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 byproduct 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
Stability	48hrs, refrigerated
Storage Requirements	2-8°C (refrigerated)
Criteria for rejection and follow up action	Waters: <10mL and/or >48hrs old

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 potentially 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 where there is a known or potential risk of exposure to 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.

QUALITY CONTROL:

Performed per shipment or Lot number –

- Please refer to Procedure **MIC60400WaterQC**
- 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 under the appropriate name – see LIS Water list. The LIS test code should already populate as waters are set up as Standing Orders. If this is a new water client – see Tech2 **LIS CODE: TSWAT, DIAST or DIAFS(Fort Smith only)**
2	Select Source(where required) – if Source is **HOT TUB** or **SPA WATER** – HPC will automatically order and a MacConkey (MAC) plate label will generate
3	If water is chlorinated – treat with sodium thiosulfate prior to analysis. Sample containers from Stanton contain sodium thiosulfate
4	Label requisition and samples
5	Label sterile media tube (green top), SimPlate and MAC plate (where required)
6	Mix water sample
7	Using 10mL syringe – aliquot off 10mL. Add to green top tube and shake. Allow powder in tube to dissolve
8	Remove SimPlate lid and pour in contents of green top tube onto the center of the plate base.
9	Replace lid and gently swirl to distribute the sample **NOTE:** Air bubbles do not interfere with test
10	Tip the plate at a 90 degree angle so the excess water will drain into the absorbent at the bottom
11	Invert the plate onto the plastic lid and incubate at **35°C for 48hrs.** Results can be read from 45-72 hours from the start of incubation
12	While viewing under the **UV lamp** - count the number of wells showing fluorescence
13	Refer to the MPN table to determine the Most Probable Number of heterotrophic plate count bacteria in the original sample – see attached chart. THE LIS SYSTEM CALCULATES THIS FOR YOU – CHOOSE THE NUMBER OF POSITIVE WELLS IN THE KEYPAD
14	**HOT TUB/SPA WATERS –** Aliquot off an additional 1mL of water and flood a labeled MAC plate - incubate along with the SimPlate (35°C for 48hrs) • 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"

REFERENCES:

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

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
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).

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 formate; New document number (old number MIC52615)	L. Steven

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Signed by: Jennifer G. Daley Bernier