# Relating Microbiological Criteria to Food Safety Objectives and Performance Objectives

**M van Schothorst[a], MH Zwietering[b*], T Ross[c], RL Buchanan[d], MB Cole[e],**

**International Commission on Microbiological Specifications for Foods (ICMSF)**

[a]Chemin du Grammont 20
La Tour- de- Peilz
CH-1814 Switzerland

[b]Laboratory of Food Microbiology
Wageningen University
6700 EV Wageningen
The Netherlands

[c]Tasmanian Institute of Agricultural Research
School of Agricultural Science
University of Tasmania
Hobart, Tasmania 7001
Australia

[d]Center for Food Systems Safety and Security
College of Agriculture and Natural Resources
University of Maryland
College Park, MD, USA 20742

[e]National Center for Food Safety and Technology (NCFST),
Illinois Institute of Technology,
6502 S. Archer Road,
Summit-Argo, Illinois 60501, USA

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[*] Author for Correspondence:

Dr. Marcel Zwietering
Laboratory of Food Microbiology
Wageningen University
6700 EV Wageningen
The Netherlands
Marcel.Zwietering@wur.nl
-31-317-482233

33 # Relating Microbiological Criteria to Food Safety Objectives and
34 Performance Objectives

35 **M. van Schothorst, M.H. Zwietering, T. Ross, R.L. Buchanan, M.B. Cole,**

36 **International Commission on Microbiological Specifications for Foods (ICMSF)***

37

38 **ABSTRACT**

39 Microbiological criteria, Food Safety Objectives and Performance Objectives, and the
40 relationship between them are discussed and described in the context of risk-based food
41 safety management. A modified method to quantify the sensitivity of attributes sampling
42 plans is presented to show how sampling plans can be designed to assess a microbiological
43 criterion. Examples presented show that testing of processed foods for confirmation of
44 safety is often not a practical option, because too many samples would need to be analysed.
45 Nonetheless, in such cases the classical "ICMSF cases" and sampling schemes still offer a
46 risk-based approach for examining food lots for regulatory or trade purposes.

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48 **Key Words; food safety objective, sampling plan, microbiological criteria**

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* Author for Correspondence:

Dr. Marcel Zwietering
Laboratory of Food Microbiology
Wageningen University
6700 EV Wageningen
The Netherlands
Marcel.Zwietering@wur.nl
-31-317-482233

49 **1. Introduction**

50

51 The Risk Analysis framework described by Codex Alimentarius (CAC, 2007a) provides a
52 structured approach to the management of the safety of food. In the Codex document on
53 Microbiological Risk Management (CAC, 2007a) and in ICMSF's "Microorganisms in
54 Foods 7: Microbiological Testing in Food Safety Management" (ICMSF, 2002), the
55 establishment of a Food Safety Objective (FSO) is described as a tool to meet a public
56 health goal such as an Appropriate Level of Protection (ALOP). More recently, an
57 FAO/WHO expert consultation re-emphasised the original definition for ALOP that was
58 part of the Sanitary and Phytosanitary (SPS) Measures Agreement (WTO, 1994), namely
59 that it is the "expression of the level of protection in relation to food safety that is currently
60 achieved. Hence, it is not an expression of a future or desirable level of protection"
61 (FAO/WHO, 2006). An FSO specifies the maximum permissible level of a microbiological
62 hazard in a food at the moment of consumption. Maximum hazard levels at other points
63 along the food chain are called Performance Objectives (POs). The current definitions for
64 FSO and PO (CAC, 2007b) are that an FSO is: "the maximum frequency and / or
65 concentration of a hazard in a food at the time of consumption that provides or contributes
66 to the appropriate level of (health) protection (ALOP)" while a PO is: "the maximum
67 frequency and / or concentration of a hazard in a food at a specified step in the food chain
68 before consumption that provides or contributes to an FSO or ALOP, as applicable".

69 Safe food is produced by adhering to Good Hygienic Practices (GHP), Good
70 Manufacturing Practices (GMP), Good Agricultural Practices (GAP) etc. and
71 implementation of food safety risk management systems such as Hazard Analysis Critical
72 Control Points (HACCP), but the level of safety that these food safety systems are
73 expected to deliver has seldom been defined in quantitative terms. Establishment of FSOs

74 and POs provides the industry with quantitative targets to be met. When necessary,
75 industry may have to validate that their food safety system is capable of controlling the
76 hazard of concern, i.e., to provide evidence that control measures can meet the targets. In
77 addition, industry must periodically verify that their measures are functioning as intended.
78 To assess compliance with FSOs and POs, control authorities rely on inspection
79 procedures (e.g., physical examination of manufacturing facilities, review of HACCP
80 monitoring and verification records, analysis of samples) to verify the adequacy of control
81 measures adopted by industry. In the context of the SPS Agreement (WTO, 1994), national
82 governments may also need to quantitatively demonstrate the equivalence of their
83 inspection procedures to ensure that food safety concerns do not result in an inappropriate
84 barrier to trade. Similarly, a control authority may require individual manufacturers to
85 provide evidence of equivalence of control measures, particularly when non-traditional
86 technologies are being used to control a hazard.

87 Although FSOs and POs are expressed in quantitative terms, they are not Microbiological
88 Criteria which are defined as the acceptability of a product or a food lot, based on the
89 absence/presence or number of microorganisms including parasites, and/or quantity of
90 their toxins/metabolites, per unit(s) of mass, volume, area or lot (CAC 1997; ICMSF
91 2002). A more detailed description of the elements and uses of Microbiological Criteria is
92 presented in Section 2, below.

93 Microbiological testing is one of the potential tools that can be used to evaluate whether a
94 food safety risk management system is providing the level of control it was designed to
95 deliver. It is one of a number of tools that, when used correctly, can provide industry and
96 regulatory authorities with tangible evidence of control.

97 A number of different types of microbiological testing may be used by industry and
98 government (e.g., within lot, process control, investigational). One of the forms of testing

99 most commonly used in relation to microbiological criteria is within-lot testing, which
100 compares the level of a microbiological hazard detected in a food against a pre-specified
101 limit, i.e., a Microbiological Criterion ('MC'; ICMSF, 2002). Microbiological criteria are
102 designed to determine adherence to GHPs and HACCP (i.e., verification) when more
103 effective and efficient means are not available. FSOs and POs are targets to be met. In this
104 context, microbiological criteria based on within-lot testing are meant to provide a
105 statistically-designed means for determining whether these targets are being achieved.
106 Such sampling plans need to consider either:

107 i) the 'consumer's risk', i.e., the chance that a lot will be accepted that exceeds a level that
108 has been determined, usually by government, to pose an unacceptable risk to public health
109 and which, for convenience here, we will call 'Acceptable Level for Safety' ('ALS', *see*
110 Appendix 1), or

111 ii) the 'producer's risk', i.e. the possibility that an acceptable lot will be rejected by the
112 sampling scheme (*see also* Section 5, below), recognizing that both 'risks' are
113 interdependent.

114 The current paper provides information on the data that are necessary, and the types of
115 decisions that have to be made, to develop meaningful sampling plans and ensure that
116 microbiological criteria based on within-lot microbiological testing are being used
117 appropriately. For the purposes of this paper, a *lot* is considered a grouping of a product
118 manufactured during a certain period of time or under the same conditions, or a
119 consignment of a food arriving at a border. A *sample* is taken from that lot to assess the
120 concentration of the hazard in that sample. A sample may comprise the entire *analytical*
121 *unit,* or the analytical unit may be an aliquot derived from the sample. It is assumed that
122 the concentration of the hazard in an aliquot of the sample is representative of the

123 concentration in the whole sample, but that different samples can have different
124 concentrations.

125

126 **2. Nature and Use of Microbiological Criteria**

127 Developing meaningful within-lot microbiological criteria for a food or ingredient is a
128 complex process that requires considerable effort. Furthermore, their application demands
129 considerable resources. Therefore, microbiological criteria should be established only
130 when there is a need and when it can be shown to be effective and practical. The criterion
131 must be capable of accomplishing one or more clearly defined objectives, such as to
132 assess:

133 - the safety of a food;

134 - adherence, on a lot-by-lot basis, to GHP and/or HACCP requirements;

135 - the acceptability of a food or ingredient from another country or region for which the
136 history of the product is unknown or uncertain, i.e., evidence of adherence to GHP or
137 HACCP-based control systems is not available;

138 - compliance of a food with an FSO and/or a PO

139 An MC consists of:

140 - a statement of the microorganism(s) of concern and/or their toxins/metabolites and the
141 reason for that concern;

142 - the food to which the criterion applies;

143 - the specific point(s) in the food chain where the MC should be applied;

144 - microbiological limits considered appropriate to the food at that specified point(s) of the
145 food chain, and

146 - a sampling plan defining the number and size of samples to be taken, and the method of
147 sampling and handling,

148 - the number and size of the analytical units to be tested. For the purposes of this
149 manuscript a sample refers to the portion of a batch that is collected and sent to a
150 laboratory for testing. Part, or all, of the sample is analyzed. The actual amount of the
151 sample that is analysed is the "analytical unit". For example, if a product was sold in
152 100 g packages, and one package of a lot was sent to the laboratory for analysis, this
153 would be the sample. If 50g was removed from the package and then divided into two
154 25-g aliquots that were then tested separately, then one would have two 25-g analytical
155 units (n = 2).

156 - the analytical methods to be used to detect and/or quantify the microorganism(s) or their
157 toxins/metabolites;

158 - the number of analytical units that should conform to these limits; and

159 - any actions to be taken when the criterion is not met.

160 An MC can be used to define the microbiological quality of raw materials, food
161 ingredients, and end-products at any stage in the food chain, or can be used to evaluate or
162 compare the stringency of alternative food control systems and product and process
163 requirements. Three classes of MC are distinguished based on regulatory consequences
164 (ICMSF, 2002):

165 - 'Standards' are microbiological criteria that are written into law or government
166 regulations, e.g., an MC specified by government to protect public health.

167 - 'Specifications' are microbiological criteria established between buyers and producers
168 that define product quality and safety attributes required by the buyer; failure to meet
169 the MC could result in rejection of the product or a reduction in price.

170 - 'Guidelines' are microbiological criteria that provide advice to industry about
171 acceptable or expected microbial levels when the food production process is under
172 control. They are used by producers, to assess their own processes and by government
173 inspectors when conducting audits.

174 To develop an MC, the following information is needed:

175 - the distribution of the microorganism within the lot

176 - the sensitivity and specificity of the test method

177 - the randomness and efficacy of the sampling scheme (i.e., number and size of samples,
178 that samples are randomly drawn from the batch)

179 and several decisions have to be made, e.g.

180 - the quality/safety level as expressed in an FSO or PO, that is required, e.g., absence of
181 *E. coli* O157 in 99% of 100 ml packages of apple juice,

182 - the expected standard deviation of counts in samples taken from the lot. (From these
183 first two decisions, the microbiological status of a lot that is just acceptable can be
184 inferred)

185 - the statistical confidence required for the acceptance or rejection of a non-conforming
186 lot (see Appendix 1)

187 - the required level of benefit derived from the application of an MC compared to cost of
188 testing or the potential consequences of not applying and enforcing an MC.

189 It should be emphasized that statistical interpretation of test results can be misleading if the
190 representativeness of the samples taken from the lot as a whole, or homogeneity of
191 contamination within a lot, cannot be assumed. Historical data relating to that product
192 and/or process are often relied upon when knowledge about the distribution and variability

193 of microorganisms in a *specific* lot of food is unknown. Several of the points mentioned
194 above will be further elaborated in the following sections.
195
196 **3. Distribution of the pathogen of concern**
197 The distribution of pathogens within the lot must be understood if informed decisions are
198 to be made concerning the applicability of within-lot microbiological testing to verify
199 compliance with GHP/HACCP or to determine whether a food lot meets an FSO or PO.
200 Often, however, this is not known and, to enable comparison of the relative stringency of
201 sampling plans, an assumed distribution is used. Furthermore, the level and standard
202 deviation associated with a microbial population is often dynamic as a food proceeds along
203 the food chain. A pathogen may be present in the raw material, but it may be partly or
204 totally eliminated during processing or preparation. It may be reintroduced as a result of
205 subsequent contamination, or increase its concentration over time in products that support
206 its growth. This can influence the prevalence and/or concentration in any specific lot. In an
207 "ideal" situation, microorganisms would be homogeneously distributed throughout the lot,
208 so that whatever sample is taken, it would have the same level of contamination. Apart
209 from liquid foods or after mixing processes, this is usually not the case and, instead, the
210 pathogens are heterogeneously distributed. In many situations the frequency distribution of
211 the contamination levels across samples can be described as log-normal (Jarvis, 1989), i.e.,
212 having a normal distribution when expressed as log CFU values, and characterised by a
213 mean log concentration and a standard deviation. Ideally, to apply statistical interpretations
214 of non-stratified sampling plans (i.e. when there is no reason to assume systematic
215 differences between different samples), samples should be taken at random if the hazard is
216 heterogeneously distributed in the lot. Random sampling cannot always be assured, nor the
217 distribution assumed always to be log-normal. However, experience has indicated that in

218 most instances these assumptions are appropriate for certain microorganisms or groups of
219 microorganisms. For illustration purposes in this paper a log normal distribution of the
220 pathogen of concern in a food is assumed because it provides the basis for establishing a
221 mathematical relationship between FSOs, POs and Microbiological Criteria.
222

223 **4. Performance of Microbiological Criteria**

224 The 'operating characteristic' (OC) curve is a graph that relates the probability of
225 accepting a lot, based on the number of units tested, to the proportion of units, or aliquots
226 in the lot that *do* exceed some specified acceptable level, i.e., the maximum tolerated
227 defect rate. The OC curve depends on both the number of samples tested, '*n*', and the
228 maximum number, '*c*', of those samples that *may* exceed the specified level.

229 While not the usual situation, if the distribution of a pathogen in a lot of food is known, an
230 OC curve can be generated to characterize the performance of an MC (*see* Appendix 1)
231 and to translate information about the proportion of units that are defective into an estimate
232 of the concentration of the contaminant in the lot. OC curves can be used to evaluate the
233 influence that parameters of the MC, i.e. number of samples (*n*), microbiological limit (*m*),
234 number of samples in excess of '*m*' that would lead to rejection of the batch or lot, (*c*), and
235 the mean and standard deviation of the underlying lot distribution, have on the efficacy of
236 the microbiological testing program. This information quantifies the confidence that we
237 can have that a 'defective' lot will be rejected. If one were able to test every unit of food
238 within the lot, the OC curve would change from 100% probability of acceptance to a 100%
239 probability of rejection exactly at the proportion of defective units that distinguishes an
240 acceptable from a defective lot. At the other extreme, taking a single sample, particularly if
241 negative, has virtually no ability to discriminate between conforming and non-confirming

242 lots. Increasing the number of samples ($n$) examined is one of the primary means for
243 increasing the ability of a sampling plan to discriminate 'acceptable' from 'defective' lots.

244 Evaluation of the OC curves for the proposed MC is a critical step in ensuring that the MC
245 is able to assess whether food lots satisfy an FSO or PO. Thus, when an MC has to be set, a
246 number of decisions have to be made. These will be illustrated below.

247

248 **5. Probabilities of accepting or rejecting lots.**

249 In the design of sampling plans it is necessary to define the probability that a "defective"
250 lot will be rejected.

251 The choice of this value has public health implications and is, thus, a risk manager's task.
252 In the examples selected for illustration purposes in Section 8 we have chosen a value of
253 95% probability of rejection of defective lots. In the following text, the consumer's ALS is
254 the mean log concentration level or the proportion defective that would result in lots
255 contaminated at this level being rejected 95% of the time. This implies, however, that 5%
256 of the non-conforming batches contaminated at this level would be accepted. This is called
257 Type II error (i.e., a lot was accepted when it should have been rejected), and is referred to
258 as "the consumer's risk".

259 Of concern to food producers is the possibility that, under the sampling plan, acceptable
260 lots are rejected. If a producer operated at the level of control required to just meet the
261 consumer ALS, there would be a substantial number of lots that would fail the
262 microbiological criterion despite the lot actually meeting the FSO or PO. This is sometimes
263 called "Type I error", and describes the producer's risk. Thus, the producers are interested
264 in determining the lot quality that would need to be achieved so that there is a high
265 probability (e.g., 95%) that lots would be accepted and adjust their production processes

266 accordingly. In this manuscript, it is assumed that the producer is operating with a degree
267 of control that is greater than that needed to achieve the consumer's ALS. Thus, the
268 producer's ALS is the mean log concentration level or that proportion defective that
269 ensures that lots are accepted 95% of the time. This percentage could be set at other levels
270 depending on the willingness of the producer to accept rejection of conforming lots.
271 Setting either the consumer's ALS or the producer's ALS, implies the other. On the other
272 hand, it is not possible to elaborate statistically-based microbiological criteria unless either
273 the consumer's, or producer's, ALS is specified.

274

275 **6. Nature of an FSO or PO in statistical terms**

276 FSOs are maximum frequencies or levels of pathogens that are considered tolerable at the
277 moment of consumption; POs specify frequencies or levels of pathogens at any other point
278 in the food chain. Ideally, FSOs and POs for a given product/pathogen combination will be
279 related mathematically in a manner revealed by, for example, a risk assessment, or
280 exposure assessment.

281 A PO for a ready-to-eat food that does not support growth of the pathogen of concern may
282 have the same value as the FSO. If a food supports multiplication of the pathogen before
283 consumption the PO will usually be lower than the FSO. Analogously a PO may, in
284 principle, be higher than the FSO in pathways where the hazard level will be reduced after
285 production and prior to consumption, e.g., such as due to cooking during preparation. In
286 some instances the PO may be only indirectly related to the FSO. For example, consider
287 the association between raw poultry and salmonellosis. This typically involves cross
288 contamination in the kitchen leading to the transfer of *Salmonella* to ready-to-eat foods. In
289 this instance the PO would be the frequency of contaminated carcasses entering the home
290 (e.g. <15% of fresh poultry carcasses are contaminated with *Salmonella*), and the FSO

291 would be the frequency/levels of *Salmonella*-contaminated meals served in the home. This
292 could then be used to reflect the potential control points, i.e., reduce the frequency of
293 contaminated carcasses entering the home and reduce the incidence of cross-
294 contamination.

295 In principle, an FSO communicates the level of a hazard that is unacceptable in any serving
296 of a food at the moment of consumption while a PO communicates a (related) limit at some
297 other defined point in the food chain. However, depending on the FSO or PO value chosen,
298 some servings will exceed the specified limits because of the expected distribution of
299 contamination levels in the food. It does not necessarily indicate, however, that the system
300 is out of control as long as the proportion of such units is within the limits expected for the
301 distribution (characterised by the standard deviation) around the mean contamination level
302 required to achieve the FSO/PO. Thus, to establish a sampling plan that allows an MC to
303 be specified to verify an FSO (or PO) the proportion of the lot that may be above the
304 nominally 'acceptable' level must be specified. In examples given below this value is set at
305 1%, but other values could have been chosen. This value means that provided that no more
306 than 1% of the lot exceeds the FSO (or PO) then the food safety risk management system
307 is operating as intended. The FSO (or PO) is then understood as being the $99^{th}$ percentile of
308 a cumulative frequency distribution of log concentrations. Choosing this tolerance is a risk
309 management decision, because it clearly has an influence on the levels of the hazard that
310 are considered acceptable. It also establishes the level of Type II error that risk managers
311 consider acceptable if the manufacturer produces product that just achieves the consumer
312 ALS. It is not possible to elaborate an MC for FSO or PO confirmation purposes without
313 specifying this tolerance.

314

315

316 **7. Relating the performance of attributes plans to concentration**

317 Previous evaluation of the performance of ICMSF sampling plans (Legan, Vandeven,
318 Dahms & Cole, 2001) did not consider the possibility that, even if the concentration of the
319 microorganism of concern in the sample is homogenous, the sampling and enumeration
320 method may over- or underestimate the actual concentration of microorganisms,
321 particularly if involving enrichment. As the concentration of microorganisms in the food
322 sample increases, there is less likelihood that the sample will produce a false negative.
323 Conversely, as the true concentration decreases there is less likelihood of obtaining a false
324 positive through sampling 'chance', i.e. detecting a target cell even though the overall
325 concentration is below the level considered just acceptable. Therefore, in situations in
326 which microbiological testing of samples involves enrichment and presence/absence
327 testing rather than enumeration, a modification to the approach described by Legan et al.
328 (2001) is appropriate. In the modified method described in Appendix 2, the overall
329 probability of detecting a positive sample in a lot, characterised by a log-normal
330 distribution, is estimated as the total probability of detecting a cell in any of the samples
331 taken from that batch, i.e. by integrating over all possible concentrations in the batch. The
332 probability of sampling any particular concentration in the batch is given by the lognormal
333 distribution and is combined with the Poisson sampling process (and size of the analytical
334 unit) to calculate the probability that a cell will be present in the sample taken and lead to a
335 "positive" result after enrichment. The choice of this Poisson-lognormal is based on the
336 assumption that at both lower and higher contamination levels the concentration of cells is
337 log-normally distributed. This model is only one of several models that could be used, and
338 might not be generally applicable.

339

340

341 **8. Establishment of Microbiological Criteria intended to confirm an FSO/PO**

342 As explained above, to establish an MC to assess compliance with the FSO/PO for a
343 pathogenic microorganism, a series of assumptions/decisions must be made.

344 1. An assumption must firstly be made regarding the distribution of the pathogens in
345 the lot of food. In the examples provided, we assume that the pathogens of concern
346 are log-normally distributed and that the standard deviation (s.d.) is known. In the
347 absence of available data, a log-normal distribution is often assumed and a default
348 value for the standard deviation applied. For the purposes of the current examples,
349 a standard deviation = 0.2 $\log_{10}$CFU $g^{-1}$ is used to describe a food in which
350 microbes would be expected to be rather homogenously distributed within a batch
351 (e.g., for liquid food with a high degree of mixing). A standard deviation of 0.4
352 $\log_{10}$CFU $g^{-1}$ is assumed for a food of intermediate homogeneity (e.g., ground beef)
353 and a standard deviation = 0.80 $\log_{10}$CFU $g^{-1}$ for an inhomogenous food (e.g., solid
354 food). It could be that in certain cases even larger inhomogeneity could occur, e.g.,
355 if clumping occurs (Wilson et al., 1935) or if the contamination is restricted to
356 surface contamination of a food.

357 2. The second requirement is to define the "maximum frequency and/or
358 concentration" of the hazard that will be used to specify the FSO/PO, including
359 what proportion (e.g., 95%, 99%, 99.9% etc.) of the distribution of possible
360 concentrations must satisfy the test limit so that the FSO/PO is met.

361 3. The third decision is to specify the level of confidence needed that a non-
362 conforming lot is detected and rejected (i.e. the consumer's ALS; examples below
363 consider 95% or 99% confidence). Alternatively, the probability of rejecting a
364 conforming lot (i.e. the producer's risk) may be considered.

365 4. The fourth decision is the analytical methodology that should be employed.

366 The following examples illustrate the consequences of such decisions on the number of
367 samples and/or sample sizes and analytical methodology required to assess compliance
368 with the FSO/PO.

369

370 *8.1.* Salmonella *in ice cream*

371 Ice cream is a product that, when properly handled, does not support microbial growth.
372 Thus, the PO for ice cream can be the same as the FSO. In the examples, three FSOs/POs
373 are considered (one *Salmonella* per 100g, one per kg and one per 10kg; *see* Table A2.1)
374 and two possible standard deviations (0.4 and 0.8) are selected to illustrate the calculations.
375 The maximum mean concentrations that can be tolerated, corresponding to each
376 assumption about the standard deviation, are determined by subtracting a certain number of
377 standard deviations from the hazard concentration nominated as the FSO so that the
378 required percentage of the lot will have concentrations below the FSO/PO. The required
379 number of standard deviations is called the z score. For example, to deduce a mean
380 concentration in the lot such that 99% of the units are at or below the target FSO requires
381 that 2.33 standard deviations are subtracted from the FSO. Determination of the number of
382 samples to be examined to assess compliance is illustrated for three analytical sample sizes
383 (25g, 100g and 250g)[a]. The number of analytical units that need to be tested to have 95%
384 confidence of rejection of non-conforming batches (with non-conforming batches defined
385 as a lot with more than 1% of the units above the FSO) is shown in Table A2.1.

386 Table A2.1 illustrates that with increasing stringency of the FSO/PO, i.e., from 1
387 *Salmonella*/100g to 1/10kg, the number of samples that need to be analysed for

[a] Note that the analytical sample size can be different from the unit size on which the FSO is based

388 confirmation becomes unacceptable for practical reasons. Table A2.1 also shows the
389 changes in the number of samples that must be tested to assess compliance when the
390 standard deviation (of the distribution of concentrations of *Salmonella*) increases from 0.4
391 to 0.8 $\log_{10}$CFU.g$^{-1}$. A higher standard deviation also means that the mean log
392 concentration that must be achieved so as not to exceed the FSO/PO must be decreased.

393 The effect of the size of the analytical unit on the number of samples that must be
394 analysed, using the modified method described in Appendix 2, is demonstrable when one
395 of the examples from Table A2.1 is considered in more detail. For the example, let us
396 assume that the FSO/PO is set at one *Salmonella* per 100g and the concentration of
397 *Salmonella* is described as having a mean $\log_{10}$ concentration of -2.93 with a standard
398 deviation of $\log_{10}$ 0.4. If the analytical unit is 25g, 69 samples need to be analysed to
399 determine compliance with the FSO/PO. If units of 100g are taken, 19 samples need to be
400 analysed and, in the case of 250g analytical units, this number is reduced to 9. Clearly with
401 an increase in the size of the analytical units a reduction in number of samples can be
402 achieved, although the validity of enrichment methods involving increased samples sizes
403 (and potentially lower concentrations of the hazard) should also be considered (Jarvis,
404 2007). In general, however, if the PO is set at a level lower than one *Salmonella* per 100 g,
405 testing may not be a practical option for assessing compliance.

406

407 *8.2.* Listeria monocytogenes *in cold-smoked salmon.*

408 If a product permits the growth of the pathogen of concern during its shelf life, the PO will
409 be lower than the FSO to take into account the growth that may be expected to occur
410 between the point to which the PO relates and the point of consumption. Using as an
411 example management of the risk from *L. monocytogenes* in cold smoked salmon, we
412 illustrate here how one might derive a PO at point of manufacture from a specified FSO.

413 For the sake of the illustration a number of (untested) simplifying assumptions have been

414 made. Thus, it is assumed that:

415 i) the product has a shelf life of two weeks from point of manufacture to point of

416 consumption when stored at or below 4°C,

417 ii) the specified FSO is 3.3 $\log_{10}$CFU.$g^{-1}$,

418 ii) that all the product is eaten on the last day of its shelf life, and

419 iii) that during this period a 0.6 $\log_{10}$CFU.$g^{-1}$ increase in *L. monocytogenes* would occur.

420 The required PO would be 2.7 (3.3 - 0.6, the maximum increase of *L. monocytogenes*

421 during shelf life) $\log_{10}$CFU.$g^{-1}$. To ensure that the PO would be met by 99 % of the food in

422 the lot, the maximum permitted level should be 2.33 × SD below the calculated PO value

423 of 2.7. Accordingly, the corresponding log-normally distributed population with an s.d. of

424 0.4 $\log_{10}$CFU.$g^{-1}$ should have a mean concentration of 1.77 $\log_{10}$CFU.$g^{-1}$ or less (2.7 – 2.33

425 x 0.4). Using statistical calculations it can be established that in order to reject a non-

426 conforming lot with a 95% probability, requires that 10 samples (*n*) be tested: if any

427 sample has ≥100 *L. monocytogenes* $g^{-1}$ the lot does not conform.

428 To illustrate the effect of detection levels, three methods of analysis for *L. monocytogenes*

429 are considered:

430 • a plate count technique able to determine ≥100 *L. monocytogenes* $g^{-1}$,

431 • an 1/0.1/0.01 g 3-tube MPN procedure with a lower limit of detection of 0.3

432 *L. monocytogenes* $g^{-1}$, and

433 • an enrichment technique that examines a single analytical unit of 25g.

434 In the example above, if the test used was presence/absence in 25g, only one sample needs

435 to be analysed to assure rejection (with >95% probability) of the lot. This is because the

436 sample size is large relative to the required mean concentration that is commensurate with

437 an acceptable batch, i.e., 1.77 $\log_{10}$CFU.$g^{-1}$. Thus, a 25 g sample from a batch with
438 acceptable mean concentration would almost certainly contain *L. monocytogenes* and
439 return a positive result. However, using this presence/absence test or using the lowest level
440 of detection with an MPN method has a substantial type I error; i.e., the risk of
441 unnecessarily rejecting lots, as well as sometimes incorrectly accepting lots because
442 sampling plans using only a single sample have limited discriminatory ability unless the
443 sampling involves the compositing of randomly selected subsamples, e.g., a 25 g analytical
444 unit consisting of the compositing of 25 1-g samples.

445 In Table A2.2 the key figures for the consumer's ALS and the producer's ALS (number of
446 samples required and mean concentrations) for three distributions (s.d.'s of 0.2, 0.4 and
447 0.8) are presented, calculated to meet three FSOs. These figures show, for instance, that as
448 the s.d. increases, the mean concentration needs to be reduced so as not to exceed the
449 FSO/PO. The figures for the producer's ALS demonstrate that the mean concentration of
450 the pathogen in the lot should be lower than that calculated to be required to satisfy the
451 consumer's ALS. The number of samples that are required to be analysed show the same
452 trends as discussed above. The figures also show that at the lowest values of the FSO/PO
453 the *m* value can no longer be (realistically) set at 100 CFU.$g^{-1}$.

454

455 *8.3.* Salmonella *in frozen poultry.*

456 In this example we illustrate the establishment of microbiological criteria designed to
457 satisfy POs. Frozen poultry will be cooked before consumption, thus the PO will differ
458 from the FSO (and may be higher than it). In Table A2.3 three POs were chosen to
459 illustrate the effect these levels have on the number of samples that need to be analysed.
460 The analytical unit in all three cases is the same, e.g. 5g of neck-skin (Notermans,
461 Kampelmacher & Van Schothorst, 1975). If the PO is formulated as: "not more than 15%

462 of chicken carcasses in a lot may test positive for *Salmonella"* and the consumer's ALS is
463 set at 95% probability, the analysis of 19 samples is sufficient to assess compliance of the
464 lot. If a 10% contamination level is chosen, 29 samples are needed to assess compliance; if
465 5% is specified as the PO then 59 samples must be tested. Thus, as illustrated in Table
466 A2.3, to produce lots that have a 95% probability of complying with these consumer ALS
467 requirements, i.e., that no more than 15%, no more than 10% or no more than 5% of
468 carcasses are contaminated with *Salmonella*, the producer needs to ensure that not more
469 than 0.27%, 0.18% and 0.09%, respectively, of the carcasses are contaminated.

470

471 **9. Developing Microbiological Criteria for pathogens when no FSOs/POs have been**
472 **established and when no historical data are available**

473 Ideally, verifying whether an FSO/PO is met is done at the site where the food is produced.
474 However, in practice this is not always possible, or other circumstances require that control
475 authorities have to assess the safety of lots of food and have to undertake testing
476 themselves in the absence of historical data about contamination levels, and variation in
477 contamination levels, in lots of that product. For this purpose ICMSF (1986, 2002)
478 developed a series of "cases", and proposed sampling plans. Although, these sampling
479 plans were not designed to assess compliance with an FSO/PO, using the analytical
480 approach presented here it is possible to explore the numerical limits that correspond to the
481 'cases', i.e., FSOs/POs that are implicit in the sampling schemes corresponding to the
482 'cases'. Appendix 3 illustrates how one can derive an FSO/PO from a particular sampling
483 plan.

484 Following the approach as set out in Appendix 2, the recommended sampling plan for
485 *Salmonella* in ice cream can be analysed. In this example it is assumed that random
486 sampling can be applied and that the standard deviation (s.d.) is 0.8 $\log_{10}$CFU.g$^{-1}$. The

487 product/hazard combination is best described by case 11 for which no *Salmonella* should

488 be detected in 10 samples of 25 gram (i.e. $c$=0, $n$=10, $m$=0/25g). When the probability of

489 rejection (consumer's ALS) is set at 95%, lots with a mean log concentration of ≥-2.25,

490 which corresponds to ≥ 6 *Salmonella* per kg (or one per 179g), will be rejected with at least

491 95% probability. With this sampling plan it would be possible to ensure, with 95%

492 confidence, that a lot of food in which ≥1% of servings have a concentration of *Salmonella*

493 ≥ -0.39 $\log_{10}$CFU.g$^{-1}$ (*ca*. 0.4 *Salmonella* g$^{-1}$) would be rejected. For a producer who wants

494 to ensure that that this food meets the MC with 95% probability (producer's ALS: mean

495 log count accepted with 95% probability), the mean log concentration would need to be ≤

496 -4.4 $\log_{10}$CFU.g$^{-1}$ of *Salmonella* (<4 CFU.100kg$^{-1}$).

497

498 It is important to note that the s.d. of 0.8 log units was based on data from national

499 surveillance programmes for *Salmonella*, *E. coli* O157:H7 and some other pathogens in

500 mainly raw products (Legan *et al*., 2001). The mean and s.d. associated with these data

501 reflect the variability among a large number of different processors and practices. Even a

502 few lots with higher concentrations can have a large effect on the s.d. value. Establishing

503 an MC based on the standard deviation for all products of a certain category at a specified

504 point in the food chain may be unnecessarily stringent for "good" producers, i.e. those that

505 produce at a more consistent standard. Lots from a single producer will typically have a

506 smaller s.d. A producer who knows the s.d. of his/her products, could recalculate the

507 likelihood that their product would be rejected. This is an advantage of using a PO based

508 MC where the outcome (e.g., 95% confidence that 99% of the servings do not contain ≥ X

509 $\log_{10}$CFU.g$^{-1}$) can be used by an individual manufacturer, in conjunction with their within-

510 lot s.d., to develop the appropriate MC for their products. Alternatively, national

511 surveillance programmes should measure both the between-lot and within-lot variability

512 for the food category and use the s.d. associated with within-lot variability for developing
513 MC and that associated with between-lot variability for conducting risk assessments and
514 establishing the FSO/PO.

515

516 **10. Practical aspects of these considerations**

517 In this publication we have not discussed how to establish an FSO because this was
518 considered in ICMSF (2002). Instead, we have demonstrated how, in certain cases, a PO
519 can be derived from an FSO and, in turn, be used to develop an MC based on the PO. We
520 have also demonstrated how articulating an MC could lead to an implied PO. In Section 8
521 the examples presented showed how the distribution of the numbers of the pathogen in a
522 lot influences the number of samples that must be examined to verify that a food lot meets
523 a prescribed PO. Moreover, it was shown that the level of the PO greatly influences the
524 practicality of using microbiological testing for this purpose. The size of the analytical
525 unit, the standard deviation, the probability of acceptance and the statistical definition of
526 the PO are other factors that determine the practicality of testing lots for the presence of
527 specified levels of pathogens for confirmation purposes. Whether testing for pathogens
528 across lots may present better possibilities needs to be further explored. In certain
529 situations testing for indicator microorganisms may offer an alternative (ICMSF, 2002).

530 When the practicality of testing and the interpretations of the results are considered, a few
531 observations can be made. Firstly, the situation regarding fresh or raw foods may be
532 different from foods processed for safety. In the case of such processed foods pathogens
533 should, in principle, not be present or their presence (due to survival or unavoidable
534 recontamination and growth) should be at levels that present a negligible risk to public
535 health. Unacceptable levels of recontamination should not occur, or should be detected,
536 and such incriminated batches should not be put on the market. Microbiological testing

should detect such lots when no other means are available. However, while the distribution of the pathogens in the lot is not known it is most likely that they are not homogeneously distributed throughout a consignment. Moreover, random sampling is often not possible for reasons of accessibility of units in consignments on trucks, ships, etc. Consequently, in these cases, the calculations and interpretations of pathogen testing data have only limited validity: in simple terms it can be argued that a positive finding (i.e. presence of a pathogen) means something, while a negative one means very little. Even when the necessary data are available to allow statistical interpretation of the test results, the number of samples needed to obtain a meaningful result may be too large to be practical, as was shown in the *Salmonella* in ice-cream example.

The situation may be different for foods that are not processed for safety, that are raw or that may originate from polluted environments. In these situations, testing may be useful because contamination levels and/or frequencies would be expected to be higher, and it is recommended to design sampling plans in a manner, such as demonstrated in this paper, to determine whether the POs set for such products are met.

## Summary

FSOs and POs are targets to be met for pathogen/food product combinations. To assess whether consignments of foods conform to such targets, background information is needed. The best information is obtained during inspection/audits of the site of processing of the food. If information is available concerning the distribution of the pathogen of concern in the food batch (i.e. a log-normal distribution with a certain standard deviation) and if samples can be randomly taken, statistical methods can be used to specify microbiological criteria intended to verify achievement of an FSO or PO. However, this requires that a number of risk management decisions be made as described in this paper.

562 The examples presented also demonstrate that when foods have received treatments that
563 greatly reduce microbiological levels, or that have very low prevalence due to other
564 reasons, testing may not be the most effective means to verify microbiological status, and
565 other approaches should be considered. When information to develop FSO/PO derived
566 microbiological criteria is not available, the classical “ICMSF cases” and sampling
567 schemes still offer a risk-based approach for examining lots for acceptance or rejection.

**Acknowledgements**

This paper is dedicated to Susanne Dahms who sadly lost her battle with cancer on July 23rd 2007.

Susanne was educated in Germany where she earned a university degree in economics and doctorate at the University of Bielefeld, and qualification for full professorship at the Free University of Berlin. Her research interests included biometrical and epidemiological approaches to evaluate dependencies between animal health management and food safety, statistical aspects of microbiological sampling plans and their relation with food safety objectives, and biometrical methods for validation of microbiological analytical techniques. Susanne was elected to serve as a member of the International Commission on Microbiological Specifications for Foods (ICMSF) in 1998, the advisory board of the German Region of the International Biometrical Society in 2002 and the Council of the International Biometrical Society in 2006.

She was a brilliant and talented mathematician who also had a unique ability to get difficult concepts across in simple ways. This was especially important to the ICMSF in order to carry out a number of important projects such as the various papers (including this one) and books produced by the Commission. Through this work Susanne has made a significant contribution to public health and food safety.

Susanne contributed much more than technical expertise to our group, she brought friendship, warmth and a great sense of humor. One of our endearing memories of Susanne will be the many lectures she used to give us, where she would take great delight and fun in teasing the mathematically challenged microbiologists amongst us, yet she would do this with great mutual respect and in a way that endeared her to the rest of the group. We feel privileged that Susanne was a member of our group and grateful for the time she spent with us. We will miss her as a colleague but especially as a very dear and special friend.

598 # Appendix 1

599 ## Relating an OC-curve to Concentrations of Pathogens

600

601 If bacteria are log-normally distributed in a product and if the standard deviation of the
602 distribution is known, an "operating characteristic" curve (OC-curve) can be established.
603 For example, for *Listeria monocytogenes* the acceptable concentration in a lot could be set
604 at less than 100 $CFU.g^{-1}$ according to the ICMSF sampling scheme (ICMSF, 1994): $m$ =
605 100 $CFU.g^{-1}$ (= 2.0 $log_{10}CFU.g^{-1}$). If the average concentration in the lot is 10 $CFU.g^{-1}$ (log
606 $N$ = 1 $log_{10}CFU.g^{-1}$) and the standard deviation is 0.2 $log_{10}CFU\ g^{-1}$, the expected
607 distribution of concentrations of *L. monocytogenes* in samples taken from the lot is as
608 presented in Figure A1.1. If one sample is taken, for example a 1 g sample, and the total
609 number of *L. monocytogenes* in the sample enumerated by spreading the 1 g sample over
610 multiple selective agar plates, the probability of accepting the lot will be virtually 100%.
611 The probability of acceptance can be calculated in Microsoft ® Excel using the "Normdist"
612 function, as shown:

613 P(acceptance for 1 sample) = Normdist($x$, $\mu$, $\sigma$, cumulative) (1)

614 Where $x$ is the value for which the probability of occurrence is wanted (in this case $x = m$
615 in $log_{10}CFU.g^{-1}$)

616 $\mu$ is the mean $log_{10}$concentration of the distribution, either a theoretical value ($\mu$) or
617 an estimated value ($m$)

618 $\sigma$ is the standard deviation of the log-normal distribution, either a theoretical value
619 ($\sigma$) or an estimated value ($s$)

620 ‘cumulative’ is a logical value. If this is set to 1, ‘Normdist’ will return the
621 cumulative distribution. If this is set to 0, it returns the probability mass function
622 (frequency distribution)

623

624 If one sample is taken from the lot, the probability of acceptance for a distribution with
625 mean concentration 1 $\log_{10}$CFU.$g^{-1}$ and standard deviation 0.2 $\log_{10}$CFU $g^{-1}$ with a limit at
626 2 $\log_{10}$CFU.$g^{-1}$ ($m$=2) is then: P(acceptance) = Normdist(2,1,0.2,1) = 99.9999713%.

627 The same procedure ($m$ = 2 $\log_{10}$CFU.$g^{-1}$) can be used for other mean $\log_{10}$concentrations
628 (e.g, 1.0, 1.5, 2.0, 2.5) with the same standard deviation of 0.2 $\log_{10}$CFU $g^{-1}$ to illustrate the
629 effect of mean microbial levels on the probabilities of acceptance (Figure A1.2).

630 Once the probabilities of acceptance are determined for various mean log concentrations,
631 they can be plotted in a graph to obtain an OC-curve as indicated in Figure A1.3.

632 The probability of acceptance for more then one sample can be calculated as follows:

633 $$P(\text{acceptance for } n \text{ samples}) = P(\text{acceptance for 1 sample})^{n} \quad (2)$$

634 For $n$ = 10, the probability of acceptance for a mean log concentration of 1.5 $\log_{10}$CFU.$g^{-1}$
635 and standard deviation 0.2 $\log_{10}$CFU $g^{-1}$ is then:

636 $$P(\text{acceptance for 10 samples}) = \text{Normdist}(2,1.5,0.2,1)^{10} = (0.994)^{10}=94\% \quad (3)$$

637 In the same way as indicated in Figure A1.3, the probability of acceptance can be
638 determined for $n$ = 10 for various mean log concentrations resulting in an OC-curve for $n$ =
639 10, $c$ = 0 and $m$ = 100 CFU.$g^{-1}$. From this OC-curve, the concentration at which the lot will
640 be accepted with 95% probability (producer’s ALS) and the concentration at which a lot
641 will be rejected with 95% probability = consumer’s ALS) can be determined. These

642 concentrations are shown in Figure A1.4 for $\sigma = 0.2$. It can be seen by comparing Figure
643 A1.4 with Figure A1.3 that the more samples are taken, the steeper the OC-curve becomes.

644

645 The OC-curve can be obtained for other standard deviations as well. Two examples are
646 given in Figure A1.5 for $\sigma = 0.4$ and Figure A1.6 for $\sigma = 0.8$. It can be seen that the larger
647 the standard deviation, the flatter the OC-curve becomes.

648 **Appendix 2**

# 649 A modified approach for estimation of the number of 'analytical units' 650 that need to be tested to have 95% confidence of rejection of non- 651 conforming batches (for enrichment tests).

652 In previous publications (Legan et al., 2001; ICMSF, 2002) an approach (described in
653 Appendix 1) was developed to determine the number of analytical units and their size that,
654 for a given distribution of microbial counts, must be tested to provide 95% confidence that
655 a batch with unacceptable levels will be rejected by attributes sampling. In the following, a
656 refinement of that approach is described for 2-class attribute testing where a specified
657 number of analytical units are cultured *via* enrichment and then assessed for
658 presence/absence of the microorganism.

659 In either approach, a *lot* has the usual meaning, i.e. a grouping of a product manufactured
660 during a certain period of time or under the same conditions, or a consignment of a food
661 arriving at a border. A *sample* is taken from that lot to assess the concentration of cells in
662 that sample. A sample may comprise the entire *analytical unit*, or the analytical unit may
663 be an aliquot derived from the sample. In certain cases a sample is made up of a composite
664 of various mixed subsamples, in order to increase homogeneity in the sample, but this does
665 not of course change the underlying distribution in the lot. It is assumed that the
666 concentration in an aliquot of the sample is representative of the concentration in the whole
667 sample, but that different samples can have different concentrations (it should be realised
668 that in very heterogeneous samples this might not be correct). Also, in both approaches it is
669 assumed that a log-normal distribution characterises the microbiological status of the lot
670 but in this modified approach a further consideration, concerning the likelihood of
671 sampling a contaminant in an analytical unit, is implemented.

672 **Modified Approach: Poisson-Log-normal Distribution**

673 Microorganisms are discrete particles that are very small relative to the size of analytical
674 units typically employed. Thus, even if the microorganisms were completely evenly
675 distributed in the sample and were present at the level of one cell per sample unit, one
676 would not expect every sample to be positive for growth; some samples selected at random
677 would contain one or more cells and produce a positive result, while others would not.
678 Moreover, if the concentration of target cells was just less than the PO, even if the cells
679 were perfectly homogenously distributed in the sample, some samples would produce a
680 positive result leading to the inappropriate rejection of the batch (in a sampling scheme that
681 specifies $c$=0). Thus, it is necessary to consider the consequences of sampling
682 'coincidences' (i.e. the detection of a cell in a set of samples even when such detection is
683 highly improbable based on the mean concentration of the organism in the batch) on the
684 interpretation of the results of analytical methods, particularly when inferring the
685 concentration of cells in a sample.

686 The probability of detecting cells, by randomly sampling from a well-mixed system can be
687 described by a Poisson distribution:

688 $$P_{detection} = 1 - \exp^{-(\text{number of cells in sample})}$$

689 $$= 1 - \exp^{-([\text{concentration of cells}] \times \text{sample size})} \quad (4)$$

690 For the Poisson distribution the variance is the same as the mean and, consequently, the
691 standard deviation is approximated by the square root of the mean count. Thus, for a
692 concentration of 100 cells per analytical unit, we would expect 95% of test results to fall in
693 the range from 80 to 120 cells, i.e. within ~2 standard deviations of the mean.

694 In other words, we need to recognise that even if the concentration of target cells in the
695 sample is perfectly homogenous, our sampling and enumeration method will sometimes
696 over-estimate the concentration and some-times under-estimate it. We could calculate the

697 total effect of the probabilities of over- and under-estimation of the concentration due to
698 sampling coincidences, i.e. samples that have a concentration greater than the PO but that
699 generate an estimate less than the target (i.e. a false-negative), and those samples that have
700 a concentration less than the target that produce an estimate greater than the PO due to
701 sampling coincidences (i.e. a false-positive). Clearly, as the true concentration in the
702 sample increases, there is less likelihood that the sample will produce a false negative and,
703 conversely, as the true concentration in the sample decreases there is less likelihood of
704 obtaining a false-positive through sampling coincidences.

705 As the target concentration of cells declines, our confidence in the result of our test method
706 also declines. From the above equations, it can be seen that when the target concentration
707 is on average one cell per analytical unit, our chance of detecting a cell in a single sample
708 is ~63% only, while a sample that contains half that concentration of cells has ~40%
709 probability of yielding a (false) positive result. To explain this further, if the concentration
710 of organisms is on average one cell per kilogram and we take samples of 100 g there will
711 be, on average, one cell in every tenth sample. Thus, there is a probability that we will
712 detect a positive even though, on average, the amount of cells is smaller than one per
713 sample unit. This arises because, as noted earlier, cells are discrete units. Thus, calculating
714 a mean concentration of 0.1 cells per 100g is somewhat misleading – it is more helpful to
715 think of any 'fractional' cell concentration as being equivalent to one cell in a suitably
716 large analytical unit, e.g. when we say "0.1 cells per 100g" we mean "one cell per kg". We
717 can also express this as a proportion of positive analytical units e.g. "one in 10 units of 100
718 g are positive".

719 Our aim, however, is to determine by sampling whether the mean log concentration in the
720 lot is such that less than 1% of the lot exceed the PO. While one approach is to test a
721 sufficient number of samples to determine whether 1% of the lot exceed this limit, equally

722 we could base our sampling strategy on assessing the proportion of samples that exceeded
723 any other concentration on the required distribution curve because we can determine the
724 probability of detecting a cell in a sample of any concentration. Thus, as long as the
725 distribution of concentrations is known, it is possible to calculate the overall probability of
726 detecting a cell from any sample drawn from a lot. This is because the overall probability
727 of obtaining a positive sample is the product of the probability of that concentration
728 occurring in the lot, and the probability of detecting a cell in the sample based on the size
729 of the analytical unit and the concentration of cells in the sample. For example, if on
730 average the amount of cells in a sample is exactly one cell, one would intuitively expect
731 that the value in a sample would be one. However, the probability for any single sample to
732 be positive is not equal to one, since there is only, on average, one cell in the sample.
733 Sometimes there will be really one organism in the sample, sometimes zero, and
734 sometimes two, or even occasionally more than two. The probability that there is no
735 organism in a sample (although on average the expected number would be one organism in
736 a sample) is exp(-1)=0.368. Therefore the probability that a sample will be positive (one or
737 more organisms in the sample) is one minus the probability of no organism in the sample:
738 1-0.368=0.632 (since P(0)=0.368). Alternatively the probability of a positive sample can
739 be calculated as the sum of the individual probabilities, i.e. the Poisson probabilities for 1,
740 2, 3, 4, etc. cells per sample are 0.368, 0.184, 0.0613, 0.0153, etc. respectively, so the
741 probability of obtaining 1 or more cells/sample is given by 0.368 + 0.184 + 0.0613 +
742 0.0153 + …. = 0.632.

743 The above concept can be used to estimate the overall probability of detecting a positive
744 sample in a lot characterised by a known, or assumed, distribution, i.e. by integrating, over
745 all possible concentrations in the batch, the overall probability of detecting a cell in any of
746 the samples taken. The probability of sampling any particular concentration in the batch is
747 given by the lognormal distribution and is combined with the Poisson sampling process

748 (and sampling size) to calculate the probability that a cell is present in the sample taken

749 and leads to detection of a 'positive'. This can be expressed mathematically as:

750 $$p(+) = \int_{-\infty}^{\infty} Pnormal(\log C, \mu, \sigma) \cdot (1 - Poisson(0, C \cdot samplesize)) \mathrm{d} \log C \quad (5)$$

751 Note that the concentration *C* for the Poisson distribution is 10^log*C*.

752 Since Poisson(0, *x*)=exp(-*x*), this results in:

753 $$p(+) = \int_{-\infty}^{\infty} Pnormal(\log C, \mu, \sigma) \cdot (1 - \exp(-C \cdot samplesize)) d \log C \quad (6)$$

754 Returning to the example of Line 3 from Table A2.1 the above leads to:

755 $$p(+) = \int_{-\infty}^{\infty} Pnormal(\log C, -2.931, 0.4) \cdot (1 - \exp(-C \cdot 250)) d \log C \quad (7)$$

756 Calculating this integral results in $p = 0.3068$, i.e. the probability of acceptance of the batch

757 based on a single sample is: 1-0.3068 = 0.6932. This is well below the required 95%

758 confidence, so that more (negative) samples are required to achieve 95% confidence that

759 the lot meets the PO.

760 If we take eight samples, the probability that all eight are acceptable (i.e. that no sample

761 contains a cell) is $(0.6932)^8 = 0.0533$, and with nine samples that probability is $(0.6932)^9 =$

762 0.0369. Thus, to reject with 95% certainty a batch that has greater than the desired log

763 mean concentration (and with the specified standard deviation), requires 9 negative

764 samples, ($n = 9$, $c = 0$) given this calculation scheme.

765 This approach differs from earlier analyses (e.g., Legan et al., 2001) because it specifically

766 considers the likelihood of *any* concentration of cells within the limiting distribution

767 generating a 'positive' result, rather than basing the sampling scheme on a specific

768 concentration only. In other words, in this approach if a sample contains on average 0.1

769 cells, it can result in a positive outcome (i.e. with a probability of 10%). Therefore,

770 samples are more often predicted to be positive and, consequently, fewer samples are

771 needed to assure rejection at a specified level of confidence.

772 **Appendix 3**

## 773 FSOs/POs based on sampling plans using the original ICMSF approach

774

775 For the purposes of this Appendix, it is assumed that an FSO/PO is interpreted as the 99%
776 or 99.5%-point of the exposure distribution that is accepted, i.e., that up to 1% or 0.5%,
777 respectively, of units with concentrations exceeding this limit can be tolerated. As an
778 example, a sampling plan for *Listeria monocytogenes* with $n$=10, $c$=0, $m$=100 $CFU.g^{-1}$ (or
779 $m$=2 in $log_{10}$-units) is used.

780 If log-normal distributions can be used to describe the frequencies of concentrations and
781 experience shows that assumption of a standard deviation = 0.4 $log_{10}$-units can be justified,
782 the situation as depicted in Figure A3.1a applies. The Figure shows the OC curve for this
783 sampling plan and shows which mean log-concentrations are rejected and those that are
784 accepted with 95% probability (consumer's and producer's ALS). Lots with mean $log_{10}$-
785 concentrations of 1.74 $log_{10}CFU.g^{-1}$ will be rejected with a 95% probability (consumers
786 ALS) and lots with mean $log_{10}$-concentrations of 0.97 $log_{10}CFU.g^{-1}$ (producers ALS) will
787 be accepted with a 95% probability. This is shown in more detail in Figures A3.1.

788 Using the $log_{10}$-concentration distribution in Figure A3.1b, which has a mean value
789 corresponding to the consumer's ALS (1.74), the 99% or 99.5%-point (i.e. the FSO/PO
790 value) can be determined. Application of this sampling scheme for this distribution of
791 *L. monocytogenes* in a lot would mean that the FSO/PO would need to be set at 2.67
792 $log_{10}CFU.g^{-1}$ to ensure that 99% of units from the lot did not exceed the FSO/PO, or 2.77
793 $log_{10}CFU.g^{-1}$ to ensure that 99.5%of units were below the FSO/PO.

794 If experience showed that an s.d. of 0.2 $log_{10}$units was more appropriate to describe the
795 distribution, the situation would be as depicted in Figure A3.2. In that case lots with mean
796 log-concentrations at 1.87 will be rejected with 95% probability when the same sampling
797 plan is applied (consumer's ALS). The producer's ALS, has a mean concentration of 1.49
798 $log_{10}CFU.g^{-1}$. The 99%-point reflecting the FSO/PO is at 2.34 and the 99.5%-point is at
799 2.39 $log_{10}CFU.g^{-1}$ as depicted in the second graph.

800 Experience may also show that an s.d. of 0.8 $log_{10}CFU.g^{-1}$ is more appropriate to describe
801 the distribution, as depicted in Figure A3.3. In this case, lots with mean log-concentrations
802 at 1.48 will be rejected with a 95% probability, and those with a mean log-concentration of
803 –0.054 $log_{10}CFU.g^{-1}$ accepted with a 95% probability. The 99%-point would be at 3.34 and
804 the 99.5%-point would be at 3.54 $log_{10}CFU.g^{-1}$.

805 These examples show the implied PO/FSO given a certain sampling plan and a given
806 standard deviation. For a specified PO/FSO the procedure would be the other way around,
807 to calculate the microbiological criterion based on the PO/FSO and the standard deviation.

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847

848 **Figure Legends**

849 **Figure A1.1** Probability density (frequency distribution) of a log-normal distribution
850 with mean = 1 $\log_{10}$CFU.g$^{-1}$ and $\sigma$ = 0.2. The probability of acceptance
851 when taking 1 sample is 100% if $m$ = 2 $\log_{10}$CFU.g$^{-1}$.

852 **Figure A1.2.** Probability densities for log-normal distributions with mean = 1
853 $\log_{10}$CFU.g$^{-1}$, 1.5 $\log_{10}$CFU.g$^{-1}$, 2 $\log_{10}$CFU.g$^{-1}$ and 2.5 $\log_{10}$CFU.g$^{-1}$ and $\sigma$
854 = 0.2. The probability of acceptance for each of these distributions is given
855 for the limit $m$ = 2 $\log_{10}$CFU.g$^{-1}$ when a single one g sample is taken ($n$ = 1).

856 **Figure A1.3.** The probability of acceptance for various mean $\log_{10}$ concentrations with a
857 standard deviation of 0.2 and a limit of $m$ = 2 $\log_{10}$CFU.g$^{-1}$; i.e., the OC-
858 curve for $n$ = 1, $c$ = 0 and $m$ = 100 CFU.g$^{-1}$.

859 **Figure A1.4.** The probability of acceptance for various mean $\log_{10}$ concentrations with a
860 standard deviation of 0.2 and a limit of $m$ = 2 $\log_{10}$CFU.g$^{-1}$, i.e., the OC-
861 curve for $n$ = 10, $c$ = 0 and $m$ = 100 CFU.g$^{-1}$.

862 **Figure A1.5.** The probability of acceptance for various mean $\log_{10}$ concentrations with a
863 standard deviation of 0.4 and a limit of $m$ = 2 $\log_{10}$CFU.g$^{-1}$, i.e., the OC-
864 curve for $n$ = 10, $c$ = 0 and $m$ = 100 CFU.g$^{-1}$.

865 **Figure A1.6.** The probability of acceptance for various mean $\log_{10}$ concentrations with a
866 standard deviation of 0.8 and a limit of $m$ = 2 $\log_{10}$CFU.g$^{-1}$, the OC-curve
867 for $n$ = 10, $c$ = 0 and $m$ = 100 CFU.g$^{-1}$.

868 **Figure A3.1.** a) Probability of acceptance as a function of mean $\log_{10}$CFU.g$^{-1}$ (assuming
869 s.d. = 0.4), for sampling plan of $n$ = 10, $c$ = 0, $m$ = 100 CFU.g$^{-1}$.

870 b) Distribution of counts in a lot *rejected* with 95% probability (assuming
871 s.d. = 0.4), for sampling plan of $n = 10$, $c = 0$, $m = 100$ CFU.g$^{-1}$.

872 c) Distribution of counts in a lot *accepted* with 95% probability (assuming
873 s.d. = 0.4), for sampling plan of $n = 10$, $c = 0$, $m = 100$ CFU.g$^{-1}$.

874 **Figure A3.2.** a) Probability of acceptance as a function of mean $\log_{10}$CFU.g$^{-1}$ (assuming
875 s.d. = 0.2), for sampling plan of $n = 10$, $c = 0$, $m = 100$ CFU.g$^{-1}$.

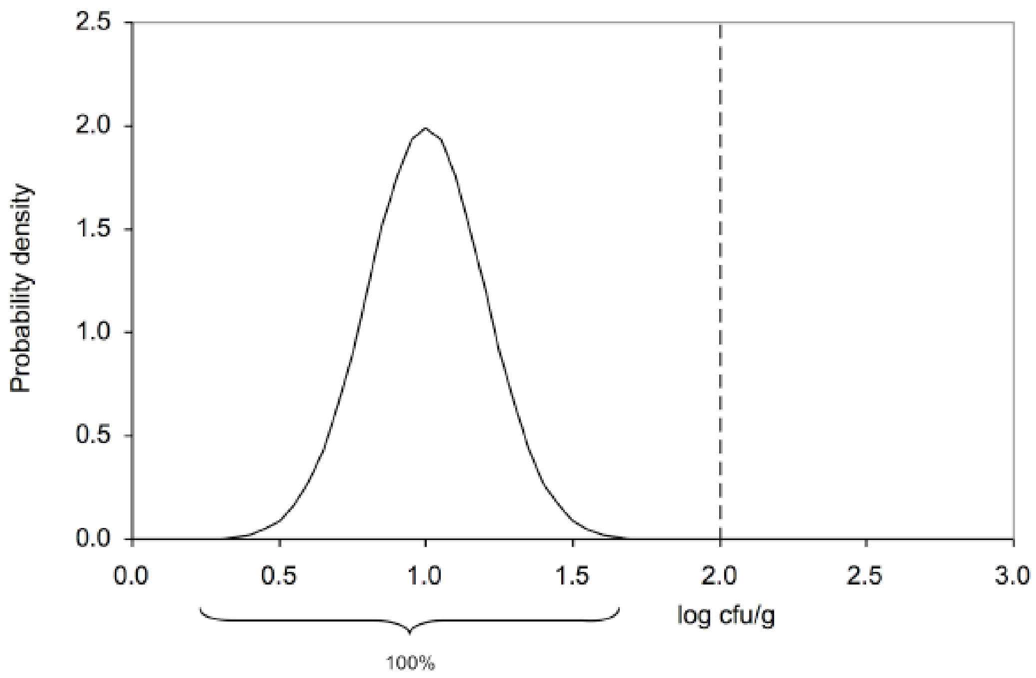
876 b) Distribution of counts in a lot *rejected* with 95% probability (assuming
877 s.d. = 0.2), for sampling plan of $n = 10$, $c = 0$, $m = 100$ CFU.g$^{-1}$.

878 c) Distribution of counts in a lot *accepted* with 95% probability (assuming
879 s.d. = 0.2), for sampling plan of $n = 10$, $c = 0$, $m = 100$ CFU.g$^{-1}$.

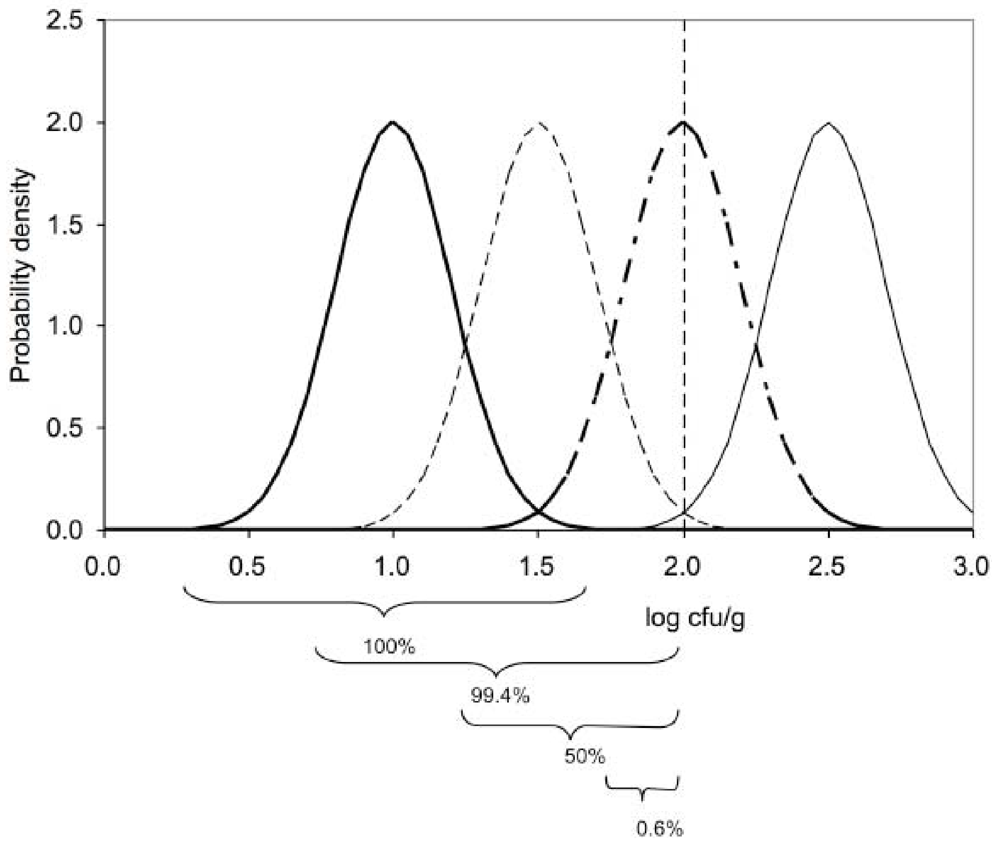
880 **Figure A3.3.** a) Probability of acceptance as a function of mean $\log_{10}$CFU.g$^{-1}$ (assuming
881 s.d. = 0.8), for sampling plan of $n = 10$, $c = 0$, $m = 100$ CFU.g$^{-1}$.

882 b) Distribution of counts in a lot *rejected* with 95% probability (assuming
883 s.d. = 0.8), for sampling plan of $n = 10$, $c = 0$, $m = 100$ CFU.g$^{-1}$.

884 c) Distribution of counts in a lot *accepted* with 95% probability (assuming
885 s.d. = 0.8), for sampling plan of $n = 10$, $c = 0$, $m = 100$ CFU.g$^{-1}$.




886 **Fig A1.1**

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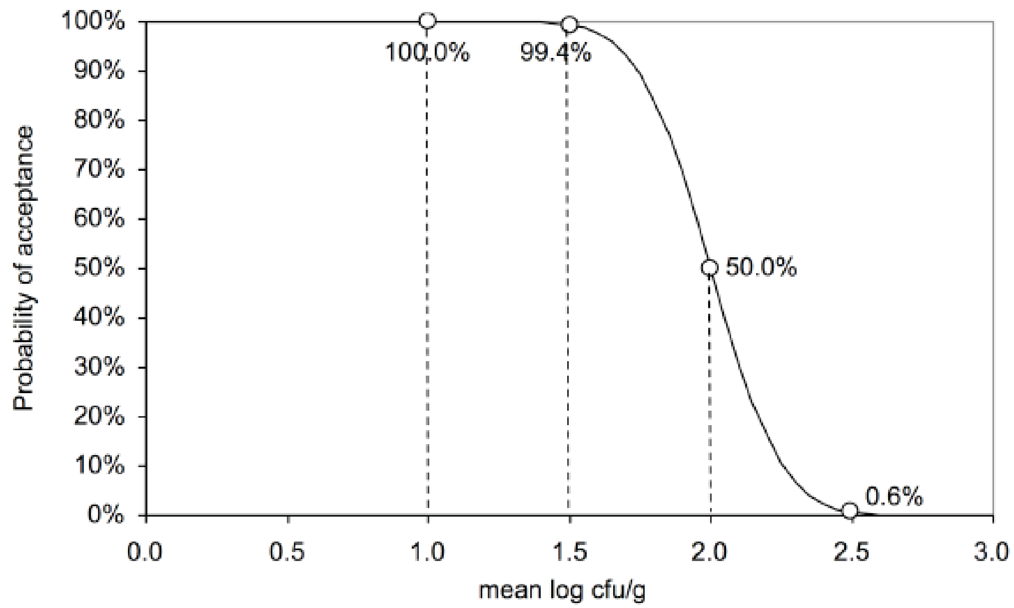


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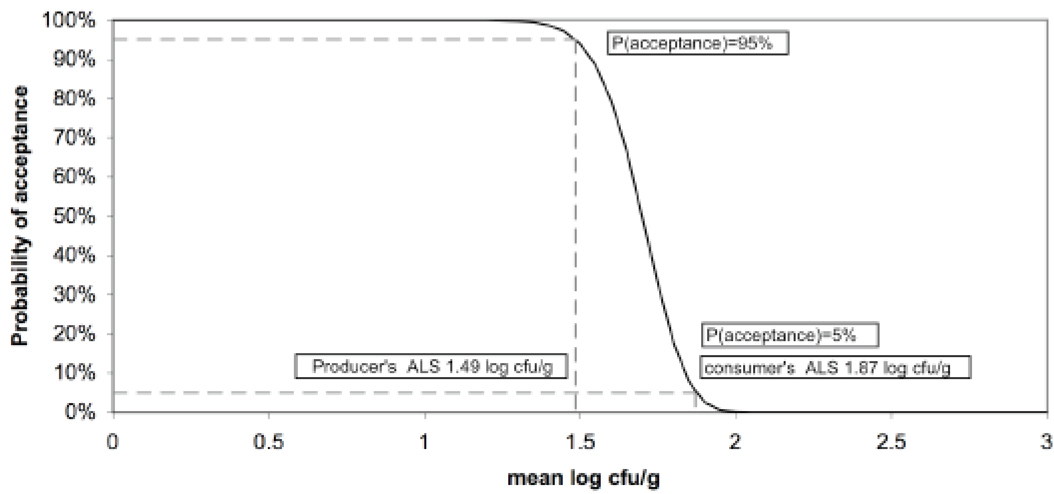


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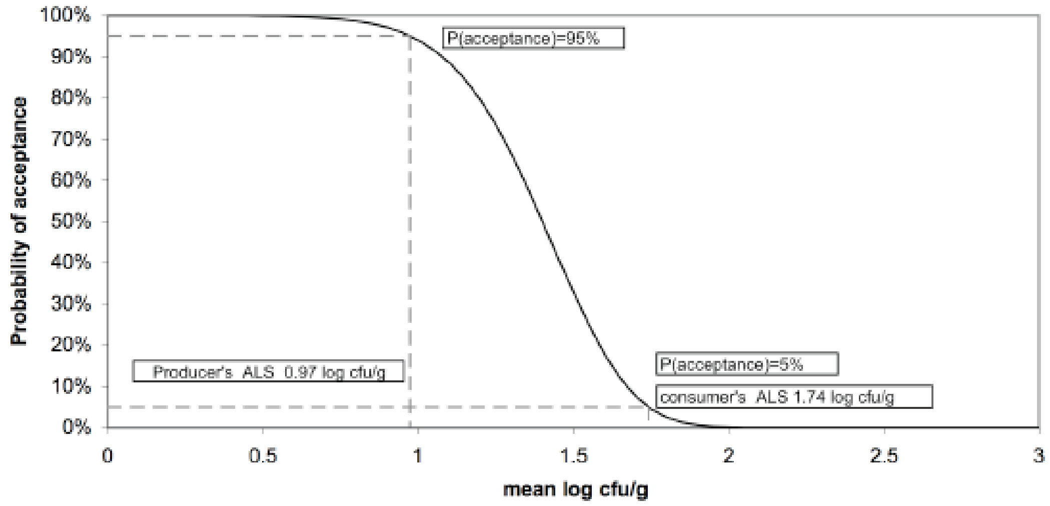
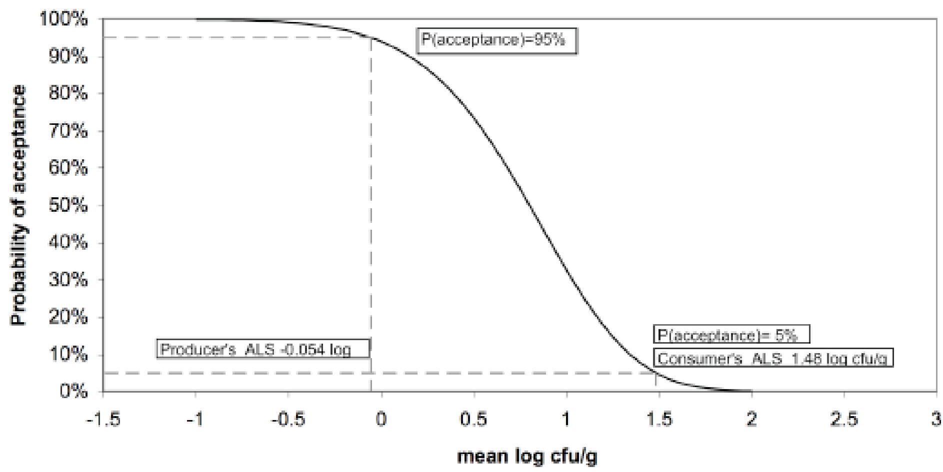



890 Fig. A1. 5

891

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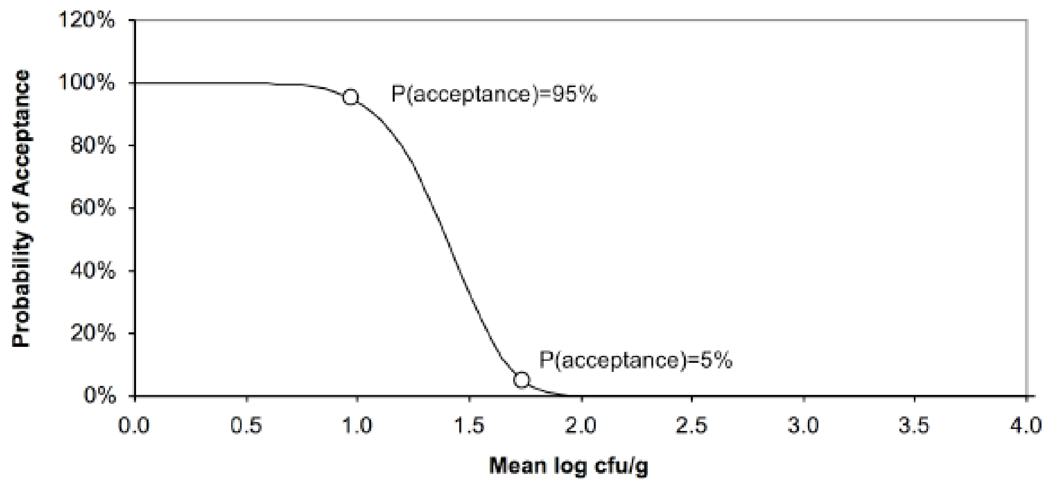


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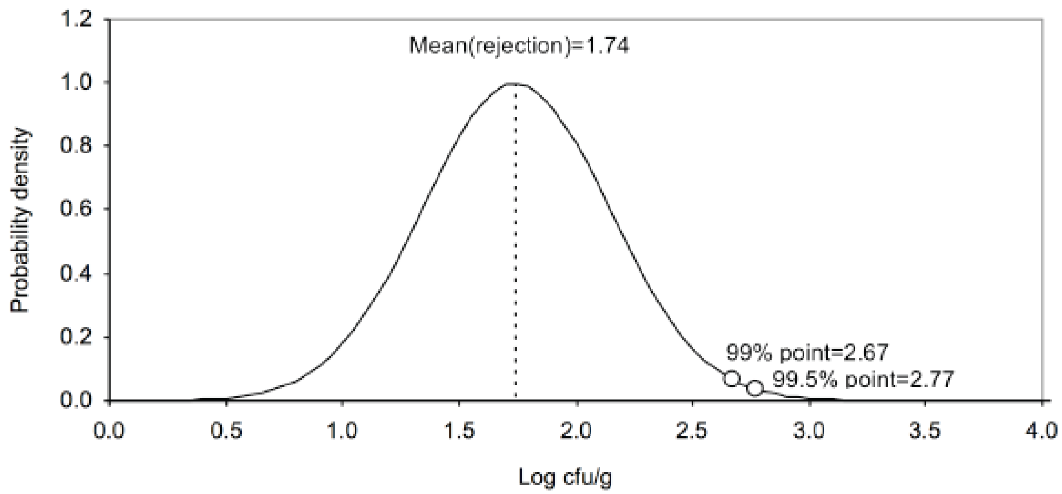


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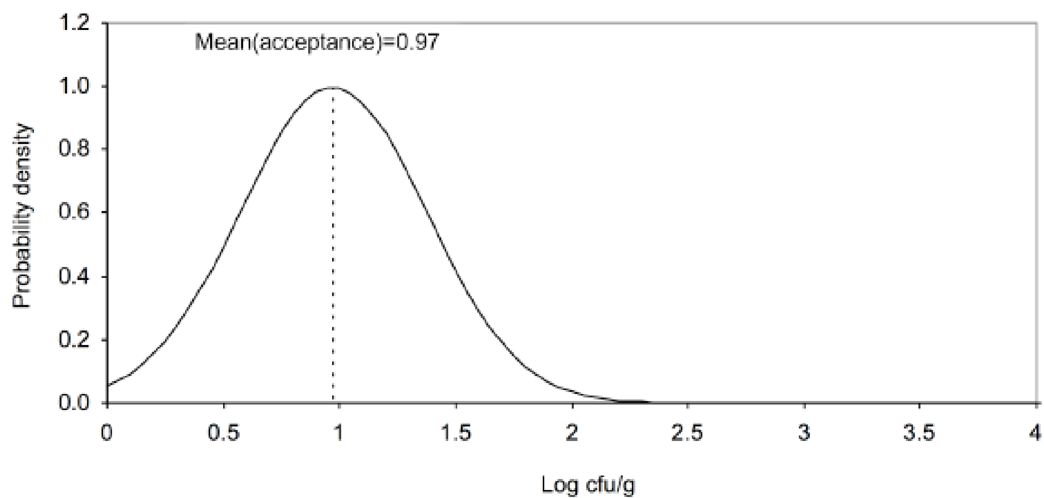


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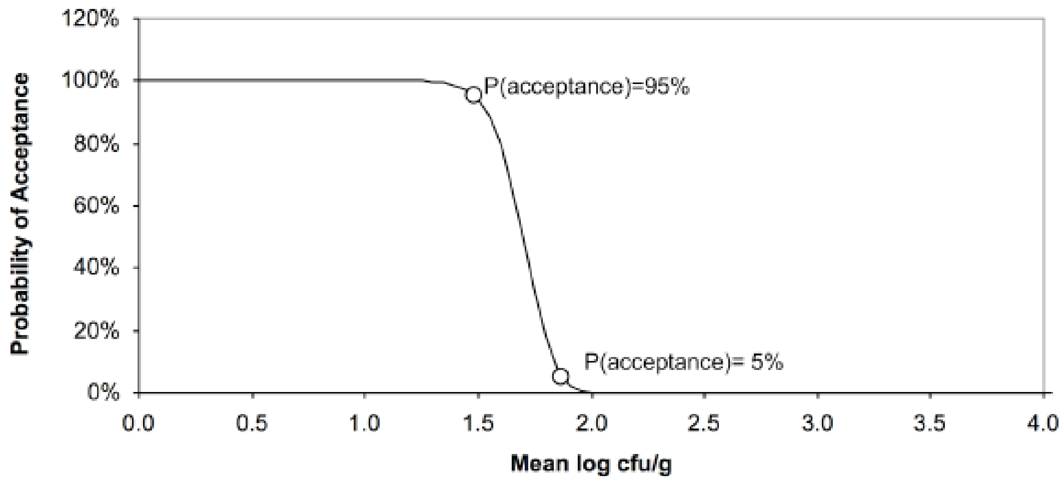


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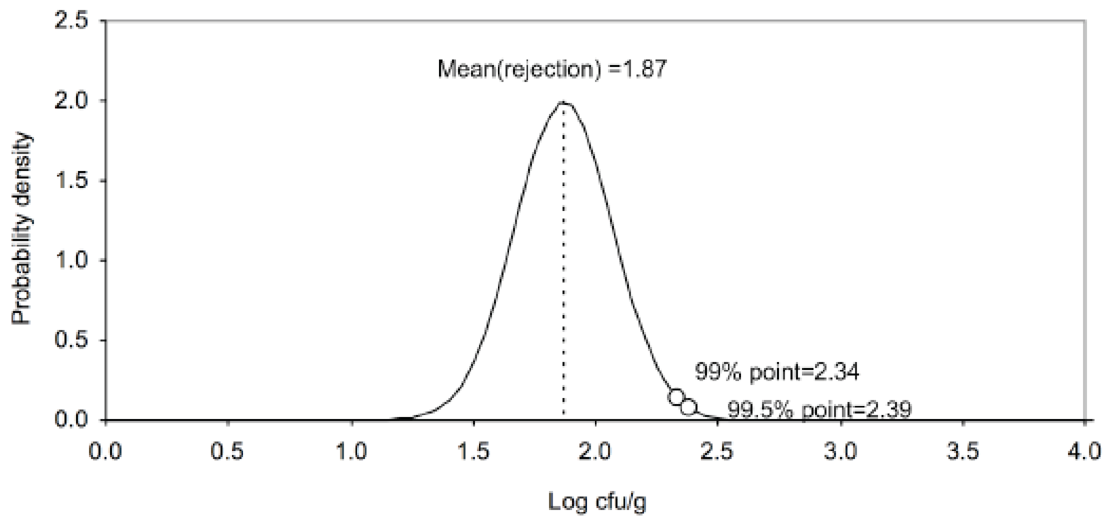


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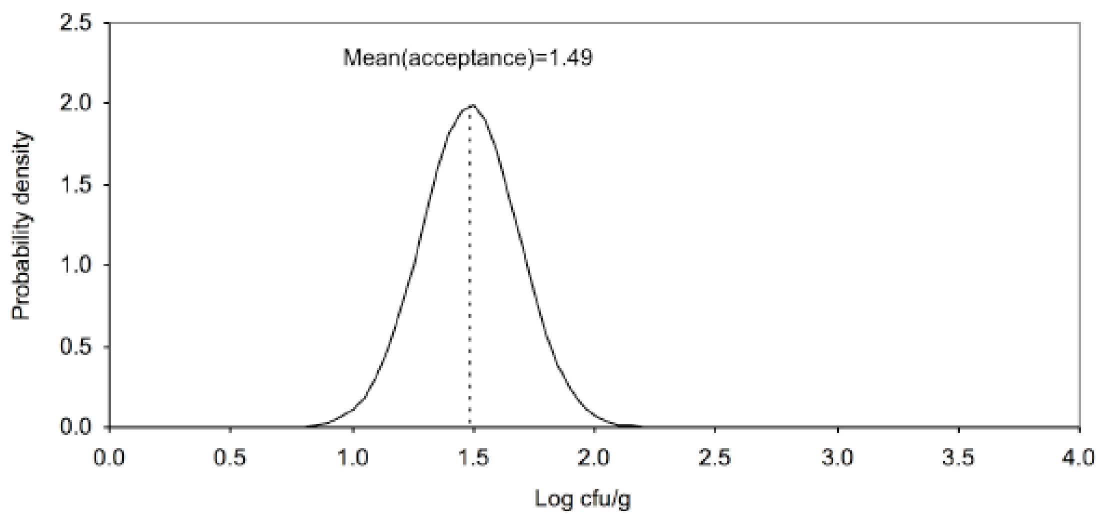


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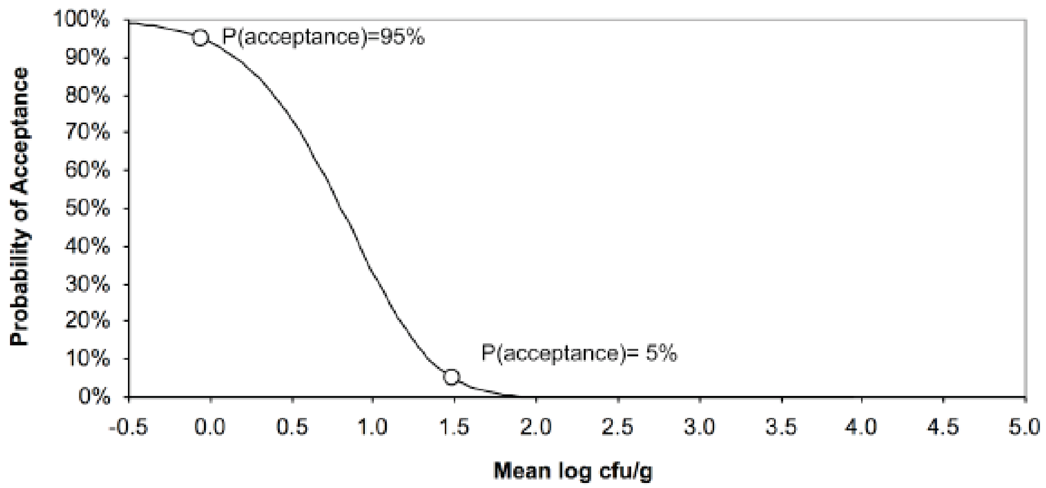


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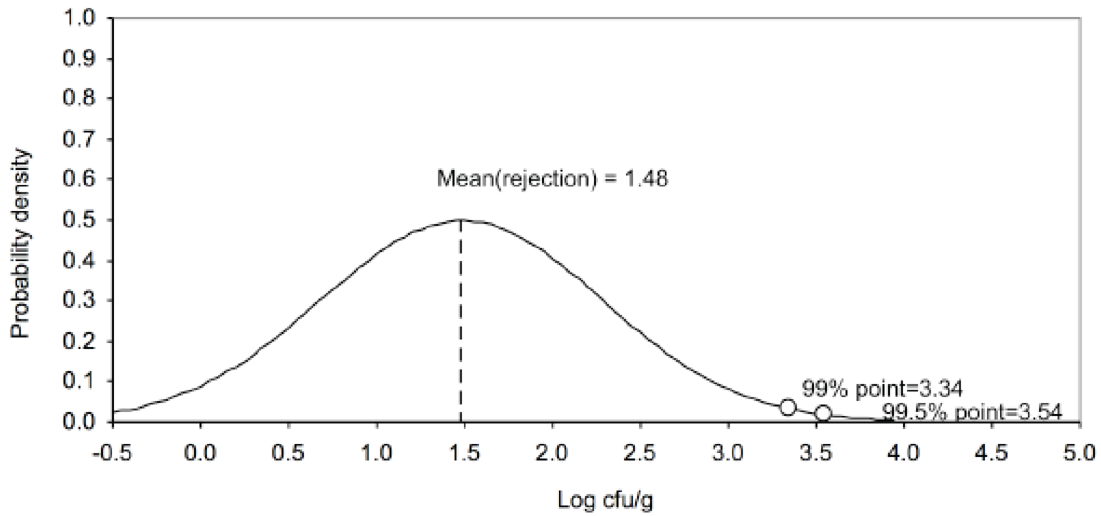


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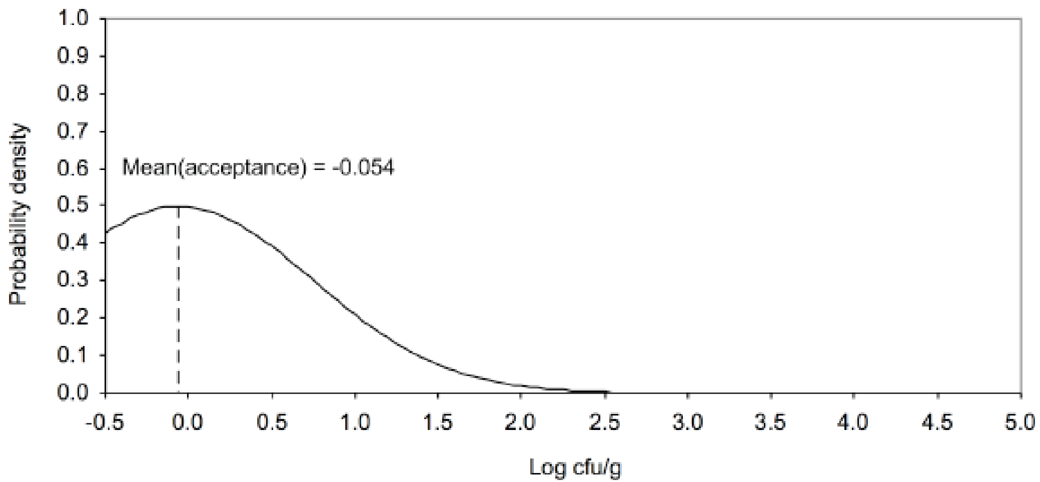


**Fig. A3.3c**

901

902 **Table A2.1**

903 **Sampling plans derived from the modified approach for *Salmonella* in ice**
904 **cream with different distributions intended to test compliance with**
905 **different FSOs/POs**

| ***m*** **absence in** | **FSO/PO** | **Log mean** (cfu.g$^{-1}$) **± s.d.**[*] | ***n*** **(*c*=0)**[**] |
|---|---|---|---|
| 25 g | 1 / 100g | -2.93 ± 0.4 | 69 |
| 100 g | | | 19 |
| 250 g | | | 9 |
| 25 g | | -3.86 ± 0.8 | 183 |
| 100 g | | | 55 |
| 250 g | | | 27 |
| 25 g | 1 / 1000g | -3.93 ± 0.4 | 671 |
| 100 g | | | 170 |
| 250 g | | | 69 |
| 25 g | | -4.86 ± 0.8 | 1631 |
| 100 g | | | 427 |
| 250 g | | | 183 |
| 25 g | 1 / 10.000g | -4.93 ± 0.4 | 6684 |
| 100 g | | | 1673 |
| 250 g | | | 671 |
| 25 g | | -5.86 ± 0.8 | 15994 |
| 100 g | | | 4027 |
| 250 g | | | 1631 |

906 These figures are based on a “99% point definition” of the PO and 95% probability of
907 rejection
908
909 [*] The maximum mean log concentration of a lot with the stated standard deviation that
910 would permit the lot to meet the FSO. log(FSO)-z(0.01)·s.d.=log(FSO)-2.326s·d (where z is
911 the ‘standard score’ and indicates how many standard deviations an observation is above or
912 below the mean).
913
914 [**] Number of samples that would need to be tested to ensure 95% confidence that a lot was not
915 exceeding the stated FSO. P=Poisson-lognormal(log(1/*m*),logmean,s.d.); $P^n$=0.05; nlogP=log0.05
916 P(+) calculated with Equation 6
917 $P(-) = 1 - P(+)$
918 $\therefore (P(-))^n = 0.05$
919 $\therefore n\log(P(-)) = \log(0.05)$
920 $\therefore n = \log(0.05)/\log(P(-))$

921 **Table A2.2: Sampling plans derived from the approach of Legan et al. (2001) for *L.*
922 *monocytogenes* in smoked salmon with different distributions intended to test
923 compliance with different FSOs and POs**

| The lower limit of detection for three analytical techniques used to examine the samples (see text) | FSO Log(cfu.g$^{-1}$) | PO Log(cfu.g$^{-1}$) | Mean log(cfu.g$^{-1}$) ± s.d.[a] | *n* (*c*=0)[b] | Mean log(cfu.g$^{-1}$) ± s.d.[c] accept |
|---|---|---|---|---|---|
| 100/g | 2.3 | 1.7 | 1.2 ± 0.2 | 4.6 x $10^4$ | 1.1 ± 0.2 |
| 0.3/g | | | | 1 | -0.8 ± 0.2 |
| Abs. in 25 g | | | | 1 | -1.7± 0.2 |
| 100/g | | | 0.8 ± 0.4 | 2.9 x $10^3$ | 0.35 ± 0.4 |
| 0.3/g | | | | 1 | -1.1± 0.4 |
| Abs. in 25 g | | | | 1 | -2.1± 0.4 |
| 100/g | | | -0.2 ± 0.8 | 867 | -1.1 ± 0.8 |
| 0.3/g | | | | 3 | -2.2± 0.8 |
| Abs. in 25 g | | | | 2 | -3.0± 0.8 |
| 100/g | 3.3 | 2.7 | 2.2 ± 0.2 | 2.0 | 1.6 ± 0.2 |
| 0.3/g | | | | 1 | -0.8 ± 0.2 |
| Abs. in 25 g | | | | 1 | -1.7 ± 0.2 |
| 100/g | | | 1.8 ± 0.4 | 10 | 1.0 ± 0.4 |
| 0.3/g | | | | 1 | -1.1 ± 0.4 |
| Abs. in 25 g | | | | 1 | -2.1 ± 0.4 |
| 100/g | | | 0.8 ± 0.8 | 40 | -0.4 ± 0.8 |
| 0.3/g | | | | 1 | -1.8 ± 0.8 |
| Abs. in 25 g | | | | 1 | -2.7 ± 0.8 |
| 100/g | 4.3 | 3.7 | 3.2 ± 0.2 | 1 | 1.7 ± 0.2 |
| 0.3/g | | | | 1 | -0.8 ± 0.2 |
| Abs. in 25 g | | | | 1 | -1.7 ± 0.2 |
| 100/g | | | 2.8 ± 0.4 | 1 | 1.3 ± 0.4 |
| 0.3/g | | | | 1 | -1.1 ± 0.4 |
| Abs. in 25 g | | | | 1 | -2.0 ± 0.4 |
| 100/g | | | 1.8 ± 0.8 | 6 | 0.1± 0.8 |
| 0.3/g | | | | 1 | -1.8 ± 0.8 |
| Abs. in 25 g | | | | 1 | -2.7 ± 0.8 |

924 a. **The maximum mean log concentration of a lot with the stated standard deviation that**
925 **would permit the lot to meet the FSO.**
926 b. **Number of samples that would need to be tested to ensure 95% confidence that a lot**
927 **was not exceeding the stated FSO.**
928 c. **The maximum mean log concentration of a lot that would be accepted with 95%**
929 **probability given this number of samples.**
930

931 **Table A2.3**

932 **Sampling plans derived for *Salmonella* in poultry carcasses intended to**
933 **test compliance with different POs**

| Proportion of contaminated carcasses tolerated (PO) | Number of samples ($n$) required to reject defective lots with 95% probability ($c$=0)[a] | Proportion of contaminated carcasses accepted with 95% probability[b] |
|---|---|---|
| 15% | 19 | 0.27 % |
| 10% | 29 | 0.18 % |
| 5% | 59 | 0.09 % |
| 1% | 298 | 0.02% |

934 [a] $(1\text{-P})^{n}=0.05$
935 $\therefore$ $n\log(1\text{-P})=\log(0.05)$
936 $\therefore$ $n=\log(0.05)/\log(1\text{-P})$
937 [b] $(1\text{-P})^{n}=0.95$
938 $\therefore$ $\log(1\text{-P})=\log(0.95)/n$
939 $\therefore$ $1\text{-P}=0.95^{1/n}$
940 $\therefore$ $P=1-0.95^{1/n}$
941 This could also be calculated with the negative binomial distribution:
942 NEGBINOMIAL(0;19;1-0.15=0.05); NEGBINOMIAL(0;19;1-0.0027=0.95);
943