# USER'S GUIDE

# Tools to Estimate the Impact of Microbiological Sampling Plans: Lot-by-Lot Testing

*June 21, 2011*

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# Introduction

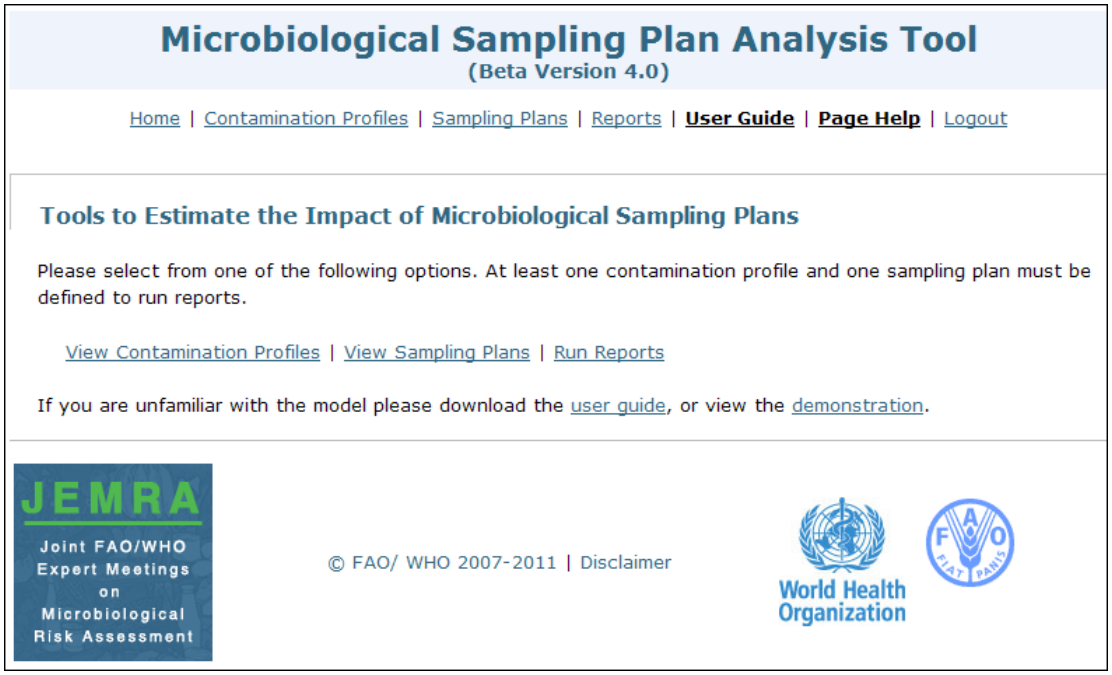
The risk of pathogens in the food supply can be reduced by the implementation of effective sampling plans. In conjunction with an appropriate microbiological criterion, a well-designed sampling plan can identify unacceptable lots of food in the supply chain, and actions can then be taken to mitigate the risk.

One goal of this web-based tool is to evaluate the performance of sampling plans with respect to acceptance sampling. Essentially, this involves calculating the probability of rejecting a lot of product under a specified sampling plan, given the level and pattern of contamination of the product. Sensitivity Analysis permits exploration of the effect of varying the value of individual sampling plan parameters.

The tool is also intended to help users design effective sampling plans, by providing appropriate values for various parameters that will result in desirable rejection rates for particular hazard concentrations.

Finally, using the assumption that rejected lots are removed from commerce, the tool calculates the risk reduction that results from the imposition of a given sampling plan, for a selected food contamination profile.

The plan types that can be considered are Presence/Absence plans, as well as either two-class or three-class Concentration-based plans.



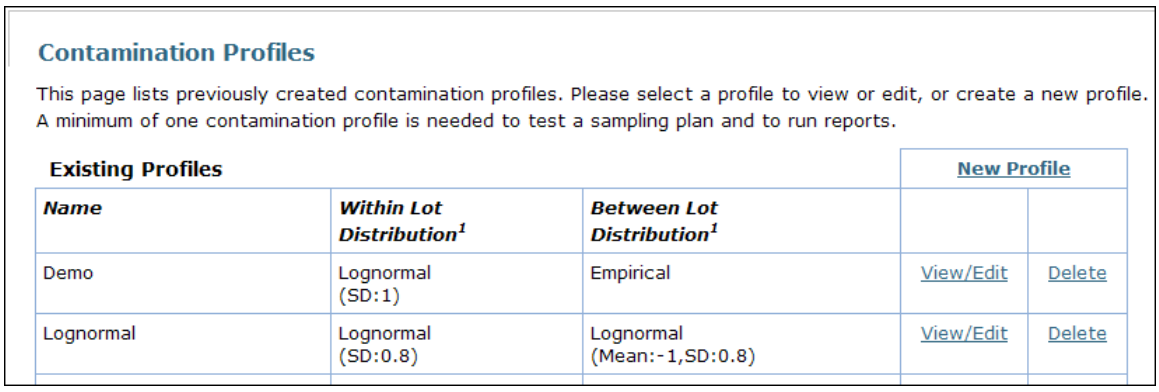

The main components of the tool are available from the menu of hyperlinks, either at the top or the middle of the home page. Analyses can be conducted via the "Sampling Plans" link, where a specific plan can be defined or selected.

For first-time users, please follow the three steps described below. The permitted ranges of input values are tabulated at the end of this document. Restrictions are necessary in order to provide a balance between convenience for the user and computing power and run-time of the model.

A glossary is also provided.

## Step 1: Define Contamination Profile(s)

Upon login, the user may edit an already existing concentration profile by using the button "View/Edit", or create a new profile by clicking "New Profile".



**Contamination Profiles**

This page lists previously created contamination profiles. Please select a profile to view or edit, or create a new profile. A minimum of one contamination profile is needed to test a sampling plan and to run reports.

**Existing Profiles** — New Profile

| *Name* | *Within Lot Distribution*[1] | *Between Lot Distribution*[1] | | |
|---|---|---|---|---|
| Demo | Lognormal (SD:1) | Empirical | View/Edit | Delete |
| Lognormal | Lognormal (SD:0.8) | Lognormal (Mean:-1,SD:0.8) | View/Edit | Delete |

Each profile can be named and given a description. Once the list of profiles is populated, the name of the profile with a summary of the defined parameters is included in the list of Existing Profiles.

**Define Contamination Profile: *Name and Description***

Overview | **Profile Name** | Within Lot | Between Lot (optional)

**Current Profile: New Profile**

Please enter a unique name for this contamination profile as well as description.

**Profile Name:** New Profile

**Description:**

Once a profile is created (or one is chosen to edit), the user describes the variation in the concentration of the hazard within a lot, and the variability in the mean concentration of the hazard between lots of product.

### Background

The distribution of arithmetic mean hazard concentration among the lots makes up the between-lot distribution (Fig 1). In Figure 1, most of the lots have an arithmetic mean hazard concentration of -0.8 $\log_{10}$ cfu/g (represented by within-lot distribution "A"). The most highly-contaminated lots (represented by within-lot distribution "B") have an arithmetic mean hazard concentration close to 2.2 $\log_{10}$ cfu/g.

Notice that the within-lot distributions of different lots have the same standard deviation and are distinguished only by their mean hazard concentration.

In the example shown, the within-lot distributions and between-lot distributions happen to be normal (on the log scale); in fact there is no requirement for them to be the same.

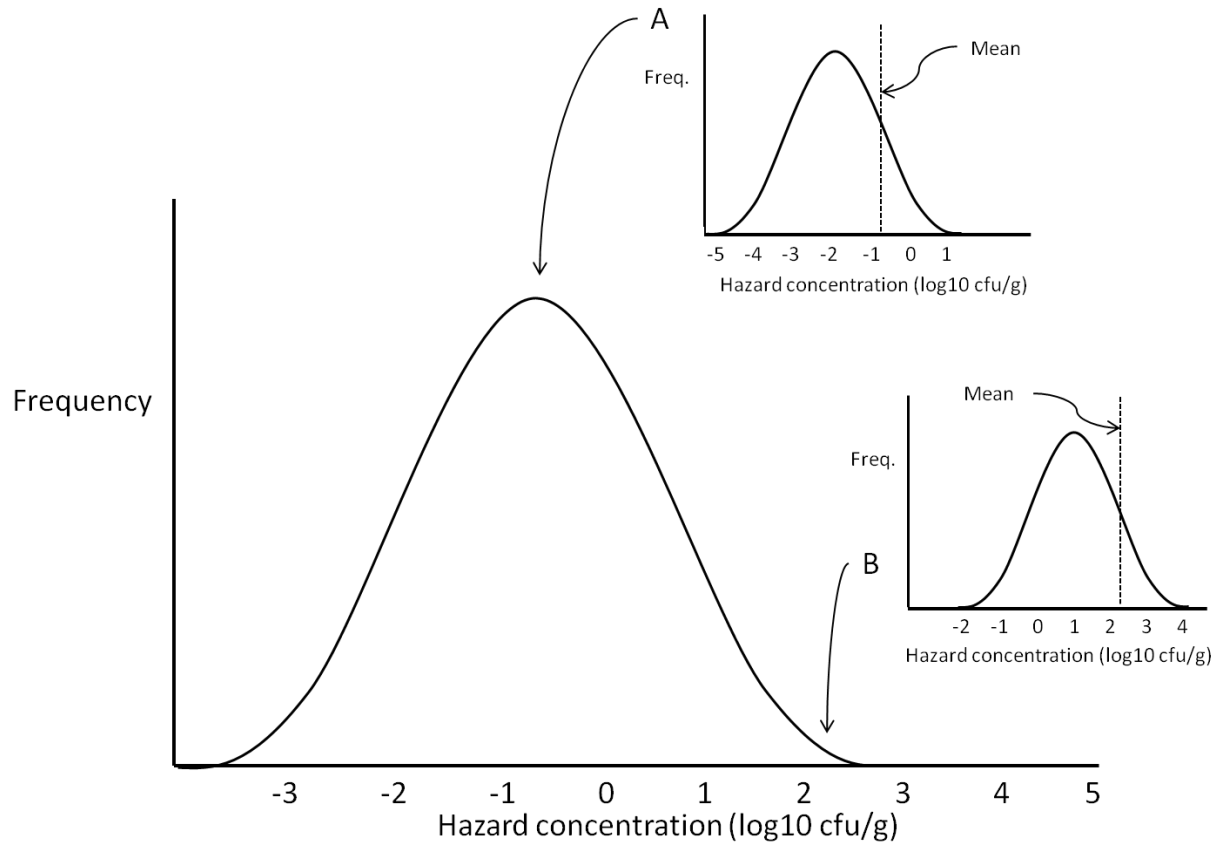



**Figure 1. Relationship between selected within-lot distributions and a between-lot distribution of hazard concentration**

## Within-Lot Distribution

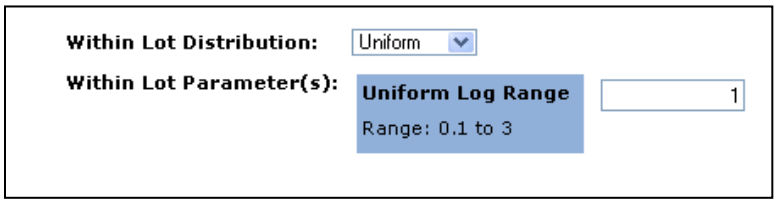
The ***within-lot*** distribution refers to the variability in the concentration in different regions or volumes within a lot. It answers the question, "When considering a single lot, how different is the concentration at different points within that lot?"

Distribution choices in the drop-down menu include: lognormal, log-uniform, log-triangular and gamma. Once a distribution is chosen, the required inputs for that distribution appear for the user to define.

All concentrations are specified on the log-transformed scale. Since the $\log_{10}$ scale is assumed, the log-uniform and log-triangular distributions are simply referred to as uniform and triangular, respectively.

To describe the extent of dispersion around the mean concentration that would be expected within a lot:

- The lognormal requires specification of the standard deviation of the distribution of concentration within a lot, on the $\log_{10}$ scale.
- The uniform requires specification of the range of hazard concentration, essentially the difference between the maximum and minimum concentrations as expressed on the $\log_{10}$ scale.
- The triangular also requires specification of the range of hazard concentration. Note that in the case of within-lot distributions, the triangular distribution is assumed to be symmetric about its mode on the $\log_{10}$ scale.
- The gamma requires the specification of the standard deviation of the distribution of concentration within a lot, on the $\log_{10}$ scale.




The within-lot distribution is required for calculation of the detectable microbial load, proportion of microbial load remaining in released (final) product, mean contamination in the accepted product and in the final product, operating characteristic curve and the desired sampling plan characteristics. These outputs are further explained in the "Analyze Sampling Plan Performance" section of this guide.

## Between-Lot Distribution (Optional)

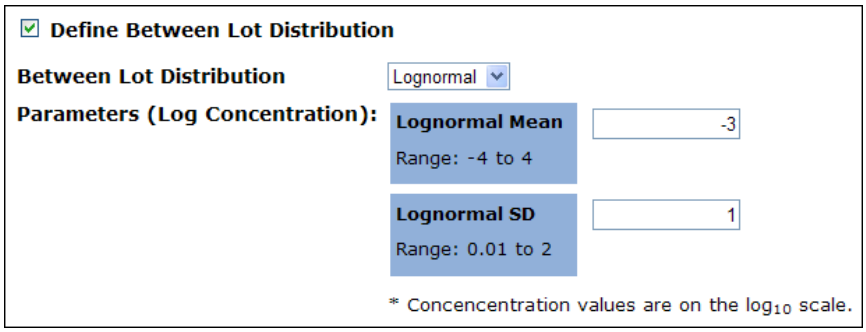
The ***between-lot*** distribution refers to the variation in the arithmetic mean concentration among different lots (see Figure 1, above). It answers the question, "When considering the mean level of contamination of a hazard in a lot, how variable is this mean among different lots of product?"

Defining the between-lot distribution is optional and is only required if the user is interested in knowing any of the following results:

- Mean Concentration Before Sampling,
- Mean Concentration in Tested Accepted Product,
- Overall Mean Concentration in All Accepted Product,
- Percentage of Tested Lots Rejected,
- Percentage of All Lots Rejected,
- Percentage Microbial Load Remaining.

The options to describe the distributions are the lognormal, log-uniform, log-triangular and log-empirical distributions. All concentrations are specified on the log-transformed scale. Since the $\log_{10}$ scale is assumed, for simplicity, the log-uniform log-triangular and log-empirical distributions are simply referred to as uniform, triangular and empirical (or "Cumulative"), respectively.

As was the case in defining the within-lot distribution, a drop down menu allows the user to select the desired distribution. After the selection, the required inputs appear for the user to define.

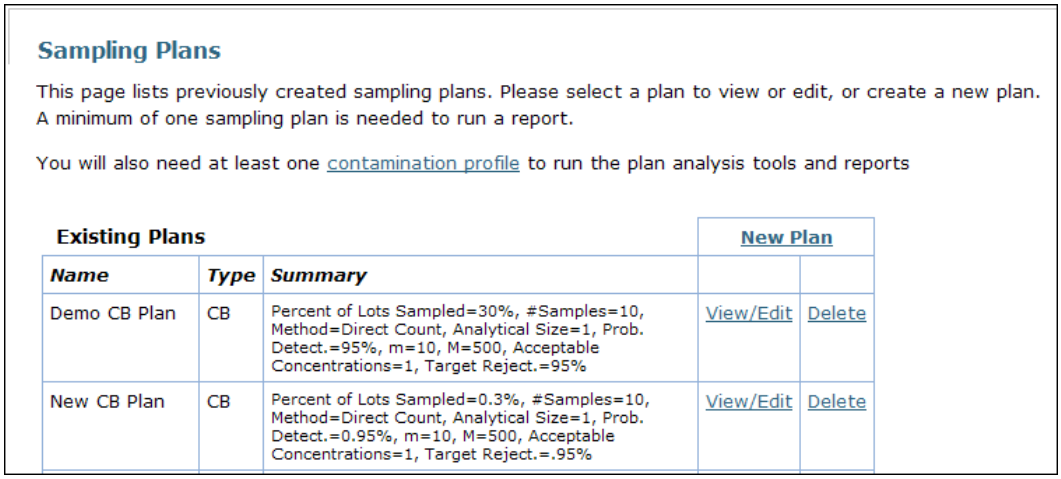



The choice of the type of distribution yields different input requirements, associated with the typical parameters for each distribution type.

- The lognormal requires specification of the mean hazard concentration among lots and the standard deviation of the distribution of the mean concentration among lots.
- The uniform requires specification of the minimum and the maximum hazard concentration among the lots. The values represent the **mean** concentration in the least and most contaminated lots, respectively.
- The triangular requires specification of the minimum, mode, and maximum mean hazard concentrations among the lots.
- The empirical is a user-specified cumulative distribution defined by paired values of concentration and cumulative probability. The range is defined by including minimum (cumulative probability = 0) and maximum (cumulative probability = 1) concentration values.

## Step 2: Define the Sampling Plans

The user may choose to model presence/absence plans or concentration-based plans. To view or edit an existing plan click "View/Edit", or click "New Plan" to create a new plan. If creating a new plan, choose Presence/Absence or Concentration-based.



**Sampling Plans**

This page lists previously created sampling plans. Please select a plan to view or edit, or create a new plan. A minimum of one sampling plan is needed to run a report.

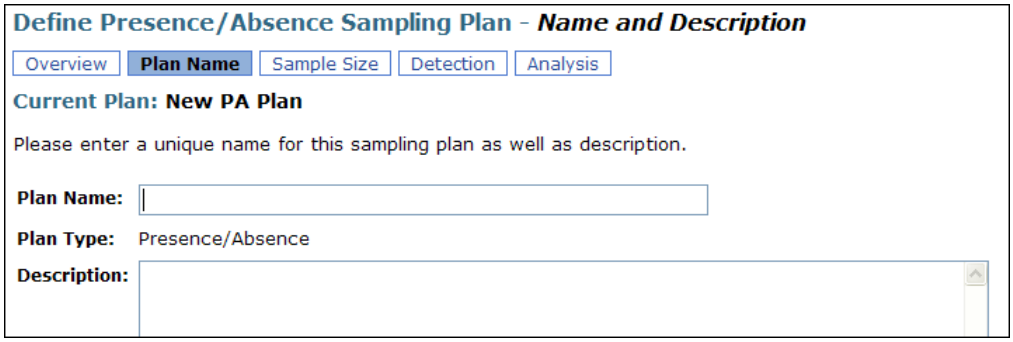
You will also need at least one contamination profile to run the plan analysis tools and reports

**Existing Plans** — New Plan

| *Name* | *Type* | *Summary* | | |
|---|---|---|---|---|
| Demo CB Plan | CB | Percent of Lots Sampled=30%, #Samples=10, Method=Direct Count, Analytical Size=1, Prob. Detect.=95%, m=10, M=500, Acceptable Concentrations=1, Target Reject.=95% | View/Edit | Delete |
| New CB Plan | CB | Percent of Lots Sampled=0.3%, #Samples=10, Method=Direct Count, Analytical Size=1, Prob. Detect.=0.95%, m=10, M=500, Acceptable Concentrations=1, Target Reject.=.95% | View/Edit | Delete |

If you click on New Plan you will be taken to a page describing the two plan options, Presence/Absence and Concentration-based, and prompted to choose.


After making the choice and clicking "Create Plan" the "Plan Name" page opens, and you will be guided through pages defining Sample characteristics, Detection, and, if desired, Analysis. These are also available directly from buttons along the top of the page.




## Background

A presence-absence plan is defined by whether contamination with any number of organisms in a single sample is detected. In such a plan, there is no operational difference between a sample that contains one organism and another which contains 1,000 organisms. They are both simply considered a "positive" sample. Presence-absence plans are most likely to be used in applications where most or all of the samples would be expected to contain no organisms, and also in cases where the available enumeration procedures are deemed impractical for routine testing.

In a concentration-based plan, individual sample results are evaluated not only with respect to the presence or absence of an organism, but also with respect to the estimated concentration of the organism in the sample taken.

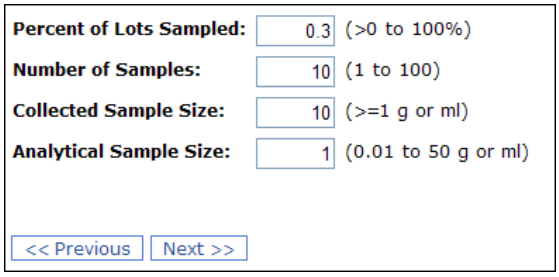
## Presence-Absence Sampling Plans

The sampling plans defined under this option are based on the detection of the organism in the tested sample. Samples are described as either "positive" or "negative." Concentration is not measured, so it is not appropriate to refer to any particular concentration measurement threshold when specifying the plan.

### Sample Characteristics

To characterize the sample(s) of the sampling plan, the model requires the user to define the *Percent of Lots Sampled*, the *Number of Samples* (n) tested from a single lot, the *Collected Sample Size* (in grams or ml) and the *Analytical Sample Size* (in grams or ml).

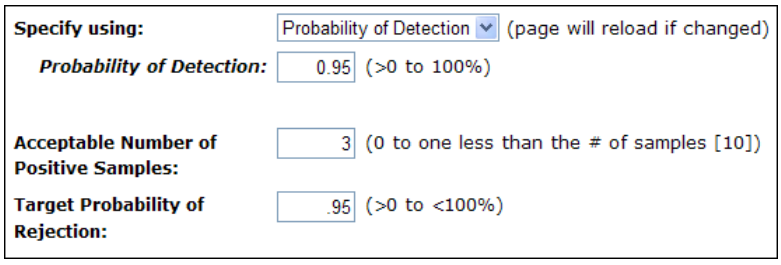
The *Analytical Sample Size* is the mass or volume of the product that is actually tested, regardless of how much is initially collected (the *Collected Sample Size*). The value for *Collected Sample Size* does not participate in the calculations, and this field is included for transparency only.




### Detection and Lot Acceptance Criteria

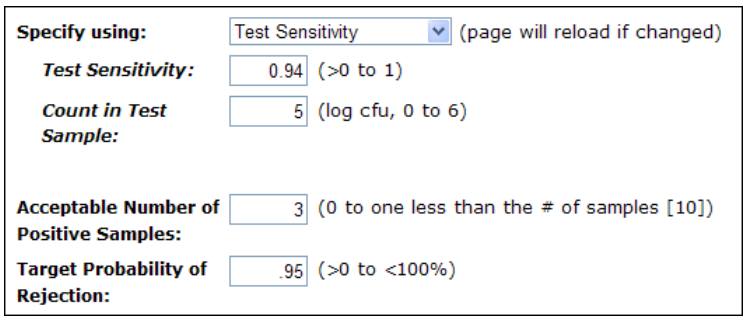
The user must also specify the parameters of the sampling plan associated with the detection of contamination. These parameters include the *Probability of Detection* (or *Test Sensitivity* with an associated *Count in Test Sample*), *Acceptable Number of Positive Samples* and the *Target Probability of Rejection*.

The *Probability of Detection* is defined as the probability that a test will detect a single organism, and influences the probability of any given sample being positive. This value may be inferred from the fraction of positive test samples known to be contaminated and the associated count in the test samples.




*Test Sensitivity* is the probability of returning a "positive" finding, given a particular number of organisms present that is specified as the *Count in Test Sample*. *Test sensitivity* with its associated *Count in Test*

*Sample*, can be an alternate input to the *Probability of Detection*. These inputs are assumed to be related by a binomial distribution as outlined in the document "Mathematical Characterization".




*Acceptable Number of Positive Samples*, denoted 'c', is the maximum number of positives permitted among the sample for a tested lot that is accepted.

Any given sampling plan can be associated with a *Detectable Microbial Load* (DML), for a given probability of lot rejection and within-lot variability. When the user specifies a *Target Probability of Rejection*, the tool can calculate the $\log_{10}$-scale mean concentration in a lot that results in that specified probability of rejection; this concentration is shown in the PDF report and in the Senstivity Analysis results, under the heading "Detectable Microbial Load". Lots with concentrations above this DML will be rejected at a higher probability than the target.

Clicking the "Next" button on this page takes the user to the "Analysis Tools" page, where the user can choose one or more Contamination Profiles to be subjected to the described sampling plan.

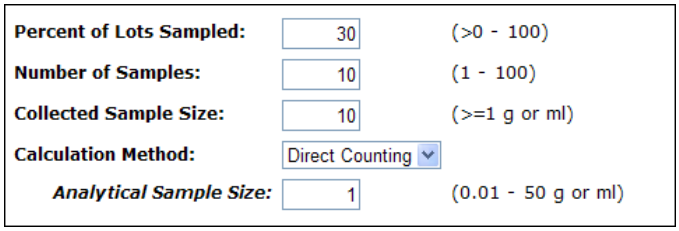
## Concentration-Based Sampling Plans

Sampling plans defined under this option are based on the estimation of concentration of organisms in the sample (and therefore the lot). Concentration-based sampling plans can be defined as either two- or three-class plans, the distinction being the inclusion of an additional threshold of concentration in three-class plans. This additional threshold distinguishes "marginal" from "outright" unacceptable concentrations.

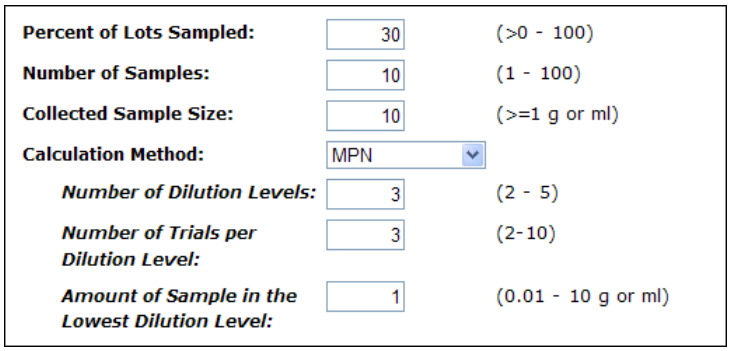
### Sample Characteristics

As in Presence/Absence testing, the user must define *Percent of Lots Sampled*, *Number of Samples* and *Collected*- and *Analytic Sample Size*.

In contrast to Presence/Absence sampling, Concentration-based sampling requires that the user chooses either *Direct Counting* or *MPN* method as the "*Calculation Method*" used to determine the number of organisms present in a sample.



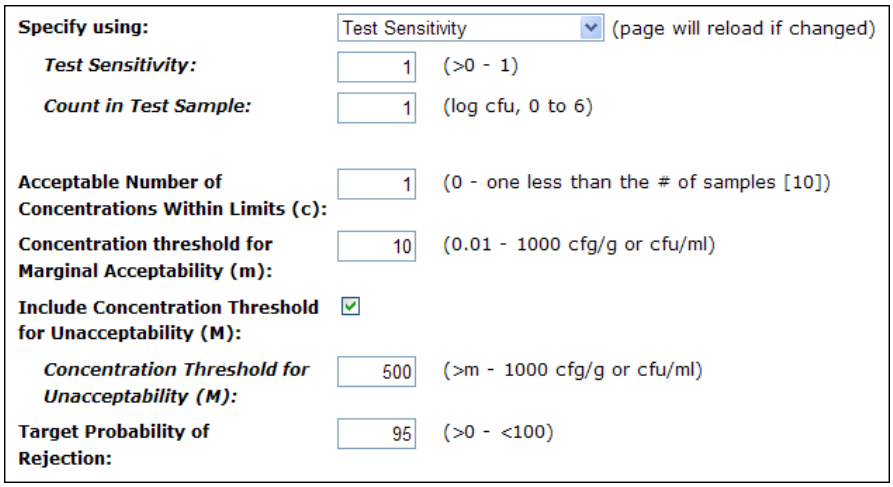

For the *Direct Counting* method the user must specify the *Analytical Sample Size*. This is the mass or volume that is tested and on which organism counts are based.




If the *MPN* method is chosen the tool assumes the use of serial decimal dilutions to calculate the "most probable number" (MPN) of organisms present per gram or per ml in the region of the lot where the sample was taken. For this method the required parameters are *Number of Dilution Levels*, *Number of Trials per Dilution Level* and *Amount of Sample in the Lowest Dilution*. The MPN method also requires inputs for small-m and big-M as described in the next section.

### Detection and Lot Acceptance Criteria

The *Test Sensitivity* and *Count in Test Sample* are required inputs when "Direct Counting" has been selected as the method of calculation. See 'Presence/Absence' plans (above) for definitions.



The *Analytical Recovery Fraction* is required when an MPN method has been chosen. This is the fraction of organisms expected to be successfully counted, out of all of those contaminating the sample.

| | | |
|---|---|---|
| **Specify using:** | Analytical Recovery Fraction (page will reload if changed) | |
| ***Analytical Recovery Fraction:*** | 95 | (>0 - 100) |
| **Acceptable Number of Concentrations Within Limits (c):** | 1 | (0 - one less than the # of samples [10]) |
| **Concentration threshold for Marginal Acceptability (m):** | 10 | (0.01 - 1000 cfg/g or cfu/ml) |
| **Include Concentration Threshold for Unacceptability (M):** | ☑ | |
| ***Concentration Threshold for Unacceptability (M):*** | 500 | (>m - 1000 cfg/g or cfu/ml) |
| **Target Probability of Rejection:** | 95 | (>0 - <100) |

Parameters which are specific to concentration-based sampling plans include *Acceptable Number of Concentrations within Limits*, *Concentration Threshold for Marginal Acceptability*, *Include Concentration Threshold for Unacceptability* and the *Concentration Threshold for Unacceptability*.

The lower bound for marginal acceptance is the *Concentration Threshold for Marginal Acceptability (m).* If the estimated concentration of organisms detected is less than *m* then a sample is deemed acceptable.

Samples having an estimated concentration of organisms above m are considered "marginally acceptable". The tolerable number of such samples from a lot is the *Acceptable Number of Concentrations within Limits (c)*. This is a 2-class plan.

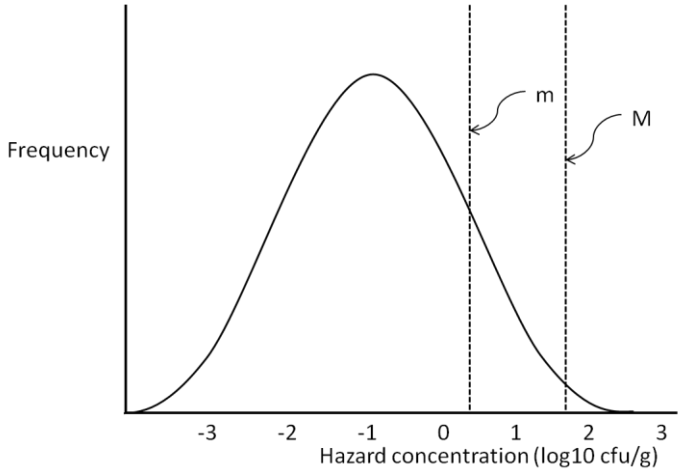



**Figure 2. A within-lot distribution subject to a three-class sampling plan. Lots containing samples with concentrations estimated to be 2 log10 cfu/g (i.e. above "M") would immediately be rejected.**

To indicate a 3-class plan (see Figure 2), the option *Include Threshold for Unacceptability* must be selected and the *Concentration Threshold for Unacceptability (M)* defined (permitted values up to 1000 cfu/g or per ml). This is the upper bound for the marginal acceptability of a sample and if the estimated concentration of organisms in any one sample is greater than *M*, then the entire lot is deemed to be

unacceptable (i.e., the result leads to "outright" rejection). Otherwise the lot is acceptable as long as the number of samples having estimated concentrations between m and M does not exceed "c", the *Acceptable Number of Concentrations within Limits*.

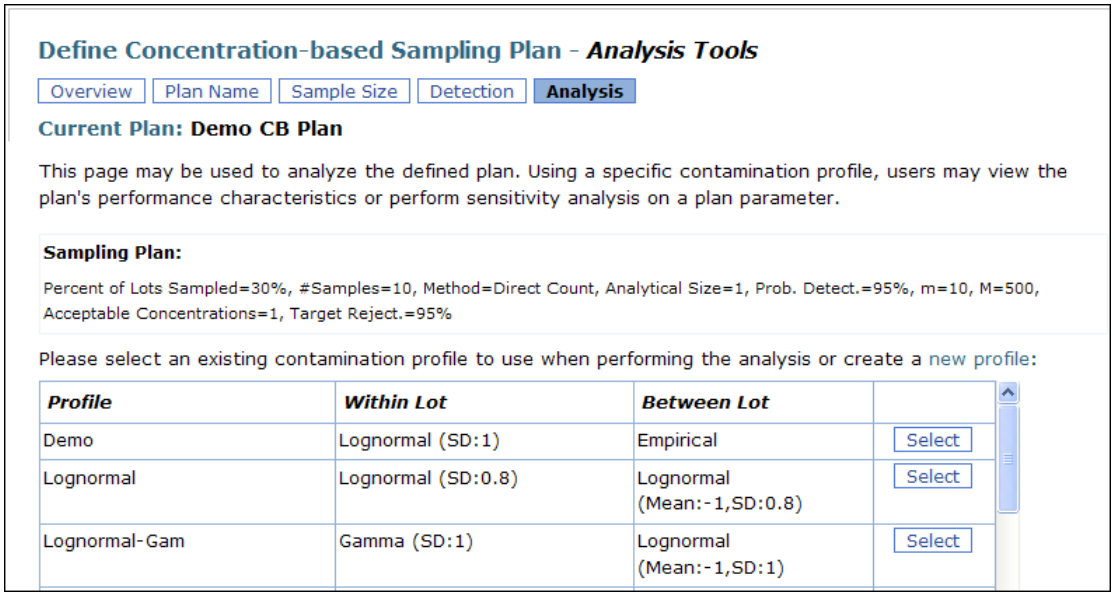
As discussed above for Presence/Absence plans, a sampling plan can be associated with a *Detectable Microbial Load* (DML), for a given probability of lot rejection and within-lot variability. When a *Target Probability of Rejection* is specified, the tool calculates the corresponding DML. This is the mean concentration in a lot that results in that specified probability of rejection, and the value obtained is shown in the PDF report and the Sensitivity Analysis results, under the heading "Detectable Microbial Load". Lots with mean $\log_{10}$ concentrations above this DML will be rejected at a higher probability than the target.

Clicking the "Next" button on this page takes the user to the "Analysis Tools" page, where the user can choose one or more Contamination Profiles to be subjected to the described sampling plan.

## Step 3: Analysis

### Choose Concentration Profile

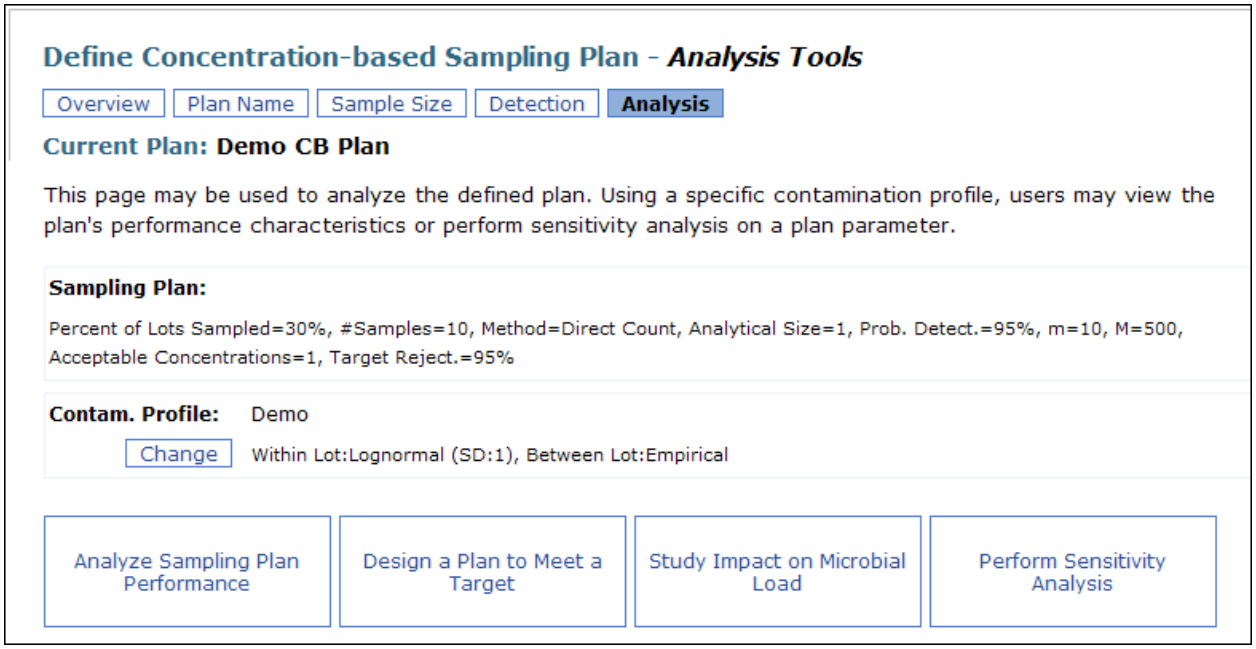
Once the sampling plan is fully defined, the user chooses one or more previously defined contamination profile(s) to pair with that sampling plan. This is done by clicking the "Analysis" button on the "Analysis Tools" page and then the "Select" button beside the desired contamination profile.




After making the selection, the user has a choice of four types of analyses. These are:

- Analyze Plan Performance: used to obtain an Operating Characteristic curve and view DML-$P_{accept}$ pairs
- Design a Plan to Meet a Target: used to find Sampling Plan characteristics that will produce a desired TDML at a specified $P_{reject}$

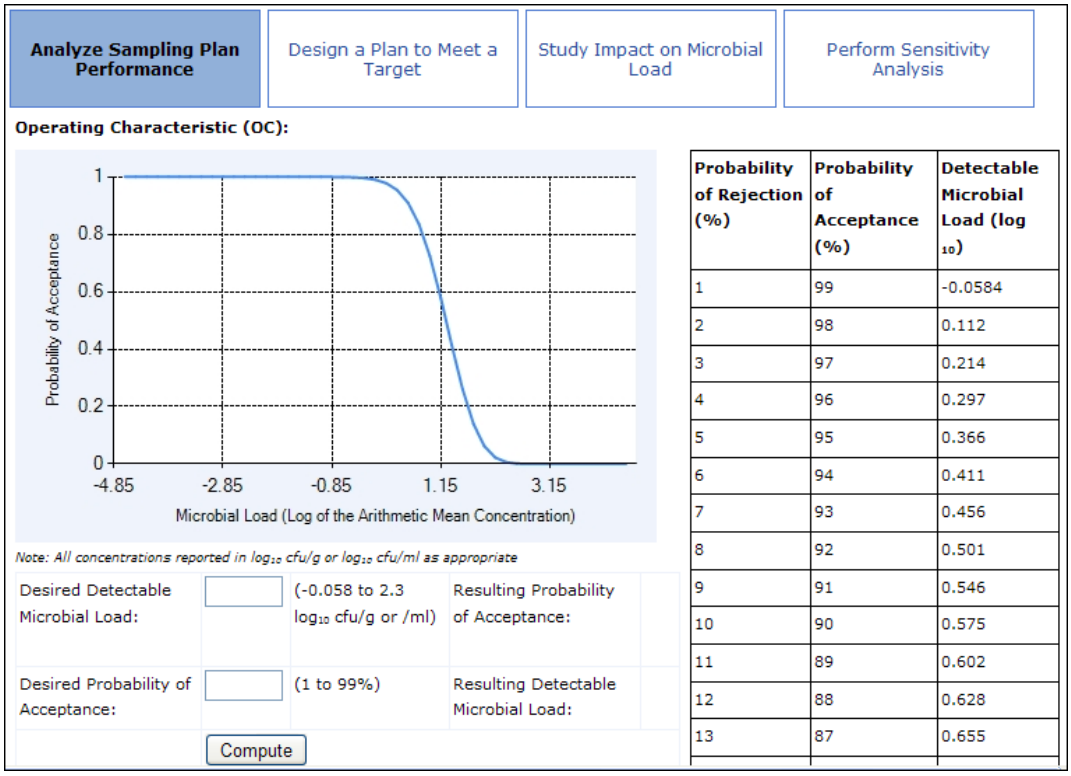
- Study Impact on Microbial Load: used to estimate the impact of the sampling plan on the microbial load in released product (i.e. the risk reduction)
- Perform Sensitivity Analysis: used to evaluate the effect of individual parameter values on the results




Selecting any of these will open a new page from which the appropriate model can be run. The tool will then calculate the results. Note that this process may take several minutes after which the results are displayed on the same page.

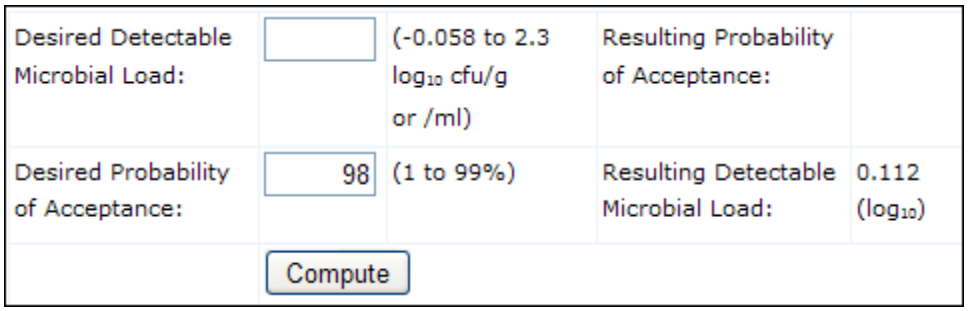
## Analysis Option A: Analyze Sampling Plan Performance

The "Analyze Sampling Plan Performance" button prompts the tool to produce an Operating Characteristic Curve (OC curve), given the within-lot distribution and the sampling plan imposed. Since the OC curve relates each mean (within-lot) hazard concentration to a specific probability of rejection, it can be used to find $P_{reject}$ for a particular mean concentration, or conversely, to find the value of a mean concentration that will be rejected at a given rate.




| Probability of Rejection (%) | Probability of Acceptance (%) | Detectable Microbial Load ($log_{10}$) |
|---|---|---|
| 1 | 99 | -0.0584 |
| 2 | 98 | 0.112 |
| 3 | 97 | 0.214 |
| 4 | 96 | 0.297 |
| 5 | 95 | 0.366 |
| 6 | 94 | 0.411 |
| 7 | 93 | 0.456 |
| 8 | 92 | 0.501 |
| 9 | 91 | 0.546 |
| 10 | 90 | 0.575 |
| 11 | 89 | 0.602 |
| 12 | 88 | 0.628 |
| 13 | 87 | 0.655 |

These values can either be read from the graph, obtained from the "look-up" table provided (for common percentiles), or obtained by specifying either the Desired Probability of Acceptance, or the Desired Detectable Microbial Load and prompting the tool to fill in the corresponding value.




In the example shown above, the user typed in "98" and clicked "Compute". The tool returned 0.112 $log_{10}$ cfu/g as the resulting Detectable Microbial Load. In other words, if a manufacturer needs an acceptance rate of at least 98% of lots under the sampling plan in question, then the mean microbial concentration across those lots needs to be lower than or equal to 0.112 $log_{10}$ cfu/g.

Conversely, if the manufacturer can attain a mean concentration across lots of 0.2 $log_{10}$ cfu/g without additional measures, this value can be entered in order to obtain the predicted rate of acceptance under the sampling plan in question, as shown below.

| | | | | |
|---|---|---|---|---|
| Desired Detectable Microbial Load: | 0.2 | (-0.058 to 2.3 $\log_{10}$ cfu/g or /ml) | Resulting Probability of Acceptance: | 97% |
| Desired Probability of Acceptance: | | (1 to 99%) | Resulting Detectable Microbial Load: | |
| | Compute | | | |

The manufacturer learns that a 97% probability of lot acceptance is expected when the mean concentration of hazard across lots is 0.2 $\log_{10}$ cfu/g.

**Background**

The *Operating Characteristic Curve* illustrates the Probability of Acceptance of *tested* lots as a function of the actual mean of the $\log_{10}$–scale concentrations, according to the specifications of the sampling plan. Note that the fraction of lots tested is not included in this characterization, as this calculation applies only to tested lots. Each mean concentration that is paired with a probability of acceptance (or rejection) is termed the "Detectable Microbial Load" (represented as the logarithm of the arithmetic mean) associated with that probability.

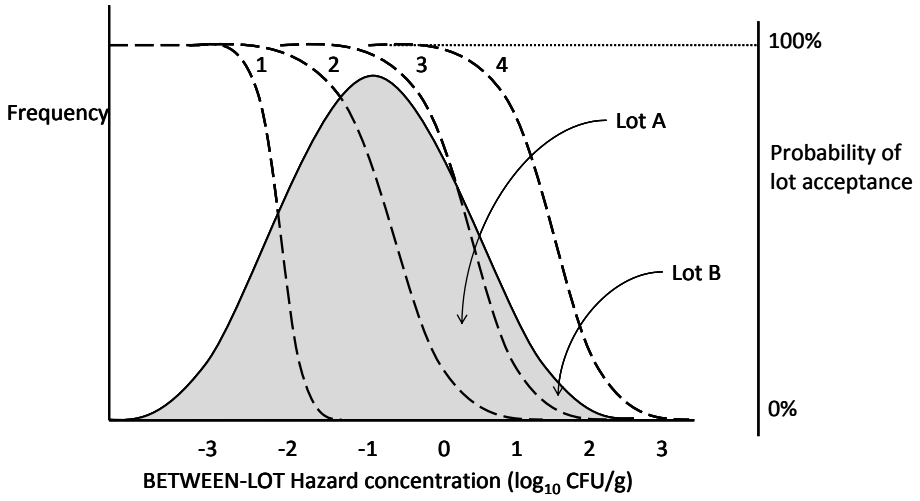



**Figure 3. Four different OC curves, representing four different sampling plans, are shown here. Lots from the distribution pictured will have a higher probability of acceptance under the sampling plan indicated by OC curve 4, than under the other 3 plans.**

In the OC curves pictured in Figure 3, lots having a mean hazard concentration of 0.2 log10 cfu/g (such as Lot A) have a higher probability of acceptance (and a lower probability of rejection) than lots with a mean concentration of 1.7 log10 cfu/g (such as Lot B). It is also true that Lot A has a higher probability of acceptance (approximately 60%) under the sampling plan represented by OC curve number 3 than under the plan represented by OC curve number 2 (approximately 5%)

### *Desired Detectable Microbial Load and Desired Probability of Acceptance*

Each OC curve consists of a series of points defined by hazard concentrations and their associated probability of acceptance as determined by the sampling plan. Therefore each probability of acceptance (or rejection) is associated with a particular "Detectable Microbial Load", and vice versa.

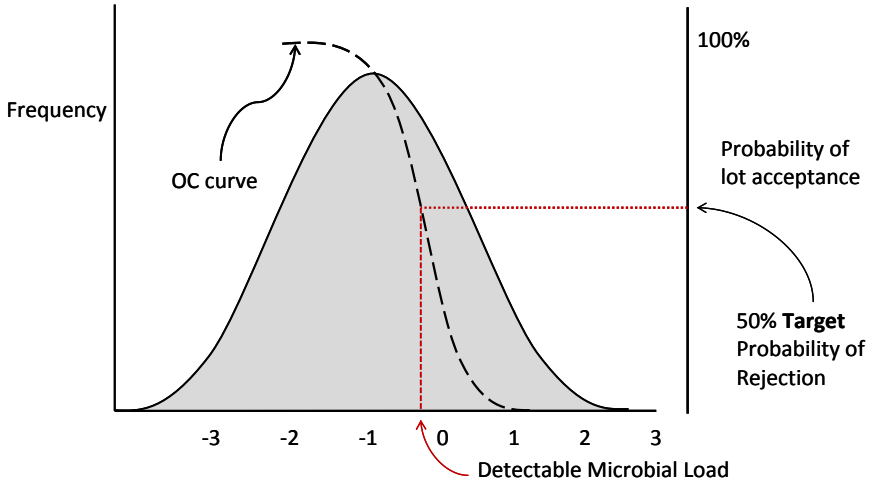



**Figure 4. The user can also specify a Desired Probability of Acceptance, and the tool will return the associated Detectable Microbial Load, under the specified sampling plan.**

For example in Figure 4, the user has specified 50% as the Target Probability of Acceptance, describing a scenario in which half of tested lots are rejected. From the OC curve, the result is provided that the Detectable Microbial Load associated with this rate of rejection is -0.2 $\log_{10}$ cfu/g.

## Analysis Option B: Design a Plan to Meet a Target

### Choosing Target Detectable Microbial Load and Desired Rejection Rate

The user may specify a *Target Detectable Microbial Load* (TDML) *and* an associated probability of rejection (*Desired Rejection Rate*). Again, the detectable microbial load is measured by the arithmetic mean on the $\log_{10}$ scale of the within-lot distribution.

### Background

When a TDML and a specified probability of rejection are held fixed, the OC curve must be altered to include that pair of values; in other words the sampling plan itself must be varied. Under this analysis option then, the user chooses which sampling plan characteristic to vary, and the tool calculates the value of this characteristic that will need to be imposed, given the other attributes of the sampling plan, to ensure that lots whose mean is at the TDML will be rejected at the desired probability (see Figure 5).

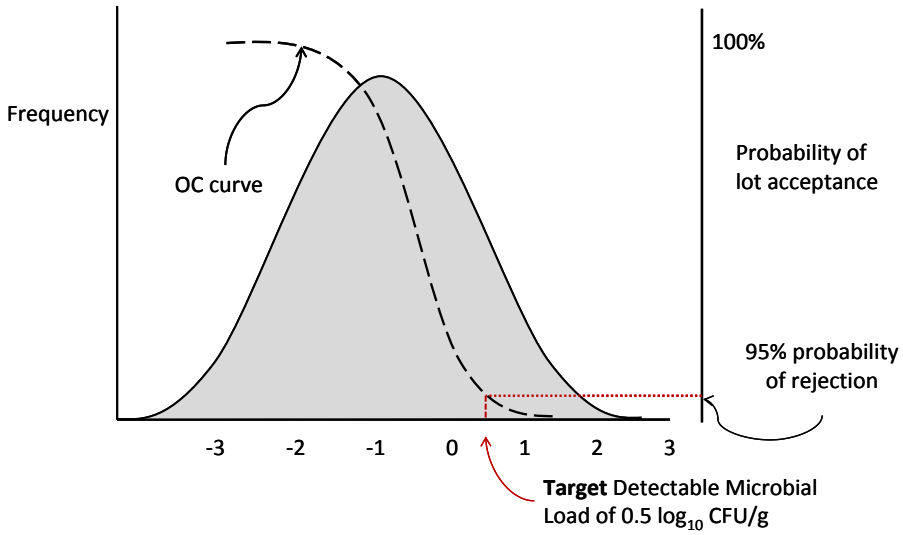
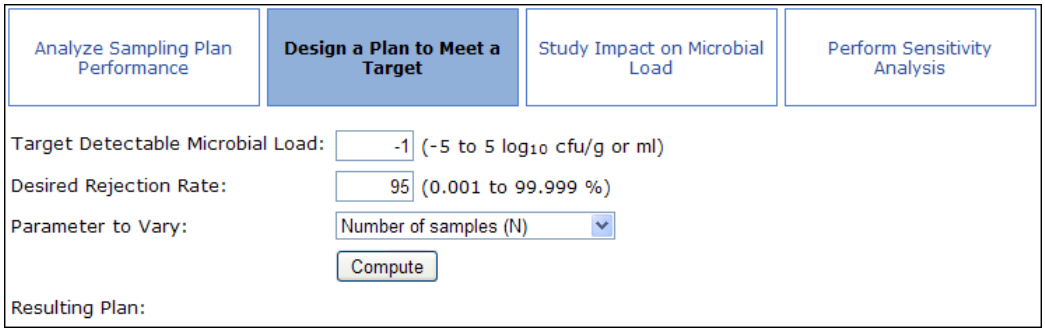


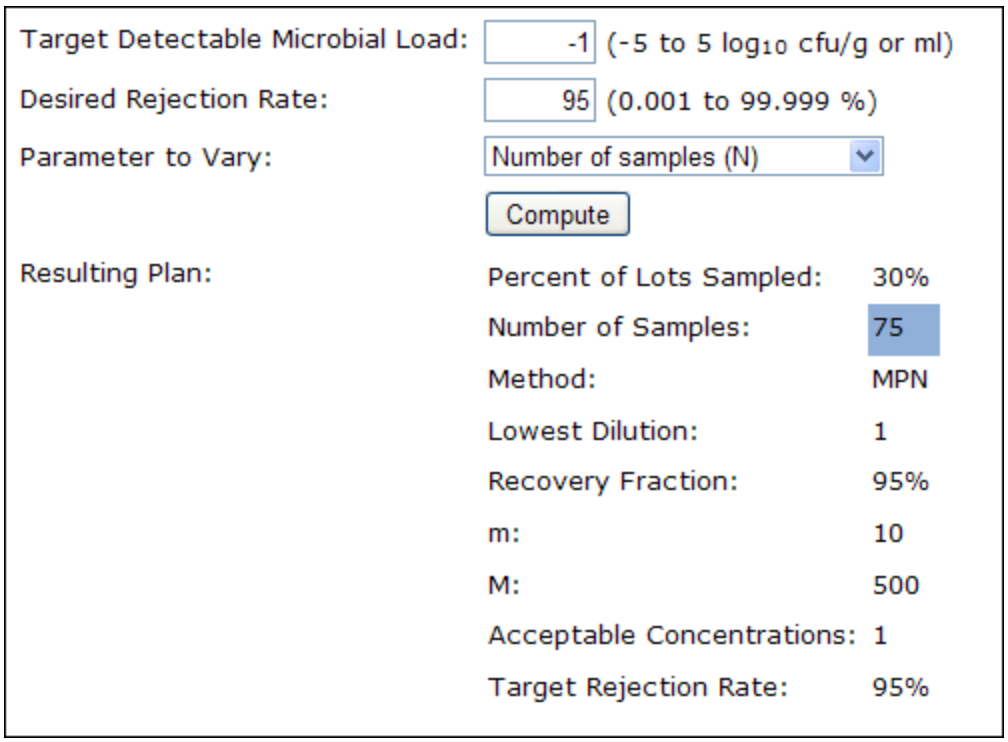

**Figure 5. A between-lot distribution and a TDML set at 0.5 $\log_{10}$ cfu/g with a probability of rejection of 95%. Under this sampling plan, 5% of lots having a mean concentration of 0.5 log10 cfu/g would be accepted.**

### Choosing a parameter to vary

The options available for the calculation of the sampling plan characteristic which meets the TDML with a desired $P_{reject}$ are different for presence-absence and concentration-based plans, and are offered in a drop-down menu. In the case of a presence-absence plan the user may choose to calculate one of: the *Number of Samples*, *Acceptable Positives* or *Analytical Sample Size* required to meet the TDML, while all other plan characteristics remain as they were defined in the plan. For a concentration-based plan the user may choose one of: *Number of Samples*, *Analytical Sample Size*, *Acceptable Number of Concentrations within Limits*, *Small-m (m)* or *Big-M (M).*




For example, having specified a TDML of -1 $\log_{10}$ cfu/g and a $P_{reject}$ of 95%, the user may request that the tool returns the required number of samples, 'N', given the other sampling plan characteristics previously defined. The tool will calculate 'c' to ensure that lots with a mean concentration of -1 $\log_{10}$ organisms per gram (1 organism per 10 grams), will be rejected 95% of the time.




## Analysis Option C: Study Impact on Microbial Load (in released product)

In this option the user can obtain measures of the impact of the sampling program on the load of the hazard in the product released to commerce. In other words, the risk reduction that results from the given sampling program can be estimated. This calculation is based in part on the percentage of all lots that undergo testing, specified by the user under "Sampling Plan".

Simply clicking on the button is sufficient; no further input is required.

**Background**

The Mean Concentration Pre-Sampling is the mean of the between-lot distribution, prior to rejection.

Given the OC curve that is associated with the specified sampling plan, the tool can calculate the Percentage of Tested Lots Rejected, and the Mean Concentration in (tested and) Accepted Product. Next, given the user-specified value for the intensity of sampling (i.e. the percentage of lots tested), the tool can calculate the Percentage of All Lots Rejected, and the Mean Concentration in Final Product (tested and untested). The latter is the post-sampling mean shown in Figure 7, below.

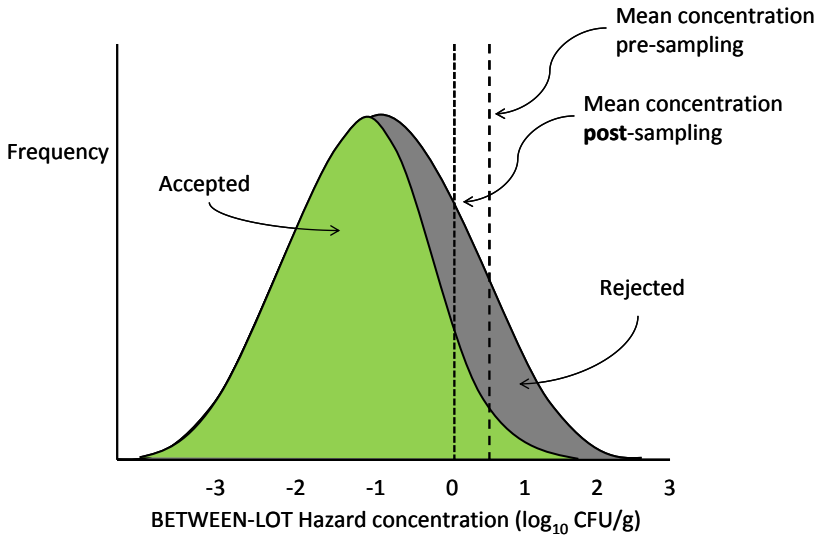


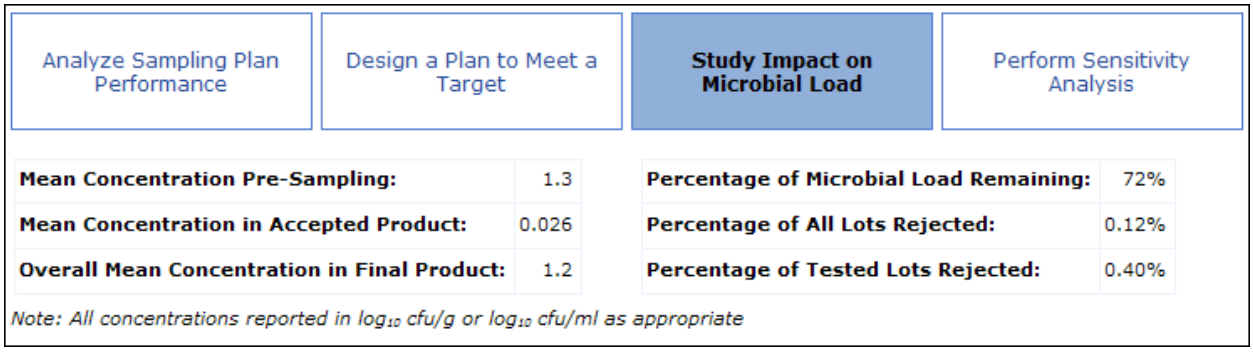

**Figure 6. The ratio of the post-sampling mean to the pre-sampling mean (in non-log units) reflects the proportion of the microbial load that remains among tested lots.**

The ratio of the post-sampling mean to the pre-sampling mean indicates the risk reduction that has occurred following the sampling program. For example a post-sampling mean of 0 compared to a pre-sampling mean of 0.5, indicates a ratio of 1 cfu/g to 3.16 cfu/g, or just under a third of microbial load remaining. This would be the risk reduction if the proportion of lots tested were 100%. In practice this risk reduction only applies to the percentage of lots that underwent testing. The proportion of microbial load remaining in the untested product is 100%.

With this information the Percentage of Microbial Load Remaining is obtained, according to the formula

Fraction untested x100% + Fraction tested x ($10^{\text{post-sampling mean}}/10^{\text{pre-sampling mean}}$)

Thus if the arithmetic mean concentration over all lots pre-sampling is -1, and the concentration post-sampling is -2, then the Proportion of Microbial Load Remaining after sampling would be 0.01/0.1 or 0.1 if all lots were tested, but if only 10% of lots were tested it would be 0.9(1) + 0.1(0.1) or 0.91.



## Analysis Option D: Perform Sensitivity Analysis:

Sensitivity Analysis allows the user to select a sampling plan parameter to vary, to define a range over which to vary that parameter, and see the following results:

- Detectable Microbial Load
- Mean Concentration Pre-sampling
- Mean Concentration in (tested and) Accepted Product
- Mean Concentration in Final Product
- Percentage of Microbial Load Remaining
- Percentage of All Lots Rejected
- Percentage of Tested Lots Rejected

Parameters which may be chosen for the sensitivity analysis are *Number of Samples*, *Acceptable Positives* or *Analytical Sample Size* in the case of presence-absence and *Number of Samples*, *Analytical Sample Size*, *Acceptable Number of Concentrations within Limits*, *Small-m* ("m") or *Big-M* ("M") in the case of concentration-based plans.

For the parameter chosen, define the minimum and maximum values to be considered as well as the number of steps to be taken over that range. For example, if a sensitivity analysis varying the number of samples over the range of 5 to 9 samples is desired, the minimum value is 5 and the maximum is 9 with 5 steps to be taken. You may choose whether to see all resulting OC curves on a single plot or individual OC curves on separate plots.

In the screenshots below, various parameters were chosen on which to perform a sensitivity analysis using a concentration-based plan: first "c" (the acceptable number of "marginally acceptable"), then "m", and finally "M". In each case the accompanying OC curve displays the impact on the probability of acceptance at different mean lot concentrations, and a table provides the DML for the specified Target Probability of Rejection. In addition, the table provides risk reduction values; these require that inputs for between-lot contamination have been specified.

Analyze Sampling Plan Performance | Design a Plan to Meet a Target | Study Impact on Microbial Load | **Perform Sensitivity Analysis**

**Parameter to Vary:** Acceptable number

Min: 1

Max: 5

# Steps: 5

☑ Include OC Charts

☐ Separate OC Charts

Run Analysis

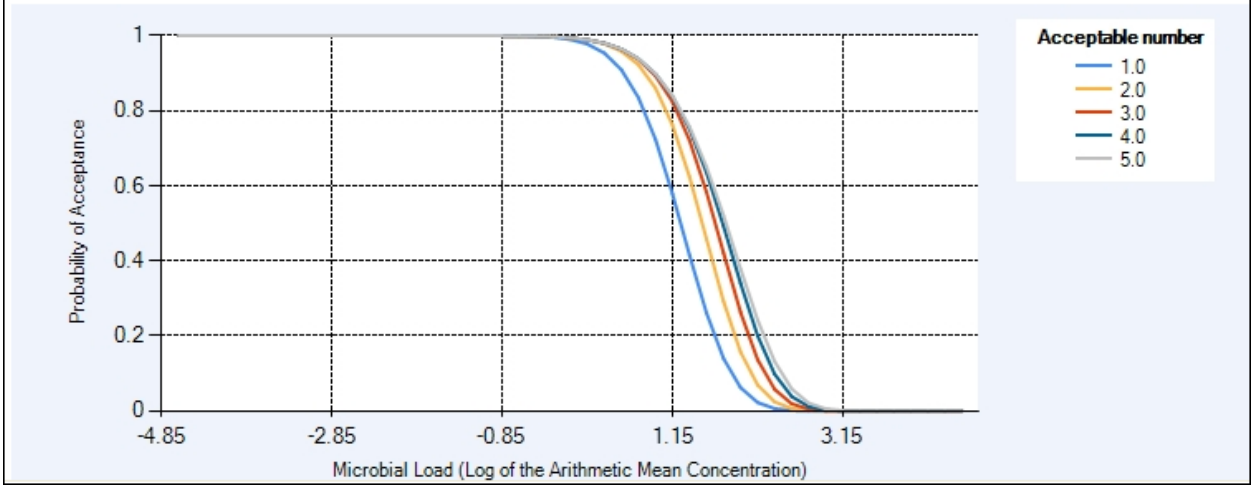
| Currently Defined Values | |
|---|---|
| Percentage of Lots Sampled: | 30% |
| Number of Samples: | 10 |
| Method: | MPN |
| Dilution Levels: | 3 |
| Diluation Trials: | 3 |
| Amout at Lowest Dilution: | 1 |
| Probability of Detection: | 95% |
| Acceptable Number: | 1 |
| Small m: | 10 |
| Big M: | 500 |

**Results**

| Acceptable number | Detectable Microbial Load | Mean Concentration | | | Percentage of | | |
|---|---|---|---|---|---|---|---|
| | | Pre-sampling | Accepted Product | Final Product | Microbial Load Remaining | All Lots Rejected | Tested Lots Rejected |
| 1.0 | 2.01 | 1.3 | 0.026 | 1.2 | 72% | 0.12% | 0.40% |
| 2.0 | 2.24 | 1.3 | 0.11 | 1.2 | 72% | 0.073% | 0.24% |
| 3.0 | 2.39 | 1.3 | 0.15 | 1.2 | 72% | 0.058% | 0.19% |
| 4.0 | 2.51 | 1.3 | 0.16 | 1.2 | 72% | 0.054% | 0.18% |
| 5.0 | 2.60 | 1.3 | 0.17 | 1.2 | 72% | 0.053% | 0.18% |

*Note: All concentrations reported in log cfu/g or log cfu/ml as appropriate*

**Operating Characteristic (OC):**



Analyze Sampling Plan Performance | Design a Plan to Meet a Target | Study Impact on Microbial Load | **Perform Sensitivity Analysis**

**Parameter to Vary:** Small m

Min: 10

Max: 100

# Steps: 5

☑ Include OC Charts

☐ Separate OC Charts

Run Analysis

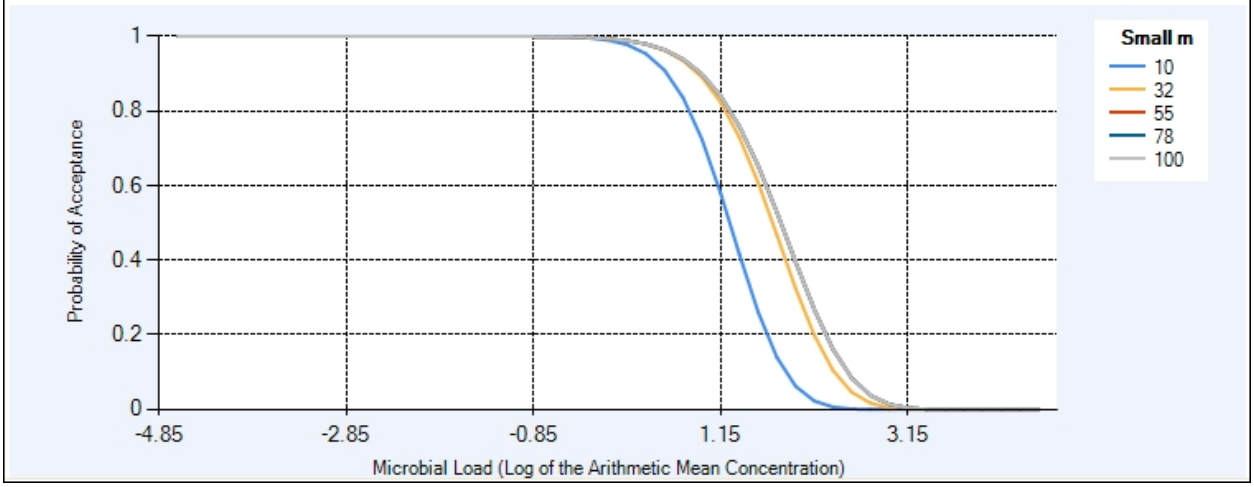
| Currently Defined Values | |
|---|---|
| Percentage of Lots Sampled: | 30% |
| Number of Samples: | 10 |
| Method: | MPN |
| Dilution Levels: | 3 |
| Diluation Trials: | 3 |
| Amout at Lowest Dilution: | 1 |
| Probability of Detection: | 95% |
| Acceptable Number: | 1 |
| Small m: | 10 |
| Big M: | 500 |

**Results**

| Small m | Detectable Microbial Load | Mean Concentration | | | Percentage of | | |
|---|---|---|---|---|---|---|---|
| | | Pre-sampling | Accepted Product | Final Product | Microbial Load Remaining | All Lots Rejected | Tested Lots Rejected |
| 10 | 2.01 | 1.3 | 0.026 | 1.2 | 72% | 0.12% | 0.40% |
| 32 | 2.54 | 1.3 | 0.16 | 1.2 | 72% | 0.057% | 0.19% |
| 55 | 2.70 | 1.3 | 0.17 | 1.2 | 72% | 0.053% | 0.18% |
| 78 | 2.70 | 1.3 | 0.17 | 1.2 | 72% | 0.053% | 0.18% |
| 100 | 2.70 | 1.3 | 0.17 | 1.2 | 72% | 0.053% | 0.18% |

*Note: All concentrations reported in log cfu/g or log cfu/ml as appropriate*

**Operating Characteristic (OC):**



Analyze Sampling Plan Performance | Design a Plan to Meet a Target | Study Impact on Microbial Load | **Perform Sensitivity Analysis**

**Parameter to Vary:** Big m

Min: 11

Max: 100

# Steps: 5

☑ Include OC Charts

☐ Separate OC Charts

Run Analysis

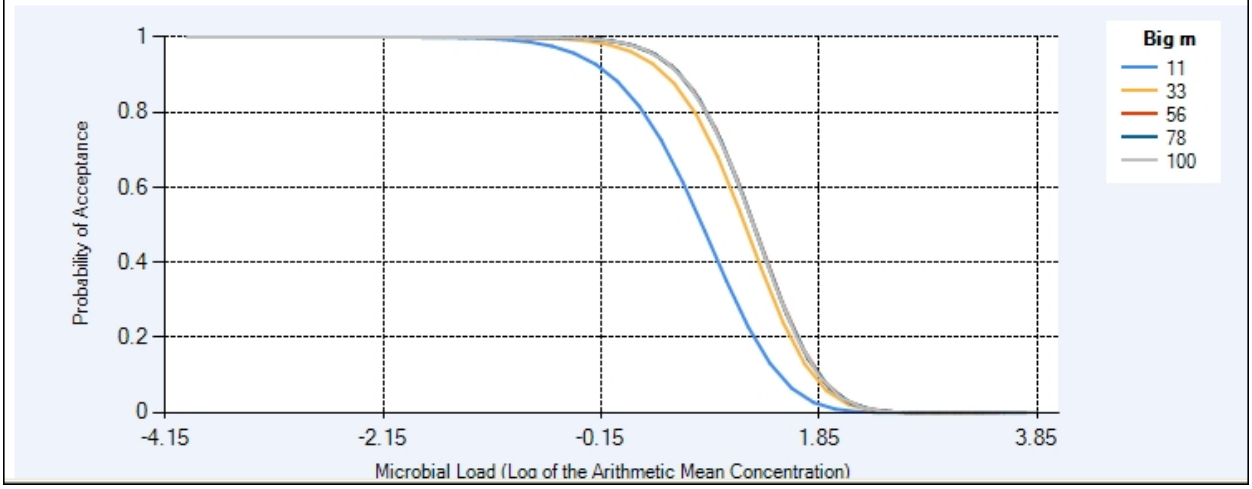
| Currently Defined Values | |
|---|---|
| Percentage of Lots Sampled: | 30% |
| Number of Samples: | 10 |
| Method: | MPN |
| Dilution Levels: | 3 |
| Diluation Trials: | 3 |
| Amout at Lowest Dilution: | 1 |
| Probability of Detection: | 95% |
| Acceptable Number: | 1 |
| Small m: | 10 |
| Big M: | 500 |

**Results**

| Big m | Detectable Microbial Load | Mean Concentration | | | Percentage of | | |
|---|---|---|---|---|---|---|---|
| | | Pre-sampling | Accepted Product | Final Product | Microbial Load Remaining | All Lots Rejected | Tested Lots Rejected |
| 11 | 1.68 | 1.3 | -0.16 | 1.2 | 71% | 0.31% | 1.0% |
| 33 | 1.96 | 1.3 | -0.0040 | 1.2 | 71% | 0.15% | 0.50% |
| 56 | 2.03 | 1.3 | 0.026 | 1.2 | 72% | 0.12% | 0.40% |
| 78 | 2.02 | 1.3 | 0.026 | 1.2 | 72% | 0.12% | 0.40% |
| 100 | 2.01 | 1.3 | 0.026 | 1.2 | 72% | 0.12% | 0.40% |

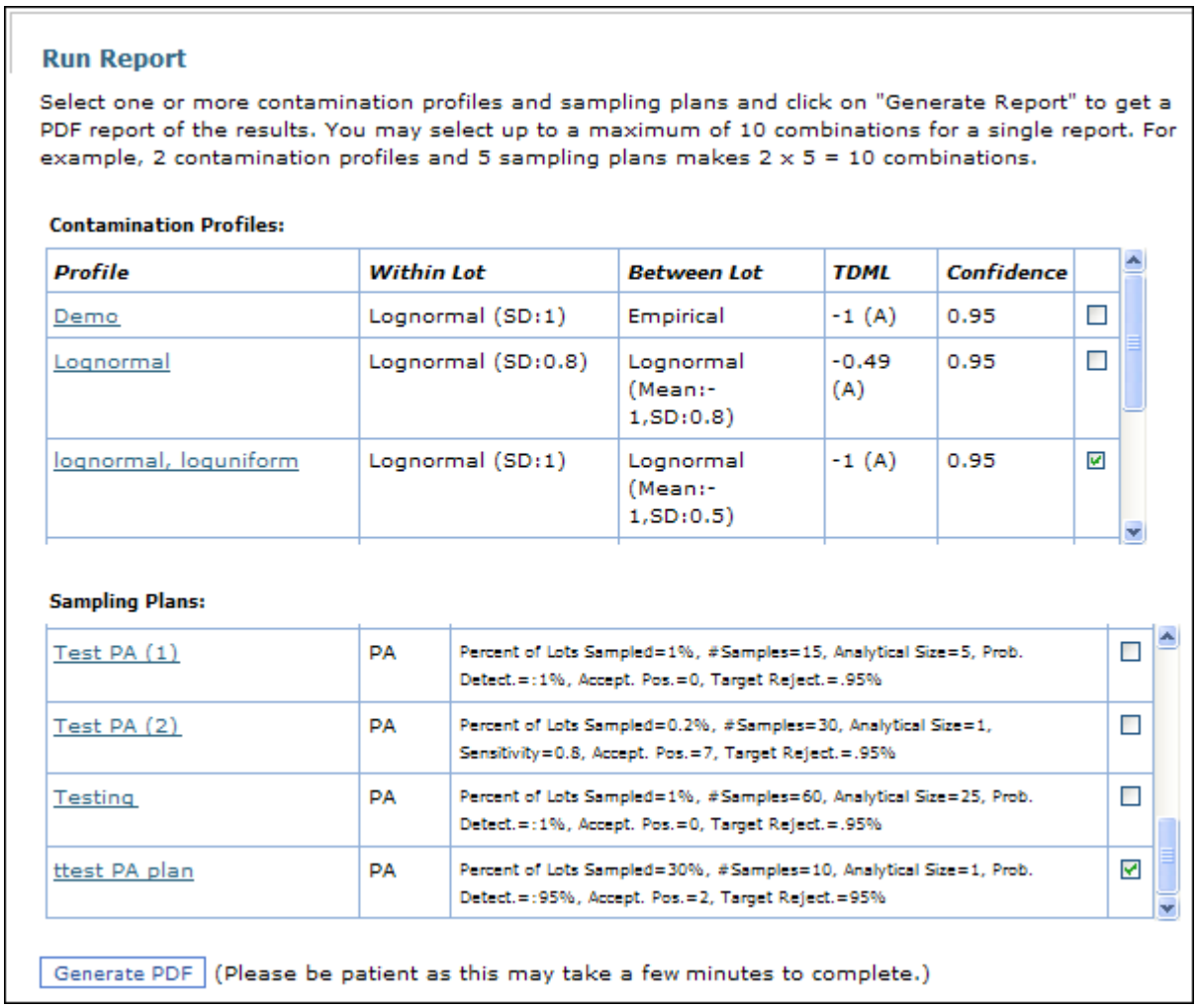
*Note: All concentrations reported in log cfu/g or log cfu/ml as appropriate*

**Operating Characteristic (OC):**



# Reports

The results may be viewed in an Adobe Acrobat (PDF) report. Simply select the "Reports" tab, choose the desired Contamination Profiles and Sampling Plans, and click "Generate PDF"; a PDF file will be generated that can be viewed, saved or printed. Up to ten (10) combinations of sampling plans and contamination profiles may be included in a report. Depending on how many combinations are chosen it may take several minutes to generate the PDF report.



**Run Report**

Select one or more contamination profiles and sampling plans and click on "Generate Report" to get a PDF report of the results. You may select up to a maximum of 10 combinations for a single report. For example, 2 contamination profiles and 5 sampling plans makes 2 x 5 = 10 combinations.

**Contamination Profiles:**

| *Profile* | *Within Lot* | *Between Lot* | *TDML* | *Confidence* | |
|---|---|---|---|---|---|
| Demo | Lognormal (SD:1) | Empirical | -1 (A) | 0.95 | ☐ |
| Lognormal | Lognormal (SD:0.8) | Lognormal (Mean:-1,SD:0.8) | -0.49 (A) | 0.95 | ☐ |
| lognormal, loguniform | Lognormal (SD:1) | Lognormal (Mean:-1,SD:0.5) | -1 (A) | 0.95 | ☑ |

**Sampling Plans:**

| | | | |
|---|---|---|---|
| Test PA (1) | PA | Percent of Lots Sampled=1%, #Samples=15, Analytical Size=5, Prob. Detect.=:1%, Accept. Pos.=0, Target Reject.=.95% | ☐ |
| Test PA (2) | PA | Percent of Lots Sampled=0.2%, #Samples=30, Analytical Size=1, Sensitivity=0.8, Accept. Pos.=7, Target Reject.=.95% | ☐ |
| Testing | PA | Percent of Lots Sampled=1%, #Samples=60, Analytical Size=25, Prob. Detect.=:1%, Accept. Pos.=0, Target Reject.=.95% | ☐ |
| ttest PA plan | PA | Percent of Lots Sampled=30%, #Samples=10, Analytical Size=1, Prob. Detect.=:95%, Accept. Pos.=2, Target Reject.=95% | ☑ |

Generate PDF (Please be patient as this may take a few minutes to complete.)

If between-lot contamination was not defined for the Contamination Profile selected, the associated results are shown as N/A and only Detectable Microbial Load (DML) can be shown. This is the log of the arithmetic mean hazard concentration within a lot that will result in the rejection of the lot at the rate specified by the user as the "Target Probability of Rejection".

If the Contamination Profile to which the Sampling Plan was applied did include inputs for between-lot contamination distribution, then the following results can be calculated: *Mean Concentration Pre-*

*Sampling, Mean Concentration in Accepted Product, Mean Concentration in Final Product, Percentage of Microbial Load Remaining, Percentage of All Lots Rejected,* and *Percentage of Tested Lots Rejected.*

**Results:**

| Sampling Plan | Contamination Profile | Type | Detectable Microbial Load | Mean Concentration | | | Percentage of | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Pre-sampling | Accepted Product | Final Product | Microbial Load Remaining | All Lots Rejected | Tested Lots Rejected |
| Plan 1 | Profile 1 | PA | 0.98 | -0.71 | -0.78 | -0.73 | 96% | 1.4% | 4.6% |

*Note: All concentrations reported in log cfu/g or log10 cfu/ml as appropriate.*

From the glossary, these values are defined as:

- *Mean Concentration Pre-Sampling:* The arithmetic mean concentration (in $\log_{10}$ cfu/gram) in the product prior to sampling.
- *Mean Concentration in Accepted Product:* The arithmetic mean concentration (in $\log_{10}$ cfu/gram) across the lots of tested product that are accepted under the sampling plan.
- *Mean Concentration in Final Product:* The arithmetic mean concentration (in $\log_{10}$ cfu/gram) across the lots of product that are accepted (including untested lots) following implementation of the sampling plan.
- *Percentage of Tested Lots Rejected:* The proportion of the lots which are tested that fail to pass the criteria of the sampling plan.
- *Percentage of All Lots Rejected:* The proportion of lots that fail to pass the criteria set out by the sampling plan, and are assumed to be removed from commerce.
- *Percentage of Microbial Load Remaining:* The effectiveness of a sampling plan is presented here in terms of a proportion of contamination remaining. This is determined based on the fraction of contamination prevented from entering commercial distribution and is obtained from the ratio of the arithmetic mean concentration of organisms before and after sampling (among sampled lots), and the proportion of lots sampled. Note that it assumes that risk reduction is proportional to the arithmetic mean of the hazard concentration in the product.

# Range Restrictions on Variable Inputs

| Category | Variable Name | Range Permitted | Units |
|---|---|---|---|
| Contamination Profile: Within-lot | SD (lognormal) | 0.01 to 2 | $\log_{10}$ cfu/g |
| | log range (uniform) | 0.1 to 3 | $\log_{10}$ cfu/g |
| | log range (triangular) | 0.1 to 3 | $\log_{10}$ cfu/g |
| | SD (gamma) | 0.01 to 3 | $\log_{10}$ cfu/g |
| Contamination Profile: Between-lot | mean (lognormal) | -4 to 4 | $\log_{10}$ cfu/g |
| | SD (lognormal) | 0.01 to 2 | $\log_{10}$ cfu/g |
| | min (uniform) | -5 to 5 | $\log_{10}$ cfu/g |
| | max (uniform) | -5 to 5 | $\log_{10}$ cfu/g |
| | mean (triangular) | -5 to 5 | $\log_{10}$ cfu/g |
| | mode (triangular) | -5 to 5 | $\log_{10}$ cfu/g |
| | max (triangular) | -5 to 5 | $\log_{10}$ cfu/g |
| | concentration (cumulative) | -5 to 5 | $\log_{10}$ cfu/g |
| | probability (cumulative) | >0 and <1 | |
| Sampling Plans: Presence/Absence | Percent of lots sampled | >0 to 100 | % |
| | Number of Samples | 1 to 100 | |
| | Collected Sample size | 1 or more | g or ml |
| | Analytical Sample size | 0.01 to 50 | g or ml |
| | Probability of Detection | >0 to 100 | % |
| | Test Sensitivity | >0 to 1 | |
| | Count in Test Sample (for Test Sensitivity) | 0 to 6 | $\log_{10}$ cfu |
| | Acceptable Number of Positive Samples | 0 to n-1 | |
| | Target Probability of Rejection | >0 to <100 | % |
| Sampling Plans: Concentration-based | Percent of lots sampled | >0 to 100 | % |
| | Number of Samples | 1 to 100 | |
| | Collected Sample size | 1 or more | g or ml |
| | Analytical Sample size (for Direct Counting) | 0.01 to 50 | g or ml |
| | Number of Dilution Levels (for MPN) | 2 to 5 | |
| | Number of Trials per Dilution Level (for MPN) | 2 to 10 | |
| | Amount of Sample in the Lowest Dilution Level (for MPN) | 0.01 to 10 | g or ml |
| | Analytical Recovery Fraction | >0 to 100 | % |
| | Test Sensitivity | >0 to 1 | |
| | Count in Test Sample | 0 to 6 | $\log_{10}$ cfu |
| | Acceptable Number of Concentrations Within Limits ( c) | 0 to n-1 | |
| | Concentration threshold for Marginal Acceptability (m) | 0.01 to 1000 | cfu/g or /ml |
| | Concentration Threshold for Unacceptability (M) | >m to 1000 | cfu/g or /ml |
| | Target Probability of Rejection | >0 to <100 | % |

# Glossary

***Acceptable Positives:*** (Denoted "c"). For presence/absence plans, the maximum number of positive samples above which the lot is deemed unacceptable.

***Acceptable Number of Concentrations Within Limits*:** (Denoted "c"). For concentration-based plans, the maximum number of "marginally acceptable" sample results, above which the lot is deemed unacceptable. A marginally acceptable sample is defined as having a concentration higher than little-m in two-class plans, or between little-m and big-M in three-class plans.

***Analytical Recovery Fraction:*** The fraction of organisms present which are expected to be successfully counted. Less than 100% detection is expected due to the inability to detect or count some percentage of the organisms.

***'Big - M':*** (Denoted "*M'*') The concentration of hazard in a food sample above which the lot will be classified as automatically unacceptable in a three-class concentration-based sampling plan. It is also referred to here as the "Threshold of Unacceptability."

***'Small - m'*:** (Denoted "m"). The concentration of hazard in a food sample, above which a sample result is considered "marginally acceptable." A specified number of marginally acceptable samples (c) will be permitted in an accepted lot, as long as no sample concentration exceeds M, when a value for M has been specified. See: "Acceptable Number of Concentrations Within Limits".

***Detectable Microbial Load (DML):*** The DML is an arithmetic mean of the concentration distribution of hazard in a food lot, expressed in the $\log_{10}$-scale. When a Target Probability of Rejection is specified by the user for a particular sampling plan, the mean concentration that is rejected with that probability is termed the Detectable Microbial Load. Alternatively, a "Desired Detectable Microbial Load" can be specified during Analysis, in order to obtain the corresponding Probability of Rejection at that concentration, given the sampling plan.

***$Log_{10}$ Range:*** The magnitude of the range (in $\log_{10}$ cfu/g) that encompasses the within-lot distribution of concentrations.

***Lot Rejection Rate:*** The Lot Rejection rate is the probability that a lot will be rejected because of the sampling plan. This is therefore indicative of the amount of product that would be removed as a result of the sampling plan, assuming that lots failing to meet the microbiological criteria are removed.

***Mean $Log_{10}$ Concentration:*** The mean, or average, of the distribution of concentrations on the $\log_{10}$ scale that occurs between different lots. Note that the log-transformation is performed before the average is calculated. This is not the same as the logarithm of the arithmetic mean concentration.

***Maximum $Log_{10}$ Concentration:*** The maximum of the distribution of mean concentrations (in $\log_{10}$ cfu/g) among different lots.

***Minimum $Log_{10}$ Concentration:*** The minimum of the distribution of concentrations (in cfu/g) on the $\log_{10}$ scale that occurs between different lots.

***Mode $Log_{10}$ Concentration:*** The modal, or most likely, of the distribution of concentrations (in cfu/g) on the $\log_{10}$ scale that occurs between different lots.

***Number of Samples:*** (Denoted 'n'). The number of samples from a single lot that are tested.

***Operating Characteristic Curve ("OC" curve):*** The relationship between Detectable Microbial Load and probability of rejection, where DML is on the $\log_{10}$ scale. The shape of the OC curve is determined by the sampling plan and the within-lot distribution of hazard concentration.

***Percent of Lots Sampled:*** The percent of all lots destined for market that undergo sampling, and thus are liable to be withdrawn from commerce.

***Probability of Detection:*** The probability that a test is able to detect a single organism.

***Collected Sample Size:*** The size, in grams or ml, of each of the samples from a single lot.

***Analytical Sample Size:*** The size, in grams or ml, of the sample which is actually used in the test.

***Number of Dilution Levels:*** The number of dilutions considered for the Most Probable Number (MPN) method . Typically 3 dilutions are used in calculating the MPN. In practice, if more than 3 dilutions levels are used, the 3 highest dilutions are considered for the MPN calculation with the understanding that the true MPN is greater than the result.

***Number of Trials per Dilution Level***: In the MPN calculation method, multiple trials are performed at each level of dilution. Typically 3, 5, or 10 trials are used at each level. The same number of trials is used for each dilution.

***Amount of Sample in the Lowest Dilution Level:*** For the MPN method, the size of the collected sample which is placed in the lowest dilution in each of the trials.

***Sample Characteristic:*** A chosen characteristic of the sampling plan whose value is adjusted to ensure that lots having a hazard concentration at the Target Detectable Microbial Load will be rejected with the indicated probability. This is part of the "Design a Plan to Meet a Target" type of analysis.

***Standard Deviation Between Lots:*** The log-transformed standard deviation of the mean concentration measured among individual lots of product.

***Standard Deviation Within a Lot:*** The standard deviation of concentration (in $\log_{10}$ cfu/gram) about the mean concentration within a single lot of product.

***Target Probability of Rejection:*** The target probability of rejection is a value specified by the user in conjunction with a sampling plan. The tool calculates the Detectable Microbial Load that will be rejected at the Target Probability of Rejection, under that plan.

***Risk Reduction from Sampling:*** The results of the sampling program are presented in terms of the following statistics:

- *Mean Concentration Pre-Sampling:* The arithmetic mean concentration (in $\log_{10}$ cfu/gram) in the product prior to sampling.
- *Mean Concentration in Accepted Product:* The arithmetic mean concentration (in $\log_{10}$ cfu/gram) across the lots of tested product that are accepted under the sampling plan.
- *Mean Concentration in Final Product:* The arithmetic mean concentration (in $\log_{10}$ cfu/gram) across the lots of product that are accepted (including untested lots) following implementation of the sampling plan.
- *Percentage of Tested Lots Rejected:* The proportion of the lots which are tested that fail to pass the criteria of the sampling plan.
- *Percentage of All Lots Rejected:* The proportion of lots that fail to pass the criteria set out by the sampling plan, and are assumed to be removed from commerce.
- *Percentage of Microbial Load Remaining:* The effectiveness of a sampling plan is presented here in terms of a proportion of contamination remaining. This is determined based on the fraction of contamination prevented from entering commercial distribution and is obtained from the ratio of the arithmetic mean concentration of organisms before and after sampling (among sampled lots), and the proportion of lots sampled. Note that it assumes that risk reduction is proportional to the arithmetic mean of the hazard concentration in the product.