Supplementary Guidance on the Derivation of Most Probable Number (MPN) for Official Control Testing of Bivalve Molluscan

Introduction

Publication of the amendment to ISO 7218:2007[1], ISO 7218:2007/Amd.1:2013(E)[2] changes include guidance with respect to derivation of MPN values. Instructions on derivation of MPN values are given in Clause 10 of ISO 7218:2007/Amd.1:2013(E). The amendment replaces Clause 10 on Enumeration of ISO 7218:2007 in its entirety and all previous ISO guidance on interpretation of tube combinations. Laboratories should note that it includes important changes to the way in which MPN values are derived, amended guidance is described below.

Current guidance

ISO 7218:2007/Amd.1:2013(E), Clause 10.5.6.2.2 Tables for multiple-dilution systems: three successive dilutions

'In any circumstance when more than three dilutions are made, it is essential that all measured data values are used. It is not scientifically correct to "select" any combinations of values on the premise that these values are more "correct" than other combinations. The results from all possible combinations of positive tubes should be recorded and the MPN calculator (10.5.6.3) used to derive MPN values.'

Therefore, laboratories undertaking more than three dilutions for either Official Control testing of bivalve molluscs or when participating in associated proficiency testing as Official Control or National Reference Laboratories, are advised to enter all results into the MPN calculator to derive the MPN value, even if the highest dilution returns a 0 result[3] (e.g. 5, 2, 2, 0 should be used against 1.0g, 0.1g, 0.01g and 0.001g and this tube combination reported, where appropriate, and not 5, 2, 2 against 1.0g, 0.1g and 0.01g). Whilst for the most part the resultant MPN value will be identical, there are some instances where this is not the case (Table 1), it is also considered less scientifically valid to report partial data.

Official Control Laboratories that do not derive MPN values according to this guidance may lose marks in EURL/PHE proficiency testing schemes.

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[1] ISO 7218:2007 - Microbiology of food and animal feeding stuffs – General requirements and guidance for microbiological examinations
[2] ISO 7218:2007/Amd.1:2013(E) - Microbiology of food and animal feeding stuffs – General requirements and guidance for microbiological examinations
[3] Where 4 dilutions are analysed, Table 2 in the EURL generic protocol Issue 13 can be used to derive MPN value.

Table 1: Examples of derived MPN values where 3 and 4 measured data values are used, highlighted rows showing discrepancies between derived MPN values

1g	0.1g	0.01g	MPN/100g	Category		1g	0.1g	0.01g	0.001g	MPN/100g	Category	
4	2	1	260	2		4	2	1	0	260	2	√
4	3	0	270	1		4	3	0	0	270	1	√
4	4	0	340	2		4	4	0	0	330	2	X
5	0	1	310	1		5	0	1	0	310	1	√
5	1	0	330	1		5	1	0	0	330	1	√
5	1	1	460	1		5	1	1	0	450	1	X
5	1	2	630	2		5	1	2	0	620	2	X
5	2	0	490	1		5	2	0	0	490	1	√
5	2	1	700	1		5	2	1	0	690	1	X
5	2	2	940	1		5	2	2	0	930	1	X
5	3	0	790	1		5	3	0	0	780	1	X
5	3	1	1100	1		5	3	1	0	1100	1	√
5	3	2	1400	1		5	3	2	0	1400	1	√
5	3	3	1700	2		5	3	3	0	1700	2	√
5	4	0	1300	1		5	4	0	0	1300	1	√
5	4	1	1700	1		5	4	1	0	1700	1	√
5	4	2	2200	1		5	4	2	0	2200	1	√
5	4	3	2800	1		5	4	3	0	2700	1	X
5	4	4	3500	2		5	4	4	0	3400	2	X
5	5	0	2400	1		5	5	0	0	2300	1	X
5	5	1	3500	1		5	5	1	0	3300	1	X
5	5	2	5400	1		5	5	2	0	4900	1	X
5	5	3	9200	1		5	5	3	0	7900	1	X
5	5	4	16000	1		5	5	4	0	13000	1	X
5	5	5	>18000	1		5	5	5	0	24000	1	X