BTS BIOTECHNOLOGY SOLUTIONS

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MPN METHOD

1.0 PURPOSE

This method presents a procedure for determining the Most Probable Number of bacteria from a fluid sample or dilution. The method is applicable for any broth media dispensed as 9 ml aliquots.

2.0 REFERENCES

W F Harrigan, M E McCance – Laboratory methods in food and dairy microbiology.

NACE Standard TMO194-2014

3.0 SUMMARY OF METHOD

A 1 ml sample of the fluid under test is inoculated, in triplicate, into 9 ml aliquots of selective enrichment broth media selective for the organism under study. The sample is serially diluted to extinction in the broth media by successive tenfold dilutions. The broths are then incubated at the specified temperature for the desired incubation time before positive growth bottles are scored. The Most Probable Number (MPN) of bacteria in the sample is then determined from statistical tables.

4.0 APPARATUS

4.1 Flat of broth media with 24 vials (9 ml vials) arranged in 1 set (1-8) of three (A, B & C)

4.2 1 ml sterile syringes

4.3 Sterile needles

4.4 Alcohol pads

4.5 Sharps Container

4.6 Incubator

5.0 SAMPLING

5.1 For field visits, refer to the appropriate sample method in the Quality Work Instructions.

5.2 For client supplied material, initial sampling will have been performed by a third party and it is assumed that the sample has been collected employing a suitable sampling method.

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6.0 PROCEDURE

6.1 Draw 1 ml sample into a sterile syringe and needle and inoculate bottle 1A. Using the same syringe and needle inoculate bottles 1B and 1C. Following inoculation of the sample into the broth bottle, repeatedly (3 times) draw the broth culture into the syringe and expel back into the broth bottle to thoroughly flush the syringe prior to inoculating the next replicate. Discard the needle and syringe carefully, and shake the bottles vigorously to ensure good mixing.

6.3 With a new syringe and needle, draw 1 ml of the broth from bottle 1A and inoculate bottle 2A. Using the same syringe and needle inoculate bottles 2B and 2C from 1B and 1C respectively. Following inoculation of the sample into the broth bottle, repeatedly (3 times) draw the broth culture into the syringe and expel back into the broth bottle to thoroughly flush the syringe prior to inoculating the next replicate. Discard the needle and syringe carefully, and shake the bottles vigorously to ensure good mixing.

6.4 With a new syringe and needle, draw 1 ml of the broth from bottle 2A and inoculate bottle 3A. Using the same syringe and needle inoculate bottles 3B and 3C with the broth from 2B and 2C respectively. Following inoculation of the sample into the broth bottle, repeatedly (3 times) draw the broth culture into the syringe and expel back into the broth bottle to thoroughly flush the syringe prior to inoculating the next replicate. Discard the needle and syringe carefully, and shake the bottles vigorously to ensure good mixing.

6.5 With a new syringe and needle, draw 1 ml of the broth from bottle 3A and inoculate bottle 4A. Using the same syringe and needle inoculate bottles 4B and 4C with the broth from 3B and 3C respectively. Following inoculation of the sample into the broth bottle, repeatedly (3 times) draw the broth culture into the syringe and expel back into the broth bottle to thoroughly flush the syringe prior to inoculating the next replicate. Discard the needle and syringe carefully, and shake the bottles vigorously to ensure good mixing.

6.6 Repeat step 6.5 for the 5th, 6th, 7th and 8th set.

6.7 Place the inoculated media in the incubator at the specified temperature and read after the specified incubation time.

6.8 At the end of the incubation period, remove media flat on bench top.

6.9 REMEMBER each set of three bottles (1A, 1B, and 1C) represents a tenfold dilution of the original inoculum.

6.10 Taking each dilution in turn 1.e.1 to 8, read the media for positive bottles.

6.11 Record the number of positive bottles in each dilution eg. If after incubation a sample with an eight fold dilution was found to have dilutions 1 to 4 with three positive vials in each dilution, dilution five with two positive vials (one negative), dilution six with one positive vial (two negative and dilutions seven to eight with no positive bottles. Record the above pattern as follows:

Dilution	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Positive Bottles	3	3	3	3	2	1	0	0

6.12 The MPN method tries to dilute the original sample to zero therefore the aim is to find the lowest dilution where no growth was detected i.e dilution 7. This is the Z reading.

6.13 Using the two proceeding results (dilutions 5 and 6) generate a three number pattern (X, Y, Z) e.g. 210. This result will be used to determine the MPN value from the table provided.

6.14 Read the three number pattern from the table provided e.g. 210 gives an MPN reading of 1.5.

6.15 Looking back at the pattern of positive vials it can be seen that four dilutions prior to our X, Y, Z pattern contained all positive vials (dilutions 1, 2, 3 and 4). Therefore the MPN reading of 1.5 must be multiplied by 10^4 (as the original sample is diluted tenfold each dilution series) to give the final result as cells per ml in original sample.

NOTE: you can only multiply back by a factor of ten if the previous dilution is all positive i.e a 3. Therefore if you find the lowest dilution that gives you a 0 value and you calculate your X, Y, Z pattern from this point, if the number immediately prior to X is not a 3 then you must move your Z number up one dilution e.g. 3331 1100 using an X, Y, Z pattern 110 would not allow you multiply back by ten as the number prior to X is 1. The pattern to use would be 111.

6.16 SEE APPENDIX FOR ADDITIONAL EXAMPLES

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7.0 **REPORT**

7.1 Report the growth pattern and the MPN result per one millilitre of initial sample inoculated into the first dilution vial.

7.2 Take into account any pre-dilution or sample preparation required prior to reporting result on test certificate.

8.0 **PRECISION AND BIAS**

8.1 The limit of detection of the method is < 0.3 cells per ml.

9.0 **HEALTH AND SAFETY**

9.1 Protective Equipment: Wear lab coat and safety glasses.

9.2 Dispose needles and syringes in sharps container.

9.3 Disposal: All of BTS bacterial growth media products are considered to be non-hazardous materials. Media may be discarded according to your local, state and federal regulations. To find out more about these regulations please refer to the environmental, health and safety staff at your facility.

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TABLE 1 MOST PROBABLE NUMBER TABLE **Pattern of positives in triplicate 10-fold dilutions.**

NOTE: This table is to be used for planktonic counts. When performing sessile counts use the appropriate correction factor to calculate the number of bacteria per cm^2.

1ST	2ND	3RD	MPN/ml
0	0	0	<0.3
0	0	1	0.3
0	1	0	0.3
0	1	1	0.6
0	2	0	0.6
1	0	0	0.4
1	0	1	0.7
1	0	2	1.1
1	1	0	0.7
1	1	1	1.1
1	2	0	1.1
1	2	1	1.5
1	3	0	1.6
2	0	0	0.9
2	0	1	1.4
2	0	2	2.0
2	1	0	1.5
2	1	1	2.0
2	1	2	3.0
2	2	0	2.0
2	2	1	3.0
2	2	2	3.5
2	2	3	4.0
2	3	0	3.0
2	3	1	3.5
2	3	2	4.0

1ST	2ND	3RD	MPN/ml
3	0	0	2.5
3	0	1	4.0
3	0	2	6.5
3	1	0	4.5
3	1	1	7.5
3	1	2	11.5
3	1	3	16.0
3	2	0	9.5
3	2	1	15.0
3	2	2	20.0
3	2	3	30.0
3	3	0	25.0
3	3	1	45.0
3	3	2	110.0
3	3	3	140.0+

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APPENDIX ONE

The grids below represent the media vials used for the MPN method. A + sign indicates that the media vial is positive for bacterial growth. A – sign indicates that the media vial is negative for bacterial growth.

EXAMPLE 1:

A	-	-	-	-
B	+	+	+	-
C	+	-	-	-
	1	2	3	4

Box 1 of 1

Pattern of positive bottles = 2110

x y, z, reading = 211

MPN Reading = 2.0

Final Result = 2.0 x 10^0 cells per ml

EXAMPLE 2:

A	+	+	+	+
B	+	+	+	-
C	+	+	+	-
	1	2	3	4

Box 1 of 1

Pattern of positive bottles = 3331

x, y, z, reading = 331

MPN Reading = 45.0 equal to 4.5 x 10^1

There is 1 positive dilution in front of our x, y, z,

pattern therefore the final Result = 4.5 x 10^2 cells per ml

EXAMPLE 3:

A	+	+	+	+	+	+	+	-
B	+	+	+	+	+	+	-	-
C	+	+	+	+	+	+	-	-
	1	2	3	4	5	6	7	8

Pattern of positive bottles = 3333 3310

x, y, z, reading = 310

MPN Reading = 4.5

There are 5 positive dilutions in front of our x, y, z, pattern therefore

the final result = 4.5 x 10^5 cells per ml

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EXAMPLE 4:

Pattern of positive bottles = 3331 1100

x, y, z, reading = 111

MPN Reading = 1.1

There are 3 positive dilutions in front of our x, y, z, pattern therefore

the final result = 1.1 x 10^3 cells per ml

A	+	+	+	-	-	-	-	-
B	+	+	+	+	+	+	-	-
C	+	+	+	-	-	-	-	-
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**

EXAMPLE 5:

Pattern of positive bottles = 3333 3222

x, y, z, reading = 222

MPN Reading = 3.5

There are 5 positive dilutions in front of our x, y, z, pattern therefore

the final result = 3.5 x 10^5 cells per ml

A	+	+	+	+	+	-	-	-
B	+	+	+	+	+	+	+	+
C	+	+	+	+	+	+	+	+
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**

EXAMPLE 6:

Pattern of positive bottles = 0000 0000

There are no positive dilutions in front of our X, Y, Z

x, y, z, reading = 000

MPN Reading = < 0.3

Therofore the final result = < 0.3 x 10^0 cells per ml

A	-	-	-	-	-	-	-	-
B	-	-	-	-	-	-	-	-
C	-	-	-	-	-	-	-	-
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**