of observing or measuring system performance, analyzing the results generated in order to define a set of control criteria, and taking action when necessary to ensure that the system continues to perform within the control criteria. FSIS has since updated those original performance standards. FSIS relies on
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qualitative (presence/absence) pathogen sampling data to apply performance standards for Salmonella in poultry products. These standards are based on quantitative microbiological risk assessments (50, 179) and are designed to achieve the national food safety goals. The Healthy People 2020 food safety goal was a 25 percent reduction in foodborne Salmonella illnesses, to achieve fewer than 11.5 Salmonella infections per 100,000 population per year. In 2020, the case rate for Salmonella infection was 13.3 per 100,000 population (120). FSIS has proposed performance standards for beef products and intends to propose performance standards for pork products.

Since the implementation of Salmonella performance standards for poultry, FSIS has measured a substantively lower occurrence of this pathogen in raw poultry products, but the incidence of human illness associated with consumption of poultry products has not decreased (177). FSIS is interested in developing microbiological criteria (i.e., an alternative type of performance standard(s)) that will result in a substantively reduced level of human illness from Salmonella in poultry. FSIS is considering microbiological criteria such as the quantity of Salmonella in products, and the presence of Salmonella serotypes more frequently associated with human illness, rather than presence/absence of any Salmonella. Criteria could be set at various points in the food safety system to better assess industry control over Salmonella in these products, for example, prior to establishment interventions and after establishment interventions, to evaluate the effectiveness of an establishment’s food safety system in mitigating Salmonella in products during the slaughter process.

Several recent studies showed a correlation between indicator organisms and Salmonella in poultry. Specifically, a correlation was shown between a change in the quantity of an indicator (i.e., Aerobic Plate Count (APC)) from the carcass to finished product and the occurrence of Salmonella in beef, pork, and poultry (175, 176). However, the university research on biomapping and the poultry industry internal testing has evaluated the use of indicator bacteria and found that the populations of indicators offer little to no predictive value for the presence of non-Typhoidal Salmonella. Regardless, based on FSIS findings, FSIS is considering developing a quantitative “log-reduction” microbiological criterion (e.g., performance standard) to measure the effectiveness of an establishment’s food safety system in controlling Salmonella in products. FSIS believes this type of performance standard would allow for continued monitoring of industry performance in achieving the Healthy People national food safety goals, thereby improving public health outcomes, while providing better insights on pathogen control throughout the food safety system. As part of this consideration, FSIS would also like to know how the Agency could address Salmonella serotypes more frequently associated with human illness, strain characteristics (e.g., virulence factors), and/or the quantity of Salmonella in a subset of products tested, prior to and after interventions, when evaluating industry’s control of Salmonella.

FSIS is considering the following microbiological criteria to assess industry control:

1. the presence of any Salmonella or only specific serotypes more likely to cause illness, at preharvest (e.g., as measured at the receiving step in the slaughter process)
2. the amount of Salmonella and/or presence of serotypes more likely to cause illness, throughout the slaughter process; and
3. a relevant indicator for Salmonella, throughout the slaughter process.
CHARGE QUESTIONS

FSIS is seeking guidance on the overarching risk management question: What types of microbiological criteria (e.g., Salmonella performance standards) might FSIS use to encourage reductions in Salmonella in poultry products so that they are more effective in preventing human Salmonella infections associated with these products?

Specific risk management questions posed to NACMCF are:

1. Can we assess the public health impact (e.g., reduction in salmonellosis) of controlling specific Salmonella serotypes and/or amount (levels) in poultry products? What types of approaches could be used?

2. What types of microbiological criteria could be established to encourage control of Salmonella at preharvest (i.e., in live birds on-farm)?
   a) Should FSIS consider qualitative microbiological criteria for control of the presence of Salmonella in a flock when they are presented for slaughter?
   b) How could FSIS use these criteria to address Salmonella serotypes most frequently associated with human illness?
   c) What industry data would provide evidence of control?

3. What types of microbiological criteria could be established for poultry carcasses, parts, and comminuted products prior to applying interventions and after interventions, considering current technology?
   a) Could the quantity of Salmonella or quantity of microbiological indicator organisms (e.g., APC) be used? What are the key parameters that need to be considered? What data analysis techniques could be used? How would these criteria be linked to human illness?
   b) How could serotypes frequently associated with human illness be considered in the development of microbiological criteria?

4. How might foodborne illness surveillance data on human Salmonella illnesses, data from foodborne outbreaks associated with Salmonella in poultry, and data on Salmonella serotypes in poultry products be used to identify the Salmonella serotypes of greatest public health concern associated with specific poultry products?
   a) Should only the most current data (e.g., 5-years) of foodborne illness surveillance, outbreak and/or pathogen testing data be used?
   b) Going forward, what methodology and criteria would focus on those Salmonella serotypes most frequently associated with human illness and attributable to poultry products?
   c) How frequently should the priority Salmonella serotypes associated with poultry be revised considering changes in their occurrence while still ensuring continuity in industry and regulatory testing?
5. There is a documented correlation between a reduction in the quantity of APC between carcasses and finished products and the occurrence of *Salmonella* in finished products for beef, pork, and poultry. How might this information be used to set microbiological criteria to assess process (pathogen) control in poultry?

6. What rapid methods and technologies are available for the quantification of *Salmonella*? How should FSIS make the best use of these methods?

7. Are there particular approaches that would result in selective identification of the serotypes of public health concern?
   a) For example, are there approaches to mitigate a potential strain selection bias introduced by the laboratory method?
   b) If needed, what type of research could be conducted to ensure performance characteristics of current laboratory methods (e.g., enrichment, incubation, pre-screening) do not result in a biased serotype detection?

8. How should pathogen characteristics derived from whole genome sequencing (e.g., serotype, virulence, antimicrobial resistance) be considered in the development of microbiological criteria?

9. What research is needed to support FSIS’ new *Salmonella* strategy in terms of setting microbiological criteria?
Approach by the Committee: The Committee organized the charge questions into three groups: (1) The impact of Salmonella and poultry on public health (Q1 and 4); (2) The role of microbial testing at the preharvest and post-harvest levels for process control (Q2, 3, and 5); and (3) Methodologies for detection and enumeration of Salmonella (Q6, 7, and 8). The working groups considered Q3-a and Q5 on the use of aerobic plate counts as indicators for Salmonella contamination similar and are merged for response. Data gaps for each individual charge question are summarized in response to Q9. The charge did not specifically request an evaluation of efficacy of various intervention strategies on the farm or post-harvest, but the Committee briefly discusses practices that may affect presence, levels or serotypes of Salmonella throughout the chain.

Question #1: Can we assess the public health impact (e.g., reduction in salmonellosis) of controlling specific Salmonella serotypes and/or amount (levels) in poultry products? What types of approaches could be used?

Summary of Question 1 Response: Two perspectives are deemed necessary to answer this question: (1) how to predict the public health impact of hypothetical changes in Salmonella control strategies in poultry products prior to their implementation, and (2) how to assess the effectiveness of the standards in reducing salmonellosis once they are implemented.

Part 1: Well-established quantitative microbial risk assessment (QMRA) methods can be used to predict salmonellosis reductions resulting from changes in microbial criteria in poultry products. Incorporating and comparing prevalence, level, and subtype in QMRAs might require the incorporation of emerging Omics methodologies while acknowledging data quality/availability issues.

Part 2: Several types of public health surveillance data are available with which to assess the efficacy of standards in reducing salmonellosis. Allowing for some year-to-year variability, case-based serotype- and genotype-based surveillance may reflect important changes within 2-5 years. The goal of reducing poultry-associated salmonellosis by 25% is an important step towards meeting the HHS Healthy People 2030 goal of reducing domestically acquired salmonellosis by 25% by 2030.

The objective of microbiological criteria (MC) (i.e., performance standards) is to establish control limits for the contamination of pathogens in foods or food production environments along the supply chain based on a specified measurement method and sampling plan, and ultimately reduce foodborne illnesses. The Salmonella Poultry Performance Standards currently employed by USDA FSIS for different poultry products follow a prevalence-based approach, regulating a fraction of maximum allowable positives over the large number of samples collected and analyzed in a specified time window (161). As an alternative to the current method, controlling specific Salmonella serotypes and/or amount (levels) on poultry and in poultry products may potentially further reduce the Salmonella exposure through poultry product consumption and therefore mitigate the public health risks. To effectively guide the changes in MC, the associated public health impact needs to be comprehensively evaluated before their implementation, which can be achieved using quantitative microbiological risk assessment (QMRA) approaches.
QMRA approaches have been applied to assess the potential impact of MC in other commodities and hazards in support of risk management decisions, in the U.S. and other countries, for example for *Campylobacter* in poultry (53, 107, 141) and *Salmonella* in pork and comminuted beef (162, 167). While this existing body of work does not fully account for the current U.S. situation and the range of possible MC to consider, it provides relevant approaches, tools, and application examples for how risk-based models can be used to support the establishment of level/serotype-oriented MC for poultry products (53, 107, 141) and *Salmonella* in pork and comminuted beef (162, 167).

Evaluating the public health impact of a MC that controls for prevalence and amount of *Salmonella* on poultry products is feasible using traditional QMRA approaches. QMRAs use a “bottom-up” mechanistic approach to modeling the change in amount of *Salmonella* on poultry products through the production and processing stages relevant to the considered MC, possibly including primary production, slaughter, processing, distribution, through consumer handling and cooking until consumption. Variability and uncertainty in the parameters considered at this stage can be represented. As a result, QMRA models allow estimating the probability distribution of exposure dose (number of *Salmonella* cells ingested) as well as the associated distribution of risk (e.g., illnesses, hospitalizations, deaths in a population) using a dose-response relationship, if a suitable one is available.

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Part 1: Using quantitative microbial risk assessment methods to predict the public health impact of changes in MC in poultry products

The public health impact of controlling specific *Salmonella* serotypes and/or amount (levels) in poultry and poultry products can be estimated using QMRA. QMRA approaches have been applied to assess the potential impact of MC in other commodities and hazards in support of risk management decisions, in the U.S. and other countries, and can provide useful templates for additional assessments. Examples of recent QMRA studies relevant to the charge question are reviewed and discussed in this section.

MC can be classified into two main categories: (1) MC defined as a threshold (prevalence and/or concentration) of a target organism in the finished product (or other specified production stage), also called food safety criteria, and (2) as a threshold in a target variable associated with processing conditions conducive to the control of the target organism, also called process hygiene criteria. While QMRA can be used to assess the impact of both, most assessments have focused on food safety criteria.

MC can have an impact on public health only if they are associated with risk control actions that keep or bring a process back into control/compliance, and if such actions are enforced. Models used to estimate the impact of a MC must include these risk control actions. For example, in the case of a MC on finished product, a batch of product not meeting the MC may undergo additional validated processing that reduce contamination by a certain number of Log CFU/g; such Log reduction will be accounted for in the model. Risk control actions involving the processing environment can also be modelled, although they are generally more complex to represent in detail. For example, an establishment not meeting a MC may undergo in-depth sanitization, validated within the establishment to bring the MC variable below the MC threshold, or to impart a known reduction. While most commonly product-level MC are paired with product-level risk control measures, and establishment-level MC are paired with establishment-level risk control measures, MC and measures can also be associated differently. For example, an establishment whose product does not meet a MC may undergo in-depth sanitization, possibly in addition to product treatment.

QMRA has been conducted to support the development or assessment of MC for *Salmonella* prevalence and/or levels, with some consideration of serotypes of public health concern, in poultry products (post-chill), including to align a MC with a desired target reduction in attributable illness (48, 50, 84, 85, 113, 175, 178) (Table 1).

MC and associated risk management measures can be set for different and multiple stages of the production and processing chain. Risk-based models can be developed to assess the impacts of such MC, by including the production steps downstream of the MC. Hence, to model MC upstream of the finished product a higher number of variables, as well as their uncertainty and assumptions, would need to be included. Most published studies on MC in postharvest poultry processing have considered MC on the finished product. While none of the developed models were applied to assess MC for *Salmonella* at rehang, they provide approaches to do so, and to model the growth and decline of *Salmonella* on poultry throughout the slaughter process, processing, and subsequent exposure pathway steps,
assuming all subtypes have the same predictive microbiology (or accounting for differences, to the extent supported by data).

QMRAs often treat all *Salmonella* subtypes or serovars equally, for lack of serovar-specific data, in particular dose-response relationships. That is, these models implicitly assume that all serovars have the same probability of infection following an exposure (infectivity) and same probability of a severe illness resulting from an infection (virulence). Infectivity is usually modeled with a dose-response function or curve (69), whereas virulence is often modeled as proportions of severe illnesses based on different disease severity outcomes such as extraintestinal infections, hospitalizations, and/or deaths.

Ebel et al. (48, 50) provide an approach for establishing a prevalence-reducing MC *Salmonella* in poultry products. This is based on a finding that at lower *Salmonella* prevalence, there is a linear dose-response relationship between prevalence in product and salmonellosis in humans. The advantage of this QMRA approach is that it simplifies the modeling by reducing the number of parameters and eliminates the uncertainty common to exposure pathways associated with consumer handling and preparation, cross-contamination, and *Salmonella* dose-response. The limitation of this approach is that it assumes the amount of *Salmonella* on contaminated products is independent of its presence, and it treats all serovars as equally infective and virulent.

Lambertini et al. (84, 85) provided a modeling framework to evaluate the potential relative reduction in public health impact associated with prevalence-based and concentration-based MC for *Salmonella* in poultry products (comminuted turkey and chicken parts), tested at the end of processing. These QMRAs use a “bottom-up” approach to modeling the change in amount of *Salmonella* on poultry products from the end of the production process, where sampling is assumed to occur, through consumer handling and consumption to estimate exposure. Considered corrective actions included simplified lot-level *Salmonella* reduction measures and establishment-wide hygiene improvements. The comparison of prevalence- and concentration-based MC, as well as a combination of the two, suggests that both approaches can lead to a significant illness burden reduction, when 100% sensitive testing is frequent and when MC are enforced, i.e., an effective corrective action is implemented upon non-compliance (84). These studies identified significant gaps in data on prevalence and concentration levels representative of the range of U.S. processing establishments, concentration variability within and between product lots, conditions during transport and retail, and consumer handling and preparation practices, including transfer coefficients to model cross-contamination in kitchens. Due to the scope of the studies, these two models did not assess the impact of different infectivity or virulence associated with different serovars, assuming that control measures applied at processing in case of non-compliance would have a similar impact on all serovars.

In another study, researchers proposed a QMRA model for *Salmonella* control in ground turkey where both the amount of *Salmonella* and hypothetical differences in serovar infectivity were incorporated. For this model, the authors grouped serovars in high and low infectivity groups and used dose-response models from experimental and outbreak data as proxy for the infectivity in the high and low groups. The model further assumed that all lots with high contamination (>1MPN/g) would be detected and removed using a test with 100% sensitivity and predicted up to 50 million annual salmonellosis cases
from ground turkey in the US. Recently, significant issues were reported with this approach (185) that resulted in the retraction of the Sampedro et al. article (126).

Oscar (112-114) also used a comparable framework to that of Sampedro et al. to contrast enumeration vs. prevalence standards in ground turkey. The approach relied on a series of untested modeling assumptions such as adjusting the dose-responses by serovar that resulted in broad infective doses (ID50) ranging from 1 to 10 log\(_{10}\) (CFU/g).

None of the existing QMRAs that evaluate Salmonella MC for poultry products fully consider the public health impact of controlling all three dimensions considered in this question (prevalence, level, and subtype) while also considering realistic scenarios of frequency and sensitivity of testing of the product and its resulting impact on the contamination of the final product. For example, one scenario recommended for investigation is the testing of all finished product batches for both presence and concentration of the pathogen, and contrast it to the less frequent sampling currently done by FSIS. Also, none evaluated the inclusion of indicator organisms as a proxy of presence or of process vulnerability to Salmonella contamination (see also Q5). Evaluating the public health impact of a MC that controls for prevalence and amount of Salmonella on poultry products is feasible using QMRA approaches. However, evaluating the public health impact of controlling certain Salmonella subtypes (i.e., serotypes and genetic factors related to virulence) would require their identification, information on their presence in poultry products, and the ability to predict differences in the probability of illness from foodborne exposure to these Salmonella subtypes.

Parameters needed to model MC

Possible inputs and parameters needed to model a MC, considering the specific processing stage where a MC is set (i.e., not including all stages needed to assess exposure to consumers), are shown in Table 2. A model may include only some of these parameters, depending on its structure and scope. These parameters can be kept in mind when evaluating MC options.

This answer does not include a comprehensive review of available data in relation to QMRA model needs. However, this committee agrees on the importance of adequate data to support risk estimates and estimate the impact of a MC with sufficient precision. For instance, current data on Salmonella prevalence or concentration collected by USDA-FSIS as part of routine monitoring or targeted data collection efforts only partially support QMRA efforts. Presence/absence data are collected infrequently, in particular for smaller establishments. Until recently, concentration data were not routinely collected; the last data collection effort on chicken products, representing only a portion of establishments, was carried out in 2012 (158). However, FSIS has launched a 4-month program for collecting paired chicken samples collected at rehang and post-chill (169). These samples are tested for Salmonella presence, levels (CFU/g), and serotypes. FSIS plans to continue testing chicken carcasses at post-chill for Salmonella levels, which would make this routine testing. The industry has also been sampling product and processing environments. Combining industry and government data could allow for improvements in how Salmonella contamination is modeled.
Including indicator organisms in models

Models to estimate the impact of MC can also include indicator organisms different from the target pathogen, complementing information on the occurrence of the target pathogen, or possibly instead of it. For the inclusion of indicators to be appropriate, a reliable quantitative relationship needs to be available that ties the presence and/or level of the indicator organism to the presence and/or level of the target pathogen. This relationship can be present in the processing environment and/or in the product, depending on the type of MC, and may be general or specific to each establishment. Question 5 further discusses possible indicators relevant to the control of *Salmonella* in poultry in the U.S. At the time of writing, such indicator/Salmonella relationships are not established, although preliminary data exist. When validated data are available, the status of an establishment vis-à-vis the selected indicator can be used in models to estimate the likelihood that the target is present at an unacceptable level at a certain processing stage, or the likelihood that a risk-relevant event may occur (e.g., cross-contamination). Examples of models and standards including indicator organisms exist in other domains; for example, drinking water standards are often based on indicators of fecal contamination, since that is a primary contamination route and presence of fecal indicators at unacceptable levels indicates lack of process control (104).

Identifying and incorporating serovar virulence into QMRAs

Very recent developments in WGS and bioinformatics have resulted in several potential approaches to use genomics to identify genetic markers of *Salmonella* of public health concern. This information can be used to create groups of isolates or serovars, which can then be used in QMRAs.

Karanth et al. (79) provided an exploratory approach to use genomics and machine learning to identify severe *Salmonella* serovars. The authors collected sequence reads for 150 *Salmonella* isolates from humans, poultry, and swine sources, and used the pangenome (i.e., all genes from all isolates) in various statistical models to predict disease presentation, categorized as gastrointestinal vs extraintestinal. The best model had a predictive accuracy of 76%. As the methodology used the pangenome, their methodology was computationally intensive, which might explain why only 150 isolates were used. The need for intense computation limits its scalability at present time. Also, disease presentation information was not present for non-human isolates (e.g., a swine isolate could not have a human disease presentation unless it was related to a traced foodborne outbreak so it had to be imputed.

Chen et al. (33) used *Salmonella enterica* subsp. enterica Ser. Saintpaul (S. Saintpaul) as a model to identify potentially hypervirulent strains. Single nucleotide polymorphism (SNP) clusters (i.e., groupings of isolates with low number of point mutations) were queried on NCBI for SNP clusters with either a high proportion of human clinical isolates (HA) or a low proportion of human clinical isolates (NHA). A total of 211 S. Saintpaul isolates were combined with 313 *S. enterica* isolates from other serovars to produce a phylogeny of *S. Saintpaul* illustrating the serovar is polyphyletic. Nine SNP clusters from group I (single monophyletic clade) were chosen for further analysis with 5 HA and 4 NHA isolates. Ten isolates were sampled from each SNP cluster to generate an analysis set of 90 contiguous (contig) assemblies.

Differential carriage of plasmid, prophage, and individual gene carriage (pangenome-wide associate study) were assessed between the HA and NHA clusters. Seven isolates (4 HA, 3 NHA) were tested for cell invasion and intracellular survival (both in vitro) to quantify phenotypic differences between the SNP...
clusters. As S. Saintpaul is polyphyletic (i.e., same taxonomic grouping, different evolutionary paths), this serovar may not be the best model to test for differences between human and non-human isolates.

Also recently, Fenske et al., (63) reported a methodology to group isolates based on genetic virulence markers and validated the groups against epidemiological data using beef isolates and outbreaks as a model. For this, contig assemblies (de novo genome assemblies from short reads) of Salmonella isolates from beef, cattle, and humans were collected from FSIS and FDA and surveillance programs (n = 12,337). Putative virulence factors were predicted, representing a virulence gene catalogue for each respective isolate. The pairwise similarity between each virulence gene catalogue (measure of genomic relatedness between isolates in regions which may impact phenotypic virulence) was estimated using unsupervised random forest and used in a hierarchical clustering algorithm to group isolates by similarity. Serovar designation was provided post-hoc and was not used to generate genomic clusters to control for the impact of polyphyletic serovars. The five groups were externally validated against epidemiological foodborne illness data (overall, and beef attributed) to explore if the genetic-based groups correlated with observed virulence at the serovar level. The isolates in the “high virulence” cluster were most often implicated in foodborne outbreaks of any cause and in beef-associated outbreaks but were less common in beef than all the other 4 groups combined. They also resulted in 1.5 times higher incidence of disease and higher hospitalization rates than all other groups combined.

The studies above illustrated the potential of genomic methodologies in the application for QMRAs in poultry where differential MC for serovars are possible, with the Karanth et al. (79) method using the most genes, and the Fenske et al. (63) method being the most scalable as it used only the subset of genes in a virulence catalog and removed all repeated, core Salmonella genes.

Using potential genetic serovar or isolate groups to adjust dose-response (DR) models

Only a few DR models exist for specific Salmonella serovars. These models have been derived based on animal studies, volunteer feeding trials, and outbreak investigations, so their application to entire populations has resulted in large disconnects between model-predicted illnesses and observed foodborne illnesses recorded in national surveillance systems such as FoodNet (see Question 4). For this reason, QMRAs often use adjustment factors to calibrate the model predictions so that they match surveillance data.

A QMRA that is designed to assess MC based on specific target serovars and corresponding levels should thus be able to incorporate dose-response models that apply to the serovars of interest and be able to predict annual salmonellosis cases that are in agreement with surveillance data. Teunis et al. (144) provide a mathematical framework to adjust different dose-response curves by serovars based on outbreak data where the mean CFU/gr of Salmonella in the food vehicle, number of exposed individuals, and numbers ill (and/or infected) was available. Although the Teunis framework was developed for individual serovars, this methodology could be expanded to accommodate genomics-based serovar groupings such as those described by Fenske et al. (63). A modification of the Teunis framework allowing for serovar groupings would also have to provide a way to map the exposure from poultry to the resulting dose-response, which could be done using calibration methods.
Note that these approaches have the advantage of working directly on the genetic source explaining possible differences in infectivity and virulence. Such methods can be paired with surveillance-based methods for source attribution—such as those covered in Q4—to provide necessary adjustments to the dose-response curves.

**Incorporating quantitative molecular methods into QMRAs**

A variety of emerging molecular assays to detect or quantify *Salmonella* are reviewed in Question 6. The sensitivity and specificity parameters (and hence level of detection (LOD) and level of quantification (LOQ)) of these assays are key to understand the efficacy of MC standards in reducing foodborne illnesses as incorporated in QMRA models.

In addition, models need to account for the interplay between LOD and LOQ of quantitative assays, MC threshold, and number of samples collected. For instance, quantitative methods such as qPCR are less sensitive to detect presence of *Salmonella* than qualitative PCR methods because they have higher LOD. A lower sensitivity would result in more samples required to determine with high confidence if a quantitative threshold has been exceeded. Generally, sensitivity is concentration-dependent, with an assay being more likely to reliably detect the pathogen if the target is present at higher concentrations. For quantitation, an assay’s ability to quantify a target precisely (i.e., low variability in the quantitative estimate) is also generally concentration dependent. It is crucial that a quantitative assay is sufficiently precise in the range of the MC threshold. Thus, depending on the MC threshold concentration versus the assay’s LOQ, quantitative standards may require a higher number of samples per product unit in order to correctly classify a batch or an establishment as compliant. This would be more so for standards based on the level of specific serovars, since each serovar would be less frequently present than *Salmonella* spp.

There is evidence that a large proportion of poultry product samples harbor low *Salmonella* concentrations. For example, in the 2012 Nationwide Microbiological Data Collection Program, 31% of screening-positive chicken part samples were found to be below the MPN assay LOD of 0.030 MPN/ml (rinsate), corresponding to 0.0066 MPN/g of product, and 79% were below 0.30 MPN/ml. Similar results were also observed in previous years (2007-08), for carcass rinse post-chill (155). Numerical examples of the impact of these results are provided under the Assay Parameters heading in the answer to Question 3, but the practical implication is that if the LOD of an assay is set to a level to only target high loads in chicken products (e.g., 10 CFU/g), the subsequent reduction in sensitivity would result in a large increase in the number of samples required to estimate the proportion of samples contaminated with that CFU/g level of *Salmonella*. The number of samples to be tested would also have to be further increased if only a subset of serovars of concern are included.

In conclusion, both in risk models and cost-benefit models, it is important to include sensitivity and specificity parameters of the sampling assay, and the sample size needed to detect positives with a high level of confidence. For currently available assays to support quantitative MC, they have to perform well at all concentrations (low and high) commonly found in poultry meat, as currently established using MPN assays.
Q1 – Part 2. Assessing the efficacy of microbiological criteria (MC; e.g., standards) in reducing salmonellosis once they are implemented

(a). What is the operational definition of success for a MC?
Can we devise an operational definition of success of one or more microbiological standards, in terms of reduced health burden? If so, what should or could be measured?

The Healthy People 2030 Food Safety goals agreed upon by FSIS, FDA, and CDC included a specific target of reducing domestically-acquired human salmonellosis by 25% by the year 2030, as measured through FoodNet, and using a baseline period of cases reported in 2016-2018 (148). If between 25 and 50% of domestically acquired salmonellosis is from poultry (see Q4) then this target is congruent with the FSIS goal under evaluation, of reducing poultry-associated salmonellosis by 25%. Prevention targeting other sources of salmonellosis will also be important to ensure that we reach the overall Healthy People goal.

The goal for FSIS pertains to domestically acquired salmonellosis, as the actions taken in the US to control salmonellosis may have little impact on exposures occurring in other countries. According to FoodNet, approximately 10% of salmonellosis in 2017-19 was associated with foreign travel (142).

(b). What existing public health surveillance data are available for salmonellosis?

Several types of public health surveillance data are available to help track salmonellosis (see Table 3). Basic case surveillance in the US is based on reporting to CDC of cases of salmonellosis through state epidemiology departments, and the serotypes and strains isolated from patients’ specimens through public health laboratories. Routine serotyping in those laboratories began in 1963 with reporting to CDC (13). Among the approximately 2,600 serotypes described (30), 883 were reported to the National Salmonella Surveillance System to have been isolated from humans during 2006 through 2016 (22). The top 20 serotypes accounted for 80.7% of isolates with a reported serotype. The same 5 serotypes have persisted as the most common causes of human illness for the last decade (Enteritidis, Newport, Typhimurium, Javiana, 1,4,5,[12]:I: - ) (36). In 1996, state public health laboratories began conducting molecular subtyping, using pulsed-field gel electrophoresis (PFGE) patterns to identify clusters with similar patterns and submitting the subtyping information to CDC through PulseNet, the national network for molecular subtyping; this became routine for Salmonella isolates in all states by the early 2000’s (66, 140). Routine subtyping has greatly improved detection and investigation of dispersed outbreaks, and source attribution. In mid-2019, PulseNet switched to genome sequencing for subtyping Salmonella (multilocus core genome sequence typing, cgMLST), which offers substantially greater precision in measuring relatedness than did PFGE. Whole-genome sequencing (WGS) data allow the systematic prediction of serotype and antibiotic resistance profile from sequence data (92, 186).

PulseNet, which includes all state and some large city health department public health laboratories, and both FSIS and FDA as members, previously used PFGE and now use WGS to characterize regulatory isolates as well. The sequences of approximately 40,000 isolates of Salmonella from humans are reported to PulseNet each year (BEAM Dashboard) (25).

FoodNet. More specialized surveillance includes FoodNet, CDC’s sentinel site surveillance system, supported by FSIS and FDA, which collects standardized information on all laboratory-diagnosed infections with Salmonella and seven other pathogens in a panel of 10 states or multi-county areas, encompassing 15% of the US population (73). Human salmonellosis incidence of laboratory-diagnosed
cases reported to FoodNet has not decreased since the 1990’s (Figure 2). Approximately 8,000 Salmonella infections are reported to FoodNet sites each year, and are summarized on the public-facing website FoodNet Fast (24). Data on long-term health sequelae are not collected. Travel history data are collected; if 10% of those infections are travel-associated, FoodNet gathers reports of approximately 7,200 domestically acquired cases per year. Standardized case exposure data have been collected in FoodNet since 2014, that are now being linked to PulseNet genomic data for future analysis.

NARMS. The National Antimicrobial Resistance Monitoring System (NARMS) is another tri-agency surveillance collaboration. Since 1997, as part of NARMS, CDC has determined the antibiotic resistance by phenotypic methods in 1 in 20 isolates of Salmonella from human infections, referred by state public health departments (23, 28). As part of NARMS, these human samples are tested in parallel with isolates collected from various meats at slaughter by FSIS (166), and from retail food samples of meat and poultry collected by FDA (149, 150). CDC PulseNet now provides resistance predictions obtained by WGS to NARMS.

Data on Salmonella serotypes in poultry products: FSIS participates in PulseNet and NARMS, submitting characterization of Salmonella isolates obtained at slaughter from cecal samples (samples from individual animals before extensive processing), as well as from carcass rinsates, cultures of poultry parts and comminuted poultry. FDA tests samples of chicken breasts obtained at retail as part of NARMS, and also sequences the Salmonella they isolate, entering the results into the NCBI database, and PulseNet. Noteworthy for its absence from NARMS is the systematic sampling and characterization of Salmonella from poultry at earlier stages of production, such broiler breeder flocks, or grandparent flocks, and from feeds or feedstuffs.

Reports of foodborne outbreaks associated with Salmonella in poultry: Possible outbreaks of cases that appear to be related can be detected because they occur in a local group with an obvious place or other exposure in common, or because a dispersed group or cluster of cases is identified that have the same molecular or genomic subtype. Epidemiological investigation of these possible outbreaks may identify a common source, confirming them as outbreaks. Investigated outbreaks are reported by public health departments and CDC to CDC’s Foodborne Disease Outbreak Surveillance System (FDOSS) using the National Outbreak Reporting System (NORS) platform (173). FDOSS collects details on implicated food type, characteristics of illnesses, and location of exposure (26). To aid in source attribution, CDC applies the categories of implicated foods developed by IFSAC to the free text fields reported through NORS (121). Approximately 150 foodborne salmonellosis outbreaks were reported to FDOSS in NORS annually between 2013 and 2019 (NORS Dashboard) (31).

(c). What can be done to improve the power and discrimination of current foodborne illness datasets? Successful control of a major source of salmonellosis would be expected to reduce both outbreak-associated cases and sporadic cases. The impact on human domestic salmonellosis can be tracked using case-surveillance data. Most reported cases are sporadic, and not part of identified outbreaks; Food Net has reported that in 2017-2019, 91% of reported salmonellosis was not part of recognized outbreaks (120).
There are challenges to surveillance data collection, and opportunities for improvement. The utility of case surveillance for source attribution and trend tracking is increased by FoodNet interviews, particularly by the more detailed Case Exposure Ascertainment interviews which began to be collected in 2014, when they can be linked to NGS (next generation sequencing) of isolates. Streamlined data gathering and reporting for FoodNet may make those data available and analyzed more quickly.

The COVID19 pandemic of 2020-2022 affected Salmonella surveillance, as it did many other public health efforts. Reported cases of salmonellosis were 22% lower in 2020 than in the preceding 3 years (120). Contributing factors likely included the steep decline in international travel, changes in where and what people ate, and changes in health care seeking behavior as some avoided direct contact with the medical facilities. These effects may not be permanent after the pandemic is resolved, and it may be prudent to exclude data from the years 2020 and 2021 in future assessments of longer-term trends.

Surveillance based on serotyping and genetic subtyping currently depends on having the actual isolate (i.e., bacterial cells cultured from the original sample) in hand, so that serotype and antimicrobial resistance can be predicted from sequence data. The growing use of culture-independent diagnostic tests (CIDTs) in clinical laboratories means that doctor and patient may more quickly learn the cause of the illness, though without antibiotic resistance determination. However, unless an isolate can be obtained, public health will not have a sequence for tracking, outbreak detection, or source attribution. The current solution is to encourage following up a CIDT positive test with a “reflex” culture of the specimen that was positive in the CID test to retrieve an isolate which can then be characterized and sequenced (8). A longer-term solution is to develop and deploy more advanced metagenomic methods to retrieve the Salmonella genome from the specimen that is positive by CIDT (8). A pilot test of one such approach began in two states in 2021 (personal communication, Heather Carleton, CDC). Recently, a high-throughput quantitative PCR assay has been validated that is able to identify 40 Salmonella serotypes and distinguish different genomic lineages and polyphyletic profiles (13). Once a metagenomic replacement for culture can be developed and implemented across the 50 states, it will bridge current PulseNet surveillance to a culture-independent future for public health. Until this as achieved, state health departments will need to obtain isolates from reflex cultures and to sequence those isolates.

(d). What analyses of datasets can help inform progress towards the FSIS goal?

Annual summaries of the frequency of Salmonella infections by serotype have been part of national surveillance reports for many years. Reducing the lag in publishing them would make them more immediately useful, for example by predicting them from PulseNet and providing them as provisional data through the BEAM Dashboard (25). Annual FoodNet surveillance summaries are produced the following year, and the data in FoodNet Fast are also updated at the same time. These case surveillance data are robust enough to permit annual assessments of progress in reducing the frequency of the most common serotypes in human infections, using established statistical approaches to detect significant differences from previous years. Based on these data, attribution models (see next section and Q4) can estimate the proportion of cases attributable to poultry or subcategories thereof.

Serotype data on non-human Salmonella isolates collected from poultry sources as part of NARMS and in the regulatory sampling of USDA could be summarized each year and compared with trends in human
infections. A time trend model that smoothed surveillance data across multiple years, adjusting for seasonal and cyclical differences would be helpful so that the estimation of success is not biased by the vagaries of a single year.

FDOSS outbreak data may also be summarized at regular intervals, likely annually, once the pressure of the COVID pandemic on state and local public health departments allows them to report outbreak investigations more quickly to NORS.

Other regular analyses that would be helpful would include 1) Outbreak data such as the annual number of outbreaks and outbreak-associated illnesses associated with chicken, with turkey, and with all poultry combined. 2) More detailed data routinely provided on the type of chicken product that was the source, e.g., parts, whole, comminuted, frozen breaded, frozen breaded stuffed, etc. 3) Annual ranking, by frequency, of serotypes by assessment of the burden of outbreak illnesses transmitted by poultry, and whether that burden is decreasing, stable or increasing. 4) Poultry sampling data, such as the annual ranking of serotypes obtained from FSIS poultry sampling overall, from raw poultry products destined for restaurants or institutions separately from those destined for grocery stores, and 5) Annual ranking of serotypes obtained from NARMS FDA retail chicken samples.

CDC produces an updated estimate of the overall health burden of salmonellosis and other foodborne infections at more frequent intervals (last estimated in 2011)(128, 129). This would depend on conducting more frequent, ideally annual FoodNet population survey to assess current care-seeking and laboratory specimen submission behaviors.

(e). Available source attribution methodology and how it can be used to sufficiently discriminate poultry-caused illnesses from both outbreak and sporadic salmonellosis cases.

This question is answered in Q4.

(f). Are year-to-year changes, trends, and lags captured in the existing foodborne datasets and source attribution?

Year-to-year changes are captured in case-based surveillance with large numbers reported each year in each of the major systems, allowing for robust comparisons. As noted above, some smoothing across several years may be useful to limit the impact of single large events. PulseNet data in particular are analyzed continuously to look for clusters of related cases and may in the future be used to produce regular updates of sequenced-based source attribution estimates. (See answer to Q4 for more discussion on this topic.)

The CDC burden estimates published in 2011 (128, 129) which updated the previous 1999 estimates (95), still serve as the best available general estimates of the health burden of foodborne diseases in the United States, by pathogen. The reported incidence of infections with four priority bacterial pathogens (E. coli O157, Listeria monocytogenes, Salmonella, and Campylobacter) have changed little in the last decade (24), and thus the health burden may also be presumed to have changed little. Should the incidence of these and other infections decrease substantially, the burden estimates will become
increasingly dated. The process of updating the estimates takes several years, so some lag between
current disease incidence is likely to persist and needs to be considered.

Foodborne illness surveillance and monitoring systems only detect a fraction of all cases that occur, and
most reported cases do not have a known source. Also, infrequent large events can have a marked
effect on the case count in a given year. This adds statistical “noise” to the numerical signal needed to
detect temporal changes in foodborne salmonellosis trends and its main food sources. Using FoodNet as
an example, Ebel et al. (51) estimated a 4% chance of detecting a true 10% change in salmonellosis one
year after a risk reduction change has taken effect. In a different scenario, a sustained reduction of 30%
in salmonellosis for four years still resulted in only approximately 50% chance of detecting such change.

In a separate study, a similar calculation was used to show that a 25% reduction in salmonellosis cases
from comminuted chicken would not be detected by FoodNet alone as this annual reduction would be
smaller than the expected year-to-year variability of the surveillance system (49, 51).

Because of the statistical power limitations of individual Salmonella surveillance and monitoring
systems, for assessments of changes (or stability) in the incidence of salmonellosis, this committee
advises the use of data from more than one system. For example, though FoodNet has high quality
data, it comes from only 15% of the population, while other Salmonella surveillance systems are
nationwide in scope. We also advise the use of genomic data and models for both incidence and source
attribution, as described in answers to questions 1 and 4.

Because the data in FDOSS/NORS are more limited, they will need to be combined across several years
to define and analyze trends for specific food categories. Currently the IFSAC outbreak-based source
attribution model estimates (described in Question 4) are updated annually, though the data are
smoothed over several years.

(g). What new approaches are being developed and could provide more accurate/precise burden data
in the future?

The health burden estimates developed by CDC depend on accounting for the sequential steps in
diagnosis and reporting of specific infections. These include assessment of frequency of acute
gastrointestinal illness in the population, of health care seeking behavior, of the frequency with which
diagnostic tests are ordered and performed, and of the frequency of reporting. Future estimates could
be improved by re-assessing these probabilities as practices change, evaluating the effects of variation in
clinical severity on the likelihood of hospitalization, and could also incorporate estimates of the burden
of long-term health sequelae.
Question 4

A) How might foodborne illness surveillance data on human Salmonella illnesses, data from foodborne outbreaks associated with Salmonella in poultry, and data on Salmonella serotypes in poultry products be used to identify the Salmonella serotypes of greatest public health concern associated with specific poultry products?

B) Should only the most current data (e.g., 5-years) of foodborne illness surveillance outbreak and or pathogen/testing be used?

C) Going forward, what methodology and criteria would focus on those Salmonella serotypes most frequently associated with human illness and attributable to poultry products?

D) How frequently should the priority Salmonella serotypes associated with poultry be revised considering changes in their occurrence while still ensuring continuity in industry and regulatory testing?

Summary of Question 4 Response

Several approaches have been used to attribute human salmonellosis to specific foods and sources. These include case-control studies, analysis of reported foodborne outbreaks, and most recently, source attribution based on whole genome sequence genotyping. Attribution based on outbreak data and on genotype both give the greatest weight to data from the most recent years. Like attribution based on reported outbreaks, genotype-based attribution indicates that poultry is the leading source of human salmonellosis. It also indicates that a small number of serotypes account for most poultry-associated salmonellosis in recent years, led by Enteritidis, Typhimurium, 4,5,12:i:-, Infantis, and Heidelberg, and even fewer serogroups: groups O:4 (formerly group B), O:7 (group C1), and O:9 (group D1). The effectiveness of a prevention strategy that includes serogroup or serotype targets should be evaluated annually with case-based surveillance; changes in the attribution model would likely take several years to observe. The most current data should be used for all analyses, but the number of years to be included depends on the rate at which new information is added. Combining data from several recent years in a trend model can smooth out the effects of single annual perturbations, such as large outbreaks. It is anticipated that targeted intervention strategies in the industry will affect predominant serotypes found to be associated with human illness. Subsequently, as other serotypes become predominant in illness, this in turn will affect industry responses.

Before answering the charge question on the predominant serotypes associated with poultry-related salmonellosis, we considered several of the serotype-focused interventions used by industry for live birds. These control programs (described below) have resulted in various levels of success, but ultimately affect and help explain changes in predominant serotypes responsible for foodborne illness attributed to poultry products (also see response to Question 2).
Effect of serotype-targeted interventions to reduce Salmonella in the live bird populations:

- In the 1990s, the United Kingdom experienced a rapid increase in *Salmonella* Enteritidis (SE) infections related to both eggs and poultry meat, much of it caused by phage type 4; at the peak SE comprised 70% of salmonellosis (86, 109). With a voluntary industry effort based on vaccination and other flock-based measures, first targeting layer flocks, then including broiler breeders, the number of SE phage type 4 infections reported in the UK dropped dramatically, from ~18,000 in 1997 to 459 in 2010, and the overall number of *Salmonella* infections reported annually fell from ~33,000 in 1997 to ~9,000 in 2010 (54, 86, 109) (Figure 3). No other serotype emerged to “replace” SE.

- The United States has experienced a profound decline in the incidence of infections caused by Typhimurium and Heidelberg over the last 20 years (24) (see Figure 4). The reasons for these declines have not been well documented, but they were corresponding to when a commercial poultry vaccine against Typhimurium became available. Highly publicized recalls in 2013 of retail chicken from a West Coast producer whose products were implicated in an outbreak of Heidelberg infections may have accelerated wider vaccine use (67). Typhimurium vaccines in poultry appear to decrease colonization and infection not only of Typhimurium but of other serotypes that share the same O group antigens, such as Heidelberg, as both share antigenic O formula: I:4,5,12:i:- (38, 100). Some producers have also implemented targeted environmental testing with a focus on breeder flocks and increased other prevention measures as well. With a mix of strategies, by 2019, Typhimurium had dropped from its long-standing position as the most common serotype isolated from humans as late as 2007, to third most common. The incidence of Typhimurium infections decreased by 70%, from 3.7 per 100,000 in 1999 to 1.3 in 2019 (24). Heidelberg had dropped from the 4th most common serotype isolated from humans in the 1990s to the 24th most common in 2019. The incidence decreased 92%, from 1.07 per 100,000 in 1999 to 0.08 in 2019 (24).

- In 2003, the European Union (EU) issued regulations for breeder flocks requiring stringent control measures for specific serotypes, based on comparing serotypes found in food animal reservoirs with those most commonly causing human infections. They chose Enteritidis, Typhimurium (including the monophasic variant I:4,5,12:i:-), Hadar, Virchow, and Infantis as targets for prevention (54). This was based on their frequency in human infections, their transmissibility through food, evidence of recent increase or spread, and increases in resistance to treatment. In 2019, based on changes in prevalence (180), the suggested group of serotypes to target was updated to Enteritidis, Typhimurium (including the monophasic variant), Infantis, and another to be determined – perhaps Heidelberg or Kentucky (54). Updates to the list may vary by country (87). Serotyping is carried out via culture methods (White-Kauffmann-Le Minor scheme) (58).

Q4.A Parts 1 and 2 and Q4.C. How might foodborne illness surveillance data on human Salmonella illnesses, data from foodborne outbreaks associated with Salmonella in poultry, and data on Salmonella serotypes in poultry products be used to identify the Salmonella serotypes of greatest public health concern associated with specific poultry products? What methodology and criteria would focus on those Salmonella serotypes most frequently associated with human illness and attributable to poultry products.
**Question 4.A1:** Surveillance data on human illnesses caused by Salmonella: Several surveillance and monitoring systems provide data on individual Salmonella infections, on outbreak events, on antimicrobial resistance in human and non-human isolates, and on frequency in meat and poultry (see response to Q1 Part 2b and Table 3).

**Question 4.A2:** How can [these data] be used to identify the Salmonella serotypes of greatest public health concern associated with specific poultry products? and **Question 4.C:** What methodology and criteria would focus on those Salmonella serotypes most frequently associated with human illness and attributable to poultry products.

The relevant serotypes of greatest public health concern are those that are common causes of reported human illness, are present in poultry, and are transmitted through foods. CDC surveillance provides data on the frequency of diagnosed illness caused by each serotype. Human illness reporting tends to be skewed towards more severe illnesses as milder cases are less likely to be diagnosed or reported, so models accounting for this are used to estimate the true number of illnesses in burden studies (129, 130). If the use of culture-independent diagnostic tests increases without an increase in associated reflex culturing, a greater proportion of reported infections will lack serotype data (120). FSIS data from slaughterhouses and retail surveys can confirm the presence and frequency of serotypes in raw poultry.

One way to quantify the importance of poultry as a source of human infection has been through case-control studies of sporadic infections. For example, a FoodNet study conducted in 1996-1997 identified eating chicken, and specifically chicken prepared outside the home, as a specific risk factor for serotype Enteritidis (SE) infections (81). This study attributed 27% of SE infections to that source; before this study, SE infections had been attributed mostly to eggs. However, a parallel study of Typhimurium infections did not identify a specific source, although it did clarify the importance of preceding antibiotic exposure in increasing host susceptibility (68). Such broad studies of sporadic cases can provide important insights and help to determine the overall burden of illness. Case-control studies attribute disease to the point of consumption, in contrast to some other methods of attribution which can include attribution to reservoir as well, thereby capturing cross-contamination of foods by an upstream source. Moreover, conducting case-control studies requires considerable time to design, execute and analyze, is expensive, and has often yielded sparse new information (64). Thus, FoodNet has conducted few case-control studies in recent years. However, improved methods and data could make case-control analyses an important component of the assessment of major sources in the future. Nearest neighbors matching for analysis, with calculation of odds ratios and population attributable risks (39), is being explored as a less resource intensive approach to case-control studies. These methods may be applied to cases from FoodNet’s case exposure ascertainment data, using the recent FoodNet Population Survey, conducted during 2018-2019 as a source for control data. The future possibility of an ongoing FoodNet Population Survey to provide timely data from control persons about exposures paired with matching questions posed to cases could provide more timely estimates of the major sources and their changes over time.

Outbreak investigations of foodborne salmonellosis can provide direct evidence that foodborne transmission of a particular serotype occurs. Outbreak data in FDOSS can provide information by year on food categories that have been reported as sources of particular serotypes. Detailed analysis of
FDOSS data also can indicate changes over time in the serotypes and broad categories of poultry products linked to outbreaks, e.g., parts, whole chickens, and frozen meals. These data can help assess the importance of various serotypes attributed to particular foods. For example, in the mid-1980s, CDC epidemiologists noted that although few SE outbreaks were attributed directly to shell eggs, most of the foods implicated contained eggs; they proposed that eggs were the source of the global increase in SE infections (137).

For the past decade, the Interagency Food Safety Analytics Collaboration (IFSAC) has annually updated results of an attribution model that estimates the percentage of all salmonellosis that can be attributed to 17 food source categories (76). The model uses outbreak data reported to FDOSS; it gives less weight to outbreaks that occurred more than 5 years earlier (back to 1998) and accounts for other factors, such as whether the outbreak was very large or occurred in multiple states (9, 121). The estimates for poultry were 10% for chicken and 7% for turkey in 2012 (17% collectively), and 17% for chicken and 7% for turkey (24% collectively) as of 2019 (75, 76). These estimates are based on vehicles identified in foodborne outbreaks, and do not capture the original source when Salmonella in raw products such as poultry cross-contaminates other foods which are then the source of an outbreak. They currently provide the best ongoing estimates of the sources of domestically acquired salmonellosis in the U.S. These IFSAC estimates do not include serotype because data on individual serotypes are sparse; however, a pilot analysis of data on SE, the most common cause of salmonellosis, has been attempted (CDC unpublished data).

Source attribution can also be predicted by comparing isolates from humans with isolates from a variety of animal and other sources. In Denmark this was done using multi-locus variable number tandem repeat typing (43). In the United States, a current IFSAC effort is using a genome sequence-based approach for source attribution. This approach may address some limitations of outbreak vehicle-based source attribution, as it can directly assess sporadic cases, and is likely to reflect original source or reservoir before cross-contamination, if those sources are represented in the non-human isolates. Led by CDC’s Analytics team, this IFSAC study has compared isolates from humans with those from a variety of foods and animals (124). The model was initially trained using the non-human isolates to identify the alleles that best differentiate these strains by animal and food source. Then human isolates were evaluated in the trained model. The initial assessment used non-human isolates collected over the past two decades, and human isolates collected 2014 - 2017 in FoodNet sites after excluding those known to be associated with foreign travel. The model predicts likely food category sources for these infections. Overall, 26% of infections were predicted to have a poultry source (Figure 1). Restricting analysis to the subset of isolates with >50% probability of attribution to a single food source, 46% of such infections were predicted to be due to chicken and 3% to turkey. The model was also able to predict food sources for the most common serotypes: Chicken was the predicted source for 86% of SE infections, 66% of Typhimurium, 55% of Infantis, 53% of Heidelberg, 29% of 1,4,5,12:i:-, and <1% of Newport and Javiana infections. Turkey was the predicted source for 9% of Heidelberg infections and <1% of infections caused by the other serotypes listed above for chicken. These percentages can be applied to estimates of the incidence or number of illnesses caused by each serotype to obtain an estimate of chicken-associated illnesses caused by each serotype or a ranking of the most important serotypes. Doing this results in an estimate that the serotypes that cause the most chicken-associated illnesses are, in order, SE,
Typhimurium, Infantis, and 4,5,12:i-; together these four account for an estimated 70.4% of chicken- and turkey-associated illnesses. Although a large percentage of Heidelberg infections are attributed to chicken, that serotype has now become uncommon, so results in relatively few attributed illnesses.

 Retaining Heidelberg as a serotype of concern may be justified by concern that it could increase if targeted control measures already in place were lifted.

Some of the poultry-associated serotypes identified by the IFSAC WGS source attribution model share serogroup specific O-antigens, relevant to vaccine choice: Typhimurium, I,4,5,12:i- and Heidelberg are part of serogroup 4, (previously known as Group B), SE is part of serogroup 9 (Group D), and Infantis is part of serogroup 7 (Group C1)(30). In the future, the serotypes chosen as targets for control could also include other poultry-associated serotypes with important antimicrobial resistance, or likelihood of hospitalization as additional criteria.

One can also compare the frequency of serotypes in different food animal reservoirs with those that cause human infections. An advanced version of this method has been used in Europe to develop a Salmonella source attribution model across 24 countries (42). Because animals can be reservoirs of non-typhoidal Salmonella, one would expect a change in the presence of a serotype in poultry to precede a change in the same direction in illnesses caused by that serotype in humans. Moreover, one would expect the changes in serotype frequency in poultry and food to be detected from the variety of sampling programs done pre-and-post harvest, such as FSIS’ cecal samples for testing in the NARMS program, and carcass samples for regulatory purposes. Measures of illness in humans may lag behind animal and food sampling because of the time it takes for a new strain to spread through many flocks, the time it takes for animals to be raised and slaughtered, and the resulting food distributed; and since most chicken is cooked sufficiently to kill Salmonella. Therefore, assessing the frequency of serotypes in live poultry and poultry at slaughter would help to monitor those serotypes most likely to cause illness now or in the near future. Similarly, routine sampling of poultry feed and feed ingredients could be relevant because this is a documented way that serotypes enter the food supply, where even low levels of contamination could be important because pathogens can multiply in moist environments on farms and because animals consume large volumes of feed. The routine practice of choosing one isolate to represent the Salmonella population present in a sample may mean that important serotypes present at lower frequency may be missed (134). In the future, it may be helpful to adopt metagenomic methods able to detect and characterize multiple serotypes that may be present in a sample.

Acomplementary approach to the source attribution methods described here also involves identifying serovars of concern using genomics. These emerging methods are reviewed on the answer to question #1. By directly targeting profiles of virulence genes or the pangenome, these methods can provide an early warning of possible serovars or indeed isolate-level pathogen subpopulations that might be responsible for an increased risk of salmonellosis burden. Since these methods can be regularly updated using all relevant Salmonella isolates submitted to NCBI, they could be combined with other foodborne surveillance and monitoring systems to provide a more rapid response to evolving changes in Salmonella infectivity and virulence. This would address some of the statistical power and resulting time lag limitations described earlier for the Salmonella surveillance and monitoring systems.
**Question 4.B: Should only the most current data (e.g., 5-years) of foodborne illness surveillance outbreak and or pathogen/testing be used?**

The most current data should be used for all analyses, but the number of years to be included depends on the rate at which new information is added regarding the spectrum of serotypes responsible for illness changes. Combining data from several recent years in a trend model can smooth out the effects of single annual perturbations, such as large outbreaks. When data from the most recent 3-5 years do not provide sufficient sensitivity to detect change, data from the most recent years could be combined with discounted data from preceding years; such analyses can still be updated each year with new data, as is done in the IFSAC annual source attribution report. PulseNet collects data from approximately 40,000 isolates each year; FoodNet has data on about 7,200 domestically–acquired illnesses each year, as the surveillance area covers 15% of the U.S. population, while FDOSS collects about 16 poultry–associated outbreaks each year. For poultry samples many isolates are obtained from carcasses, fewer from ceca and other sources, resulting in thousands of sequenced isolates submitted to FDA’s GenomeTrakR. Data from food samples are more limited; they include isolates from the NARMS retail food program and isolates submitted to NCBI by state agriculture departments.

There is also an opportunity to study the results from 2020 and 2021, when the COVID19 pandemic perturbed many aspects of the food supply. Whether there was serotype- or food source-specific effects remains to be examined in detail. If there were, then future estimations may need to consider excluding those years from more general models of attribution.

**Question 4.C: How frequently should the priority Salmonella serotypes associated with poultry be revised considering changes in their occurrence while still ensuring continuity in industry and regulatory testing?**

Ideally, a serotype, serogroup, or subtype-based control strategy would be reevaluated at annual intervals for evidence of the effectiveness of control methods, and to provide a timely alert for the need for possible changes in control measures based on a decline, or lack of decline, in illnesses, or emergence of other serotypes. It would seem prudent to retain targets even if those serotypes become rare, as halting the control measures that led to their decrease may provide an opportunity for a rebound.

The frequency of reported infections with targeted serotypes or subtypes can be reviewed annually using case surveillance data. Outbreak-based attribution can also be analyzed annually, but the sparsity of data will limit the sensitivity for detecting change quickly, particularly at the serotype level. In the last decade, approximately 16 chicken- or turkey- associated outbreaks of salmonellosis were reported each year. To detect meaningful change at the level of a common serotype for one food category, at least 5 years or ~80 such outbreaks may be needed to provide reasonable sensitivity to detect a change. Successful prevention that reduced the number of outbreaks could stretch this time span further.

Ideally, source attribution analyses based on the IFSAC WGS model would be repeated annually so progress can be measured by the estimated overall number of illnesses that can be attributed to chicken as well as the number caused by targeted serotypes, and other serotypes emerging as sources. This
method depends on two analytic datasets, one of non-human isolate sequences, and the other of human isolate sequences. The dataset of non-human isolate sequences may increase at a rate that would allow the training data analysis to be repeated at regular intervals, e.g., 1-3 years, and data representing older sources are minimized or excluded. More strain sequences could be added from other sources, e.g., the APHIS NAHMS studies or state agricultural databases. Sequences of *Salmonella* strains isolated from humans are now being added to the PulseNet database at the rate of ~40,000 each year, of which ~7,000 are in FoodNet sites. Once appropriate sequencing workflows and automation can be established, it will likely be possible to repeat the WGS-based IFSAC source attribution predictions at annual intervals.

Subtype-specific prevention strategies will likely need to be modified as the incidence of infection with specific serotype or subtype targets change. It would be appropriate to include regular assessment of the need for modification of targets, ideally annually, as part of a regulation, as well as guidance on likely methods to be used and a mechanism to evaluate the role of improved methods and data sources in making assessments. For example, re-evaluating the prevention strategy could be indicated if: (1) no change was observed in incidence of infections caused by a serotype (as tracked in FoodNet surveillance) at the end of at least two years of full implementation (or other time window indicated by a power analysis, accounting for the expected rate of reduction) compared with a chosen baseline, such as the pre-pandemic one (2016-2018) used to assess progress towards the Healthy People 2030 Goal, and (2) the WGS-based IFSAC model and possibly other analyses indicate no significant change in the percentage of infections caused by that serotype attributed to chicken. A statistically significant or major (e.g., >15% increase or decrease) change in illnesses attributed to chicken overall or in a targeted serotype, serogroup, or subtype, should merit re-assessment of the prevention strategy. A method should be clearly defined for the target metrics (e.g., confidence metric, p-value, % change) required changes in guidance or policy based on the results of these assessments. Because of data limitations, re-assessment may also be deemed appropriate to protect public health even if the changes do not reach formal statistical significance, for example due to applicable lessons learned from other contexts or advances in research.
Question 2: What types of microbiological criteria could be established to encourage control of Salmonella at preharvest (i.e., in live birds on-farm)?

a) Should FSIS consider qualitative microbiological criteria for control of the presence of Salmonella in a flock when they are presented for slaughter?

b) How could FSIS use these criteria to address Salmonella serotypes most frequently associated with human illness?

c) What industry data would provide evidence of control?

Summary of Question 2 Response
Before deciding whether qualitative vs. quantitative data is best for determining control of Salmonella in a flock, substantially more data (including industry and university research data) should be analyzed to ascertain correlation between Salmonella prevalence, levels, and serovars, either in flocks presented at slaughter or on farm shortly before presenting for slaughter. Power of detection and enumeration needs to be sufficient to provide evidence on both process control and long-term association of contamination trends in flocks with farm features and farm management measures (including any potential associations with indicator organisms), i.e., the real-world cost-effectiveness of on-farm control measures.

In considering possible MC or testing programs to encourage Salmonella control at preharvest, the following conclusions emerged:

- US producers voluntarily implement several Salmonella control practices on farms, and use qualitative testing to monitor effectiveness of management in breeder flocks, hatchery, grow out and transport. This approach has resulted in lower prevalence on chilled carcasses, but not resulted in fewer illnesses associated with poultry.

- Multiple routes of contamination have been observed, and no single control measure had been found to be effective in controlling Salmonella on farms. Hence, farms should be incentivized to apply rigorous multiple-barriers approach that combine a suite of best practices, including testing at multiple points (such as feed, litter, etc.) to inform farm management operations.

- Qualitative Salmonella testing of breeder flocks is recommended to avoid using contaminated eggs for hatching.

- Feed treatments (e.g., heat), preventing recontamination, and testing for presence of Salmonella is recommended to control an important entry route.

- Testing flocks for Salmonella 1-2 weeks prior to slaughter can inform management on level of contamination to affect processing schedule, transport, remediation of houses or incorporating other mitigation strategies. Qualitative testing of Salmonella levels in broiler and turkey flocks (cecal), immediate environment (litter, feed, water), or in birds at receiving for slaughter could inform control actions on farms. These tests could be complemented by testing for a subset of high-concern serovars. However, complex serovar occurrence patterns are likely to complicate interpretation and actionability.

- Sampling protocols and assays exist to test farm samples (e.g., litter, feed, water) for both detection and enumeration. In designing sampling protocols, frequency and number of samples need to account for currently achievable sensitivity.
• More research is needed to quantify the effectiveness of preharvest control measures and combinations thereof, which would also improve the ability of risk models to estimate impacts and hence the cost-effectiveness of *Salmonella* control actions.

• Assays to detect specific serovars of concern exist but are currently cumbersome. New molecular methods to rapidly identify multiple serovars are promising but require additional validation and are not yet commercially available.

• Vaccines are likely the only serovar-specific control strategy at preharvest but will not eliminate all *Salmonella* from flocks.

The Joint FAO/WHO Expert Meeting on Microbial Risk Assessment (JEMRA) recently reviewed data on control measures for *Salmonella* in the broiler production chain (181). The panel concluded that no single control measure is sufficient to control contamination prevalence or level in poultry meat. Rather, utilizing multiple strategies, such as those outlined below as best practices for the poultry industry, is most effective to ultimately reduce carriage of *Salmonella* through to the food supply. Testing of flocks producing eggs for hatching for *Salmonella* is recommended to exclude eggs from infected flocks being transported for hatching.

Current US practices include testing for the presence of *Salmonella* in hatcheries, at breeders, broiler house to inform voluntary management actions by the company (see Figure 5 for description of US commercial broiler production and (145, 153). The overall compliance with the USDA FSIS performance standard during processing by the poultry industry is high and further reduction in prevalence may need an incentive for the industry to increase the testing of live birds before processing. The proposed requirement to test flocks before entering the processing plant by FSIS USDA would increase available information to base risk management decisions on, but it is unclear what the impact would be, or what incentives the industry will respond comply with these proposed requirements.

Testing the flocks before processing for scheduling at the processing establishment based on negative/positive flocks is a tool with limited known impact (108, 119). Testing for specific serotyping could help target vaccine development and focus their use where needed, but focusing solely on the currently highly prevalent serotypes may miss shifts in the strains that cause illness. In addition, the fact that serovars detected at processing or in the product might show different patterns from those most commonly observed on farms, complicates the use of serovar-specific on-farm testing information to inform risk control in the product. Thus, the industry should consider utilizing testing approaches that test overall *Salmonella* as a means to measure effectiveness of different interventions at preharvest, but in combination with serovar-specific monitoring to determine if the interventions select for or exclude virulent strains.

**What have we been testing?**

In the U.S., there are currently no required industry-wide routine on-farm quality indicator sampling plans or sampling techniques. Hatchery sampling most often relies on testing settle plates in hatcheries (open plates for a given period of time for air monitoring) and/or filter paper under newborn chicks, which are tested for presence of *Salmonella*. For primary breeders, breeders or broilers, *Salmonella* testing is most often done with boot socks (socks worn over shoes as the technician walks around the
Potential Microbiological Criteria at preharvest

Potential MC applied at preharvest, discussed in this section and the following, include:

- *Salmonella* testing of the farm environment [e.g., litter, feces, water, dust (7, 12, 16, 99)] to assess contamination of a specific flock and the farm; tests can be qualitative or quantitative, complemented by serovar identification to assess the presence of high-concern serovars; this
test, if sufficiently sensitive, could inform both processing actions for the flock as well as farm
management actions;
• Indicator testing of the farm environment, if an association with Salmonella presence or levels is
demonstrated, with similar purpose as above;
• Salmonella testing of a flock presented at slaughter, to provide feedback on farm management
actions and verify on-farm process control;
• Salmonella testing of breeder flocks (qualitative, possibly quantitative), to control vertical
transmission to broiler flocks.

MC at any stage rely on the availability of a sampling and testing protocol with adequate sensitivity and
specificity (as well as other parameters, such number of samples collected and time to results) so that
results can inform the management actions associated with the MC.

At this time, there is no clear correlation between non-Salmonella populations (i.e., indicator
microorganisms) that can be used to predict flocks that are highly contaminated, and which should be
targeted for special processing; testing specifically for Salmonella appears to be the best strategy (see
also Question 5).

While there are currently no routine quantitative sampling programs being utilized industry wide, an
appropriate qualitative Salmonella method (see Question 6) could be used to test flocks one to two
weeks before processing, to inform risk management at processing (as well as during transport, and on
farm to prepare for the next flock). For instance, test results from pre-slaughter flocks could potentially
be used to reduce the risk of cross-contamination across flocks during processing, e.g., by scheduling
more heavily contaminated flocks at the end of a processing day or potentially on days with other
contaminated flocks. It should be noted that many factors would need to be considered if changing
scheduled processing days for flocks. For example, trucks may need to be filled from multiple houses; it
could be costly for trucks to visit multiple houses or farms; and growers are paid based on weight from a
house/farm. However, there is evidence that changes in processing schedules to account for higher or
lower flock contamination (so called “logistical slaughtering”) are unlikely to provide benefits (108, 119).

Other risk-reduction measures could be implemented on higher-risk flocks when received for processing
(e.g., increased Salmonella reduction treatments during processing, where applicable), or on farms with
frequent highly contaminated flocks. For this purpose, quantitative testing for generic Salmonella is
expected to provide the most cost-effective and actionable information, although complementary
identification of serotypes of concern could allow for a more accurate estimate of risk from a flock and
hence of the degree of Salmonella reduction necessary at processing, if processing conditions allow for
such flexibility. While voluntary testing at this stage would be informative, coordination across agencies
(FSIS and APHIS) would be needed to implement an MC of regulatory relevance.

More research is needed to determine if qualitative and/or quantitative testing on farm and resulting
changes in processing scheduling (or other control action during processing, see also Question 3) would
significantly affect Salmonella prevalence and levels on carcasses and in finished products. In addition,
we highlight the research need to determine if testing for non-Salmonella indicator(s) could provide
guidance to companies on which houses have a higher probability of *Salmonella* contamination (or level of contamination).

Considering how testing results could be acted upon throughout the production and processing chain, qualitative and/or serotyped *Salmonella* spp. data collected at flock receiving (and potentially at other early processing stages) may be used to create an incentive for targeted control mechanisms applied during live production. For example, as an illustrative scenario where a flock is tested for *Salmonella* levels at receiving, the producers whose flocks are, over a set time window, performing worse than the industry average or worse than a specified threshold for the prevalence or levels of *Salmonella* (or select serovars) could be subjected to additional preventive or remedial actions, or be subjected to financial disincentives from the processor. Independently from potential regulatory actions, the information would provide feedback to producer farms about their process control or the need for increased control actions. For this purpose, *Salmonella* testing, possibly complemented by serotyping if not already carried out in previous preharvest stages, could provide the most valuable information to focus on-farm measures. Conversely, testing at flock receiving is unlikely to be sufficiently fast to inform processing actions on that flock; testing on farm may provide more timely and actionable information on the contamination status of a flock, albeit possibly less accurate. We recommend comparing the value of information vs. cost of testing flocks on farm vs at presenting for slaughter, using statistical modeling approaches.

Industry data could be leveraged to better understand how a MC at flock receiving could be used to inform management actions on farms. For example, matching data on APC (or other indicator organisms) and *Salmonella* at receiving, would provide evidence supporting (or not) the enumeration of indicators as a predictor of the likelihood of *Salmonella* occurrence or *Salmonella* levels. Data (Salmonella enumeration or presence of high-concern serovars) by flock and farm, collected at receiving ideally matched by data on farms, could provide insights on what farm or flock features are most associated with high levels or high-concern serovars.

Testing feed and breeders (and possibly other) could provide information on specific *Salmonella* entry or spread routes known to be associated with broiler farm contamination. For example, feed testing is implemented in Sweden and has contributed to the success of the *Salmonella* control program (105). Breeding flocks have been found to often be the likely source of *Salmonella* in broilers (54). For the purpose of controlling contamination from these routes, qualitative or quantitative testing for generic *Salmonella*—carried out before feed or eggs/chicks reach the next stage—is likely to provide sufficient information to inform process control on farm. Some serovars can be closely associated with some routes, e.g., *S. Enteritidis* was back-traced from broilers to breeders more often than other serovars, in some studies (18, 80). In these cases, testing and controlling for relevant serovars of concern in a stage/route (upstream of the grow-out farm) could be more cost-effective than testing for generic *Salmonella*. However, in general a serovar does not consistently map to one source only, and hence serovar testing on farm is unlikely to be cost-effective for on-farm process control.
Management Practices for Controlling Salmonella at preharvest

Any MC, as well as other approaches to risk management, needs to be associated with effective risk control measures. MC can verify that control measures are effective and can trigger additional actions in case the MC is not met. In this section we provide a brief summary of available Salmonella control actions that could be used in conjunction with a MC, or on their own. In the following section, we discuss serovar-specific MC and control strategies.

Several strategies to control Salmonella at preharvest stages exist and have been implemented. Currently, in the U.S., these measures are voluntary; many are recommended as best practices (15, 145, 153, 181). Evidence of effectiveness varies by measure, and it is recognized that no individual measure alone can control Salmonella. A non-exhaustive list of control measures at different preharvest stages and potential testing points include:

** Breeders:
- Cull visibly soiled eggs that may be contaminated with Salmonella-containing feces (65)
- Salmonella-free chicks
- Competitive exclusion treatments
- Vaccination program (including serotypes from Groups B, C, and D) (65)
- Biosecurity (40)
- Rodent and insect control program
- Footbaths / movement of workers
- Testing for Salmonella in chicks could be used to verify process control.
- Qualitative or quantitative testing in breeder flocks can be used to prevent eggs from contaminated flocks from reaching the hatchery.

** Feed:
- Attempt to control quality of ingredients
- Sufficient time in conditioner to give time/temperature/moisture for Salmonella kill
- Control post pelleting (processing) recontamination. Pay particular attention to cooling area to avoid conditions favorable to Salmonella growth.
- Testing for Salmonella in feed could verify that feed does not introduce Salmonella on farms.

** Hatchery:
- Cleaning/sanitation programs
- Control air movement in hatchery
- Chemical disinfection program in hatch cabinets during hatch period
- Do not reuse tray liners
- Testing for Salmonella in chicks could verify process control and inform Salmonella control actions at the hatchery and/or on a chick flock (e.g., not shipping contaminated chick flocks to grow-out farms).
Grow-out (broilers):

• Only *Salmonella*-free chicks
• Competitive exclusion treatments
• Moisture control (no leaking nipple drinkers)
• Proper working ventilation system (reduce stress on birds – litter amendments if necessary)
• Rodent and insect control program
• Limit movement of workers / visitors
• Testing of flock or flock environment for *Salmonella* before transport to slaughter could determine the contamination level of a specific flock and inform risk mitigation actions at slaughter and processing.

Transport:

• Proper feed and water withdrawal time
• Clean transport coops
• To extent possible, limit time in transport cages
• Testing a flock at receiving or at slaughter, summarized by farm over a time window, could provide feedback to producer farms and inform farm management actions. However, test results on individual flocks received are unlikely to be useful to inform management actions for that flock at processing (unless, in the future, a real-time test is devised and used).

As summarized above, a range of *Salmonella* control strategies are implemented at preharvest. A recent Joint FAO/WHO Expert Meeting on Microbiological Risk Assessment (JEMRA) reviewed evidence on the effectiveness of *Salmonella* control measures in poultry, both on farms and during processing (181). Sweden instituted a rigorous *Salmonella* control program to reduce the prevalence of *Salmonella* on birds grown and processed in Sweden (62) (see Appendix B Case Study for details and comparison with US practices). Some practices used to reduce *Salmonella* on U.S. farms such as biosecurity and control of feed to exclude *Salmonella* are also incorporated in the Sweden program. The Sweden model further relies on microbial testing for presence of *Salmonella* in fecal droppings or cecum. If any serotype of *Salmonella* is isolated, the flock is destroyed (172). Because the method of control depends on eradication of *Salmonella*-positive breeders or broilers, replication of this system in US operations has been thus far considered controversial, and potentially too costly to implement. While the economic burden associated with poultry-related salmonellosis has been estimated (131), a cost assessment of implementing an eradication model, compared to other approaches, has not been carried out for the U.S.

**Serotype-specific control strategy and MC**

Vaccines can target a specific serotype or serogroup and are likely the only serotype-specific intervention strategies. Almost all broiler breeders in the US are vaccinated against some *Salmonella* (98). Vaccines can be live deletion mutant strains that are derived from a specific serovar of *Salmonella*, i.e., *S. Typhimurium* or autogenous killed-cell vaccines against a cocktail of strains found in a local area. Commercially available vaccines have been developed for *Salmonella* in the poultry industry. For food safety purposes, they are meant to reduce infective pressure in a flock. Vaccines are commonly administered in-ovo, stimulating the innate and adaptive immune responses for zoonotic poultry.
diseases and may be administered post-hatch as a spray or eye drop. *Salmonella* vaccinations of broilers are generally considered impractical as the bird’s immune system is not fully competent until the birds are a week or older and the handling of birds in the broiler house is problematic.

Vaccines have likely contributed to a significant reduction in *S. Enteritidis* in eggs and fin *S. Typhimurium* for the broiler industry; they may have contributed to the reduction in *S. Typhimurium* compared to other serotypes (47, 184). The vaccine industry is currently developing new vaccines against additional serotypes of *Salmonella*. Safety and efficacy requirements of commercial vaccine development take three or more years. Therefore, changes in target serovars – if implemented - should consider vaccine development times.

Serovar-specific control strategies such as vaccines could be paired with generic *Salmonella* MC to monitor the cumulative effectiveness of a suite of on-farm control measures. While serovar-specific MC should be considered, serovar shifts over time, as well as inconsistent occurrence patterns between preharvest and processing (there is initial evidence of shifts in the relative serovar abundance going from breeders to carcasses and products (134) may complicate the interpretation of test results and risk control decisions. A recent study by EFSA pointed out the expected higher effectiveness of an all-*Salmonella* target for breeding hens, compared to a target including five high-concern serovars (54). On the other hand, an additional serovar-specific MC at preharvest could allow focusing resources on strains of higher concern (i.e., be more risk-based) using more targeted measures (primarily vaccines, targeting known sources of specific serovars). Combinations of all-Salmonella and serovar-specific MC, at preharvest or throughout the preharvest-processing chain in multiple-barrier fashion, could offer cost-effective trade-offs. However, since vaccines alone do not eliminate a *Salmonella* strain (15, 184), and a suite of control measures are needed, quantitative Salmonella testing is likely to offer the most actionable information.

Several examples of serovar-specific control efforts implemented at national level, including MC implemented as part of these efforts, are summarized in Question 1 (part 2), showing various degrees of success.

The Working Group for Questions 3 and 5 felt that Questions 3 and 5 were related, and in fact Question 5 may be a sub-part of Question 3. The consensus was to develop a knowledge base of the information currently available and use that to further develop an answer to these questions.

**Q3. What types of microbiological criteria could be established for poultry carcasses, parts, and comminuted products prior to applying interventions and after interventions, considering current technology?**

a) Could the quantity of *Salmonella* or quantity of microbiological indicator organisms (e.g., APC) be used? What are the key parameters that need to be considered? What data analysis techniques could be used? How would these criteria be linked to human illness?

b) How could serotypes frequently associated with human illness be considered in the development of microbiological criteria?
Summary of Question 3 Response While indicator organisms may be useful for process control to verify that an intervention step has been applied (such as an antimicrobial carcass rinse), several unpublished studies (university and industry personal communications) suggest a very weak correlation between quantity of indicator organisms (e.g., APC, Enterobacteriaceae) and presence or level of Salmonella post-chill. Rather, enumeration of Salmonella (not serotype specific) with a set limit of quantification (limit to be determined pending further research) can be used to identify highly contaminated lots to be diverted for further processing. Removing materials that are highly contaminated will reduce consumer exposure to infectious levels. This is even more important for comminuted, mechanically separated, and similar products with high degree of handling that can disseminate Salmonella through commingling of contaminated poultry meat.

Salmonella prevalence testing does not differentiate between carcasses with low levels of Salmonella vs. those with high levels that are more likely to cause illness (93, 127, 135). Risk analyses suggest that while the prevalence of Salmonella, which is currently used in FSIS’ performance standards for poultry, affects the risk of salmonellosis, neither prevalence alone nor total bacteria populations is correlated with the risk of salmonellosis (84, 113). Rather, prevalence could be used in conjunction with Salmonella enumeration. In addition, levels of virulent strains and undercooking/improper food handling also impact risk. Given the limitations of current strategies to control Salmonella on carcasses, parts and comminuted products, quantification of Salmonella as a performance standard may have greater impact on identifying products with higher (infectious) levels that can be diverted for alternate processing. Further refinement of standards may be possible as additional data and interventions become available.

Currently, the Pathogen Reduction Performance Standards does not require that poultry processors conduct pathogen testing. Rather, monitoring compliance with the Pathogen Reduction regulatory requirements is conducted by FSIS. Statistically demonstrable performance that is worse than a set standard (or, in the future, not meeting a selected Key Performance Indicator (KPI)(165)) could trigger a Comprehensive Food Safety Assessment by FSIS (or other measure to be established). Establishments that fail to maintain the required documentation, including documented efforts to improve performance, would be subjected to the Rules of Practice at 9 CFR, Section 500 (35), including, as appropriate, the Suspension of Inspection. There is some evidence that public posting of establishment’s performance may have contributed to prevalence reduction across the industry (111).

Potential MC approaches applicable to postharvest poultry processing
In poultry processing establishments, data on Salmonella occurrence and/or levels could be collected at multiple in-process steps (Figure 5): stunned / slaughtered / “feather on” carcasses, post picked carcasses, carcasses at rehang, post-evisceration / pre-chill carcasses (both on-line processed carcasses and off-line reprocessed carcasses and parts), post-chill carcasses and parts (off-line processed parts), and post-processing (immediately prior to packaging) finished products (e.g., cut portions, deboned portions, giblets, and comminuted product). Such monitoring data could be used in several ways to inform process control and risk management actions and used for performance standards in lieu of the current Salmonella prevalence model.
When choosing MC, the feasibility and usefulness of serovar-specific approaches should be considered. For example, the lack of enumeration techniques for specific serovars limits the option of serovar-specific MC to qualitative ones. Several options for MC during processing could be considered, including:

- Prevalence-based (qualitative) MC on the pathogen in the finished product
- Concentration-based (quantitative) MC on the pathogen in the finished product
- Concentration-based (quantitative) MC on the pathogen during processing (e.g., at one stage, or reduction between two or more stages)
- Concentration-based (quantitative) MC on an indicator organism during processing (e.g., at one stage, or reduction between two or more stages)
- A combination, e.g., a quantitative indicator MC at processing and a quantitative or qualitative pathogen MC in the finished product
- A combination, e.g., of a qualitative MC on selected serovars and a quantitative MC on all Salmonella.

If a concentration-based MC is established, once the MC threshold concentration is defined, it is necessary to identify with sufficient accuracy whether a sample is above the threshold (i.e., a semi-quantitative approach) in order to prove that the MC has been met. This approach is already used by some companies to assess their processes (135). At the same time, the full quantification information provides additional information that can be used for trend and root cause analyses in support of process control protocols. The tradeoffs of test sensitivity and quantitative target are discussed in answer to Question 1.

For the purposes of evaluating the microbiological reductions achieved during processing, appropriate indicator microorganisms may be monitored, e.g., Enterobacteriaceae (EB) or Aerobic Plate Count (APC) (14, 44, 127). The data deriving from such testing are relatively inexpensive and, given there is no need for a pathogenic positive control culture, the testing may be conducted using simple, standard microbiological methods at onsite laboratories -- avoiding the need for expensive and time-consuming sample shipment to centralized, bio-secure testing laboratories. However, at this time, data on the association between the occurrence or levels of indicator organisms and presence or levels of Salmonella in poultry during processing is scarce and inconclusive (see Question 5).

MC combining Salmonella thresholds (or thresholds in selected serotypes) and indicator microorganism thresholds are possible and could provide complementary information on both process control and public health risk. For example, more frequent indicator testing combined with relatively less frequent Salmonella testing might provide cost-effective information to assess whether additional risk control measures are necessary, in a timely fashion. Although more evidence is needed to estimate the effectiveness of such approaches, QMRAs based in existing evidence to derive different scenarios of efficacy could inform the public health impact of such approaches and inform more targeted data collection efforts.
Examples of Canadian and EU Microbial Criteria for *Salmonella* in finished poultry products, and

*Salmonella* control programs:

- In Canada, a surge in SE infections occurred in 2016-2018, and many outbreaks were linked to raw breaded processed poultry products, including chicken nuggets, chicken strips, and chicken tenders (personal communication, Kate Thomas, Canadian Food Inspection Agency). The outbreaks prompted regulatory changes requiring that such products either be cooked before being sold or tested and shown to be *Salmonella*-free; the rules were finalized in early 2019 (20). SE cases reported in Canada fell by more than 50%, from ~13 per 100,000 in 2017 to 5.8 per 100,000 in 2019 (20).

- The EU adopted a similar approach for finished poultry products that were adopted for flocks. The EU regulation (57) was set in 2003 to take effect in December 2011 targeting the absence of *Salmonella* spp. in carcasses and fresh poultry meat (broilers, laying hens, turkeys). Since by December 2011 most poultry flocks would have been out of compliance from a target <=1% flock-level prevalence, the EU created a new regulation (59), also starting in December 2011, where only S. Typhimurium and S. Enteritidis would have needed to be absent from fresh poultry meat and carcasses, based on an EFSA report citing those two serovars as causing approximately 80% of all salmonellosis in the EU (60). Serotyping is carried out via culture methods (White-Kauffmann-Le Minor scheme) (58). In contrast, poultry meat preparations intended to be eaten cooked (e.g., salted raw poultry meat) were regulated under a different law (58) that required absence of all *Salmonella* in 25 grams and remained unmodified. Despite these measures, no consistent decline has been observed in human salmonellosis in the EU as a whole (Figure 7).

One possible approach relevant to MC – as well as to other risk management frameworks - involves using process control (71, 88, 89) where the standard to be met is phrased as the consistent maintenance of a microbial (or other) parameter at or below a specified level over time, within a specified tolerance. Data from each monitoring point would be statistically analyzed to assess process trends, e.g., utilizing Statistical Process Control Charts (e.g., Xi & MR charts) with both quantitative values at the monitoring point and the reduction achieved from the previous monitoring point in time, plotted and analyzed for statistically significant trends. In addition, other relevant measures may be recorded, such as source of the flock being slaughtered, season of the year, line speed, temperature of scalding, and any relevant metrics of interventions being employed. All indications of a statically significant trend in the data (utilizing standard SPC rules to assess “out of control” signals) could trigger a documented root cause analysis to determine the reason for the process change, and efforts to continuously improve process performance by implementing documented process changes to restore the target performance level or to capture the improved performance level towards such target. Corrective actions could be informed by trend analyses, which could identify factors associated with deviations from target metrics, including occurrence/levels of the pathogen to control. Deviation from process control could also trigger risk control actions in the case that product had been processed while not in-control, for example possibly including -in the future- treatments of proven effectiveness such as high-pressure processing or irradiation (136).
This approach would require the definition of an acceptable risk-based target level of the adopted metric, similarly to a MC assessed at each sampling time, but process control or compliance could also be evaluated over longer time spans, hence capturing longer-term processes including the impact of corrective actions that take longer times to be implemented or take effect. Quantitative microbiological analyses would provide substantially improved data to assess the status of process control. Documenting corrective actions would be needed to both demonstrate that such action was taken, and to build further evidence of its effectiveness in real settings. This approach involves extensive data collection and analysis, and hence could be resource-intensive. However, advances in management and rapid analysis of large datasets could increase feasibility. Issues of equity across large and small establishments should be considered.

**b) What are the key parameters that need to be considered?**

Parameters of the sampling and testing protocol can impact the value of the resulting information, for instance the point of sampling, the frequency of sampling in relation to product flow rate, sample volume or weight, assay sensitivity and specificity, and how test results are analyzed (e.g., summarized over which time frame or moving window, or by product flow rate). Key parameters may vary depending on the scope of a MC, e.g., characterizing the performance of an establishment ("process hygiene criteria") vs. assessing a batch of product ("food safety criteria"). A summary of MC parameters impacting risk, and hence also QMRA estimates of MC impacts, are outlined in Question 1. Selected aspects are illustrated below.

**Assay parameters.** If considering molecular quantification methods for *Salmonella* instead of culture-based methods such as MPN, the assays should be able to provide consistent, sensitive quantification at low bacterial loads (e.g., well under 1 CFU/g, depending on which MC threshold would result in the target illness reduction) in order to capture the majority of the bacterial concentrations observed post-chill. For example, in the 2007-2008 poultry baseline study (155), FSIS collected 3,275 samples post-chill, and 267 (8.15%) were positive. From those, the most common level was 0.036 CFU/g, while only 18 samples (6.7%) exhibited concentrations above 1 CFU/g. Extrapolating those figures to the current *Salmonella* prevalence in poultry of roughly 4.5% and assuming that bacterial concentrations has remained the same (it is likely lower, since prevalence has declined since), if 10,000 poultry samples were taken annually, over 93% of the 450 expected positive samples would have concentrations under 1 CFU/g. In contrast, an average of 2-3 samples a month would have loads above that threshold. This stresses the importance of quantification methods reliable at low concentrations, in particular in the range of considered MC thresholds. The different performances of quantitative tests are further discussed in Question 6.

The point above is also true for serotype-specific MC, which would also require high specificity to the considered serotype. In addition, biases in the detection and quantification of different serotypes may be introduced by different assays, as discussed in Question 7.

**Metrics demonstrating effective process control.** Key parameters include the absolute quantitative microbiological performance or other Key Performance Indicator (KPI) demonstrating sequential microbiological reduction (as measured by the indicator microorganisms, as the expected low
quantitative levels of *Salmonella* spp. results may not result in a statistically meaningful reduction between sequential processing steps) through each step of the process. Process control could be demonstrated by statistical stability demonstrating consistent microbiological reductions through the process and/or indications of statistical trends that are evaluated via root cause analyses to drive continuous process improvement. A quantitative threshold in *Salmonella* levels at a specific stage (e.g., finished product) would also be needed to assess whether the process consistently results in sufficient bacterial reduction to protect public health, based on risk targets (while still in the context of continuous improvement).

**How would these criteria be linked to human illness?** Any MC and associated risk control measures should be risk-based, that is linked to human illness burden targets, and/or associated *Salmonella* levels in finished products. As discussed in Question 1, if MC result in a sustained reduction of human salmonellosis, the combination of measures implemented to control *Salmonella* would be reflected in epidemiological data. However, given the year-to-year variability in salmonellosis reporting, a consistent reduction in salmonellosis might still take several years to be evident in the epidemiological data. QMRA models can be used to estimate the impact of individual measures and help determine the extent of testing and corrective actions necessary to reduce burden by a target amount. For example, QMRA models indicate that reduced concentrations of *Salmonella* on consumer-ready poultry products is likely to yield a reduction in human cases of salmonellosis (see Question 1).

Through process control and associated continuous improvement methodologies, the poultry industry could be required to demonstrate processes are consistently capable of meeting the quantitative microbiological performance standards/MC combined with efforts to continuously improve process performance and capability.

**Qualitative vs quantitative MC**

Qualitative MC are based on presence/absence (detection/non-detection) of the pathogen in a sample and are thus represented in terms of the proportion of positives out of the total samples (i.e., prevalence). Quantitative MC instead are based on the concentration of the pathogen found in a sample. But both prevalence and concentration metrics stem from the same underlying distribution of concentration, i.e., cell numbers per unit weight in the product. Hence, prevalence and concentration MC are not completely separated approaches. As described in Q1 and under Assay parameters in this chapter, *Salmonella* concentrations in U.S. poultry are low, with only 6.7% of samples above 1 CFU/g post-chill in the 2007-2008 poultry baseline study performed by FSIS (155), so in order to implement a quantitative MC, assays should be able to characterize all concentrations found in poultry products, and at the very least at and above a candidate MC threshold of public health significance. Complexity is added by variability across lots, flocks, establishments, regions, etc., which is not well characterized. Some authors have proposed that a linear relationship between prevalence and illness incidence exists for *Salmonella* at low concentrations (48, 50), which would make it sufficient to use prevalence in low concentration settings. However, the current qualitative performance standards and subsequent prevalence reduction in poultry meat and products have not resulted in a corresponding decrease in human salmonellosis from poultry in the US. Current evidence supports that a quantitative MC on finished product, if set at an appropriately protective threshold and using a rapid test, could identify
product batches that require additional processing before being sent to retail (in addition to informing process control). More evidence would be needed to support a quantitative MC upstream during processing, e.g., carcasses at rehang, which would allow for more time to adapt processing operations based on test results. Evidence relevant to MC options including levels of indicator organisms is discussed in Question 5.

It is possible that a quantitative MC for other/all serovars, in combination with a qualitative MC targeting select serovars of high public health concern, might result in a more marked reduction in human salmonellosis, if a stricter MC were to be applied for serovars of high concern compared to current qualitative performance standards. The efficacy of such MC options and specific MC thresholds should be evaluated using QMRAs, as further described in Question 1. Relevant target metrics would be the concentration of generic Salmonella in a sample of set weight, and the frequency of detection of high-concern serovars. Even more important than the distinction between qualitative and quantitative targets is the implementation of risk control measures associated with a MC, either voluntarily or via enforcement. Question 1 provides a brief review of risk-based studies that illustrate how both prevalence-based and concentration-based MC, associated with effective control actions, can potentially reduce illness burden, if control actions are implemented.

Serotype-specific MC approaches based on process control

Considerations outlined above, including both MC on finished products as well as MC approaches at establishment level that adopt a process control approach, are also applicable to serovar-specific MC.

Adopting a process control approach, the poultry processing industry could potentially be required to assess the serotype of each Salmonella spp. sample positive result against a FSIS-maintained list of serotypes of public health concern and maintain failure rate statistics or other KPIs from tracking trends in the frequency of detection of those serotypes. To provide a hypothetical example, a process control metric/KPI could be required to track and trend the frequency of isolation of serotypes of public health concern, reported per standard production unit of measure (e.g., per 1,000 head slaughtered). Such KPI could be calculated annually by FSIS from accumulated establishment-specific data for each establishment size (as defined in the PR-HACCP regulations). Frequency of sampling is a key parameter to obtain adequate detection power and actionable information and should be included in assessments of MC impact (see Q1). Continuing with this example, all statistically significant trends above a threshold, signaling recurring loss of process control, could trigger a Comprehensive Food Safety Assessment by FSIS, or other measures to be established. Establishments that fail to maintain the required documentation demonstrating efforts to improve performance and reduce the frequency of the target Salmonella serotypes, could be subjected to the Rules of Practice at 9 CFR, Section 500, including, as appropriate, the Suspension of Inspection. Trends signaling loss of process control could also be evaluated (voluntarily or not) using root cause analysis tools to determine the cause, to facilitate efforts to ensure the reduced frequency of detection is sustainably maintained in the future, hence driving continuous process improvement efficiently. In assessing the potential impact of different MC, the effectiveness of establishment-level corrective actions (e.g. an FSIS Food Safety Assessment) should be compared to the effectiveness of risk control measures demonstrated to reduce Salmonella (or the
target serovars) on the product, as well as combinations of establishment-level and product batch-level approaches.

The answer to **Question 4** provides a reasoned review of the factors and available data and approaches to consider in vetting *Salmonella* serovars of priority public health interest linked to poultry consumption, for the potential design of a serovar-specific MC.

**Q5. There is a documented correlation between a reduction in the quantity of APC between carcasses and finished products and the occurrence of Salmonella in finished products for beef, pork, and poultry. How might this information be used to set microbiological criteria to assess process (pathogen) control in poultry?**

The objective of microbiological performance standards or MC is to control the contamination of pathogens in foods along the supply chain, and ultimately mitigate associated public health risks. From this perspective, a pathogen-based performance standard provides the most relevant information on which to base risk management actions. However, its implementation in practice can be laborious, highly resource-demanding, and sometimes problematic, for instance considering the very large sample size needed for an acceptable statistical power to correctly classify a food production establishment into either high or low contamination groups in particular, when the rate of occurrence of contaminated food units is low (175). Hence, it is worthwhile to explore methods to supplement the pathogen-based system for the development and implementation of microbiological performance standard by regulatory agencies and the food industry in a more feasible and effective manner.

Employing indicator organisms to reflect the microbiological status in water and foods can be traced back to more than one century ago. Since then, their use has become widespread in microbiological testing programs employed by regulatory agencies and the industry. Albeit the most common use is to evaluate the microbiological quality of food and predict the product shelf life, indicator organisms are frequently used as safety indicators to determine the presence of foodborne pathogens, identify the insufficiency in meeting GMPs requirements, and assess the integrity of process control. The long-established usage of indicators suggests the potential of an indicator-based performance standard in foodborne pathogen control, representing an analytical substitute for the detection of the target pathogen. Indicator organisms do not directly represent the specific target pathogen but are used to suggest the possible presence of a specific organism or source of contamination, or the occurrence of low hygiene conditions.

In the U.S., several indicators have been used for foods in specific applications, including aerobic plate count (APC), coliform group, generic *Escherichia coli* (*E. coli*), and *Enterobacteriaceae*. These commonly tested nonpathogenic bacteria can be considered as the candidates to evaluate their suitability in the use of indicator-based performance standard for *Salmonella* control, for several reasons. These indicators naturally share ecological association with *Salmonella*, such as their common enteric origin and similarity in taxonomic classification. In addition, efforts have been made and continued for data collection to support such an investigation. Relevant to poultry products, USDA FSIS uses *E. coli* as the
major determinant for the development of process control verification criteria in meat and poultry (9 CFR 310.25). Nationwide, FSIS conducted the baseline sampling of the four indicator bacteria through Young Chicken Survey (155) and Young Turkey Survey (156) to inform the establishment of statistical process control limits and procedures. In addition, a substantial amount of data is generated by poultry industry and/or academic researchers. Federally inspected establishments may collect samples on a routine basis at more processing steps and a higher frequency in comparison to the regulatory sampling programs (44). With the availability of the nation-representative and extensively collected data, it offers the opportunities to address the important question if an alternative performance standard built upon indicator organisms can be effective in process control improvement and food safety protection against *Salmonella*.

When considering candidate indicator organisms, several attributes should be considered. Based on the International Commission on Microbiological Specifications for Foods, the following attributes are suggested for the selection of a safety indicator suitable for the use in an indicator-based performance standard (77, 147):

- Characteristics of the indicator can be stably identified over time and through food production and processing steps.
- The presence or level of the indicator organism indicates (e.g., semi-quantitatively or quantitatively) the degree of the potential for contamination, or lack of process control. Testing results provide actionable feedback for process control.
- The detection and/or quantification of the indicator should be easy, rapid, inexpensive, sensitive, reliable, and safe for an in-establishment use.
- The responses of the indicator to the intrinsic properties of foods and extrinsic conditions of the food production and processing environment should behave in a similar but slightly more conservative manner in comparison to the target pathogen (e.g., inactivation, growth, and survival kinetics). For example, growth of the indicator should be slightly faster, while survival of the indicator should be greater in response to the food safety hurdles.
- Quantitative/qualitative correlation between the level of the indicator and the level of target pathogen can be established.

The usefulness of an indicator depends on the purpose of its testing. The reliability of an indicator to predict the presence or amount of a target pathogen, needed in a risk-based MC, is related to the strength of the quantitative correlation between the presence or amount of the two. In many cases, there may not be a strong statistical correlation between the presence or amount of an indicator and the presence or amount of a specific target organism. However, this does not rule out the usefulness of the indicator for the industry as a method of monitoring process control, where indicator trends and patterns can highlight deviations from normal or target conditions and inform adjustments (56). Trends in the levels of the indicator organism may be associated with other process variables, which may ultimately contribute to the presence of the target organism. This is an area where biomapping research and industry data on *Salmonella* levels could provide insights.
Mesophilic aerobic populations may be useful to indicate the degree of process control, with the understanding that there are many variables which may affect the outcome. Mesophilic aerobic bacterial populations, commonly referred to as Aerobic Plate Count (APC), is simply a reflection of the bacteria present in a sample which will grow under the proscribed conditions of plating media, incubation temperature and incubation time. As such, the initial composition of the microbiota in the sample has a significant effect on the outcome of the analyses and the APC in no way reflects the “total” microbial population in the sample.

There is limited and inconclusive evidence on the correlation between indicator organisms and *Salmonella* at specific stages during processing. For instance, using FSIS data a lack of meaningful correlation was found between generic *E. coli* (GEC) and *Salmonella* (174), while one study (broadly summarizing data from 20 establishments) suggests a potentially high association (1).

The predictive value of indicator patterns and trends (spatial and temporal) in providing process control information should also be considered, in addition to indicator-*Salmonella* association at a specific point. Currently many poultry establishments are sampling poultry carcasses at the re-hang step and after chilling using a whole carcass rinse (154). Based on retrospective analyses of regulatory data, one study found a weak but non-negligible correlation between *Salmonella* prevalence and the log_{10} difference in APC levels from rehang to post-chill carcass samples (correlation coefficient of -0.4) (175). The “true” correlation of this relationship may vary from establishment to establishment, but the change in population may be useful as a means of monitoring process control. If these findings were confirmed by additional data, a MC based on a minimum APC reduction between two processing stages, quantifiable via existing assays, could be a viable MC candidate. An assessment of the potential performance of a similar MC was carried out for U.S. beef carcasses (176). Conversely, one study compared two groups of establishments implementing two different intervention approaches. Differences in indicator levels were observed between the two groups at multiple processing stages. Both groups had significant reduction in APC and EB levels throughout processing. However, these differences in indicators between the two groups did not correspond to different *Salmonella* levels in the product, suggesting a lack of or weak correlation (44).

Evidence is insufficient on the association of indicators with specific *Salmonella* serotypes. Data from the poultry industry has shown a quantifiable reduction in *Salmonella* concentration during processing, from hot rehang to the subsequent processing stages to the finished product (chicken parts), consistent with the decline in *Salmonella* prevalence along the process (10, 83). These data illustrate that, while quantification at the low concentrations observed in finished product can be problematic, quantifications at earlier processing stages for the purposes of a process control target/MC is feasible, as an alternative to process control metrics based on indicator organisms.

Given the limitations and evidence gaps described above, APC may or may not correlate with the presence of non-Typhoidal *Salmonella*. That is, *Salmonella* may be present in samples which contain low, intermediate, and high populations of indicator bacteria. In addition to the evidence summarized above, unpublished data provided by the poultry industry and university researchers suggests that indicator bacteria have very limited predictive value for the prevalence of *Salmonella* [unpublished
industry data, A. Siemens, personal communication, R. Kalinowski, personal communication, E. Moorman, personal communication and ref. (127).] However, a change in APC from an early sampling point on the slaughter line to a final sampling point on the processing line, as well as absolute levels at the final point, may provide useful information about the effectiveness of the process in maintaining hygienic conditions. Therefore, APC may be useful to indicate process control even though it is not a true indication of the presence, level, or virulence of Salmonella. In the future, once Salmonella prevalence and/or levels are low enough that testing assays are not able to reliably detect its presence, i.e., the proportion of non-detects increases, the value of information from indicator organisms to indicate departures from process control may increase.

Additional considerations on how indicator organisms can be included in MC is provided in Q3.

Additional consideration on how indicator organisms can be included in QMRA models to estimate the impact of MC is provided in Q1.

Q6. What rapid methods and technologies are available for the quantification of Salmonella? How should FSIS make the best use of these methods?

6.1 Background: traditional Most Probable Number (MPN) used today

Methods based on cell cultures have historically been used to determine the concentration of microorganisms in a sample (i.e., for bacteria, cells per unit of volume or weight). The population is enumerated or estimated by either using plate count methods or Most Probable Number (MPN). MPN is utilized when organisms may be at lower levels (i.e., <10 CFU/g or mL) or when an enrichment is needed to address resuscitation, possibly from injury. Bacterial injury can occur in response to processing stresses (i.e., heat, sanitizers, storage conditions, etc.) (116).

MPN analysis requires multiple test portions to be analyzed from the same sample, requiring significant resources of time and supplies (media and laboratory disposables) and associated costs. Time-to-result is dependent upon the detection system used for determining the presence of Salmonella. After incubation, a reference cultural method or a rapid detection system can be used to determine positive growth. The USDA Microbiology Laboratory Guidebook presents a dilution scheme for MPNs in Appendix 2.05 (159) analyses following the method in Chapter 4.12 for the detection of Salmonella (163). This method describes both the cultural approach and molecular detection system (152). Using a rapid detection system following enrichment of multiple samples for MPNs shortens the time-to-result (159, 163).

6.2 Available technologies

In recent years, methods have been developed using quantitative PCR (qPCR) for enumerating Salmonella in poultry. The cycle threshold (CT) value, which is inversely related to the amount of Salmonella in the sample, is used to quantify the number of colony-forming units (CFUs). (Hygiena MWP-2005-Rev_A, https://cdn.brandfolder.io/KA71VJV5/at/q7tmon-8ui1s0-2w7zld/BAX-System-SalQuant-New-Era.pdf, accessed October 29, 2022). Many of these methods require that the sample is enriched in specified media for a prescribed time and temperature (See Appendix D Tables 6 and 7). One
method has been developed without enrichment that uses centrifugation of the sample to concentrate
cells (bioMerieux Gene-Up Quant Salmonella). Two of these methods, Hygiena’s SalQuant™ and
bioMérieux’s GENE-UP® QUANT Salmonella, have been validated by AOAC Performance Tested Method
certification for enumeration of Salmonella in 2021 and 2022 as presented in Appendix D Table 6.

Other enumeration assays that have been developed or are expected to be commercialized with varying
levels of evaluations from internal data or collaborations with service laboratories or poultry producers
are presented in Table 7 in Appendix D. These methods include dd-Check Salmonella, iQ-Check
Salmonella, SalLimits, Molecular Detection Assay 2- Salmonella Quantification, CASE 2 Salmonella, and
SureCount Salmonella Species. Enumeration of Salmonella has been studied by using digital droplet PCR
(ddPCR) that uses partitioning live encapsulated bacterial cells (Bio-Rad). This approach can be achieved
without enrichment. Other methods are being developed including the use of a chromogenic agar plate
counts (Neogen), and Loop-mediated isothermal amplification (LAMP, see Appendix D Table 7).
ThermoFisher developed a multiplex PCR method for enumeration of S. Typhimurium and S. Enteritidis
that is in the process of being validated at the time of print.

6.3 How can USDA FSIS use these methods
Table 6 shows two current validated enumeration methods commercially available and their
 specifications. These two methods, Hygiena’s SalQuant™ and bioMérieux’s GENE-UP Quant Salmonella, have AOAC Performance Tested Method (PTM) approval being evaluated for robustness and tested in
an independent laboratory according to guidelines for selective matrices (2). The methods were
compared to the USDA FSIS MLG MPN method as presented in Chapter 4.12 (152) and according to
Appendix 2.05 (159). Since these are enumeration methods, the quantitative validation guidelines were
used for the comparison (2). The limits of quantification as shown in Table 6 present both the claim
based on the target levels as defined in the guideline and the actual limits from the validation study. It
should be noted that, as part of AOAC’s PTM, concentrations as low as 37 CFU/mL in poultry carcass
rinse were tested (3, 5). Given that most concentration observed in poultry products are lower,
additional validation may be warranted before new assays are adopted for poultry testing.

USDA FSIS can consider the validation status of each method, the impact of bias during the test
methods, the workflow when selecting Salmonella enumeration methods, and the ability of the
methods to detect the pathogen concentrations commonly observed in poultry. An enumeration assay
should also be considered in the broader context of the sampling protocol, including number and
frequency of samples collected in relation to product flow rate. Assay parameters relevant to MC are
also discussed in Question 1 (Table 2).
Q7. Are there particular approaches that would result in selective identification of the serotypes of public health concern?

Is there strain selection bias introduced by laboratory methods? And if so, what strategies can be used to mitigate this bias?

a. For example, are there approaches to mitigate a potential strain selection bias introduced by the laboratory method?

b. If needed, what type of research could be conducted to ensure performance characteristics of current laboratory methods (e.g., enrichment, incubation, pre-screening) do not result in a biased serotype detection?

Is there strain selection bias introduced by laboratory methods? And if so, what strategies can be used to mitigate this bias?

Culture-based quantitative methods, as well as enrichments carried out prior to molecular assays, provide an estimate of the number of organisms in a food according to rely on the medium employed and the time and temperature of incubation (116). This is also the case for qualitative methods for determining presence or absence. Both testing approaches depend upon the conditions for growth and whether they are optimal for the target organism or group of organisms. Failure of the target organism or group of organisms to be detected (or enumerated) can be attributed to intrinsic or extrinsic factors (e.g., time, temperature, media) influencing growth. For the purposes of this response, the focus will remain on Salmonella and strain selection bias.

It is well-known that sampling, method choice, and enrichment practices have inherent biases due to a variety of reasons. These biases impact detection of Salmonella and selective identification of serotypes. Some examples include:

- Whole carcass rinse preenrichment released more Salmonella cells than standard rinse aliquot preenrichment. However, false negatives were observed at times and the approach failed to detect all serotypes present; nonetheless, it was found to be the best method to determine true prevalence by Cox et al, 2019 (37). Preenrichment bias impacts all serovars, although it is possible that different serovars are impacted to different degrees.

- Non-selective media recipe may affect detection of Salmonella. Williams et al. (177) reported that approximately 0.02 to almost 0.06 when the rinse changed from BPW to BPW with a neutralizing agent. This was not the case for detection of Salmonella in chicken parts though. Media formulation impacts all serovars, although it is possible that different serovars are impacted to different degrees depending on their individual growth characteristics, such as generation time under otherwise ideal conditions, utilization of nutrients, and ability to equally compete with other microbes in the enrichment.

- Selective media may affect the growth of Salmonella. It was reported that S. Enteritidis is selected in Tetrathionate (TT) broth and S. Schwarzengrund is selected in Rappaport-Vassiliadis (RV) broth (37). The shift in serotypes throughout the enrichment time limits the ability to identify Salmonella by culture methods. The use of multiple media recipes (i.e., selective, non-selective broths and agar
plates) mitigates the bias by accounting for variation in sensitivity to selective ingredients and atypical biochemical profiles that affect growth on the media.

- Approaches to confirm presumptive *Salmonella* may only require to sample 1 typical colony from a selective plate, to be confirmed (152). Colonies from other selective plates may not be selected if the initial colony confirms as *Salmonella*. Even when a method requires up to 12 colonies to be confirmed across three selective plates representing two selective enrichments (152), routine laboratory practice may only identify 1-2 colonies. It only requires one isolate to confirm and report a sample as positive for *Salmonella* saving on labor and supplies (37, 110).

- The shift in serotypes throughout the enrichment time limits the ability to identify *Salmonella* serovars by culture-based approaches. This shift over time may be due to different growth kinetics of different serovars in selective media, or possibly to competitive inhibition. Cox et al., 2019 (37) using clustered regularly interspaced short palindromic repeats (CRISPR) analyses to differentiate individual serovars demonstrated that the *Salmonella* serovar profile shifted when original isolates from poultry carcasses were compared with the serovar profile after enrichment.

- This last example of CRISPR demonstrates one approach to mitigating bias, although this approach is not readily available for routine testing in laboratories.

- There are many methods currently and historically available that can differentiate serotypes that are commonly associated with disease. Example platforms include PCR-based approaches (e.g., TaqMan, MLST), WGS, Riboprinting, and classic serotyping. However, serotypes are typically identified after selective enrichment for *Salmonella*, which introduces selection bias as discussed above (152).

A current study characterizing *Salmonella* is being conducted at USDA Agriculture Research Service (ARS) that will investigate a novel molecular assay for the detection of select *Salmonella* serovars in raw meat enrichments that would identify Highly Pathogenic *Salmonella* (HPS) preventing the bias of serotyping from an isolate ([72] and https://www.ars.usda.gov/research/project/?accnNo=437924]). The work has potential to be applied to poultry samples for culture isolation using *Salmonella* specific immunomagnetic separation (IMS) to determine prevalence, direct plating enumeration of *Salmonella* present within an enrichment to examine limit of detection, the HPS molecular detection assay, and the Neogen (legacy 3M) *Salmonella* Molecular detection assay for comparison to the FSIS approved molecular detection method. This study will also characterize all *Salmonella* isolated for determination of serotype and antibiotic resistance (https://www.ars.usda.gov/research/project/?accnNo=437924), which is an example of how both identification and detection can be achieved.

Methods for qualitative detection of multiple targets are commercially available. As serotypes of concern change over time, multiplex PCR assays can target specific serotypes, as ThermoFisher has with their SureCount *Salmonella* Species, Typhimurium and Enteritidis Multiplex PCR Assay (Table 7). Others can be expected to be developed. For example, ANSES (the French Agency for Food, Environmental and Occupational Health & Safety) has recently developed and validated a high-throughput qPCR assay able to identify 40 Salmonella serotypes, and distinguish different genomic lineages and polyphyletic profiles (19).
After detection and isolation of Salmonella, commercial Salmonella serotype identification methods can be utilized. Two include:

- The Neogen NeoSeek Salmonella serotyping it a technology that utilizes a targeted amplicon sequencing approach to identify the serotype(s) of Salmonella present in a sample. Samples may be submitted as isolates or enrichment broths. If a broth is submitted, up to three Salmonella serotypes can be identified if present. Briefly, sample processing involves extraction and purification of DNA, amplification of targets using multiplex PCR, and sequencing on an Illumina MiSeq. Serotypes are called using proprietary software to analyze sequencing results, including MLST (Multilocus sequence typing) targets. (https://www.neogen.com/categories/bacterial-sequencing/neoseek-salmonella-serotyping/?recommendationId=2128188658911; accessed October 29, 2022).

- Check & Trace Salmonella (CTS) is a rapid genetic test based on a microarray platform to identify Salmonella serotypes (46). Each position on the microarray represents a specific DNA marker associated with a unique Salmonella target sequence. Spots only become visible if the DNA markers exactly match the corresponding DNA sequences of the Salmonella isolate. The combination of present and absent spots yields a pattern. The database includes top serotypes from outbreaks although is limited and is periodically updated.

Research is needed to address the items listed above to mitigate bias in Salmonella detection and specifically changes in the serotypes of concern due to virulence. A list of select research needs is reported in Question 9.

8. How should pathogen characteristics derived from whole genome sequencing (e.g., serotype, virulence, antimicrobial resistance) be considered in the development of microbiological criteria?

8.1 Recent WGS-based advancements in Salmonella characterization and current use case

Numerous studies support that Whole Genome Sequencing (WGS) is a critical element in establishing microbiological criteria for Salmonella. WGS can define serotype, predict antimicrobial resistance profiles, and offer considerable insight into virulence capacity of an isolate as thoroughly reviewed in Cheng et al., 2019 (34). The greatest value of WGS is at the convergence of serotype, epidemiological, and phenotypic data to differentiate Salmonella with high public health relevance from Salmonella of limited public health relevance. Some serotypes (e.g., S. Cerro, S. Kentucky) (22) are much less likely to cause severe, invasive human disease than others (e.g., S. Choleraesuis, S. Dublin (78)). WGS can be used to differentiate hypo- and hypervirulent serovars and clades by leveraging data beyond serotyping; thus, WGS can contribute to identifying and weighting relevant strain characteristics, including virulence, that inform best targets for control resulting in the greatest public health benefit. For example, WGS studies found that S. Cerro isolates have a premature stop-codon in sopA (82, 123), which contributes to host cell entry (118). S. Kentucky is the most commonly isolated serovar from broiler chickens in the United States (151), yet constitutes 0.1% of reported human salmonellosis cases (22). S. Kentucky isolates lack the virulence genes (e.g., grvA, sseI, sopE, and sodCI (11, 34) and other metabolism-associated genes (143) that may reduce its ability to cause severe disease; presence and absence of such genes can and have been defined by WGS.
Recent phylogenetic analyses have determined that commonly isolated serovars (Newport, Montevideo, Kentucky, Paratyphi B, Derby, Nchanga, Cerro, Bareilly, Stanleyville, Dusseldorf, Livingstone, and others) are polyphyletic (21, 45, 132, 146, 182, 183). This underscores that serotype alone cannot define the relative virulence potential of a strain. There are also important clinical implications of polyphyletic Salmonella serovars whereas clades of the same serovar differ in the virulence factors that they encode (96). One example of this is Salmonella Kentucky where two distinct clades have been defined by WGS. (32)

8.2 Current limits to WG sequencing to characterize pathogen virulence

There are several limitations to WGS to characterize pathogen virulence. First, there is a need to expand the database of sequenced isolates associated with illness to determine if eliminating a serotype by intervention strategies implemented by the poultry industry will simply give rise to another serotype. Research on polyphyletic serovars will offer more insight.

Numerous virulence factors have been identified that contribute to Salmonella pathogenicity (41, 125). The interactions of these factors and the resulting strain virulence and pathogenicity has not been completely elucidated, but single genes and pathogenicity islands have been identified as key virulence traits. However, there is currently no agreed-upon definition of virulence genes presence/absence profile that can reliably predict severity of disease.

Host-interactions (e.g., environmental conditions) further complicate the ability to predict virulence. For example, in a study examining the pathogenicity islands (SPI) from Salmonella isolated from different environments that included warm blooded mammals (porcine, bovine, equine, and avian), environment, and human clinical isolates, it was observed that SPI-1, SPI-3, and SPI-5 had genetic variation across the 13 Salmonella serovars tested, while SPI-2 and SPI-4 were well conserved across the same serovars examined.

There is emerging potential for WGS data to be incorporated into quantitative microbial risk assessments (QMRA), but it is currently limited by lack of standardization in assembly, processing, and integration of WGS and metadata (32). The application of WGS to further inform QMRAs and selectively assess serovars of concern is further discussed in Question 1.

Nevertheless, WGS subtyping adds an additional level of discriminatory power that can be used to aid epidemiologic investigations of traceback studies. With continued WGS, a better understanding of important virulence traits and their association with foodborne illness outbreaks may be possible.
Research needs: Data and analytic gaps:

Question 1 and 4 data gaps

- Update estimates of the health burden of salmonellosis and other foodborne diseases at regular intervals
- Analysis of CEA FoodNet data linked with PulseNet to provide next generation of attribution.
- With appropriate anonymization, add sequences of *Salmonella* isolates from live animal sampling from poultry labs, APHIS field studies and NVSL to national databases, as well as from sampling of feed and feedstuffs.
- Conduct a risk assessment that determines the public health impacts of different risk-based *Salmonella* control strategies to enhance our ability to select and implement risk-based *Salmonella* control strategies. This will require substantially more data to inform which strategy will have the largest public health impact. For example, it remains unknown whether a reduction in *Salmonella* levels in food or a more targeted reduction in specific serotypes or subtypes would result in fewer cases of salmonellosis.
- Assess the role of antimicrobial use for *Salmonella* control at preharvest (and its change as a result of implementing a MC and associated *Salmonella* control measures) in the burden of poultry-attributable human salmonellosis, and more widely to the spread of antimicrobial-resistant *Salmonella* and genes.
- Assess health impacts associated with long-term health sequelae of *Salmonella* infection, and account for such burden and costs in cost-benefit analyses.
- Collect quantitative information needed for QMRAs including *Salmonella* prevalence, levels and subtypes of concern on poultry within a product lot, between product lots, product form (ground vs. parts, etc.) and between slaughter and processing establishments.
- Refine attribution models that differentiate illness associated with specifically with comminuted, mechanically separated, parts or whole carcasses to focus resources for intervention on highest risk forms of poultry
- Identification of those *Salmonella* serotypes or subtypes that pose the greatest concern (e.g., most virulence) and their presence in specific poultry products
- Dose-response data and fitted relationships for *Salmonella* serotypes of concern
- Evaluate the frequency of consumer handling and type of preparation practices for specific types of poultry products that contribute to potential for cross-contamination with other foods or undercooking
- Conduct studies on transfer coefficients for transfer of the amount of *Salmonella* to other foods and food contact surfaces during consumer preparation of poultry products as part of meals
- Study human behavior as a route of transmission during normal consumer preparation of poultry products
- Develop public awareness campaign to educate consumers on proper handling, preparation, cooking and storage practices for carcasses, parts, and ground poultry products
• Increase availability of isolates from produce and other non-meat/poultry sources to strengthen the IFSAC Source attribution model.

• Expand NARMS or other sampling to include animal feeds and feed ingredients, as well as poultry-based pet foods.

• Examine the results from 2020 and 2021, when the COVID19 pandemic perturbed many aspects of the food supply. If there were food source-specific effects, then future estimations may need to consider excluding those years from more general models of attribution.

• When metagenomic models are available, assess the presence of even low numbers or relevant serotypes in samples from farm or slaughterhouses.

Question 2 data gaps

• Continue research to determine if non-Salmonella quality indicator sampling could be established, that would provide guidance to companies on which houses have a higher probability of Salmonella contamination.

• Develop a cost-benefit assessment for the U.S., accounting for both intervention implementation costs and cost of illness.

• Identify industry practices that alter the presence and variability of Salmonella serovars between lots of product.

• Eliminate conditions in house that encourage Salmonella.

• Develop cultures for Competitive Exclusion that are capable of controlling more than a single serotype.

Question 3 data gaps

• Conduct research to determine if qualitative and/or quantitative testing and processing scheduling would significantly affect the number of Salmonella-positive processed carcasses.

• Increase the FSIS sampling frequency and sequencing of Salmonella isolates from different types of poultry, including comminuted poultry products, the mechanically separated meat, breaded raw stuffed chicken, and other highly processed products.

• Expand training and knowledge for plant personnel in statistical process control and Continuous Improvement methodologies.

• Continue research to determine if qualitative and/or quantitative testing and resulting changes in processing scheduling would significantly affect the number of positive processed Salmonella positive carcasses.

• Develop predictive microbiology models (i.e., growth, survival and thermal inactivation) and/or biomapping studies characterizing the responses of Salmonella prevalence, concentration, and subtypes to operation/handling conditions during processing and post-processing steps.

• Collect evidence on public health impacts of preharvest vs postharvest MC (or an integrated combination). (Q2 and Q3)

• Collect Evidence on incentives and BCA on preharvest vs postharvest MC (or an integrated combination). (Q2 and Q3)
Question 5 data gaps:

- Provide incentive for industry to deposit data (anatomized, nonpunitive) on the presence or concentration of an indicator (such as APC or others), and the presence/prevalence or concentration of *Salmonella* (possibly by serotype), in the finished product and/or at critical processing stages in the establishment. Use pooled data to develop a comprehensive analysis of quantitative relationship between indicators and *Salmonella*.
- Develop trend analyses and root cause analyses quantitatively linking indicators levels (or other process control metrics, such as variability, trends, or changes between processing stages) to presence or concentration of *Salmonella* in the finished product and/or at critical processing stages in the establishment.

Question 6 data gaps

- Develop detection and enumeration methods that are based on virulence rather than serotypes that are changing over time.
- Develop enumeration methods that are more laboratory friendly considering operational workflow.
- Develop a probabilistic evaluation of the accuracy of qPCR methods at the concentrations found in poultry and poultry products.
- Estimates of sensitivity and specificity of quantitative *Salmonella* assays.

Question 7 data gaps

- Develop and evaluate methods that reduce or eliminate the preenrichment step of methods. Investigate the power of whole genome sequencing to accomplishment this.
- Evaluate media recipes specifically with relevant serotypes from poultry and competition within the matrices of concerns.
- Determine the significance of atypical phenotypical and biochemical strains of *Salmonella* that routine laboratories may be down selecting during analyses.
- Evaluate enrichment conditions impacting growth of strains of concern (time, temperature, media formulation, detection).
- Develop metrics to evaluate and mitigate bias in detection, isolation, isolation and identification methods.
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(formatting for JFP to be completed before submission to journal)


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FOR COMMITTEE REVIEW NOT FOR ATTRIBUTION


### Table 1. Risk assessments of the impact of microbiological criteria (MC) for *Salmonella* and poultry.

<table>
<thead>
<tr>
<th>Article/Report</th>
<th>Main topic</th>
<th>Country</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>EFSA (2010)</td>
<td>Link between <em>Salmonella</em> MC at different stages of the poultry chain.</td>
<td>E.U.</td>
<td>(52)</td>
</tr>
<tr>
<td>Williams et al. (2022)</td>
<td>Risk-based assessment of the effectiveness of performance standards based on APC for <em>Salmonella</em> contamination of chicken parts.</td>
<td>U.S.</td>
<td>(177)</td>
</tr>
</tbody>
</table>

**Additional supporting work on MCs in poultry OR *Salmonella* (examples)**

<table>
<thead>
<tr>
<th>Article/Report</th>
<th>Main topic</th>
<th>Country</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>EFSA (2011)</td>
<td>Includes risk-based modelling assessment of performance targets for <em>Campylobacter</em> in broiler meat; includes impact of different control options.</td>
<td>E.U.</td>
<td>(53)</td>
</tr>
<tr>
<td>Article/Report</td>
<td>Main topic</td>
<td>Country</td>
<td>Reference</td>
</tr>
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<td>---------------------</td>
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<tr>
<td>Swart (2013)</td>
<td>Risk-based <em>Campylobacter</em> MC in poultry.</td>
<td>NL</td>
<td>(141)</td>
</tr>
<tr>
<td>Williams et al.</td>
<td>Assessing impacts of a MC based on indicator organisms, for <em>Salmonella</em> (and <em>E. coli</em> O157:H7) in beef carcasses.</td>
<td>U.S.</td>
<td>(176)</td>
</tr>
</tbody>
</table>
**Table 2.** Key variables to model microbiological criteria (MC) using quantitative microbial risk assessment (QMRA) approaches.

<table>
<thead>
<tr>
<th>Parameter/Variable</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Production/processing stage</td>
<td>Stage where samples are collected (may be multiple)</td>
</tr>
<tr>
<td>Proportion of establishments</td>
<td>Proportion or number of establishments on which the MC assessment is based. May be 100% or a subset, by type of establishment.</td>
</tr>
<tr>
<td>Number of lots/units produced per time unit (e.g., per week)</td>
<td>Production may differ by establishment category.</td>
</tr>
<tr>
<td>Lot size</td>
<td>Weight of product in each lot (lbs./lot)</td>
</tr>
<tr>
<td>Prevalence of <em>Salmonella</em>, by serovars</td>
<td>Number of positives out of total samples tested. If MC selectively targets serovars of concern, results should be provided by serovar.</td>
</tr>
<tr>
<td>Concentration distribution, within lots</td>
<td>Parameters needed to describe the distribution of concentration within each lot (CFU/g)</td>
</tr>
<tr>
<td>Concentration variability across lots</td>
<td>If applicable; represents variability in concentration across lots. Modeling approach may vary.</td>
</tr>
<tr>
<td>Concentration variability across establishments</td>
<td>If applicable; represents variability in contamination for different establishment groups.</td>
</tr>
<tr>
<td>Number of lots/units sampled per time unit</td>
<td>Number or proportion of lots sampled per time unit</td>
</tr>
<tr>
<td>Lot sampling algorithm</td>
<td>Testing protocol within MC determines which lots are sampled, e.g., systematic, randomized, tier randomized.</td>
</tr>
<tr>
<td>Pooled sampling approach (if applicable)</td>
<td>Which samples are pooled, how many and weight of each.</td>
</tr>
<tr>
<td>Number of samples per lot</td>
<td>MC parameter. Number of individually analyzed samples (not pooled).</td>
</tr>
<tr>
<td>MC concentration threshold</td>
<td>MC parameter. Multiple thresholds can be established. In presence/ absence MC, m is the detection limit of the assay.</td>
</tr>
<tr>
<td>Number of samples allowed to be above the MC threshold, among the n samples.</td>
<td>If applicable. MC parameter.</td>
</tr>
<tr>
<td>Assay sensitivity</td>
<td>Ability of the assay to detect the target if the target is present. Tied to the rate of false negatives.</td>
</tr>
<tr>
<td>Assay specificity</td>
<td>Ability of the assay to exclusively detect the target instead of other organisms. Associated with the rate of false positives.</td>
</tr>
<tr>
<td>Assay limit of detection (LOD)</td>
<td>Number of cells in a sample (or per g or ml) that would yield the assay to reliably detect the sample as “positive”. See glossary.</td>
</tr>
<tr>
<td>Parameter/Variable</td>
<td>Description</td>
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<tr>
<td>-----------------------------------------------------------------------------------</td>
<td>------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Assay limit of quantification (LOQ)</td>
<td>Number of cells in a sample (or per g or ml) above which the assay can reliably and accurately quantify concentration. See glossary.</td>
</tr>
<tr>
<td>Batch-level risk management action in case MC is not met (food safety criteria), and associated reduction in target organism in product</td>
<td>E.g., a lot not meeting a MC may undergo additional processing that results in a known Log CFU/g reduction. Different tiers of action can be established if there are multiple tiers of MC compliance.</td>
</tr>
<tr>
<td>Establishment-level risk management action in case MC is not met (process hygiene criteria), and associated reduction in target organism in product (directly or indirectly)</td>
<td>Quantitative impact of establishment-level corrective action. E.g., establishment whose product or environmental/process control samples have not met the MC undergoes in-depth cleaning known to bring the process variable within control, or to or target to a specified level, or yield a specified reduction.</td>
</tr>
<tr>
<td>Level of implementation of risk management actions</td>
<td>Proportion of establishments or lots where corrective action is implemented; may be by establishment category.</td>
</tr>
</tbody>
</table>
Table 3. U.S. Sources of *Salmonella* surveillance information.

<table>
<thead>
<tr>
<th>Surveillance Type</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Case-based surveillance</strong></td>
<td></td>
</tr>
<tr>
<td>National <em>Salmonella</em> Surveillance System</td>
<td>Case-based reporting from State and local health department epidemiology offices to CDC. This includes serotype for the great majority. Summarized in 2016 46,623 cases were reported (of which 39,980 were serotyped) (22)</td>
</tr>
<tr>
<td>PulseNet</td>
<td>Individual clinical isolates of <em>Salmonella</em> are sequenced in local and state public health laboratories and reported to CDC’s PulseNet database. They are summarized in BEAM Dashboard, starting with data from 2017. Between 2017 and 2019 a mean of 41,278 <em>Salmonella</em> isolates were reported to PulseNet (25). CDC and state health departments test human isolates, FSIS and FDA test non-human strains.</td>
</tr>
<tr>
<td>National Notifiable Disease Surveillance System (NNDSS)</td>
<td>This is information reported to CDC about diagnosed salmonellosis cases and other notifiable diseases that does not include serotype. It is summarized annually on the NNDSS website (29)</td>
</tr>
<tr>
<td><strong>Outbreak-based surveillance</strong></td>
<td></td>
</tr>
<tr>
<td>Foodborne Disease Outbreak Surveillance System (FDOSS)</td>
<td>Foodborne Disease Outbreak Surveillance System (FDOSS)(26), as reported to the National Outbreak Reporting System (NORS)(31) by local and state health departments and investigating offices at CDC. Summarized on NORS Dashboard website (31). Between 2016-2019, an annual mean of 143 foodborne salmonellosis outbreaks were reported.</td>
</tr>
<tr>
<td><strong>Specialized surveillance</strong></td>
<td></td>
</tr>
<tr>
<td>FoodNet</td>
<td>Part of the CDC’s Emerging Infections Network, and supported by FSIS and FDA, 10 state and large county group sites report all diagnosed infections of 7 types of bacteria, including salmonellosis, since 1996. Since 2014, a subset of patients has been interviewed about a standardized set of exposures (“Case Exposure Ascertainment”) and these data are linked with corresponding PulseNet, NARMS, and outbreak data. FoodNet data are summarized in annual and periodic publications and at FoodNet Fast (24). Between 2016 and 2019, a mean of 8472 salmonellosis cases were reported to FoodNet, of which 10% were travel-associated, 7% were outbreak associated, and 10% were diagnosed by culture-independent methods alone (24).</td>
</tr>
<tr>
<td>National Antimicrobial Resistance Monitoring System (NARMS)</td>
<td>A tri-agency collaboration since 1997. CDC measures the antimicrobial susceptibility of a sample of 1 out of 20 of <em>Salmonella</em> isolates from humans, referred by State public health labs. FDA tests retail samples of meat and poultry for <em>Salmonella</em> and other organisms, and FSIS tests samples gathered during inspection (poultry cecal samples since 2013). Human NARMS is summarized at the NARMS NOW website (23). Between 2016 and 2019, NARMS CDC tested an annual mean of 2543 <em>Salmonella</em> strains per year. Results for testing by FDA and FSIS are also available on respective websites (149, 166)</td>
</tr>
</tbody>
</table>
### Table 4. *Salmonella* Poultry Performance Standards (161)

<table>
<thead>
<tr>
<th>Product</th>
<th>Performance Standard*</th>
<th>Maximum Acceptable Percent Positive</th>
<th>Minimum Number of Samples to Assess Process Control**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Broiler Carcasses</td>
<td>5 of 51</td>
<td>9.8%</td>
<td>11</td>
</tr>
<tr>
<td>Turkey Carcasses</td>
<td>4 of 56</td>
<td>7.1%</td>
<td>14</td>
</tr>
<tr>
<td>Comminuted Chicken</td>
<td>13 of 52</td>
<td>25%</td>
<td>10</td>
</tr>
<tr>
<td>Comminuted Turkey</td>
<td>7 of 52</td>
<td>13.5%</td>
<td>10</td>
</tr>
<tr>
<td>Chicken Parts</td>
<td>8 of 52</td>
<td>15.4%</td>
<td>10</td>
</tr>
</tbody>
</table>

*The performance standard is represented as a fraction of maximum allowable positives over the target number of samples collected and analyzed in a 52-week window.

**FSIS must analyze at least this number of samples in a single 52-week window in order to categorize an establishment for the standard listed.
## Appendix A. Glossary.

<table>
<thead>
<tr>
<th>Term</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colony-forming Units (CFUs)</td>
<td>CFUs is an estimation of the number of viable microbial cells in a sample. CFUs are typically expressed as a rate per unit of volume or mass such as CFU/g or CFU/ml.</td>
</tr>
<tr>
<td>Fit-for-purpose</td>
<td>Demonstration (validation) that a specific method delivers expected results in a specific matrix or conditions, in relation to the purpose of the information/results.</td>
</tr>
<tr>
<td>Infectivity</td>
<td>The ability of an organism to cause infection. In risk assessments, this is incorporated as the probability of human infection following oral exposure to any amount of <em>Salmonella</em>. This probability can vary depending on pathogen factors such as the serovar or subtype, and host susceptibility.</td>
</tr>
<tr>
<td>Limit of Detection (LoD)</td>
<td>LoD is the lowest concentration of microbial cells that can be reliably detected using a standard test.</td>
</tr>
<tr>
<td>Limit of Quantification (LoQ)</td>
<td>LoQ is the lowest concentration of microbial cells that can be quantified based on predefined goals for of confidence in the estimation. LoQ is typically higher than the LoD as estimating a numerical value requires more information than requiring a positive/negative result.</td>
</tr>
<tr>
<td>Key Performance Indicators (KPI)</td>
<td>Critical (key) indicators of progress toward an intended result or goal. Per FSIS: “The KPI will measure the percent reduction in raw poultry samples contaminated with Salmonella serotypes commonly associated with human illness as a percentage of all samples analyzed for all types of Salmonella contamination. The samples considered in the KPI are those collected from products subject to a performance standard (i.e., from chicken parts, chicken and turkey carcasses, and comminuted chicken and turkey).”(165)</td>
</tr>
<tr>
<td>Mean Time Between Failure (MTBF)</td>
<td>Mean period of time between two “failures”, i.e., the inverse of the frequency of failure, where failure is defined as an episode of departure from a set target or standard (which may or may not have regulatory relevance).</td>
</tr>
<tr>
<td>Microbiological criteria (MC)</td>
<td>Also called performance standards, MC defines the acceptability of a product based on presence (absence) or number of microorganisms per unit of the product. It can also be based on a biomarker of the microorganism, such as toxins, metabolites, or genetic components. The product unit can be defined as a lot, volume, mass, or area.</td>
</tr>
<tr>
<td>Omics</td>
<td>Characterization and quantification of pools of biological molecules that translate into the structure, function, and dynamics of an organism or organisms. Includes disciplines such as genomics, proteomics, metabolomics, metagenomics, phenomics and transcriptomics.</td>
</tr>
<tr>
<td>Pathogenicity</td>
<td>The ability of an organism to cause disease. In risk assessments, this is usually modeled as the probability of clinical disease given infection. This probability</td>
</tr>
<tr>
<td>Term</td>
<td>Definition</td>
</tr>
<tr>
<td>------</td>
<td>------------</td>
</tr>
<tr>
<td>can vary depending on pathogen factors such as the serovar or subtype, and host susceptibility</td>
<td><strong>Performance standards</strong></td>
</tr>
<tr>
<td>An AOAC program that provides a third-party review and certification for proprietary test method performance</td>
<td><strong>Performance Tested Methods (PTM)</strong></td>
</tr>
<tr>
<td>Quantitative microbial risk assessment (QMRA)</td>
<td>Quantitative microbial risk assessment is a mathematical modeling approach used to estimate the risk of infection and/or illness when a population is exposed to microorganisms from a variety of sources, in this case in foods. QMRA estimates can be used to predict the potential reduction (increase) in foodborne illnesses resulting from the implementation of strategies to mitigate foodborne pathogens in foods.</td>
</tr>
<tr>
<td>The term serovar is used to distinguish groups within a <em>Salmonella</em> species that share distinctive surface structures, namely the O surface antigen and the H antigen that is part of the flagella (7). Consequently, serovars represent phenotypical differences between individual bacteria belonging to a <em>Salmonella</em> species, and do not necessarily represent evolutionary differences as elucidated in the <em>Salmonella</em> genome. Note that in this report, the term serovar and serotype are used interchangeably.</td>
<td><strong>Serovar</strong></td>
</tr>
<tr>
<td>The ability of an organism to cause severe illness. In risk assessments, this is usually modeled as the probability of severe illness given infection. Virulence in bacteria is mediated by genes often called “virulence factors”. Both pathogen and host factors contribute to whether disease occurs and to disease severity</td>
<td><strong>Virulence</strong></td>
</tr>
</tbody>
</table>
Appendix B. Supporting resources for Questions 1-7

Figure 1. Estimated percentage of foodborne Salmonella illnesses (with 90% credibility intervals) for 2019, in descending order, attributed to each of 17 food categories, based on multi-year outbreak data,* United States (76).

*Based on a model using outbreak data that gives equal weight to each of the most recent five years of data (2015-2019) and exponentially less weight to each earlier year (1998-2014).

Over 75% of illnesses were attributed to seven food categories: Chicken, Fruits, Pork, Seeded Vegetables (such as tomatoes), Other Produce (such as fungi, herbs, nuts and root vegetables), Turkey and Eggs.

The credibility intervals for each of the seven food categories that account for 75.9% of all illnesses overlap with some of the others.
Figure 2. Trends in salmonellosis incidence in the U.S., as detected by culture-independent diagnostic test (CIDT) only vs. CIDT plus culture confirmation. (24)

*Salmonella* infections by year; 1996-2021

Incidence per 100,000 population – FoodNet sites: all test methods

* Culture-confirmed includes those infections confirmed by culture only or by culture following a positive CIDT.

Source: FoodNet, Centers for Disease Control and Prevention
Figure 3. Trends in the reporting of incidents of Salmonella enterica in chickens in Great Britain versus laboratory reporting of human S. enterica serovar Enteritidis infection, England and Wales, 1985–2011, S. enterica serovar Enteritidis phage type 4 (SE4). Figure shows the most recent trends from the UK, showing a systematic decline in S. Enteritidis post intervention (86)
Figure 4. Trends in salmonellosis incidence associated with S. Typhimurium and S. Heidelberg in the U.S. Salmonella infections by year: 1966-2019. Incidence per 100,000 population – FoodNet sites; cultured confirmed (27).
**Figure 5.** Commercial Broiler Production in the USA. Assumption 1M birds/week complex (many complexes are 2.5 – 4 x larger)

Diagram Courtesy S. Stillwell, 2022
**Figure 6.** *Salmonella* survival through broiler production (133).
Figure 7. Evaluation of the incidence of *Salmonella* infection in poultry populations and the number of reported human cases of salmonellosis. (54). Figure shows recent trends in EU member states, highlighting no clear decline in salmonellosis post intervention. The intervention implemented in 2011 consists of MC prescribing absence of *S. Typhimurium* or *Enteritidis* in 25g fresh poultry meat, and for all *Salmonella* in poultry meat preparations (intended to be eaten cooked).
Q2-Case Study: Sweden’s Salmonella Control Program and comparison with US production practices

The Swedish Board of Agriculture (SBA) began a Salmonella control program for poultry in 1970 whereby broiler producers participating in the program were required to meet SBA’s requirements and agree to governmental monitoring of all aspects of broiler production. All flocks that tested positive for Salmonella are destroyed and houses are remediated. For those broiler producers participating in SBA’s initial program, the government paid the costs related to destroying the infected flock(s). The concept of control in the Sweden program is that if broilers are never exposed to Salmonella, then they cannot become colonized and subsequently they will not be contaminated after processing. Therefore, no chemical treatments are used in the processing plant due to excluding Salmonella positive birds from entering the plant.

In 1984, Sweden made Salmonella testing compulsory 10-14 days before slaughter, using a boot-sock method. The government also stopped payments to broiler producers for infected flocks, with private insurers paying 90% of the losses if the producer was a participant in Sweden’s SBA Salmonella control program. As a result of the on-farm Salmonella control program, the European Food Safety Authority reported in 2010 that less than 0.1% meat samples collect at the slaughterhouse or cutting plant were positive for Salmonella (62). The latest report also shows very low prevalence (0.27% in chicken meat at production stage; 0% in turkey meat at production stage) [REF]. It should be noted, in contrast with other programs, that vaccines are not used in Sweden.

Aside from the mandatory aspect of testing and destroying positive flocks, other aspects of Sweden’s control program for Salmonella free broiler production are used in the U.S. to reduce Salmonella in poultry, poultry feed and the poultry environment, but on a voluntary basis. The programs do not specifically address Campylobacter, which remains a predominant foodborne bacterial pathogen in the EU and the US.

The Sweden case study is also useful to illustrate not only options and potential impact of MC and multiple-barrier Salmonella control measures, but also, adopting a food system approach, the crucial role of a strong enabling environment (e.g., insurance, government program, etc.) to support a feasible and sustainable implementation. While the poultry industry in Sweden is quite different from that in the US (e.g., in the number of establishments defined as “very large” according to US standards), the US also has a strong enabling environment that could be leveraged.

Sweden’s Five Principles of Salmonella-Free Broiler Production (90)

1) Start with Salmonella-free day-old chickens
2) Rear chicks in a Salmonella-free environment
3) Provide feed and water that is free from Salmonella
4) Regularly monitor and test for Salmonella in the whole production chain
5) Take immediate action whenever Salmonella is detected
To implement these principles, Sweden adopted the following practices and regulations (122):

- All imported birds are quarantined until it is determined that they are *Salmonella* free.
- All feed is tested for *Salmonella* and has strict regulations regarding fertilizing, harvesting, transportation and storage.
- All breeder and broiler houses must be free of rodents and wild birds, and the houses must have an “ante room” for personal hygiene control -- some ante rooms include showers.
- Most of Sweden broiler houses have cement floors, not dirt floors, to improve cleaning and sanitation.
- When a flock is moved out of a house, all the litter must be removed within 24 hours. The broiler house must then be cleaned and sanitized and must sit idle for a minimum of 2 weeks.
- If *Salmonella* is found, the flock is destroyed, and all the litter is removed and composted for at least 6 months to prevent contamination of the surrounding environment(s).
- Twice a year, a state veterinarian visits each facility and takes environmental samples to test for *Salmonella*. Broiler houses and feed mills must be *Salmonella* free to operate.
- Sweden also requires “Neck Skin” samples to be collected twice a day every day (after carcass air chilling) in broiler slaughterhouses.
- When *Salmonella* is detected, a veterinarian is appointed by the SBA to investigate.
- Whenever *Salmonella* is verified, regardless of prevalence or serotype, the flock is destroyed.

**Benefits/Cost Analysis (BCA)**

In Sweden, a BCA compared Sweden’s *Salmonella* regulations to the less-rigorous controls in Denmark and the Netherlands. Sundstrom et al. (139) found the expected increase in human salmonellosis cases and the associated increase in reactive arthritis and irritable bowel syndrome (IBS) to be more costly than any reduction in *Salmonella*-control costs in those countries. Evaluating the costs and benefits of *Salmonella*-free broilers in the United States involves tallying up the externalities that broiler production currently borne by the U.S. public. Externalities are defined as costs such as the acute and long-term health costs, productivity losses, and pain and suffering due to illness from eating or working with *Salmonella*-contaminated foods. Scharff (131) estimates that salmonellosis costs for the U.S. are $5-16 billion annually, with poultry estimated to be responsible for 23% of salmonellosis cases (115).

In addition to medical costs and loss of life, *Salmonella* in poultry contributes to environmental losses and potential of spread of antibiotic resistant *Salmonella* strains. For example, the nutrient-rich runoff from broiler facilities has caused algae blooms and “dead zones” in U.S. bays and oceans – the Chesapeake Bay contamination was extremely notable in causing losses to both the fishing and crabbing industries (55). Another concern is the spread of multi-drug resistant bacteria. The impact of adding antibiotics to animal feed can be significant. -- For example, Ambruster and Roberts (2018) suggest that 60-80% of tetracycline used in food animals is excreted in feces or urine, ending in animal waste, which in turn can be applied as a farm fertilizer and lead to the development of more antibiotic resistant bacterial strains (6).
### Table 5 Comparison of Broiler Production in Sweden and the United States

<table>
<thead>
<tr>
<th>Feature</th>
<th>Sweden (90)</th>
<th>United States (91)</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genetics</td>
<td>Ross &amp; Cobb 99% (fast)</td>
<td>Fast growing birds</td>
<td>Sweden harvests birds earlier.</td>
</tr>
<tr>
<td></td>
<td>4.3 lbs. in 33 days</td>
<td>6.13 lbs. in 49.5 days</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Feed Conversion=1.6</td>
<td>Feed Conversion=1.9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>7 grow outs/year</td>
<td>5.5 grow outs/yr.</td>
<td></td>
</tr>
<tr>
<td>Grow out house size SIMILAR</td>
<td>Aver. size 15,780 sq. ft.</td>
<td>Aver. size 18,618 sq. ft.</td>
<td>US slaughters 162 million broilers/week vs. Sweden 90 million/yr.</td>
</tr>
<tr>
<td></td>
<td>Aver. 4.4 houses/farm</td>
<td>Aver. 4 houses/farm</td>
<td>ERS estimates of 33 billion pounds of chicken produced in the United States in 2016 (70)</td>
</tr>
<tr>
<td></td>
<td>100 broiler growers</td>
<td>233,770 poultry farms including broilers, eggs, and turkeys (102)</td>
<td></td>
</tr>
<tr>
<td>Housing &amp; cleanout between flocks DIFFERS</td>
<td>Enclosed with sanitary perimeters, as hygiene barrier, with change of footwear and coveralls. 100% total cleanout and sanitation between flocks</td>
<td>Enclosed, fan ventilation 77% not cleaned out &amp; sanitized between flocks, 56% no change of clothing, 17 days between flocks</td>
<td>US has no Salmonella control regulations in housing design or cleanout regulations between flocks.</td>
</tr>
<tr>
<td>Litter disposal DIFFERS</td>
<td>All litter removed between flocks &amp; sold as fertilizer to farmers. But if test Salmonella positive, on- farm composting of litter.</td>
<td>33% of litter sold for use by other farms in 2011</td>
<td>US has no Salmonella requirements for litter. US contamination of soil and water with excess nutrients and pathogens (55)</td>
</tr>
<tr>
<td>Feed DIFFERS</td>
<td>Farm-grown wheat. Purchased feed must be S- Fishmeal is not allowed in feed.</td>
<td>Provided by contractor- mostly corn &amp; soybeans. No regulations prohibiting Salmonella in feed.</td>
<td>US has no regulations on Salmonella in feed. Sweden allows no fishmeal in feed because of sustainability concerns.</td>
</tr>
<tr>
<td>Antibiotic use in feed DIFFERS</td>
<td>Not allowed.</td>
<td>Antibiotics important in human health being phased out of animal feed by FDA.</td>
<td>US antibiotics used on-farm add to antibiotic resistance of human pathogens.</td>
</tr>
<tr>
<td>Feature</td>
<td>Sweden (90)</td>
<td>United States (91)</td>
<td>Comments</td>
</tr>
<tr>
<td>--------------------------</td>
<td>--------------------------------------------------</td>
<td>-------------------------------------------------------------------------------------</td>
<td>----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Vaccination</td>
<td>Not used</td>
<td>Vaccinations against select serogroups used by more than 90% of broiler houses (98)</td>
<td>Vaccine development lags behind shifting of predominant serotypes found in US broiler populations.</td>
</tr>
<tr>
<td></td>
<td>DIFFERS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>On-farm Salmonella tests</td>
<td>Negative Salmonella test in GP stock, hatchery, breeders, feed. Flock tested before slaughter: must be S- to get into slaughterhouse</td>
<td>No on-farm regulations for Salmonella control</td>
<td>US has no on-farm regulations for Salmonella control and no on-farm tests required but conducted on a voluntary basis. On-farm control highest likelihood of controlling Salmonella (117)</td>
</tr>
<tr>
<td></td>
<td>DIFFERS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Slaughterhouse Salmonella tests</td>
<td>Neck Skin tests daily for Salmonella, gas kill, blast air chill, no chlorine or other chemicals allowed. If test is S+, flock is depopulated.</td>
<td>Performance standard allows 10% Salmonella rate in whole carcass rinse test (161).</td>
<td>US does not test daily; allows 10% Salmonella-positive tests (161). Chlorine, water absorption &amp; US kill method has potential to reduce flavor &amp; tenderness (Smart Chicken organizational communication at <a href="https://www.smartchicken.com/ourstory">https://www.smartchicken.com/ourstory</a>)</td>
</tr>
</tbody>
</table>
### Table 6. Summary of Approved Methods for Quantitation of *Salmonella* in Poultry Products (see individual supplier websites for updates)

<table>
<thead>
<tr>
<th></th>
<th>bioMérieux</th>
<th>Hygiena</th>
</tr>
</thead>
<tbody>
<tr>
<td>Product Name</td>
<td>GENE-UP® QUANT Salmonella</td>
<td>SalQuant™</td>
</tr>
<tr>
<td>Technology/ platform</td>
<td>qPCR Non-enrichment concentration</td>
<td>qPCR Enrichment</td>
</tr>
</tbody>
</table>
| Approvals                 | AOAC Research Institute (RI) Performance Tested Method (PTM) #061801  
GENE-UP QUANT Salmonella is an application of the EnviroPro Sal (PTM #061801) (5) | AOAC RI PTM #081201, (3)  
Official Methods of Analysis submitted in October 2022 (5) |
| Applicable matrices and test portion sizes | AOAC (5)  
chicken carcass rinsates (40mls) (163)  
raw ground turkey (100g)  
Microtally® (40mls taken from 200mls enrichment volume)  
Internal studies: boot swabs, poultry organs, intestinal tract (bioMérieux, personal communication) | Validated matrix extensions at AOAC in 2021 – comminuted chicken (325g) and Turkey (325g) (3)  
Validated matrix extensions at AOAC in 2022 – poultry rinsates (30ml) (3)  
Internal studies: Boot Swabs, Dust swabs, Feet Swabs, Cloacal Swabs, Poultry Pads (cardboard and Straw), Feed, Trailer Drag Swabs, Cecal Tonsils, Crop, Lungs, Liver, Spleen, Low Level Rinsates, Chicken Pieces, Poultry Carcass Swabs, Breaded Stuffed Raw Chicken Products |
<p>| Time to result (TTR)     | TTR = &lt;4h                                       | TTR = 7.5 – 9.5h                             |
| LOQ Limit of Quantification | Non enrichment: 10 CFU/ g or ml (AOAC PTM study 99 CFU/g or mL) | 1 CFU/mL 6h rinsate enrichment (AOAC PTM study results present as low as 10 CFU/g or mL) |</p>
<table>
<thead>
<tr>
<th></th>
<th>bioMérieux</th>
<th>Hygiena</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Claim (actual validation study)</strong></td>
<td>1 CFU/g 8h comminuted ground enrichment (AOAC PTM study results present as low as 10 CFU/g or mL)</td>
<td>1-10,000 CFU/g or mL</td>
</tr>
<tr>
<td><strong>Range of quantification</strong></td>
<td>10 – 10,000,000 CFU/g or ml</td>
<td>10-10,000 CFU/g or ml</td>
</tr>
<tr>
<td><strong>Accuracy</strong></td>
<td>Within 0.5 log CFU when compared to MPN result</td>
<td>Within 0.5 log CFU when compared to MPN result</td>
</tr>
<tr>
<td><strong>References</strong></td>
<td>User guide available from vendor upon request AOAC RI # 061801 (5)</td>
<td>User guide <a href="https://cdn.brandfolder.io/KA71VJV5/at/gxkwprp5rs9n8kmwhsr5g5/BAX-System-Q7-Users-Guide.pdf">link</a> AOAC RI #081201 (3) Quantification Guidebook Poster Presentation (138, 171)</td>
</tr>
<tr>
<td><strong>Advantages</strong></td>
<td>Non enrichment reduces bias by eliminating culture-based enrichment and concentrating cells initially and provides faster time to result &lt;4h same shift procedure Universal workflow and data analysis algorithm Proven compatibility with primary production samples (on farm, boot, internal work)</td>
<td>Universal workflow is utilized with RT <em>Salmonella</em> Assay that was AOAC Official Method of analysis (OMA) approved in 2012 (170) <a href="https://cdn.brandfolder.io/KA71VJV5/at/v5rkgjq8cbsqhk3s8hqk5n/ins-bax-q7-assay-salmonella-rt.pdf">link</a> Prevalence sample and quantitative sample come from same sample enrichment. The quantitative sample has a shorter enrichment time and keeps incubating for the prevalence sample at a later time Large dynamic range (1-10,000 CFU/g or mL) or 5 log range Regulatory sample size validated</td>
</tr>
<tr>
<td><strong>Limitations</strong></td>
<td>Procedure not the same as the standard qualitative method for presence / absence Sample prep method requires centrifugation Data to show capability distinguishing live versus dead cells to be provided</td>
<td>Individual curve per matrix requires validation when adding a new matrix (i.e., there are 20 curves today) Culture based bias from the impact of natural flora and determination of lag and log phase for each strain.</td>
</tr>
</tbody>
</table>
**Table 7. Examples of Methods in Development for Quantification of *Salmonella* (check individual supplier websites for updates on validation and approvals)**

<table>
<thead>
<tr>
<th>Website for updated information</th>
<th>Bio-Rad</th>
<th>Bio-Rad</th>
<th>Hygiena</th>
<th>Neogen (Legacy 3M)</th>
<th>Neogen</th>
<th>Thermo Fisher</th>
</tr>
</thead>
<tbody>
<tr>
<td>Product Name</td>
<td>dd-Check Salmonella*</td>
<td>iQ-Check Salmonella</td>
<td>SalLimits*</td>
<td>3M™ Molecular Detection Assay 2-Salmonella Quantification*</td>
<td>CASE 2 <em>Salmonella</em> Species, Typhimurium and Enteritidis Multiplex PCR Assay*</td>
<td></td>
</tr>
<tr>
<td>Technology/platform</td>
<td>ddPCR</td>
<td>qPCR</td>
<td>RT PCR threshold testing (positive or negative at 10 cells)</td>
<td>Loop-mediated isothermal amplification (LAMP) &amp; bioluminescence, along with cloud-based software</td>
<td>Chromogenic Agar Plate for rapid detection and enumeration of <em>Salmonella</em></td>
<td>qPCR</td>
</tr>
<tr>
<td>Approvals</td>
<td>Internal studies supporting the use of the method</td>
<td>Internal studies supporting the use of the method AOAC 081904 (4)</td>
<td>Internal studies supporting the use of the method</td>
<td>In development</td>
<td>In development</td>
<td>Internal studies supporting and submission for approval at AOAC in process as of October 2022</td>
</tr>
<tr>
<td>Bio-Rad</td>
<td>Bio-Rad</td>
<td>Hygiena</td>
<td>Neogen (Legacy 3M)</td>
<td>Neogen</td>
<td>Thermo Fisher</td>
<td></td>
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<td>--------------------</td>
<td>--------</td>
<td>---------------</td>
<td></td>
</tr>
<tr>
<td>Applicable matrices</td>
<td>Internal work by collaborating with outside service laboratory and poultry producers:</td>
<td>Internal work by collaborating with outside service laboratory and poultry producers:</td>
<td>Internal studies by collaborating with outside service laboratory and poultry producers:</td>
<td>Internal data: poultry rinse (30mL) raw poultry (325g) primary production (i.e., boot swabs/socks, others)</td>
<td>Product in development and studied in Europe</td>
<td></td>
</tr>
<tr>
<td>Poultry rinsates (30mL) Ground poultry Microtally®</td>
<td>Ground turkey (325g) Poultry rinsate (30mL)</td>
<td>Comminuted chicken (325g) Turkey (325g) Poultry rinsates (30ml)</td>
<td>raw poultry (325g) primary production (i.e., boot swabs/socks, others)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time to result (TTR)</td>
<td>TTR = 5.5-6 h</td>
<td>TTR = 4.5-5 h</td>
<td>TTR = 4-8h at LOD10</td>
<td>Total = 9.5-10h</td>
<td>16-18h incubation TTR = 16 – 18h</td>
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<tr>
<td>LOQ</td>
<td>Poultry Rinse 1 CFU/mL</td>
<td>Table showing in Bio-Rad’s technical note (need reference) based on strain/serotype and matrix 10 CFU/g</td>
<td>LOD10 based on threshold 4h rinsate enrichment 6h comminuted ground enrichment *validated at LOD1 for 6h and 8h, respectively</td>
<td>At least as low as currently commercialized methods. LOQ may vary by matrix. LOQ for poultry rinse: 10 CFU per original 30 mL rinse sample</td>
<td>The LOQs for finished goods: 1 CFU per 30 mL rinse from chicken parts or carcasses; 1 CFU per 30 mL homogenized ground meat sample, including turkey, pork and beef. Environmental 30 CFU per 30 mL</td>
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<td><strong>Bio-Rad</strong></td>
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<td><strong>Range of quantification</strong></td>
<td>$1 - 1,000,000$ CFU/mL</td>
<td>$1$ CFU/mL or $10$ CFU/mL limit</td>
<td>Threshold above $10$ CFU/g (mL) ... qual result $&lt;10$ = negative at this LOD $&gt;10$ = positive</td>
<td>$10$-1000 CFU / 30 mL</td>
<td>$10 &gt; 10,000,000$ CFU/g (dependent upon number of dilutions performed)</td>
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<td><strong>Accuracy</strong></td>
<td>2-fold difference (personal communication, BioRad)</td>
<td>Tech note</td>
<td>within $0.5$ log CFU (est.) compared to MPN result</td>
<td>To be determined in development</td>
<td>Within 1 log $99.8%$ agreement Within 0.5 log $80%$ agreement</td>
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<tr>
<td><strong>References</strong></td>
<td><a href="https://www.bio-rad.com/en-us/category/digital-pcr-food-applications?ID=PU4PWZRT8IG9">https://www.bio-rad.com/en-us/category/digital-pcr-food-applications?ID=PU4PWZRT8IG9</a></td>
<td>User guide upon request Technical note and internal research upon request</td>
<td>User guide Quantification Guidebook Peer reviewed publications available from Hygiena</td>
<td>Available at commercialization</td>
<td>Available at commercialization Instruction-For-Use AOAC-PTM study available Aug/Sept Video on the software use and results IAFP Posters 2022</td>
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</table>
| **Advantages** | Non enrichment reduces bias by eliminating culture-based enrichment and partitioning of intact bacterium cells initially and provides faster time to result | Universal workflow with same *Salmonella* assay Extensive research with a variety of strains addressing growth rate bias | Universal workflow with RT *Salmonella* Assay Shorter enrichment time compared to SalQuant (faster) | Universal workflow with same *Salmonella* assay Technology utilized is recognized and utilized by USDA as in the MLG 4.12 | -Easy traceability -Cost and time saving -RTU, DCM, RTR (µPrep) and Remelt formats -BPW, MRD or equivalent diluent validated -Enumeration of *Salmonella* spp. Simultaneous detection, quantitation and differentiation of the three target analytes, i.e., *Salmonella* spp., *S. Enteritidis*, *S. Typhimurium* Universal workflow with same *Salmonella*
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<tr>
<td><strong>Limitations</strong></td>
<td>Working on improvement of the throughput allowing more samples to be analyzed</td>
<td>Individual curve per matrix requiring validation when adding a new matrix</td>
<td>Look at SalQuant list and use related</td>
<td>Implementation process to verify method and application as expected (i.e., impact)</td>
<td>Might require dilutions in case of high level of Enterobacteriaceae contamination</td>
<td>Defining finished product vs environment samples in the process (working with clients)</td>
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<td>End point PCR when the droplet lights up (positive)</td>
<td>Sample sizes up to 375g including regulatory sizes</td>
<td>Regulatory sample size validated</td>
<td>Shorter enrichment time compared to qualitative assay;</td>
<td>-Easy confirmation using latex, MALDI or molecular assay from an isolated colony</td>
<td>assay with non-proprietary media that runs with the Applied Biosystems QuantStudio 5, maximizing multiplexing</td>
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<td>Shortened time to result 4.5 – 5h total</td>
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<td>Can maintain enrichments for prevalence</td>
<td>-No CAPEX needed</td>
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<td>Same as qualitative</td>
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<td>Able to detect low values at 8h enrichment</td>
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<td>Hands-on technician time not optimized and making some improvements with the filter and centrifuge steps. Additional instrumentation needed as compared to qPCR.</td>
<td>Impact of flora due to enrichment to be considered.</td>
<td>of background flora, degree of injury. Enrichment even 8h is over a shift (tight schedule). US focused. Offered in Q3 (to be determined).</td>
<td>Time to result is longer than other methods in the market. Sensitivity and potential injured cell quantification. Expected to be commercialized in 2023.</td>
<td>on this and knowing the difference for the different al) Q5 new (talk about 7500 being in many labs now). Not universal – is this possible for any method with enrichment?</td>
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2830 *include emerging technologies*