MASSEY UNIVERSITY

SCHOOL OF MATHEMATICAL AND COMPUTER SCIENCES

# Contributions to Food Safety Acceptance Sampling Plans

*Author:*

T. Mayooran

*Supervisors:*

Dr K. Govindaraju

Prof Mark Bebbington

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# Abstract

An appropriate sampling inspection method is an essential tool for risk assessment in the food industry. A representative sampling approach will be helpful to reduce risk while minimising the sampling costs. Consequently, food manufacturers are employing efficient sampling approaches to assure food safety. In the food safety field, microbiological or other contamination often spreads unevenly across the production. Many factors are involved in the microbial risk assessment, such as (1) the amount of sample used for inspection, (2) what sampling methods were applied, (3) laboratory testing procedures, (4) physical sampling of materials from lots/batches of products and (5) the mixing of initially collected samples. This study focusses on improved sampling inspection approaches to reduce microbiological risk in food products.

Part of this research also included developing open-source R packages to generate graphical displays for probabilistic risk assessment for practitioners. A single "wrapper" package is also provided to install all the newly developed packages in a single step.

**Keywords:** grab sampling, auto-sampler, presence-absence tests, microbiological risk, OC curve, Poisson mixture distributions, dilution, plate counting, beta binomial, truncated distributions, risk control

# Declaration

I affirm that all contents of this dissertation are original, unless otherwise specified through specific reference to the work of others. Furthermore, this dissertation is solely my own work and has not been submitted in full or in part for any other degree or qualification at this or any other university. I declare that no part of this work was produced in collaboration with others, except as explicitly acknowledged within the text and Acknowledgements section. Finally, the dissertation consists of fewer than 65,000 words, which includes appendices, bibliography, footnotes, tables, and equations, and has fewer than 150 figures.

Mayooran Thevaraja

April, 2023

# Acknowledgements

After finishing my Master's degree, I made the bold decision to relocate from the United States to New Zealand to pursue my PhD. Some might have deemed it a drastic and risky choice, but now that my PhD journey has culminated, I can unequivocally state that it was the best decision I could have made. Although it was a challenging experience, completing my PhD thesis proved to be incredibly fulfilling, and I attribute much of my success to the unwavering support and guidance of my supervisors.

I owe a debt of gratitude to my primary supervisor, Dr K. Govindaraju, and co-supervisor, Prof. Mark Bebbington, for their unwavering academic, social, and emotional support throughout my journey towards finishing my PhD. It is with utmost confidence that I can attest to the pivotal role that Raj and Mark have played in shaping my research career.

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**Declaration**

This thesis complies with the 'Guidelines for Doctoral Thesis by Publications' and with the requirements from the Handbook for Doctoral Study by the Doctoral Research Committee (DRC), Massey University. January 2011. Version 7.

**Disclaimer**

The opinions, findings and conclusions in this thesis are solely those of the author(s). Under no circumstances will the author(s) be responsible for any loss or damage of any kind resulted from the use of these techniques. The software codes and the apps produced by this research are licensed under GPL 2.0 and it comes without warranty of any kind.

# Chapter 1

# Introduction

This chapter aims to describe the influence of sampling inspection methods in the field of food safety. The second aim is to establish a basis for the research that follows. An overview of the research questions and objectives is provided in Section 1.2.

## 1.1 Motivation

Food contamination is at best unpleasant, but at its worst, can be deadly. Chemicals such as melamine, or microbial organisms such as *Escherichia coli*, *Campylobacter* spp., *Listeria monocytogenes* and *Salmonella* spp., can contaminate food, and contamination can occur at any time in the food production process, i.e., at any point in the farm-to-fork supply chain. South Africa experienced the largest ever recorded outbreak of *Listeria monocytogenes* from contaminated deli meats, with almost 1,060 confirmed cases and 216 deaths between 1 January 2017 and 26 July 2018; see NICD (2018). A report from the World Bank stated that the impacts of contaminated food cost low and middle income countries approximately USD110 billion per annum in productivity losses and medical expenses (Jaffee et al., 2018). According to the current fact sheet on food safety published by the World Health Organization (WHO), worldwide, one in ten people fall sick every year because of contaminated food, and 420, 000 people die each year, resulting in the loss of 33 million healthy life-years worldwide; see AL-Mohaithef (2021).

Current study focuses on microbial food contamination because its impacts are so devastating. For example, two out of five food-borne microbial diseases are carried by children under five years of age, and 125,000 children in this age group die from food poisoning every year; see Gizaw (2019) and Lang and Sant'Ana (2021). Clearly, food production processes must be robust enough to prevent, or at least control, microbial contamination to ensure food safety.

Over the years, the International Commission on Microbiological Specifications for Foods (ICMSF) has published many recommendations, guidelines and tools for microbiological sampling of food (ICMSF, 1986, 2002, 2009, 2011). Similar guidelines, policies, recommendations and standards related to food safety, particularly the use of sampling inspection plans in the food trade, have been provided by the Codex Alimentarius (CAC, 1997, 2004). The Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) regularly promote joint meetings and publish recommended sampling plans for various micro-organisms of interest (FAO/WHO, 2016).

However, due to the high cost of microbiological sampling inspection, food manufacturers continually call for sampling efficiency over complex, sometimes inscrutable methodologies (Phil et al., 2019), and this problem of translating guidelines into practical methodologies influenced the research work presented in this thesis.

## 1.2 Research Questions and Objectives

The most challenging problem in the food safety field is that current food safety sampling protocols do not comprehensively address the effect of variation between samples. When compositing primary increments as a representative analytical sample, variation between primary increments must be deliberated. Consequently, variations that inevitably occur in a batch impact on all associated statistical measures and subsequent sampling procedures. It is common for a small portion of a batch to be contaminated with microbes while most portions are not.

Clearly, the effect of how test samples are prepared needs to be considered for risk assessment. Therefore, the first part of this research is designed to answer the following research questions:

1. What are the limitations of current sampling protocols used in managing the safety of a food production process?

2. Do we need additional sampling to detect contamination when grab samples are collected from the production process, i.e., does grab sampling elevate the risk of non-detection?

3. To detect microorganisms, how exactly the analytical sample must be prepared from a mix of primary samples ?

4. To test *Salmonella* spp. in powdered products, companies commonly use 1,500g sub samples taken from the daily composite sample, which is approximately 7.5 kg. Is this method representative of the whole batch?

5. Is plate count-based testing an efficient way to assess risk when microorganisms un-evenly contaminate a batch?

6. Based on current regulatory guidance, how does the sample quantity used to prepare the diluted solution impact microbial detection?

In answering the questions above, this research explores how sampling inspection methods can be modified to control microbial contaminants, also known as hazards, in the production process. In particular, the current work examines the use of practical sampling procedures to help food manufacturers and importers reduce their sampling inspection costs, and to provide higher protection to consumers.

The eventual objective is to offer the food industry a series of food safety sampling plan templates with better performance than those currently available. The steps to reach this objective are:

1. To investigate the effect of grab sampling on detection tests undertaken in powdered food products (based on the two-state Markov chain sampling model for serial correlation).

2. To investigate the effect of perfect vs. imperfect mixing of primary samples in the preparation of analytical samples.

3. To investigate the effect of the quantity of material sampled on non-detection.

4. To study the probability distribution-based modelling of plate counts in dilution testing.

5. To investigate the effect of the diluted sample quantities on risk assessments.

6. To develop an open-source R package based on the results of this research for use by food safety practitioners.

## 1.3 Thesis Structure

This thesis has seven additional Chapters. Chapter 2 provides a critical review of the literature related to this topic and explains how the research fills the gaps identified. Chapter 3 studies the efficacy of model based sampling inspection methods on microbial detection in powdered products. Next, in Chapter 4, the effectiveness of mixing primary samples of powdered products is discussed. Chapter 5 investigates how the quantity of material sampled impacts risk assessment, and Chapter 6 deals with the risk assessment of plate counts in dilution testing. Chapter 7 addresses the impact of diluted sample quantities on risk assessments. Finally, conclusions and future research recommendations are proposed in Chapter 8.

Part of this research involved developing four open-source R packages, each associated with a specific research problem. All four packages are wrapped up as a single package, called "*mRatool*", which contains four individual R packages ( "*grabsampling*", "*mixingsimulations*", "*uneqmixr*" and "*dilutionrisk*") to construct graphical displays of probabilistic risk measures for microbiological safety. A full description of our open-source *R* package is available at `https://mayooran1987.github.io/mRatools`.

Each R package is equipped with individual web pages dedicated to the maintenance of help files for every function associated with the package. These help files offer thorough and comprehensive documentation for each function, simplifying the process for users to

grasp and employ these functions effectively in the context of each chapter. Consequently, readers can readily access extensive information regarding the primary functions of the R package from its documentation, guaranteeing they possess the essential resources needed to apply these functions in their work. In this thesis, a variety of algorithms are employed for simulations presented in each chapter. Detailed information about these algorithms is available in the help section of the corresponding package.

# Chapter 2

# Literature Review

This chapter presents an overview of acceptance sampling inspection methods. Also included is a critical review of related research, i.e., microbiological sampling inspection methods for food safety. Furthermore, this chapter explains the gaps in some of the popular food safety sampling protocols.

## 2.1 Background of Acceptance Sampling

Acceptance sampling was popularised by Dodge and Romig, who were veterans of the Bell Laboratories quality assurance department. Initially, the U.S. military employed acceptance sampling to examine bullets during World War II (Littauer, 1950). Later, Wald (1945) published the comprehensive principles of sequential sampling, and Wallis (1947) subsequently introduced an approach for creating a variables inspection plan for lot disposition. These significant contributions were milestones in the development of acceptance sampling theory. Thereafter, the field of study grew apace, and a considerable amount of literature has since been published in this field.

Acceptance sampling is "the middle of the street" strategy between no inspection and 100% inspection; see Montgomery (2020). In the quality control field, a specified quantity of manufactured homogeneous product is defined as a "lot" or "batch". Generally, samples are drawn from a lot, and then, a decision on whether to accept or reject the lot is made based on the sample(s). If testing is destructive, or the cost of 100% inspection is very high,

acceptance sampling is used to decide whether or not the lot is acceptable, but the primary objective is not to estimate the quality of the lot.

There are various ways to classify acceptance sampling plans. Sampling plans are usually classified according to how the inspection process is carried out, such as single sampling, double sampling, multiple sampling or sequential sampling. In food safety, two significant classifications of acceptance sampling plans are recommended by the ICMSF (2009), which are plans for attributes (presence/absence or multi-class) or plans for variables. As suggested by Schilling and Neubauer (2010), a mixed plan is a more robust sampling plan because it is a combination of the two types.

The type of sampling plan is chosen according to the microbiological criteria, and the choice depends on the type of microorganism and the type of data; see FDA (1998) and Institute of Medicine and National Research Council (1985). Attribute acceptance sampling plans are used to evaluate qualitative data (presence/absence) or quantitative data that have been grouped (good/marginal/bad). Variables acceptance plans evaluate ungrouped quantitative data assuming a probability distribution for microbial data. Variables acceptance plans are less popular due to qualitative (presence/absence) testing procedures employed in the food industry (Ross et al., 2011). On the other hand, attribute acceptance plans are more popular for food safety assessment criteria, such as the presence of *Salmonella* spp. in 30 samples of 25$g$ milk powder, or a limit of 100 colony forming units (CFUs) of *Cronobacter* spp. in 10$g$ of powdered infant formula.

## 2.2 Operating Characteristic (OC) Curve

The performance of a sampling plan is revealed by the operating characteristic (OC) curve, which is a graphical display of the probability of acceptance versus the proportion of contaminated product, or the mean level such as the mean concentration level of microorganisms. The OC curve clearly describes the performance of the sampling plan in detecting good and bad food quality. The producer's risk $(\alpha)$ and consumer's risk $(\beta)$ are traditional terms specified in the acceptance sampling literature, including the CAC (2004) guidelines for acceptance sampling. The probability that the sampling plan will reject a satisfactory lot is defined as the producer's risk. The probability that the sampling plan will accept

an unsatisfactory lot is defined as the consumer's risk. In this study, "better than safe" is defined as no contamination cases compromising safety characteristics. "Worse than unacceptable" is defined as contamination levels exceeding the specified sanitary characteristics limits. Lots with contamination levels worse than unacceptable fail to meet the necessary sanitary standards and should be rejected. The acceptable quality level/limit $(AQL)$ and the limiting quality level $(LQL)$ are fixed so that they have a high probability of accepting lots with safe levels of contamination (or better), and a low probability of accepting lots with unacceptable levels of contamination (or worse), respectively.

An example of an OC curve is given in Figure 2.1. The vertical axis shows the probability of acceptance as well as the producer's and the consumer's risks; the horizontal axis shows the fraction nonconforming levels such as the $AQL$ and $LQL$. In practice, nominal values are set for $AQL, \alpha, LQL$ and $\beta$. For example, the plans recommended by the ICMSF (2002) have $AQL$s in the range of 0.09% to 1%.

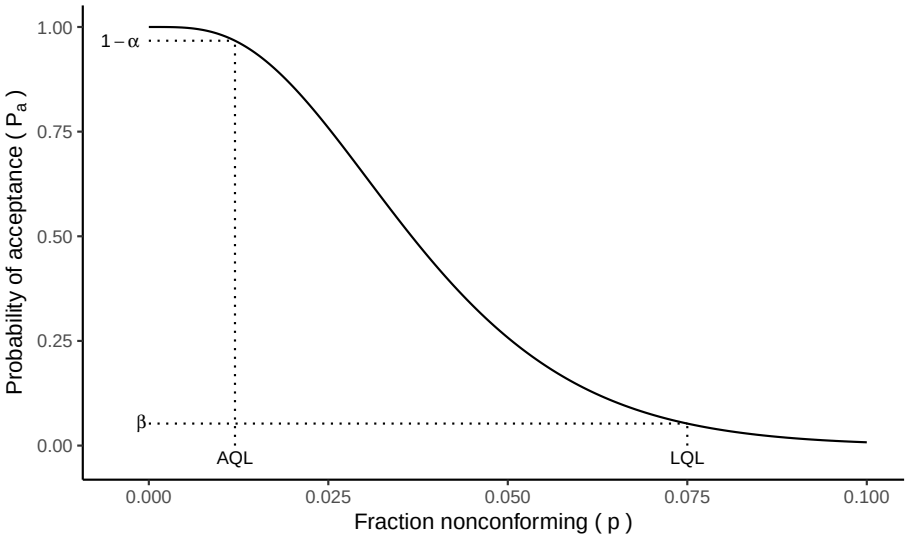


Figure 2.1: An example of OC curve.

## 2.3 Other Statistical Methodologies for Performance Assessment

Beyond acceptance sampling plans and OC curves, various statistical methodologies exist for assessing performance in different industries, including manufacturing and beyond. These methods encompass a range of strategies used in the food industry and can help maintain the quality and safety of food products. Some of these methodologies include:

- Statistical Process Control (SPC): SPC is a critical tool in the food industry for monitoring and regulating production processes to ensure they adhere to specified safety standards. Utilizing control charts and data analysis, SPC aids in identifying fluctuations and deviations from the desired safety parameters; see Montgomery (2020).

- Six Sigma: Six Sigma, when applied to food safety, utilizes the DMAIC framework to decrease defects and variability in food production processes. This methodology assists in defining, measuring, analyzing, improving, and controlling aspects of the production process to enhance food safety; see Pyzdek and Keller (2014).

- Design of Experiments (DOE): Food safety can benefit from the systematic manipulation of variables within processes to comprehend their influence on safety outcomes. DOE enables the optimization of processes and identification of key factors impacting food safety; see Box et al. (2005).

- Regression Analysis: By employing regression analysis, the food industry can model the connections between various factors and predict outcomes related to food safety. This technique can be invaluable in identifying elements contributing to safety hazards or variations in food production; see Kutner (2004).

- Reliability Analysis: Food safety requires the assessment of the probability that food products will remain safe and free from contamination over a specified duration. This analysis is particularly crucial in industries where safety and shelf-life are of utmost importance; see Lawless (1982).

- Capability Analysis: Evaluating the ability of a process to consistently produce food products meeting safety specifications is paramount. Capability analysis, including metrics like Cp and Cpk, is instrumental in ensuring that food production processes remain within safety limits; see Breyfogle (2003).

OC curve-based food safety assessment offers a real-time, dynamic monitoring approach, enabling early detection of safety issues and proactive prevention measures. Tailored to

specific safety standards and critical control points, this method fosters continuous improvement and is underpinned by rigorous statistical principles, ensuring a solid foundation for safeguarding food safety throughout the production process.

## 2.4 Food Safety Sampling

### 2.4.1 Probability Calculations and Risk Estimates

Since microbial counts are generally expressed on a logarithmic scale (base $e$ or base 10), the traditional theory of acceptance sampling for variables can be utilised. This approach has been discussed by number of authors, e.g., Legan et al. (2001) described a single attribute plan when the underlying plate count follows the lognormal distribution. A more comprehensive description can be found in Dahms (2004), who studied the performance of sampling plans under the assumption of normality of the log concentration of microorganisms. Subsequent studies of microorganism counts in food production processes suggested Poisson gamma and Poisson lognormal distributions in order to account for the non-homogeneous microbial contamination; see Gonzales-Barron and Butler (2011a) and Mussida et al. (2013a).

Jongenburger et al. (2012) suggested that distributional mixtures are generally useful for characterising microbial counts in powdered products. In practice, the major limitation to this idea is that it requires knowledge of incoming log concentration variability.

A composite sample can be defined as "the physical mix of individual sample units or a batch of unblended individual sample units that are tested as a group" (Patil, 2006). In recent years, interest in composite sampling for food safety purposes has increased; Jarvis (2007) and Ross et al. (2011). As stated by the ICMSF (2002), an "increase in the stringency of examination, without correspondingly increasing laboratory effort" can be obtained through composite sampling. However, the efficacy of composite sampling remains debatable because of variation is masked when primary increments are combined. The CAC (2004) recommends composite sampling only for economic reasons "given the loss of information on sample-to-sample variation due to the combination of primary samples".

Many researchers used a mixed Poisson distribution for risk estimation; for example, a standard risk computation formula for increasing the analytical amount with the assumption of no correlation in the microbial contamination was studied by Haas et al. (2014) and Mussida et al. (2013b). Another risk estimation approach has been posited by Santos–Fernández et al. (2016). Santos–Fernández et al. (2016) approach represents a scenario where contamination is most likely present as a single cluster. Also, Monte Carlo simulations were employed to obtain the probability of detection. Unfortunately, both approaches underestimate the risk of highly correlated contamination across samples. To address this shortcoming, the current research attempts to use Markov chain-based probability calculations to provide more realistic risk assessment. The first part of this research introduces serial correlation into the detection probability calculation when a combination of primary increments forms the test sample of powdered products.

### 2.4.2 Effect of Mixing and Dilution

In food production, mixing/blending of primary samples is crucial in preparing analytical samples. Very few studies deal with probability distribution-based risk assessments for analytical sample preparation. To the best of our knowledge, no studies consider the number of revolutions as one of the parameters in the mixing process. The current study introduces the number of revolutions as a mixing parameter for microbiological risk assessment.

Also, this study concentrates on the quantity of material sampled for risk assessment. Five different sampling scenarios are described. All samples of contaminated material are assumed to be taken from a production line batch.

Only a few considered the probability modelling of dilution procedures in food safety; see Jarvis (2016), Herigstad et al. (2001) and Jongenburger et al. (2010). In practice, an analytical sample is prepared by mixing primary samples from which a diluted analytical sample is prepared for testing (dilution test). The main limitation of the mixing/blending of primary samples is the risk of dilution. For example, if only one of the primary samples in a composite sample is contaminated, the concentration of the contaminant in a mixed analytical sample is reduced. Consequently, results could be a false negative, with contamination

values below the limit of detection. In addition, Jarvis (2007) has claimed that sensitivity might be affected by mixing samples in a dilution test.

For dilution tests, substantial extra laboratory manipulation is needed to prepare an analytical sample from mixed primary samples. Consequently, the arduous preparation of composite sampling might increase the risk of cross-contamination; see Jarvis (2007) and Morawicki (2017). Therefore, the current research also focuses on risk assessment in plate count-based testing. Based on regulatory guidance governing plate count procedures (e.g.,(CAC, 2008; ISO 4833-1, 2003), the current work introduces a truncated mixed Poisson distribution to assess microbiological risk and to compare sampling schemes used in the dilution process.

The final component of this research studies risk assessment based on the sample quantity used for diluted solution preparation. Dilution procedures for microbiological testing of food products provided by the International Organization for Standardization (ISO) are described in ISO 7218 (2007). However, due to extra binomial variation after incubation, ISO 7218 (2007) protocols are not robust in some situations. Therefore, the current work attempts to overcome this challenge by introducing beta-binomial distribution to cope with extra variation in the dilution process.

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# STATEMENT OF CONTRIBUTION
# DOCTORATE WITH PUBLICATIONS/MANUSCRIPTS

We, the student and the student's main supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the student's contribution as indicated below in the Statement of Originality.

| | |
|---|---|
| Student name: | Mayooran Thevaraja |
| Name and title of main supervisor: | DR. K. Govindaraju |

| | |
|---|---|
| In which chapter is the manuscript/published work? | Chapter 3 |
| What percentage of the manuscript/published work was contributed by the student? | 80% |

Describe the contribution that the student has made to the manuscript/published work:

The candidate did the necessary research, statistical modeling, code writing and preparation of the manuscript.

Please select one of the following three options:

- (●) **The manuscript/published work is published or in press**

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  Thevaraja, M, K Govindaraju, and M Bebbington (2021). Modelling the effect of sampling methods on detection tests for powdered products. Food Control 120, 107512, https://doi.org/10.1016/j.foodcont.2020.107512.

- ( ) **The manuscript is currently under review for publication**

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# Chapter 3

# Modelling the effect of sampling methods on detection tests for powdered products

Thevaraja, M, K Govindaraju, and M Bebbington
Food control[1], 2021
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## 3.1 Abstract

Grab sampling is often a convenient and cost effective way to sample bulk food materials, such as milk powder. On the other hand, modern auto-samplers can sample very small increments directly from the production process and they can be set to collect primary increments systematically. While the quantity of sampled bulk material is important, it is also necessary to consider the impact of sampling on quantitative risk assessment. When grab samples are drawn, the principle of randomisation is only partially met because of the inability to draw small primary increments at random. Food contamination (microbiological or otherwise) does not occur uniformly, and is often patchy or heterogeneous within a batch. Hence, even random sampling of primary increments does not amount to random sampling of pathogens or contaminants. As a consequence, the consumer's risk is underestimated under the holistic assumption of complete randomisation. In this theoretical study, a correlation parameter is introduced to allow for lack of independence in the presence and absence of contamination, and then the effect of the various sampling methods on the

[1]This chapter has been published in *Food Control*, 2021, 120:107512.

consumer's risk is examined. The main conclusion from this study is that grab sampling can increase the consumer's risk by as much as 50% and hence additional sampling is necessary when grab samples are used for lot disposition when compared to directly sampling the product from the process, which can be done using auto-samplers.

## 3.2 Introduction

The International Commission on Microbiological Specifications for Foods (ICMSF) has published recommendations, guidelines and tools for microbiological sampling inspection over the years; see ICMSF (1986), ICMSF (2002), ICMSF (2009) and ICMSF (2011). Similar guidelines, policies, recommendations and standards relating to food safety, and particularly on the use of inspection plans for the food trade, have been given by the Codex Alimentarius; see CAC (1997) and CAC (2004). The Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) regularly promote joint expert meetings and publish recommendations on sampling plans for different micro-organisms of interest; see FAO/WHO (2016).

Food products are typically bulk in nature, and hence random sampling of food products is difficult unless they are packaged in small volumes. If the population of interest consists of $N$ discrete items, a simple random sample of size $n$ can be easily drawn using a random number generation tool. Even when food is packaged, random selection of packages does not amount to random selection of primary units or increments unless the quality or safety characteristic of interest is completely homogeneous. As the characteristics are microbial in nature, they cannot be randomly sampled at all. However, microbiological and other food quality sampling inspection plans are typically evaluated under the holistic assumption of random sampling. The bias caused by the non-random sampling method can be large. This bias can increase the probabilities of false negative cases and also reduce the probability of false positive and vice versa. In other words, the designated consumer's and producer's risks under the chosen sampling inspection plan may be exceeded. Hence it is important to examine the violations of random sampling assumption for risk assessment and management.

In the industrial production of powdered food products such as milk powder, usually robotic or automatic samplers are employed to sample very small quantities, such as 1g, from the production process. Auto-samplers are often set to sample systematically, such that a total quantity such as 5kg can be accumulated to provide an overall representation of the product quality. Even though auto samplers can be programmed to perform random sampling, the common practice is to set them to sample systematically, for example every 5 minutes.

The term *grab sampling* refers to the draw of a specified quantity of material, and this sampling procedure is common not only for packaged bulk products but also the sampling of water, soil etc in environmental, agricultural, and geophysical studies. A grab sample is basically a "block" of successive or clustered unit amounts of the material drawn from flowing production process material at a particular time point or drawn as a block amount from a packaged material. Each grab sample can be viewed as a block of several primary increments. Even though grab samples can be selected both randomly and systematically, the principle of randomisation does not extend to the selection of primary increments. In other words, there is bound to be within grab sample variability and such variation is unlikely to be random. There is a scarcity of literature dealing with grab sampling theory. Gy's Theory Of Sampling (TOS) literature is critical of grab sampling methods, because it ignores the irreducible fundamental error, and other material subsampling errors in the test sample preparation; see Gy (1979), Pitard (1993) and Minkkinen and Esbensen (2009). The TOS literature warns that the grab sampling is the worst performing approach among all of the mass reduction approaches; see Minkkinen and Esbensen (2009). Mathematical modelling of the grab sampling method is scarce in the TOS literature because comparison is largely done using empirical methods.

When food products are sampled for regulatory and export inspections, the underlying product is in packaged form. Hence most samples drawn from the batch must be treated as random grab samples. On the other hand, the producer's quality and safety inspection schemes are based on grab or auto-samples drawn directly from the production process. Even though random grab sampling from the flow of the products over time is desirable, regulatory inspection cannot rely on the producer's samples due to legal and consumer

protection issues. The main aim of this article is to quantify the inefficiency of the grab sampling method when compared to systematic auto sampling and how to possibly compensate for this inefficiency with higher sample sizes.

Whether one small incremental sample is sufficient to detect contamination in a grab sample of large incremental samples depends on several factors. These include the distribution of contaminants, the detection method's sensitivity, and the risk tolerance level. If the contamination is uniformly distributed and the detection method is highly sensitive, a single small incremental sample may be adequate for detection. However, in cases where contaminants are heterogeneously distributed, or the detection method is less sensitive, multiple small incremental samples may be required to enhance the likelihood of detection. Additionally, it's essential to consider the potential consequences of false negatives and any regulatory guidelines.

In Section 3.3, an underlying mathematical formulation of the production process incorporate sequential dependence via the primary increments is given. Four possible methods of sampling are then described. The comparison of four sampling approaches based on presence - absence testing is discussed in Section 3.4. Finally, additional operating characteristic properties are studied in Section 3.5.

## 3.3 Methodology

Most powdered food products are produced in large volumes, such as 20 tons, through a single production run. Even though the produced material is continuous and not discrete like nuts and bolts, we may define a very small quantity or primary increment such as 1 g and then conceptually discretise the production volume (say, 20 tons) into a very long chain made of 1g primary increments. This approach of discretising bulk material production was employed in Govindaraju et al. (2017). Let the bulk material production process be modelled as a series $\{X_1, X_2, \ldots, X_N\}$ where $N = \text{int}(M/s)$ for known total production quantity $M$ and a small primary incremental amount $s$. Usually, $N$ is a very large number (in the billions) for food production processes but can be in millions of primary increments for kilogram scale pharmaceutical production processes.

The incremental sampling serves two purposes: as a theoretical limit or baseline for comparison, and as a mechanism for modelling the autocorrelation of contamination. It is not intended as a sampling strategy.

Let $X_j = 1$ or $0$ depending on the presence or absence of contamination. By our construction, the $X_j$'s are serially correlated. Contamination is often spotty even when the main quality characteristics of the product (such as percentage protein and fat) are relatively homogeneous. We assume that the bulk material production process can be modelled as a one step two-state Markov chain with the transition matrix,

$$\mathbf{P} = \begin{array}{c} \\ 0 \\ 1 \end{array} \begin{array}{c} \begin{array}{cc} 0 & 1 \end{array} \\ \begin{bmatrix} 1-a & a \\ b & 1-b \end{bmatrix} \end{array} \tag{3.1}$$

$(0 \leq a, b \leq 1)$, where $a$ is the probability of contamination in the primary increment when it is absent in the previous increment. The difference $d = 1 - a - b$ is interpreted as the *serial correlation* of the $X_j$'s in the statistics literature; see Bebbington and Lai (1998), Govindaraju et al. (2017). The limiting fraction of contaminated increments $P(X_j = 1) = p$ is given by $a/(a+b)$. An alternative parameterization of $\mathbf{P}$ in terms of $p$ and $d$ is given by,

$$\mathbf{P} = \begin{array}{c} \\ 0 \\ 1 \end{array} \begin{array}{c} \begin{array}{cc} 0 & 1 \end{array} \\ \begin{bmatrix} 1-(1-d)p & (1-d)p \\ (1-d)(1-p) & p+(1-p)d \end{bmatrix} \end{array}, \text{ see Govindaraju et al., 2017} \tag{3.2}$$

Detection of contamination not only depends on the prevalence $p$ but also relies on the method of sampling. We consider four different scenarios. The first one is the direct simple random sampling of primary increments, while the second one is systematic sampling of primary increments. The other two methods are, respectively, random and systematic sampling of grab samples instead of primary increments. Table 3.1 gives the glossary of various notation and terminology employed for these four methods of sampling for contamination detection.

Table 3.1: Chapter 3: Glossary of symbols and abbreviations

| | |
|---|---|
| $M$ | total production quantity |
| $s$ | primary incremental amount |
| $N$ | length of the production in primary increments |
| $X_i$ | contamination status of the $i^{\text{th}}$ primary increment |
| $a$ | probability of contamination of the primary increment when it is absent in the previous increment |
| $b$ | probability of contamination of the primary increment when it is present in the previous increment |
| $c$ | acceptance number |
| $p$ | limiting fraction or proportion of contaminated increments |
| $d$ | serial correlation of contamination between the primary increments |
| $d_g$ | serial correlation of contamination between blocks or grab samples |
| $k$ | sampling interval of the systematic sampling procedure |
| $f$ | sampling frequency of the systematic sampling procedure |
| $D$ | serial correlation between the systematically sampled primary increments |
| $D_g$ | serial correlation between the systematically drawn grab samples |
| $\mathbf{P}$ | transition matrix for $X_i$ |
| $\mathbf{P_k}$ | transition matrix for systematic primary increment sampling of $X_i$ |
| $\mathbf{P_{G_k}}$ | transition matrix for systematic grab sampling of $X_i$ |
| $P_{\text{D}}$ | probability of detection |
| $P_{\text{ND}}$ | probability of non-detection |
| $r$ | grab sample size or the number of primary increments in a grab sample |
| $t$ | number of grab samples |
| $n$ | number of primary increments in selected samples ($n = rt$ for grab samples) |
| $y_i$ | $i^{\text{th}}$ primary increment |
| $y_{i(j)}$ | $j^{\text{th}}$ primary increment in $i^{\text{th}}$ block (grab sample) |
| $p_d$ | probability of detection in any block (grab sample) |
| $p_{nd}$ | probability of non-detection in any block (grab sample) |
| $a^*$ | probability of contaminated block given that the previous block is not contaminated |
| $b^*$ | probability of uncontaminated block given that the previous block is contaminated |
| $\mu$ | location parameter (mean log) of the Lognormal and Poisson-lognormal distributions on the log10 scale |
| $\sigma$ | standard deviation of the lognormal and Poisson-lognormal distributions on the log10 scale (default value 0.8) |
| $\lambda$ | arithmetic mean of the cell counts |
| $K$ | dispersion parameter of the Poisson gamma distribution |
| $m$ | microbiological limit |
| $\lceil f \rceil$ | ceiling or least integer function giving the smallest integer that is not smaller than $f$ |
| $TOS$ | Theory Of Sampling |
| $ND$ | Non-Detect |
| $D$ | Detect |
| $OC$ | Operating Characteristic |
| $OQ$ | Outgoing Quality |
| $AOQ$ | Average Outgoing Quality |
| $AOQL$ | Average Outgoing Quality Limit |

### 3.3.1 Simple random sampling of primary increments

Even though it is in practice very difficult to perform, we consider the simple random sampling of primary increments for comparative risk evaluation purposes. Under simple random sampling, serial correlation can be ignored and only the limiting fraction matters. Hence, if $n$ primary increments are selected at random for a presence-absence type test on each sampled increment, the detection probability under the simple random sampling method is given by

$$P_D = 1 - (1-p)^n \tag{3.3}$$

### 3.3.2 Systematic sampling of primary increments

Systematic sampling of primary increments can be implemented using a high-tech robotic auto-sampler. Let $k = \lceil 1/f \rceil$ where $\lceil 1/f \rceil$ is the ceiling or least integer function for upward rounding of $1/f$ to an integer, and $f$ is the sampling frequency of the systematic sampling procedure. If one primary increment is selected for every $k^{\text{th}}$ increment produced, the two-state Markov chain model described above applies. Systematic sampling from this process model is discussed in Vellaisamy and Sankar (2001) and Govindaraju et al. (2017). Let $\{y_1, y_{k+1}, y_{2k+1}, \ldots, y_{(n-1)k+1}\}$ for some integer $k \geq 1$, be the systematic selected auto-samples of $n$ primary increments. Then the one step transition matrix for the two-state Markov chain of the presence and absence of contamination in selected auto-samples is given by,

$$\mathbf{P_k} = \begin{array}{c} \\ 0 \\ 1 \end{array} \begin{array}{c} \begin{array}{cc} 0 & 1 \end{array} \\ \begin{bmatrix} 1-A & A \\ B & 1-B \end{bmatrix} \end{array} = \begin{array}{c} \\ 0 \\ 1 \end{array} \begin{array}{c} \begin{array}{cc} 0 & 1 \end{array} \\ \begin{bmatrix} 1-p(1-d^k) & p(1-d^k) \\ (1-p)(1-d^k) & p+(1-p)d^k \end{bmatrix} \end{array} \tag{3.4}$$

where $A = p(1-d^k)$, and $B = (1-p)(1-d^k)$; see Govindaraju et al. (2017). The steady state probabilities of $\mathbf{P_k}$ are the same as that of the original Markov chain $\mathbf{P}$ in Equation (3.2). The serial correlation between the auto samples, $D$, is equal to $D = 1 - A - B = d^k$.

Following Vellaisamy and Sankar (2001), the probability of non-detection in two consecutive systematic auto-samples is given by $(1-A)(1-p)$ and hence the probability of non-detection in a set of systematic auto-samples becomes

$$P_{\text{ND}} = (1-p)(1-A)^{n-1} = (1-p)(1-p(1-d^k))^{n-1} \tag{3.5}$$

Alternatively we can express this as the probability of detection

$$P_{\text{D}} = 1-(1-p)\Big[(1-p(1-d^k))\Big]^{n-1} \tag{3.6}$$

where $k = \lceil N/n \rceil$; see Appendix 3.A for further details.

### 3.3.3 Random selection of grab samples

We now consider random selection of grab samples, which are basically blocks of primary increments. Each block has two possible outcomes ND (non-detect) or $D$ (detect) depending on whether a contaminated primary increment is absent or present in a particular block. The state space $\{\text{ND, D}\}$ also becomes a two-state Markov chain, and the serial correlation between blocks is given as $d_g = \left[dp(1-p(1-d))^{r-1}\right]/p_d$ where $r$ is the number of consecutive primary increments which form the grab sample (see Appendix 3.A).

Let the $i^{\text{th}}$ block be $\{y_{i(1)}, y_{i(2)}, \ldots, y_{i(r)}\}$. The probability of non-detection for the $i^{\text{th}}$ grab sample is given by

$$\begin{aligned} P(\text{non-detection in block } i) = P(y_{i(1)} = 0, y_{i(2)} = 0, \ldots, y_{i(r)=0}) = \\ P(y_{i(1)} = 0)P(y_{i(2)} = 0|y_{i(1)} = 0)P(y_{i(3)} = 0|y_{i(2)} = 0, y_{i(1)} = 0)\cdots \\ P(y_{i(r)} = 0|y_{i(1)} = 0, y_{i(2)} = 0 \ldots y_{i(r-1)} = 0) \end{aligned} \tag{3.7}$$

This probability can be simplified as

$$p_{nd} = (1-p)(1-p(1-d))^{r-1} \tag{3.8}$$

which is valid for any block $i$, of size $r$, and hence the probability of detection in any block is given by

$$p_d = 1 - (1-p)(1-p(1-d))^{r-1} \tag{3.9}$$

Under random grabsampling, serial correlation between blocks can again be ignored, and hence the underlying Markov process converges to a series of independent Bernoulli trials. Consequently, if $t$ grab samples are randomly selected and tested, the detection probability becomes

$$P_{\mathrm{D}} = 1 - (1-p_d)^t \tag{3.10}$$

### 3.3.4 Systematic selection of grab samples

Systematic selection of grab samples is difficult with packaged food product. Only the packaged unit of product can be sampled systematically and hence sub-sampling becomes necessary to obtain the regular analytical test sample amount, such as 10g. On the other hand, time oriented systematic selection of grab samples can be easily done using robotic samplers. For example, the auto samplers can be set to sample 10g of samples every half an hour instead of selecting a very small amount such as 1g at every 3 minutes. Under this sampling method, grab samples (blocks) are taken systematically with sampling frequency $f$ from the production process in a given period; where $f = rt/N$. Let $k = \lceil 1/f \rceil$ be the systematic grab sampling interval, which means every $k^{\text{th}}$ lump of primary increments (block) is periodically collected from the production process. The results given in Vellaisamy and Sankar (2001) and Govindaraju et al. (2017) are valid for systematic primary increments selection, but these results can be modified for systematic block selection, substituting for $p$ with $p_d$ and $d$ with $d_g$. The resulting transition matrix for the one-step two-state Markov chain describing the systematic selection of grab samples is given by,

$$\mathbf{P}_{\mathbf{G_k}} = \begin{array}{c} \\ \text{ND} \\ \text{D} \end{array} \begin{array}{c} \begin{array}{cc} \text{ND} & \text{D} \end{array} \\ \begin{bmatrix} 1-E & E \\ F & 1-F \end{bmatrix} \end{array} \tag{3.11}$$

where $E = p_d(1 - d_g^k)$, and $F = (1 - p_d)(1 - d_g^k)$. The vector of steady state probabilities is equal to $[1 - p_d, p_d]$ which is different from the vector $[1 - p, p]$ valid for the original series $X$'s. Also, the serial correlation between the systematic grab samples becomes $D_g = 1 - E - F = d_g^k$. The probability of non-detection with the $t$ systematic grab samples is then given by,

$$P_{\text{ND}} = (1 - p_d)\Big[(1 - p_d(1 - d_g^k))\Big]^{t-1} \tag{3.12}$$

Hence the detection probability with the $t$ selected grab samples is

$$P_{\text{D}} = 1 - (1 - p_d)\Big[(1 - p_d(1 - d_g^k))\Big]^{t-1} \tag{3.13}$$

where $k = \lceil N/rt \rceil$. If $r = 1$ and $t = n$ then $\mathbf{P}_{\mathbf{G_k}}$ becomes $\mathbf{P_k}$ and $D_g$ becomes $D = d^k$, which means that systematic grab sampling and systematic primary increment sampling methods become identical.

## 3.4 Comparison of the four sampling approaches

In order to compare the four sampling methods, we assume that the same analytical testing protocol will be followed irrespective of the method of sampling. For example, detection of *Salmonella* is based on incubation of the sampled material (grab or otherwise).

For detection of *Salmonella* in milk powder intended for general consumption, the International Organization for Standardization (ISO) recommends to gather 30 grab samples of 25g each forming a total sample amount of 750g; see ISO 6579 (2017). Let us assume that the primary increment amount is 1g of material (in order to be commensurate with the analytical sample amount for specific microorganism testing such as 10g for *Cronobacter* spp.). Let the batch quantity be $M = 10^7$g (or 10 metric tonnes) which are packaged in

400 bags of 25kg each. There is an obvious cost advantage in sampling the product during production before it is packaged, particularly using auto samplers. If 30 grab samples are to be taken after packaging the product, it requires the bags to be opened and hence such a method of sampling may not be economical for small batch sizes.

For the batch volume of $N = 10^7$, and sample size of $n = 750$ primary increments of 1g, Equations (3.3) and (3.6) produce almost identical probabilities of detection values because $k$ is very large for large $N$ and $d = 0.99$, which is graphically illustrated in Figure 3.8 of Appendix 3.B. The value of $d$ adopted here is realistic for *Salmonella* spp. because its occurrence is rather rare; see Morlay et al. (2016). Other words, probability of contamination of the primary increment when it is absent in the previous increment is expected to be close to zero in *Salmonella* spp. testing. On the other hand, contamination such as foreign matter or chemical in the milk powder would be more consistent with a less extreme $d$ value such as 0.9 which can result in a small difference between systematic and random sampling of primary increments; see Qin et al. (2017). The likelihood of *Cronobacter* spp. (formerly called *Enterobacter sakazakii*) is higher than the likelihood of *Salmonella* spp. or *Listeria* in milk powder. For such pathogens, a value of $d = 0.9$ may be adopted.

In other words, the systematic or random sampling of primary increments will involve the same risk of non-detection of *Salmonella* spp. in milk powder for the total sampled amount of 750g. It is easier to configure robotic auto-samplers to draw systematic samples of primary increments and hence this strategy is desirable. Auto-samplers can also be configured to draw systematic grab samples of 25g instead of 1g primary increments and also detection probability can be calculated by using Equation 3.13. This strategy is not desirable because of the drop in probability of detection as illustrated in Figure 3.1.

Figure 3.2 compares the systematic grab sampling method for a total sample amount of 750g formed by different combinations of $r$ and $t$. It is clear from this figure that drawing many systematic grab samples of smaller amount is a better strategy to improve detection when compared to drawing fewer grab samples of a more substantial amount. However the probability of detection for the grab sample method will still be inferior to the systematic autosampling of primary increments.

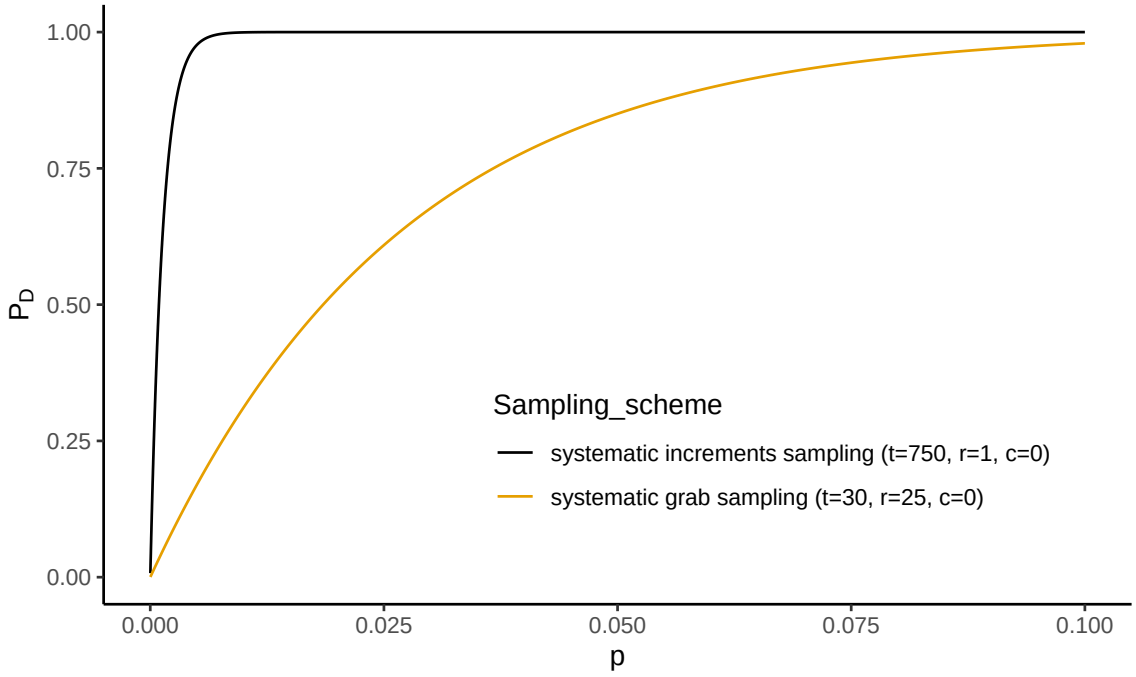


Figure 3.1: Comparison of systematic grab sampling and systematic increments selection using autosamplers

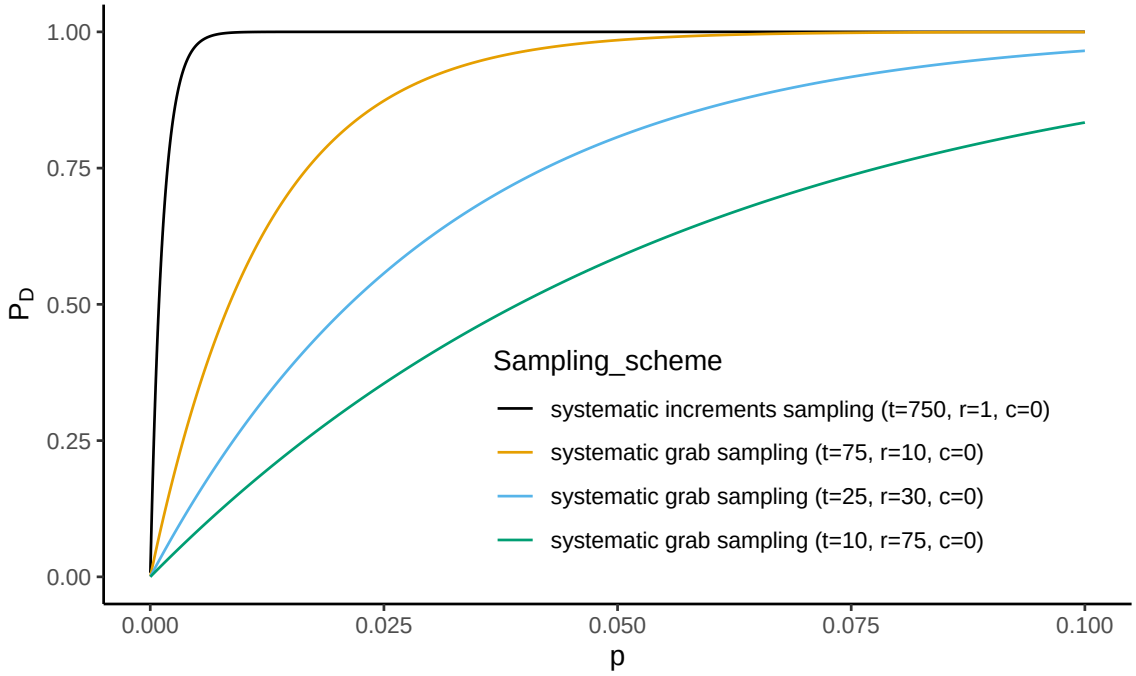


Figure 3.2: The effect of number of systematic grab samples on probability of detection

In the absence of auto-samplers, random grab sampling is the option commonly adopted. For $t = 30$ random grab samples and $r = 25$g, the probability of detection (of *Salmonella*

spp. in milk powder) remains poor when compared to systematic sampling of primary increments but at the same level of detection under systematic grab sampling. Testing for *Salmonella* spp. is usually done using a subsample taken from the composite of the grab samples taken. Hence the probability of non-detection $P_{\text{ND}}$ is the same as the probability of lot acceptance $P_a$ (assuming that there are no false positive or negative errors). Figure 3.3 (Case I) shows the $P_{\text{D}}$ under random grab sampling and systematic increments selection methods for various $p$ and given $t = 30$ and $r = 25$g.

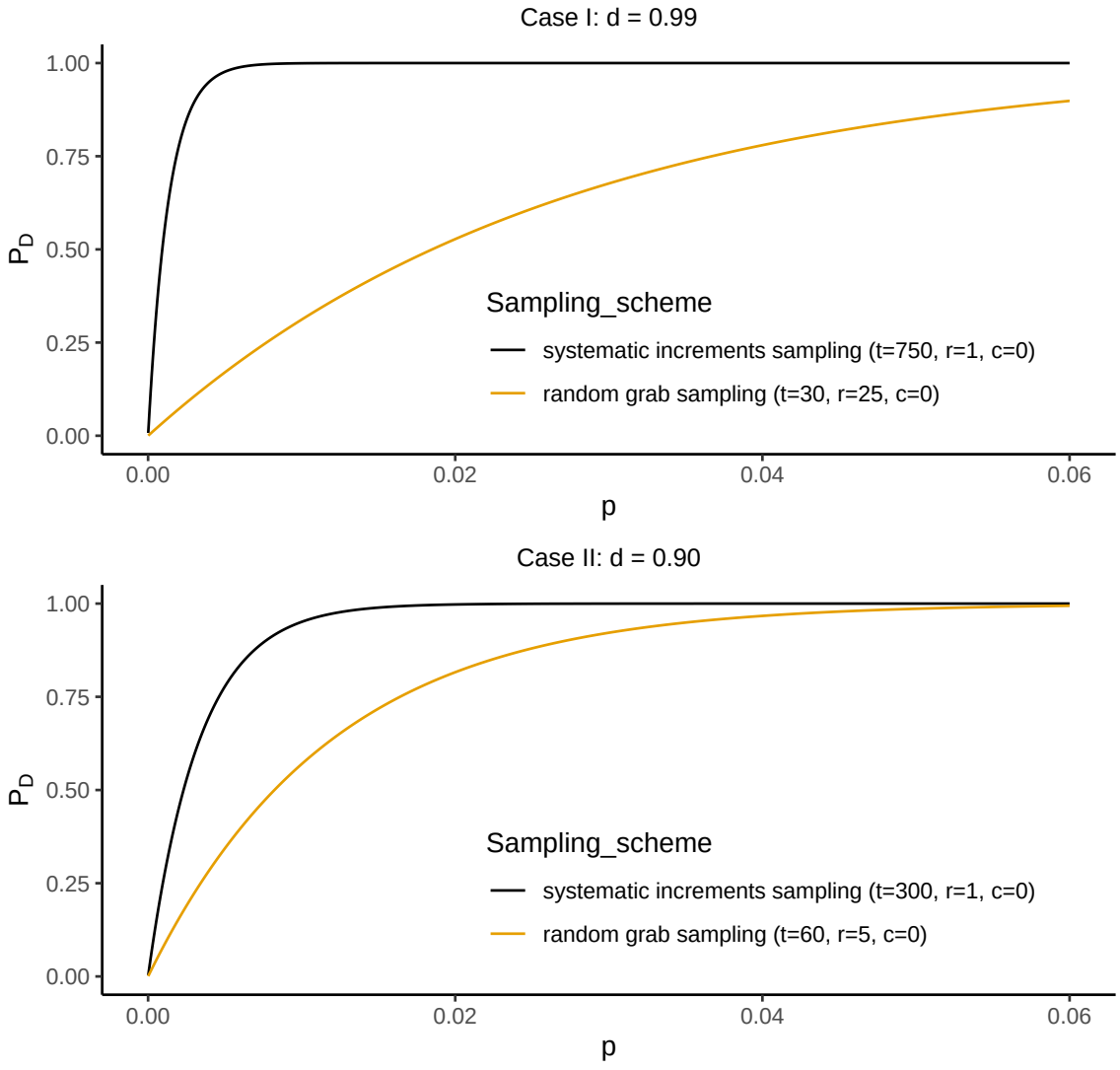


Figure 3.3: Comparison of random grab sampling and systematic increments selection methods

The detection capability of the grab sampling method is improved with the increase in the number of grab samples taken as well as sample amount. Assume that 30 grab samples of

10g are taken for the detection of *Cronobacter* spp. in milk powder. These grab samples are often treated as random samples for risk modelling. Even though 60 grab samples of 5g ensure better detection when compared to 30 grab samples of 10g, they still fall short of the detection under systematic and random sampling from the process as seen from Figure 3.3 (Case II).

The sampling methods employed for the detection plans can also be compared for their outgoing quality $(OQ)$ performance. Only batches that pass the detection tests are cleared for customers. Since $P_{\text{ND}}$ is the probability of non-detection, the outgoing contaminated proportion of primary increments is given by the product $pP_{\text{ND}}$; see McShane and Turnbull (1991). The quantity $AOQL$ is defined as the maximum proportion of outgoing contaminated primary increments and is given by

$$AOQL = \max_{0 \leq p \leq 1} pP_{\text{ND}} \tag{3.14}$$

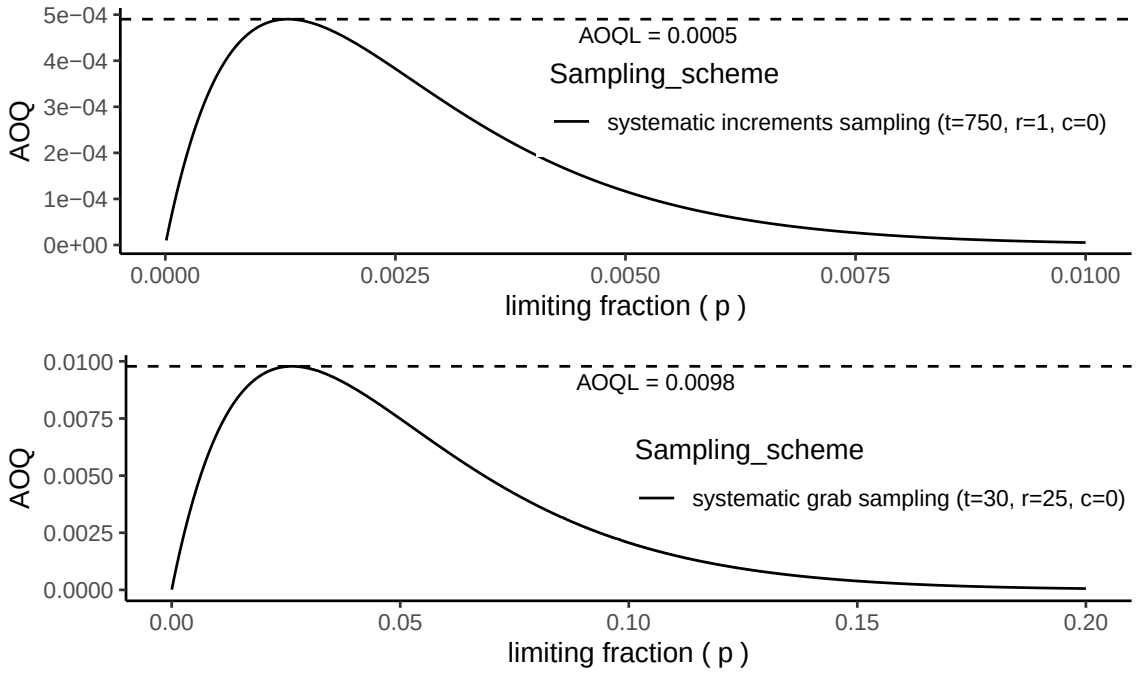


Figure 3.4: Average outgoing quality (AOQ) versus $p$ for sampling methods with $d = 0.99$

We plotted $AOQ$ for two different sampling schemes: 750 of 1g samples and 30 of 25g samples, as displayed in Figure 3.4. The average outgoing quality limits of contaminated

primary increments for the two sampling schemes are approximately 0.05% and 0.98% nonconforming respectively. Furthermore, systematic increments sampling achieves an AOQL of 0.05%, assuring that the worst fraction nonconforming the consumer receives as a long-term average is no more than 0.05%. Therefore, primary increment sampling, or in general, sampling small amounts more frequently, is more effective than the less frequent grab sampling method for protecting the consumer.

## 3.5 Further Operating Characteristic Properties

Single sampling plan by attributes is the most commonly employed sampling inspection plan. The cumulative binomial distribution function gives the probability of acceptance:

$$P_a = \sum_{x=0}^{c} \binom{n}{x} p^x (1-p)^{n-x} \tag{3.15}$$

where $P_a$ is the probability of acceptance, $p$ is the proportion of contaminated increments, $\binom{n}{x}$ is the binomial coefficient, $n$ is the number of primary increments in selected samples, $c$ is the acceptance number and $x$ is the number of contaminated increments. For the presence-absence testing for detection of food contamination, particularly pathogens, the acceptance number is generally zero $(c = 0)$, so the probability of acceptance becomes

$$P_a = (1-p)^n \tag{3.16}$$

For risk evaluation in terms of microbial counts, the Operating Characteristic (OC) curve which plots the probability of acceptance against the underling concentration level is useful. Poisson mixture distributions are commonly employed for modelling the underlying microbial counts in the literature. Schothorst et al. (2009) and Gonzales-Barron and Butler (2011b) suggested that the Poisson-lognormal and Poisson-gamma distributions are particularly suitable for high and low microbial concentrations respectively.

Let $Y$ be the random variable representing the count of microorganisms in a primary increment and $m$ be the microbiological limit, then the probability of detection in a single primary increment is given by $p_d = P(Y > m)$.

We can then compute the probability of acceptance in $t$ samples as single plan by attribute which can be calculated from Equation (3.15). It is known that $E(Y)$, the arithmetic mean of the cell counts, is equal to $10^{\mu+0.5\ln(10)\sigma^2}$; see Mussida et al. (2013b).

Let $Z$ be the total count of microorganisms in the grab sample. Following Mussida et al. (2013b), the count $Z$ is nothing but the sum of identically distributed Poisson-lognormal random variables $Y$, thus the distribution of $Z$ is also approximately Poisson-lognormal with mean $\mu_z$, standard deviation $\sigma_z$ where $E(Z) = rE(Y)$. The Poisson-lognormal distribution is appropriate when the number of microorganisms follows a Poisson distribution with rate $\lambda$ which is lognormally distributed. Then the probability mass function in terms of log mean concentration $\mu_z$ and standard deviation $\sigma_z$ is given by,

$$P(Z = z|\mu_z, \sigma_z) = \int_0^\infty P(z|\lambda) f(\lambda|\mu_z, \sigma_z) d\lambda \tag{3.17}$$

where the parameter $\mu_z$, as the average count of microorganisms in the grab sample, can be estimated (assuming a fixed value of $\sigma_z$ ) by

$$\mu_z = \log_{10}(\lambda r) - \frac{\sigma_z^2}{2}\ln(10), \tag{3.18}$$

see Mussida et al. (2013b).

The probability of detection in a primary increment is given by $p_d = 1 - P(Z = 0|\mu_z, \sigma_z)$ and so if we select $t$ samples with zero acceptance sampling plan, the probability of acceptance is given by $P_a = (1 - p_d)^t$.

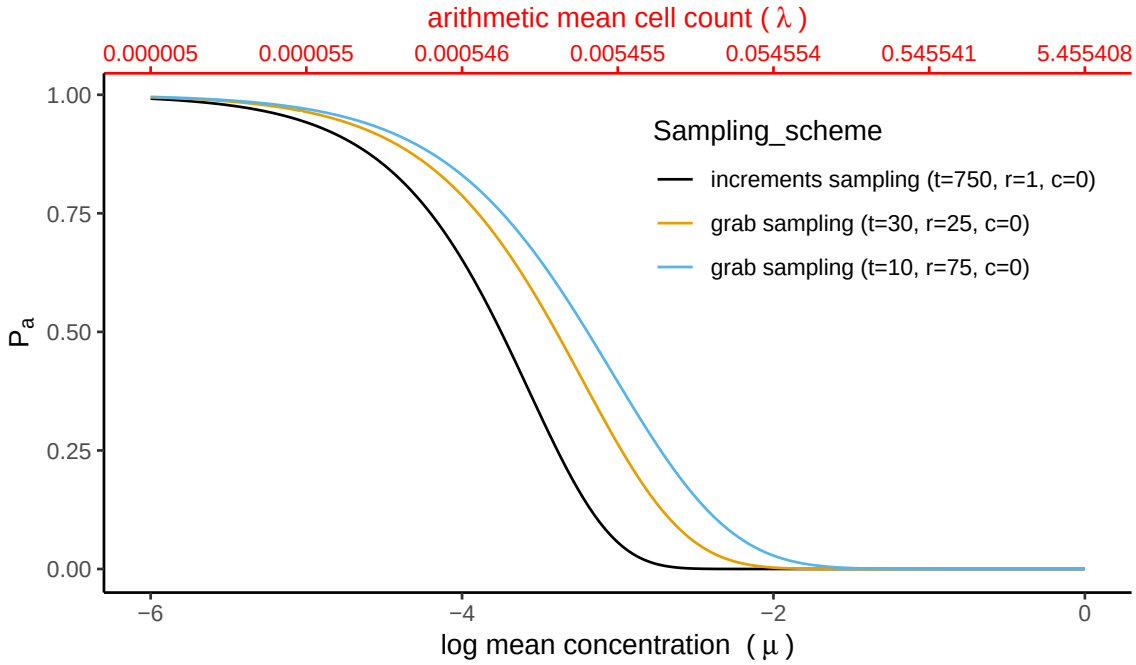


Figure 3.5: Operating Characteristic (OC) curves of the sampling methods based on Poisson lognormal distribution with $\sigma_z = 0.8$

In this paper, we fixed $\sigma_z = 0.8$ following Gonzales-Barron et al. (2013, p. 370), Jongenburger et al. (2015, p. 490) and others for the Poisson-lognormal case; see Dahms (2004), Schothorst et al. (2009), Mussida et al. (2013b) and Powell (2015). However, an OC curve can be constructed for different standard deviations as well. OC curves with different standard deviations such as $\sigma_z = 0.2, 0.4$ and $0.8$ are also shown in Figure 3.9 of Appendix 3.B. From this, it can be seen that the OC curve becomes flat for large standard deviations.

The Poisson gamma distribution is another suitable mixture distribution for food safety management, and whose probability mass function in terms of arithmetic mean of the cell counts $\lambda$ and dispersion parameter $K$ is given by,

$$P(Z = z|K, \lambda, r) = \frac{\Gamma(z+K)}{\Gamma(K)z!}\left(\frac{K}{K+\lambda r}\right)^K\left(\frac{\lambda r}{K+\lambda r}\right)^z \tag{3.19}$$

where $\Gamma$ is the gamma function. If $r$ primary increments form the total quantity of the sample, the probability of detection is given by

$$p_d = 1 - P(Z = 0|K, \lambda, r) = 1 - \left(\frac{K}{K + \lambda r}\right)^K \tag{3.20}$$

If $t$ samples are tested under a single sampling plan with zero acceptance number, the probability of acceptance is given by $P_a = (1 - p_d)^t$. Following Gonzales-Barron and Butler (2011a), the dispersion parameter $K$ for the Poisson-gamma case can be fixed in the range 0.044 and 0.401 and we used $K = 0.05$ for illustrative purposes; see Mussida et al. (2013a).

Figure 3.5 and 3.6 show the design effect of various sampling schemes on the probability of acceptance for various contamination levels. It is clear that drawing 750 samples of 1g increments provides superior protection to drawing the same total weight such as 30 samples of 25g or 10 samples of 75g each.

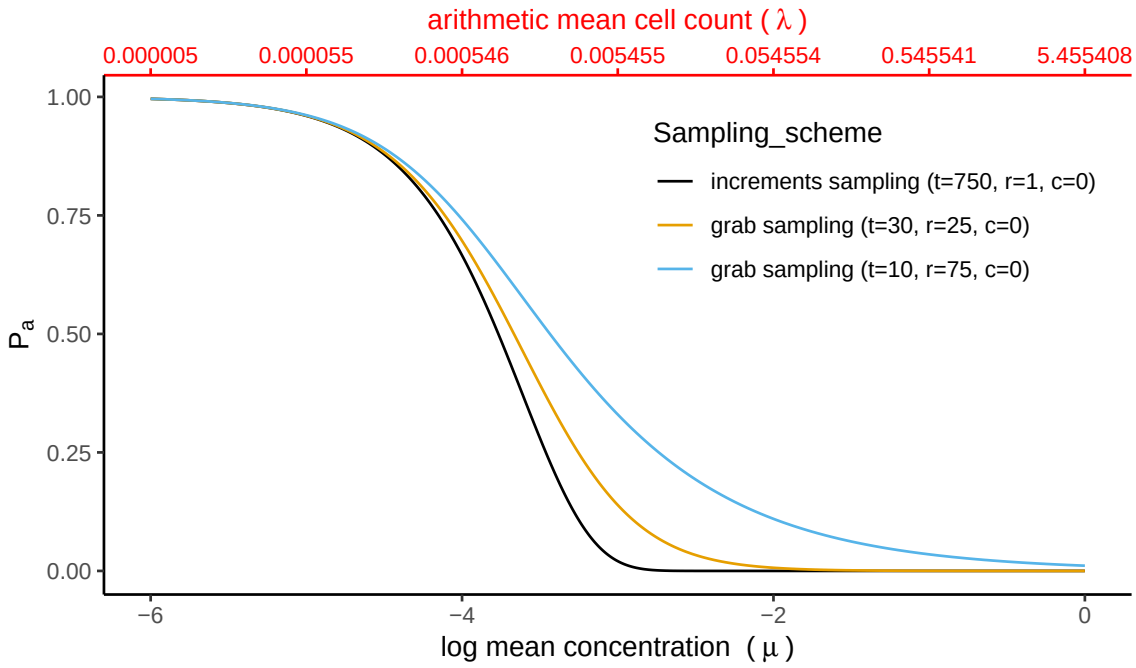


Figure 3.6: Operating Characteristic (OC) curves of the sampling methods based on Poisson gamma distribution with $K = 0.05$

Another way to monitor the risk is to examine the outgoing quality $(OQ)$ performance as a function of the process microbial count. Only batches that pass the inspection are

cleared for customers. Since $P_a$ is the probability of acceptance, $\lambda$ is the arithmetic mean of the cell count and the outgoing contaminated arithmetic mean of cell count of primary increments is given by $AOQ$ as the product $\lambda P_a$ which is slightly different to the classical $AOQ$ formula. The quantity $AOQL$ is defined as the maximum of outgoing contaminated primary increments and is given by

$$AOQL = \max_{\lambda \geq 0} \lambda P_a \tag{3.21}$$

The $AOQL$ limits of contaminated primary increments for the two sampling schemes such as 750 samples of 1g increments and 30 samples of 25g subsamples each are approximately 0.06% and 0.14% nonconforming respectively. These limits of outgoing quality mirror the conclusion reached with the presence-absence testing based outgoing quality in 750g sampled as shown in Figure 3.4 and Figure 3.7. Therefore, we can conclude that sampling primary increments is more effective than grab sampling, resulting in higher protection to consumers.

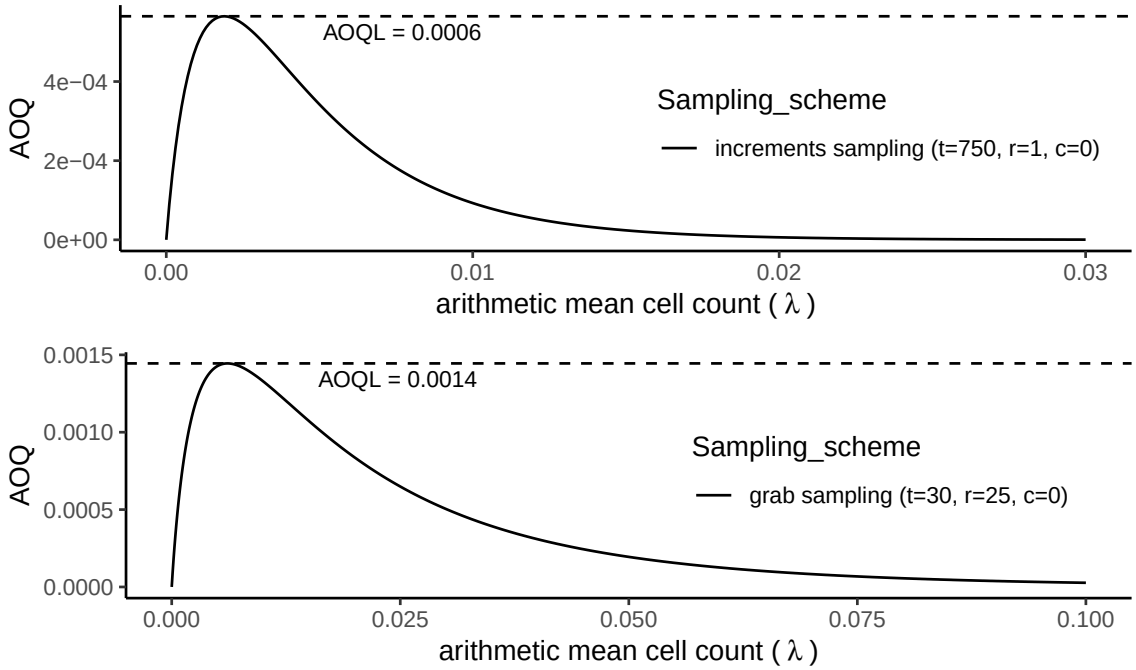


Figure 3.7: Average outgoing quality (AOQ) versus arithmetic mean of cell count $(\lambda)$ for sampling methods based on Poisson lognormal distribution with $\sigma_z = 0.8$

## 3.6 R Package "*grabsampling*"

We developed an *R* (R Core Team, 2023) software package *grabsampling* (available at https://github.com/Mayooran1987/grabsampling) for the probability of detection calculation for systematic or random grab sampling using a two-state Markov chain model. This package also draws the the OC curves under various methods of sampling so that the efficacy of grab sampling for a different set of parameters can be assessed. The user can specify the parameters of the single sampling plan $n$ and $c$. The package also allows for $c > 0$ even though the examples covered here are mainly based on $c = 0$. Description of our new package functions is available at https://mayooran1987.github.io/grabsampling.

## 3.7 Conclusion

This theoretical study has scrutinised the risk of non-detection when grab samples are employed, comparing it with the statistical 'gold standard' method of randomly sampling primary increments. Our approach allows for correlation of contamination in primary increments and the probability calculation is based on a two-state Markov chain. It was shown that the grab sampling method has a higher probability of non-detection when compared to sampling primary increments directly. We also presented a brief evaluation based on the OC and AOQ curves. The grab sampling methods exhibited enhanced risk of non-detection in general. So additional sampling is needed when grab samples are used for lot disposition when compared to direct sampling of the product from the process, with auto-samplers.

## 3.A Mathematical proofs

1. The transition probability matrix between blocks ($\mathbf{P_G}$).

$$
\begin{aligned}
&P(\text{ND in block } (i+1)|\text{ND in block } i)\\
&= \frac{P(\text{ND in block } (i+1), \text{ND in block } i)}{P(\text{ND in block } i)}\\
&= \frac{(1-p)(1-a)^{2r-1}}{(1-p)(1-a)^{r-1}}\\
&= (1-a)^r = (1-p(1-d))^r = 1-a^*(\text{say})
\end{aligned}
$$

$$
\begin{aligned}
&P(\text{ND in block } (i+1)|\text{D in block } i)\\
&= \frac{P(\text{ND in block } (i+1), \text{D in block } i)}{P(\text{D in block } i)}\\
&= \frac{P(\text{ND in block } (i+1)) - P(\text{ND in block } (i+1),\ \text{ND in block } i)}{P(\text{D in block } i)}\\
&= \frac{p_{nd} - (1-p)(1-a)^{2r-1}}{p_d}\\
&= \frac{p_{nd}(1-(1-a)^r)}{p_d} = \frac{a^* p_{nd}}{p_d} = b^*(\text{say})
\end{aligned}
$$

Transition probability matrix between blocks is given by,

$$
\mathbf{P_G} = \begin{array}{c} \\ \text{ND} \\ \text{D} \end{array}\!\!\begin{array}{c} \begin{array}{cc} \text{ND} & \text{D} \end{array} \\ \begin{bmatrix} 1-a^* & a^* \\ b^* & 1-b^* \end{bmatrix} \end{array} = \begin{array}{c} \\ \text{ND} \\ \text{D} \end{array}\!\!\begin{array}{c} \begin{array}{cc} \text{ND} & \text{D} \end{array} \\ \begin{bmatrix} 1-(1-d_g)p_d & (1-d_g)p_d \\ (1-p_d)(1-d_g) & p_d+(1-p_d)d_g \end{bmatrix} \end{array}
$$

where $a^* = 1-(1-p(1-d))^r$, $b^* = a^* p_{nd}/p_d$, $a^*$ is the probability of contaminated presence in the block when it is absent in the previous block and serial correlation between blocks is $d_g = 1-a^*-b^*$.

2. Serial correlation between blocks.

$$\begin{aligned} d_g &= 1 - a^* - b^* \\ &= 1 - \frac{a^*}{p_d} \\ &= 1 - \frac{1-(1-p(1-d))^r}{p_d} \\ &= \frac{(1-p(1-d))^r - (1-p)(1-p(1-d))^{r-1}}{p_d} \\ &= \frac{dp(1-p(1-d))^{r-1}}{p_d} \end{aligned}$$

Therefore,

$$d_g = \frac{dp(1-p(1-d))^{r-1}}{\left[1-(1-p)(1-p(1-d))^{r-1}\right]}$$

3. Probability of detection for systematic selection of grab

$P(\text{non-detection in } t \text{ number of selected grab samples})$

$= P(\text{ND in block 1}, \text{ND in block 2}, \cdots, \text{ND in block } t)$

$= P(\text{ND in block 1})P(\text{ND in block 2}|\text{ND in block 1})P(\text{ND in block 3}|\text{ND in block 1}, \text{ND in block 2})$

$\cdots\cdots P(\text{ND in block } t|\text{ND in block 1}, \text{ND in block 2} \ldots \text{ND in block } (t-1))$

By the Markov property,

$= P(\text{ND in block 1})\ P(\text{ND in block 2}|\text{ND in block 1})P(\text{ND in block 3}|\text{ND in block 2})$

$\cdots\cdots P(\text{ND in block } t|\text{ND in block } (t-1))$

$= p_{nd}(1-E)(1-E)\cdots\cdots(1-E)$

$= p_{nd}\,(1-E)^{t-1}$

$= p_{nd}\left[(1-p_d(1-d_g^k))\right]^{t-1}$

Therefore, the detection probability of all of the selected grab samples is given by,

$$P_{\mathrm{D}} = 1-(1-p_d)\left[(1-p_d(1-d_g^k))\right]^{t-1}$$

where $k = \lceil N/rt \rceil$.

For the probability of detection in all selected systematic auto-samples, fix $p_d = p$, $d_g = d$ and $t = n$. Therefore, the probability of detection in all selected systematic auto-samples is given by,

$$P_{\mathrm{D}} = 1 - (1 - p)\Big[(1 - p(1 - d^k))\Big]^{n-1}$$

## 3.B Additional graphical displays

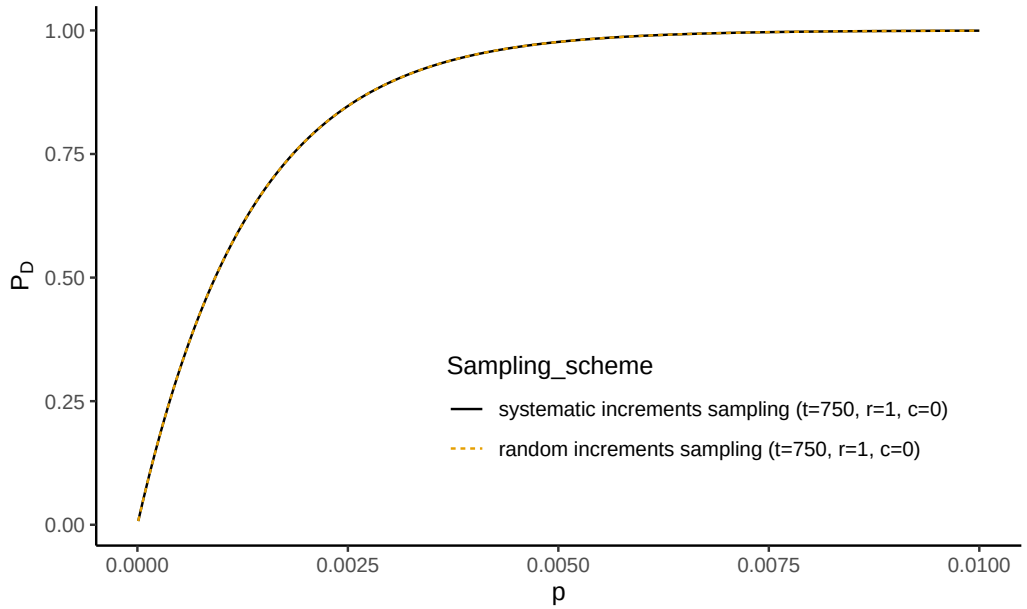


Figure 3.8: Comparison of systematic and random primary increments selection sampling

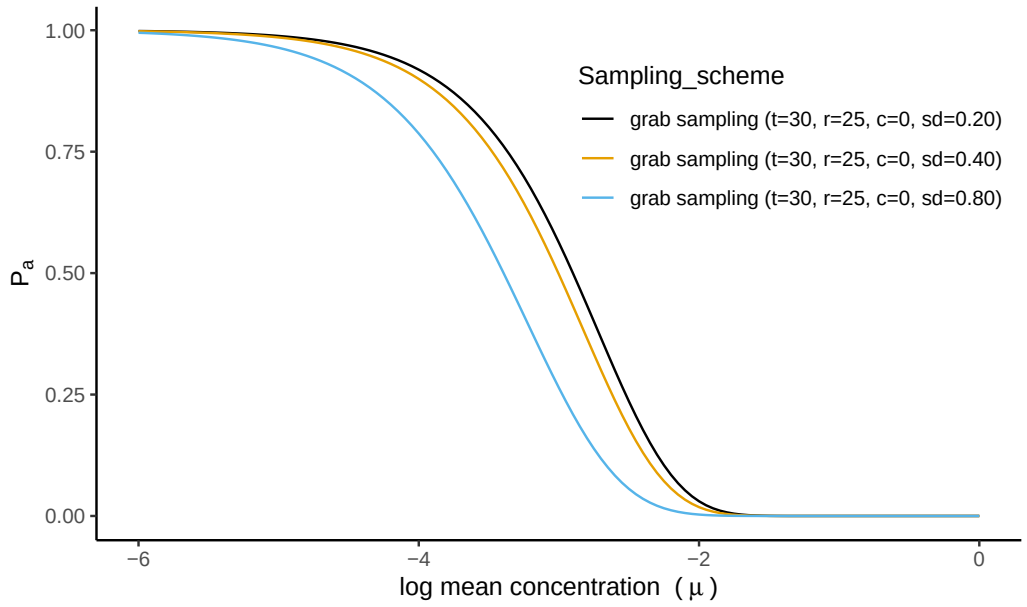


Figure 3.9: Comparison of Operating Characteristic (OC) curves based on Poisson lognormal distribution with $\sigma_z = 0.2, 0.4, 0.8$

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# STATEMENT OF CONTRIBUTION
# DOCTORATE WITH PUBLICATIONS/MANUSCRIPTS

We, the student and the student's main supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the student's contribution as indicated below in the Statement of Originality.

| | |
|---|---|
| Student name: | Mayooran Thevaraja |
| Name and title of main supervisor: | DR. K. Govindaraju |
| In which chapter is the manuscript/published work? | Chapter 4 |
| What percentage of the manuscript/published work was contributed by the student? | 90% |

Describe the contribution that the student has made to the manuscript/published work:

The candidate did the necessary research, statistical modeling, code writing and preparation of the manuscript.

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- ○ **The manuscript/published work is published or in press**
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| Student's signature: | Mayooran Thevaraja Digitally signed by Mayooran Thevaraja Date: 2023.04.26 04:21:34 +05'30' | Main supervisor's signature: | Dr. K. Govindaraju Digitally signed by Dr. K. Govindaraju DN: cn=Dr. K. Govindaraju, c=NZ, o=Massey University, ou=School of Mathematical and Computational Sciences, email=k.govindaraju@massey.ac.nz Date: 2023.04.26 11:21:13 +12'00' |

*This form should be placed at the beginning of each relevant thesis chapter.*

# Chapter 4

# Efficacy of Mixing Primary Samples for Powdered Products

## 4.1 Abstract

In many of the food safety assurance protocols, the first step is to collect several primary samples or increments and then mix them thoroughly to form composite samples. This action is called the *mixing procedure* in this thesis. Analytical samples may also be taken from the composite samples and tested as such or after dilution. The mixing procedure is crucial for detection of hazards such as *Salmonella* spp. detection in powdered products such as milk powder. Indeed, detecting microbial or any contamination in the powder mixing operation is highly complicated due to the cohesive nature of the powder. In practice, microorganisms are not homogeneously scattered in the primary samples of powdered products. Therefore, the mixing operation is helpful to produce a homogenized analytical sample. The efficacy of homogenization depends on the mixing parameters, such as the number of revolutions when mixing is done using a motorised device. While the number of mixing revolutions is low, the risk of non-detection in the analytical sample is expected to increase. In food safety, a limited number of studies have used the number of revolutions in the mixing operation as one of the parameters for risk assessment. This study fills this gap and uses the number of stages of mixing or revolutions as a mixing parameter for modeling non-detection and thereby risk evaluation. An R software package is provided to assess the risk using measures such as OC curves.

## 4.2 Introduction

It is implausible that each primary increment will contribute equally to the final blended sample or composite. When the underlying contamination is clustered, a poor or imperfect mixing operation will not result in a homogeneous composite.

The sampling literature has already established that the primary samples contribute unequally towards the composite sample preparation. Microbiological or other contamination does not occur uniformly over the primary increments. Therefore, the impurity level in each increment will not contribute proportionately to the final blended or mixed sample. The contamination level in the mixed or composited sample is usually modelled as a linear combination of the individual contributions of each primary increment, which are themselves random variables. The compositing process can be modelled using the Dirichlet distribution or the multivariate hypergeometric distribution (Elder et al., 1980; Patil et al., 2010; Rohde, 1976).

Composite sample preparation for discrete solid products, such as grains and coal is well studied in the literature (Elder et al., 1980; Lancaster and Keller-McNulty, 1998). However, this is not the case with powdered products. Clustering of microorganisms is a particular problem when the particle size is tiny. In this study, the term 'cluster' is defined as a contiguous block of impure particles or harmful organisms in the sample. The problem of clustering is more pronounced when the primary samples are simply grab samples.

This study aims to focus on research related to the detection of contamination such as *Salmonella* spp. in homogenized products such as milk powder. The mixing mechanism and mixing regimes of standard industrial mixtures are studied in the literature (Ponomarev et al., 2009; Gyenis et al., 2000). In this study, the focus is centred on statistical risk assessment rather than product specific hazard determination.

When mixing is done using a motorised blender, the number of stages of mixing can be equated to simply the number of motor revolutions set for mixing the primary samples. In this study, we define the term 'revolution' as a single rotation of the mechanical motor and the term 'stage' of the mixing operation to indicate a mixing state after a single mixing

of the material. Homogenization occurs progressively when powders are mixed, which is illustrated in Figure 4.1. The concentration or clustering of contamination is lowered with each revolution of the mixture because the contribution of each contaminated primary increment toward the mixed sample undergoes a random change when mixing progresses. In practice, the powder compositing requires numerous mixing stages or revolutions in order to obtain a fully homogenized sample. Hence, the powder-mixing operation can be viewed as breaking clusters in a long chain of contaminants. The efficacy of the mixing or blending can be measured based on how completely clusters are broken while mixing. In other words, it is reflected in how the number of clusters changes with the number of stages of mixing.

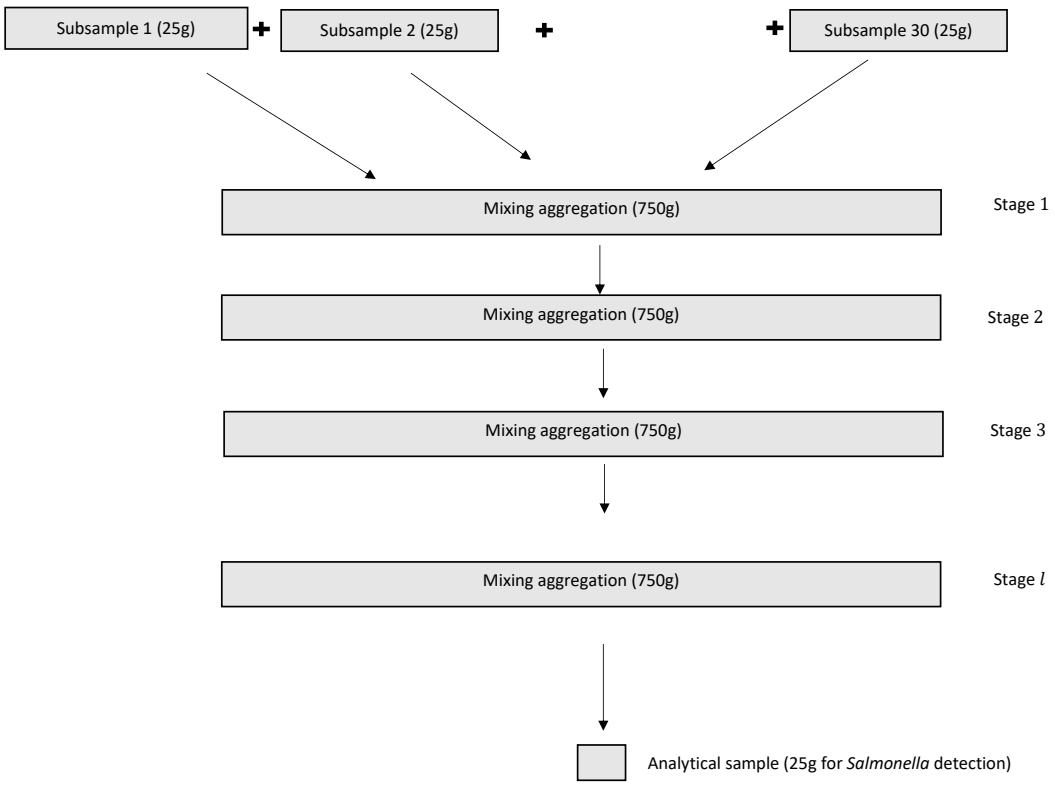


Figure 4.1: An analytical sample preparation process for microorganisms testing.

Assuming that the mixing operation diminishes the cluster lengths in the sample, the number of clusters increases with the number of mixing revolutions or stages of mixing.

Therefore, the contamination level in the mixed sample measurements at the $j^{\text{th}}$ stage can be modelled by:

$$Z_j = \sum_{i=1}^{k} w_{ij} x_i \tag{4.1}$$

where $k$ is the number of primary samples contributing to the mixed sample, $w_{ij}$ are the stochastic weights subject to $\sum w_{ij} = 1$ at the $j^{\text{th}}$ stage of the mixing and $x_i$ is the contamination level in the $i^{\text{th}}$ primary sample.

For food safety, presence-absence type testing of microorganisms using the analytical sample is popular. For example, according to the International Organization for Standardization (ISO) sampling guidelines, industries have to test one or more 25g analytical samples selected from the 750g composite sample for testing *Salmonella* spp. in milk powder ISO 6579 (2017). For illustrative purposes, this Chapter assumes that the analytical sample is of size 25g drawn from a 750g aggregate of thirty 25g (grab) samples. To precisely detect the presence of a pathogen, a 25g analytical sample must be representative of the 750g composite sample. If sufficient homogenization is achieved, the 25g analytical sample can be deemed to be representative of the 750g composite sample.

In the ideal mixing operation, clusters are repeatedly broken and sometimes recombined until there is an equal concentration level of contamination in every part of the mixed sample or final composite. Different primary increments are not expected to have the same concentration level of contamination and hence the mixing operation needs to be repeated many times to achieve homogeneity.

This study provides a microbiological risk assessment for the powder-mixing operation, based on a Markov chain incorporating the Poisson lognormal probability distribution used in the literature to model the microbial counts. All scientific symbols and abbreviations used in this Chapter are defined in Table 4.1.

Table 4.1: Chapter 4: Glossary of symbols and abbreviations

| | |
|---|---|
| $Z_l$ | contamination level in 750g aggregate sample in the $l^{\text{th}}$ stage of the mixing operation |
| $x_i$ | contamination level in the $i^{\text{th}}$ primary sample |
| $N$ | total primary increments in the mixed sample |
| $w_{ij}$ | stochastic weights which sum to one |
| $l$ | number of rotations or revolutions of the mixer |
| $N_i$ | number of colony-forming units in the $i^{\text{th}}$ primary sample of given size (e.g 30g) |
| $N'$ | total number of colony-forming units in the mixed sample (new large unit, e.g 750g) |
| $\mu$ | location parameter (mean log) of the Poisson-lognormal or lognormal distribution on the logarithmic scale |
| $\sigma$ | standard deviation of the Poisson-lognormal or lognormal distribution on the logarithmic scale (default value 0.8) |
| $y_i$ | number of microorganisms in the $i^{\text{th}}$ primary increment |
| $k$ | number of primary samples |
| $P_{\text{D}}$ | probability of detection |
| $P_{\text{ND}}$ | probability of non-detection |
| $\boldsymbol{\alpha_j}$ | the vector of concentration parameter at $j^{\text{th}}$ stage |
| $\alpha_{\text{initial}}$ | initial value of elements in the vector of the concentration parameter |
| $r$ | the rate of the parameter $\boldsymbol{\alpha_j}$ changes at each mixing stage |
| $P_{d_i}$ | probability of detection at $i^{\text{th}}$ stage of the mixing operation |
| *UDL* | Upper Decision Limit |
| *pmf* | probability mass function |
| *CDF* | Cumulative Distribution Function |
| *CFU* | Colony-Forming Unit |

## 4.3 Materials and methods

The mixing operation can be modelled as breaking clusters in a long series of primary increments. Following Nauta (2005), let $N_i$ be the number of colony-forming units (CFUs) in the $i^{\text{th}}$ primary sample, and let $N'$ be the total number of CFUs in the mixed sample (new large unit). Then, the total number of CFUs can be calculated as $N' = \sum N_i$ when $N_i$'s are known counts. However, each subsample contains numerous primary increments and the sampling interval for selecting primary samples impacts the concentration level in the sampled material. For modelling purposes, in powdered products each primary sample is basically a grab sample, as analysed by Thevaraja et al. (2021).

Powder mixing is achieved by combining all primary samples and mechanically mixing them. Mechanical mixing involves a single revolution, which is controlled by a motorised mixing instrument, is the main mixing parameter. The level of contamination in the mixed

sample, rather than the number of primary samples involved in the mixing operation, is crucial. However, the contribution of contamination from the primary samples to the aggregate may not be equal due to the inherent heterogeneity in primary samples. In the following section, we first consider an ideal (equal) contributions case, and then a more realistic unequal contributions case.

### 4.3.1 Equal contributions

For the equal contributions case, we assume that each primary increment contributes equally to the mixed sample. Therefore, the total contribution is equal to the sum of each primary increment contribution. Regulatory authorities recommend the Poisson lognormal and Poisson gamma distributions as the most suitable options for modeling microbial counts; see Schothorst et al. (2009) and Gonzales-Barron and Butler (2011b). Let $Y$ be the number of microorganisms in a primary increment, and assume that each primary increment has equally contributed to the mixed sample. The Poisson lognormal distribution is commonly employed to model the microbial counts (Schothorst et al., 2009; Gonzales-Barron and Butler, 2011b), where $Y$ is a Poisson random variable whose rate parameter follows a lognormal distribution with parameters $\mu_y$ and $\sigma_y$. In this case, if $N'$ is the number of micro-organisms in the mixed sample, then $N'$ follows the Poisson lognormal distribution with parameters $\mu_{N'}$ and $\sigma_{N'}$.

Under the assumption of equal contributions of contamination, the expected value is $E(N') = NE(Y)$; where $N$ is the total number of primary increments. Following Gonzales-Barron et al. (2013), Jongenburger et al. (2015) and others for the Poisson lognormal case, we assume that there is a known or empirically fixed value of $\sigma_{N'} = 0.8$ which is a measure of the inhomogeneous microbial contamination (Dahms, 2004; Schothorst et al., 2009; Mussida et al., 2013b; Powell, 2015).

Therefore, the probability of detection in the mixed sample is given by:

$$P_D = 1 - P_{ND} = 1 - P(N' = 0|\mu_{N'}, \sigma_{N'}) \tag{4.2}$$

It is not assumed that the contamination will always be detected. In this Chapter, particularly when dealing with very low contamination levels, the probability of detection can indeed approach zero. This underscores the significance of understanding CFUs (Colony Forming Units) and the limitations in detecting and quantifying them, especially when contamination levels are extremely low.

A detection probability estimation for contamination can be made by using individual detection probabilities as per Thevaraja et al. (2021), where a lump of primary increments is defined as a grab sample. With grab sampling, overall detection probability for the total sample is estimated from individual detections derived from grab samples, and by using different sampling methods, such as systematic and random selections. For example, if $25g$ increments from 30 grab samples are combined to make a $750g$ sample, the probability of detection in the $750g$ sample can be estimated using individual detections from the $30 \times 25g$ grab samples. This means that, for detection probability estimation, Thevaraja et al. (2021) assumed each grab sample contributed equally to the total sample.

With the assumption of equal contributions, the derivations given in Thevaraja et al. (2021) are valid for our mixture modelling because the combination of primary samples forms the mixed sample. Furthermore, Thevaraja et al. (2021) derived the detection probability formula, based on a two-state Markov chain model for grab sampling adopting different sampling methods.

In most circumstances, primary samples will not equally contribute to the mixed sample. The next case discusses the unequal contributions of contamination while mixing.

### 4.3.2 Unequal contributions

The number of revolutions, or the number of times primary samples are blended, is an important parameter in evaluating the mixing efficiency. When primary increments are not expected to make equal contributions to the final mixed sample, the number of revolutions must increase with underlying heterogeneity in the microbial contamination.

Unequal contributions can be modelled by multivariate Dirichlet distribution based on the literature (Elder et al., 1980; Nauta, 2005; Patil et al., 2010). Moreover, the contribution of

each primary sample to a mixed sample can be linked to the unequal weights of primary samples using the multivariate Dirichlet distribution. Therefore, the probability model of the weights is given by:

$$(w_{1j}, w_{2j}, \cdots w_{kj}) \sim Dir(\alpha_{1j}, \alpha_{2j}, \cdots \alpha_{kj}); \tag{4.3}$$

where $w_{ij}$ is defined as the weight of the contribution from the $i^{\text{th}}$ primary sample to the mixed sample at the $j^{\text{th}}$ stage and $\alpha_{ij}$ is the corresponding concentration parameter.

Nauta (2005) studied a microbiological risk assessment model for the mixing operation based on the relative size of different animal carcass parts and this work does not involve the number of revolutions as one of the parameters for modelling the mixing operation.

Let $k$ be the number of primary samples in the mixing operation, and suppose the $i^{\text{th}}$ primary sample each contains $N_i$ CFUs for all $i = 1, 2, \cdots, k$, and let $N'$ be the total number of colony forming units. Thus, each weight can be defined by the number of CFUs in the particular primary sample divided by the total number of CFUs in all the primary samples. Using Equation 4.3, the contribution by each primary sample can be defined as the random variable $w_{ij}$, whose joint probability distribution of $(w_{1j}, w_{2j}, \cdots w_{kj})$ follows the Dirichlet distribution with a vector of concentration parameter $\boldsymbol{\alpha_j}$.

The probability mass function (pmf) of the weights is given by:

$$f(w_{1j}, w_{2j}, \cdots w_{kj}; \boldsymbol{\alpha_j}) = \frac{\Gamma(\sum_{i=1}^{k} \alpha_{ij})}{\prod_{i=1}^{k} \Gamma(\alpha_{ij})} \prod_{i=1}^{k} w_{ij}^{\alpha_{ij}-1} \tag{4.4}$$

where $\boldsymbol{\alpha_j} = [\alpha_{1j}, \alpha_{2j}, \cdots \alpha_{kj}]$ is the vector of concentration parameters, and the sum of weights must be equal to one.

If we assume that the samples are exchangeable, the concentration parameters become identical (say $\alpha_j$) in all the primary samples. The probability mass function of the weights is given by:

$$f(w_{1j}, w_{2j}, \cdots w_{kj}; \boldsymbol{\alpha}) = \frac{\Gamma(k\alpha_j)}{\Gamma(\alpha_j)^k} \prod_{i=1}^{k} w_i^{\alpha_j - 1} \tag{4.5}$$

In practice, the contribution of primary samples toward the mixed sample is high for those with large concentration parameter values because the underlying density curve becomes highly peaked or leptokurtic. This property is illustrated in Figure 4.2, which shows the smoothed density of the probability mass function (pmf) of weights with various concentration parameters. Due to the lack of theoretical results for the distribution of the sum of correlated Dirichlet random variables, this study utilised simulation techniques. The resulting open-source *R* package (R Core Team, 2023) *mixingsimulation* was developed to obtain simulation results as part of this research.

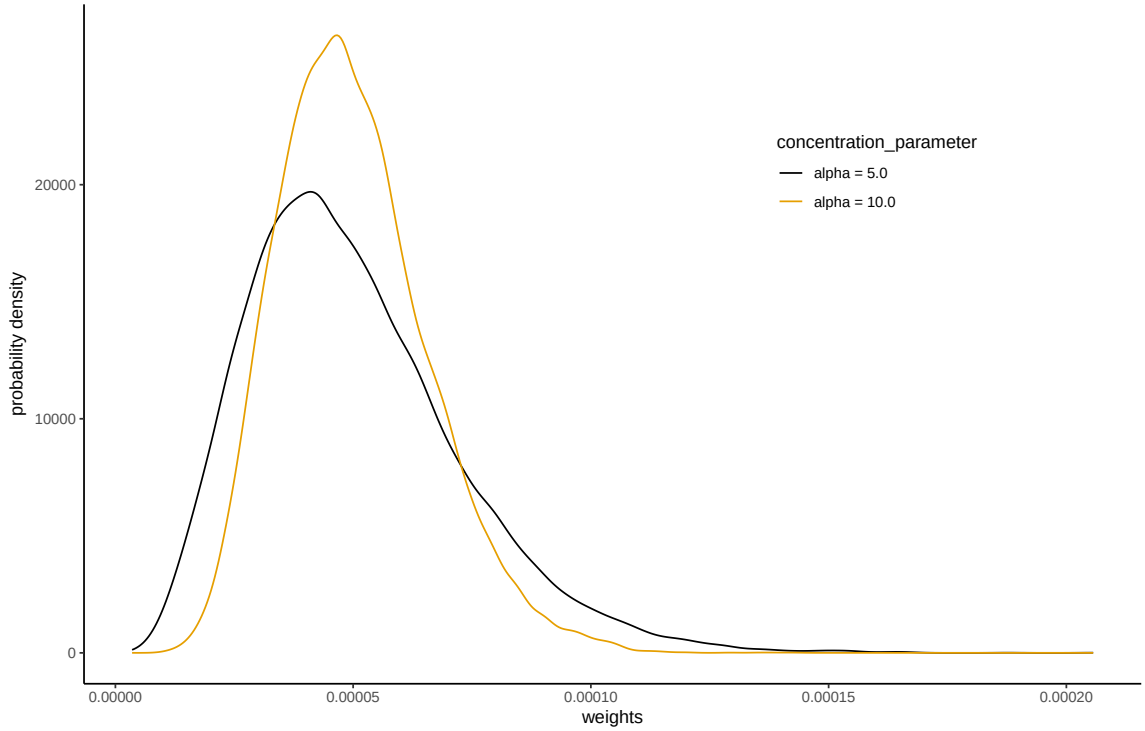


Figure 4.2: Comparison of probability densities of weights for two concentration parameters $(\alpha)$ (20,000 weights randomly generated from Dirichlet distribution).

## 4.4 Results and discussion

Let $l$ be the number of stages, or mixing revolutions, and assume that the vector of the concentration parameter is fixed and identical across all the primary samples at the initial phase of the mixing operation. The powder mixing operation can be defined as breaking clusters, stage by stage. Usually, cluster breaking occurs systematically at each stage

of a standard powder mixing operation. Following the literature (Nauta, 2005; Santos-Fernández et al., 2015), we assume that mixing capability increases in synch with the concentration parameter. Accordingly, with all elements being identical, the vector of the concentration parameter can be assumed to increase at every mixing stage. Also, this increase happens at a constant rate when the mixing instrument has a mechanical motor and runs at a stable speed.

As alpha increases in each stage, the mixture tends to become more homogenous. This means that the components of the mixture are distributed more evenly, leading to a more consistent and uniform final test sample. Increasing alpha allows for better mixing of the components, reducing the likelihood of clumps or unmixed portions within the powder mixture. This results in improved product uniformity and reduces the risk of "hotspots" where certain components may be over-represented.

The vector of the concentration parameter at the $j^{\text{th}}$ mixing stage is given by:

$$\boldsymbol{\alpha_j} = [\alpha_j, \alpha_j, \cdots \alpha_j] \quad \text{for all } j = 1, 2, \cdots, l \tag{4.6}$$

If we assume that the initial value of elements in the vector of the concentration parameter is $\alpha_{\text{initial}}$, then elements of the vector of the concentration parameter at the $j^{\text{th}}$ mixing stage can be described by:

$$\alpha_j = \alpha_{\text{initial}} + \sum_{i=1}^{j} r_i \quad \text{for all } j = 1, 2, \cdots, l \tag{4.7}$$

where $r_i$ is the increase in rate of the parameter at the $i^{\text{th}}$ mixing stage. In practice, the $r_i$ must be established empirically, and as a result, the mixing stage variable can be converted to a linear equation. For this study, we assume $r_i$ is a fixed value for all mixing stages.

The initial concentration parameter values are proportionate to the weights of the primary samples. However, initial concentration parameter values do not depend on the number of primary samples. For this research, we assumed 25g primary sample aggregates were added to each stage of mixing, no matter the different sizes of mixed samples, which were

250g, 750g and 1500g. Therefore, we set the initial concentration parameter $(\alpha_{\text{initial}})$ at 0.01 for all mixing schemes.

Each primary grab sample contains numerous increments. For example, if we consider thirty 25g samples for the mixing operation, then each primary sample contains $N/30$ primary increments, where $N$ is the total number of primary increments in a grab sample. The expected number of microorganisms contributed by the $i^{\text{th}}$ primary sample at $j^{\text{th}}$ stage is equal to $w_{ij}x_i$, where $w_{ij}$ is a weight of the contribution from the $i^{\text{th}}$ primary sample at $j^{\text{th}}$ stage and $x_i$ is the number of microorganisms in the $i^{\text{th}}$ primary sample.

Each subsample can be considered in a combination of small amounts, e.g., 1g, which can be called a primary increment. This situation can be modelled so that the contribution of each unit amount to the primary sample is governed by a linear model of unequal contributions. However, the current study does not describe the modelling of primary increment contributions. Instead, the focus is on the contributions of primary samples toward the aggregated sample.

For modelling of the powder mixing operation, the expected total number of CFUs in the mixed sample after the mixing operation becomes $N' = \sum N_i$; where $N_i$ follows a specific probability distribution. Nauta (2005) considered four different distributional cases for modelling:

- case 1 (Poisson-Type A): $N_i$ follows $Poisson(\mu/k)$
- case 2 (Poisson-Type B): $N_i$ follows $Poisson(\mu w_i)$
- case 3 (Lognormal-Type A): $N_i$ follows $Binomial(M_i, 1/k)$; where $M_i$ follows $Lognormal(\mu, \sigma)$ and $k$ is the number of primary samples
- case 4 (Lognormal-Type B): $N_i$ follows $Binomial(M_i, w_i)$; where $M_i$ follows $Lognormal(\mu, \sigma)$

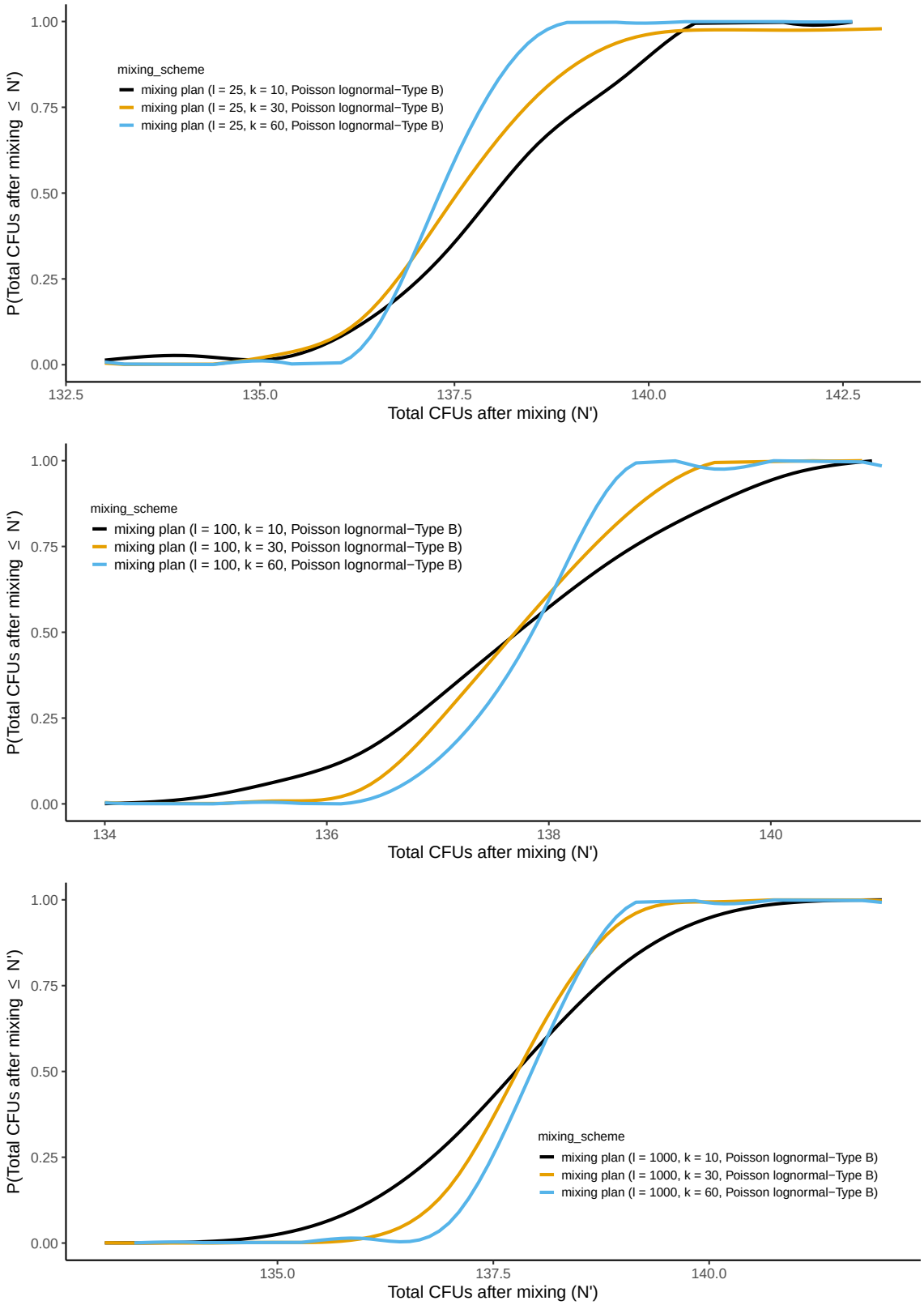


Figure 4.3: Cumulative probability function of different mixing schemes (based on 500 simulations).

For this research, we employed the convention "Type-A" and "Type-B" to indicate the types of distribution, which were termed as "fair" and "beta", respectively, as in Nauta (2005). Poisson mixture distributions are the most commonly employed models in food safety assessments (Gonzales-Barron et al., 2013; Gonzales-Barron and Butler, 2011b; Jongenburger et al., 2015; Mussida et al., 2013b; Powell, 2015; Schothorst et al., 2009).

Therefore, this study introduces two Poisson lognormal distributions Type A and Type B, as further cases:

- case 5 (Poisson lognormal-Type A): $N_i$ follows $Binomial(M_i, 1/k)$; where $M_i$ follows $Poisson\ lognormal(\mu, \sigma)$
- case 6 (Poisson lognormal-Type B): $N_i$ follows $Binomial(M_i, w_i)$; where $M_i$ follows $Poisson\ lognormal(\mu, \sigma)$

Figure 4.3 shows a comparison of cumulative distribution function (CDF) curves for three different mixing schemes with various number of mixing stages, such as 10 of 25g samples (250g total), 30 of 25g samples (750g total) and 60 of 25g samples (1500g total) blended to form a large composite with an initial concentration parameter of 0.01. In this comparison, it is assumed that there are equal average CFUs in the large unit (mixed unit) in each mixing scheme. The cumulative probability curves of total expected CFUs after mixing intersect at a particular point as shown in Figure 4.3. This point of intersection shifts slightly depending on the number of mixing stages. This indicates that the variability is different for each mixing stage, while the average of total expected CFUs is approximately the same.

Additionally, Figure 4.3 illustrates that, the overall slope of the CDF curve becomes steeper when the number of stages of mixing increase. Hence, it is better to avoid excessive mixing, such as 25,000 revolutions. On the other hand, inadequate mixing will not decrease inhomogeneity, and hence, microbiological non-detection risk will increase.

The mixing operation must continue until a homogenous composite is obtained, so that an analytical sample drawn from it represents the whole batch. Figure 4.4 illustrates that the mean and variance of CFUs in the primary sample are nearly equal after the number of revolutions becomes large such as 200. This implies that the mean converges quickly, but

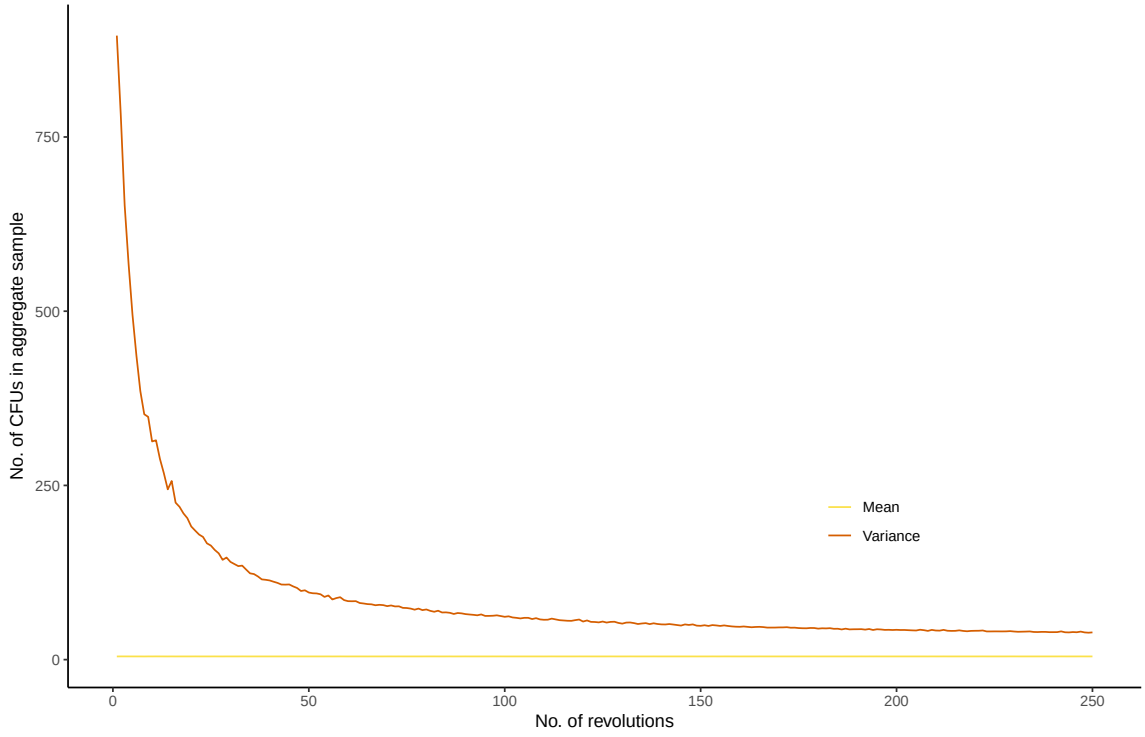


Figure 4.4: Mean and variance of CFUs in the aggregate sample at each mixing stage (number of simulations = 20000, k = 30, $\boldsymbol{\alpha}_{\text{initial}} = \mathbf{0.01}$).

the variance does not; hence, a later stage determines the required number of revolutions. In practice, a minimum, optimised number of mixing revolutions can be stipulated when variance reaches stability. However, stability is slightly influenced by the inconsistent spread of microorganisms in powdered products. In theory, variability will decrease as the number of clusters increases; see Lauer et al. (2015).

Also, Figure 4.4 reveals that variability decreases with the number of revolutions and becomes stable after a specific number of mixing revolutions. This means that the number of clusters increases with revolutions due to clusters breaking at each stage of the mixing.

Figure 4.5 illustrates changes occurring to the expected total number of CFUs at each stage of mixing. Figure 4.5 also displays the simple running average of the total number of CFUs indicated by the red line. The expected total number of CFUs fluctuates with the number of revolutions, but based on the running average curve, the overall trend seems to be stable. Therefore, the next part of this study focuses on other statistical tools for risk assessment.

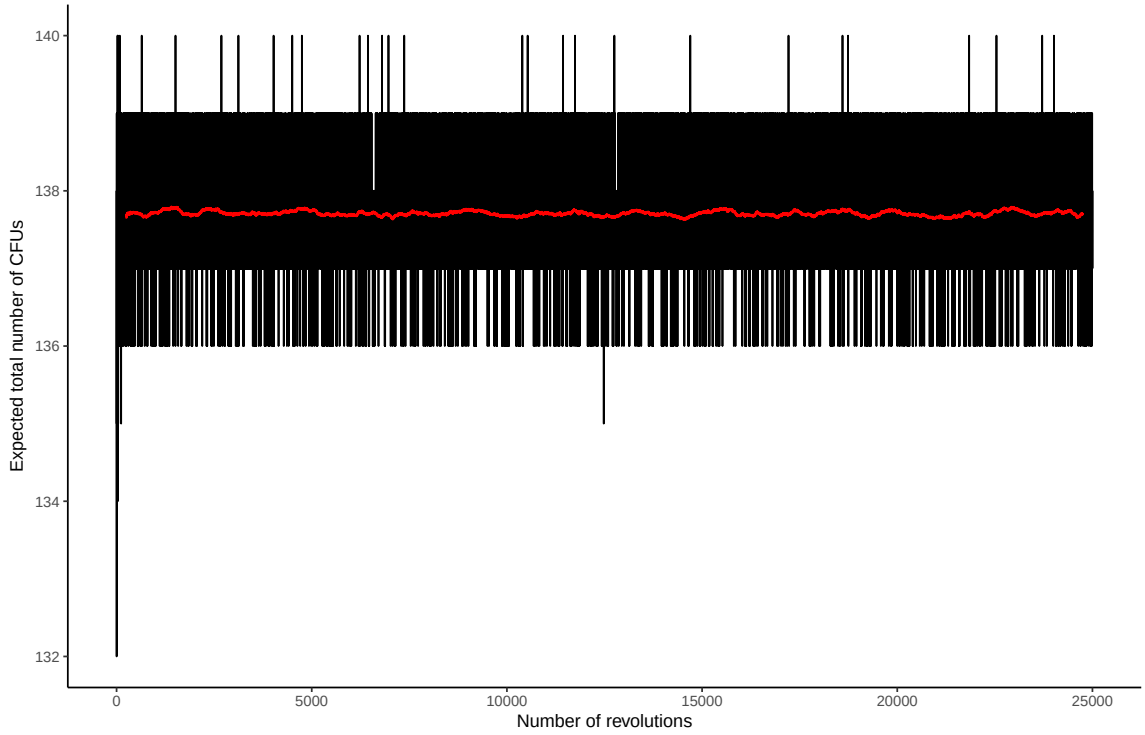


Figure 4.5: The expected total number of CFUs after each mixing revolution based on Poisson lognormal-Type B distribution (number of simulations = 20000, k = 30).

For each mixing revolution, the probability of detection is defined as the number of primary samples containing CFUs greater than the upper decision limit (UDL) out of the total primary samples. Therefore, the probability of detection at $j^{\text{th}}$ stage of mixing $(P_{d_j})$ is given by:

$$P_{d_j} = \frac{\text{number of primary samples which contain CFUs greater than } UDL \text{ at } j^{\text{th}}\text{stage}}{\text{number of primary samples}} \tag{4.8}$$

where UDL is the upper decision limit which depends on the type of pathogens/microorganisms, the amount of primary sample, and testing protocols. For example, according to ISO guidance ISO 6579 (2017), no *Salmonella* spp. must be detected in the 25g sample. Therefore, we used an UDL equal to zero in this study.

In practice, estimating $P_{d_j}$ often necessitates the use of historical data when the number of samples containing CFUs greater than the Upper Detection Limit (UDL) is too numerous to count. This approach requires to address the reliance on historical data and the associated challenges, including the effect that the parameters can only be estimated by trial samples.

We should focus on how the historical data are collected, validated, and utilised in estimating $P_{d_j}$ and the potential limitations and uncertainties involved in relying on such data. Additionally, it is crucial to highlight the importance of ensuring the representativeness and relevance of historical data for accurate estimation, making it an integral aspect of setting the number of mixing revolutions in practical applications.

For this modelling, the probability of detection for each mixing scheme is obtained through simulations by using the following algorithm:

- Step 1: define the mixing parameters $k$, type of distribution, $\sigma$ and $l$.
- Step 2: set the number of simulations. Large scale simulation, such as 20,000, will yield a precise estimation.
- Step 3: set the vector of concentration parameters with all identical values ($\boldsymbol{\alpha} = [\alpha_1, \alpha_1, \cdots \alpha_1]$).
- Step 4: generate the number of CFUs in the primary samples, which comes from a random distribution (such as Poisson lognormal-Type B) at each stage of mixing.
- Step 5: apply the formula (Equation 4.8) to obtain probability of detection $P_{d_j}$, which is the probability of detection at $j^{\text{th}}$ stage of mixing. Using the set upper decision limit, obtain the proportion of non-detects.

Figure 4.6 illustrates the probability of detection vs. the number of mixing revolutions. After a specific number of mixing revolutions, the detection probability is nearly stable. The probability of detection reaches a desirable level at about 500 stages.

Figure 4.7 reveals that fewer primary samples such as 10 to 30 cannot achieve good detection. The cumulative running average detection probability does not change significantly after a particular point in the mixing (Figure 4.7). This means that the mixing method must stipulate a minimum number of revolutions to control the risk of non-detection based on the set $k$. As the number of primary samples $(k)$ increases, the likelihood of capturing a subsample that contains higher level of contamination may decrease, leading to a lower probability of detection (averaging effect) for a fixed level of overall contamination.

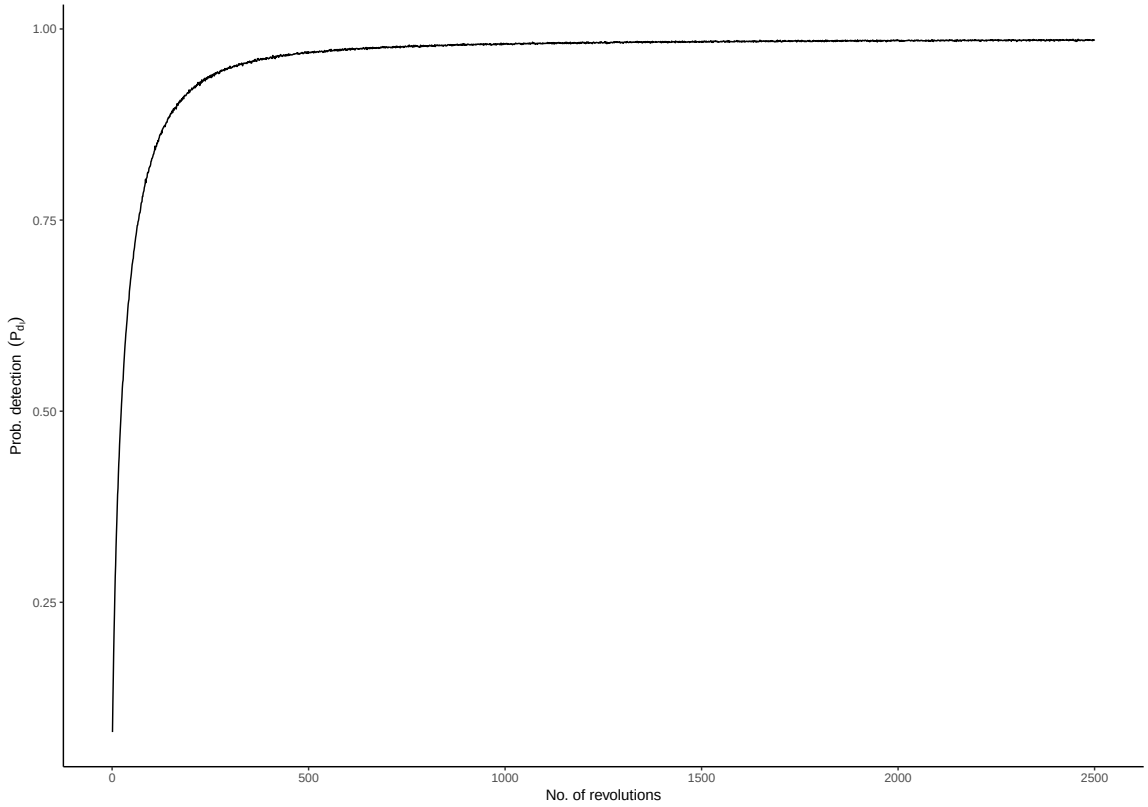


Figure 4.6: Probability of detection at each mixing revolution (number of simulations =2000).

Figure 4.8 illustrates a comparison based on the probability of detection at the end of mixing for two different mixing schemes that underwent vastly different mixing revolutions while other specific parameters remained the same. The probability of detection becomes higher when the number of mixing revolutions is increased for all mean concentration levels. Based on these simulation results, we recommend that blending (mixing) procedures in popular food sampling inspection guidelines also stipulate a minimum number of revolutions to obtain a representative analytical sample, thereby reducing the risk of non-detection.

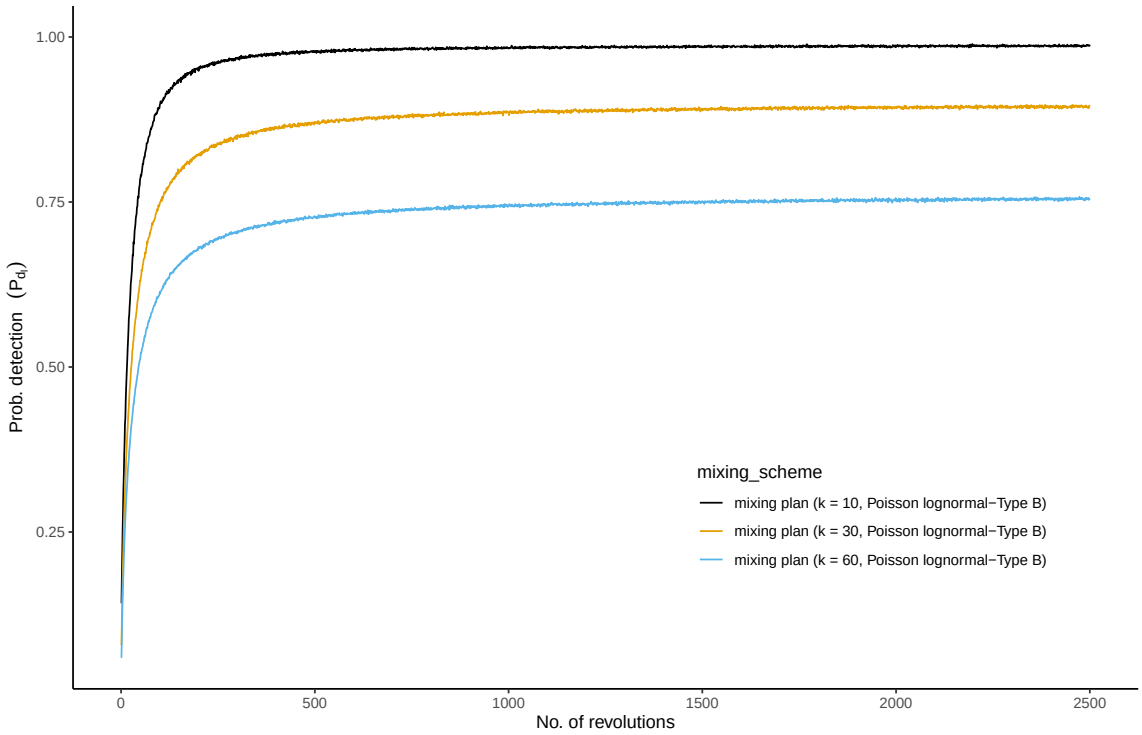


Figure 4.7: Probability of detection versus the number of revolutions of a mixing plan (number of simulations = 2000).

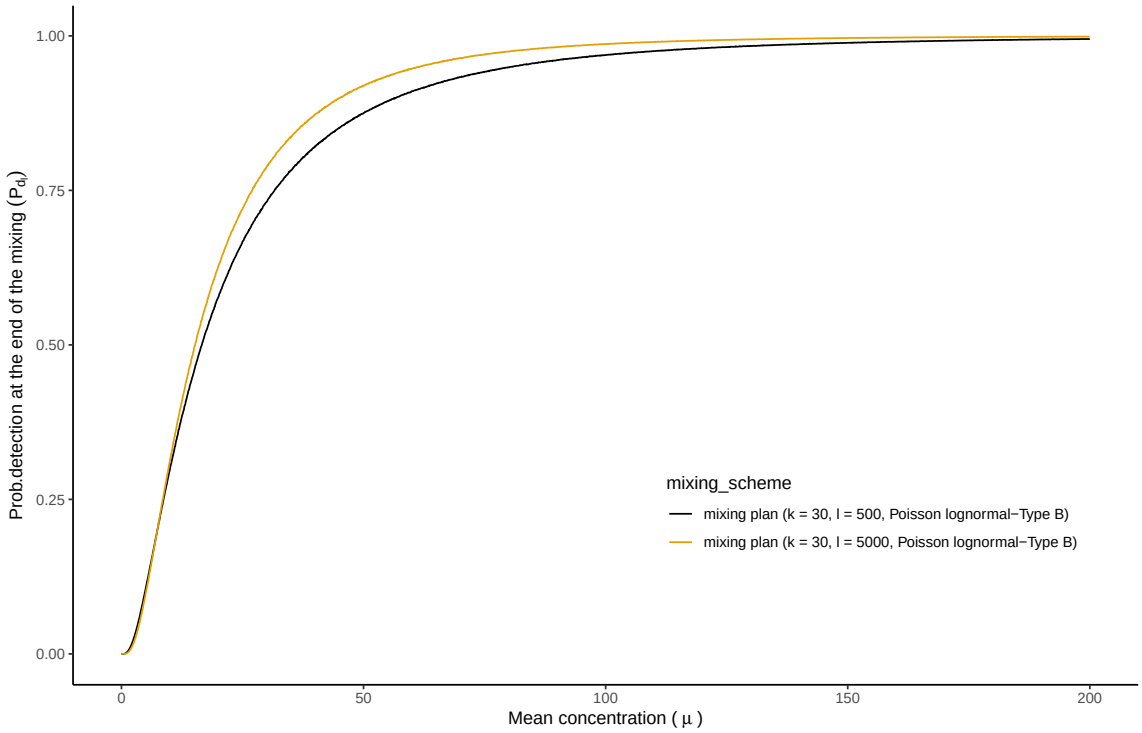


Figure 4.8: Comparison of mixing plans based on estimated detection probability at the end of the mixing (number of simulations = 2000).

## 4.5 R Package "*mixingsimulation*"

We developed an *R* (R Core Team, 2023) software package *mixingsimulation*, (available at https://github.com/Mayooran1987/mixingsimulation) for implementing the results discussed herein. This package composes several graphical displays under various scenarios so that the efficacy of mixing or blending protocols could be assessed. The package allows the user to specify mixing parameters such as the number of revolutions, primary sample size and the type of underlying probability distribution. A description of this package is available at https://mayooran1987.github.io/mixingsimulation.

## 4.6 Conclusions and limitations

The main findings of this chapter are that when mixing/blending does not degrade the product, the number of revolutions in the mixtures is highly associated with the probability of detection. The limitations of this study are that it assumes that each revolution is synonymous to the stage of the mixing procedure; also, we assume that the quantity of the primary sample remains the same at each mixing stage for manual mixing. While mechanical mixing can efficiently achieve a uniform mixture, several issues can arise such as product degradation, which is not addressed in this Chapter. However, we should consider whether the size of CFUs in the primary sample could constitute another significant factor influencing the probability of detection. For instance, primary samples with CFU sizes that cannot be further divided during the mixing process may potentially lead to a reduced probability of detection. This raises an important aspect to explore when examining the intricacies of contamination detection.

We employed the Poisson lognormal distribution-based simulations for the risk assessment. By choosing an optimised number of mixing stages, or revolutions, the risk of non-detection can be reduced.

MASSEY UNIVERSITY
TE KUNENGA KI PŪREHUROA
UNIVERSITY OF NEW ZEALAND

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# STATEMENT OF CONTRIBUTION
# DOCTORATE WITH PUBLICATIONS/MANUSCRIPTS

We, the student and the student's main supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the student's contribution as indicated below in the Statement of Originality.

| | |
|---|---|
| Student name: | Mayooran Thevaraja |
| Name and title of main supervisor: | DR. K. Govindaraju |
| In which chapter is the manuscript/published work? | Chapter 5 |
| What percentage of the manuscript/published work was contributed by the student? | 90% |

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The candidate did the necessary research, statistical modeling, code writing and preparation of the manuscript.

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*This form should be placed at the beginning of each relevant thesis chapter.*

# Chapter 5

# Modelling the Quantity of Material Sampled in the Risk Assessment

## 5.1 Abstract

Under food safety sampling regulations (CAC, 2004; European Commission, 2007; ISO 2859-2, 2020; ISO 3951-1, 2022), the sample size as number of items or amount (in grams, or ml) depends on the lot size. Lot sizes are unequal in bulk production processes such as milk powder production, due to differing customer requirements. Consequently, the quantity of the selected samples from lots is also unequal. Therefore, this Chapter focuses on risk evaluation when unequal size (quantity) primary samples are used to form the composites.

## 5.2 Introduction

It is well known that the microbiological or other contaminants occur unevenly in the production lots; see European Commission (2005), Jongenburger et al. (2012). Generally, lots are supplied based on characteristics of products, specified by the customer. Therefore, the quantity of the material sampled may also vary when large lots are formed using sub-lots produced on different days. The term sublot is defined as the part of a large lot; see ISO 3534-2 (2006). However, if lot sizes are not too large, incremental samples can be selected from production lots (not necessarily after packaging). According to European Commission (2005), the incremental (or primary) samples are collected from lots or sublots. Then, aggregates comprising of incremental samples are used to form a laboratory sample

(or sub-sample) preparation. Under European Commission (2009) guidelines, the sampled amount must be at least 500g for solid feeds, such as the milk powder.

The number of incremental samples for inspection depends on the lot size; see European Commission (2007). In practice, sampling from lots or sublots can be easily done using autosamplers during production but before packaging. The stationary lots of bulk materials cannot be easily sampled and auto-samplers cannot be employed. Consequently, unequal incremental sample sizes arise when sub-lot sizes are not the same.

Mixing unequal incremental samples will cause extra variability in laboratory sample preparation. Typically, extra variability is expected to cause increased risk. This property has been widely studied; see Bann et al. (2022), Cerqueti et al. (2022). Although the risk of microbiological contamination involves many factors, a large portion of the risk can be reduced using an appropriate sampling technique.

However, the non-detection of contaminants may not be pronounced when perfectly mixed sub-samples are used to prepare an analytical sample. Nevertheless, perfectly mixed sub-samples are rarely feasible due to the heterogeneity of contaminants in powdered products. For food safety purposes, microbial counts, and presence/absence testing are commonly used as the quality/safety measure in microbiological risk assessments. For this study, the total amount of material sampled for the $j^{\text{th}}$ sublot is defined as:

$$M_j = \sum_{i=0}^{k-1} m_{ij}, \tag{5.1}$$

where $m_{ij}$ is the quantity of the $i^{\text{th}}$ incremental sample from the $j^{\text{th}}$ sublot, $m_{0j}$ is the minimum initial sample quantity set, and $k$ is the total number of incremental samples, including the initial or first sample.

Figure 5.1 illustrates the sample selection in the first inspection stage for bulk materials. For this study, we assumed that an equal number of incremental samples would be selected from each lot. Also, we assumed that the minimum amount of the incremental samples collected from each lot are identical (i.e., $m_{0j}$'s values are equal for all $j$).

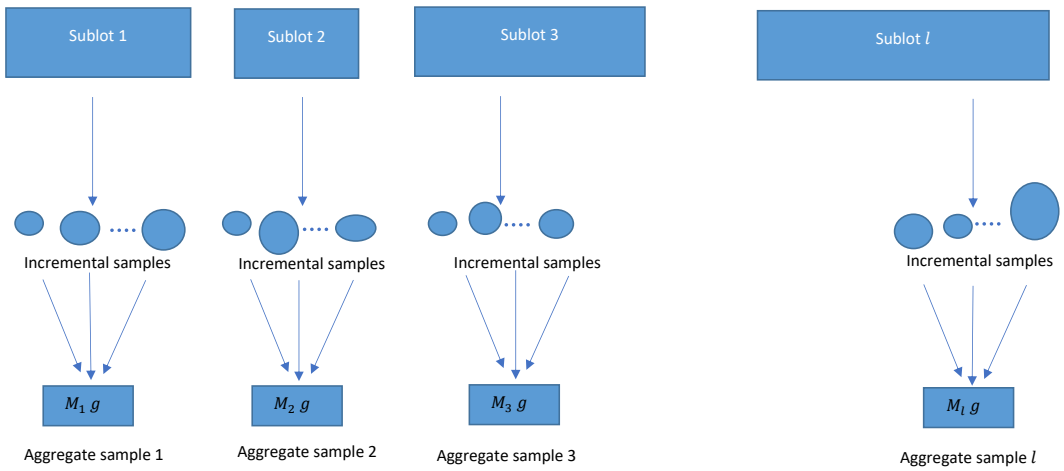


Figure 5.1: Sampling selection in the initial stage of the bulk production process.

In practice, the minimum amount of the incremental sample may be approximately half of the maximum possible amount in the aggregated sample. Santos–Fernández et al. (2016) used $m-$fold increasing amounts of the analytical sample to study the effect of increasing analytical sample amounts. $m-$fold technique means just one of the incremental samples can be proxied $m$ times for forming an aggregate sample. This approach saves computational time in simulations.

When equal incremental amounts form the aggregated sample, risk evaluation can be based on the $m-$fold technique. When unequal amounts form the aggregated sample, the $m-$fold technique fails because the number of microorganisms in each incremental sample would be different. However, contaminant level in the aggregate sample can be estimated based on the corresponding ratio of weights when incremental samples are unequal; see Lovison et al. (1994, p. 152). Therefore, the overall contaminant level can be modelled as a linear combination of contaminant levels of each incremental sample. Depending on the safety parameter, aggregated samples are mandated. For example, incubation methods call for aggregated samples. On the other hand, testing for protein percentage is done using individual samples.

Let $X_{ij}$ be the number of microorganisms in the $i^{\text{th}}$ incremental sample which is used to form $j^{\text{th}}$ aggregate sample, and let $Y_j$ be the number of microorganisms (or contaminant level) in the $j^{\text{th}}$ aggregate sample. Then, $Y_j$ is given by:

$$Y_j = \sum_{i=0}^{k-1} w_{ij} X_{ij}, \tag{5.2}$$

where $w_{ij}$ is the weighting corresponding to the $i^{\text{th}}$ incremental sample of $j^{\text{th}}$ aggregate sample, and $k$ is the total number of incremental samples. According to commonly accepted methods available in the composite sampling literature, the appropriate weighting is proportional to the physical size of the sample; see Lovison et al. (1994, p. 152). Therefore, for the purposes of this study, $w_{ij}$ was defined as the relative weight of $i^{\text{th}}$ incremental sample, which is equal to $m_{ij}/M_j$.

While the proportional representation of contaminants in $m_{ij}/M_j$ assumes a proportional distribution, it does not guarantee a uniform spread throughout the sample. Microorganisms can cluster, leading to non-uniform distributions. The applicability of this assumption varies based on microorganism types, sampling environments, and techniques. Future research can explore when it is likely to hold or require adjustments for real-world scenarios, providing a more nuanced perspective on its validity in different contexts.

Many researchers have studied risk assessment in the food safety area when aggregating equal incremental samples; for example Jarvis (2007). However, the case of unequal incremental samples is not well addressed in the literature. The type of contamination need not be homogeneous irrespective of whether equal or unequal amounts are sampled. Homogeneous contamination is defined as the contamination spread uniformly throughout the lot, and heterogeneous contamination is defined as non-uniformly spread contamination in the lot.

Risk assessment based on the mixing of equal size primary samples was discussed in the previous Chapter. The Poisson mixture distributions are commonly used in microbiological risk assessment for the case of heterogeneous contamination; see Gonzales-Barron et al. (2013), Gonzales-Barron and Butler (2011b), Jongenburger et al. (2015), Jongenburger et al.

(2012), Mussida et al. (2013b), Powell (2015), Schothorst et al. (2009). This chapter focuses on risk assessment of unequal sample amounts under various mixing scenarios employing models, such as Poisson lognormal distribution, or Poisson gamma distribution which are suitable for the case of heterogeneous contamination.

The concentration level is a measure used to report contaminant concentrations, such as microbial count per ml. Following Santos–Fernández et al. (2016) and Jongenburger et al. (2015), the following five possible scenarios are considered for this Chapter:

- Scenario 1 — lots with homogeneous contamination;
- Scenario 2 — lots with heterogeneous, high-level contamination;
- Scenario 3 — lots with heterogeneous, low-level contamination;
- Scenario 4 — lots with homogeneous contamination and concentration levels fluctuating from sublot to sublot; and
- Scenario 5 — lots with heterogeneous contamination and concentration levels fluctuating from sublot to sublot.

This study investigated two important issues (1) the effect of unequal contaminant quantities in incremental samples; and (2) the quantity of the incremental samples. This Chapter considers the probability of detection, operating characteristic (OC) curve-based comparison for risk assessment in each scenario. Also, we developed an open-source R package to evaluate OC curves and other risk measures.

We also considered example sampling schemes (sets of incremental samples) as per the relevant regulatory authority's guidance (European Commission, 2009). Decisions on the number of incremental samples depend on lot size for both solid and powdered products. For example, every aggregate sample is required to be a minimum of 0.5kg (the weight of the final sample), and seven is the minimum number of incremental samples mandated by the European Commission (2009) if the lot size does not exceed 2.5 metric tonnes.

Small incremental amounts can be sampled more frequently using industrial autosamplers; see Govindaraju et al. (2017). For example, 10g of milk powder can be selected every five minutes from a bulk production process. Based on European Commission (2009) guidelines, we used 10g × 50 samples in our initial sampling scheme, and the second scheme consisted of 50 samples of different weights, e.g., 10g, 15g and 20g . A total amount of 0.5kg formed the final aggregated sample. Detailed descriptions of the two sampling schemes are given in Appendix 5.C.

This Chapter concentrates on the theoretical risk assessment in certain commonly occurring scenarios. Simulation technique is again used in this study to validate the theoretical results derived, or when the derivations were complex. Mathematical proofs are shown in Appendix 5.A. Algorithms for simulations are provided in the main body of this Chapter. Simulation results can be obtained using the open-source R package provided. All symbols and abbreviations are illustrated in Table 5.1.

This Chapter is organised in the following way. After the introduction, Section 5.3 discusses five scenarios based risk assessments based on presence/absence testing using a two-class attribute sampling plan. For probability of detection calculations, compound Poisson or Poisson mixture distribution models are utilised in each of the five scenarios. Also, Section 5.3 describes two-class-attribute risk assessments based on average outgoing quality (AOQ) limits and explores the impact of variability in risk assessment when using two different sampling schemes. Section 5.4 discusses the results of this theoretical study; the associated R package is described in Section 5.5 and finally conclusions are given in 5.6.

## 5.3 Microbial Count-Based Risk Assessment

This section focuses on risk assessment based on presence/absence testing using a two-class attribute sampling plan. Presence/absence two-class testing forms the basis for a detection test. If we inspect $n$ samples, the probability of acceptance $(P_a)$ is given by:

$$P_a = \sum_{x=0}^{c} \binom{n}{x} {P_d}^x (1 - P_d)^{n-x}, \tag{5.3}$$

where $P_d$ is the probability of detection in a single test, and $c$ is the acceptance number. The zero-acceptance sampling plan $(c = 0)$ is commonly employed in microorganism detection plans. If $c = 0$, then the probability of acceptance $P_a$ is given by:

$$P_a = (1 - P_d)^n \tag{5.4}$$

When rejected lots are discarded, risk assessment can be based on the average outgoing quality $(AOQ)$ performance. Only lots that pass inspection are cleared because rectification is often infeasible for bulk products. Since $P_a$ is the probability of acceptance, $\lambda$ is the expected mean of cell counts for incremental samples, and the average outgoing mean of cell counts is given by the product of $\lambda P_a$. The average outgoing quality limit $(AOQL)$ is defined as the maximum outgoing contaminated primary increment, and is given by:

$$AOQL = \max_{\lambda \geq 0} \lambda p_a \tag{5.5}$$

In practice, the $\lambda$ value is not a constant when incremental samples are collected from heterogeneously contaminated lots. However, each $\lambda$ can be written in terms of the mass (of the incremental samples and the minimum incremental quantity) adjusted mean of cell counts as shown in Equation 5.7 given in the next section.

### 5.3.1 Scenario 1: Homogeneous Contamination

Microbiological risk assessments for lots with homogeneous contamination were conducted using presence/absence testing and based on a minimum quantity of incremental samples per sampling scheme. If the contamination in a lot is homogeneous, sublots and selected incremental samples can be presumed as homogeneously contaminated. Due to the homogeneous contamination across the lots, the concentration of contamination would be expected to be the same.

Incremental samples are selected from lots, or sublots, for laboratory sample preparation. For $k$ incremental samples taken from a lot, the formula for total amount of the aggregate sample was given in Equation 5.1. Unequal incremental samples would have different levels of contamination proportional to their quantity or mass.

For this study, we define the minimum quantity of the incremental samples as $m_{0j}$, selected from the $j^{\text{th}}$ sublot. Let $X_{ij}$ be the number of microorganisms in the $i^{\text{th}}$ incremental sample, selected from the $j^{\text{th}}$ sublot. The quality of lots (or sublots) are assumed to be independent. Consequently, we can assume that spatial correlations between incremental samples to be absent. Based on the literature, we can model this homogeneous contamination scenario by using Poisson distribution; see Fernando (2021). Thus, the probability mass function of $X_{ij}$ is given by:

$$P(X_{ij} = x_{ij}) = \frac{{\lambda_{ij}}^{x_{ij}} e^{-\lambda_{ij}}}{x_{ij}!}; \quad x_{ij} = 0, 1, 2, \cdots\cdots \tag{5.6}$$

where $\lambda_{ij}$ is the expected cell count in the $i^{\text{th}}$ incremental sample selected from the $j^{\text{th}}$ sublot. Suppose we assume that the expected cell count for the $i^{\text{th}}$ incremental sample is $\lambda_{ij}$, then we can write $\lambda_{ij}$ in terms of $m_{ij}, m_{0j}$ and $\lambda_{0j}$, which can be calculated as:

$$\lambda_{ij} = \lambda_{0j} \left( \frac{m_{ij}}{m_{0j}} \right); \quad i = 0, 1, 2, \cdots\cdots, k-1 \tag{5.7}$$

Let $Y_j$ be the number of microorganisms in the $j^{\text{th}}$ aggregate sample, which can be estimated from the $j^{\text{th}}$ sublot (Equation 3.2). Therefore, the probability of detection $(P_{d_j})$ in a single aggregate sample can be defined as

$$P_{d_j} = 1 - \exp\left( -\frac{M_j \lambda_{0j}}{m_{0j}} \right) \tag{5.8}$$

where $M_j$ is the weight of the $j^{\text{th}}$ aggregate sample, and $m_{0j}$ is the minimum quantity of the incremental sample; and $\lambda_{0j}$ represents expected cell count. Therefore, if we inspect $n$ number of aggregate samples, the probability of acceptance when using a zero-acceptance sampling plan $(c = 0)$ is given by:

$$P_{a_j} = (1 - P_{d_j})^n \tag{5.9}$$

where $P_{d_j}$ is the probability of detection in the $j^{\text{th}}$ aggregate sample. All mathematical proofs are provided in Appendix 5.A.

Recall that $Y_j$ is the number of cell counts in an aggregate sample. Expected values of $Y_j$ are given by:

$$E(Y_j) = \frac{\lambda_{0j}}{M_j m_{0j}} \sum_{i=0}^{k-1} m_{ij}^2 \tag{5.10}$$

Variances are

$$Var(Y_j) = \frac{\lambda_{0j}}{M_j^2 m_{0j}} \sum_{i=0}^{k-1} m_{ij}^3 \tag{5.11}$$

In the food safety area, expected cell count $E(X_{ij})$ becomes $\lambda_{ij}$ and can be linked to the log mean concentration $(\mu_{ij})$ parameter as $\lambda_{ij} = 10^{\mu_{ij}+0.5\log(10)\sigma_w^2}$ under the Poisson-lognormal model; see FAO/WHO (2016), Santos–Fernández et al. (2016), Thevaraja et al. (2021).

**Algorithm 5.1** Computation of probability of detection in the $j^{\text{th}}$ aggregate sample under scenario 1

1. Initialise the number of incremental samples $(k)$ and the vector of incremental samples $(m_{1j}, m_{2j}, \cdots m_{kj})$.
2. Set the weights according to the respective quantity (e.g., in g) of incremental samples, where $w_{ij} = m_{ij} / \sum(m_{ij})$.
3. Set the number of simulations, $n_{sim}$. Use a large value (e.g., $100,000$) to obtain a more precise result.
4. Generate number of microorganisms by using Poisson distribution, with parameter $\lambda_{ij} (= 10^{\mu_{ij}+0.5\log(10)\sigma^2})$ with $n_{sim}$ rows and $k$ columns.
5. Multiply each column element by its corresponding weight.
6. Sum by rows to obtain the number of microorganisms in the aggregate sample.
7. Calculate the probability of detection $(P_{d_j})$ as the proportion of $Y_j$ units with one or more microorganisms.

In the food safety assurance, the value of standard deviation within lot $(\sigma_w)$ is set empirically. We used $\sigma_w = 0.8$ for inhomogeneous contamination following Gonzales-Barron et al. (2013, p. 370), Dahms (2004), Schothorst et al. (2009), Mussida et al. (2013b) and Powell (2015).

Theoretical results derived in this Chapter are the detection probability, expectation and variance of the number of cell counts in an aggregate sample, which were also verified using simulation. We estimated the probability of detection by using a simulation algorithm (Algorithm 1), and the probability of acceptance was derived as per Equation 5.9. The probability of detection according to our theoretical results $(0.42047)$ and simulation $(0.42026)$ were close when 2,000,000 simulations were run.

However, the scenario of homogeneous contamination is infrequent in practice. Therefore, the following sections investigate more realistic scenarios, which are (1) heterogeneous contamination (Scenario 2 and Scenario 3) and (2) contamination levels fluctuating from sublot to sublot (Scenario 5).

### 5.3.2 Scenario 2: Heterogeneous, High-Level Contamination

In practice, microbial contamination is unevenly distributed over a lot. Sampling aims to ensure that each incremental sample represents the sublot. According to the literature, the microbial count can be modelled by Poisson lognormal distribution when the level of contaminations is high; see Schothorst et al. (2009) and Gonzales-Barron and Butler (2011b).

In Scenario 2, we assume that $X_{ij}$ follows Poisson lognormal distribution with parameters $(\mu_{ij}, \sigma_w)$. This means that $X_{ij}$ follows Poisson distribution with parameter $\lambda_{ij}$, which follows lognormal distribution, along with parameters $(\mu_{ij}, \sigma_w)$. Following Bulmer (1974) methodology, the probability mass function of $X_{ij}$ is given by:

$$P(X_{ij} = x_{ij}|\mu_{ij}, \sigma_w) = \frac{1}{\sqrt{2\pi}\sigma_w x_{ij}!} \int_0^\infty \exp(-\lambda_{ij}) \lambda_{ij}^{x_{ij}-1} \exp\left(-\frac{(\log(\lambda_{ij}) - \mu_{ij})^2}{2\sigma_w^2}\right) d\lambda_{ij} \tag{5.12}$$

where $\mu_{ij}$ is the mean concentration level, and $\sigma_w$ is the standard deviation within a lot. The number of microorganisms in the aggregate sample is equal to the sum of all microorganisms in the incremental samples. The probability of detection in an aggregate sample equals the proportion of aggregate units with one or more microorganisms. Therefore, the probability of detection in the $j^{\text{th}}$ aggregate sample is given by:

$$P_{d_j} = 1 - \left[\frac{1}{\sqrt{2\pi}\sigma_w}\right]^k \prod_{i=0}^{k-1} \left[\int_{-\infty}^{\infty} \exp\left(-\exp(z) - \frac{(z - \log_{10}(m_{ij}/m_{0j}) + \mu_{0j})^2}{2\sigma_w^2}\right) dz\right] \quad (5.13)$$

where $z = \log(\lambda_{ij})$ is the substitution variable used to simplify the formula. The mathematical proof is illustrated in Appendix 5.A. A closed form solution for probability detection from Equation 5.13 could not be obtained, but we can solve it numerically. Also, we used simulation techniques to verify this result.

The simulation algorithm we used for computation of probability of detection is shown in Algorithm 2. For example, the theoretical probability of detection estimation $(0.99872)$ and simulation $(0.99872)$ were the almost same with $2,000,000$ simulations.

**Algorithm 5.2** Computation of probability of detection in the $j^{\text{th}}$ aggregate sample under scenario 2

1. Initialise the number of incremental samples $(k)$ and the vector of incremental samples $(m_{1j}, m_{2j}, \cdots m_{kj})$.
2. Set the weights of the incremental samples, where $w_{ij} = m_{ij} / \sum(m_{ij})$.
3. Set the number of simulations, $n_{sim}$. Use a large value (e.g., $100,000$) to obtain a more precise result.
4. Generate number of microorganisms by using Poisson lognormal distribution with parameters $\mu, \sigma$ and with $n_{sim}$ rows and $k$ columns.
5. Multiply each column element by its corresponding weight.
6. Sum by rows to obtain the number of microorganisms in the aggregate sample.
7. Calculate the probability of detection $(P_{d_j})$ as the proportion of $Y_j$ units with one or more microorganisms.

The probability of acceptance formula was given as Equation 5.9. Expected values and variances of $Y_j$ can be derived by using the Poisson lognormal probability mass function. All derived mathematical proofs are given in Appendix 5.A. Expected values are given by:

$$E(Y_j) = \left(\frac{\exp\left(0.5\sigma_w^2\right)}{M_j}\right) \sum_{i=1}^{k} m_{ij} \exp\left(\log_{10}\left(m_{ij}/m_{0j}\right) + \mu_{0j}\right) \tag{5.14}$$

Variances are given by:

$$\begin{aligned} Var(Y_j) = &\left(\frac{\exp\left(0.5\sigma_w^2\right)}{M_j^2}\right) \sum_{i=0}^{k-1} m_{ij}^2 \exp\left(\log_{10}\left(m_{ij}/m_{0j}\right) + \mu_{0j}\right) \\ &+ \left(\frac{\left(\exp(\sigma_w^2) - 1\right)\exp(\sigma_w^2)}{M_j^2}\right) \sum_{i=0}^{k-1} m_{ij}^2 \exp\left(2\log_{10}\left(m_{ij}/m_{0j}\right) + \mu_{0j}\right) \end{aligned} \tag{5.15}$$

### 5.3.3 Scenario 3: Heterogeneous, Low-Level Contamination

When lot contamination is heterogeneous, low-level contamination scenarios are examined first. The total number of microorganisms in the aggregate sample can be obtained from Equation 5.2. Following the literature, low level of contaminations can be modelled using Poisson gamma distribution (Gonzales-Barron and Butler, 2011b; Schothorst et al., 2009). We assumed no correlation between the number of microorganisms in the selected incremental samples. Therefore, $X_{ij}$ follows Poisson gamma distribution with parameters $(\lambda_{ij}, K)$. Thus, the probability mass function is obtained by:

$$P(X_{ij} = x_{ij}|\lambda_{ij}, K) = \frac{\Gamma(x_{ij} + K)}{\Gamma(K)x_{ij}!}\left(\frac{\lambda_{ij}}{1+\lambda_{ij}}\right)^K \left(\frac{1}{1+\lambda_{ij}}\right)^{x_{ij}} \tag{5.16}$$

where $\Gamma$ is the gamma function, $\lambda_{ij}$ is the arithmetic mean of cell counts, and $K$ is the dispersion parameter. Then, the probability of detection in the $j^{\text{th}}$ aggregate sample is given by:

$$P_{d_j} = 1 - \prod_{i=0}^{k-1}\left[\frac{m_{ij}\lambda_{0j}}{m_{0j} + m_{ij}\lambda_{0j}}\right]^K \tag{5.17}$$

The expected value is obtained as:

$$E(Y_j) = \left(\frac{K}{M_j}\right) \sum_{i=0}^{k-1} m_{ij} \left(\frac{m_{ij}\lambda_{0j}}{m_{0j}}\right) \tag{5.18}$$

The variance formula is:

$$Var(Y_j) = \left(\frac{K}{M_j^2}\right) \sum_{i=0}^{k-1} m_{ij}^2 \left(\frac{m_{ij}\lambda_{0j}}{m_{0j}} + \left[\frac{m_{ij}\lambda_{0j}}{m_{0j}}\right]^2\right) \tag{5.19}$$

All mathematical proofs are provided in Appendix 5.A. Following the literature, the parameter $K$ for Poisson gamma distribution can be fixed empirically in the range of 0.044 and 0.401 (Gonzales-Barron and Butler, 2011a; Mussida et al., 2013a). We used $K = 0.05$ for illustrative purposes.

Probability of detection can also be well approximated via simulation (Algorithm 3), which is useful as a validation of theoretical results. For example, the theoretical probability of detection estimation $(0.02684)$ and simulation $(0.02688)$ were close when $2,000,000$ simulations were run.

**Algorithm 5.3** Computation of probability of detection in the $j^{\text{th}}$ aggregate sample under scenario 3

1. Initialise the number of incremental samples $(k)$ and the vector of incremental samples $(m_{1j}, m_{2j}, \cdots m_{kj})$.
2. Set the weights according to the respective quantity (e.g., in g) of the incremental samples, where $w_{ij} = m_{ij} / \sum(m_{ij})$.
3. Set the number of simulations, $n_{sim}$. Use a large value (e.g., $100,000$) to obtain a more precise result.
4. Generate number of microorganisms by using Poisson gamma distribution with parameters $K, \lambda$ with $n_{sim}$ rows and $k$ columns.
5. Multiply each column element by its corresponding weight.
6. Sum by rows to obtain the number of microorganisms in the aggregate sample.
7. Calculate the probability of detection $(P_{d_j})$ as the proportion of $Y_j$ units with one or more microorganisms.

### 5.3.4 Scenario 4: Homogeneous Contamination and Concentration Levels Fluctuating from Sublot to Sublot

Scenario 4 considers lots with homogeneous contamination, but concentration levels fluctuate from sublot to sublot. This means that each sublot has a different concentration level of contaminants, but it is assumed that the contamination has spread uniformly within the sublot. Each primary sample is selected from the corresponding sublot, which has a specific concentration level. Suppose we assume that $k$ incremental samples are collected from each sublot, and $l$ sublots are used.

In this scenario, we used an approach based on contaminant concentration via the minimum quantity of the incremental sample to assess risk. Following Santos–Fernández et al. (2016), the mean concentration level of microorganisms in the minimum quantity sampled $\mu_{0j}$ follows a normal distribution with parameters $\mu$ and $\sigma_b$, where $\sigma_b$ is the standard deviation between sublots. The number of sublots does not affect the risk when contamination does

not fluctuate. Nevertheless, in this scenario, each set of incremental samples (taken from the corresponding sublot) arise from a source with a unique level of contamination. Therefore, the number of microorganisms in each incremental sample can be modelled according to Poisson distribution with parameter $\lambda_{ij}$, where $\lambda_{ij} = 10^{\mu_{ij}+0.5log(10)\sigma_w^2}$. Also, $\mu_{0j}$ follows normal distribution with parameters $\mu$ and $\sigma_b$.

The probability mass function of $X_{ij}$ is given by:

$$P(X_{ij} = x_{ij}|\lambda_{ij}) = \frac{\lambda_{ij}^{x_{ij}} \exp(-\lambda_{ij})}{x_{ij}!} \; ; \; x_{ij} = 0, 1, 2, \cdots \tag{5.20}$$

But $\lambda_{ij} = \lambda_{0j}(m_{ij}/m_{0j})$, $\lambda_{0j} = 10^{\mu_{0j}+0.5\log(10)\sigma_w^2}$ and $\mu_{0j} \sim Normal(\mu, \sigma_b)$. Therefore, the probability mass function of $X_{ij}$ is given by:

$$\begin{aligned} &P(X_{ij} = x_{ij}|\boldsymbol{\mu_{0j}} = \mu) \\ &\qquad = \frac{(10^{\mu+0.5\log(10)\sigma_w^2})^{x_{ij}} \exp(-10^{\mu+0.5\log(10)\sigma_w^2}(m_{ij}/m_{0j}))}{x_{ij}!} \; ; \; x_{ij} = 0, 1, 2, \cdots \end{aligned} \tag{5.21}$$

Relevant results for this scenario can be deduced from Scenario 1, which has the same distribution. Therefore, we can obtain the probability of detection expressions in a similar manner as shown below. In scenario 1 concentration level of contamination does not fluctuate over the sublots, but concentration levels fluctuate from sublot to sublot in this scenario (Scenario 4). Let $l$ be the number of sublots from which incremental samples are selected. Then, the probability of detection $(P_d)$ in an aggregate sample is given by

$$\begin{aligned} P_d = 1 - P_{nd} &= 1 - P(\text{ND in sublot 1 and ND in sublot 2} \cdots \text{ND in sublot l}) \\ &= 1 - \prod_{j=1}^{l} P(\text{ND in sublot j}) \end{aligned} \tag{5.22}$$

where ND denotes non-detection.

Hence, we can get

$$P_D = 1 - \prod_{j=1}^{l} \left[ 1 - \exp\left( -\frac{M_j}{m_{0j}} \times \lambda_{0j} \right) \right] \tag{5.23}$$

where $M_j$ is the weight of the $j^{\text{th}}$ aggregate sample, and $m_{0j}$ is the minimum quantity of the incremental samples which is used to form $j^{\text{th}}$ aggregate sample. Then, $\lambda_{0j} = 10^{\mu_{0j}+0.5\log(10)\sigma_w^2}$ is the expected cell count, and $\mu_{0j}$ follows normal distribution with parameters $\mu$ and $\sigma_b$.

The expected value of $Y_j$ is given by:

$$E(Y_j) = \frac{\lambda_{0j}}{M_j m_{0j}} \sum_{i=0}^{k-1} m_{ij}^2 \tag{5.24}$$

The variance of $Y_j$ is given by:

$$Var(Y_j) = \frac{\lambda_{0j}}{M_j^2 m_{0j}} \sum_{i=0}^{k-1} m_{ij}^3 \tag{5.25}$$

For probability of detection estimation, we used the simulation Algorithm 4, which is implemented in the R package decribed in Section 5.5.

**Algorithm 5.4** Computation of probability of detection in scenario 4.

1. Initialise the number of incremental samples $(k)$ and the vector of incremental samples $(m_{1j}, m_{2j}, \cdots m_{kj})$ pertaining to $j^{\text{th}}$ sublot.
2. Set the weights of the incremental samples, where $w_{ij} = m_{ij} / \sum(m_{ij})$.
3. Set the number of simulations, $n_{sim}$. Use a large value (e.g., $100,000$) to obtain a more precise result.
4. Generate $l$ number of the $\mu_{0j}$s by using normal distribution with parameters $\mu, \sigma_b$ and estimate corresponding $\lambda_{0j}$.
5. Generate number of microorganisms by using Poisson distribution with parameters $\lambda_{0j}$ and with $n_{sim}$ rows and $k$ columns.
6. Multiply each column element by its corresponding weight.
7. Sum by rows to obtain the number of microorganisms in the aggregate sample.
8. Calculate the probability of detection $(P_d)$ as the proportion of $Y$ units with one or more microorganisms in the aggregate sample.
9. Repeat steps 1 to 8 for all $j$ to estimate the probability of detection.
10. Obtain overall probability of detectin by using the Equation 5.22.

### 5.3.5 Scenario 5: Heterogeneous Contamination and Concentration Levels Fluctuating from Sublot to Sublot

Scenario 5 considers lots with heterogeneous contamination, and concentration levels are allowed fluctuate from sublot to sublot. Therefore, each incremental sample selected from a sublot has a specific expected concentration level of contaminants.

Since sublots are heterogeneous in their contamination, let $X_{ij}$ represent the number of microorganisms in the $i^{\text{th}}$ incremental sample. This is modelled using compound Poisson lognormal distribution and previously derived theoretical results (in Scenario 2) for the probability of detection, expectation and variance estimations when concentrations are identical in all sublots. Therefore, we can deduce the results for Scenario 2, which are

based on the minimum quantity of the incremental sample; the mean concentration in the minimum quantity sampled follows a normal distribution for $\mu$ and $\sigma_b$. The probability mass function of $X_{ij}$ can be expressed as:

$$P(X_{ij} = x_{ij}|\boldsymbol{\mu_{0j}} = \mu, \sigma_w) = \frac{1}{\sqrt{2\pi}\sigma_w x_{ij}!} \int_0^\infty \exp(-\lambda_{ij})\lambda_{ij}^{x_{ij}-1} \exp\left(-\frac{\left(\log(\lambda_{ij}) - \left(\log_{10}\left(m_{ij}/m_{0j}\right) + \mu\right)\right)^2}{2\sigma_w^2}\right) d\lambda_{ij} \quad (5.26)$$

Therefore, the probability of detection in the $j^{\text{th}}$ aggregate sample at $\boldsymbol{\mu_{0j}} = \mu$ can be derived as given the following equation:

$$P_{d_j} = 1 - \left[\frac{1}{\sqrt{2\pi}\sigma_w}\right]^k \prod_{i=0}^{k-1} \left[\int_{-\infty}^{\infty} \exp\left(-\exp(z) - \frac{\left(z - \log_{10}\left(m_{ij}/m_{0j}\right) + \mu\right)^2}{2\sigma_w^2}\right) dz\right] \quad (5.27)$$

The expected value of $Y_j$ at $\boldsymbol{\mu_{0j}} = \mu$ is given by:

$$E(Y_j) = \left(\frac{\exp\left(0.5\sigma_w^2\right)}{M_j}\right) \sum_{i=1}^{k} m_{ij} \exp\left(\log_{10}\left(m_{ij}/m_{0j}\right) + \mu\right) \quad (5.28)$$

The variance of $Y_j$ at $\boldsymbol{\mu_{0j}} = \mu$ is given by:

$$Var(Y_j) = \left(\frac{\exp\left(0.5\sigma_w^2\right)}{M_j^2}\right) \sum_{i=0}^{k-1} m_{ij}^2 \exp\left(\log_{10}\left(m_{ij}/m_{0j}\right) + \mu\right) + \left(\frac{\left(\exp(\sigma_w^2) - 1\right)\exp(\sigma_w^2)}{M_j^2}\right) \sum_{i=0}^{k-1} m_{ij}^2 \exp\left(2\log_{10}\left(m_{ij}/m_{0j}\right) + \mu_{0j}\right) \quad (5.29)$$

In Scenario 5, we assessed the risk based on probability of detection across two different sampling schemes. Probability of detection, found using Equation 5.22, and Algorithm 5 are implemented in the contributed R package *"uneqmixr"*.

**Algorithm 5.5** Computation of probability of detection in scenario 5.

1. Initialise the number of incremental samples $(k)$ and the vector of incremental samples $(m_{1j}, m_{2j}, \cdots m_{kj})$ pertaining to $j^{\text{th}}$ sublot.
2. Set the weights of the incremental samples, where $w_{ij} = m_{ij} / \sum(m_{ij})$.
3. Set the number of simulations, $n_{sim}$. Use a large value (e.g., $100,000$) to obtain a more precise result.
4. Generate the $\mu_{0j}$ by using normal distribution with parameters $\mu, \sigma_b$.
5. Generate number of microorganisms by using Poisson lognormal distribution with parameters $(\mu_{0j}, \sigma_w)$ and with $n_{sim}$ rows and $k$ columns.
6. Multiply each column element by its corresponding weight.
7. Sum by rows to obtain the number of microorganisms in the aggregate sample.
8. Calculate the probability of detection $(P_d)$ as the proportion of $Y$ units with one or more microorganisms in the aggregate sample.
9. Repeat steps 1 to 8 for all $j$ to estimate the probability of detection.
10. Obtain overall probability of detectin by using the Equation 5.22.

## 5.4 Results and Discussion

### 5.4.1 Probability of Detection Based Risk Assessment

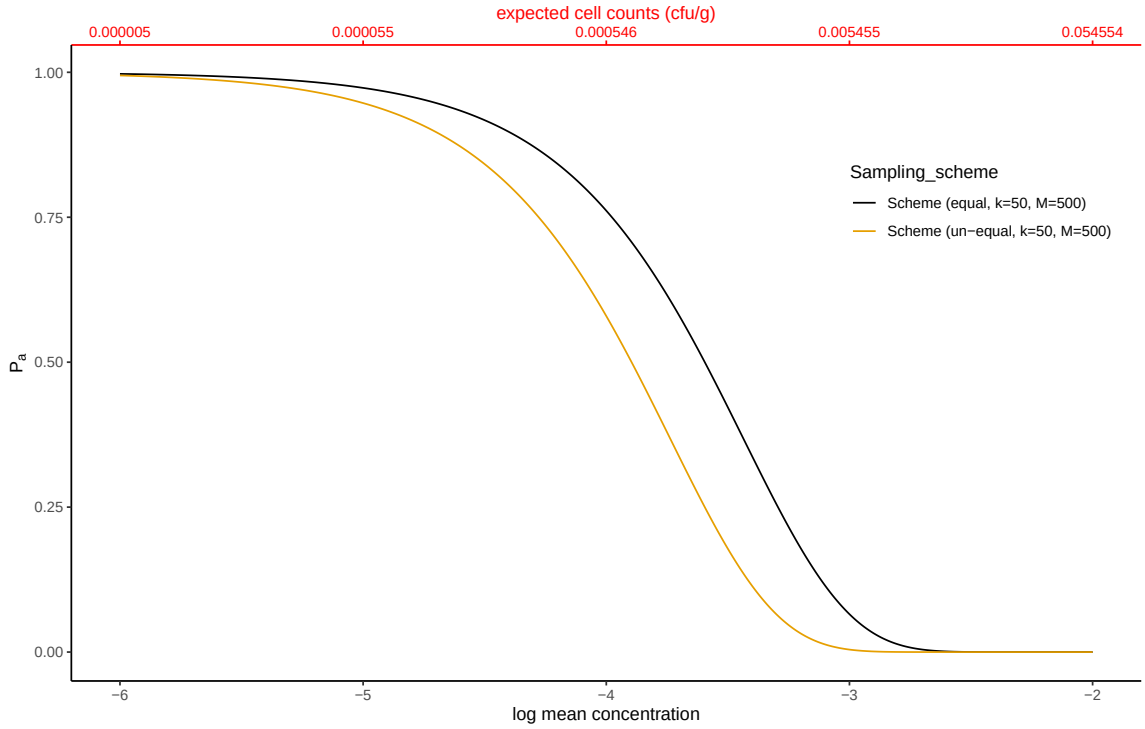


Figure 5.2: Operating characteristics (OCs) for two different sampling schemes in Scenario 1 ($n = 10$).

Figure 5.2 illustrates a comparison based on OC curves for equal and unequal sample quantities but the total amount of aggregated samples remains the same. Two sampling schemes are considered in Figure 5.2. Each scheme has a minimum quantity of incremental samples of $10g$ and $5g$, respectively, but the total quantity of the aggregate sample is $500g$ in each scheme. The first sampling scheme considered 50 $\times$ $10g$ incremental samples, and the second scheme considered 50 $\times$ unequal incremental samples, i.e., $5g, 10g, 20g$ and $30g$. Also, for the purposes of comparison, 10 $\times$ aggregate samples were considered. The probability of acceptance was consistently high when using equal incremental samples (curve shown in black in Figure 5.2). Therefore, in the homogeneous contamination scenario, incremental samples with unequal amounts lowers the probability of acceptance and hence the consumer's risk. However the producer's risk is increased due to lowering the probability of acceptance.

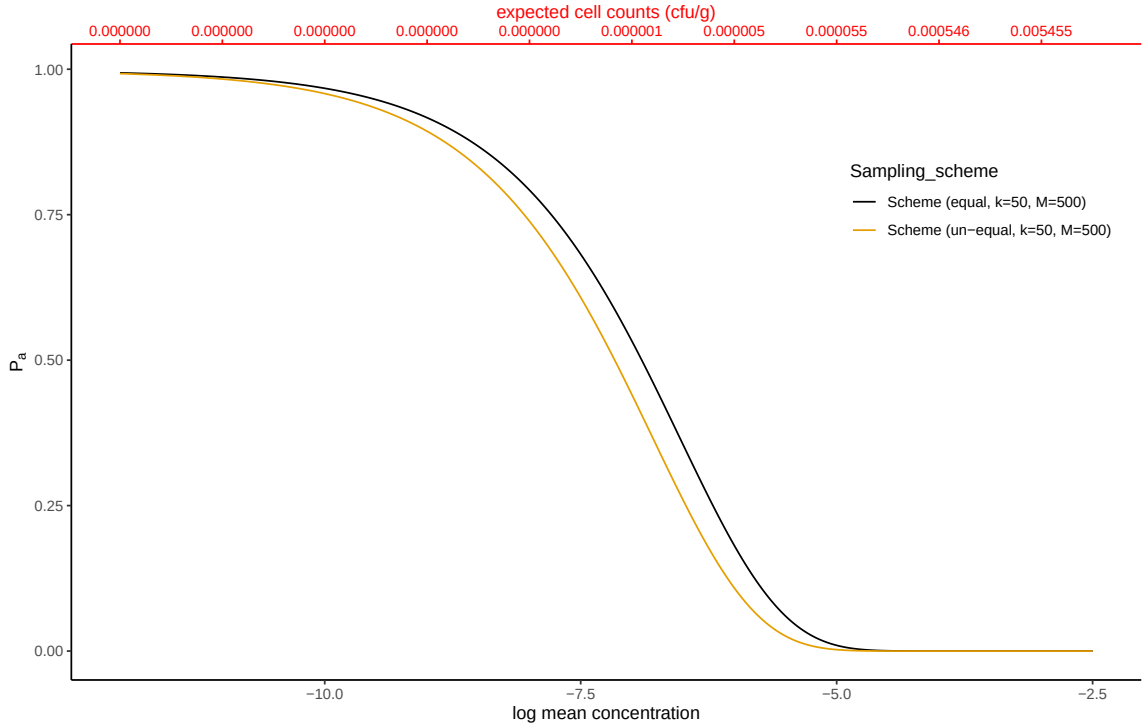


Figure 5.3: Operating characteristics (OCs) for two sampling schemes in Scenario 2, based on Poisson lognormal distribution ($\sigma_w = 0.8, n = 10$).

Figure 5.3 illustrates two different incremental sampling schemes when samples were collected from a lot with heterogeneous, high-level contamination. In Scenario 2, we used the same sampling scheme as we used in Scenario 1. The sampling schemes were: (1) 50 $\times$ 10$g$ incremental samples (equal weights); and (2) 50 $\times$ incremental samples of varying amounts, i.e. 5$g$, 10$g$, 20$g$ and 30$g$. Also, for the purposes of comparison, 10 $\times$ aggregate samples were considered. This scenario led to a similar conclusion as the previous scenario because the probability of acceptance remained high when using equal incremental samples. However the unequal increments did not lower the probability of acceptance that much compared to the previous scenario.

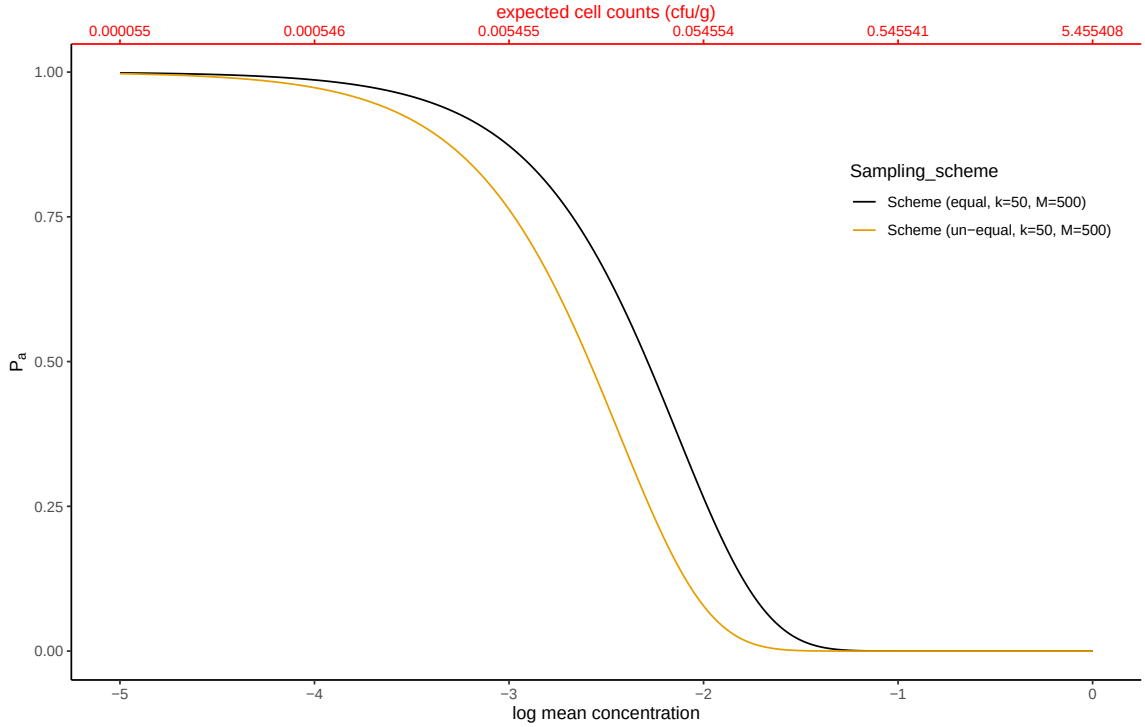


Figure 5.4: Operating characteristics (OCs) for two sampling schemes in Scenario 3, based on Poisson gamma distribution$(\sigma_w = 0.8, K = 0.05, n = 10)$

Figure 5.4 shows a comparison between two different incremental sampling schemes when samples with heterogeneous, low-level contamination are collected from the lot. Scenario 3 follows the same sampling schemes as in Scenarios 1–2. Figure 5.4 shows that the conclusions are similar to the previous scenarios when it comes to unequal amounts lowering the $P_a$.

Risk assessment based on the OC curve and the probability of detection, relies heavily on the set minimum amount of incremental samples when the total amount and number of incremental samples remain the same in both schemes. Therefore, risk can be significantly reduced by drawing several small amount of incremental samples. This type of sampling procedure can be executed using autosamplers but this approach can get harder with manual sampling.

### 5.4.2 Effect of $Var(Y_j)$

When aggregating equal and unequal incremental samples, we investigated the impact of variance of $Y_j$ with $\mu_0$ in each scenario. The effect of $Var(Y_j)$ in Scenarios 1, 2 and 3 is illustrated in Figures 5.5, Figure 5.6 and Figure 5.7, respectively.

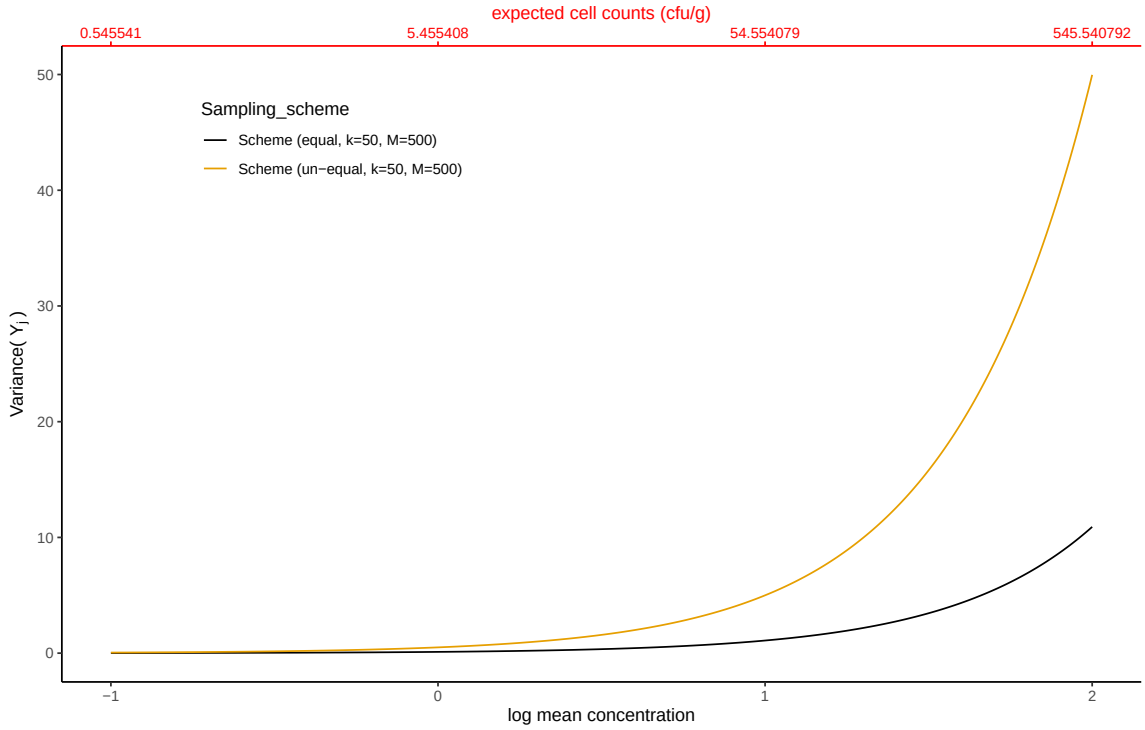


Figure 5.5: Variability in observed cell counts as a function of the mean concentration level of contamination in Scenario 1, based on Poisson distribution.

Figure 5.5 demonstrates that variance of observed cell counts is sensitive to the higher contamination levels even when lot contamination is homogeneous. When using unequal incremental samples to form an aggregate sample, variance becomes higher when compared to the variance for the case of equal amount sampled from sublots. In this scenario, we employed the Poisson distribution. Thus, it can be inferred that a sudden large increase in cell counts would also result in a disproportionate increase in its variance.

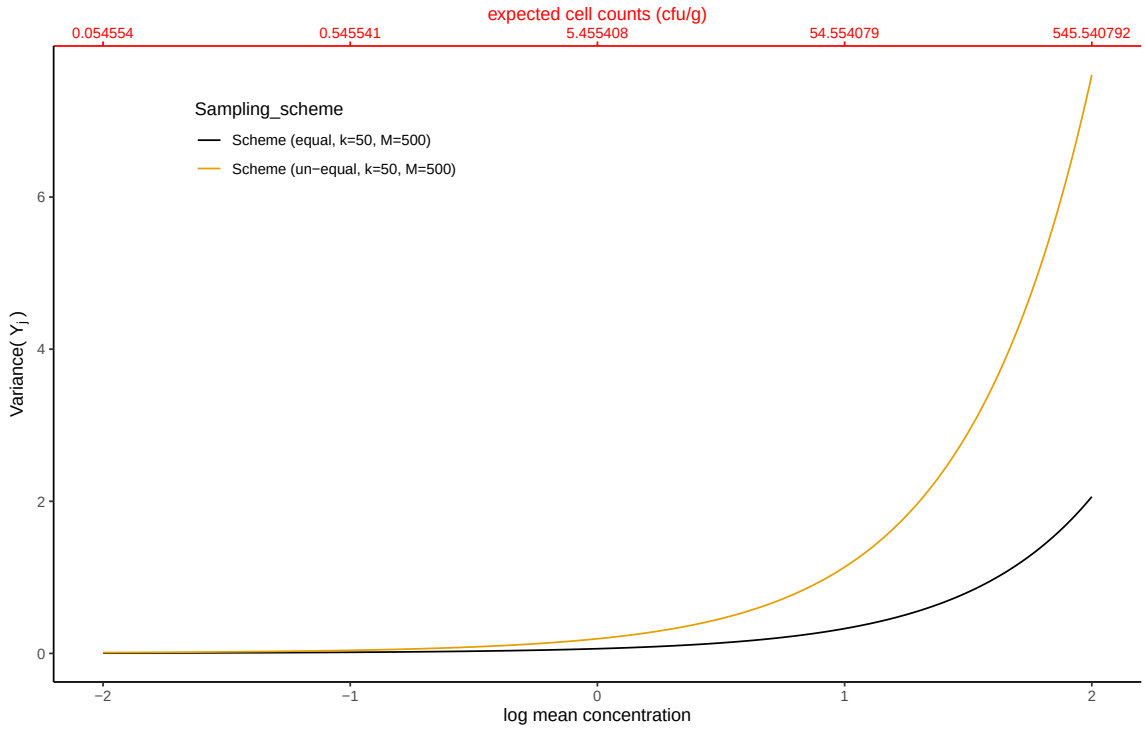


Figure 5.6: Variability in observed cell counts as a function of the mean concentration level of contamination in Scenario 2, based on Poisson lognormal distribution.

Figures 5.6 and 5.7 illustrate the variability in Scenarios 2 and 3, respectively, which are more representative of real-world situations involving heterogeneous contamination. Figures 5.6 and 5.7 show that variances are higher when using unequal quantities of incremental samples to form an aggregate sample in lots with high level of heterogeneous contamination (Scenario 2) and low level of heterogeneous contamination (Scenario 3) respectively.

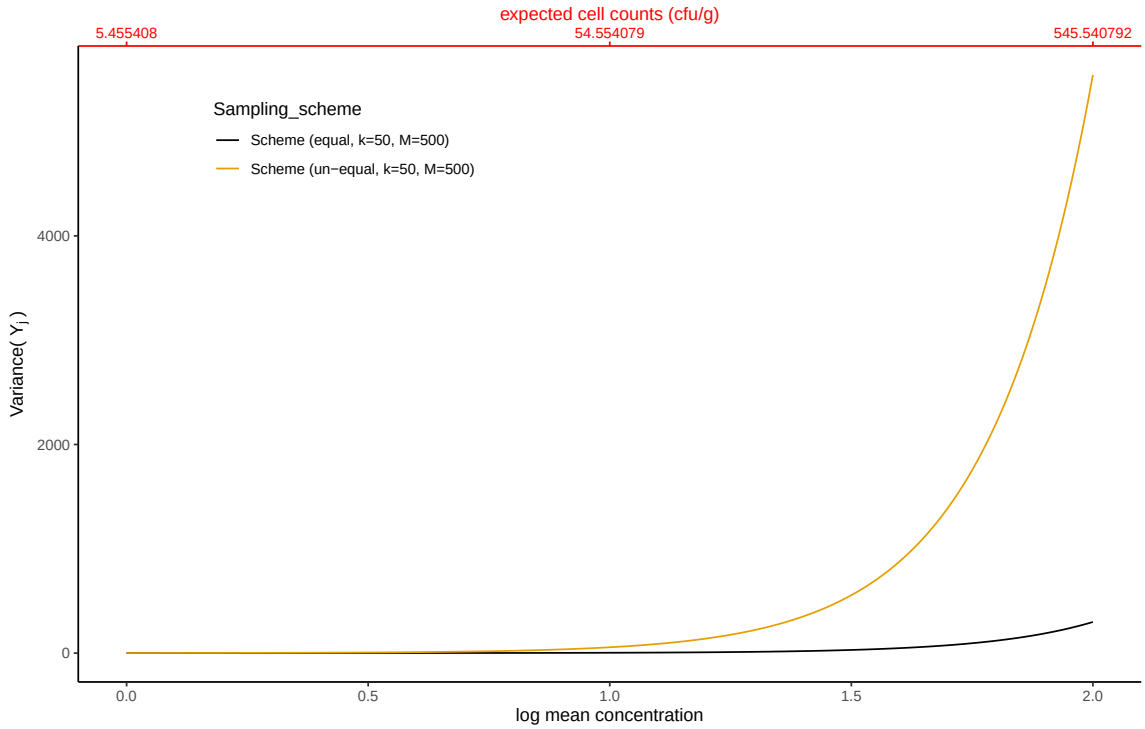


Figure 5.7: Variability in observed cell counts as a function of the mean concentration level of contamination in Scenario 3, based on Poisson gamma distribution.

Based on Figures 5.5, 5.6 and 5.7, it is evident that opting for unequal incremental samples introduces additional variability in the risk assessments, and as a consequence, the probability of acceptance is uniformly lowered and increased variability raises the risk levels in sampling procedures.

### 5.4.3 Average Outgoing Quality Performance-Based Risk Assessments

Risk assessment based on outgoing quality performance is also an appropriate tool for disposition of a series of lots produced in a production process. Only lots that pass the acceptance criteria are cleared and those fail are either discarded or screened. Lot screening is difficult for bulk materials but suitable for discrete units. The AOQL procedure provides the maximum value of average outgoing quality, which is helpful for consumer protection.

Figure 5.8 illustrates AOQ for the two different sampling schemes with both unequally and equally weighted incremental samples forming aggregate samples as in Scenario 1. The average outgoing quality limits for the two sampling schemes were approximately 0.04% and 0.07% contaminated, respectively. Furthermore, aggregating unequally weighted

incremental samples achieved an AOQL of 0.04%, thus assuring the worst average fraction of contamination the consumer will receive. Therefore, to protect the consumer, selecting small amounts of unequal incremental samples is desirable than selecting equal but large amounts.

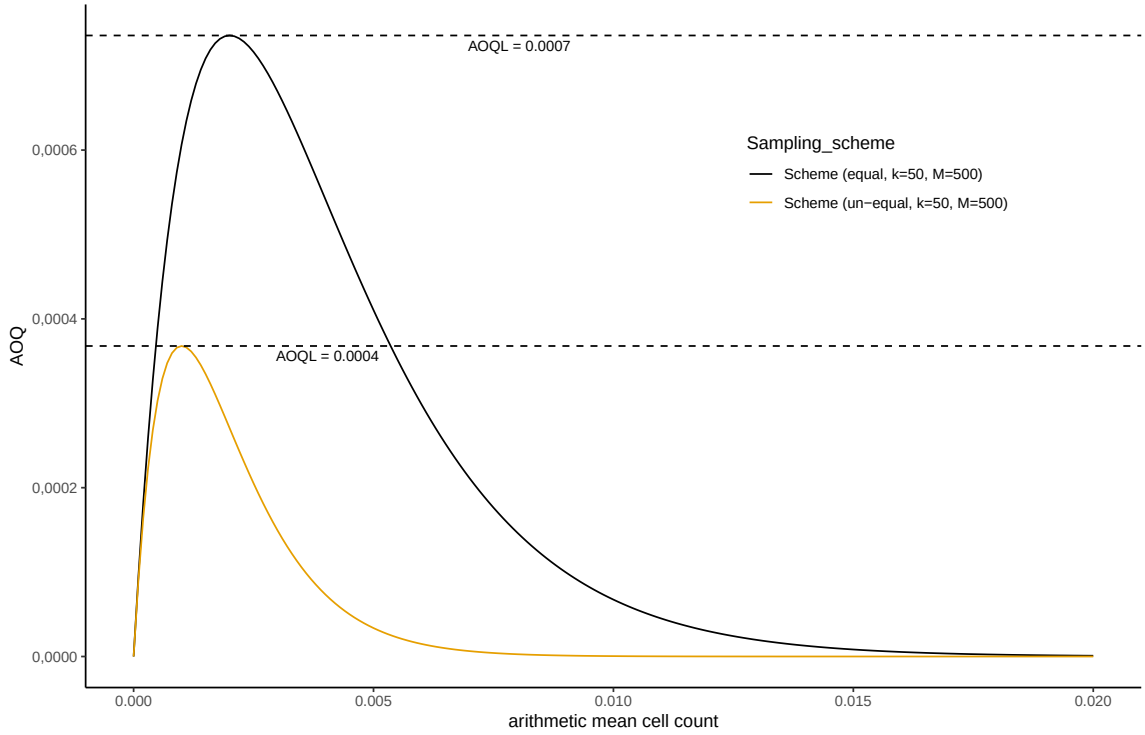


Figure 5.8: Average outgoing quality $(AOQ)$ versus arithmetic mean cell count for sampling schemes with $\sigma = 0.8$ in Scenario 1.

The fall in AOQ (and hence in AOQL) is due to lowering of $P_a$ with unequal incremental samples particularly at higher contamination levels.

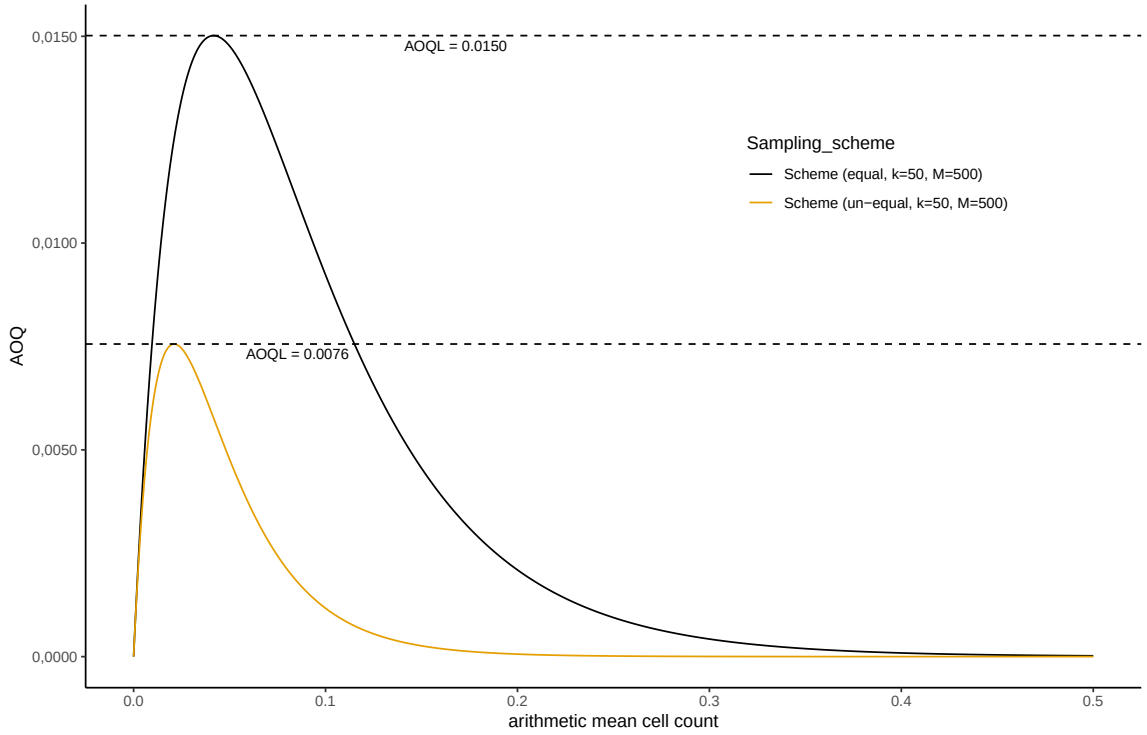


Figure 5.9: Average outgoing quality $(AOQ)$ versus arithmetic mean cell count for sampling schemes with $\sigma = 0.8$ in Scenario 3.

Figure 5.9 illustrates AOQ using two different sampling schemes discussed under Scenario 3. The average outgoing quality limits of contaminated materials in two aggregating sampling schemes, for both the unequally and equally amount incremental samples, were approximately 0.74% and 1.52%, respectively. These limits of outgoing quality performance coincided with the conclusion we reached in Scenario 1 (Figure 5.8). Therefore, we reaffirm the conclusion that small amounts of unequal amounts of incremental sample selection are more effective to lower the AOQ than equal but large amounts of incremental sampling.

### 5.4.4 Risk Assessment in Scenarios 4 and 5

When contamination levels (e.g. $\mu_{0j}$) fluctuate from sublot to sublot, each sublot has a specific concentration level of contamination. Following Santos–Fernández et al. (2016), the mean concentration level of microorganisms in the minimum quantity sampled, $\mu_{0j}$, follows a normal distribution with parameters $\mu$ and $\sigma_b$, where $\sigma_b$ is the standard deviation between sublots. For this study, incremental samples will be collected from five sublots, in

both equal and unequal amounts. The sampling schemes used for these two scenarios are illustrated in Appendix 5.C.

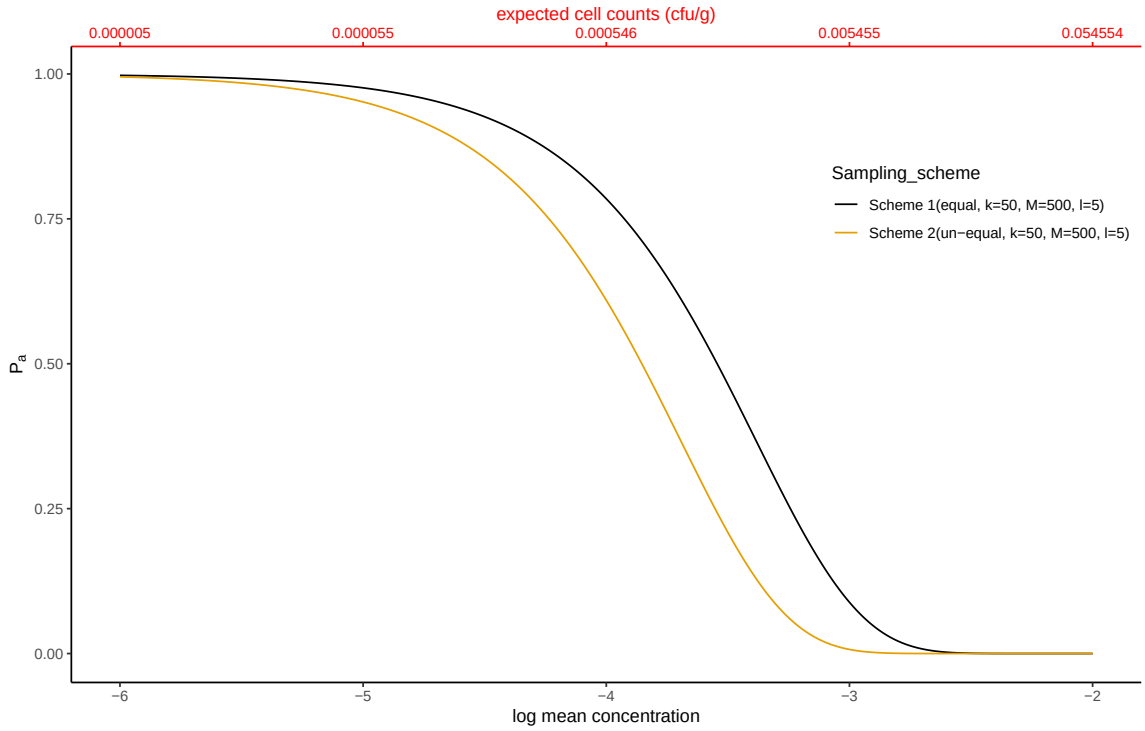


Figure 5.10: Operating characteristics (OCs) for two sampling schemes in Scenario 4, based on Poisson distribution ($\sigma_w = 0.8, n = 10, l = 5$).

Figure 5.10 plots the probability of acceptance against concentration level for the case of sublots with homogeneous contamination but the contamination level fluctuates from sublot to sublot. For equal incremental samples, the probability of acceptance is higher for all $\lambda$; where $\lambda = 10^{\mu+0.5\log(10)\sigma_b^2}$. It is also assume that $\mu_{0j}$ follows the normal distribution with parameters $\mu, \sigma_b$. We again find that unequal increments lower the probability of acceptance $P_a$. While this leads to a smaller consumer's risk, the associated producer's risk becomes greater.

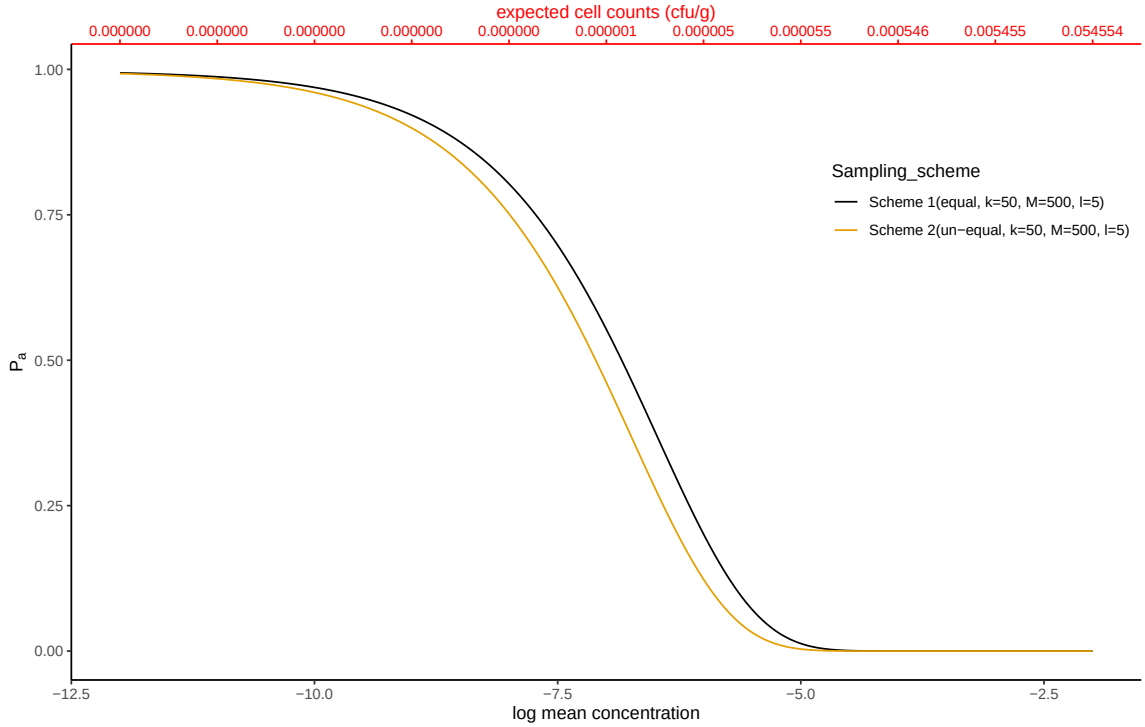


Figure 5.11: Operating characteristics (OCs) for two sampling schemes in Scenario 5, based on Poisson lognormal distribution ($\sigma_w = 0.8, n = 10, l = 5$).

Figure 5.11 illustrates that probability of acceptance versus concentration level for the case of heterogeneous contamination with the contamination level fluctuating from sublot to sublot. In Scenario 5, compound Poisson lognormal distribution was employed to model the number of microorganisms in the incremental samples. Figure 5.11 also shows a higher probability of acceptance when equal incremental samples were taken. The results obtained in this scenarios 4 and 5 match those obtained in the first three scenarios.

## 5.5 R Package "*uneqmixr*"

An open-source *R* (R Core Team, 2023) software package, known as *"uneqmixr"* (available at `https://github.com/Mayooran1987/uneqmixr`) was developed for probability of acceptance and other calculations done in this Chapter. This package is capable of drawing graphical displays such as OC curves, and probability of detection curves, for user specified scenarios. A full description of this *R* package is available at: `https://mayooran1987.github.io/uneqmixr`. Even though this Chapter illustrated two specific sampling schemes, the package allows users to compare various other scenarios.

## 5.6 Conclusion

This Chapter established that the quantity of incremental samples is an important factor for microbiological risk assessment. Our findings indicate that selecting unequal incremental samples introduces additional variability in the observed cell count. Unequal sample selection leads to increased producer's risk due to the resulting extra variability. On the other hand, the strategy of taking several but smaller amount of incremental samples also provides higher protection to consumers. Consequently, we suggest that equally weighted smaller incremental samples will be able to provide protection to both consumers and producers. The chosen scenarios mimic those given in regulatory guidelines such as European Commission (2009); however, results can be reproduced for other scenarios using the R package presented.

Table 5.1: Chapter 5: Glossary of symbols and abbreviations

| | |
|---|---|
| $M_j$ | total quantity of $j^{\text{th}}$ aggregate sample |
| $m_{ij}$ | quantity of $i^{\text{th}}$ incremental sample which is used to form $j^{\text{th}}$ aggregate sample |
| $k$ | number of incremental samples |
| $X_{ij}$ | number of microorganisms in $i^{\text{th}}$ incremental sample used to form $j^{\text{th}}$ aggregate sample |
| $\lambda_{ij}$ | expected cell counts in the $i^{\text{th}}$ incremental sample used to form $j^{\text{th}}$ aggregate sample |
| $Y_j$ | number of cell counts in the aggregate sample, a random variable |
| $P_d$ | probability of detection |
| $P_{nd}$ | probability of non-detection |
| $n$ | number of aggregate samples used for inspection |
| $P_a$ | probability of acceptance |
| $\mu_{ij}$ | location parameter (mean log) of the Poisson-lognormal distribution on the logarithmic scale |
| $\sigma_w$ | standard deviation of concentration level within sublot of the Poisson-lognormal distribution on the logarithmic scale (default value 0.8) |
| $\sigma_b$ | standard deviation between sublots of the Poisson-lognormal distribution on the of concentration level logarithmic scale (default value 0.8) |
| $n_{sim}$ | number of simulations |
| $K$ | shape parameter |
| $w_{ij}$ | weights corresponding to $i^{\text{th}}$ incremental sample which is used to form $j^{\text{th}}$ aggregate sample |
| $c$ | acceptance number |
| $C$ | concentration level |
| $l$ | number of lots |
| $OC$ | operating characteristics |
| $AOQ$ | average outgoing quality |
| $AOQL$ | average outgoing quality limit |
| $pmf$ | probability mass function |
| $CFU$ | Colony-Forming Unit |

## 5.A Mathematical proofs

### 5.A.1 The proof of mean and variance of $Y_j$ in each scenario

The number of microorganisms in the aggregate sample is given by:

$$Y_j = \sum_{i=0}^{k-1} w_{ij} X_{ij},$$

where $w_{ij} = \frac{m_{ij}}{M_j}$, $M_J = \sum_{i=0}^{k-1} (m_{ij})$ and $k, M_j$ are fixed for all $j$. For example, a $500g$ aggregate sample can be produced by 50 equally weighted samples or 50 unequally weighted samples.

$$\begin{aligned} E(Y_j) &= E\left(\sum_{i=0}^{k-1} w_{ij} X_{ij}\right) \\ &= \sum_{i=0}^{k-1} w_{ij} E(X_{ij}) \\ &= \sum_{i=0}^{k-1} \left(\frac{m_{ij}}{M_j}\right) E(X_{ij}) \end{aligned}$$

$$\begin{aligned} Var(Y_j) &= Var\left(\sum_{i=0}^{k-1} w_{ij} X_{ij}\right) \\ &= \sum_{i=0}^{k-1} w_{ij}^2 Var(X_{ij}) \\ &= \sum_{i=0}^{k-1} \left(\frac{m_{ij}}{M_j}\right)^2 Var(X_{ij}) \end{aligned}$$

**Scenario 1**

Since $X_{ij}$ follows Poisson distribution with parameter $\lambda_{ij}$, the probability mass function of $X_{ij}$ is given by:

$$P(X_{ij} = x_{ij}) = \frac{{\lambda_{ij}}^{x_{ij}} e^{-\lambda_{ij}}}{x_{ij}!}; \quad x_{ij} = 0, 1, 2, \cdots\cdots$$

Also, we can write $E(X_{ij}) = Var(X_{ij}) = \lambda_{ij}$. This implies that,

$$
\begin{aligned}
E(Y_j) &= \sum_{i=0}^{k-1} \left(\frac{m_{ij}}{M_j}\right) \lambda_{ij} \\
&= \sum_{i=0}^{k-1} \left(\frac{m_{ij}}{M_j}\right) \left(\frac{m_{ij}}{m_{0j}}\right) \lambda_{0j} \\
&= \frac{\lambda_{0j}}{M_j m_{0j}} \sum_{i=0}^{k-1} m_{ij}^2
\end{aligned}
$$

$$
\begin{aligned}
Var(Y_j) &= \sum_{i=0}^{k-1} \left(\frac{m_{ij}}{M_j}\right)^2 \lambda_{ij} \\
&= \sum_{i=0}^{k-1} \left(\frac{m_{ij}}{M_j}\right)^2 \left(\frac{m_{ij}}{m_{0j}}\right) \lambda_{0j} \\
&= \frac{\lambda_{0j}}{M_j^2 m_{0j}} \sum_{i=0}^{k-1} m_{ij}^3
\end{aligned}
$$

**Scenario 2**

Let $X_{ij}$ follows Poisson lognormal distribution with parameters $\mu_{ij}, \sigma_w$. This means that $X_{ij}$ follows Poisson distribution with parameter $\lambda_{ij}$, which further follows lognormal distribution with parameters $\mu_{ij}, \sigma_w$. Following Bulmer (1974) methodology, the probability mass function of $X_{ij}$ can be written as:

$$P(X_{ij} = x_{ij}|\mu_{ij}, \sigma_w) = \int_0^\infty P(x_{ij}|\lambda_{ij}) f(\lambda_{ij}|\mu_{ij}, \sigma_w) d\lambda_{ij}$$

where

$$P(X_{ij} = x_{ij}|\lambda_{ij}) = \frac{e^{-\lambda_{ij}} {\lambda_{ij}}^{x_{ij}}}{x_{ij}!}\ ; \quad x_{ij} = 0, 1, 2, \cdots\cdots$$

and

$$f(\lambda_{ij}|\mu_{ij}, \sigma_w) = \frac{1}{\lambda_{ij}\sigma_w\sqrt{2\pi}} \exp\left(-\frac{(log(\lambda_{ij}) - \mu_{ij})^2}{2\sigma_w^2}\right)\ ; \quad \lambda_{ij} \in (0, \infty),\ \sigma_w > 0,\ \mu_{ij} \in (-\infty, +\infty)$$

**Derivation of** $E(X_{ij})$

$$\begin{aligned} E(X_{ij}) &= E\left[E(X_{ij}|\lambda_{ij})\right] = E(\lambda_{ij}) \quad \text{(by using law of total expectation)} \\ &= \exp\left(\mu_{ij} + 0.5\sigma_w^2\right) \end{aligned}$$

**Derivation of** $Var(X_{ij})$

$$\begin{aligned} Var(X_{ij}) &= E\left(Var(X_{ij}|\lambda_{ij})\right) + Var\left(E(X_{ij}|\lambda_{ij})\right) \quad \text{(by using law of total variance)} \\ &= E(\lambda_{ij}) + Var(\lambda_{ij}) \\ &= \exp\left(\mu_{ij} + 0.5\sigma_w^2\right) + \left[(\exp(\sigma_w^2) - 1)\exp(2\mu_{ij} + \sigma_w^2)\right] \end{aligned}$$

If random variable $X \sim LN(\mu, \sigma)$ then $aX \sim LN(\mu + \log_{10}(a), \sigma)$; where $a$ is constant. Hence,

$$\mu_{ij} = \mu_{0j} + \log_{10}(m_{ij}/m_{0j})$$

**Derivation of** $E(Y_j)$

$$\begin{aligned} E(Y_j) &= \sum_{i=0}^{k-1} \left(\frac{m_{ij}}{M_j}\right) E(X_{ij}) \\ &= \sum_{i=1}^{k} \left(\frac{m_{ij}}{M_j}\right) \exp\left(\mu_{ij} + 0.5\sigma_w^2\right) \\ &= \left(\frac{\exp\left(0.5\sigma_w^2\right)}{M_j}\right) \sum_{i=1}^{k} m_{ij} \exp\left(\mu_{ij}\right) \\ &= \left(\frac{\exp\left(0.5\sigma_w^2\right)}{M_j}\right) \sum_{i=1}^{k} m_{ij} \exp\left(\log_{10}(m_{ij}/m_{0j}) + \mu_{0j}\right) \end{aligned}$$

**Derivation of** $Var(Y_j)$

$$
\begin{aligned}
Var(Y_j) &= \sum_{i=0}^{k-1} \left(\frac{m_{ij}}{M_j}\right)^2 Var(X_{ij}) \\
&= \sum_{i=0}^{k-1} \left(\frac{m_{ij}}{M_j}\right)^2 \left\{\exp\left(\mu_{ij} + 0.5\sigma_w^2\right) + \left[(\exp(\sigma_w^2) - 1)\exp(2\mu_{ij} + \sigma_w^2)\right]\right\} \\
&= \left(\frac{\exp\left(0.5\sigma_w^2\right)}{M_j^2}\right) \sum_{i=0}^{k-1} m_{ij}^2 \exp\left(\mu_{ij}\right) + \left(\frac{(\exp(\sigma_w^2) - 1)\exp(\sigma_w^2)}{M_j^2}\right) \sum_{i=0}^{k-1} m_{ij}^2 \exp\left(2\mu_{ij}\right) \\
&= \left(\frac{\exp\left(0.5\sigma_w^2\right)}{M_j^2}\right) \sum_{i=0}^{k-1} m_{ij}^2 \exp\left(\log_{10}\left(m_{ij}/m_{0j}\right) + \mu_{0j}\right) \\
&\qquad + \left(\frac{\left(\exp(\sigma_w^2) - 1\right)\exp(\sigma_w^2)}{M_j^2}\right) \sum_{i=0}^{k-1} m_{ij}^2 \exp\left(2\log_{10}\left(m_{ij}/m_{0j}\right) + \mu_{0j}\right)
\end{aligned}
$$

**Scenario 3**

Let $X_{ij}$ follows Poisson gamma distribution with parameters $\lambda_{ij}$ and $K$. That is, the random variable $X_{ij}$ follows Poisson distribution with parameter $\mu_{ij}$, which further follows gamma distribution with shape and scale parameters $\lambda_{ij}$ and $K$, respectively. The probability mass function of $X_{ij}$ can be written as:

$$P(X_{ij} = x_{ij}|\lambda_{ij}, K) = \int_0^\infty P(x_{ij}|\mu_{ij}) f(\mu_{ij}|\lambda_{ij}, K) d\mu_{ij}$$

where

$$P(X_{ij} = x_{ij}|\mu_{ij}) = \frac{e^{\mu_{ij}} {\mu_{ij}}^{x_{ij}}}{x_{ij}!}\ ; \quad x_{ij} = 0, 1, 2, \cdots\cdots$$

and

$$f(\mu_{ij}|\lambda_{ij}, K) = \frac{1}{\Gamma(K)\lambda_{ij}^K} \mu_{ij}^{K-1} \exp\left(-\mu_{ij}/\lambda_{ij}\right)\ ; \quad \lambda_{ij} \in (0, \infty),\ \mu_{ij} \in (-\infty, +\infty)$$

After simplification, the *pmf* is:

$$P(X_{ij} = x_{ij}|\lambda_{ij}, K) = \frac{\Gamma(x_{ij} + K)}{\Gamma(K)x_{ij}!} \left(\frac{\lambda_{ij}}{1 + \lambda_{ij}}\right)^{x_{ij}} \left(\frac{1}{1 + \lambda_{ij}}\right)^K$$

**Derivation of** $E(X_{ij})$

$$\begin{aligned} E(X_{ij}) &= E\left[E(X_{ij}|\mu_{ij})\right] = E(\mu_{ij}) \quad \text{(by using law of total expectation)} \\ &= K\lambda_{ij} \end{aligned}$$

**Derivation of** $Var(X_{ij})$

$$\begin{aligned} Var(X_{ij}) &= E\left(Var(X_{ij}|\mu_{ij})\right) + Var\left(E(X_{ij}|\mu_{ij})\right) \quad \text{(by using law of total variance)} \\ &= E(\mu_{ij}) + Var(\mu_{ij}) \\ &= K\lambda_{ij} + K\lambda_{ij}^2 \end{aligned}$$

**Derivation of** $E(Y_j)$

$$\begin{aligned} E(Y_j) &= \sum_{i=0}^{k-1} \left(\frac{m_{ij}}{M_j}\right) E(X_{ij}) \\ &= \sum_{i=0}^{k-1} \left(\frac{m_{ij}}{M_j}\right) K\lambda_{ij} \\ &= \left(\frac{K}{M_j}\right) \sum_{i=0}^{k-1} m_{ij}\lambda_{ij} \\ &= \left(\frac{K}{M_j}\right) \sum_{i=0}^{k-1} m_{ij} \left(\frac{m_{ij}\lambda_{0j}}{m_{0j}}\right) \end{aligned}$$

**Derivation of** $Var(Y_j)$

$$\begin{aligned} Var(Y_j) &= \sum_{i=0}^{k-1} \left(\frac{m_{ij}}{M_j}\right)^2 Var(X_{ij}) \\ &= \sum_{i=0}^{k-1} \left(\frac{m_{ij}}{M_j}\right)^2 \left(K\lambda_{ij} + K\lambda_{ij}^2\right) \\ &= \left(\frac{K}{M_j^2}\right) \sum_{i=0}^{k-1} m_{ij}^2(\lambda_{ij} + \lambda_{ij}^2) \\ &= \left(\frac{K}{M_j^2}\right) \sum_{i=0}^{k-1} m_{ij}^2 \left(\frac{m_{ij}\lambda_{0j}}{m_{0j}} + \left[\frac{m_{ij}\lambda_{0j}}{m_{0j}}\right]^2\right) \end{aligned}$$

### 5.A.2 The proof of probability of detection $P_{d_j}$ in each scenario

The probability of detection $(P_{d_j})$ at $j^{\text{th}}$ aggregate sample is given by:

$$\begin{aligned}
P_{d_j} &= P(Y_j > 0) = 1 - P(Y_j = 0) \\
&= 1 - P\left(\sum_{i=0}^{k-1} w_{ij} X_{ij} = 0\right) \\
&= 1 - P\left(\sum_{i=0}^{k-1} X_{ij} = 0\right) \qquad \text{(by } w_{ij} \neq 0\text{) for all } i, j \\
&= 1 - \left(\prod_{i=0}^{k-1} P(X_{ij} = 0)\right) \qquad (X_{ij}\text{'s are independent})
\end{aligned}$$

**Scenario 1**

$$\begin{aligned}
P_{d_j} &= 1 - \prod_{i=0}^{k-1} \exp\left(-\lambda_{ij}\right) \\
&= 1 - \exp\left(-\sum_{i=0}^{k-1} \lambda_{ij}\right) \\
&= 1 - \exp\left(-\sum_{i=0}^{k-1} \left(\frac{m_{ij} * \lambda_{0j}}{m_{0j}}\right)\right) \\
&= 1 - \exp\left(-\frac{M_j}{m_{0j}} * \lambda_{0j}\right)
\end{aligned}$$

**Scenario 2**

Since the *pmf* is given by:

$$P(X_{ij} = x_{ij} | \mu_{ij}, \sigma_w) = \frac{1}{\sqrt{2\pi}\sigma_w x_{ij}!} \int_0^\infty \exp(-\lambda_{ij}) \lambda_{ij}^{x_{ij}-1} \exp\left(-\frac{(\log(\lambda_{ij}) - \mu_{ij})^2}{2\sigma_w^2}\right) d\lambda_{ij}$$

So, we obtain $P(X_{ij} = 0)$ as:

$$P(X_{ij} = 0) = \frac{1}{\sqrt{2\pi}\sigma_w} \int_0^\infty \exp(-\lambda_{ij}) \lambda_{ij}^{-1} \exp\left(-\frac{(\log(\lambda_{ij}) - \mu_{ij})^2}{2\sigma_w^2}\right) d\lambda_{ij}$$

Substitute $\log(\lambda_{ij}) = z$ then, $\lambda_{ij} dz = d\lambda_{ij}$.

$$P(X_{ij} = 0) = \frac{1}{\sqrt{2\pi}\sigma_w} \int_{-\infty}^\infty \exp\left(-\exp(z) - \frac{(z - \mu_{ij})^2}{2\sigma_w^2}\right) dz$$

Hence, the probability of detection is given by:

$$\begin{aligned}
P_{d_j} &= 1 - \prod_{i=0}^{k-1} P(X_{ij} = 0) \\
&= 1 - \prod_{i=0}^{k-1} \frac{1}{\sqrt{2\pi}\sigma_w} \int_{-\infty}^{\infty} \exp\left( - \exp(z) - \frac{(z - \mu_{ij})^2}{2\sigma_w^2} \right) dz \\
&= 1 - \left[ \frac{1}{\sqrt{2\pi}\sigma_w} \right]^k \prod_{i=0}^{k-1} \left[ \int_{-\infty}^{\infty} \exp\left( - \exp(z) - \frac{(z - \mu_{ij})^2}{2\sigma_w^2} \right) dz \right] \quad ; \text{where } \mu_{ij} = \log_{10}\left(m_{ij}/m_{0j}\right) + \mu_{0j}
\end{aligned}$$

**Scenario 3**

Since the *pmf* is given by:

$$P(X_{ij} = x_{ij} | \lambda_{ij}, K) = \frac{\Gamma(x_{ij} + K)}{\Gamma(K) x_{ij}!} \left( \frac{\lambda_{ij}}{1 + \lambda_{ij}} \right)^{x_{ij}} \left( \frac{1}{1 + \lambda_{ij}} \right)^K$$

Hence

$$P(X_{ij} = 0) = \left( \frac{1}{1 + \lambda_{ij}} \right)^K$$

Therefore, the probability of detection is given by:

$$\begin{aligned}
P_{d_j} &= 1 - \prod_{i=0}^{k-1} P(X_{ij} = 0) \\
&= 1 - \prod_{i=0}^{k-1} \left( \frac{1}{1 + \lambda_{ij}} \right)^K \\
&= 1 - \prod_{i=0}^{k-1} \left[ \frac{m_{0j}}{m_{0j} + m_{ij}\lambda_{0j}} \right]^K
\end{aligned}$$

## 5.B Further graphical displays

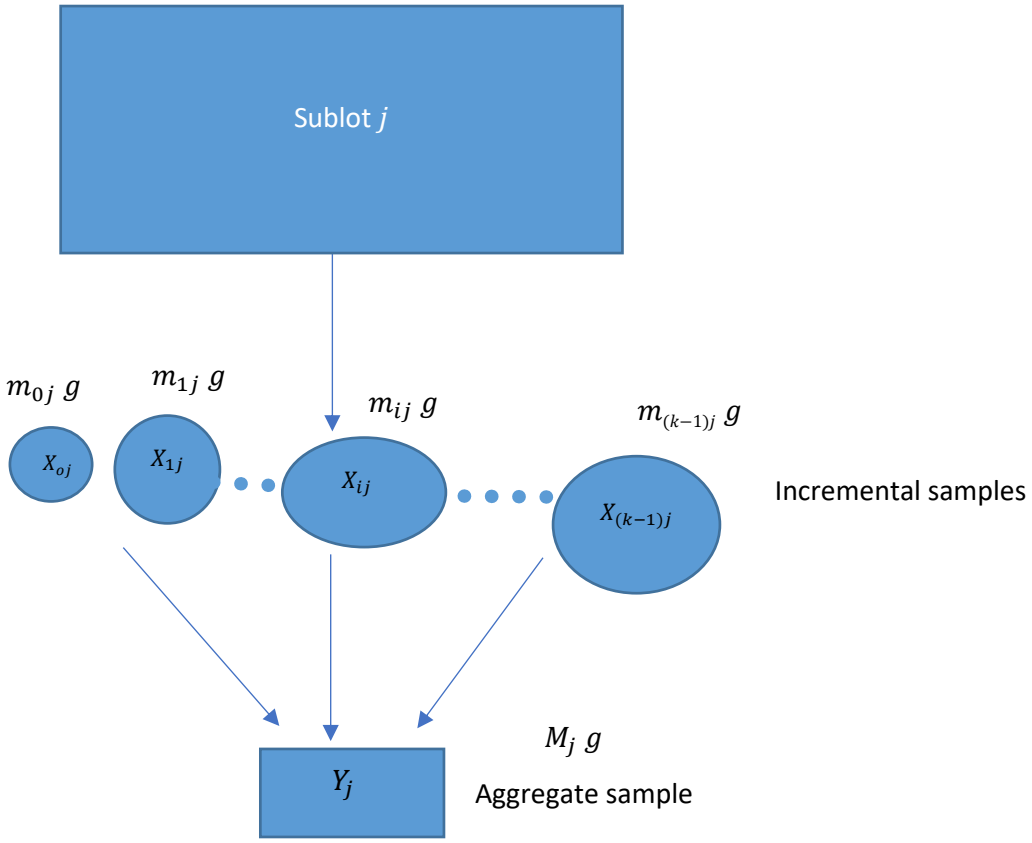


Figure 5.12: The $j^{\text{th}}$ aggregate sample selection procedure from the $j^{\text{th}}$ sublot.

## 5.C Sampling schemes and results validation

Scheme 1 (50 equally weighted samples with total amount equal to $0.5kg$.):

$$[10g, 10g, 10g, 10g, 10g, 10g, 10g, 10g, 10g, 10g, 10g, 10g, 10g, 10g, 10g, 10g,$$
$$10g, 10g, 10g, 10g, 10g, 10g, 10g, 10g, 10g, 10g, 10g, 10g, 10g, 10g, 10g, 10g,$$
$$10g, 10g, 10g, 10g, 10g, 10g, 10g, 10g, 10g, 10g, 10g, 10g, 10g, 10g, 10g, 10g]$$

Scheme 2 (50 un-equally weighted samples with total amount equal to $0.5kg$.):

$$[15g, 5g, 5g, 5g, 10g, 5g, 10g, 5g, 15g, 10g, 5g, 10g, 5g, 25g, 10g, 5g, 10g, 5g, 5g, 10g, 5g,$$
$$15g, 10g, 5g, 5g, 20g, 5g, 10g, 5g, 10g, 20g, 5g, 10g, 30g, 5g, 20g, 5g, 10g, 5g, 10g, 20g,$$
$$15g, 10g, 15g, 10g, 10g, 5g, 10g, 15g, 5g]$$

| **Scenarios** | **Distribution** | **Type** | $\mathbf{P_d}$ $(\mu_0 = -3)$ | $\mathbf{p_a}$ $(\mu_0 = -6)$ | $\mathbf{E(Y)}$ $(\mu_0 = 2)$ | $\mathbf{Var(Y)}$ $(\mu_0 = 2)$ |
|---|---|---|---|---|---|---|
| Scenario 1 | Poisson | theory | 0.238732 | 0.997276 | 545.5408 | 2.060024 |
| | | simulation | 0.238267 | 0.997253 | 545.5404 | 2.060234 |
| Scenario 2 | Poisson lognormal | theory | 0.964221 | 0.181552 | 10.91082 | 27.27704 |
| | | simulation | 0.964097 | 0.181397 | 10.91416 | 27.28691 |
| Scenario 3 | Poisson gamma | theory | 0.013509 | 0.999864 | 10.17567 | 298.1603 |
| | | simulation | 0.013382 | 0.999885 | 10.17564 | 298.4599 |

Table 5.2: Validation of theoretical results through simulation (number of simulations = 2,000,000), based on sampling Scenario 1, $\sigma_w = 0.8$.

## 5.D Sampling schemes for Scenarios 4 and 5

Assuming that incremental samples are selected from five sublots, with an equal number of samples in each selection, it can be assumed for scenarios 4 and 5 that the concentration levels fluctuate from sublot to sublot.

Scheme 1 (equally weighted set of samples with total amount equal to 0.5kg.):

Sublot 1: $10g, 10g, 10g, 10g, 10g, 10g, 10g, 10g, 10g, 10g$

Sublot 2: $10g, 10g, 10g, 10g, 10g, 10g, 10g, 10g, 10g, 10g$

Sublot 3: $10g, 10g, 10g, 10g, 10g, 10g, 10g, 10g, 10g, 10g$

Sublot 4: $10g, 10g, 10g, 10g, 10g, 10g, 10g, 10g, 10g, 10g$

Sublot 5: $10g, 10g, 10g, 10g, 10g, 10g, 10g, 10g, 10g, 10g$

Scheme 2 (un-equally weighted set of samples with total amount equal to 0.5kg.):

Sublot 1: $15g, 5g, 5g, 5g, 10g, 5g, 10g, 5g, 15g, 10g$

Sublot 2: $5g, 10g, 5g, 25g, 10g, 5g, 10g, 5g, 5g, 10g$

Sublot 3: $5g, 15g, 10g, 5g, 5g, 20g, 5g, 10g, 5g, 10g$

Sublot 4: $20g, 5g, 10g, 30g, 5g, 20g, 5g, 10g, 5g, 10g$

Sublot 5: $20g, 15g, 10g, 15g, 10g, 10g, 5g, 10g, 15g, 5g$

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# STATEMENT OF CONTRIBUTION
# DOCTORATE WITH PUBLICATIONS/MANUSCRIPTS

We, the student and the student's main supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the student's contribution as indicated below in the Statement of Originality.

| | |
|---|---|
| Student name: | Mayooran Thevaraja |
| Name and title of main supervisor: | DR. K. Govindaraju |
| In which chapter is the manuscript/published work? | Chapter 6 |
| What percentage of the manuscript/published work was contributed by the student? | 90% |

Describe the contribution that the student has made to the manuscript/published work:

The candidate did the necessary research, statistical modeling, code writing and preparation of the manuscript.

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- ○ **The manuscript/published work is published or in press**
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  Please provide the name of the journal:
- ◉ **It is intended that the manuscript will be published, but it has not yet been submitted to a journal**

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| Student's signature: | Mayooran Thevaraja Digitally signed by Mayooran Thevaraja Date: 2023.04.26 04:17:22 +05'30' | Main supervisor's signature: | Dr. K. Govindaraju Digitally signed by Dr. K. Govindaraju DN: cn=Dr. K. Govindaraju, c=NZ, o=Massey University, ou=School of Mathematical and Computational Sciences, email=k.govindaraju@massey.ac.nz Date: 2023.04.26 11:21:49 +12'00' |

*This form should be placed at the beginning of each relevant thesis chapter.*

# Chapter 6

# Modelling and Assessment of Risk-Based Plate Counts in Dilution Testing

## 6.1 Abstract

The aerobic plate count (APC), or standard plate count (SPC), gives a total bacterial count in the sample or just the count of colony forming units (CFU) per ml or gram. The CFU count is commonly utilised as a quality measure in dairy products. An increased bacterial count indicates poor sanitary practices during manufacturing and handling of the product. Plate count testing is often based on a homogenate prepared after decimal dilution of the sampled material. The effect of the diluted testing is shown to be more pronounced on the disposition of the lot when the true microbiological concentration is around the regulatory limit. A procedure for adjusting plan parameters to compensate for the dilution effect on consumer's risk is addressed in this Chapter. Dilution testing is allowed only for the assessment of sanitary quality rather than food safety, and CFU count is more common than the actual microbial count. These two terms, CFU count and microbial count are interchangeably used in this Chapter, but the results will apply to both cases as long as the underlying distribution relates to the appropriate count measure. A new open-source R package, "*dilutionrisk*", is provided to examine the dilution related risk, and then establish good manufacturing practice limits.

## 6.2 Introduction

The aerobic plate count, the number of microorganisms in the test sample, is often used as an indicator of food sanitary quality. International and national bodies, such as the Association of Official Agricultural Chemists (AOAC), International Dairy Federation (IDF) and the American Public Health Association (APHA), have provided standard methods for obtaining aerobic and other types of plate counts. Only a limited number of (viable) colonies on agar plates can be counted satisfactorily, however. This range is fixed for empirical reasons, and a justification for this approach can be traced back to Breed and Dotterrer (1916). Dilution becomes necessary when the number of colony-forming units (CFUs) is expected to be too large (say, > 250) for an agar plate examination. Errors associated with the plate count method are also a consideration by many; see Jennison and Wadsworth (1940), Hedges (2002), and Chapters 6 and 7 in Jarvis (2016).

For the analysis of dairy products, a countable range of 25 to 250 CFUs per plate was suggested in Tomasiewicz et al. (1980). This original suggestion was later amended to a range of 30 to 300 CFUs per plate, which has been widely accepted; see Adams and Moss (2000) and Sutton (2006). Decimal dilution of food homogenate in the order of $10^{-1}$, $10^{-2}$, $10^{-3}$ is usually done when assessing plate counts. For determination of the APC in dairy products, the dilution level of $10^{-2}$ is generally used; see Wehr (2004). The observed plate count is multiplied by $10^2$ compared with the upper specification limit (USL) set for the undiluted product. Even with a standard dilution level, cases of "too numerous to count" (TNTC) can occur; see Blodgett (2008). In a manufacturing setting, hundred of batches are produced. Testing serially diluted samples from each batch is impractical in a large-scale manufacturing operation. Only a prefixed decimal dilution such as $10^{-2}$ can be done, and the dilution level is also dictated by the industry standards, such as Wehr (2004).

The truncated Poisson-family of distributions is employed in this Chapter to model microbial counts in dilution testing. Bancroft et al. (1983) established the theory of truncated Poisson distribution, but the truncated Poisson-mixture family distributions are not well known in the literature. Most studies are based on the theory for zero-truncated Poisson-mixture models, which are only altered lower limits of the random variable as bounded

by zero; see Valero et al. (2010), Kiani (2020). Therefore, the present study attempted to fill that research gap in the literature and also provides relevant R codes for the truncated Poisson-mixture model.

Figure 6.1 illustrates a simple dilution process for the estimation of true concentration level (CFU count) based on plate counts. The final dilution factor $(f)$ estimates how much sample is diluted for plate counting via the ratio of sample volume $(V_1)$ added to the tube to total volume $(V_1 + V_2)$ in the tube after adding the sample and $u$ be the amount (or volume) of sample on the plate which is usually in millilitres. The estimated true concentration $(\widehat{C})$ is defined as the microbial count per millilitre in the undiluted sample, which can be estimated via microbial count observed on the plate.

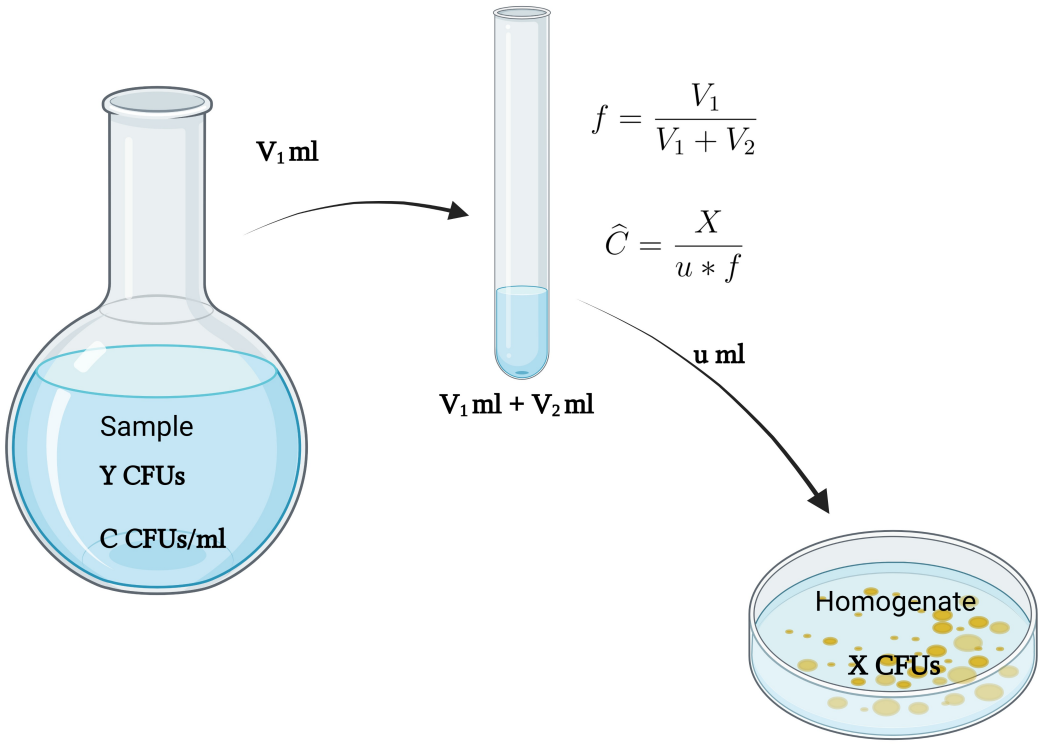


Figure 6.1: Preparation of a diluted homogenate for plating.

The aim of this chapter is to investigate the additional risk due to dilution when the specifications are set for undiluted product. Section 6.3.1 examines the effect of the dilution factor by using truncated Poisson distribution when diluted samples are collected from a

homogeneously contaminated batch. Section 6.3.2 extends the results to diluted samples drawn from a non-homogeneously contaminated batch using truncated Poisson-lognormal distribution. The last section presents the conclusions. For convenience, the observed plate counts are expressed in $\log_e$ scale in this Chapter and a fixed $\sigma$ value in the range 0.2 to 0.8 is used.

An open-source R package *"dilutionrisk"* is presented to examine the operating characteristic (OC) curves which are adjusted to the given dilution schemes. All notation used in this chapter are illustrated in Table 6.3, and mathematical proofs are available in Appendix 6.A. Theoretical results derived were validated through simulations.

## 6.3 Methodology

This Chapter focuses on risk assessment when a diluted sample is tested to obtain the aerobic plate count. In food safety, Poisson and Poisson-mixture distributions are commonly employed in the risk assessment of homogeneous and heterogeneous batches, respectively; see Gonzales-Barron et al. (2013), Gonzales-Barron and Butler (2011b), Jongenburger et al. (2015), Jongenburger et al. (2012), Mussida et al. (2013b), Powell (2015), Schothorst et al. (2009). However, for efficient plate counting, the acceptable count of CFUs is constrained in the range 30 to 300. Therefore, we employed truncated count distributions, such as truncated Poisson distribution and truncated Poisson-lognormal distribution, to assess the risk when undiluted samples were collected from homogeneously and inhomogeneously contaminated batches, respectively.

Furthermore, we assumed that the upper limit of CFUs on a plate is 300 excluding the case of TNTC. Therefore, the observed number of microbial counts per plate will vary from 0 to 300. Diluted testing is conducted specifically for the sanitary characteristic, not for safety assessment. The CFU counts are usually large for sanitary characteristics as well as the specifications. Dilution rate is determined based on the specification to avoid the 'too numerous to count' scenario. It's important to consider the effect that the parameters can only be estimated by trial samples.The contamination specifications are not the same for both diluted and undiluted samples. For instance, if we scale down specification limit of 100 in the 1:10 dilution, the new specification should be 10.

When plate counts are obtained using diluted homogenate, dilution errors occur. Jennison and Wadsworth (1940) used the term *dilution error* to refer to homogenate preparation (pipetting, etc.) errors. This type of error is distinguished from repeatability type sampling/counting errors. Jarvis (2016) provided an in-depth discussion of these two error types, and warned that these errors may not be negligible. The errors discussed by Jarvis (2016), such as pipetting errors, are rather systematic and one-off in nature, which sets them apart from random errors. Consequently, these errors can prove challenging to model accurately due to their specific nature. However, it's worth noting that while their systematic nature might make modeling complex, these errors are not immune to reduction measures. Following established Standard or 'best' practices can significantly mitigate the impact of such errors. This suggests that a comprehensive approach to quality control, which encompasses both error prevention and error detection strategies, remains essential in managing the potential consequences of these systematic errors. Dilution errors are expected to increase with the degree of dilution when dilution is done serially. Standardised plating methods aim to minimise these non-statistical or systematic errors, e.g., Herigstad et al. (2001).

This research focuses on risk assessment when using different dilution factors to assess the level of contamination. For the probability of detection estimation, this study employed bounded distributions. We also derived theoretical results as well as validated them using simulation and evaluated a sampling scheme stipulated in food safety protocols (Section 6.4.3).

The first part of this section considers diluted samples are formed from a homogeneous batch, and then, it considers the more realistic case of diluted samples formed from a non-homogeneous (heterogeneous) batch. Operational characteristics and other measures are derived to assess the producer's and consumer's risks when dilution testing is done.

### 6.3.1 Diluted samples formed from a homogeneous batch

While sampling from a homogeneous batch, the effect of dilution is corrected by scaling up the observed count or alternatively scaling down the USL. Let $Y$ be the number (count) of microorganisms in the undiluted material and $C$ is the true concentration level in the

batch usually expressed as a count per $ml$. The true concentration level can be estimated from plate counts. We assume that $Y$ follows the Poisson distribution. The probability mass function is given by:

$$P(Y = y) = \frac{e^{-\lambda}\lambda^{y}}{y!}; \quad y = 0, 1, 2, \cdots \tag{6.1}$$

where $\lambda$ in the expected microbial count in the undiluted material. Let $X$, the number of microorganisms on the plate, follows the truncated Poisson distribution, bounded in the interval $[0, 300]$. Following Bancroft et al. (1983), the probability mass function is given by:

$$P(X = x) = \frac{\lambda_d^x}{x!\left[\sum_{t=0}^{300}\left(\frac{\lambda_d^t}{t!}\right)\right]}; \quad x = 0, \cdots\cdots 300 \tag{6.2}$$

where $\lambda_d$ represents the expected microbial CFU count on the plate which can be written in terms of $\lambda$ as

$$\lambda_d = \lambda \times u \times f \tag{6.3}$$

where $f$ is the (decimal) dilution fraction such as $10^{-2}$ and $u$ is the amount of diluted material on the plate. The estimated true concentration level $\widehat{C}$ (count per $ml$) in the undiluted material is given by:

$$\widehat{C} = \frac{X}{u \times f} \tag{6.4}$$

where $\widehat{C}$ is the estimated true concentration level, $X$ is the number of CFUs counted on the plate, $f$ is the final dilution factor and $u$ is the amount of diluted sample on the plate.

This is justified, on average, because $E(X) = ufE(Y)$. The observed standard deviation (SD) of the plate counts can also be scaled up by $1/f$ to estimate $\sigma$. This affine scaling approach requires perfect mixing after dilution.

The probability of detection is evaluated as the proportion of CFU counts (per $ml$ ) are over the USL set in food safety regulations (e.g., CAC 2008; ISO 4833-1 2003). The USL usually differentiates good quality from marginally acceptable and unacceptable qualities .

Therefore, the probability of detection is given by:

$$P_d = P(\widehat{C} > USL) = 1 - \sum_{x=0}^{USL_d} \left( \frac{(\lambda uf)^x}{x! \sum_{t=0}^{300} \left( \frac{(\lambda uf)^t}{t!} \right)} \right) \tag{6.5}$$

where $\lambda$ is the expected microbial count in the undiluted sample, $USL_d$ is the upper specification limit for the diluted sample (equal to the integer part of $uf$ multiplied by USL) and $f, u$ are final dilution factor and volume of diluted material on the plate, respectively.

By using the Maclaurin series for expansion of $e^{\lambda_d}$, the approximate probability of detection is given by:

$$P_d \approx 1 - \sum_{x=0}^{USL_d} \left( \frac{\lambda_d^x}{x!(e^{\lambda_d} - 1)} \right) \tag{6.6}$$

This approximation was validated through simulation. The simulation algorithm which is used for finding the probability of detection is shown in Algorithm 6.1. Both theoretical and simulated probability of detection values were closer (shown in Figure 6.9) and again in Appendix 6.B.

The probability of acceptance $(P_a)$ is given by:

$$P_a = \sum_{x=0}^{c} \binom{n}{x} {P_d}^x (1 - P_d)^{n-x}, \tag{6.7}$$

where $n$ is the number of plates inspected, $c$ is the acceptance number, and $P_d$ is the probability of detection in the undiluted material.

**Algorithm 6.1** Computation of probability of detection when diluted samples are selected from a homogeneous batch.

1. Assume the expected microbial counts $\lambda$.
2. Set the final dilution factor $(f)$ and amount of diluted sample on the plate $(u)$.
3. Set the USL value.
4. Set the number of simulations, $n_{sim}$. Use large values (e.g., 50, 000) to achieve good precision.
5. Generate a random vector of CFU counts from the truncated Poisson distribution for given $\lambda_d = uf\lambda, a, b$ with $n_{sim}$ rows and a single column.
6. Multiply each row element by $1/uf$ to get the estimated true concentration of contaminants, which is the scaled count per $ml$ .
7. Calculate the probability of detection $(P_d)$ as the proportion of CFU counts over the USL.

### 6.3.2 Diluted samples formed from a non-homogeneous batch

Following food safety literature (Schothorst et al., 2009; Gonzales-Barron and Butler, 2011b), the microbial count is assumed to follow a compound Poisson mixture distribution when the samples are selected from a non-homogeneous batch. To be specific, it is assumed that $Y$ the follows Poisson-lognormal distribution with parameters $\mu$ and $\sigma$. This means that $Y$ follows the Poisson distribution with parameter $\lambda$, which follows lognormal distribution with parameters $\mu, \sigma$. Following Bulmer (1974) methodology, the probability mass function of $Y$ can be written as:

$$P(Y = y|\mu,\sigma) = \frac{1}{\sigma\sqrt{2\pi}} \int_0^\infty \frac{e^{-\lambda}\lambda^y}{y!} \frac{1}{\lambda} \exp\left(-\frac{(\log(\lambda)-\mu)^2}{2\sigma^2}\right) d\lambda$$
$$; \quad y = 0, 1, 2, \cdots\cdots, \lambda \in (0,\infty),\ \sigma > 0,\ \mu \in (-\infty, +\infty) \quad (6.8)$$

where $\mu$ is the average microbial count in the undiluted sample, and $\sigma$ is the standard deviation of the microbial count in the undiluted sample.

Let $X$ be the CFU count on the plated sample, and $\widehat{C}$ be the estimated true concentration level in the undiluted material; formula given in Equation 6.4.

When the maximum observable CFU count is limited to 300, $X$ is assumed to follow the truncated Poisson-lognormal distribution with parameters $\mu_d, \sigma$, with upper truncation point set at 300. The probability mass function of $X$ is given by:

$$P(X = x|\mu_d, \sigma) = \frac{1}{\sigma\sqrt{2\pi}} \int_0^{\infty} \left( \frac{\lambda_d^x}{\lambda_d x! \left[ \sum_{t=0}^{300} \left( \frac{\lambda_d^t}{t!} \right) \right]} \exp\left( -\frac{(\log(\lambda_d) - \mu_d)^2}{2\sigma^2} \right) \right) d\lambda_d$$
$$; \quad x = 0, 1, 2, \cdots 300, \lambda \in (0, \infty),\ \sigma > 0,\ \mu \in (-\infty, +\infty) \quad (6.9)$$

where $\mu_d$ is the mean microbial count in log scale for the plated amount, and it can be written in terms of $\mu$ as

$$\mu_d = \mu + \log(f) + \log(u) \quad (6.10)$$

Following Gonzales-Barron et al. (2013, p. 370) and Schothorst et al. (2009) a population $\sigma$ of 0.2 can be used for liquid foods with a high degree of mixing. This study also adopts this $\sigma$ value and assumes plating a thoroughly mixed diluted sample.

**Algorithm 6.2** Computation of probability of detection when diluted samples are formed from a non-homogeneous batch.

1. Assume the average microorganism counts (mean microbial count) $(\mu)$and standard deviation $(\sigma)$.
2. Set the final dilution factor $(f)$ and amount of diluted sample on the plate $(u)$.
3. Set the USL value.
4. Set the number of simulations, $n_{sim}$. Use large values (e.g., $50,000$) to achieve good precision.
5. Generate a random vector of CFU counts from by using truncated Poisson-lognormal distribution with parameters $\mu_d, \sigma, a, b$ with $n_{sim}$ rows and a single column.
6. Multiply each row element by $1/uf$ to get the estimated true concentration of contaminants, which is in scale counts per $ml$.
7. Calculate the probability of detection $(P_d)$ as the proportion of CFU counts over the USL.

The probability of detection is then given by:

$$P_d = 1 - \sum_{x=0}^{USL_d} \frac{1}{\sigma\sqrt{2\pi}} \int_{-\infty}^{\infty} \left( \frac{e^{zx}}{x! \left[ \sum_{t=0}^{300} \left( \frac{e^{zt}}{t!} \right) \right]} \exp\left( -\frac{(z-\mu_d)^2}{2\sigma^2} \right) \right) dz \tag{6.11}$$

The simulation algorithm for computation of probability of detection is shown in Algorithm 6.2. In order to solve $P_d$ numerically, we can use the Maclaurin series expansion of $e^{\lambda_d}$ given in Equation 6.8; therefore, the approximate probability of detection is given by:

$$P_d = 1 - \sum_{x=0}^{USL_d} \frac{1}{\sigma\sqrt{2\pi}} \int_{0}^{\infty} \left( \frac{\lambda_d^{x-1}}{x! \left[ e^{\lambda_d} - 1 \right]} \exp\left( -\frac{(\log(\lambda_d) - \mu_d)^2}{2\sigma^2} \right) \right) d\lambda_d \tag{6.12}$$

Maclaurin series expansion-based approximation worked well in the homogeneous case. Nevertheless, in homogeneous cases, a closed form solution for detection probability cannot be found. When applying approximation inside the integration, probability of detection was slightly higher compared to the simulation-based results. However, the probability of detection for given true concentration was satisfactorily approximated, as illustrated in Figure 6.10 (Appendix 6.B).

### 6.3.3 AQL and LQL based risk assessments

The term Acceptable Quality Level (AQL) refers to the acceptable or good quality level at which the probability of acceptance is kept high, such as 95%. Therefore, it is associated with the producer's risk $(\alpha)$.

When sampling inspection is done on a homogeneous batch, the producer's risk $(\alpha)$ is given by;

$$\alpha = 1 - P_a[\lambda = AQL_\lambda] \tag{6.13}$$

When sampling inspection is done on a non-homogeneous batch, the producer's risk is given by;

$$\alpha = 1 - P_a[\mu = AQL_\mu] \tag{6.14}$$

The term Limiting Quality Level (LQL) refers to the rejectable or poor quality level at which the probability of acceptance $\beta$ or the consumer's risk is kept low, such as 5% or 10%.

When sampling inspection is done on a homogeneous batch, the consumer's risk $(\beta)$ is given by

$$\beta = P_a[\lambda = LQL_\lambda] \tag{6.15}$$

When sampling inspection is done on a non-homogeneous batch, Equation 6.15 will also be valid but the underlying distributional parameters will change.

## 6.4 Results and discussion

For risk assessment, we analysed various scenarios of dilution for given expected microbial counts in the undiluted sample. Estimation of true concentration and the associated operating characteristics are studied for different dilution schemes. We also examined the mesophilic aerobic bacteria (MAB) testing in dairy products at the end of this section.

### 6.4.1 Risk assessment when diluted samples selected from homogeneous batch

Assume that $USL = 10^3$ CFU/ml, $f = 10^{-1}$ , $10^{-2}$ and $u = 0.1ml$. This is a common scenario in milk product APC testing. Figure 6.2 compares the probabilities of detection for various values of $\lambda$. The effect of $10^{-1}$ and $10^{-2}$ dilutions were found to be pronounced at rejectable quality levels $(\lambda > USL)$ with poorer detection. At acceptable quality levels $(\lambda \leq USL)$, the diluted tests yielded false positives.

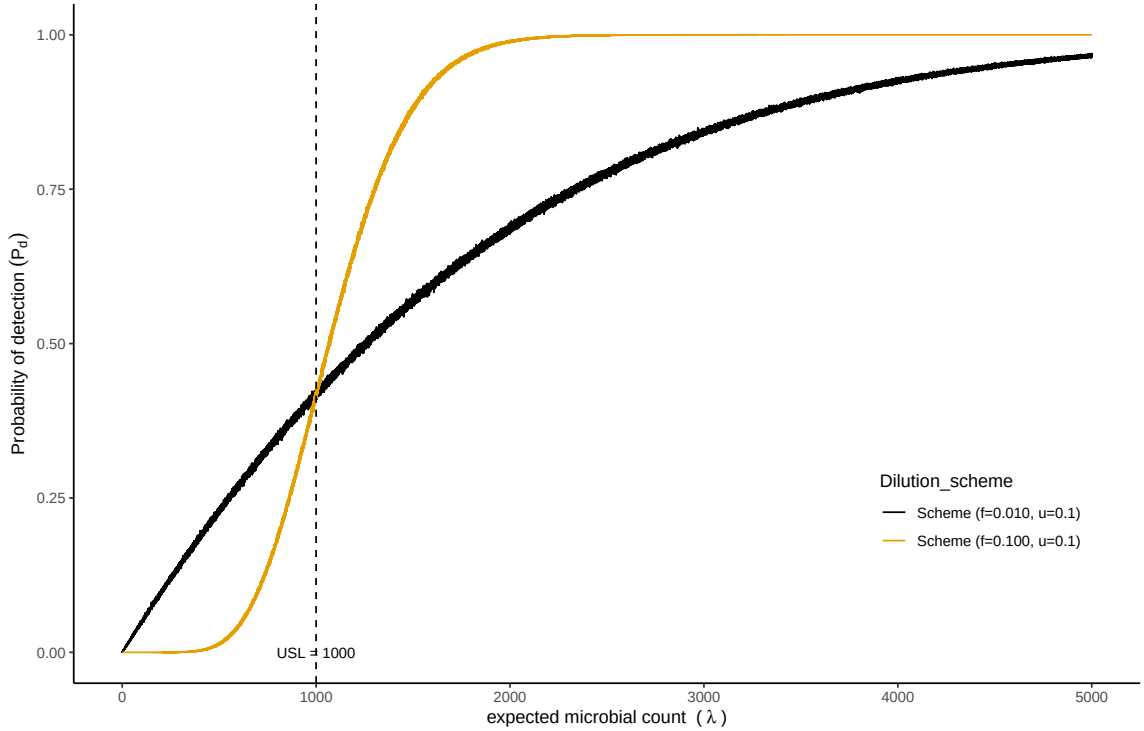


Figure 6.2: Probability of detection changes for two dilution schemes; diluted samples were formed from a homogeneous batch (based on 20,000 simulations from truncated Poisson distribution).

We assumed that a single sampling attribute plan with sample size $n = 5$ and acceptance number $c = 1$ was employed for lot disposition. This plan is, in fact, the Case 6 plan

recommended by the International Commission on Microbiological Specifications for Foods (ICMSF) and discussed in Legan et al. (2001).

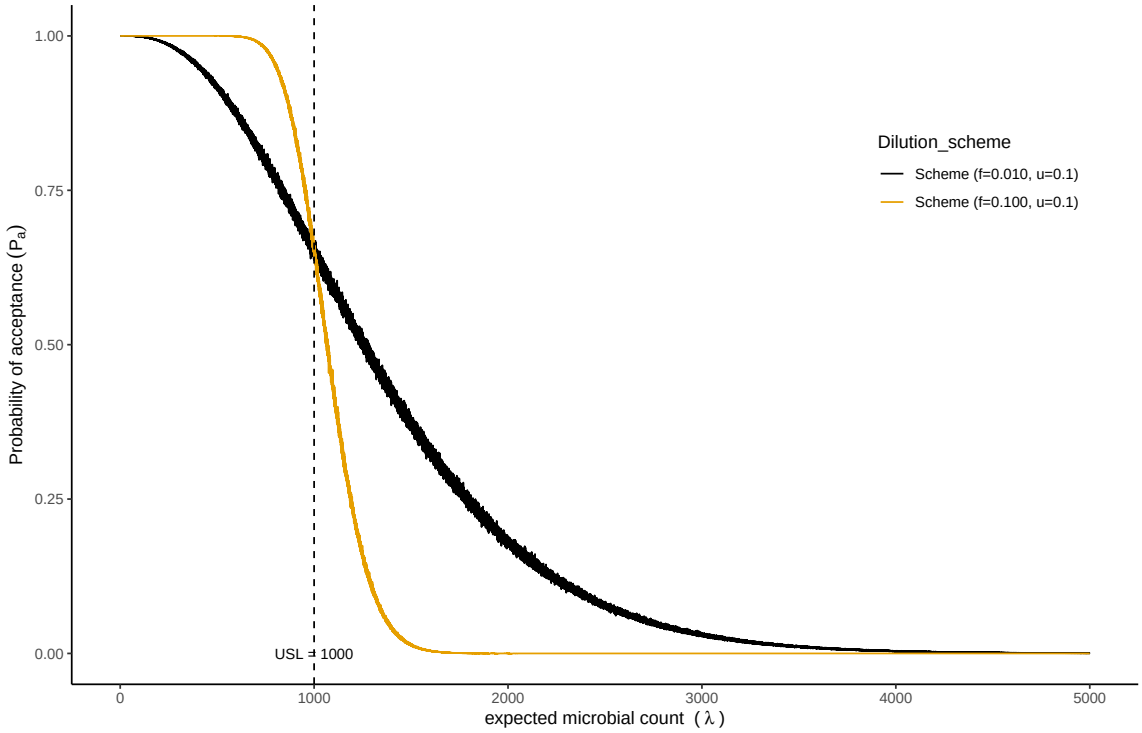


Figure 6.3: Operating characteristics (OCs) for two dilution schemes; diluted samples were formed from a homogeneous batch (based on 20,000 simulations from truncated Poisson distribution).

The operating characteristic (OC) curves for the two plans based on two different dilution tests are shown in Figure 6.3. Clearly, with diluted testing, the sampling inspection plan becomes less powerful in discriminating between acceptable and rejectable concentration rates.

### 6.4.2 Risk assessment when diluted samples were formed from a non-homogeneous batch

When a diluted sample is formed from a non-homogeneous batch, microbial contamination may also become unevenly distributed in the undiluted sample. Figure 6.4 illustrates that the probability of detection changes for various levels of CFU counts in the original sample. Two different 10-fold dilutions, such as $10^{-1}, 10^{-2}$, were employed for this comparison.

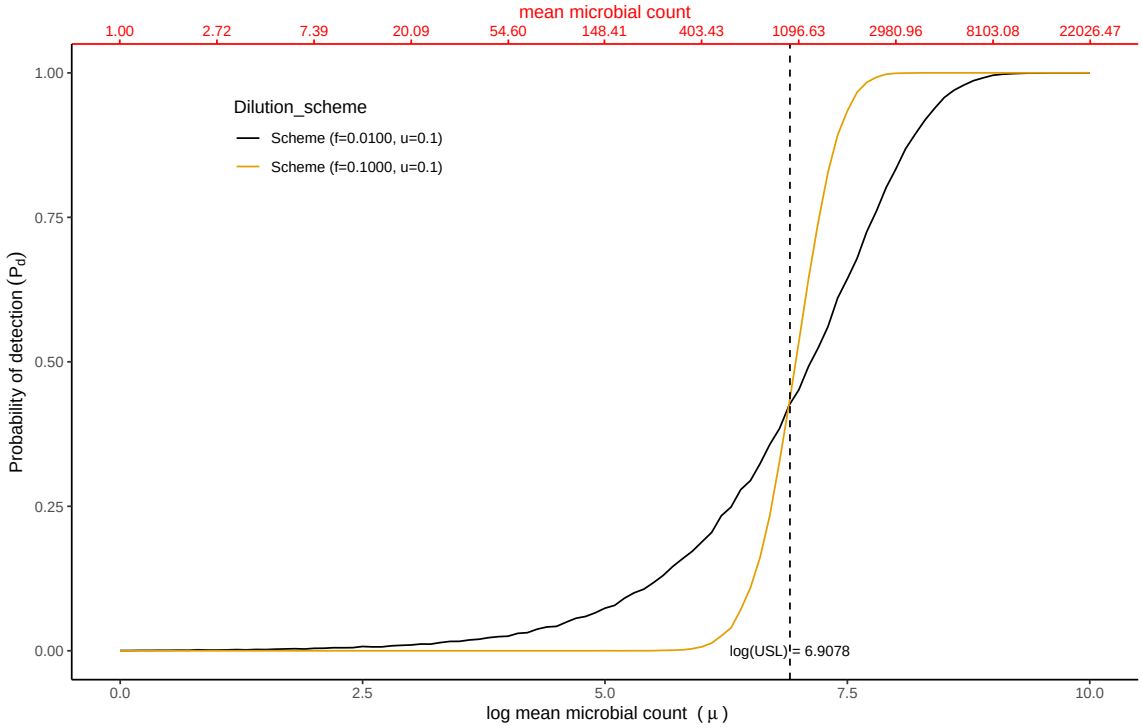


Figure 6.4: Probability of detection changes for two dilution schemes; diluted samples were formed from a non-homogeneous batch (based on 20,000 simulations from truncated Poisson-lognormal distribution with $\sigma = 0.2$)

Figure 6.5 shows the corresponding probability of acceptance under the single sampling plan $n = 5$ and $c = 1$. It is clear that the impact of dilution factor and microbial count heterogeneity is pronounced near the set regulator limit. For consumer protection, the mean microbial count during the production process must be kept well below the regulatory limit to reduce the risk of diluted testing.

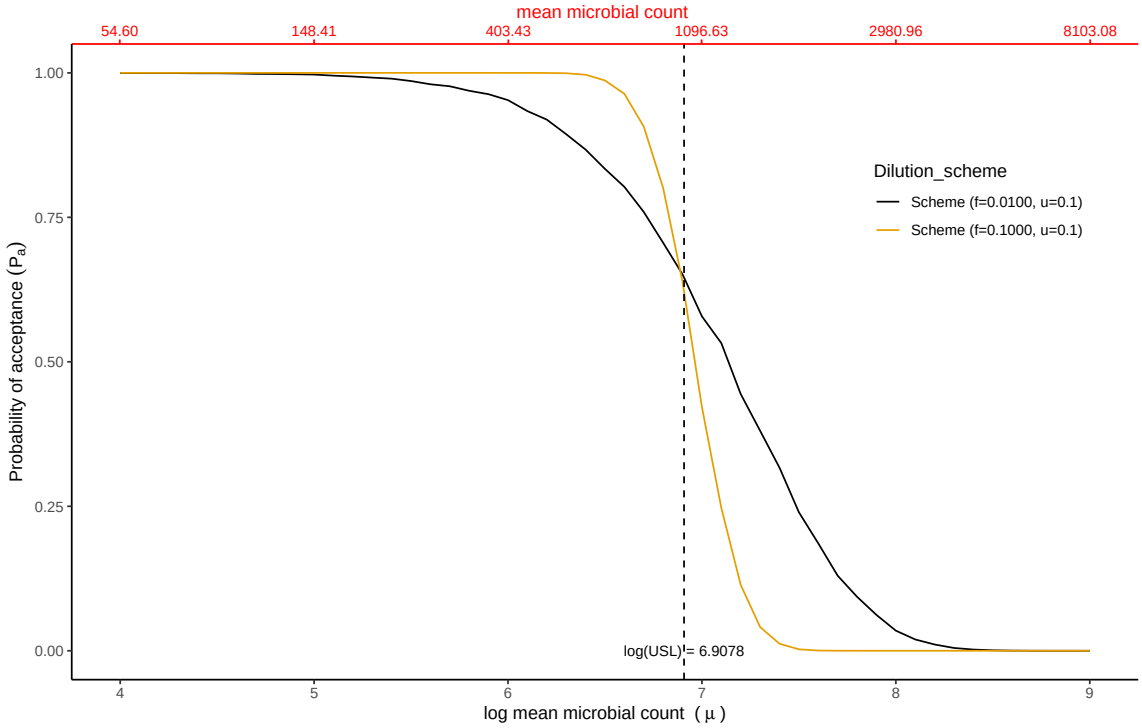


Figure 6.5: Operating characteristics (OCs) for two dilutions schemes; diluted samples were formed from a non-homogeneous batch (based on 20,000 simulations from truncated Poisson-lognormal distribution with $\sigma = 0.2$)

Both scenarios (of homogeneous and non-homogeneous) lot inspection revealed the same conclusions: the dilution testing leads to more false negative results when the true mean microbial counts exceed than the regulatory limit. At acceptable CFU count levels, higher the dilution, greater the false positives.

### 6.4.3 A case study application

We considered two 10-fold dilutions, e.g., $10^{-1}$ and $10^{-2}$, which are common in dilution testing. Aerobic bacteria grow in the presence of oxygen and at moderate temperatures (meaning they were mesophilic, and MAB constituted the total number of bacteria in the sample). This is the most widely used standard hygiene indicator used for microbiological detection in dairy products; see Freitas et al. (2009).

The Codex Alimentarius Commission (CAC) criteria recommend MAB testing in powdered infant formula (PIF) samples, for which assessors must use a three-class sampling plan with the scheme $n = 5$, $c = 2$, $m = 500$ and $M = 5000$; see CAC (2008), ISO 4833-1

(2003). The three-class sampling plan classifies a lot as acceptable, marginally acceptable or unacceptable according to microbiological limits $m$ and $M$, respectively.

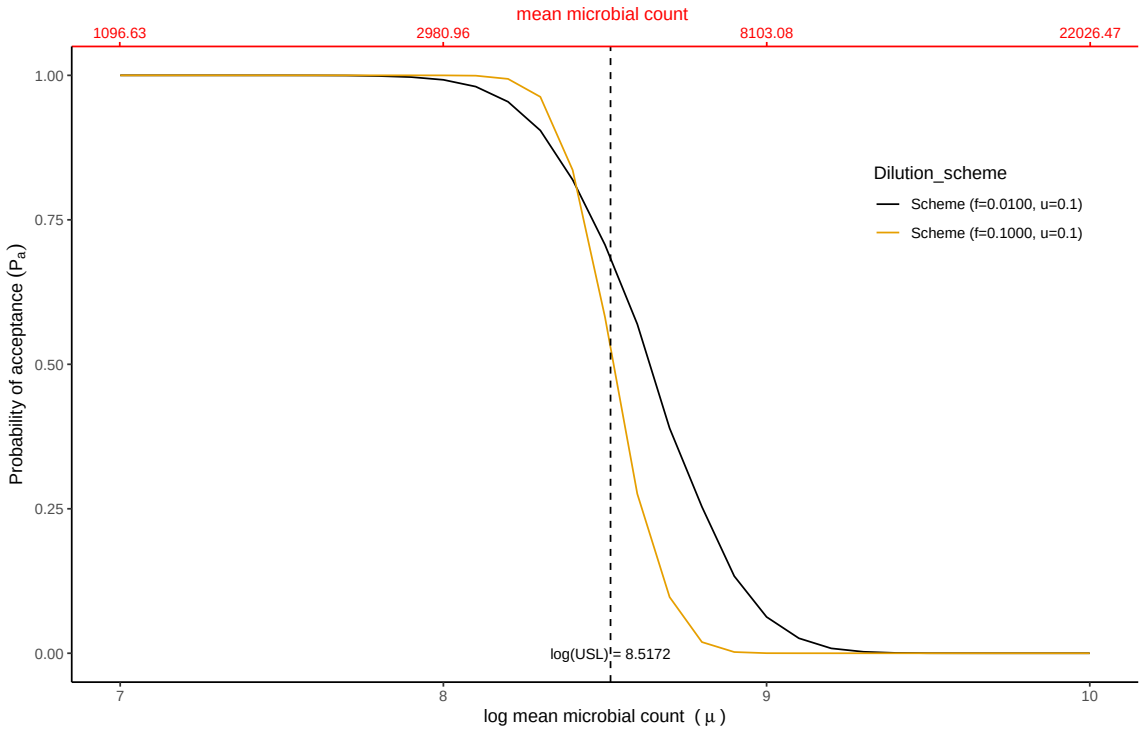


Figure 6.6: Operating characteristics (OCs) for two dilution schemes; diluted samples were formed from a non-homogeneous batch (based on 20,000 simulations from truncated Poisson-lognormal distribution with $\sigma = 0.2, n = 5$)

We obtained the probability of detection for given true concentrations at or exceeding USL. We set $M$ as USL because unacceptable quality should be higher than $M$ in the three-class sampling plan. Based on this example, we found that the dilution testing is unsuitable for MAB detection for a batch with low concentrations of levels. The reasoning follows:

Figures 6.7 and 6.8 show the OC curves when diluted homogenates were formed from homogeneous or non-homogeneous batches, respectively. Both figures also show the points corresponding to 5% producer's risk and 10% consumer's risk.

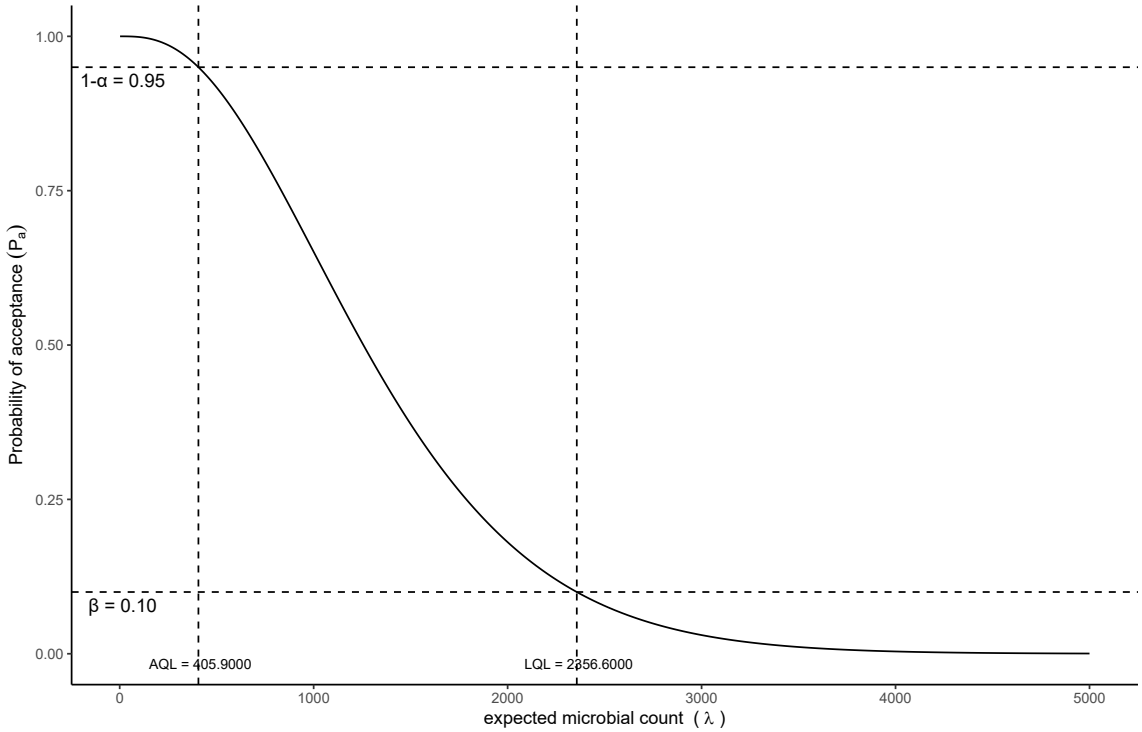


Figure 6.7: Attained AQL and LQL values for the plan with $n = 5$ (diluted samples were formed from a homogeneous batch)

In Figure 6.7, the acceptable level of $\lambda$ is shown as $\lambda_{\text{AQL}} = 405.9$. This implies that the acceptable level of $\lambda_d$ can be estimated by using Equation 6.3, as 0.4059. This value was obtained using numerical methods for given sampling inspection plan parameters such as $f = 1/100$ and fixed $\alpha = 0.05$. Typically all the underlying plan parameters can affect the OC surface but the two dimensional OC curve requires all other distributional parameters to be fixed except $\lambda$. In other words, we found, using numerical methods, that **Truncated Poisson**$(405.9, 0, 300)$ distribution corresponds to $\alpha = 0.05$, which is the acceptable process level distribution. Similarly, **Truncated Poisson**$(2356.6, 0, 300)$ distribution corresponds to $\beta = 0.10$ which is the rejectable process level distribution. $\lambda_{\text{LQL}} = 2356.6$ pertains to mean microbial or CFU count level that is rejectable.

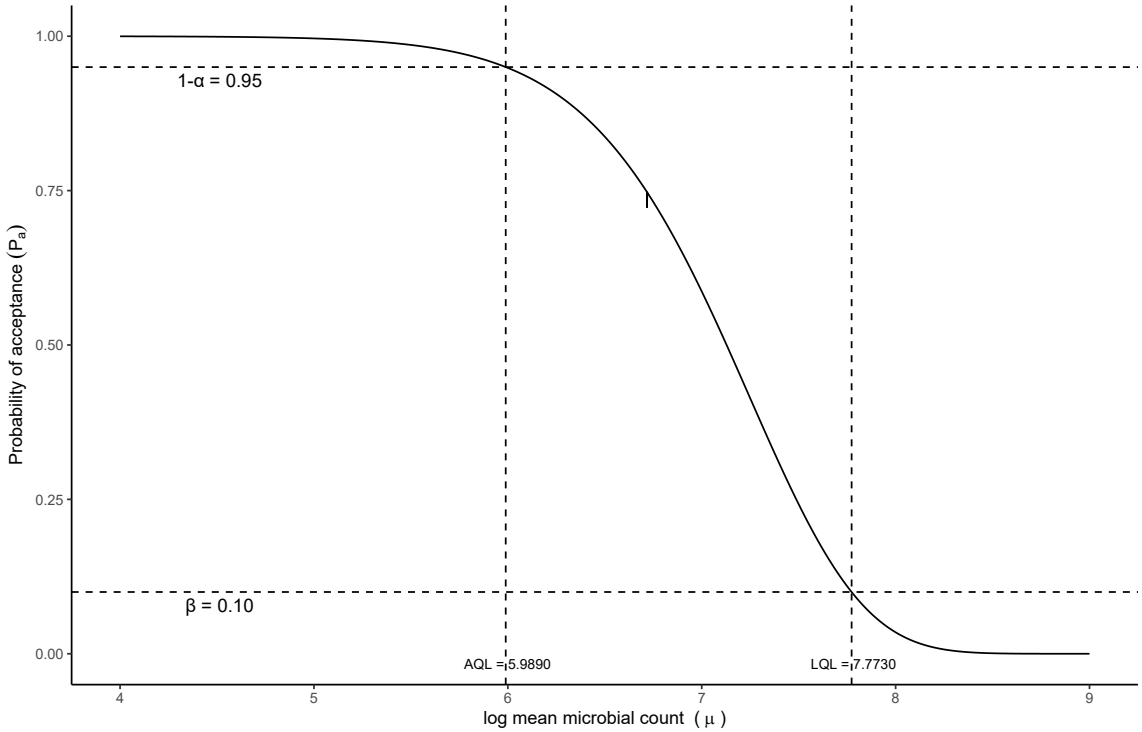


Figure 6.8: Attained AQL and LQL values for the plan with $n = 5$ (diluted samples were formed from a non-homogeneous batch)

In Figure 6.8, the acceptable level of $\mu$ is shows as $\mu_{\text{AQL}} = 5.9890$. This value was obtained using numerical methods for given sampling inspection plan parameters such as $f = 1/100$ and fixed $\alpha = 0.05$. We found, using numerical methods, that **Truncated Poissonlognormal**$(\mu = 5.9890, \sigma, 0, 300)$ distribution corresponds to $\alpha = 0.05$, which is the acceptable process level distribution. Similarly, **Truncated Poissonlognormal**$(\mu = 7.7730, \sigma, 0, 300)$ distribution corresponds to $\beta = 0.10$ which is the rejectable process level distribution. $\mu_{\text{LQL}} = 7.7730$ pertains to mean microbial or CFU count level that is rejectable.

Table 6.1 provides the AQL values attained for various values of producer's risk $(\alpha)$ and plated amounts $(u)$. It is noted that the $AQL$ values increase with plated amount for fixed dilution factor $f$ and $USL$. This means that higher the plated amount, lower the producer's risk.

Whereas Table 6.2 provides the $LQL$ values atained for various values of consumer's risk $(\beta)$ and plated amounts $(u)$. It is noted that the $LQL$ values decrease with plated amount

| | $AQL_\lambda$ | | | $AQL_\mu$ | | |
|---|---|---|---|---|---|---|
| $\alpha$ | $u = 0.1$ | $u = 0.2$ | $u = 0.5$ | $u = 0.1$ | $u = 0.2$ | $u = 0.5$ |
| 0.01 | 219.3 | 449.3 | 632.5 | 5.371 | 6.076 | 6.396 |
| 0.02 | 284.0 | 518.8 | 682.1 | 5.630 | 6.222 | 6.476 |
| 0.03 | 331.7 | 566.2 | 714.8 | 5.787 | 6.311 | 6.526 |
| 0.04 | 371.3 | 603.7 | 740.0 | 5.900 | 6.375 | 6.563 |
| 0.05 | 405.9 | 635.2 | 760.8 | 5.989 | 6.427 | 6.593 |
| 0.06 | 437.1 | 662.9 | 778.8 | 6.064 | 6.471 | 6.618 |
| 0.07 | 465.1 | 687.7 | 794.9 | 6.128 | 6.508 | 6.640 |
| 0.08 | 492.6 | 710.4 | 809.4 | 6.183 | 6.541 | 6.660 |
| 0.09 | 517.9 | 731.4 | 822.7 | 6.234 | 6.571 | 6.677 |

Table 6.1: AQL values for various values of producer's risk $(\alpha)$ and plated amounts $(u)$.

for fixed dilution factor $f$ and $USL$. This means that higher the plated amount, lower the consumer's risk as well. Lab practitioners generally used $0.1ml$ for plating in microbial testing of food samples.

| | $LQL_\lambda$ | | | $LQL_\mu$ | | |
|---|---|---|---|---|---|---|
| $\beta$ | $u = 0.1$ | $u = 0.2$ | $u = 0.5$ | $u = 0.1$ | $u = 0.2$ | $u = 0.5$ |
| 0.01 | 3541.9 | 2587.3 | 1834.1 | 8.201 | 7.899 | 7.574 |
| 0.02 | 3207.1 | 2400.9 | 1739.3 | 8.095 | 7.818 | 7.512 |
| 0.03 | 3004.4 | 2287.1 | 1681.0 | 8.027 | 7.765 | 7.473 |
| 0.04 | 2856.4 | 2203.8 | 1638.1 | 7.973 | 7.724 | 7.443 |
| 0.05 | 2739.1 | 2137.4 | 1603.8 | 7.929 | 7.691 | 7.419 |
| 0.06 | 2641.3 | 2081.9 | 1575.0 | 7.892 | 7.663 | 7.398 |
| 0.07 | 2557.2 | 2033.9 | 1550.0 | 7.858 | 7.638 | 7.380 |
| 0.08 | 2483.1 | 1991.6 | 1527.9 | 7.827 | 7.615 | 7.363 |
| 0.09 | 2416.8 | 1953.5 | 1508.0 | 7.799 | 7.594 | 7.348 |

Table 6.2: LQL values for various values of consumer's risk $(\beta)$ and plated amounts $(u)$.

This amount might have been fixed on practical grounds, but a larger amount will reduce both the producer's and consumer's risks.

## 6.5 R Package "*dilutionrisk*"

We developed an open-source *R* (R Core Team, 2023) software package, known as *"dilutionrisk"* (available at https://github.com/Mayooran1987/dilutionrisk). This package contains functions to draw graphical displays such as OC curves, and probability of detection curves, for different dilution schemes. A full description of this *R* package is available

at: https://mayooran1987.github.io/dilutionrisk. In this Chapter, only two specific dilution schemes were illustrated but the R functions in the package can handle all other schemes.

## 6.6 Conclusion

This study has demonstrated that dilution testing yielded false positives at low contamination levels in a batch; homogenous or not and the opposite was true at rejectable concentration levels resulting in false negatives. Additional testing, or other detection tests, are essential to avoid microbiological risks when diluted testing is done. Furthermore, the findings indicated that increasing the plated amount would lead to a reduction in both the producer's and consumer's risks. Microbial detection also relies heavily on the amount of homogenate tested and the amount of sample drawn. We have not studied the interaction effect of differing sampled amount with the volume of the homogenate tested and this was deferred for future research.

Table 6.3: Chapter 6: Glossary of symbols and abbreviations

| | |
|---|---|
| $Y$ | Observed CFU count in undiluted material |
| $X$ | Observed CFU count on the plate |
| $\lambda$ | expected CFU count in undiluted material |
| $\lambda_d$ | expected CFU count on the plated sample |
| $V_1$ | sample volume added to the test tube |
| $V_2$ | diluent volume added to the test tube |
| $f$ | final dilution factor |
| $u$ | amount of sample on the plate |
| $USL$ | upper specification limit |
| $C$ | true concentration (count per ml) |
| $\mu$ | mean microbial count in undiluted material on a logarithmic scale |
| $P_d$ | probability of detection |
| $n$ | number of samples inspected |
| $P_a$ | probability of acceptance |
| $\mu_d$ | mean microbial count on the plate on a logarithmic scale |
| $\sigma$ | standard deviation of microbial count on a logarithmic scale |
| $c$ | acceptance number |
| $OC$ | operating characteristics |
| $pmf$ | probability mass function |
| $CFU$ | colony-forming unit |
| $TNTC$ | too numerous to count |
| $APC$ | aerobic plate count |
| $SPC$ | standard plate count |

## 6.A Mathematical proofs

### 6.A.1 The proof of probability of detection when a diluted sample is prepared from a homogeneous batch

Since $Y \sim Poisson(\lambda)$ and $X \sim TrPoisson(\lambda_d, a, b)$, then the corresponding probability mass function is:

$$P(Y = y) = \frac{e^{-\lambda}\lambda^y}{y!}; \quad y = 0, 1, 2, \cdots$$

The relationship between $\lambda$ and $\lambda_d$ is given by:

$$\lambda_d = \lambda \times u \times f,$$

where $f$ is the final dilution factor, and $u$ is the amount of diluted sample on the plate.

By definition, the probability mass function of a truncated discrete distribution is given by:

$$P_{[a,b]}(X = x) = \frac{P(X = x)}{[F_X(b) - F_X(a-1)]}; \quad x = a, \cdots, \cdots b$$

For the Poisson case, we derive the following:

$$\begin{aligned} P_{[a,b]}(X = x) &= \frac{P(X = x)}{[P(X \leq b) - P(X \leq a-1)]} \\ &= \frac{P(X = x)}{[P(a \leq X \leq b)]} \\ &= \frac{\left[\frac{e^{-\lambda_d}\lambda_d^x}{x!}\right]}{\left[\sum_{t=a}^{b}\left(\frac{e^{-\lambda_d}\lambda_d^t}{t!}\right)\right]} \\ &= \frac{\lambda_d^x}{x!\left[\sum_{t=a}^{b}\left(\frac{\lambda_d^t}{t!}\right)\right]} \end{aligned}$$

Hence, the *pmf* is given by:

$$P_{[a,b]}(X = x) = \frac{{\lambda_d}^x}{x!\left[\sum_{x=a}^{b}\left(\frac{{\lambda_d}^x}{x!}\right)\right]}; \quad x = a, \cdots, \cdots b$$

The probability of detection in the undiluted material is calculated based on USL (which is equal to $m$ or $M$ in the food safety field). Therefore, the probability of detection $(P_d)$ is given by:

$$\begin{aligned}
P_d &= P(\widehat{C} > USL) \\
&= P\left(\frac{X}{u \times f} > USL\right) \\
&= P(X > USL \times u \times f) \\
&= 1 - P(X \leq USL \times u \times f) \\
&= 1 - \sum_{x=a}^{USL_1} P_{[a,b]}(X = x) \qquad \text{say } USL_1 = USL \times u \times f \\
&= 1 - \sum_{x=a}^{USL_1}\left(\frac{\lambda_d^x}{x!\sum_{t=a}^{b}\left(\frac{\lambda_d^t}{t!}\right)}\right)
\end{aligned}$$

And therefore, the probability of detection is given by:

$$P_d = 1 - \sum_{x=a}^{USL_1}\left(\frac{\lambda_d^x}{x!\sum_{t=a}^{b}\left(\frac{\lambda_d^t}{t!}\right)}\right)$$

This theoretical result can also be approximated for computational efficiency. By using the Maclaurin series for expansion of $e^{\lambda_d}$ and $a = 0$ and $b = 300$, an approximation is presented below:

$$
\begin{aligned}
e^{\lambda_d} &= \sum_{t=0}^{\infty} \left( \frac{\lambda_d^t}{t!} \right) \\
&= \sum_{t=0}^{300} \left( \frac{\lambda_d^t}{t!} \right) + \sum_{t=301}^{\infty} \left( \frac{\lambda_d^t}{t!} \right) \\
&= \sum_{t=0}^{300} \left( \frac{\lambda_d^t}{t!} \right) + \mathcal{O}(\lambda_d)
\end{aligned}
$$

This implies that:

$$
e^{\lambda_d} = \sum_{t=0}^{300} \left( \frac{\lambda_d^t}{t!} \right) + \mathcal{O}(\lambda_d)
$$

Therefore,

$$
\sum_{t=0}^{300} \left( \frac{\lambda_d^t}{t!} \right) \approx e^{\lambda_d} - 1
$$

Hence, the probability of detection is approximately given by:

$$
P_d \approx 1 - \sum_{x=0}^{USL_1} \left( \frac{\lambda_d^x}{x!(e^{\lambda_d} - 1)} \right)
$$

### 6.A.2 Derivation of probability of detection when a diluted sample is prepared from a non-homogeneous batch

Since $Y$ follows Poisson-lognormal distribution with parameters $\mu, \sigma$, this means that $Y$ follows the Poisson distribution with parameter $\lambda$, which follows lognormal distribution with parameters $\mu, \sigma$. Following the Bulmer (1974) methodology, the probability mass function of $Y$ can be written as:

$$
P(Y = y|\mu, \sigma) = \int_0^{\infty} P(Y = y|\lambda) f(\lambda|\mu, \sigma) d\lambda
$$

where

$$
P(Y = y|\lambda) = \frac{e^{-\lambda} \lambda^y}{y!}; \quad y = 0, 1, 2, \cdots\cdots
$$

and

$$f(\lambda|\mu,\sigma) = \frac{1}{\lambda\sigma\sqrt{2\pi}} \exp\left(-\frac{(\log(\lambda)-\mu)^2}{2\sigma^2}\right) \; ; \quad \lambda \in (0,\infty),\ \sigma > 0,\ \mu \in (-\infty,+\infty)$$

It is assumed that $X$ follows truncated Poisson distribution with parameter $\lambda_d, a, b$, and $\lambda_d$ further follows lognormal distribution with parameters $\mu_d, \sigma$. Therefore, the probability mass function of $X$ can be written as:

$$P_{[a,b]}(X = x|\mu_d,\sigma) = \int_0^\infty P_{[a,b]}(X = x|\lambda_d) f(\lambda_d|\mu_d,\sigma) d\lambda_d$$

where

$$P_{[a,b]}(X = x|\lambda_d) = \frac{\lambda_d^x}{x!\left[\sum_{t=a}^{b}\left(\frac{\lambda_d^t}{t!}\right)\right]}$$

and

$$f(\lambda_d|\mu_d,\sigma) = \frac{1}{\lambda_d\sigma\sqrt{2\pi}} \exp\left(-\frac{(\log(\lambda_d)-\mu_d)^2}{2\sigma^2}\right) \; ; \quad \lambda_d \in (0,\infty),\ \sigma > 0,\ \mu \in (-\infty,+\infty)$$

Since the relationship between $\mu$ and $\mu_d$ is given by:

$$\mu_d = \mu + \log(f) + \log(u),$$

the *pmf* becomes

$$P_{[a,b]}(X = x|\mu_d,\sigma) = \frac{1}{\sigma\sqrt{2\pi}} \int_0^\infty \left( \frac{\lambda_d^x}{\lambda_d x!\left[\sum_{t=a}^{b}\left(\frac{\lambda_d^t}{t!}\right)\right]} \exp\left(-\frac{(\log(\lambda_d)-\mu_d)^2}{2\sigma^2}\right)\right) d\lambda_d$$

We can substitute $\log(\lambda_d) = z$ at $dz = \left(\frac{1}{\lambda_d}\right) d\lambda_d$ and $\lambda_d = e^z$, and then:

$$P_{[a,b]}(X = x|\mu_d, \sigma) = \frac{1}{\sigma\sqrt{2\pi}} \int_{-\infty}^{\infty} \left( \frac{e^{zx}}{x! \left[ \sum_{t=a}^{b} \left( \frac{e^{zt}}{t!} \right) \right]} \exp\left( -\frac{(z-\mu_d)^2}{2\sigma^2} \right) \right) dz$$

The probability of detection $(P_d)$ is given by:

$$\begin{aligned}
P_d &= P(\widehat{C} > USL) \\
&= P\left( \frac{X}{u \times f} > USL \right) \\
&= P(X > USL \times u \times f) \\
&= 1 - P(X \leq USL \times u \times f) \\
&= 1 - \sum_{x=a}^{USL_1} P_{[a,b]}(X = x) \qquad \text{say } USL_1 = USL \times u \times f \\
&= 1 - \sum_{x=a}^{USL_1} \frac{1}{\sigma\sqrt{2\pi}} \int_{-\infty}^{\infty} \left( \frac{e^{zx}}{x! \left[ \sum_{t=a}^{b} \left( \frac{e^{zt}}{t!} \right) \right]} \exp\left( -\frac{(z-\mu_d)^2}{2\sigma^2} \right) \right) dz
\end{aligned}$$

Therefore, the probability of detection becomes

$$P_d = 1 - \sum_{x=a}^{USL_1} \frac{1}{\sigma\sqrt{2\pi}} \int_{-\infty}^{\infty} \left( \frac{e^{zx}}{x! \left[ \sum_{t=a}^{b} \left( \frac{e^{zt}}{t!} \right) \right]} \exp\left( -\frac{(z-\mu_d)^2}{2\sigma^2} \right) \right) dz$$

The above equation can be approximated using the Maclaurin series for expansion of $e^{\lambda_d}$ as

$$P_d = 1 - \sum_{x=0}^{USLuf} \frac{1}{\sigma\sqrt{2\pi}} \int_{0}^{\infty} \left( \frac{\lambda_d^{x-1}}{x! \left[ e^{\lambda_d} - 1 \right]} \exp\left( -\frac{(\log(\lambda_d) - \mu_d)^2}{2\sigma^2} \right) \right) d\lambda_d$$

## 6.B Additional graphic displays

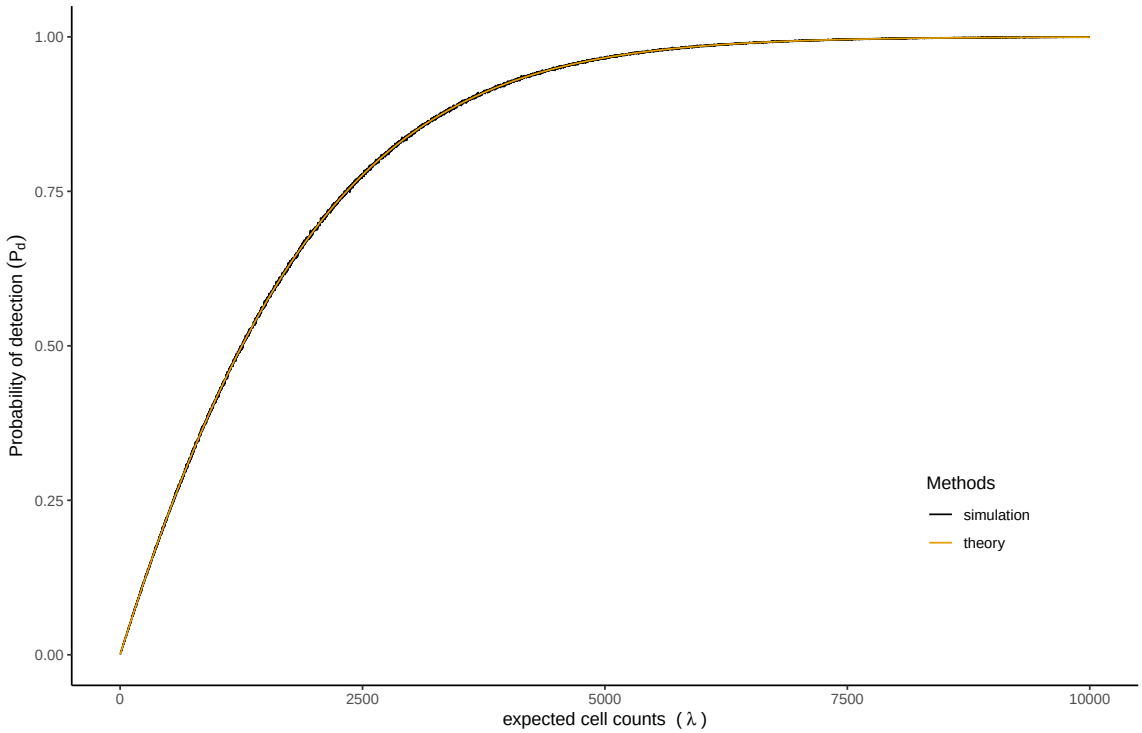


Figure 6.9: The validation of results when diluted testing pertains to a homogeneous batch (based on 20,000 simulation from truncated Poisson distribution).

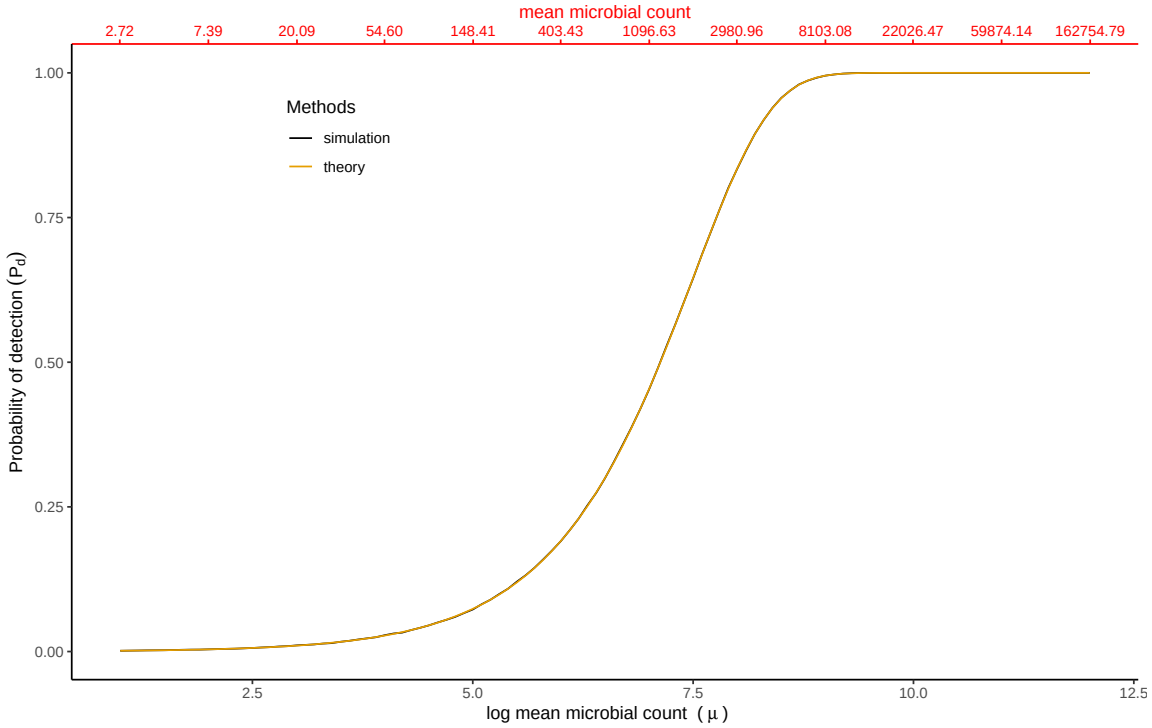


Figure 6.10: The validation of results when diluted samples were formed from a non-homogeneous batch (based on 20,000 simulations from truncated Poisson-lognormal distribution with $\sigma = 0.2$).

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# STATEMENT OF CONTRIBUTION
# DOCTORATE WITH PUBLICATIONS/MANUSCRIPTS

We, the student and the student's main supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the student's contribution as indicated below in the Statement of Originality.

| | |
|---|---|
| Student name: | Mayooran Thevaraja |
| Name and title of main supervisor: | DR. K. Govindaraju |
| In which chapter is the manuscript/published work? | Chapter 7 |
| What percentage of the manuscript/published work was contributed by the student? | 90% |

Describe the contribution that the student has made to the manuscript/published work:

The candidate did the necessary research, statistical modeling, code writing and preparation of the manuscript.

Please select one of the following three options:

- ○ **The manuscript/published work is published or in press**
  Please provide the full reference of the research output:
- ○ **The manuscript is currently under review for publication**
  Please provide the name of the journal:
- ◉ **It is intended that the manuscript will be published, but it has not yet been submitted to a journal**

| | | | |
|---|---|---|---|
| Student's signature: | Mayooran Thevaraja Digitally signed by Mayooran Thevaraja Date: 2023.04.26 04:19:19 +05'30' | Main supervisor's signature: | Dr. K. Govindaraju Digitally signed by Dr. K. Govindaraju DN: cn=Dr. K. Govindaraju, c=NZ, o=Massey University, ou=School of Mathematical and Computational Sciences, email=k.govindaraju@massey.ac.nz Date: 2023.04.26 11:22:11 +12'00' |

*This form should be placed at the beginning of each relevant thesis chapter.*

# Chapter 7

# Effect of the Quantity of Diluted Sample

## 7.1 Abstract

This chapter explores the impact of the quantity of analytical samples used for preparing diluted samples on microbial risk assessment in food safety testing procedures. Microbiologists commonly employ plate count techniques involving serial dilution and placing microorganisms on a solid medium for microbial enumeration. While existing research has focused on plate count-based risk assessment with fixed dilution factors and sample amounts, the specific quantity of the analytical sample remains a crucial but understudied aspect affecting risk assessment outcomes. To address this gap, the chapter presents theoretical findings, leveraging the beta-binomial distribution to investigate the influence of sample quantity on mitigating microbiological risk. The research yields valuable insights to enhance microbial risk management and refine food safety testing protocols.

## 7.2 Introduction

Microbiologists have developed several testing procedures in the food safety area to determine microbial counts in an analytical sample. The most commonly used testing procedures for microbial enumeration are culturing techniques followed by a plate count procedure. The plate count method consists of two separate steps: serial dilution and placement of microorganisms in a solid medium; see Tortora et al. (2010). The previous Chapter examined plate count-based risk assessment in the dilution process fixing the dilution factor

and the amount of diluted sample on the plate. However, the amount of analytical sample used for preparing diluted samples can also affect the risk assessment. Therefore this Chapter focuses on the amount of sample used for diluted sample preparation to reduce microbiological risk.

Following this introduction, Section 7.3 discusses some theoretical results, including results based on the beta-binomial distribution. The results and discussion are then briefly presented in Section 7.4. Section 7.5 discusses the R package that was developed for this study, as well as the wrapper package for all the packages developed. Finally, the conclusions and limitations of this study are provided in Section 7.6. All mathematical notation and abbreviations used in this chapter are listed in Table 7.1.

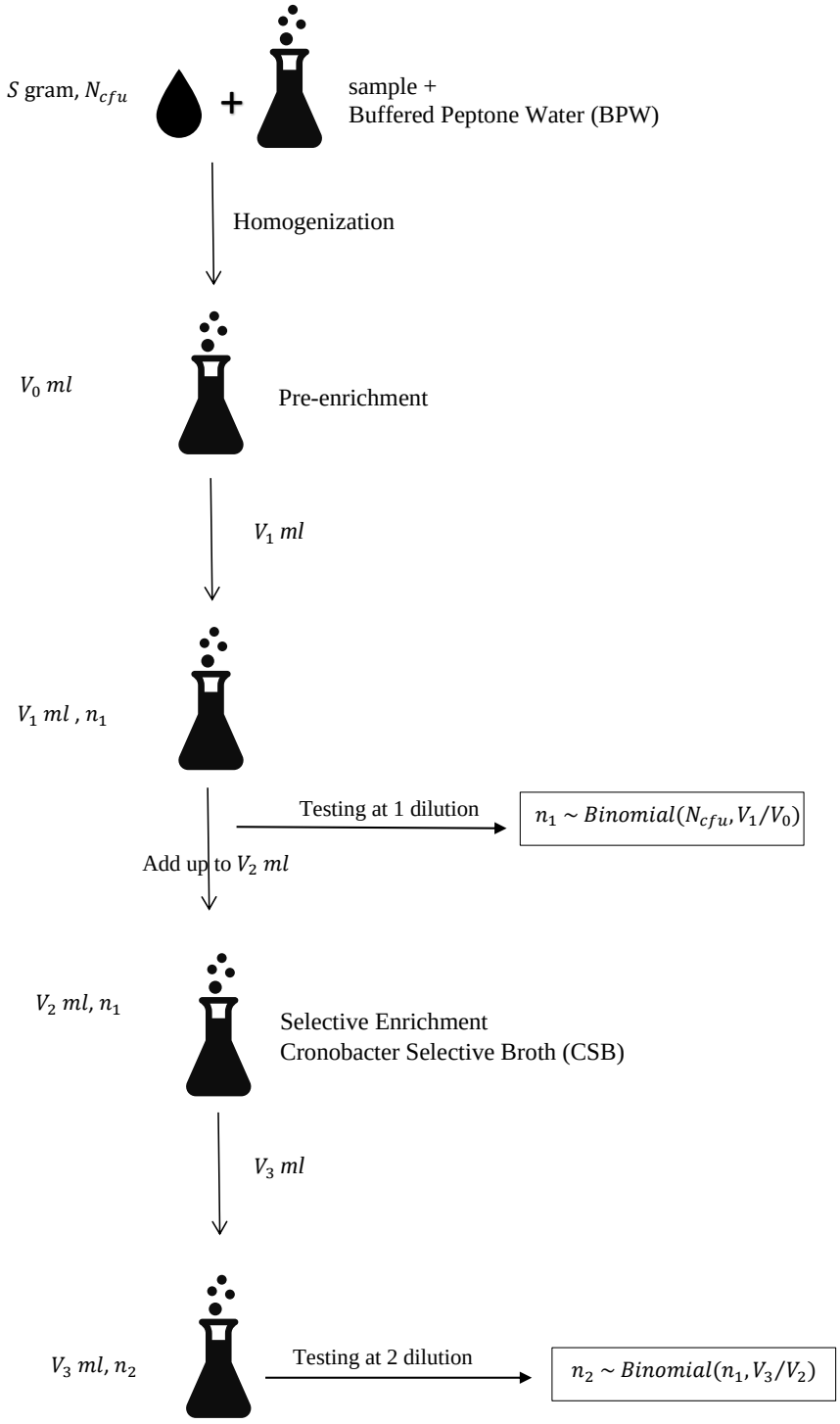
## 7.3 Methodology

This Chapter investigates the impact of sample quantity used for diluted solution preparation in the initial stage of the dilution process. Poisson and Poisson mixture distributions are the commonly employed probability distributions to model the count of microorganisms in food safety studies; see Gonzales-Barron et al. (2013), Gonzales-Barron and Butler (2011b), Jongenburger et al. (2015), Jongenburger et al. (2012), Mussida et al. (2013b), Powell (2015), Schothorst et al. (2009). In line with the published research, this chapter employs Poisson and Poisson lognormal distributions to model the number of microorganisms in the sample used for diluted solution preparation.

Let $N_{cfu}$ be the number of microorganisms in the undiluted sample. Let $N_{cfu}$ follows Poisson distribution with parameter $\lambda$, whose probability mass function is given by:

$$P(N_{cfu} = n_{cfu}) = \frac{e^{-\lambda}\lambda^{n_{cfu}}}{n_{cfu}!}; \quad n_{cfu} = 0, 1, 2, \cdots \tag{7.1}$$

where $\lambda$ is the expected microbial count per gram.




$N_{cfu}$ , $n_1$ and $n_2$ are denoted the number of Colony Forming Units (CFU) in each diluted sample

Figure 7.1: Explanation of the dilution process for microorganisms testing

If $N_{cfu}$ follows Poisson lognormal distribution with parameter $\mu, \sigma$, following Bulmer (1974) methodology, the probability mass function of $N_{cfu}$ can be written as:

$$P(N_{cfu} = n_{cfu}|\mu,\sigma) = \frac{1}{\sigma\sqrt{2\pi}} \int_0^\infty \frac{e^{-\lambda}\lambda^{n_{cfu}}}{n_{cfu}!} \frac{1}{\lambda} \exp\left(-\frac{(\log(\lambda)-\mu)^2}{2\sigma^2}\right) d\lambda$$
$$; \quad n_{cfu} = 0,1,2,\cdots\cdots, \lambda \in (0,\infty),\ \sigma > 0,\ \mu \in (-\infty,+\infty) \quad (7.2)$$

where $\mu$ is the average microbial count and $\sigma$ is the standard deviation of the microbial count. Also, $\mu$ can be estimated (assuming a fixed value of $\sigma$ ) by

$$\mu = \log_{10}(\lambda) - \frac{\sigma^2}{2}\ln(10), \quad (7.3)$$

see Mussida et al. (2013b).

A general laboratory testing procedure for the detection of *Cronobacters* in food commodities is given in ISO 22964 (2017) by using the presence-absence testing method (Silva et al., 2019, p. 222). Following ISO 22964 (2017) guidelines, the flowchart of the *Cronobacters* checking in $S$ gram powdered sample is given in Figure 7.1.

This Chapter employs the binomial and beta-binomial distributions based study to investigate the impact of the quantity of the sample in the diluted solution preparations. A simple binomial-based approach is considered first and then a more general beta binomial-based approach follows later.

### 7.3.1 Binomial distribution-based risk assessment

Let $N_1$ be the number of colony-forming units in the first diluted sample, $N_1$ follows a binomial distribution with parameters $N_{cfu}$ and $V_1/V_0$. Therefore the probability mass function is given by

$$P(N_1 = n_1) = \binom{N_{cfu}}{n_1} (V_1/V_0)^{n_1}(1 - V_1/V_0)^{N_{cfu}-n_1}; \quad n_1 = 0,1,2,\cdots N_{cfu} \quad (7.4)$$

Therefore, the detection probability is given by,

$$P_d = 1 - P(N_1 = 0) \tag{7.5}$$

This implies that

$$P_d = 1 - (1 - V_1/V_0)^{N_{cfu}} \tag{7.6}$$

where $N_{cfu}$ follows Poisson distribution with parameter $\lambda$ or Poisson lognormal distribution with parameters $\mu$ and $\sigma$.

Let $N_2$ be the number of colony-forming units in the second diluted sample, $N_2$ follows a binomial distribution with parameters $n_1$ and $V_3/V_2$. Therefore the probability mass function is given by

$$P(N_2 = n_2) = \binom{n_1}{n_2} (V_3/V_2)^{n_2} (1 - V_3/V_2)^{n_1 - n_2}; \quad n_2 = 0, 1, 2, \cdots n_1 \tag{7.7}$$

Therefore, the detection probability is given by,

$$P_d = 1 - P(N_2 = 0) = 1 - (1 - V_3/V_2)^{n_1} \tag{7.8}$$

### 7.3.2 Beta binomial distribution based risk assessment

During the incubation period, microorganisms are grown to high levels; so enough microorganisms will be present in minimal volumes, which is helpful for later detection tests. During incubation is that the sub-samples may not be truly independent, even if they are cultured separately. For example, if the original sample contains clusters of microorganisms, the sub-samples are expected to be cross-contaminated, leading to the correlation between the sub-sample results. This correlation can lead to an under-estimation of the sampling variance and covariance. Consequently, the dilution process will often lead to extra binomial variation. Beta-binomial distribution is widely used to deal with over dispersion of a binomial random variable. Therefore, this Chapter employs the beta-binomial distribution in the dilution process to study the effect of the amount of sample used for diluted sample preparation.

The beta binomial distribution is a mixture distribution of binomial and beta distributions. For first stage of the dilution testing, let $N_1$ be the number of colony-forming units in the first diluted sample, $N_1$follows binomial distribution with parameters $N_{CFU}$ and $p$ while $p$ follows beta distribution with parameters $\alpha$ and $\beta$. Then the probability mass function is given by,

$$P(N_1 = n_1|\mathbf{p}) = \binom{N_{cfu}}{n_1}(\mathbf{p})^x(1-\mathbf{p})^{N_{cfu}-n_1}; \quad n_1 = 0, 1, 2, \cdots N_{cfu} \tag{7.9}$$

where $p$ follows beta distribution with parameters $\alpha$ and $\beta$. Therefore, the probability mass function of $p$ is given by

$$P(\mathbf{p} = p|\alpha, \beta) = \frac{p^{\alpha-1}(1-p)^{\beta-1}}{\mathbf{B}(\alpha, \beta)} \tag{7.10}$$

where $\mathbf{B}(\cdot, \cdot)$ is the beta function. Therefore following Skellam (1948), the marginal distribution of $n_1$ can be written by;

$$P(N_1 = n_1|\alpha, \beta, N_{cfu}) = \binom{N_{cfu}}{n_1}\frac{\mathbf{B}(\alpha + n_1, N_{cfu} + \beta - n_1)}{\mathbf{B}(\alpha, \beta)}; \quad n_1 = 0, 1, 2, \cdots N_{cfu} \tag{7.11}$$

The probability of detection is given by

$$P_d = 1 - P(N_1 = 0|\alpha, \beta, N_{cfu}) \tag{7.12}$$

Therefore, the probability of detection is given by

$$P_d = 1 - \frac{\mathbf{B}(\alpha, \beta + N_{cfu})}{\mathbf{B}(\alpha, \beta)} \tag{7.13}$$

where $N_{cfu}$ follows Poisson distribution with parameter $\lambda$ or Poisson lognormal distribution with parameters $\mu$ and $\sigma$.

For the second stage of the dilution testing, let $N_2$ be the number of colony-forming units in the second diluted sample, $N_2$ follows a binomial distribution with parameters $n_1$ and $p$

while $p$ follows beta distribution with parameters $\alpha$ and $\beta$. Therefore following Skellam (1948), the probability mass function is given by

$$P(N_2 = n_2|\alpha, \beta, n_1) = \binom{n_1}{n_2} \frac{\mathbf{B}(\alpha + n_2, n_1 + \beta - n_2)}{\mathbf{B}(\alpha, \beta)}; \quad n_2 = 0, 1, 2, \cdots n_1 \tag{7.14}$$

Therefore, the probability of detection is given by

$$P_d = 1 - \frac{\mathbf{B}(\alpha, \beta + n_1)}{\mathbf{B}(\alpha, \beta)} \tag{7.15}$$

For computing the probability of detection, this study used the simulation technique. $n_1$ can be generated as a random beta binomial variate. The simulation algorithm used for computing the probability of detection is shown as Algorithm 7.1. The following section discusses the summary results based on these methodologies.

**Algorithm 7.1** Computing the probability of detection in the second dilution testing.

1. Initialise the amount of sample $(S)$ used for diluted solution preparation.
2. Set the values of $V_0, V_1$ and $\lambda$ (if $N_{cfu}$ follows Poisson) or $\mu$ (if $N_{cfu}$ follows Poisson lognormal).
3. Generate the $N_{cfu}$ by using corresponding distribution we used in the previous step.
4. Set the number of simulations, $n_{sim}$. Use large values to achieve precision.
5. Generate the number of CFUs $(n_1)$ in the diluted solution after pre-enrichment.
6. Obtain the probability of detection by using Equations 7.8, or 7.13 depends on what distribution (binomial or beta-binomial) we used, with $n_{sim}$ rows and a single column.
7. Compute the probability of detection as the average of $n_{sim}$ simulated cases.

## 7.4 Results and discussion

This particular physical experiment, comprehensively detailed in the literature authored by Silva et al. (2019), investigates the impact of the amount of sample used for dilution. In this example, various sample amounts, such as 25g, 125g, and 250g (assuming a primary

increment amount of 1g of material), were considered. For the first dilution test, $Sg$ of infant formula powder was added to the buffered peptone water (BPW) to reach a volume level of $V_0$ $ml$ and mixed until the powder was uniformly suspended.

After the incubation period, we assumed to use $V_1$ $ml$ for the detection test, which is defined as the first dilution stage testing. Meanwhile, another $V_1$ $ml$ transfer of the BPW culture into *Cronobacter* selective broth (CSB) up to reach the volume level of $V_2$ $ml$. Then select $V_3$ $ml$ for the detection test, which is called second dilution stage testing in this study.

This procedure is demonstrated in Figure 7.1, and we used $S = 25g,\ 125g,\ 250g, V_0 = 100ml, V_1 = 1ml, V_2 = 10ml$ and $V_3 = 1ml$ for illustrative purposes.

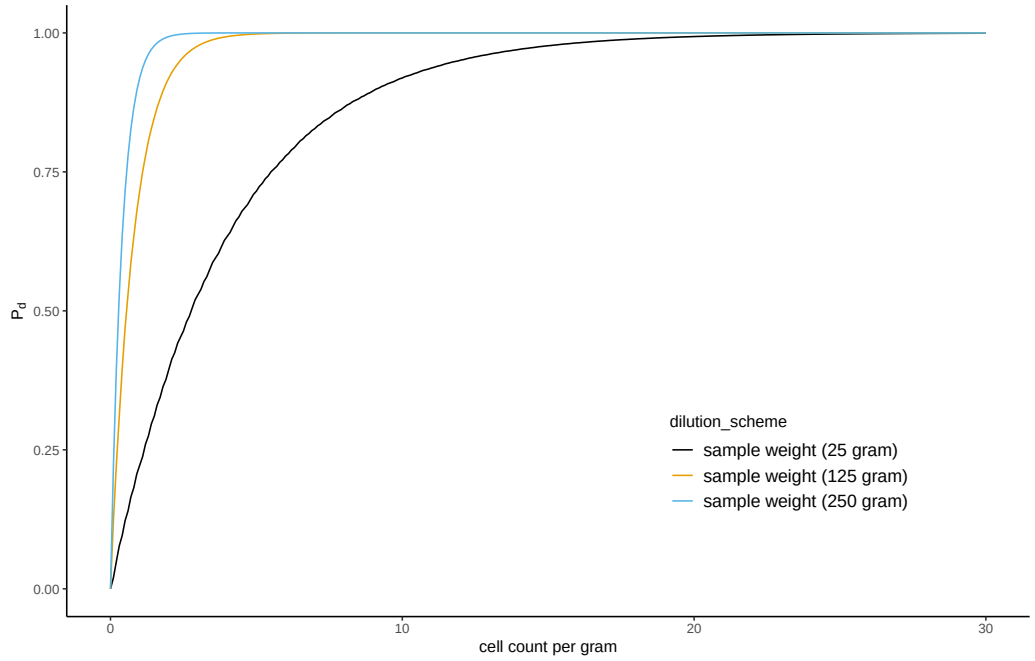



Figure 7.2: Binomial-based probability of detection for three dilution schemes in the first dilution testing, assuming $N_{cfu}$ follows Poisson.

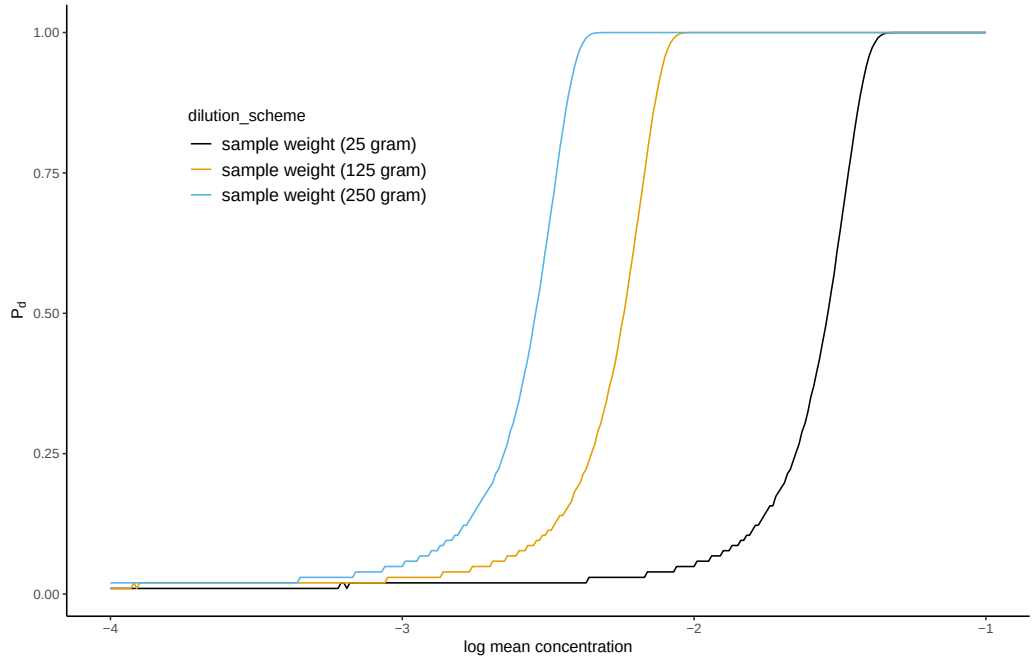



Figure 7.3: Binomial-based probability of detection for three dilution schemes in the first dilution testing, assuming $N_{cfu}$ follows Poisson lognormal.

Figures 7.2 and 7.3 compare various amounts of samples used in diluted solutions preparations in the first dilution test. These figures are generated based on binomial distribution assuming that $N_{cfu}$ follows Poisson or Poisson lognormal distributions, respectively. The probability of detection increases as a function of the expected cell count or mean concentration of the contamination. The probability of detection converges to one or gets closer to one for larger values of $\lambda$ or $\mu$. A larger sample amount means a higher probability of detection in the first dilution test.

Comparisons are made in Figures 7.4 and 7.5 for different sample amounts used in the preparation of diluted solutions for the first dilution test. These figures are based on the beta-binomial distribution assuming that $N_{cfu}$ follows either a Poisson or a Poisson lognormal distribution, respectively.

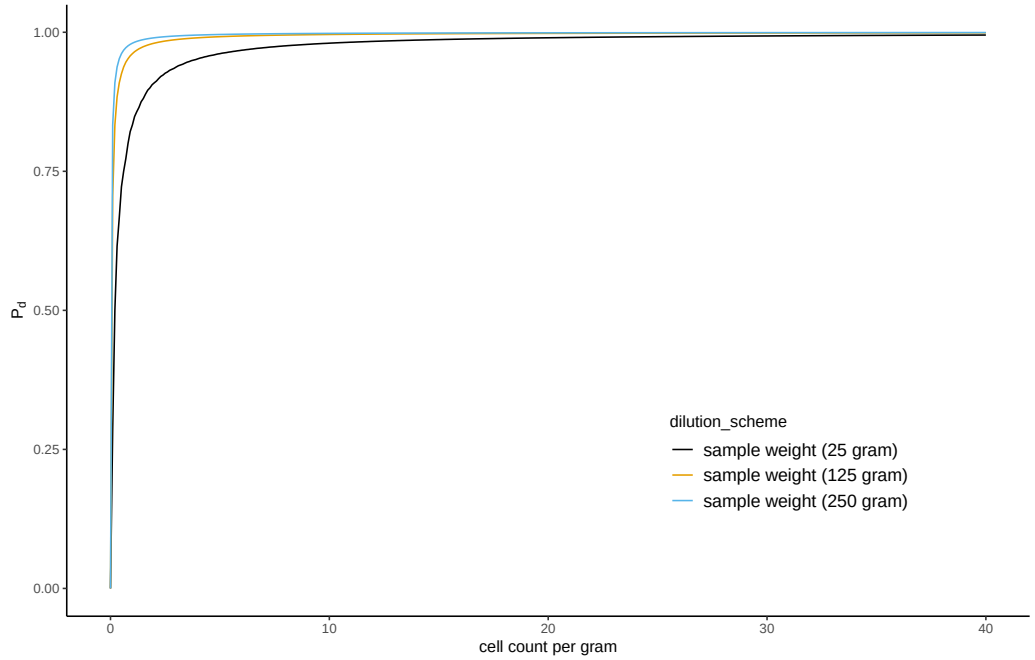



Figure 7.4: Beta binomial-based probability of detection for three dilution schemes in the first dilution testing, assuming $N_{cfu}$ follows Poisson.

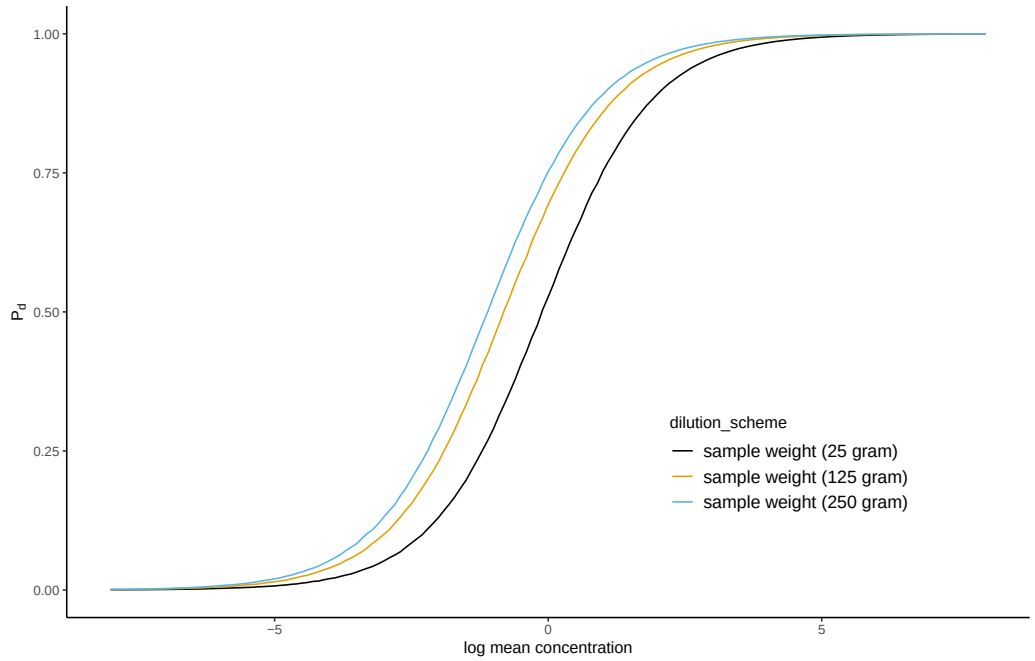



Figure 7.5: Beta binomial-based probability of detection for three dilution schemes in the first dilution testing, assuming $N_{cfu}$ follows Poisson lognormal.

Figures 7.6, 7.7 and 7.8 compare the sample amounts in the second dilution testing. These Figures are based on binomial distribution and beta binomial distributions assuming

$N_{cfu}$ follows Poisson or Poisson lognormal distribution. For computing the probability of detection in second dilution testing, this study assumes that the number of microorganisms reappear from the original count of $n_1$. This $n_1$ can be simulated by using a binomial or beta-binomial distribution. This simulation can be done using the R package *"dilutionrisk"*.

The conclusions on the second testing stage are the same as in the first testing stage. A larger sample for preparing a diluted solution improves the probability of detection. The risks are reduced with larger samples and this theoretical results hold in both single and two-stage testing. However, the second stage testing includes incubation and hence the risks are reduced greatly. The amount tested becomes somewhat less sensitive when incubation is done.

Enrichment process is necessary when the test sample contains very low levels of pathogenic microorganisms. Specifically, the risk of non-detection would be high if only the first dilution testing is alone used to make a decision.

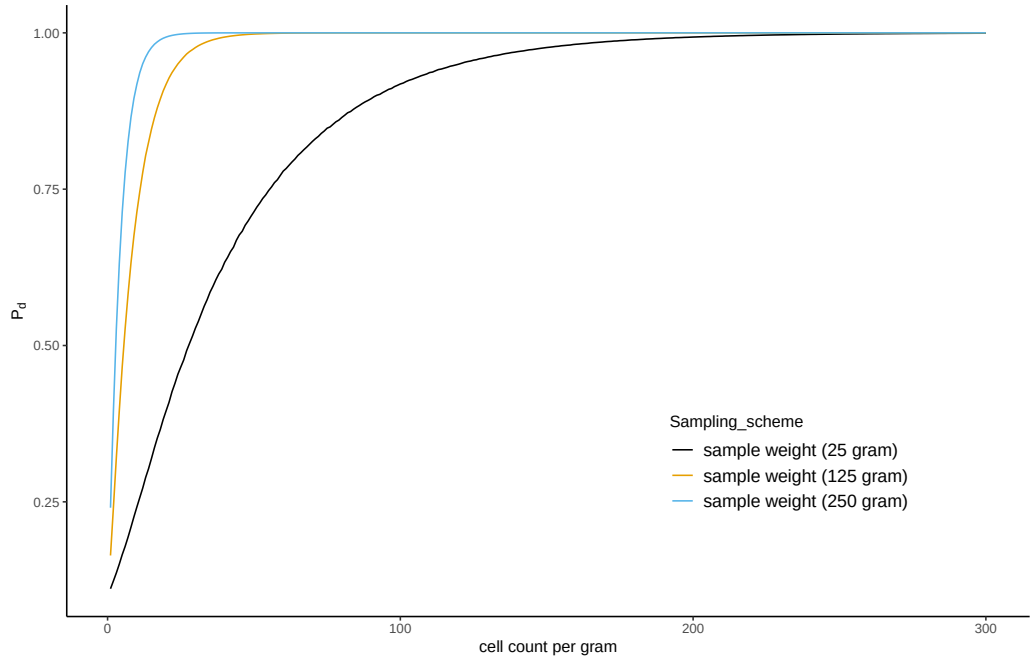



Figure 7.6: Binomial-based probability of detection for three dilution schemes in the second dilution testing, assuming $N_{cfu}$ follows Poisson (based on 20,000 simulations).

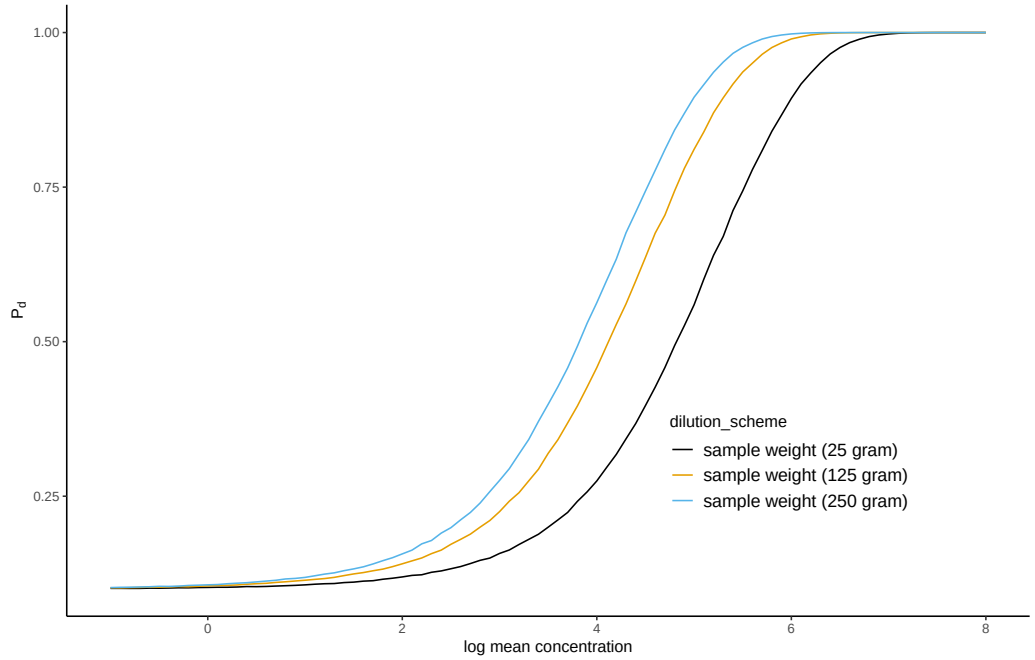



Figure 7.7: Binomial-based probability of detection for three dilution schemes in the second dilution testing, assuming $N_{cfu}$ follows Poisson lognormal (based on 20,000 simulations).

The results of the second dilution testing, which occurs after enrichment, show a significantly high detection probability, with all $P_d$ values being greater than 0.95.

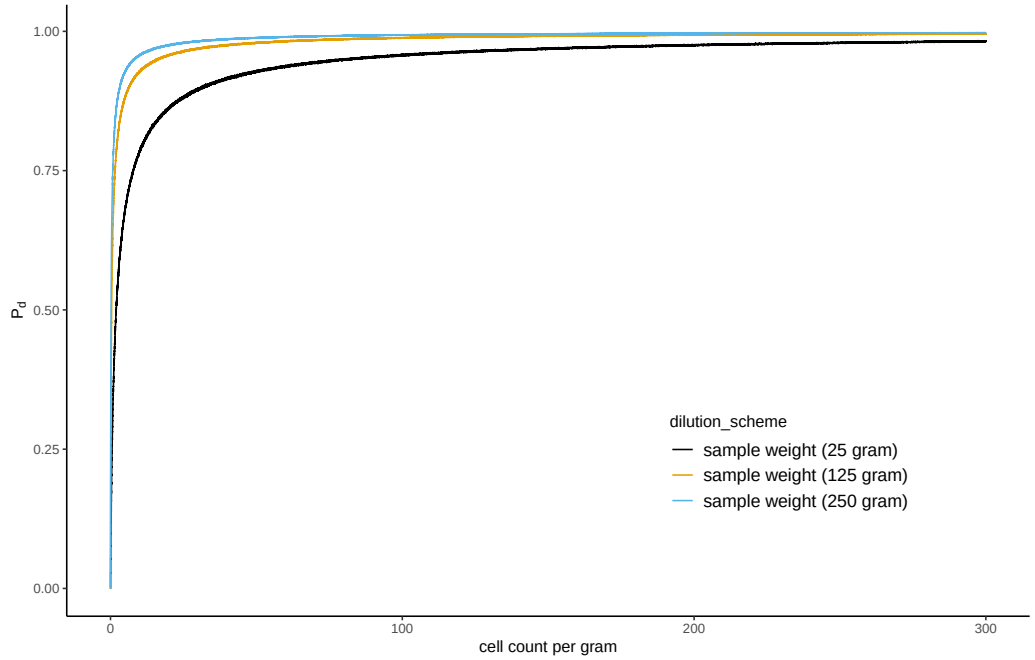



Figure 7.8: Beta binomial-based probability of detection for three dilution schemes in the second dilution testing, assuming $N_{cfu}$ follows Poisson (based on 20,000 simulations).

## 7.5 R Package "*mRatools*"

The R programming codes related to this Chapter are incorporated in the open-source R package called *"dilutionrisk"* (available at `https://github.com/Mayooran1987/dilutionrisk`), which is described in Chapter 6. Although combining the codes from Chapters 6 and 7 into a single package might appear feasible, it could result in a codebase that is less maintainable and less adaptable.

Part of this research developed four open-source R packages to compute the probability of detection and obtain graphical displays such as OC curves for risk assessment. The open-source R package as a microbiological risk assessment tool (*"mRatools"*) was developed to wrap four packages. The *mRatools* package will automatically install all four packages in a single step. A full description of this wrapper *R* package is available at `https://mayooran1987.github.io/mRatools`.

## 7.6 Conclusions and limitations

The primary conclusion drawn from this Chapter is that the quantity of sample utilized in the preparation of diluted solutions plays a pivotal role in influencing the likelihood of successful detection. It has been established that the detection efficiency improves as the sample size increases, both in cases where enrichment is employed and when it is not. Moreover, the study has yielded another significant finding: the enrichment process substantially enhances the probability of successful detection. In the second dilution test, the detection probabilities are so high that they approach near certainty. Consequently, this discovery holds the potential to revolutionize the practices of food safety professionals. By heeding the insights of this research, practitioners can streamline their testing protocols, potentially eliminating the need for superfluous detection tests. In doing so, they contribute to bolstering the protection of consumers and fortifying food safety standards.

Table 7.1: Chapter 7: Glossary of symbols and abbreviations

| | |
|---|---|
| $N_{cfu}$ | the number of microorganisms in the selected test sample |
| $V_{\text{plate}}$ | plated volume (ml) |
| $V_0$ | Volume of diluted sample in the pre-entrichment stage |
| $V_1$ | volume of diluted sample used for first-stage of testing |
| $V_2$ | Volume of diluted sample in the Selective Enrichment stage |
| $V_3$ | Volume of diluted sample used for second-stage of testing |
| $\alpha$ | beta distribution's non negative parameter |
| $\beta$ | beta distribution's non negative parameter |
| $S$ | sample quantity such as volume (ml) or weight (g) |
| $N_1$ | the number of colony-forming units in the first stage of testing |
| $N_2$ | the number of colony-forming units in the second stage of testing |
| $P_d$ | probability of detection |
| $P_{nd}$ | probability of non-detection |

# Chapter 8

# Conclusions and Future work

This research addresses crucial statistical issues concerning sample selection for microbial risk assessment in the food industry. The primary goal is to evaluate the risk of food contamination by drawing a representative analytical sample and assessing its variability. The thesis aims to achieve two key objectives: firstly, to comprehend the current sampling practices commonly employed in the food industry, and secondly, to propose improved food safety sampling plans compared to the existing ones. These objectives are extensively elaborated upon in Chapter 1. Additionally, Chapter 2 of the thesis presents a literature review focused on acceptance sampling procedures and their application in the food industry.

## 8.1 Contributions of the thesis

Chapter 3 is a theoretical study which investigated the microbiological risk of non-detection when grab samples are employed, comparing it with the statistical 'gold standard' method of randomly sampling primary increments. Due to the grab sampling methods exhibiting an enhanced risk of non-detection, additional sampling is needed. In Chapter 3, it may be more appropriate to refer to the stated probability of contamination as the probability of detectable contamination. This term accounts for the likelihood of detecting contaminants above a specific threshold or limit of detection.

Chapter 4 focused on the number of mixing stages for risk assessment. For example, suppose the number of mixing stages or revolutions is fewer during analytical sample preparation. In that case, the risk of non-detection will increase, even under the recommended international guidelines for analytical sample preparation. However, by choosing an optimised number of mixing stages, or revolutions, the risk of non-detection can be reduced. The main findings of this chapter indicate that when mixing or blending does not result in product degradation, the likelihood of detection is strongly correlated with the number of revolutions in the mixtures to a point.

Chapter 5 suggests that the quantity of incremental samples is an essential controllable factor for reducing the microbiological risk. Our results showed that unequal incremental sample selection induces extra variability in the sample selection. Therefore, the risk level will increase when we use an unequal quantity of incremental samples. On the other hand, the smaller amount of incremental sample selection provides higher protection to consumers. Therefore, microbiological risk can be reduced when using equally weighted smaller incremental sample selection.

In Chapter 6, it was demonstrated that plate count techniques relying on dilution testing produced false positive results in cases where a low level of contamination was present in the batch. Consequently, it is crucial to employ additional testing methods or alternative detection tests to mitigate microbiological risks when collecting diluted samples from batches with low contamination levels. The findings of this chapter have revealed that dilution testing resulted in false positives at low contamination levels in both homogeneous and non-homogeneous batches. Conversely, false negatives were observed at rejectable concentration levels. Furthermore, the findings indicated that increasing the plated amount would lead to a reduction in both the producer's and consumer's risks.

Chapter 7 focused on the effect of the amount of material sampled when preparing a sample for dilution testing. This study assess microbiological risk based on simple binomial and beta binomial distributions. Chapter 7 concludes that the quantity of sample utilized in preparing the diluted solutions has a significant impact on the probability of detecting

contamination. Moreover, it is observed that larger samples result in improved detection, both with and without enrichment.

In addition to the research findings, the thesis also highlights the development of five open-source R packages (i.e., *grabsampling, mixingsimulations, uneqmixr, dilutionrisk* and *mRatools*), which includes the wrapper package.

The thesis thus comprises a comprehensive examination of issues deriving from sampling that affect acceptance sampling methods, particularly in the food industry, providing valuable insights for enhancing food safety practices. To illustrate this, the next section describes how Fonterra, New Zealand's largest dairy exporter, might benefit from the work in this thesis by implementing more robust and effective microbial risk assessment and sampling strategies, which can ultimately lead to safer dairy product exports and increased consumer confidence.

## 8.2 Applications to Fonterra

The intricate examination of statistical intricacies pertaining to sample selection for microbial risk assessment materializes when applied to a significant and complex sector of the food industry. This section elaborates on the inherent strengths that characterize this concluding chapter as a wellspring of actionable insights.

Traditional sampling methods in the dairy industry often involve grab samples taken at specific points during the pasteurization process. This practice might lead to an elevated risk of non-detection of pathogens, potentially compromising the safety of the final product. By applying the principles outlined in this thesis, dairy processing plants can significantly improve their sampling strategies and mitigate microbial risks.

Utilizing the insights from Chapter 3, dairy processing plants can evaluate the impact of grab sampling on the risk of non-detection. They can then transition to a more robust sampling approach, such as the "gold standard" method of randomly sampling primary increments. Furthermore, insights from Chapter 4 can guide them in determining the optimal number of mixing stages or revolutions during sample preparation, ensuring that the risk of non-detection is minimized without compromising the product quality.

Chapters 5 and 6 of this thesis offer valuable guidance for selecting the appropriate quantity of incremental samples and testing methods. By implementing equal and smaller incremental sample selections, dairy plants can enhance consumer safety while minimizing the potential for false positives or false negatives in microbial testing, as demonstrated in Chapter 6.

The dairy processing industry can also benefit from the findings in Chapter 7, where the quantity of material sampled is shown to influence the probability of detecting contamination. Applying this knowledge, plants can adapt their sampling protocols to include larger sample sizes, thus improving the accuracy of detection.

In conclusion, the research presented in this thesis has far-reaching implications for various industries, including dairy processing. By adopting the proposed sampling practices and risk assessment methodologies, dairy processing plants can bolster food safety, minimize contamination risks, and uphold consumer trust. The developed R packages, outlined in the thesis, offer practical tools for implementing these strategies.

In essence, the research conducted in this thesis has the potential to revolutionize how the food industry approaches microbial risk assessment, leading to safer products and healthier consumers. By bridging the gap between theoretical insights and practical implementation, this work stands as a testament to the transformative power of statistical analysis in ensuring food safety.

## 8.3 Potential future work

The scope of future work offers a promising trajectory for enhancing microbial risk assessment in the food industry. A pivotal avenue for future research involves exploring Markov chain-based risk assessment studies, representing a departure from traditional statistical distribution-based approaches. This novel perspective can usher in more precise predictions of contamination risks, and future research efforts will concentrate on these Markov chain-based theoretical studies, particularly with regard to the optimization of sample sizes and dilution processes.

Part of this research focuses on understanding the risk when using primary samples of different sizes to create composites, a topic studied in Chapter 5. In future studies, we plan to compare how well it works when using an equal number of incremental samples at each stage compared to using an unequal number. The analysis will also encompass the consequences of using the same or different numbers of samples at each stage. This research aims to deepen our understanding of the connection between sample quantity and microbiological risk, potentially leading to insights for optimizing sampling strategies to enhance risk reduction and consumer protection.

In parallel, there is a commitment to further augment the efficacy of the developed R packages, enhancing their functionality and user-friendliness. These refinements are designed to empower general practitioners with the tools to apply sophisticated statistical methods within their unique contexts effortlessly. Moreover, the creation of user-friendly R Shiny apps is on the horizon, aimed at simplifying the generation of results and making these advanced statistical techniques accessible to a broader audience within the food industry. These multifaceted enhancements will make risk assessment more accurate and more approachable, setting the stage for a new era of precision and efficiency in the domain of microbial risk assessment and food safety practices.

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