PURPOSE: The presence of heterotrophic bacteria in dialysate waters can lead to the development of a Gram negative toxin mediated pyrogenic reaction, bacteremia, and chronic inflammatory response syndrome. Hot tub waters are screened for the presence of *Pseudomonas aeruginosa*, the causative agent of a superficial skin infection known as "Hot tub folliculitis". The HPC SimPlate method utilizes enzyme technology to target the most common enzymes of waterborne bacteria. The by-product of the enzymatic reactions can be seen as fluorescence using a UV light. Additionally, hot tub waters have a MacConkey plate inoculated to aid in the detection of *Pseudomonas aeruginosa.*

SAMPLE INFORMATION:

Type	Hot tub water, Dialysate water
Volume	10mL +/- 0.2mL
Storage Requirements	Refrigerated
Criteria for rejection and follow up action	• <10 mL water received • Received >48 hours after collection

REAGENTS and/or MEDIA:

Type	SimPlate for HPC kit from IDEXX
Storage Requirements	• Store at 2-30°C away from light • Expiry date is printed on the box of media tubes

SUPPLIES:

- 10mL syringe
- UV light
- 35°C incubator

SPECIAL SAFETY PRECAUTIONS:

Containment Level 2 facilities, equipment, and operational practices for work involving infectious or potential infectious materials or cultures.

- Lab gown must be worn when performing activities with potential pathogens.
- Gloves must be worn when direct skin contact with infected materials is unavoidable.
- Eye protection must be used when there is a known or potential risk of exposure of splashes.
- All procedures that may produce aerosols, or involve high concentrations or large volumes should be conducted in a biological safety cabinet (BSC).
- The use of needles, syringes and other sharp objects should be strictly limited.

All patient specimens are assumed to be potentially infectious. Universal precautions must be followed. Since viable micro-organisms are used, all cultures must be handled with appropriate precautions. All equipment in contact with cultures should be decontaminated by appropriate methods.

QUALITY CONTROL:

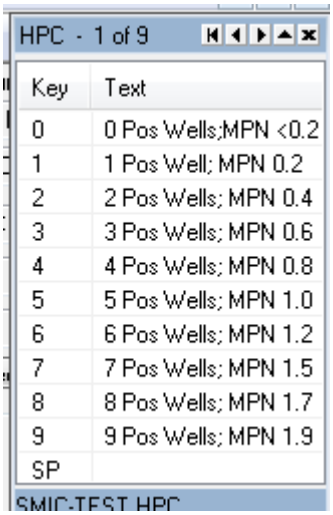
Performed per shipment or lot number:

- Refer to QC Procedure MIC60500 – Water quality control.
- A TQC order is automatically generated when new kit is entered into TQC to record the QC results.

PROCEDURE INSTRUCTIONS:

Step	Action
Performing HPC Water Testing	
1	Accession waters and select source. If source is HOT TUB or SPA WATER – HPC will automatically order and a MacConkey plate label will generate. Labels for total coliform testing will also print out for these waters.
2	Label requisition and sample containers.
3	Label sterile media tube (green top), SimPlate and MAC plate (where required)
4	Mix water. Use a 10 mL syringe to remove 10 mL of water from sample container. Add to green top tube and shake. Allow powder in tube to dissolve
5	Remove SimPlate lid and pour contents of green top tube onto the center of the plate base
6	Replace the lid and gently swirl to distribute the sample. Note: air bubbles do not interfere with test
7	Tip the plate at a 90° angle so the excess water will drain into the absorbent pad at the bottom of the plate
8	Invert the plate onto the plastic lid. On lid write R: (Date + 2 day) and time incubated
9	If water source is HOT TUB, perform total coliform testing as well using the Colilert-18 kit and processed as above.
10	If water is HOT TUB, aliquot off an additional 1mL of water and flood a labeled MAC plate - incubate along with the SimPlate (35°C for 48hrs)

INTERPRETATION AND REPORTING:

1	While viewing under the **UV lamp** - count the number of wells showing fluorescence.
2	Using the HPC keypad, select the number of wells that show fluorescence. This will automatically calculate the MPN (most probably number) of heterotrophic plate count bacteria in the original sample.  HPC - 1 of 9 Key / Text 0 0 Pos Wells;MPN <0.2 1 1 Pos Well; MPN 0.2 2 2 Pos Wells; MPN 0.4 3 3 Pos Wells; MPN 0.6 4 4 Pos Wells; MPN 0.8 5 5 Pos Wells; MPN 1.0 6 6 Pos Wells; MPN 1.2 7 7 Pos Wells; MPN 1.5 8 8 Pos Wells; MPN 1.7 9 9 Pos Wells; MPN 1.9 SP SMIC-TEST HPC Report this number as the HPC.
3	HOT TUB/SPA WATERS: • Look for growth of *Pseudomonas aeruginosa* – Non-lactose fermenter, oxidase positive – send to VITEK for GNI • Report as: "Isolated: *Pseudomonas aeruginosa*" • If MAC plate is negative- Report as: "No *Pseudomonas aeruginosa* isolated"

APPENDIX:

Unit-Dose
SimPlate For HPC
Most Probable Number (MPN) Table

# Positive Wells	MPN	95% confidence limits lower	upper
0	<0.2	<0.03	<1.4
1	0.2	0.0	1.4
2	0.4	0.1	1.6
3	0.6	0.2	1.9
4	0.8	0.3	2.2
5	1.0	0.4	2.5
6	1.2	0.6	2.7
7	1.5	0.7	3.0
8	1.7	0.8	3.3
9	1.9	1.0	3.6
10	2.1	1.1	3.9
11	2.3	1.3	4.2
12	2.6	1.5	4.5
13	2.8	1.6	4.8
14	3.0	1.8	5.1
15	3.3	2.0	5.4
16	3.5	2.2	5.8
17	3.8	2.3	6.1
18	4.0	2.5	6.4
19	4.3	2.7	6.7
20	4.5	2.9	7.0
21	4.8	3.1	7.4
22	5.1	3.3	7.7
23	5.3	3.5	8.0
24	5.6	3.8	8.4
25	5.9	4.0	8.7
26	6.2	4.2	9.1
27	6.5	4.4	9.4
28	6.8	4.7	9.8
29	7.1	4.9	10.2
30	7.4	5.1	10.6
31	7.7	5.4	10.9
32	8.0	5.6	11.3
33	8.3	5.9	11.7
34	8.6	6.2	12.1
35	9.0	6.4	12.6
36	9.3	6.7	13.0
37	9.7	7.0	13.4
38	10.0	7.3	13.9
39	10.4	7.6	14.3
40	10.8	7.9	14.8
41	11.2	8.2	15.2
42	11.6	8.5	15.7

# Positive Wells	MPN	95% confidence limits lower	upper
43	12.0	8.8	16.2
44	12.4	9.1	16.7
45	12.8	9.5	17.3
46	13.2	9.8	17.8
47	13.7	10.2	18.3
48	14.1	10.6	18.9
49	14.6	10.9	19.5
50	15.1	11.3	20.1
51	15.6	11.7	20.7
52	16.1	12.1	21.3
53	16.6	12.5	22.0
54	17.1	13.0	22.7
55	17.7	13.4	23.4
56	18.3	13.9	24.1
57	18.9	14.4	24.9
58	19.5	14.9	25.7
59	20.2	15.4	26.5
60	20.9	15.9	27.3
61	21.6	16.5	28.2
62	22.3	17.1	29.2
63	23.1	17.7	30.2
64	23.9	18.3	31.2
65	24.8	19.0	32.3
66	25.7	19.7	33.5
67	26.6	20.4	34.7
68	27.6	21.2	36.1
69	28.7	22.0	37.5
70	29.9	22.9	39.0
71	31.1	23.8	40.7
72	32.4	24.8	42.5
73	33.9	25.8	44.4
74	35.5	27.0	46.6
75	37.2	28.2	49.1
76	39.2	29.6	51.9
77	41.4	31.1	55.1
78	44.0	32.8	58.9
79	47.0	34.8	63.6
80	50.7	37.1	69.5
81	55.5	39.8	77.5
82	62.3	43.2	89.9
83	73.8	47.6	114.6
84	>73.8	>47.6	>114.6

MPN is per ml of the 10 ml sample added to the media tube (pour-off is accounted for).

REFERENCES:

- *SimPlate for HPC Water Testing Product Information*. (n.d.). Retrieved from www.idexx.ca/view/xhtml/en_ca/water/simplate.jsf.

REVISION HISTORY:

REVISION	DATE	Description of Change	REQUESTED BY
1.0	31Dec2013	Initial Release	A.Darrach
1.1	31March2016	Reviewed – No Changes	C. Russell
2.0	12-May-2017	Reviewed and revised. Safety precautions and reagent storage requirements added; Updated format; New document number (old number MIC52615)	L. Steven