

## MPNplate™ Overview

The Colitag™ MPNplate™ is a simple, field-ready method to quantify Total Coliforms and *E. coli* in the range 0 – 1600 MPN/100mL. The approach is based on the 15-tube serial tube dilution method defined in the Standard Methods for the Examination of Water and Wastewater (APHA, AWWA).

The MPNplate™ replaces the time-consuming dilution approach by providing 15 individual wells split into three defined volumes of 10mL, 1mL and 0.1mL.

Sample mixed with Colitag™ reagent is poured into the MPNplate™ to fill the wells completely. The MPNplate™ is sealed and incubated for between 16 and 48 hours, depending on time required for result.

The results are determined by counting the number and size of yellow and fluorescent wells with quantification calculated by reference to the Most Probable Number table on page 4.

In addition to Colitag™ media and the MPNplate™, the test requires a sealing tool for the MPNplate film, an incubator and a long-wavelength UV lamp.

## MPNplate™ Operating Instructions

1

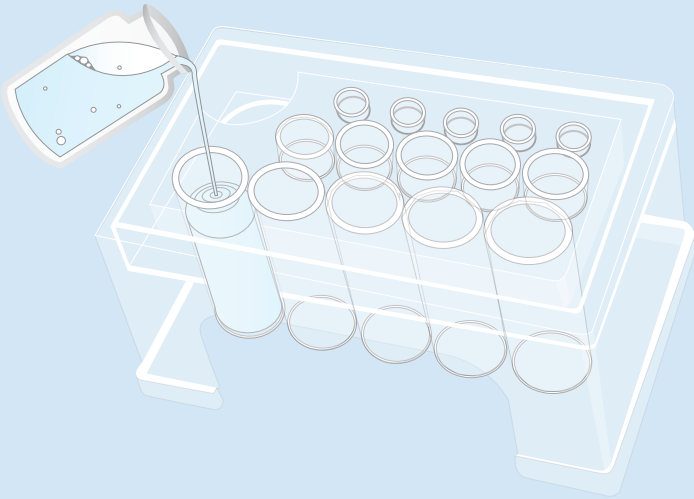


Add Colitag™ media to 100mL of sample in a sterile container and mix thoroughly to dissolve (shaking at least 25 times).

If samples are highly saline e.g. coastal recreational waters, dilute sample by a factor of 10 with sterile water before adding Colitag™ media. **Remember to adjust results accordingly!**

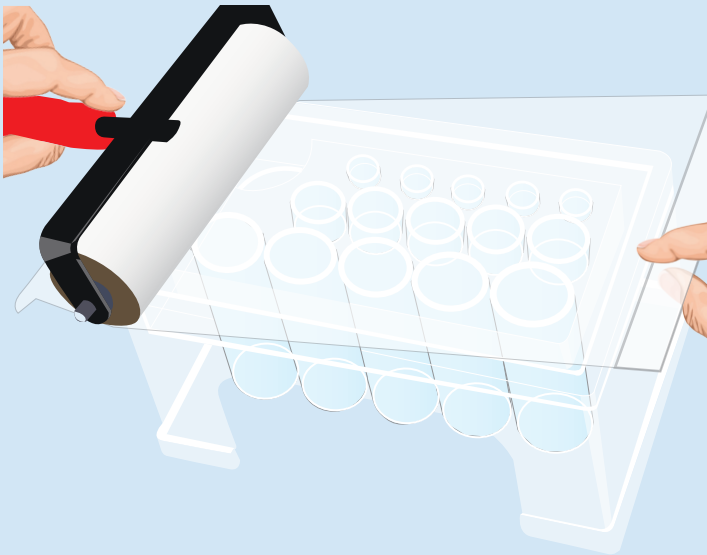
If following the **Accelerated Protocol, 16 – 48 hours**, pre-warm the sample at 44.5°C for 7 – 10 minutes, otherwise progress to Step 2.

2



Unwrap the MPNplate™ and place on a level surface. Starting with the large wells, fill the MPNplate™ wells to the very top and pour any additional sample over the top or directly into the overflow well.

3

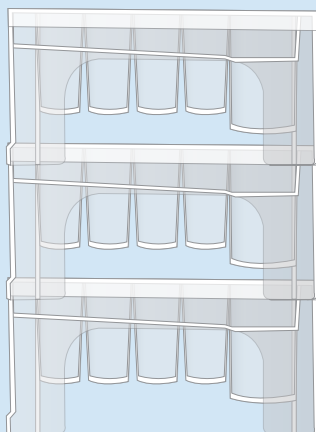


Take the sealing film and remove the thin backing strip at one end. Centre the film and attach the exposed adhesive edge of the sealing film to the side of the MPNplate™ to act as a fixing point.

Remove the remainder of the sealing film backing and, using the sealing tool, securely fix the film to the top of the MPNplate™, effectively sealing the individual wells. Avoid trapping any air bubbles in the wells. Any excess sample will flow automatically into the overflow well.

Remove the final backing strip and attach the final section of the sealing film to the opposite side to the first fixing point. The MPNplate™ is now secure and can be transported without further protection or incubated immediately.

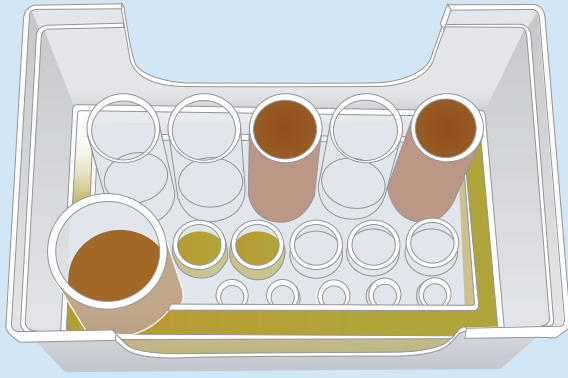
4



Place the sealed MPNplate™ into an incubator at  $35 \pm 0.5^\circ\text{C}$  for 24 – 48 hours if standard protocol has been followed, or 16 – 48 hours for accelerated protocol.

MPNplate™ devices can be stacked to save space within the incubator.

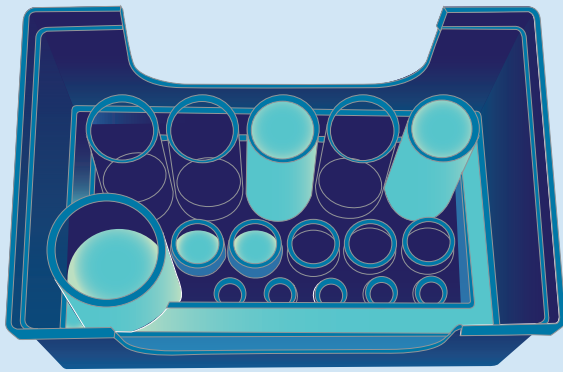
5



Invert the MPNplate™ following incubation and count the number of each well volume that show a positive yellow colouration equivalent to a control or the Colitag Comparator solution dispensed into an MPNplate™.

Positive wells indicate the presence of Total Coliforms and the number will be calculated based on the table in Step 7.

6



Illuminate the MPNplate™ with a long-wavelength UV lamp and count the number of each well volume that fluoresces at an equivalent intensity to the control standard or Colitag Comparator.

7

Use the numbers of positive wells of each volume with the table attached to report the Total Coliform and *E. coli* concentrations as MPN/100mL.

If all wells are negative, note also the yellow colour or fluorescence in the overflow well. If all wells including the overflow are negative, the result is <1 MPN/100mL. If the overflow well is positive, the result is 1 MPN/100mL.

## Spares and Consumables

Colitag™ Refill Pack (20 tests)	<b>CT220</b>
Colitag™ Refill Pack (100 tests)	<b>CT200</b>
120mL Sterile Sample Vessel with Thio (100 off)	<b>CT230</b>
120mL Sterile Sample Vessel w/out Thio (10 off)	<b>CT104</b>
120mL Sterile Sample Vessel w/out Thio (100 off)	<b>CT105</b>
Handheld UV lamp, Long Wavelength	<b>CT102</b>
Colitag™ MPNplate™ 1600 Most Probable Number device (100 off) including sealing film.	<b>CT260</b>
Sealing Tool for MPNplate™	<b>CT265</b>

# of Positive Wells				95% Confidence Limit	
10mL	1.0mL	0.1mL	MPN /100mL	Lower	Upper
0	0	0	<2		
0	0	1	2	<0.5	7
0	1	0	2	<0.5	7
0	2	0	4	<0.5	11
1	0	0	2	<0.5	7
1	0	1	4	<0.5	11
1	1	0	4	<0.5	11
1	1	1	6	<0.5	15
1	2	0	6	<0.5	15
2	0	0	5	<0.5	13
2	0	1	7	1	17
2	1	0	7	1	17
2	1	1	9	2	21
2	2	0	9	2	21
2	3	0	12	3	28
3	0	0	8	1	19
3	0	1	11	2	25
3	1	0	11	2	25
3	1	1	14	4	34
3	2	0	14	4	34
3	2	1	17	5	46
3	3	0	17	5	46
4	0	0	13	3	31
4	0	1	17	5	46
4	1	0	17	5	46
4	1	1	21	7	63
4	1	2	26	9	78
4	2	0	22	7	67
4	2	1	26	9	78
4	3	0	27	9	80
4	3	1	33	11	93
4	4	0	34	12	93

# of Positive Wells				95% Confidence Limit	
10mL	1.0mL	0.1mL	MPN /100mL	Lower	Upper
5	0	0	23	7	70
5	0	1	31	11	89
5	0	2	43	15	110
5	1	0	33	11	93
5	1	1	46	16	120
5	1	2	63	21	150
5	2	0	49	17	130
5	2	1	70	23	170
5	2	2	94	28	220
5	3	0	79	25	190
5	3	1	110	31	250
5	3	2	140	37	340
5	3	3	180	44	500
5	4	0	130	35	300
5	4	1	170	43	490
5	4	2	220	57	700
5	4	3	280	90	850
5	4	4	350	120	1000
5	5	0	240	68	750
5	5	1	350	120	1000
5	5	2	540	180	1400
5	5	3	920	300	3200
5	5	4	1600	640	5800
5	5	5	≥ 2400		