

**Supplementary Materials:** The following supporting information can be downloaded at: [www.mdpi.com/xxx/s1](http://www.mdpi.com/xxx/s1). **Video S1.** Example timelapse video of *Acanthamoeba* movement in experimental conditions during hour 18 (last hour of AC6 recovery), to demonstrate the differences in movement and possible recovery of amoebae after disinfection. Scale bar = 10mm. White arrows indicate remaining moving amoebae. Example calculations (using the representative videos in this figure) demonstrating the difference in using the average total distance moved in a frame vs. the relative units of net number of microns moved:

One-quarter Ringer's solution:

Moving amoebae: 409

Average total distance of each amoeba: 457  $\mu\text{m}$

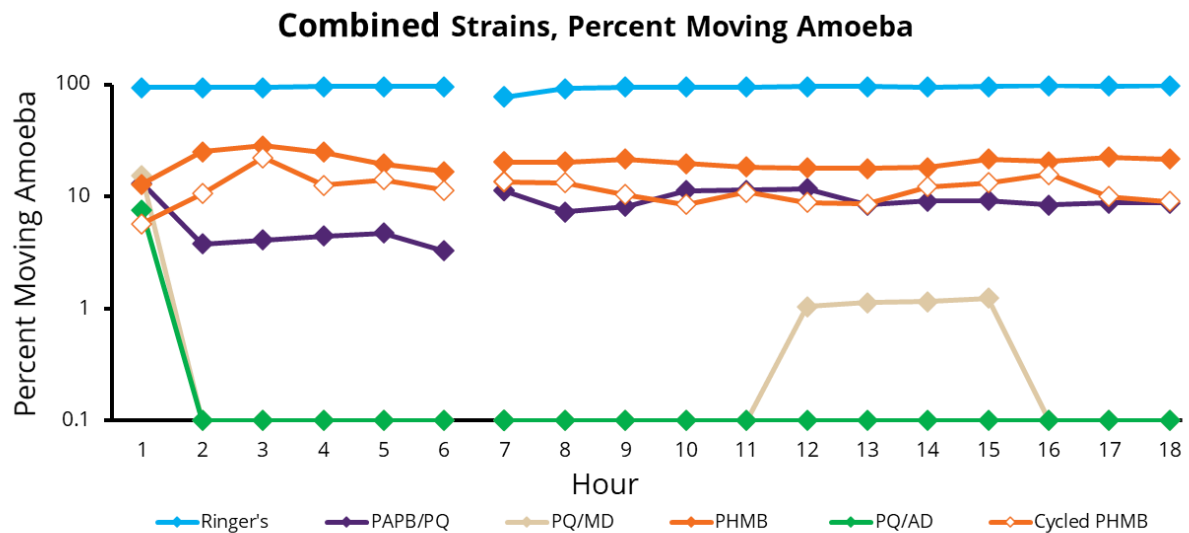
409 amoebae  $\times$  457  $\mu\text{m}$  = 186,913  $\mu\text{m}$  net microns moved by amoebae in the field of view over one hour

PAPB/PQ:

Moving amoebae: 33

Average total distance of each amoeba: 197  $\mu\text{m}$

33 amoebae  $\times$  197  $\mu\text{m}$  = 6,501  $\mu\text{m}$  net microns moved by amoebae in the field of view over one hour



**Figure S1.** Percentage of amoebae which are moving in any one field of view, with both strains combined. Amoebae were disinfected or put in one-quarter Ringer's solution for the first 6 h, followed by AC6 media for the following 12 h (total 18 h). Shown in log scale to visualize differences between conditions.  $n = 3/\text{group}/\text{strain}$ .

**A**

Total Distance					
50370	CLC			AC6	
Hour	1	3	6	12	18
Ringer's	172182.1	143914.7	149368.3	140863.5	135344.6
PHMB	9402.6	36382.8	22693.2	49843.7	73428.4
Cycled PHMB	1129.2	39336.4	32226.1	44716.0	43024.0
PAPB/PQ	213.2	425.1	1689.2	7972.8	7824.5
PQ/MD	607.9	0.0	0.0	933.2	367.3
PQ/AD	138.4	0.0	0.0	0.0	0.0

**B**

Total Distance					
30461	CLC			AC6	
Hour	1	3	6	12	18
Ringer's	156380.5	140067.0	119209.2	98284.6	92958.2
PHMB	38924.3	51017.6	18116.2	26131.0	20736.1
Cycled PHMB	50081.2	91675.0	30811.4	9112.2	8384.0
PAPB/PQ	17124.9	1722.9	166.8	8001.7	1598.4
PQ/MD	17667.4	0.0	0.0	121.9	167.7
PQ/AD	5771.6	0.0	0.0	0.0	0.0

**C**

Speed					
50370	CLC			AC6	
Hour	1	3	6	12	18
Ringer's	47.9	40.2	41.7	39.4	45.2
PHMB	2.6	10.2	6.3	13.9	20.5
Cycled PHMB	0.3	11.0	9.0	12.5	12.0
PAPB/PQ	0.0	0.1	0.5	2.2	2.2
PQ/MD	0.2	0.0	0.0	0.3	0.1
PQ/AD	0.0	0.0	0.0	0.0	0.0

**D**

Speed					
30461	CLC			AC6	
Hour	1	3	6	12	18
Ringer's	43.5	39.1	33.3	27.4	24.1
PHMB	10.8	14.3	5.1	7.3	5.8
Cycled PHMB	13.9	25.6	8.6	2.6	2.3
PAPB/PQ	4.8	0.5	0.1	2.2	0.4
PQ/MD	4.9	0.0	0.0	0.0	0.1
PQ/AD	1.6	0.0	0.0	0.0	0.0

**Figure S2.** Data tables summarizing numerical findings at key time points, from Figures 4 and 6. Amoebae were disinfected or put in one-quarter Ringer's solution for the first 6 h, followed by AC6 media for the following 12 h (total 18 h). (**A,B**) Normalized total distance is presented as mean  $\mu\text{m}$  traveled per hour, multiplied by the number of moving amoebae per field of view. (**C,D**) Speed is presented as mean  $\mu\text{m}$  per second, multiplied by the number of moving amoebae per field of view (relative units).  $n = 3/\text{group}$ . Relevant standard error and statistical comparisons presented in Figures 4 and 6.