



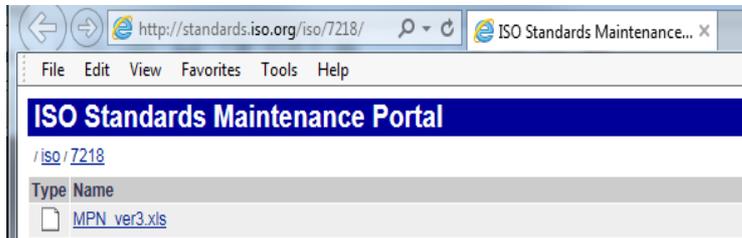
Application and use of new MPN tables

Introduction

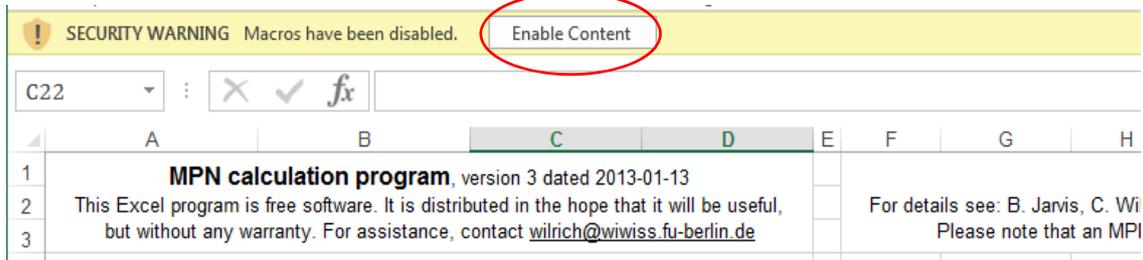
The Most Probable Number (MPN) procedure is widely used to determine the density of microorganisms in a given sample. MPN tables given in the NRL generic protocol are used to obtain the MPN value where 3 or 4 dilutions have been tested. Where more than 4 dilutions are tested a computer program is used 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 ver3'.



3. On clicking the 'MPN ver3' 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. Fill in cells C20 to C23 (shaded in yellow) as described below. Once cells C20 to C23 are filled in press the return button. The required number of data boxes will automatically appear below (one for each test series specified in C22).

- 'Name of experiment' – The name given for the work performed.
- 'Date of experiment' – The date the work was carried out.
- 'Total no. of test series / matrices' – The value recorded will determine how many sets of sample tables will be generated automatically. A total of 18 samples can be inputted into the spreadsheet at one time.
- 'Maximum no. of dilutions' – The value recorded is based on the number of dilutions examined. E.g. For the example given below 5 dilutions were examined (N , 10^{-1} , 10^{-2} , 10^{-3} and 10^{-4}).

	A	B	C	D
1	MPN calculation program , version 3 dated 2013-01-13			
2	This Excel program is free software. It is distributed in the hope that it will be useful,			
3	but without any warranty. For assistance, contact wilrich@wiwiss.fu-berlin.de			
4	How to use this program (Macros have to be enabled):			
5				
6	1. Fill in the yellow cells: Enter the total no. of test series/matrices into cell C22 and			
7	the maximum no. of dilutions for any of the test series into cell C23. A test series			
8	consists of the different dilutions for one target organism in one matrix. ► The			
9	according no. of tables for your input data will be generated automatically below.			
10	2. Enter your data into the yellow cells of the tables generated according to step 1.			
11	3. Then press "Ctrl+m" to start the calculation. ► The results will be shown in green			
12	cells (and are repeated to the right of each yellow input table).			
13				
14	4. You can change the no. of test series or dilutions in cell C22 or C23 at any time.			
15	5. You can also change the data in the input tables at any time and re-calculate by			
16	pressing "Ctrl+m" again.			
17	The results are based on the equations shown in the sheet "Equations".			
18	Examples are given in the sheet "Examples". They correspond to ISO 7218.			
19				
20	Name of experiment:		Test	
21	Date of experiment:		12.10.15	
22	Total no. of test series/matrices (up to 18):		2	
23	Maximum no. of dilutions (up to 50):		4	
24				
25	No. of test series/matrix:		1	
26	Name of test series/matrix = sample referenc			
27	Test medium or target organism:			
28	dilution factor	volume in ml or g	no. of tubes	no. of positive tubes
29	d*	w**	n	x
30				
31				
32				
33				
34				
35	No. of test series/matrix:		2	
36	Name of test series/matrix = sample referenc			
37	Test medium or target organism:			
38	dilution factor	volume in ml or g	no. of tubes	no. of positive tubes
39	d*	w**	n	x
40				
41				
42				
43				

Number of samples tested

Number of dilutions examined

5. Populate the data table as follows:

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

25	No. of test series/matrix:		1	
26	Name of test series/matrix = sample reference:		Example 1	
27	Test medium or target organism:		E.coli	
28	dilution factor	volume in ml or g	no. of tubes	no. of positive tubes
29	d*	w**	n	x
30	0.1	10.0	5	5
31	0.1	1.0	5	5
32	0.01	1.0	5	4
33	0.001	1.0	5	2
34				
35	No. of test series/matrix:		2	
36	Name of test series/matrix = sample reference:		Example 2	
37	Test medium or target organism:		E.coli	
38	dilution factor	volume in ml or g	no. of tubes	no. of positive tubes
39	d*	w**	n	x
40	0.1	10.0	5	5
41	0.1	1.0	5	4
42	0.01	1.0	5	3
43	0.001	1.0	5	1

Homogenate concentration used to inoculate MMGB tubes

Volume of inoculum added

Number of MMGB tubes inoculated

Number of positive tubes on TBGA/TBX

6. Press 'Ctrl M'. The MPN value (MPN/g) will be calculated in column H and the category in column N.

1	Example 1		No. of test series	Name of test series	MPN	log ₁₀ MPN	SD log ₁₀ MPN	95% Confidence Limits		Rarity Index	Category
	E.coli		1	Example 1	220	2.3	0.20	88	560	0.304	1
	no. of tubes	no. of positive tubes									
	n	x									
	5	5									
	5	5									
	5	4									
	5	2									
* d _i is the dilution ratio used for inoculating that series of tubes: i.e. 1.0 (undiluted), 0.1 (diluted 1 in 10), 0.01 (diluted 1 in 100), 0.001 (diluted 1 in 1000)											
** w _i is the volume of the dilution added to each tube in that series											
2	Example 2		No. of test series	Name of test series	MPN	log ₁₀ MPN	SD log ₁₀ MPN	95% Confidence Limits		Rarity Index	Category
	E.coli		2	Example 2	120	2.1	0.18	42	180	0.250	2
	no. of tubes	no. of positive tubes									
	n	x									
	5	5									
	5	4									
	5	3									
	5	1									

MPN/g

Category number

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.

No. of test series	Name of test series	MPN	log ₁₀ MPN	SD log ₁₀ MPN	95% Confidence Limits		Rarity Index	Category
					Lower	Upper		
1	Example 1	220	2.3	0.20	88	560	0.304	1
2	Example 2	33	1.5	0.18	14	75	0.010	3

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 H by 100 to give the results in MPN/100g.

No. of test series	Name of test series	MPN	log ₁₀ MPN	SD log ₁₀ MPN	95% Confidence Limits		Rarity Index	Category
					Lower	Upper		
1	Example 1	220	2.3	0.20	88	560	0.304	1
2	Example 2	33	1.5	0.18	14	75	0.010	3

For the examples given above

- Test series 1 = 220 x 100 = 1.1 x 10⁴ MPN /100g.
- Test series 2 = 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.

1		No. of test series	Name of test series	MPN	log ₁₀ MPN	SD log ₁₀ MPN	95% Confidence Limits		Rarity Index	Category
							Lower	Upper		
		1		0			0	0.66	1.000	1
no. of tubes	no. of positive tubes									
n	x									
5	0									
5	0									
5	0									
* d _i is the dilution ratio used for inoculating that series of tubes: i.e. 1.0 (undiluted), 0.1 (diluted 1 in 10), 0.01 (diluted 1 in 100)										
** w _i is the volume of the dilution added to each tube in that series										
2		No. of test series	Name of test series	MPN	log ₁₀ MPN	SD log ₁₀ MPN	95% Confidence Limits		Rarity Index	Category
							Lower	Upper		
		2		Inf			65	Inf	1.000	1
no. of tubes	no. of positive tubes									
n	x									
5	5									
5	5									
5	5									