Version 09/03/2002

Limit of Detection Program for Qualitative Microbiology Methods

NOTE There is code only for 3-, 4-, and 5-level spiking protocols

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PURPOSE

This programmed non-parametric statistical procedure (Spearman-Karber 50% Endpoint) will calculate the microbial analyte concentration (and confidence limits) in a given food matrix that corresponds to a 50 % probability of a positive result with the test method used. The microbe may be spiked or incurred.

REQUIREMENTS

1. A minimum of three different concentrations is needed but more are preferable even at the expense of the degree of replication.
2. At least one spiking level should give a partially positive response otherwise no confidence limits can be calculated.
3. One of the concentration (spiking) levels should give a 100% response.
4. One of the concentration levels should give a 0% response (= negative control).
5. Three, and preferably at least 5 or 6, replicates per concentration (spiking) level are needed. (the confidence level window narrows with increased replication)
6. A *constant* analytical portion size.
7. Equal spacing of log spiking (concentration) levels and equal numbers of replicates per spike level are preferable but not obligatory.

ACCOMMODATIONS

1. Sometimes it is possible to have only 2 replicates per concentration level.
2. An uninoculated control set of replicates is often used. In this case a concentration of 0.004 MPN/g(mL) or cfu/g(mL) can be allocated to such a set of data (since the calculations take the log of the concentration levels, 0 cannot be used, and a concentration of 0,004 is assumed to give always a negative result).
3. If no spike level has a 100% replicate growth response, use a dummy set of data. The spike level should be 10 times the uppermost level giving a partial response.

PROCEDURE

Analytical portion size (g or mL)

25

Step 1. Insert analytical portion size in the green-filled cell.

Step 2a. Insert spike sizes *either* on an analytical portion basis *or* on a per g/mL basis in the appropriate green-filled cells. Spike size must increase in the downward direction.

Note: If values are entered on a analytical portion basis they will be processed to a per g/mL basis

Step 2b. Enter number of replicates at each spiking level. (The number of spiking levels must include one with all replicates not grown and one with all replicates grown.)

[Read the guidelines in the hidden comments. They may be revised in the future.]

Caution: Do not copy from cells without color fill. They may contain hidden code and, if so, only the code will be copied.
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Step 3. Read cream color -filled Results panel.

Enter spike size cfu or MPN/portion	Enter spike size cfu or MPN / g or mL	Enter number of replicates per spiking level	Enter number of replicates grown
			0
	Total degrees of freedom =	-1	
From Table enter t value for 95% confidence level		2.03	

RESULT

LOD 50 % (cfu or MPN per g/mL)	lower limit	upper limit

TABLE t-values

df	t-value (2-tail)
3	3.182
6	2.447
9	2.262
12	2.179
15	2.131
20	2.086
25	2.06
30	2.042
40	2.021
60	2
infinite	1.96

Jan-00

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	0.001	10	0
	0.01	10	1
	0.1	10	9
	Total degrees of freedom =	27	
From Table enter t value for 95% confidence level		2.03	

df		Spike size cfu or MPN / g or mL
9	1	0
9	1	0.01
9	1	0.1
0	0	0
0	0	0
0	0	0
	3	

RESULT

LOD 50 % (cfu or MPN per g/mL)	lower limit	upper limit
0.025	0.016	0.04

TABLE t-values

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60	2
infinite	1.96

3-SPIKING LEVEL DESIGN

	spike levels MPN or cfu /g-n	number of replicates per spiking level	r of replicates grown	log spike	proportion grown
Lowest (or uninoculated)	0.001	10	0	-3	0
	0.01	10	1	-2	0.1
Highest level	0.1	10	10	-1	1

	a	b	a*b	A	B	A*B	
	-2.5	0.1	-0.25	0.01	1	0.01	
	-1.5	0.9	-1.35	0.01	1	0.01	
log median	-1.6					var	sqrt var
						0.01	0.1

Log 50% endpoint	Log lower limit	upper limit
-3.6848	-4.152309	-3.22

RESULT

50 % endpoint	lower limit	upper limit
0.025	0.016	0.04

4-SPIKING LEVEL DESIGN

	spike levels MPN or cfu /g-n	number of replicates per spiking level	r of replicates grown	log spike	proportion grown
Lowest (or uninoculated)	0.001	10	0	-3	0
	0.01	10	1	-2	0.1
	0.1	10	9	-1	0.9
Highest level	0	0	0	#NUM!	#DIV/0!

	a	b	a*b	A	B	A*B	
	-2.5	0.1	-0.25	0.01	1	0.01	
	-1.5	0.8	-1.2	0.01	#####	#####	
	####	####	#NUM!			var	sqrt var
log median	#NUM!					#####	#NUM!

Log 50% endpoint	Log lower limit	upper limit
#NUM!	#NUM!	####

RESULT

50 % endpoint	lower limit	upper limit
#NUM!	#NUM!	#NUM!

5-SPIKING LEVEL DESIGN

	spike levels MPN or cfu /g-n	number of replicates per spiking level	r of replicates grown	log spike	proportion grown
Lowest (or uninoculated)	0.001	10	0	-3	0
	0.01	10	1	-2	0.1
	0.1	10	9	-1	0.9
	0	0	0	#NUM!	#DIV/0!
Highest level	0	0	0	#NUM!	#DIV/0!

	a	b	a*b	A	B	A*B	
	-2.5	0.1	-0.25	0.01	1	0.01	
	-1.5	0.8	-1.2	0.01	#####	#####	
	####	####	#NUM!	#DIV/0!	#####	#####	
	####	####	#NUM!			var	sqrt var
log median	#NUM!					#####	#NUM!

Log 50% endpoint	Log lower limit	upper limit
#NUM!	#NUM!	####

RESULT

50 % endpoint	lower limit	upper limit
#NUM!	#NUM!	#NUM!

6 OR MORE SPIKING LEVEL DESIGNS

Not typically needed.

Generalized Spearmann-Kärber Formula (see "Karber Method" entry on p. 354ff of Encyclopedia of Statistics, volume 4)

$$\tilde{\mu} = \sum_{i=1}^{k-1} (p_{i+1} - p_i)\,(\mathbf{x}_i + \mathbf{x}_{i+1}) / 2$$

$\tilde{\mu}$ = estimator of the mean, $\boldsymbol{\mu}$

$\mathbf{x_i}$'s are the log spiking concentrations with $\mathbf{x_i} < \ldots < \mathbf{x_k}$

k is the number of spiking levels

$\mathbf{p_i}$'s are observed proportions of positive replicates in an experiment where $\mathbf{n_i}$ replicates are tested independently at spiking level $\mathbf{x_i}$ yielding $\mathbf{r_i}$ positive replicates , so $\mathbf{p_i} = \mathbf{r_i} / \mathbf{n_i}$, where $\mathbf{i} = 1, \ldots, \mathbf{k}$.
It is assumed that $\mathbf{p_i} = 0$ and $\mathbf{p_k} = 1$.

$$\text{var}(\tilde{\mu}) = \sum_{i=2}^{k-1} [p_i / q_i / (n_i - 1)]\,[x_{i+1} - x_{i-1})/2]^2$$

provided that $\mathbf{n_i} \geq 2$, and $\mathbf{i} = 1\ldots$, $\mathbf{k}$, and where $\mathbf{q_i} = 1 - \mathbf{p_i}$.

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