NORTHWEST TERRITORIESStanton Territorial HospitalNORTHWEST TERRITORIESP.O. Box 10, 550 Byrne RoadHealth and Social Services AuthorityYELLOWKNIFE NT X1A 2N1	Document Number: MIC:	Document Number: MIC10315	
	Version No: 2.0	Page: 1 of 6	
	Distribution:	Distribution:	
	Microbiology Specimen Processing		
	Effective: 12 May, 2017		
Document Name:	Date Reviewed: 12 May, 2017		
Water Testing – HPC Unit Dose SimPlate Method	Next Review: 12 May, 2019		
Approved By: Jennifer G. Daley Bernier, A/Manager, Laboratory Services	Status: APPROVED		

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:

Туре	Hot tub water, Dialysate water		
Volume	10mL +/- 0.2mL		
Stability	48hrs, refrigerated		
Storage	2-8°C (refrigerated)		
Requirements	2-0 C (reingerated)		
Criteria for rejection	Waters: <10mL and/or >48hrs old		
and follow up action			

REAGENTS and/or MEDIA:

Туре	SimPlate for HPC kit from IDEXX		
Storage	 Store at 2-30°C away from light 		
Requirements	 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
Perfo	rming HPC Water Testing
	Accession waters under the appropriate name – see LIS Water list. The LIS test code
1	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
2	automatically order and a MacConkey (MAC) plate label will generate
3	If water is chlorinated – treat with sodium thiosulfate prior to analysis.
Ŭ	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
Ū	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
	Refer to the MPN table to determine the Most Probable Number of heterotrophic plate
13	count bacteria in the original sample – see attached chart.
10	THE LIS SYSTEM CALCULATES THIS FOR YOU – CHOOSE THE NUMBER OF
	POSITIVE WELLS IN THE KEYPAD
	HOT TUB/SPA WATERS – Aliquot off an additional 1mL of water and flood a labeled
14	MAC plate - incubate along with the SimPlate (35°C for 48hrs)
	Look for growth of <i>Pseudomonas aeruginosa</i> – Non-lactose fermenter, oxidase

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	positive – send to VITEK for GNI		
Report as: "Isolated: <i>Pseudomonas aeruginosa</i> "			

• 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	MPN	95% confidence limits		
Wells		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 28	6.5	4.4	9.4	
	6.8	4.7	9.8	
29	7.1	4.9	10.2	
30 31	7.4	5.1 5.4	10.6 10.9	
31	8.0	5.6	11.3	
32	8.0	5.9	11.3	
34	8.6	6.2	12.1	
35	9.0	6.4	12.6	
35	9.0	6.4	12.6	
36	9.3	7.0	13.4	
38	10.0	7.3	13.9	
39	10.4	7.6	14.3	
40	10.4	7.9	14.8	
40	11.2	8.2	15.2	
42	11.6	8.5	15.7	
42	11.0	0.0	10.7	

Wells 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 </th <th># Positive</th> <th>MPN</th> <th colspan="2">95% confidence limits</th>	# Positive	MPN	95% confidence limits	
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82 62.3 43.2 89.9 83 73.8 47.6 114.6				
83 73.8 47.6 114.6				
	83			
		>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).

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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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Jennifer G. Daley Bernier, R.T. (CSMLS) A/ Manager, Laboratory Services Signed by: Jennifer G. Daley Bernier