

Rapid and precise dilutions for the Most Probable Number test procedure

Introduction

The Most Probable Number (MPN) or multi-tube method (McCrary)¹ is a statistical method used to estimate the number of viable cells of a particular microorganism in a sample, most commonly food or water. It is used when samples contain too few bacteria to provide reliable viable cell numbers by classical plate count.

Replicates of liquid broth are filled into tubes and inoculated with different amounts of the sample. The bacterial growth is then monitored. Microorganism growth is indicated by a color change, with the tube becoming cloudy or producing gas during

incubation. The number of viable microorganisms in the original sample is estimated according to an MPN table, based on statistical probabilities.

One of the most useful applications of MPN is to estimate coliform bacteria and *Escherichia coli* in water quality tests and screening, in particular, in drinking water. The presence of *E. coli* in water is indicative of recent fecal contamination. The DOSE IT peristaltic pump speeds up the pipetting steps of MPN procedures to test drinking water samples for coliform and *E. coli*.

Key benefits:

- The DOSE IT allows fast and accurate dispensing of broth and sample into the culture tube.
- DOSE IT's enclosed system ensures that the culture media and sample remain contained inside the tubing, minimizing the risk of contamination. The tubing can also be easily sterilized.
- The compact and lightweight design allows the DOSE IT to be moved and placed virtually anywhere in the lab, including inside a laminar flow cabinet, guaranteeing sterile working conditions.
- Hands-free control of dispensing using the optional benchtop switch or foot switch, or remote operation via PC software, offers additional flexibility for adjustment of dispense rates to the user's workflow.
- CUSTOMIZE mode enables easy and efficient creation of personalized liquid handling protocols.
- A large display and clear user guidance support fast set-up and use.

Overview: How to perform MPN tests

Before starting the MPN test, sterile single- and double-strength Phenol Red Lactose broth should be freshly made (MacFaddin).² A 10 times dilution of the water sample should also be prepared with phosphate buffer (0.5 mM K₂HPO₄, pH 7.2).

The protocol is divided into two dispensing programs, MPN-1 and MPN-2 (**Figure 1**).

Overview of the steps and corresponding programs:

Program 1: Dispensing the broth (MPN-1)

Program 2: Dispensing the samples (MPN-2)

The detailed programs are given in the appendix (Tables 2 and 3, respectively). Both programs need to be manually set up on the DOSE IT peristaltic pump.



Experimental set-up

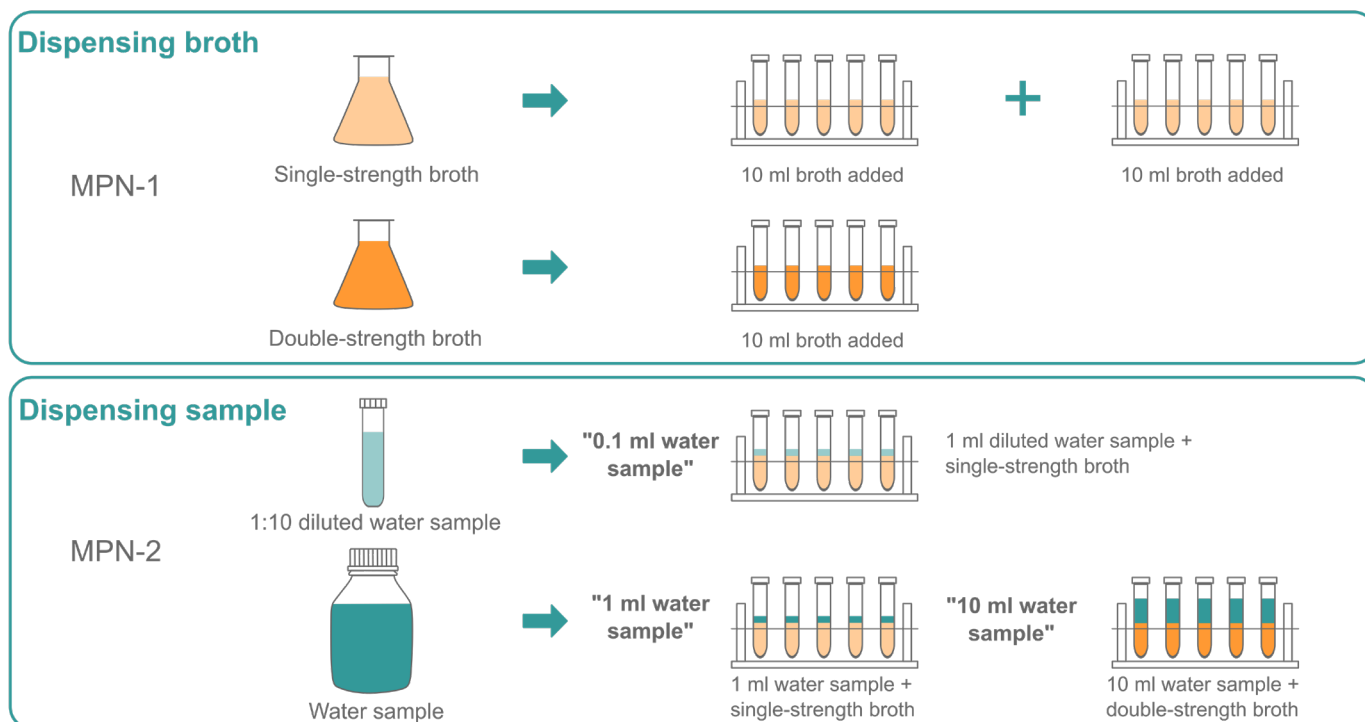


Figure 1: Experimental set-up of the MPN procedure for testing drinking water.

Step-by-step procedure:

Program 1 – Dispensing the broth

1. DOSE IT set-up

STEP: Tubing adjustment, priming and calibration for dispensing the broth

HOW TO: Open the pumphead, adjust the tubing clamps and insert the 4.0 mm tubing in the DOSE IT. Put the aspiration tube with the tube collar into the single-strength Phenol Red Lactose broth and the dispensing tube into an empty bottle in order to fill the empty tubing with liquid. Choose MPN-1 from the APPLICATIONS menu and press START to access the program window (**Figure 2**).

Perform the priming and calibration steps according to the [Operating instructions](#).



Figure 2: Program window of the DOSE IT peristaltic pump.

2. Dispensing the single-strength broth

STEP: Filling 10 tubes with 10 ml single-strength Phenol Red Lactose broth

HOW TO: Put the dispensing tube into the first test tube. Press START. DOSE IT directly dispenses 10 x 10 ml of single strength broth with 2 second intervals between tubes (Step 1). After each dispense, move the dispensing tube into the next empty test tube. 'Waiting time: manual' is displayed on the screen (Step 2) when tube filling has finished. Remove the aspiration tube with the tube collar from the single-strength broth and put the dispensing tube into an empty bottle to drain. Press NEXT STEP. The single-strength broth will be removed from the tubing (Step 3). Press PAUSE once all liquid is drained from the tubing.

3. Dispensing the double-strength broth

STEP: Filling five tubes with 10 ml double-strength Phenol Red Lactose broth

HOW TO: Put the aspiration tube with the tube collar into the double-strength broth and the dispensing tube into an empty bottle to refill the empty tubing. Press NEXT STEP (Step 4). Press PAUSE once the tubing is filled with double-strength broth. Put the dispensing tube into the first test tube. Press NEXT STEP (Step 5). DOSE IT dispenses 5 x 10 ml of broth into the tubes with a 2 second break between tubes. During each pause, move the dispensing tube into the next empty test tube. At the end, 'Custom application complete.' will appear on the screen. Press MAIN MENU to continue with program MPN-2.

Tip:

- As the less concentrated broth (single-strength broth) is dispensed before the more concentrated broth (double-strength broth), cleaning of the tubing is not required between the dispensing steps.

Program 2 – Dispensing the samples

1. DOSE IT set-up

STEP: Tubing adjustment, priming and calibration for dispensing the sample

HOW TO: Open the pumphead and remove the 4.0 mm tubing. Adjust the tubing clamps and insert the 2.0 mm tubing into the DOSE IT. Put the aspiration tube with the tube collar into the diluted water sample and the dispensing tube into an empty bottle. Select MPN-2 from the APPLICATIONS menu and press START to access the program window. Priming and calibration should be carried out as described in the [Operating instructions](#).

2. Dispensing the diluted water sample

STEP: Addition of 1 ml of diluted water sample to 10 ml of single-strength broth (corresponds to 0.1 ml of original water sample volume – see **Figure 1**)

HOW TO: Put the dispensing tube into the first test tube containing single-strength broth. Press START. DOSE IT directly dispenses 5 x 1 ml of the diluted water sample with a 2 second interval between tubes (Step 1). During each pause, move the dispensing tube into the next empty test tube. Make sure the dispensing tube doesn't touch the surface of the broth in the tube. When complete, 'Waiting time: manual' will appear on the screen (Step 2). Remove the aspiration tube with the tube collar from the diluted water sample and put the dispensing tube into an empty bottle to drain. Press NEXT STEP. The diluted water sample will be pumped out of the tubing (Step 3). Press PAUSE when it is done.

3. Dispensing the original water sample to the single-strength broth

STEP: Addition of 1 ml original water sample to 10 ml single-strength broth (1 ml sample – see **Figure 1**)

HOW TO: Put the aspiration tube with the tube collar into the undiluted water sample and the dispensing tube into an empty bottle. Press NEXT STEP (Step 4). Press PAUSE when the tubing is filled with the water sample. Put the dispensing tube into the first test tube containing single-strength broth. Press NEXT STEP (Step 5). DOSE IT dispenses 5 x 1 ml of water sample to the tubes with a 2 seconds interval between tubes. During this pause, move the dispensing tube into the next empty test tube. Make sure the dispensing tube doesn't touch the surface of the broth in the tube. Once finished, 'Waiting time: manual' will appear on the screen (Step 6).

4. Dispensing the original water sample to the double-strength broth

STEP: Addition of 10 ml original water sample to 10 ml double-strength broth (10 ml sample – see **Figure 1**)

HOW TO: Put the dispensing tube into the first test tube containing double-strength broth. Press NEXT STEP (Step 7). DOSE IT directly dispenses 5 x 10 ml of water sample to the tubes with 2 seconds between each tube. During this pause, move the dispensing tube into the next empty test tube. Once finished, 'Custom application complete.' will appear on the screen.

Tip:

- As the diluted water sample is dispensed prior to the original water sample, cleaning of the tubing is not required between the dispensing steps.

After adding broth and sample to the tubes, mix and incubate at 37 °C for 24 hours.

If you have further samples, repeat programs MPN-1 and MPN-2, or adapt the MPN-1 program to fill more tubes in one go, to speed up your process even more.

Remarks

The MPN method can be used to estimate the number of several types of microorganism. For example, it is commonly used in the food industry for estimating the number of *Clostridium tyrobutyricum* in cheese or *Listeria monocytogenes* (causing listeriosis) in meat and dairy products.

How to interpret the results of the MPN test of water samples

In this specific test, Phenol Red Lactose broth is used to detect coliforms in drinking water. It contains phenol red indicator, which is red at pH 7.4 ± 0.2, changing to yellow at acidic pH. Coliforms ferment lactose and produce an acid byproduct. Therefore, when they are present in drinking water, the Phenol Red Lactose broth turns yellow. After incubation, the number of positive test tubes per dilution step should be recorded.

How to use the MPN table

The number of positive (yellow) tubes from the five tubes in the first, second and third dilutions should be noted, then matched to the MPN table (**Appendix, Table 1**). Based on the result, an estimation is made on how many bacteria are found in 100 ml of sample.

In the example below (**Figure 3**), the pattern of positive (yellow) tubes is: 5-2-0, which means that on average approximately 49 bacteria (with the confidence interval: 17-130 bacteria) were in 100 ml original water sample.

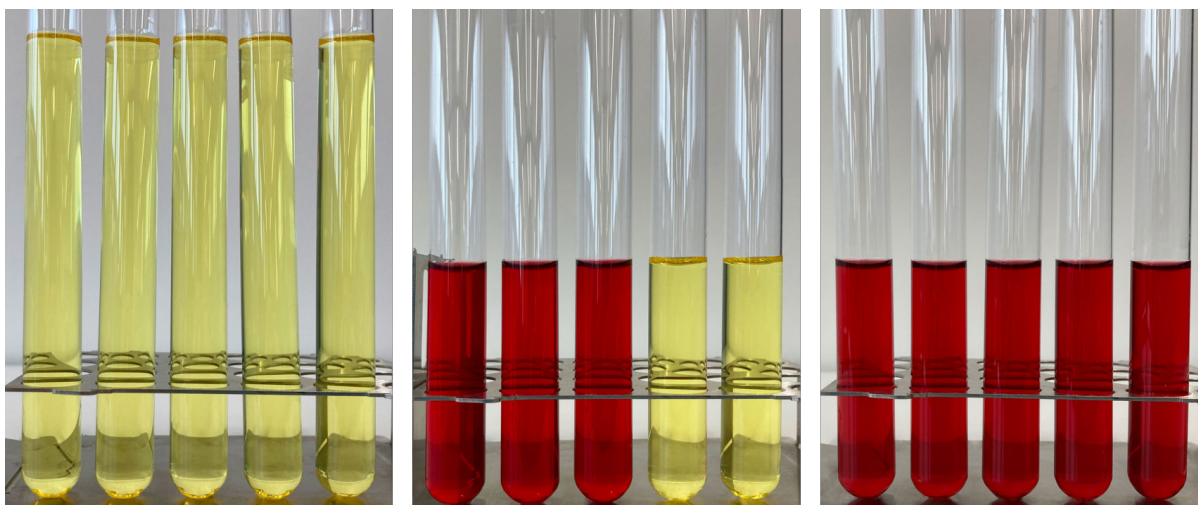


Figure 3: Result of coliform MPN test in drinking water samples.

Conclusion

- Regular testing for coliform bacteria is needed to ensure that water quality is sufficiently high. The Most Probable Number method is suitable for the estimation of coliforms in water samples, but this multi-tube process can be very time consuming and tedious if there is a large number of samples. The DOSE IT peristaltic pump makes MPN testing easy and efficient, with fast aliquoting of the culture media and samples into test tubes.
- DOSE IT offers self-explanatory dispense settings and parameters. It is very simple to create customized liquid handling protocols based on configurable parameters, such as volume, flow rate and repetitions.
- The DOSE IT is compatible with different sized tubing ranging from 1 mm for small doses to 8 mm for high-throughput pumping, making it useful for accurate dispensing of a broad selection of volumes

¹ M. H. McCrady (1918). Tables for Rapid Interpretation of Fermentation-tube Results. *The Public Health Journal* 9(5): 201-220.

² J. MacFaddin (1985): Cultivation-Identification-Maintenance of Medical Bacteria. Vol.1. Williams and Wilkins, Baltimore, MD.

Materials

Manufacturer	Part Number	Description	Link
INTEGRA Biosciences	171 000	DOSE IT P910	https://www.integra-biosciences.com/switzerland/en/peristaltic-pump/dose-it
INTEGRA Biosciences	143 200	Foot switch	https://www.integra-biosciences.com/switzerland/en/peristaltic-pump/dose-it
INTEGRA Biosciences	171 081	Benchtop switch	https://www.integra-biosciences.com/switzerland/en/peristaltic-pump/dose-it
INTEGRA Biosciences	171 021 171 024	Silicone tubing	https://www.integra-biosciences.com/switzerland/en/peristaltic-pump/dose-it
INTEGRA Biosciences	171 052 171 054	Aspiration/dispensing tubes	https://www.integra-biosciences.com/switzerland/en/peristaltic-pump/dose-it
INTEGRA Biosciences	171 071	Tube collar	https://www.integra-biosciences.com/switzerland/en/peristaltic-pump/dose-it
INTEGRA Biosciences	103 061	Test tube rack stainless steel, autoclavable	https://www.integra-biosciences.com/switzerland/en/media-preparation/mediajet
VWR	212-0031	Test tube	https://ch.vwr.com/store/product/en/576332/test-tubes-with-or-without-rim

Appendix

Table 1: MPN values per 100 ml sample and 95 % confidence limits.

No. of tubes giving a positive reaction			MPN (per 100 ml)	95 % confidence limits		No. of tubes of giving a positive reaction			MPN (per 100 ml)	95 % confidence limits	
5 of 10 ml	5 of 1 ml	5 of 0.1 ml		lower	upper	5 of 10 ml	5 of 1 ml	5 of 0.1 ml		lower	upper
0	0	0	<2	<1	7	4	3	0	27	9	80
0	1	0	2	<1	7	4	3	1	33	11	93
0	2	0	4	<1	11	4	4	0	34	12	93
1	0	0	2	<1	7	5	0	0	12	7	70
1	0	1	4	<1	11	5	0	1	31	11	89
1	1	0	4	<1	11	5	0	2	43	15	110
1	1	1	6	<1	15	5	1	0	33	11	93
2	0	0	5	<1	13	5	1	1	46	16	120
2	0	1	7	1	17	5	1	2	63	21	150
2	1	0	7	1	17	5	2	0	49	17	130
2	1	1	9	2	21	5	2	1	70	23	170
2	2	0	9	2	21	5	2	2	94	28	220
2	3	0	12	3	28	5	3	0	79	25	190
3	0	0	8	1	19	5	3	1	110	31	250
3	0	1	11	2	25	5	3	2	140	37	340
3	1	0	11	2	25	5	3	3	180	44	500
3	1	1	14	4	34	5	4	0	130	35	300
3	2	0	14	4	34	5	4	1	170	43	490
3	2	1	17	5	46	5	4	2	220	57	700
3	3	0	17	5	46	5	4	3	280	90	850
4	0	0	13	3	31	5	4	4	350	120	1000
4	0	1	17	5	46	5	5	0	240	68	750
4	1	0	17	5	46	5	5	1	350	120	1000
4	1	1	21	7	63	5	5	2	540	180	1400
4	1	2	26	9	78	5	5	3	920	300	3200
4	2	0	22	7	67	5	5	4	1600	640	5800
4	2	1	26	9	78	5	5	5	>1800	-	-

Reference: WHO: Guidelines for drinking-water quality, Annex 5: Multiple-tube method for thermotolerant (fecal) coliforms.

Table 2: MPN-1 customized program.

Parameter	Value	Description of settings
Name	MPN-1	
Type	CUSTOMIZE	
Tubing ID	4.0 mm	Define the inner diameter of the tubing inserted
1.6-marking of clamps	Completely open	Adjust the clamps for the tubing
Dispense direction	↻	The rotational direction of the rotor: clockwise
No. of heads	1	
Step 1:	Dispense	Volume: 10.0 ml Repetitions: 10 Flow rate: 200.0 ml/min Pause: 2.0 s
Step 2:	Wait	Waiting time: manual
Step 3:	Pump	Flow rate: 100.0 ml/min Time: ∞
Step 4:	Pump	Flow rate: 100.0 ml/min Time: ∞
Step 5:	Dispense	Volume: 10.0 ml Repetitions: 5 Flow rate: 200.0 ml/min Pause: 2.0 s

Table 3: MPN-2 customized program.

Parameter	Value	Description of settings
Name	MPN-2	
Type	CUSTOMIZE	
Tubing ID	2.0 mm	Define the inner diameter of the tubing inserted
1.6-marking of clamps	4.8	Adjust the clamps for the tubing
Dispense direction	↻	The rotational direction of the rotor: clockwise
No. of heads	1	
Step 1:	Dispense	Volume: 1.0 ml Repetitions: 5 Flow rate: 50.0 ml/min Pause: 2.0 s
Step 2:	Wait	Waiting time: manual
Step 3:	Pump	Flow rate: 100.0 ml/min Time: ∞
Step 4:	Pump	Flow rate: 100.0 ml/min Time: ∞
Step 5:	Dispense	Volume: 1.0 ml Repetitions: 5 Flow rate: 50.0 ml/min Pause: 2.0 s
Step 6:	Wait	Waiting time: manual
Step 7:	Dispense	Volume: 10.0 ml Repetitions: 5 Flow rate: 150.0 ml/min Pause: 2.0 s

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