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Preparation, Separation,
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Application Note

HY-LiTE® Plus – Testing of Biocide efficacy

The method described can be used to demonstrate the speed and mode of action of different Biocides.

Materials

HY-LiTE® Plus ATP pens (1.30895.0021)

HY-LiTE® Free ATP pens (1.30194.0021) – Optional

Biocides for evaluation

- Water samples: naturally contaminated or spiked with bacterial cultures.
- Disposable sample containers for HY-LiTE testing (e.g. Bijou, 2 mL Microtube or 1.5 mL microcentrifuge tube)
- Disposable plastic pipettes (optional)
- Clean Plastic bottles / jars for incubation of samples with biocide

Equipment

HY-LiTE® 2 Luminometer (1.30100.0001)

Incubator at appropriate temperature e.g. 35 °C (Optional)

Method

- Prepare appropriate stock dilutions of Biocide to be tested, e.g.
- If a biocide is to be tested at 50, 100 and 200 ppm, prepare stock solutions at 100 x these concentrations.
- Collect a sample of the water to be treated (or for general lab evaluations, use sterile, distilled water spiked with bacterial cultures). This should preferably give HY-LiTE readings between 5000 and 50000 RLU.
- Mix well and subdivide into 4 aliquots of 100 mL.
- Label these as Control, 50, 100 and 200 (or as appropriate)
- To sample Control, add 1% of water (same as used to dilute biocide). Mix well. Draw subsamples and perform test of total and free ATP. Record as Control, Time = 0.
- To each of the other samples add 1 % of appropriate stock solution. Mix well. Start stop- watch. Place at appropriate temperature.
- At appropriate time intervals (e.g. 2, 60 and 120 minutes after Time = 0), mix each of the “test” samples and with a pipette, transfer sub-samples to two separate containers for testing with Total respectively Free ATP pens. Return remaining sample to incubator (if used) and leave until next test time.

Note: It is recommended not to sample for Total and Free ATP in the same sample! If this is never the less to be done (e.g. due to limited amount of sample), the sample MUST be tested with the Free ATP pen first!

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Example:

Biocide X Temperature: 22 °C

		Concentration*							
		Control (0ppm)		50 ppm		100 ppm		200 ppm	
		Total (RLU)	Free (RLU)	Total (RLU)	Free (RLU)	Total (RLU)	Free (RLU)	Total (RLU)	Free (RLU)
Contact time (min)*	0	500	69	500	69	500	69	500	69
	0	620	90	620	90	620	90	620	90
	2	610	95	580	210	750	350	740	510
	2	670	84	660	250	670	430	560	590
	60	1800	140	240	170	300	320	400	350
	60	1200	180	280	210	240	300	280	470
	120	2500	100	140	150	170	160	260	340
	120	3700	140	180	110	210	240	280	280

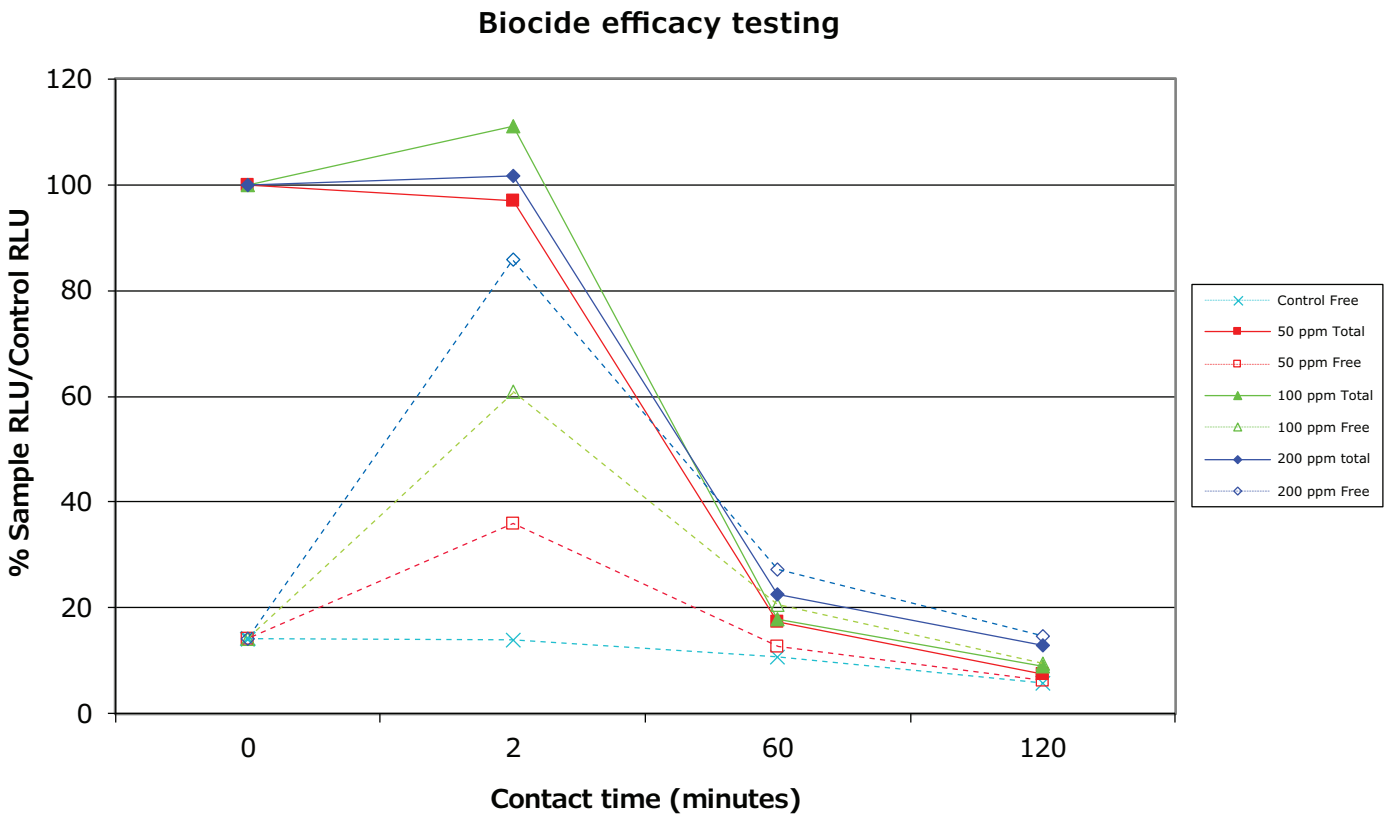
* Contact times and Biocide concentrations shown are only examples and may need adjusting.

Calculate the RLU for Control (Free) and all other samples as % of Control (Total), and plot this against the contact time (c.f. **Fig. 1**).

Fig 1:

Example of results of Biocide efficacy test.

The biggest relative change (on short contact time) is seen for 200 ppm Biocide (Free ATP), indicating that this is the most effective dose.



Notes:

- It is recommended that all ATP testing be performed in duplicate.
- If several concentrations of Biocide are tested, the start of each "test" sample should be staggered by staggering the addition of biocide, to obtain accurate contact. Alternatively, the actual contact times must be recorded.
- Concentrations and contact times will vary between different Biocides.
- ATP measurements essentially measure bacteriostatic effects, whereas Viable Count determinations measure only bactericidal effects. The results will therefore not necessarily correlate with Viable Counts.
- Biocide efficacy may be highly temperature dependant. It is therefore important to record and control the temperature.
- The effect of biocides on planktonic (free-swimming) micro-organisms are often much higher than on micro-organisms in a Biofilm. If biofilms are present in the system, the concentration of Biocide may therefore need to be adjusted accordingly.

Note:

Contact times and concentrations above are only examples.

Select appropriate dosage and contact times based on suppliers recommendations, previous experience or as dictated by processes and circumstances.

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MS_AN13236EN Ver. 1.0
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01/2024