

Letheen Stability Experiment

Aims:

1. Determine what will grow in Letheen broth of varying ages
2. Test the efficacy of the neutralization of the Letheen broths

Broth samples tested:

Broth	Date of Manufacture	Age (at time of testing)	Batch Number	Storage Conditions
Letheen 1	2nd Nov 2009	4 months	104509	4°C
Letheen 2	30th April 2009	10 months	31509	4°C
Letheen 3	27th Oct 2008	16 months	94608	4°C
Letheen 4	6th Jan 2008	26 months	05508	RT in dark
Letheen 5	1st Nov 2007	29 months	72807	RT in light
TSB Control	n/a	n/a	n/a	n/a

Method 1: What will grow in the Letheen?

1. Each batch of Letheen and the TSB control was inoculated with ~1000 of the following organisms:
 - a. Salmonella
 - b. Citrobacter
 - c. MRSA
 - d. *E. coli*
 - e. Listeria
2. The Letheen samples were then incubated at 37°C for 24 hours
3. To determine whether the organisms had been able to grow in the Letheen samples, their optical density was measured at 550nm on the spectrophotometer to indicate turbidity and therefore growth.

Method 2:

Does the age of the Letheen determine its neutralizing effect against benzethonium chloride?
At what concentration of benzethonium chloride (NRM) does the neutralizing effect become inhibited?

Benzethonium chloride (NRM) is a quaternary ammonium compound that needs to be neutralized for bacterial growth to occur.

1. Each batch of Letheen and the TSB control were inoculated with 1000 *E. coli* and a range of

- benzethonium chloride concentrations (0.009% - 0.05%)
2. These were incubated at 37°C for 24 hours to determine which concentration of benzethonium chloride would inhibit the neutralizing effect of the Letheen and therefore inhibit growth of the *E. coli*.
 3. The turbidity of the Letheen samples was measured in optical density on the spectrophotometer (550nm).
 4. 10µl of the TSB control samples were added to UltraSnap devices and read in the Ensure instrument to determine if there was any *E.coli* growth present.

Results:

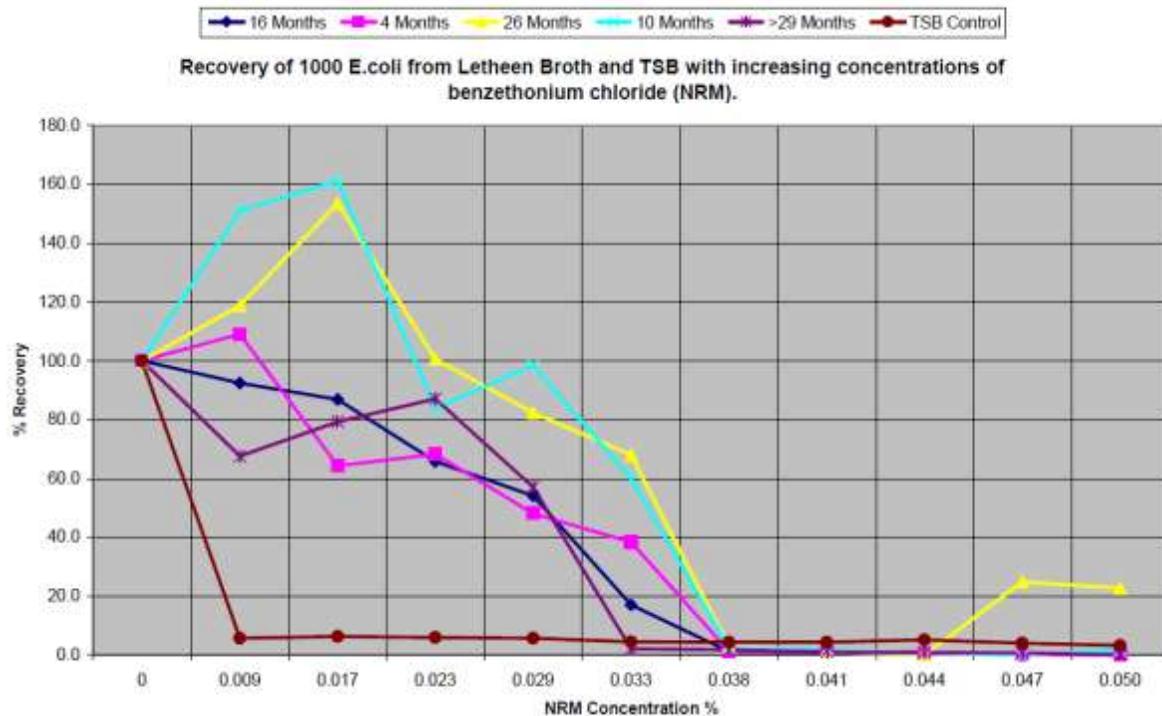
1. What will grow in the Letheen?

Optical density measured on the spectrophotometer for each inoculated Letheen batch and the TSB control:

Letheen Batch Age	Salmonella	Citrobacter	MRSA	<i>E. coli</i>	Listeria
4 Months	0.40	0.55	0.63	0.84	0.59
10 Months	0.40	0.53	0.72	0.74	0.62
16 Months	0.52	0.72	0.69	0.87	0.57
26 Months	0.47	0.51	0.66	0.71	0.59
29 Months	-0.02	0.78	-0.02	1.04	0.70
TSB Control	1.76	1.37	1.76	1.47	1.67

Only the Salmonella and MRSA were unable to grow in the oldest batch of Letheen, the other batches of Letheen grew all the organisms.

2. Does the Lethen lose its neutralizing ability as it gets older? At what concentration does the benzethonium chloride inhibit Lethen neutralization?



The purple line represents the oldest batch of Lethen that has been stored at room temperature in the light. This Lethen loses its neutralizing ability at a benzethonium chloride concentration of 0.033%.

In comparison, the pink line represents the most recent batch of Lethen which has been stored at 4°C. This Lethen loses its neutralizing ability at a benzethonium chloride concentration of 0.038%. The brown line represents the control where Lethen was replaced with TSB; this broth does not have the neutralizing effect of the Lethen as demonstrated by the lack of growth of *E. coli* in all the samples containing benzethonium chloride.

Summary:

- The oldest batch of Lethen is still viable but was unable to grow Salmonella and MRSA suggesting it is no longer suitable for use. Lethen of this age that has been stored at 4°C and not room temperature would perform better.
- The Lethen batch 4 that has been stored for 26 months at room temperature still works well, suggesting it would be viable to extend the shelf life of Lethen up to 26 months at room

temperature or 4°C.

- In the neutralizing test the oldest batch of Letheen also performed well; even though it lost its neutralizing ability sooner than the more recent Letheen batches the concentration of benzethonium chloride that causes the inhibition (0.033%) is still higher than the concentration that is commonly used (see table below). It is only when quaternary ammonium compounds are used at a higher than normal concentration and a large sample (e.g. 100µl) is picked up on the swab that the inhibition of Letheen neutralization would be a problem.

This table contains examples of other Quaternary Ammonium Compounds (QACs) and their resultant concentration (%) if 10µl and 100µl is picked up on the swab. The normal recommended dilution is 1:256; where a higher concentration is required a 1:64 dilution is recommended.

QACs	10 µL pick up on swab from surface		100µl pick up on swab from surface	
	1 in 64	1 in 256	1 in 64	1 in 256
Roccal D	0.0036%	0.0009%	0.036%	0.009%
Barquot 50	0.008%	0.002%	0.08%	0.02%
Cetylcide II	0.004%	0.001%	0.04%	0.01%

If 100µl of the QACs diluted 1:64 were picked up (highlighted in blue in the table) on a Letheen Q swab the Letheen would no longer work properly and would lose its neutralizing effect.