Application and use of new MPN tables

Introduction

The Most Probable Number (MPN) procedure is widely used to determine the density of microorganisms in a given sample. MPN tables given in the NRL generic protocol are used to obtain the MPN value where 3 or 4 dilutions have been tested. Where more than 4 dilutions are tested a computer program is used to calculate the MPN value. This document is aimed to assist laboratories on how to input data into the online MPN calculator described in ISO 7218.

Procedure

1. Open the link http://standards.iso.org/iso/7218/ (ISO 7218 – Amendment 1, section 10.5.6.3).

2. The ISO Standard Maintenance portal screen will appear. Click once the underlined name 'MPN calculator Excel programme ver5'.

3. On clicking the 'MPN calculator' link, an MPN calculator program excel spreadsheet will open. Click on the 'Enable Content' button at the top of the spreadsheet to enable all macros.

4. Enter the number of test series and dilutions in the relevant cells. Once the cells are filled, the required number of data boxes will automatically appear below (one for each test series specified).

- 'Name of experiment' – The name given for the work performed.
- 'Date of experiment' – The date the work was carried out.

- 'No. of test series' – The value recorded will determine how many sets of sample tables will be generated automatically. A total of 30 samples can be inputted into the spreadsheet at one time.
- 'Max. no. of dilutions' – The value recorded is based on the number of dilutions examined. E.g. For the example given below 3 dilutions were examined (N, 10^{-1} and 10^{-2}).

Number of samples tested

Number of dilutions

	General data and data for generating the input tables			
	Name of experiment	Date of experiment	No. of test series	Max. no. of dilutions
			2	3

Note: A test series / matrix consists of the different dilutions for one target organism / test medium.

Note: The dilution factor d is the dilution ratio used for inoculating the tubes of that row, i.e. 1.0 (undiluted), 0.1 (diluted 1 in 10), etc.   The volume w is the volume of the dilution added to each tube in that row.

Input data			
Test series 1 / Matrix 1 - Designation:			
Target organism / Test medium:			
Dilution factor d	Volume in ml or g w	No. of tubes n	No. of positive tubes x

Test series 2 / Matrix 2 - Designation:			
Target organism / Test medium:			
Dilution factor d	Volume in ml or g w	No. of tubes n	No. of positive tubes x

5. Populate the data table as follows:

- Column 1 - Insert the homogenate concentration used to inoculate each row of MMGB tubes.
- Column 2 - Insert the volume of inoculum added.
- Column 3 – Insert the number of MMGB tubes inoculated at each dilution. (For the *E. coli* generic protocol 5 tubes are inoculated for each dilution).
- Column 4 – Insert the number of positive tubes on TBGA/TBX at each dilution.

	Input data			
12				
13	Test series 1 / Matrix 1 - Designation:		Example 1	
14	Target organism / Test medium:		E.coli	
15	Dilution factor	Volume in ml or g	No. of tubes	No. of positive tubes
16	d	w	n	x
17	0.1	10.0	5	5
18	0.1	1.0	5	4
19	0.01	1.0	5	2
20				
21	Test series 2 / Matrix 2 - Designation:		Example 2	
22	Target organism / Test medium:		E.coli	
23	Dilution factor	Volume in ml or g	No. of tubes	No. of positive tubes
24	d	w	n	x
25	0.1	10.0	5	4
26	0.1	1.0	5	3
27	0.01	1.0	5	1

Homogenate concentration used to inoculate MMGB	Volume of inoculum	Number of MMGB tubes	Number of positive tubes on TBGA/TBX

6. Press 'Ctrl M' or press Calculate results (green button). The MPN value (MPN/g) will be calculated in column 8 and the category in column 14.

	Results of the MPN calculations						
Test series / Matrix		MPN	log$_{10}$ MPN	SD log$_{10}$ MPN	95% confidence limits		Rarity Index	Category
No.	Designation				Lower	Upper		
1	Example 1	22.	1.3	0.20	8.8	56.	0.304	1
2	Example 2	3.3	0.51	0.18	1.4	7.5	0.010	3

Note: An MPN that falls into category 3 is unreliable and should not be used. The respective rows are marked in blue.

MPN/g	Category

7. Check the category of each sample tested and accept only MPN results that give a category 1 or category 2 profile. Reject all MPN results that fall within a category 3.

- For example 1 - the MPN/100g can be used.
- For example 2 - the result is rejected and reported as a 'Void' result. A repeat sample must be requested.

8. The MPN calculator calculates the MPN per gram (MPN/g). *E. coli* results are reported as MPN per 100g (MPN/100g). Multiply the value given in column 8 by 100 to give the results in MPN/100g.

For the examples given above

- For example 1 - 22 x 100 = 2,200 MPN /100g.
- For example 1 - A repeat sample would need to be requested as it has a category number of 3.

9. For samples that give a tube combination of 0, 0, 0 or 5, 5, 5 the MPN calculator will calculate a value of 0 and infinity respectively (see below). An estimated MPN value given for these tube combinations of 0, 0, 0 and 5, 5, 5 should be assigned values of <18 and >18 000 per 100g.

data

Example 1	
E.coli	
No. of tubes n	No. of positive tubes x
5	0
5	0
5	0

Example 2	
E.coli	
No. of tubes n	No. of positive tubes x
5	5
5	5
5	5

	Results of the MPN calculations							
No.	Test series / Matrix Designation	MPN	log$_{10}$ MPN	SD log$_{10}$ MPN	95% confidence limits Lower	Upper	Rarity Index	Category
1	Example 1	0			0	0.66	1.000	1
2	Example 2	∞			65.	∞	1.000	1