Workshop on:

Microbiological Sampling and Testing in Food Safety Management

"Securing Global Food Safety"

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the australian institute of food science and technology incorporated

International Commission on Microbiological Specifications for Foods (ICMSF)

International Association for Food Protection.

Role of microbiological criteria and value of sampling: 2

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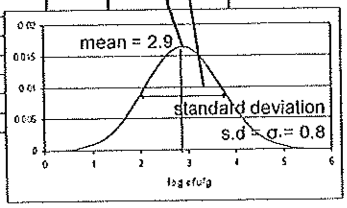
Distributions in excel

log cfu/g	P				
0	2.33E-05	=NORMDIST([log_cfu/g],2.9,0.8,0)			
0.2	5.59E-05				
0.4	1.26E-04				
0.6	2.67E-04				
0.8	5.30E-04				
1	9.90E-04				
1.2	1.74E-03				
etc.	etc.				



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What are the numbers that we see ?

- Draw 1 card from each stack
- Which number is higher: blue or pink ?

Which color has the HIGHEST number ?

Blue higher	Pink higher

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Averages

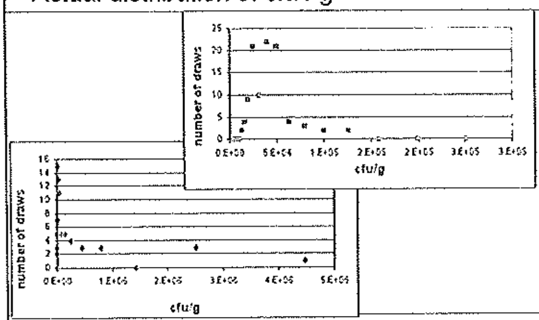
Blue higher	Pink higher

Blue:
$\bar{x}$ = 139,932

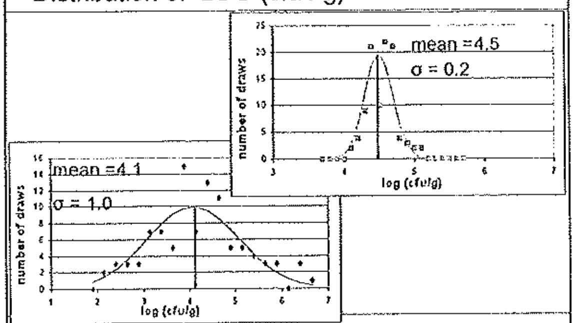
Pink:
$\bar{x}$ = 35,035

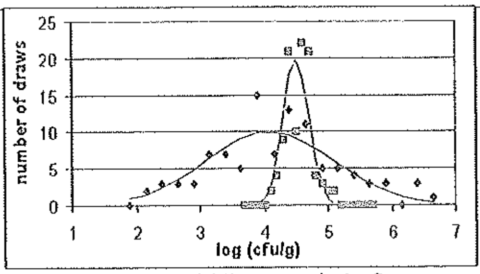
- Contradiction ?

Actual distribution of cfu / g



Distribution of LOG (cfu / g)





Where could they apply to ?

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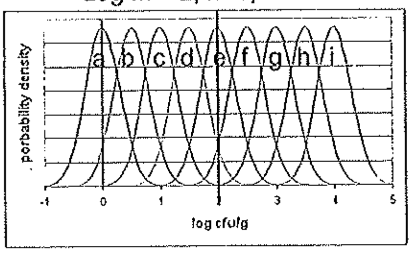
Batches to accept or reject (2 class)

Log m = 2, n=1, σ=0.3



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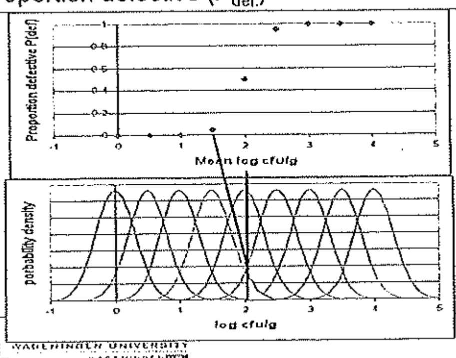
Proportion defective ($P_{def.}$)



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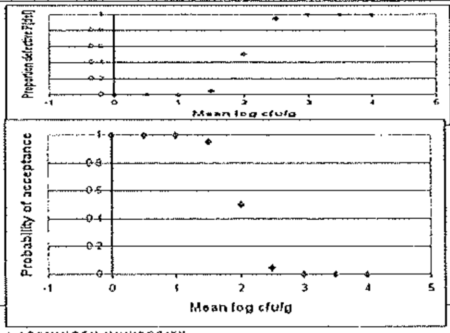
Probability of acceptance ($P_{accept.} = 1\text{-}P_{defect.}$)



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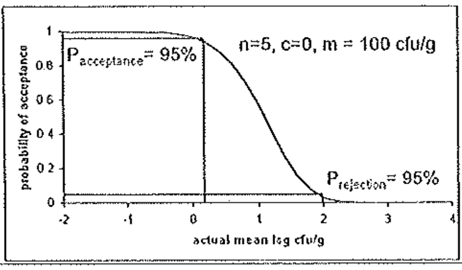
Probability of acceptance by mean log cfu

σ = 0.8



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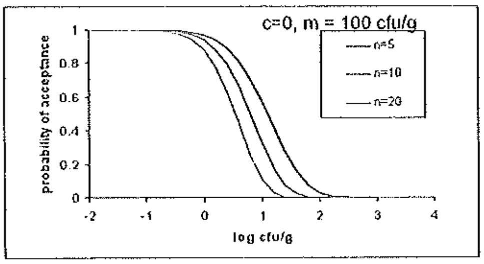
Probability of acceptance



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Microbiological sampling plans: a tool to explore ICMSF recommendations

Two-class sampling plans ("2class enrichment" and "2class counts")

The sheet has several components containing graphs, input/output fields, and calculation results. For the normal user only the graphs and input/output fields shown in lines 1 to 33 and columns A to U should be of interest.

Input fields are shown in yellow. In the following text information that can be entered in such fields is written in italics.

Graphs for two-class sampling plans (from left to right):

Plot 1 – Common type of operating characteristic (OC) curve relating probability of accepting a lot to the proportion defective it contains.

Acceptance probabilities are calculated for given number of sampling units that are examined, *n*, given microbiological limit specified by the sampling plan, *m*, and given maximum number of sampling units that are allowed to exceed the limit, *c*.

Plot 2 – Normal frequency distribution assumed for Log-transformed colony count numbers per gram to be found in sampling units drawn randomly from a lot characterized by given *mean* Log count per gram and given standard deviation *sigma*.

The pink vertical line indicates the microbiological limit, *m*, specified in the sampling plan. Right to *m* the area under the curve corresponds to the proportion defective the lot contains.

Plot 3 – Using these proportions defective and the given sampling plan specifications for *n* and *c* corresponding probabilities of lot acceptance are calculated. Results are plotted (black) to show the OC curve in relation to mean Log counts per gram.

Input fields for two-class sampling plans:

Yellow fields in the center –

- Lot characteristics: *mean* Log count per gram and standard deviation *sigma*, shown in Plot 2.
- Sampling plan specifications: the microbiological limit, *m*, (and for enrichments the sample amount), the number of sampling units, *n*, and the number of sampling units that are allowed to exceed the limit, *c*.

To change any of these values go to that field, type in a new value, and press *Enter*. Calculation results, like *P(accept)*, and graphs will be changed accordingly.

- The desired acceptance probability *P(accept)*.

The desired *P(accept)* can be changed for example to 5%, and by clicking the upper grey box the mean Log count per gram accepted with this new probability *P(accept)* is calculated and graphs are changed accordingly.

Yellow field on the left –

To calculate the acceptance probability P(accept) for a specific proportion defective type in this proportion as Pd and press *Enter*. The result will be shown in the next field to the right.

For this calculation only the given values for *n* and *c* are needed (see Plot 1).

To calculate the acceptance probability P(accept) for a specific combination of proportion defective, i.e. exceeding *M*, and proportion marginally defective, i.e. between *m* and *M*, type in these proportion as *Pd* and *Pm*, respectively, and press *Enter*. The result will be shown in the next field to the right.

For this calculation only the given values for *n* and *c* are needed (see Plot 1).

Yellow fields on the right –

Only sampling plan specifications (*n* and *m*) can be manipulated here, lot characteristics remain unchanged. When new values for *n* and/or *c* are entered the corresponding acceptance probability *P(accept)* can be calculated using the microbiological limits *m* and *M* as given. When the grey box on the right side is clicked a new value for *m* is derived that yields the same combination of *mean* Log count per gram and lot acceptance probability *P(accept)* that is achieved by the sampling plan described in the center. The microbiological limit *M* is left unchanged (as long as *M*>*m*).

Furthermore the upper percentile of the concentration distribution can be determined (implied acceptance level).

EXPLANATION OF THE SAMPLING PLAN SPREADSHEET

The **performance** of a sampling plan describes the lowest level of an organism that will be detected with a particular plan with a particular certainty. E.g. one can state that one wants to be 95% sure that a faulty lot is rejected. One has to make an assumption about the distribution of organisms in the samples; especially the standard deviation.

Example 1: Assessing the performance of a sampling plan with microbial counts.

To assess the performance of a sampling plan with m=2 $\log_{10}$ cfu/g and n=10 samples, for batches with a standard deviation *sigma* of 0.8 $\log_{10}$ cfu/g:

- Select the worksheet "2 class counts" (by clicking on the appropriate tab at the bottom of the workbook)
- Set cell J20, *sigma*, the known or assumed standard deviation of counts in the lot) at 0.8 $\log_{10}$ cfu/g
- Set J21 (m, the microbiological limit in $\log_{10}$ cfu/g) at 2
- Set J22 (n, the number of samples to be tested) at 10
- Set J23 (c, the number of samples permitted to test positive) to 0
- Set M20 (the desired acceptance level) at 5%
- Press the button "Find mean that gives desired P(accept)". Now cell J19 will change to 1.48, with cell M19 and M20 displaying 5%.

Interpretation of Example 1

This can be interpreted as follows: a batch with a mean log concentration of more than 1.48 $\log_{10}$ cfu/g and with a standard deviation *sigma* of 0.8 $\log_{10}$ cfu/g will be accepted with <5% probability, and hence rejected with >95% probability, if the microbiological limit m is 2 logs in each of n=10 samples.

Example 2: Assessing the performance of a sampling plan based on testing by enrichment.

To assess the performance of a sampling plan with m=absence in 25 g and n=10 samples, for batches with a standard deviation (*sigma*) of 0.8:

- Select the worksheet "2 class enrichment"
- Set cell J20 (*sigma*, the standard deviation) at 0.8
- Set J22 (n, the number of samples) to 10

- Set J23 (c, the number of samples permitted to test positive) to 0
- Set J25 (the sample weight, influencing the microbiological limit m) to 25 g
- Set M20 (desired acceptance level) to 5%
- Press the button "Find mean that gives...." and cell J19 will report "-2.25", with cell M19 and M20 equal to 5.0%.

Interpretation of Example 2

This can be interpreted as follows: a batch with mean log concentration greater than -2.25 with a standard deviation of 0.8 logs, will be accepted with <5% probability, and hence rejected with >95% probability, if the microbiological limit is absence in 25 g, based on 10 samples. It is clear that this sampling plan will detect batches with a much lower concentration than the sampling plan in Example 1, since, in this case the criterion, m, was 100 cfu/g.

Other examples

We could also use the spreadsheet tool to ***design*** a sampling plan that detects batches with a mean log concentration greater than -2.5, i.e. 1 cell per 316g. For this, use the setup from Example 2, and set cell J19 to -2.5, and press the button "Find n that gives...".

You will see that 15 samples ('n') are needed (cell J22). The probability that this batch will be accepted is 4.6 %, just below 5.0% or 0.05, and is not exactly 5.0% since n can only be an integer (i.e., it is not possible to assess a fraction of a sample unit). With 14 samples tested the probability is just above 5.0% (5.7%), so n=15 is the minimal number of samples required to obtain a probability of acceptance *below* 5.0%.

We could also calculate what the value of m should be (or, equivalently, the sample size) to have an equivalent plan using 30 samples. To calculate this, continue with the setup from Example 2, move to the right-hand box, enter 30 in cell Q22, press the button "For any value of ...."). This will result in a plan with 30 samples (Q22) of 9.6 g (Q25) that will have the same performance as a plan with 15 (J22) samples of 25 g (J25), that is, either of the two plans will reject batches with the same mean log concentration detected and the same probability of acceptance.

Graphical outputs

Now consider the graphs at the top of sheet "2 class enrich". The **left graph** shows the Operating Characteristic (OC) curve as a function of the proportion of the samples in the batch that are defective. That is, the graph shows the probability of detection, and therefore also rejection, of a lot for a given sampling plan defined by *n* and *c*, given the actual frequency of contaminated units (horizontal axis).

This curve only depends on the *n* and *c* values and defines the probability that the batch will be accepted, given the number of samples and the permitted number *c* of samples that test positive. Set *n*=1 (cell J22) and observe that the OC curve is a straight line. If one sample is taken and the proportion defective is 20% or 0.2, the probability that the batch will be rejected is 20% or 0.2, and the probability that it will be accepted is 1.0-0.2 = 0.8, that is, 80%. If we now set *n* to 2, the curve moves left and the probability of acceptance of 20% or 0.2 of units defective will now be 64% or 0.64 (i.e., 0.8*0.8, because both samples need to be negative for a batch to be accepted). For *n*=10, the curves moves even further to the left (set *n*=10 in cell J22).

Now look at the **middle graph** ("Probability density function …"). This curve shows the probability of certain concentrations being encountered given the mean and standard deviation of microbial levels. Press the button "Find mean that gives…". The graph will change and the graph presented will represent the distribution (for standard deviation *sigma* = 0.8 of $\log_{10}$ counts) that is just accepted with 5% probability with these 10 samples. In this case, the probability that one sample is below the *m* value is 74%, i.e., the area under the curve to the left of the vertical red line, and the probability that one sample is above *m* is 26%. This is the proportion defective, shown as "actualPd" in the left-hand box, and this is the area under the curve to the right of the vertical red line. For all 10 samples to be below the *m* value, the probability is $(0.74)^{10}$ which is equal to 0.05, or 5%. Now set *n* equal to 1 (cell J22) and press the button "Find mean that gives…". Now the distribution moves to the right, and shows that a batch is detected only with 95% probability (or accepted with 5% probability), if 95% of the distribution is actually above the *m* value.

This proportion of the distribution above this *m* value is actually the defect rate. This defect rate together with the left graph is combined to show in the **right-hand graph**, the operating characteristic (OC) curve as function of the mean log count. This graph shows for a range of mean log concentrations, the probability of acceptance of a batch, depending on *n*, *c*, *m*, and *sigma*. Batches with a low mean concentration are accepted with a probability of 1, batches with a high concentration are always rejected, and values in between depend upon the sampling plan selected. In this case, the mean log concentration resulting in a 5% acceptance can be read to be about 0 logs, as given also in cell J19. Now set *n* again to 10 samples, and press "Find mean that gives ..." You can see that the OC curve for n=10 values is much more stringent, as expected, with the OC curve moving more to the left and a mean log concentration accepted at a 5% probability, equal to about -2.5; the exact value -2.25 is in cell J19.

Example 3: Assessing the performance of a 3-class plan.

To assess the performance of a 3 class sampling plan consider a test with n=5 samples, with the limit for marginally acceptable samples m=3 $\log_{10}$ cfu/g and the limit for unacceptable samples M=4 $\log_{10}$ cfu/g. The number of acceptable marginal samples c=2:

- Select the worksheet "3 class counts"
- Set cell J20 (*sigma*, the standard deviation) at 0.8
- Set J21 (*m*, the limit for marginally acceptable samples) to 3
- Set J22 (*M*, the limit for unacceptable samples) to 4
- Set J23 (*n*, the number of samples) to 5
- Set J24 (*c*, the number of samples permitted to test in the marginal range) to 2
- Set M20 (desired acceptance level) to 5%
- Press the button "Find mean that gives...." and cell J19 will report "3.52", with cell M19 and M20 equal to 5.0%.

Interpretation of Example 3

This can be interpreted as follows: a batch with mean log concentration greater than 3.52 with a standard deviation of 0.8 logs, will be accepted with <5% probability, and hence rejected with >95% probability, if the microbiological limits are m=3, M=4, with 5 samples, and c=2. It can be seen in cells D23 and D24 that for one sample to be defective (>*M*) the probability is 27.3%, and the probability to be in the marginal area (between *m* and *M*) is and 46.8%. The overall probability with 5 samples to accept the batch is 5%, meaning that there is 95% probability that either one of the samples is in the defective region (>*M*) or more than 2 samples are in the marginal region (>*m*, <*M*).