

Chapter III

RESULTS

Inoculum Sizes

Calibrating concentrations of organisms used for the inoculum with turbidities, the results of turbidities for 1:100 dilutions of each organism harvested from agar slants were obtained and shown in Table 2, page 40. These dilutions were further diluted for counting. From the turbid measured dilutions, the bacteria were diluted 10^7 times, and the yeast and mold were diluted 10^6 times. The three last dilutions of each were counted for viable organisms. The results were calculated to be concentrations of the turbid measured dilutions. These results are shown in Table 2, page 40.

When they were in use, the microorganism suspensions were calibrated to the turbidities of those shown in the table. Then, the bacteria were diluted 10 times but the yeast and mold were used in such concentrations.

Results of the Challenge Tests of Samples

After incubation of inoculated samples at room temperature, the counts per ml of each were made. The results are shown in Table 3, page 41, to Table 37, page 75.

The results stated to be less than 100 organisms per ml means

Table 2
Test Organisms Calibration

Test Organisms	Turbidity at 546 nm.	Count per ml.
<u>Staphylococcus aureus</u>	0.183	2.2×10^9
<u>Pseudomonas aeruginosa</u>	0.095	1.8×10^9
<u>Escherichia coli</u>	0.125	1.6×10^9
<u>Aspergillus niger</u>	0.530	0.7×10^8
<u>Candida albicans</u>	0.290	1.5×10^8

that there was no growth obtained from duplicate plates of 1:10 dilutions used in counting method. If one colony was found from one plate of that dilution, the expression is 0.5×10^2 organisms per ml.

It was found from this study that all samples tested met the requirements of preservative efficiency testing. All bacteria were not found on the seventh day of test period, and yeast and mold were not found also, except in Sample No. 11, 13, 14, and 15. In these four samples, C. albicans were found but the amounts were less than initial concentrations and were not found when the samples were tested on the fourteenth, twenty first, and twenty eighth days of the test period.

Table 3
Challenge Tests of Sample No. 1 to 8 with S. aureus

Sample No.	Average count per ml after incubation for				
	0 day	7 days	14 days	21 days	28 days
1	0.9×10^6	< 100	< 100	< 100	< 100
	1.2×10^6	< 100	< 100	< 100	< 100
2	1.4×10^6	< 100	< 100	< 100	< 100
	1.2×10^6	< 100	< 100	< 100	< 100
3	1.1×10^6	< 100	< 100	< 100	< 100
	1.4×10^6	< 100	< 100	< 100	< 100
4	1.7×10^6	< 100	< 100	< 100	< 100
	1.0×10^6	< 100	< 100	< 100	< 100
5	1.2×10^6	< 100	< 100	< 100	< 100
	2.2×10^6	< 100	< 100	< 100	< 100
6	0.8×10^6	< 100	< 100	< 100	< 100
	1.3×10^6	< 100	< 100	< 100	< 100
7	0.7×10^6	< 100	< 100	< 100	< 100
	1.8×10^6	< 100	< 100	< 100	< 100
8	2.5×10^6	< 100	< 100	< 100	< 100
	1.7×10^6	< 100	< 100	< 100	< 100



Table 4

Challenge Tests of Sample No. 1 to 8 with Ps. aeruginosa

Sample No.	Average count per ml after incubation for				
	0 day	7 days	14 days	21 days	28 days
1	2.2×10^6	<100	<100	<100	<100
	2.5×10^6	<100	<100	<100	<100
2	3.1×10^6	<100	<100	<100	<100
	2.4×10^6	<100	<100	<100	<100
3	1.8×10^6	<100	<100	<100	<100
	2.7×10^6	<100	<100	<100	<100
4	1.4×10^6	<100	<100	<100	<100
	1.0×10^6	<100	<100	<100	<100
5	1.2×10^6	<100	<100	<100	<100
	2.0×10^6	<100	<100	<100	<100
6	1.1×10^6	<100	<100	<100	<100
	1.0×10^6	<100	<100	<100	<100
7	1.7×10^6	<100	<100	<100	<100
	2.2×10^6	<100	<100	<100	<100
8	0.8×10^6	<100	<100	<100	<100
	2.0×10^6	<100	<100	<100	<100

Table 5

Challenge Tests of Sample No. 1 to 8 with E. coli

Sample No.	Average count per ml after incubation for				
	0 day	7 days	14 days	21 days	28 days
1	1.7×10^6	<100	<100	<100	<100
	2.5×10^6	<100	<100	<100	<100
2	2.5×10^6	<100	<100	<100	<100
	2.9×10^6	<100	<100	<100	<100
3	1.0×10^6	<100	<100	<100	<100
	2.7×10^6	<100	<100	<100	<100
4	0.7×10^6	<100	<100	<100	<100
	3.0×10^6	<100	<100	<100	<100
5	1.4×10^6	<100	<100	<100	<100
	3.7×10^6	<100	<100	<100	<100
6	0.9×10^6	<100	<100	<100	<100
	1.1×10^6	<100	<100	<100	<100
7	0.7×10^6	<100	<100	<100	<100
	0.9×10^6	<100	<100	<100	<100
8	1.2×10^6	<100	<100	<100	<100
	3.0×10^6	<100	<100	<100	<100

Table 6

Challenge Tests of Sample No. 1 to 8 with C. albicans

Sample No.	Average count per ml after incubation for				
	0 day	7 days	14 days	21 days	28 days
1	0.8×10^6	<100	<100	<100	<100
	1.1×10^6	<100	<100	<100	<100
2	0.65×10^6	<100	<100	<100	<100
	0.9×10^6	<100	<100	<100	<100
3	1.2×10^6	<100	<100	<100	<100
	1.1×10^6	<100	<100	<100	<100
4	1.8×10^6	<100	<100	<100	<100
	2.0×10^6	<100	<100	<100	<100
5	1.2×10^6	<100	<100	<100	<100
	1.0×10^6	<100	<100	<100	<100
6	1.5×10^6	<100	<100	<100	<100
	1.9×10^6	<100	<100	<100	<100
7	2.1×10^6	<100	<100	<100	<100
	2.9×10^6	<100	<100	<100	<100
8	0.7×10^6	<100	<100	<100	<100
	2.5×10^6	<100	<100	<100	<100

Table 7

Challenge Tests of Sample No. 1 to 8 with A. niger

Sample No.	Average count per ml after incubation for				
	0 day	7 days	14 days	21 days	28 days
1	0.5×10^6	<100	<100	<100	<100
	0.7×10^6	<100	<100	<100	<100
2	0.8×10^6	<100	<100	<100	<100
	0.7×10^6	<100	<100	<100	<100
3	0.6×10^6	<100	<100	<100	<100
	0.9×10^6	<100	<100	<100	<100
4	1.0×10^6	<100	<100	<100	<100
	0.6×10^6	<100	<100	<100	<100
5	1.0×10^6	<100	<100	<100	<100
	0.5×10^6	<100	<100	<100	<100
6	1.1×10^6	<100	<100	<100	<100
	1.2×10^6	<100	<100	<100	<100
7	1.8×10^6	<100	<100	<100	<100
	0.7×10^6	<100	<100	<100	<100
8	0.7×10^6	<100	<100	<100	<100
	0.8×10^6	<100	<100	<100	<100

Table 8

Challenge Tests of Sample No. 9 to 15 with S. aureus

Sample No.	Average count per ml after incubation for				
	0 day	7 days	14days	21days	28days
9	0.8×10^6	<100	< 100	< 100	< 100
	1.5×10^6	<100	< 100	< 100	< 100
10	1.7×10^6	<100	< 100	< 100	< 100
	1.9×10^6	<100	< 100	< 100	< 100
11	1.1×10^6	<100	< 100	< 100	< 100
	1.9×10^6	<100	< 100	< 100	< 100
12	1.2×10^6	<100	< 100	< 100	< 100
	1.0×10^6	< 100	< 100	< 100	< 100
13	0.8×10^6	<100	< 100	< 100	< 100
	0.7×10^6	< 100	< 100	< 100	< 100
14	1.9×10^6	<100	< 100	< 100	< 100
	2.5×10^6	<100	< 100	< 100	< 100
15	3.0×10^6	<100	< 100	< 100	< 100
	1.1×10^6	<100	< 100	< 100	< 100

Table 9

Challenge Tests of Sample No. 9 to 15 with Ps. aeruginosa

Sample No.	Average count per ml after incubation for				
	0 day	7 days	14. days	21days	28 days
9	2.0×10^6	<100	< 100	< 100	< 100
	1.5×10^6	<100	< 100	<100	< 100
10	1.1×10^6	<100	< 100	< 100	<100
	2.9×10^6	<100	<100	<100	< 100
11	3.5×10^6	<100	< 100	<100	<100
	1.0×10^6	<100	<100	<100	<100
12	3.7×10^6	<100	<100	<100	<100
	0.9×10^6	<100	<100	<100	<100
13	1.7×10^6	<100	<100	< 100	<100
	1.4×10^6	<100	<100	<100	<100
14	1.5×10^6	<100	<100	<100	<100
	2.7×10^6	<100	<100	<100	<100
15	3.5×10^6	<100	< 100	<100	<100
	0.9×10^6	<100	<100	<100	<100

Table 10

Challenge Tests of Sample No. 9 to 15 with E. coli

Sample No.	Average count per ml after incubation for				
	0 day	7 days	14 days	21 days	28 days
9	3.3×10^6	< 100	< 100	< 100	< 100
	3.0×10^6	< 100	< 100	< 100	< 100
10	2.7×10^6	< 100	< 100	< 100	< 100
	2.1×10^6	< 100	< 100	< 100	< 100
11	3.8×10^6	< 100	< 100	< 100	< 100
	1.0×10^6	< 100	< 100	< 100	< 100
12	3.5×10^6	< 100	< 100	< 100	< 100
	3.9×10^6	< 100	< 100	< 100	< 100
13	2.4×10^6	< 100	< 100	< 100	< 100
	3.1×10^6	< 100	< 100	< 100	< 100
14	2.5×10^6	< 100	< 100	< 100	< 100
	2.0×10^6	< 100	< 100	< 100	< 100
15	1.1×10^6	< 100	< 100	< 100	< 100
	1.9×10^6	< 100	< 100	< 100	< 100

Table 11

Challenge Tests of Sample No. 9 to 15 with C. albicans

Sample No.	Average count per ml after incubation for				
	0 day	7 days	14 days	21 days	28 days
9	0.7×10^6	<100	<100	<100	<100
	0.9×10^6	<100	<100	<100	<100
10	0.5×10^6	<100	<100	<100	<100
	0.9×10^6	<100	<100	<100	<100
11	0.7×10^6	1.0×10^2	<100	<100	<100
	1.2×10^6	4.5×10^2	<100	<100	<100
12	0.9×10^6	<100	<100	<100	<100
	1.1×10^6	<100	<100	<100	<100
13	1.5×10^6	1.0×10^2	<100	<100	<100
	1.8×10^6	1.5×10^2	<100	<100	<100
14	0.8×10^6	1.0×10^2	<100	<100	<100
	1.1×10^6	2.5×10^2	<100	<100	<100
15	1.7×10^6	3.0×10^2	<100	<100	<100
	0.9×10^6	2.0×10^2	<100	<100	<100

Table 12

Challenge Tests of Sample No. 9 to 15 with A. niger

Sample No.	Average count per ml after incubation for				
	0 day	7 days	14 days	21 days	28 days
9	1.1×10^6	<100	<100	<100	<100
	0.7×10^6	<100	<100	<100	<100
10	1.0×10^6	<100	<100	<100	<100
	1.1×10^6	<100	<100	<100	<100
11	0.9×10^6	<100	<100	<100	<100
	1.1×10^6	<100	<100	<100	<100
12	0.7×10^6	<100	<100	<100	<100
	0.9×10^6	<100	<100	<100	<100
13	1.3×10^6	<100	<100	<100	<100
	0.8×10^6	<100	<100	<100	<100
14	0.6×10^6	<100	<100	<100	<100
	0.8×10^6	<100	<100	<100	<100
15	1.1×10^6	<100	<100	<100	<100
	1.8×10^6	<100	<100	<100	<100

Table 13

Challenge Tests of Sample No. 16 to 23 with S. aureus

Sample No.	Average count per ml after incubation for				
	0 day	7days	14 days	21 days	28 days
16	1.9×10^6	< 100	< 100	< 100	< 100
	3.5×10^6	< 100	< 100	< 100	< 100
17	1.7×10^6	< 100	< 100	< 100	< 100
	2.1×10^6	< 100	< 100	< 100	< 100
18	2.5×10^6	< 100	< 100	< 100	< 100
	1.5×10^6	< 100	< 100	< 100	< 100
19	0.9×10^6	< 100	< 100	< 100	< 100
	3.0×10^6	< 100	< 100	< 100	< 100
20	1.5×10^6	< 100	< 100	< 100	< 100
	3.5×10^6	< 100	< 100	< 100	< 100
21	0.8×10^6	< 100	< 100	< 100	< 100
	0.9×10^6	< 100	< 100	< 100	< 100
22	1.4×10^6	< 100	< 100,	< 100	< 100
	1.6×10^6	< 100	< 100	< 100	< 100
23	1.6×10^6	< 100	< 100	< 100	< 100
	2.0×10^6	< 100	< 100	< 100	< 100

Table 14

Challenge Tests of Sample No. 16 to 23 with Ps. aeruginosa

Sample No.	Average count per ml after incubation for				
	0 day	7 days	14 days	21 days	28 days
16	2.4×10^6	< 100	< 100	< 100	< 100
	2.0×10^6	< 100	< 100	< 100	< 100
17	2.8×10^6	< 100	< 100	< 100	< 100
	3.8×10^6	< 100	< 100	< 100	< 100
18	1.5×10^6	< 100	< 100	< 100	< 100
	2.5×10^6	< 100	< 100	< 100	< 100
19	1.5×10^6	< 100	< 100	< 100	< 100
	1.3×10^6	< 100	< 100	< 100	< 100
20	2.3×10^6	< 100	< 100	< 100	< 100
	1.0×10^6	< 100	< 100	< 100	< 100
21	2.4×10^6	< 100	< 100	< 100	< 100
	2.2×10^6	< 100	< 100	< 100	< 100
22	2.2×10^6	< 100	< 100	< 100	< 100
	2.3×10^6	< 100	< 100	< 100	< 100
23	0.8×10^6	< 100	< 100	< 100	< 100
	3.5×10^6	< 100	< 100	< 100	< 100

Table 15

Challenge Tests of Sample No. 16 to 23 with E. coli

Sample No.	Average count per ml after incubation for				
	0 day	7 days	14 days	21 days	28 days
16	1.1×10^6	<100	<100	<100	<100
	1.0×10^6	<100	<100	<100	<100
17	1.1×10^6	<100	<100	<100	<100
	1.2×10^6	<100	<100	<100	<100
18	1.5×10^6	<100	<100	<100	<100
	0.9×10^6	<100	<100	<100	<100
19	1.1×10^6	<100	<100	<100	<100
	1.2×10^6	<100	<100	<100	<100
20	3.1×10^6	<100	<100	<100	<100
	1.5×10^6	<100	<100	<100	<100
21	2.3×10^6	<100	<100	<100	<100
	1.7×10^6	<100	<100	<100	<100
22	1.8×10^6	<100	<100	<100	<100
	1.4×10^6	<100	<100	<100	<100
23	1.4×10^6	<100	<100	<100	<100
	1.4×10^6	<100	<100	<100	<100

Table 16

Challenge Tests of Sample No. 16 to 23 with C. albicans

Sample No.	Average count per ml after incubation for				
	0 day	7 days	14 days	21 days	28 days
16	2.2×10^6	<100	<100	<100	<100
	1.0×10^6	<100	<100	<100	<100
17	0.7×10^6	<100	<100	<100	<100
	1.1×10^6	<100	<100	<100	<100
18	1.2×10^6	<100	<100	<100	<100
	1.0×10^6	<100	<100	<100	<100
19	1.3×10^6	<100	<100	<100	<100
	1.3×10^6	<100	<100	<100	<100
20	1.7×10^6	<100	<100	<100	<100
	0.9×10^6	<100	<100	<100	<100
21	2.1×10^6	<100	<100	<100	<100
	2.0×10^6	<100	<100	<100	<100
22	2.0×10^6	<100	<100	<100	<100
	1.1×10^6	<100	<100	<100	<100
23	1.4×10^6	<100	<100	<100	<100
	1.7×10^6	<100	<100	<100	<100

Table 17

Challenge Tests of Sample No. 16 to 23 with A. niger

Sample No.	Average count per ml after incubation for				
	0 day	7 days	14 days	21 days	28 days
16	0.9×10^6	< 100	< 100	< 100	< 100
	0.8×10^6	< 100	< 100	< 100	< 100
17	0.8×10^6	< 100	< 100	< 100	< 100
	0.5×10^6	< 100	< 100	< 100	< 100
18	0.8×10^6	< 100	< 100	< 100	< 100
	0.8×10^6	< 100	< 100	< 100	< 100
19	0.9×10^6	< 100	< 100	< 100	< 100
	0.7×10^6	< 100	< 100	< 100	< 100
20	1.1×10^6	< 100	< 100	< 100	< 100
	0.7×10^6	< 100	< 100	< 100	< 100
21	1.0×10^6	< 100	< 100	< 100	< 100
	1.3×10^6	< 100	< 100	< 100	< 100
22	0.6×10^6	< 100	< 100	< 100	< 100
	0.5×10^6	< 100	< 100	< 100	< 100
23	0.5×10^6	< 100	< 100	< 100	< 100
	1.0×10^6	< 100	< 100	< 100	< 100

Table 18

Challenge Tests of Sample No. 24 to 29 with S. aureus

Sample No.	Average count per ml after incubation for				
	0 day	7 days	14 days	21 days	28 days
24	2.7×10^6	< 100	< 100	< 100	< 100
	3.9×10^6	< 100	< 100	< 100	< 100
25	3.0×10^6	< 100	< 100	< 100	< 100
	1.5×10^6	< 100	< 100	< 100	< 100
26	1.7×10^6	< 100	< 100	< 100	< 100
	1.6×10^6	< 100	< 100	< 100	< 100
27	1.1×10^6	< 100	< 100	< 100	< 100
	1.0×10^6	< 100	< 100	< 100	< 100
28	3.8×10^6	< 100	< 100	< 100	< 100
	1.1×10^6	< 100	< 100	< 100	< 100
29	1.2×10^6	< 100	< 100	< 100	< 100
	2.0×10^6	< 100	< 100	< 100	< 100

Table 19

Challenge Tests of Sample No. 24 to 29 with Ps. aeruginosa

Sample No.	Average count per ml after incubation for				
	0 day	7 days	14 days	21 days	28 days
24	4.0×10^6	< 100	< 100	< 100	< 100
	3.5×10^6	< 100	< 100	< 100	< 100
25	2.8×10^6	< 100	< 100	< 100	< 100
	4.1×10^6	< 100	< 100	< 100	< 100
26	3.5×10^6	< 100	< 100	< 100	< 100
	2.5×10^6	< 100	< 100	< 100	< 100
27	1.6×10^6	< 100	< 100	< 100	< 100
	2.4×10^6	< 100	< 100	< 100	< 100
28	3.0×10^6	< 100	< 100	< 100	< 100
	1.2×10^6	< 100	< 100	< 100	< 100
29	1.7×10^6	< 100	< 100	< 100	< 100
	3.2×10^6	< 100	< 100	< 100	< 100



Table 20

Challenge Tests of Sample No. 24 to 29 with E. coli

Sample No.	Average count per ml after incubation for				
	0 day	7 days	14 days	21 days	28 days
24	1.3×10^6	< 100	< 100	< 100	< 100
	2.6×10^6	< 100	< 100	< 100	< 100
25	3.0×10^6	< 100	< 100	< 100	< 100
	2.4×10^6	< 100	< 100	< 100	< 100
26	2.1×10^6	< 100	< 100	< 100	< 100
	2.7×10^6	< 100	< 100	< 100	< 100
27	1.1×10^6	< 100	< 100	< 100	< 100
	3.5×10^6	< 100	< 100	< 100	< 100
28	1.5×10^6	< 100	< 100	< 100	< 100
	3.2×10^6	< 100	< 100	< 100	< 100
29	3.0×10^6	< 100	< 100	< 100	< 100
	2.8×10^6	< 100	< 100	< 100	< 100

Table 21

Challenge Tests of Sample No. 24 to 29 with C. albicans

Sample No.	Average count per ml after incubation for				
	0 day	7 days	14 days	21 days	28 days
24	1.2×10^6	< 100	< 100	< 100	< 100
	2.0×10^6	< 100	< 100	< 100	< 100
25	2.4×10^6	< 100	< 100	< 100	< 100
	1.6×10^6	< 100	< 100	< 100	< 100
26	1.9×10^6	< 100	< 100	< 100	< 100
	2.5×10^6	< 100	< 100	< 100	< 100
27	1.3×10^6	< 100	< 100	< 100	< 100
	2.0×10^6	< 100	< 100	< 100	< 100
28	1.7×10^6	< 100	< 100	< 100	< 100
	1.9×10^6	< 100	< 100	< 100	< 100
29	1.1×10^6	< 100	< 100	< 100	< 100
	1.6×10^6	< 100	< 100	< 100	< 100

Table 22

Challenge Tests of Sample No. 24 to 29 with A. niger

Sample No.	Average count per ml after incubation for				
	0 day	7 days	14 days	21 days	28 days
24	1.0×10^6	<100	<100	<100	<100
	0.9×10^6	<100	<100	<100	<100
25	0.7×10^6	<100	<100	<100	<100
	1.1×10^6	<100	<100	<100	<100
26	1.4×10^6	<100	<100	<100	<100
	1.1×10^6	<100	<100	<100	<100
27	1.0×10^6	<100	<100	<100	<100
	0.8×10^6	<100	<100	<100	<100
28	0.9×10^6	<100	<100	<100	<100
	1.6×10^6	<100	<100	<100	<100
29	1.5×10^6	<100	<100	<100	<100
	1.7×10^6	<100	<100	<100	<100

Table 23

Challenge Tests of Sample No. 30-34 and 38-41 with S. aureus

Sample No.	Average count per ml after incubation for				
	0 day	7 days	14 days	21 days	28 days
30	3.5×10^6	< 100	< 100	< 100	< 100
	4.0×10^6	< 100	< 100	< 100	< 100
31	3.9×10^6	< 100	< 100	< 100	< 100
	2.6×10^6	< 100	< 100	< 100	< 100
32	3.0×10^6	< 100	< 100	< 100	< 100
	2.7×10^6	< 100	< 100	< 100	< 100
33	3.8×10^6	< 100	< 100	< 100	< 100
	4.1×10^6	< 100	< 100	< 100	< 100
34	2.9×10^6	< 100	< 100	< 100	< 100
	3.1×10^6	< 100	< 100	< 100	< 100
38	2.9×10^6	< 100	< 100	< 100	< 100
	3.1×10^6	< 100	< 100	< 100	< 100
39	2.7×10^6	< 100	< 100	< 100	< 100
	4.1×10^6	< 100	< 100	< 100	< 100
40	3.5×10^6	< 100	< 100	< 100	< 100
	4.2×10^6	< 100	< 100	< 100	< 100
41	1.9×10^6	< 100	< 100	< 100	< 100
	2.8×10^6	< 100	< 100	< 100	< 100

Table 24

Challenge Tests of Sample No. 30-34 and 38-41 with Ps. aeruginosa

Sample No.	Average count per ml after incubation for				
	0 day	7 days	14 days	21 days	28 days
30	2.0×10^6	< 100	< 100	< 100	< 100
	3.6×10^6	< 100	< 100	< 100	< 100
31	2.7×10^6	< 100	< 100	< 100	< 100
	3.1×10^6	< 100	< 100	< 100	< 100
32	4.5×10^6	< 100	< 100	< 100	< 100
	3.9×10^6	< 100	< 100	< 100	< 100
33	3.5×10^6	< 100	< 100	< 100	< 100
	2.0×10^6	< 100	< 100	< 100	< 100
34	1.9×10^6	< 100	< 100	< 100	< 100
	3.5×10^6	< 100	< 100	< 100	< 100
38	4.5×10^6	< 100	< 100	< 100	< 100
	4.8×10^6	< 100	< 100	< 100	< 100
39	3.9×10^6	< 100	< 100	< 100	< 100
	4.6×10^6	< 100	< 100	< 100	< 100
40	3.6×10^6	< 100	< 100	< 100	< 100
	2.9×10^6	< 100	< 100	< 100	< 100
41	3.6×10^6	< 100	< 100	< 100	< 100
	4.4×10^6	< 100	< 100	< 100	< 100

Table 25

Challenge Tests of Sample No. 30-34 and 38-41 with E.coli

Sample No.	Average count per ml after incubation for				
	0 day	7 days	14 days	21 days	28 days
30	3.3×10^6	< 100	< 100	< 100	< 100
	3.5×10^6	< 100	< 100	< 100	< 100
31	2.1×10^6	< 100	< 100	< 100	< 100
	3.9×10^6	< 100	< 100	< 100	< 100
32	2.5×10^6	< 100	< 100	< 100	< 100
	1.7×10^6	< 100	< 100	< 100	< 100
33	4.1×10^6	< 100	< 100	< 100	< 100
	1.9×10^6	< 100	< 100	< 100	< 100
34	3.5×10^6	< 100	< 100	< 100	< 100
	2.4×10^6	< 100	< 100	< 100	< 100
38	3.8×10^6	< 100	< 100	< 100	< 100
	2.7×10^6	< 100	< 100	< 100	< 100
39	1.1×10^6	< 100	< 100	< 100	< 100
	2.8×10^6	< 100	< 100	< 100	< 100
40	3.9×10^6	< 100	< 100	< 100,	< 100
	3.0×10^6	< 100	< 100	< 100	< 100
41	1.4×10^6	< 100	< 100	< 100	< 100
	3.7×10^6	< 100	< 100	< 100	< 100

Table 26

Challenge Tests of Sample No. 30-34 and 38-41 with C. albicans

Sample No.	Average count per ml after incubation for				
	0 day	7 days	14 days	21 days	28 days
30	2.0×10^6	< 100	< 100	< 100	< 100
	2.4×10^6	< 100	< 100	< 100	< 100
31	1.9×10^6	< 100	< 100	< 100	< 100
	3.0×10^6	< 100	< 100	< 100	< 100
32	2.4×10^6	< 100	< 100	< 100	< 100
	1.6×10^6	< 100	< 100	< 100	< 100
33	2.3×10^6	< 100	< 100	< 100	< 100
	2.6×10^6	< 100	< 100	< 100	< 100
34	1.4×10^6	< 100	< 100	< 100	< 100
	1.8×10^6	< 100	< 100	< 100	< 100
38	2.7×10^6	< 100	< 100	< 100	< 100
	2.3×10^6	< 100	< 100	< 100	< 100
39	2.0×10^6	< 100	< 100	< 100	< 100
	1.5×10^6	< 100	< 100	< 100	< 100
40	2.5×10^5	< 100	< 100	< 100	< 100
	1.6×10^6	< 100	< 100	< 100	< 100
41	2.6×10^6	< 100	< 100	< 100	< 100
	1.6×10^6	< 100	< 100	< 100	< 100

Table 27

Challenge Tests of Sample No. 30-34 and 38-41 with A. niger

Sample No.	Average count per ml after incubation for				
	0 day	7 days	14 days	21 days	28 days
30	0.6×10^6	< 100	< 100	< 100	< 100
	1.1×10^6	< 100	< 100	< 100	< 100
31	1.3×10^6	< 100	< 100	< 100	< 100
	1.0×10^6	< 100	< 100	< 100	< 100
32	1.1×10^6	< 100	< 100	< 100	< 100
	0.7×10^6	< 100	< 100	< 100	< 100
33	0.8×10^6	< 100	< 100	< 100	< 100
	0.5×10^6	< 100	< 100	< 100	< 100
34	1.4×10^6	< 100	< 100	< 100	< 100
	1.1×10^6	< 100	< 100	< 100	< 100
38	1.5×10^6	< 100	< 100	< 100	< 100
	0.8×10^6	< 100	< 100	< 100	< 100
39	2.9×10^6	< 100	< 100	< 100	< 100
	1.0×10^6	< 100	< 100	< 100	< 100
40	1.7×10^6	< 100	< 100	< 100	< 100
	1.7×10^6	< 100	< 100	< 100	< 100
41	2.0×10^6	< 100	< 100	< 100	< 100
	1.3×10^6	< 100	< 100	< 100	< 100

Table 28

Challenge Tests of Sample No. 35-37 and 42-44 with S. aureus

Sample No.	Average count per ml after incubation for				
	0 day	7 days	14 days	21 days	28 days
35	3.7×10^6	< 100	< 100	< 100	< 100
	3.8×10^6	< 100	< 100	< 100	< 100
36	2.9×10^6	< 100	< 100	< 100	< 100
	3.9×10^6	< 100	< 100	< 100	< 100
37	2.1×10^6	< 100	< 100	< 100	< 100
	1.4×10^6	< 100	< 100	< 100	< 100
42	1.1×10^6	< 100	< 100	< 100	< 100
	3.0×10^6	< 100	< 100	< 100	< 100
43	3.9×10^6	< 100	< 100	< 100	< 100
	2.1×10^6	< 100	< 100	< 100	< 100
44	1.0×10^6	< 100	< 100	< 100	< 100
	4.1×10^6	< 100	< 100	< 100	< 100

Table 29

Challenge Tests of Sample No. 35-37 and 42-44 with Ps. aeruginosa

Sample No.	Average count per ml after incubation for				
	0 day	7 days	14 days	21 days	28 days
35	2.7×10^6	< 100	< 100	< 100	< 100
	4.9×10^6	< 100	< 100	< 100	< 100
36	1.0×10^6	< 100	< 100	< 100	< 100
	2.1×10^6	< 100	< 100	< 100	< 100
37	2.9×10^6	< 100	< 100	< 100	< 100
	3.1×10^6	< 100	< 100	< 100	< 100
42	3.4×10^6	< 100	< 100	< 100	< 100
	4.2×10^6	< 100	< 100	< 100	< 100
43	2.0×10^6	< 100	< 100	< 100	< 100
	4.9×10^6	< 100	< 100	< 100	< 100
44	3.0×10^6	< 100	< 100	< 100	< 100
	2.6×10^6	< 100	< 100	< 100	< 100

Table 30

Challenge Tests of Sample No. 35-37 and 42-44 with E. coli

Sample No.	Average count per ml after incubation for				
	0 day	7 days	14 days	21 days	28 days
35	1.9×10^6	< 100	< 100	< 100	< 100
	2.6×10^6	< 100	< 100	< 100	< 100
36	2.2×10^6	< 100	< 100	< 100	< 100
	3.9×10^6	< 100	< 100	< 100	< 100
37	1.1×10^6	< 100	< 100	< 100	< 100
	3.9×10^6	< 100	< 100	< 100	< 100
42	5.0×10^6	< 100	< 100	< 100	< 100
	1.0×10^6	< 100	< 100	< 100	< 100
43	4.9×10^6	< 100	< 100	< 100	< 100
	2.0×10^6	< 100	< 100	< 100	< 100
44	0.7×10^6	< 100	< 100	< 100	< 100
	1.6×10^6	< 100	< 100	< 100	< 100

Table 31

Challenge Tests of Sample No. 35-37 and 42-44 with C. albicans

Sample No.	Average count per ml after incubation for				
	0 day	7 days	14 days	21 days	28 days
35	2.2×10^6	< 100	< 100	< 100	< 100
	2.9×10^6	< 100	< 100	< 100	< 100
36	3.9×10^6	< 100	< 100	< 100	< 100
	5.5×10^6	< 100	< 100	< 100	< 100
37	1.0×10^6	< 100	< 100	< 100	< 100
	1.1×10^6	< 100	< 100	< 100	< 100
42	3.0×10^6	< 100	< 100	< 100	< 100
	1.4×10^6	< 100	< 100	< 100	< 100
43	2.0×10^6	< 100	< 100	< 100	< 100
	1.1×10^6	< 100	< 100	< 100	< 100
44	0.7×10^6	< 100	< 100	< 100	< 100
	0.7×10^6	< 100	< 100	< 100	< 100

จุฬาลงกรณ์มหาวิทยาลัย

Table 32

Challenge Tests of Sample No. 35-37 and 42-44 with A. niger

Sample No.	Average count per ml after incubation for				
	0 day	7 days	14 days	21 days	28 days
35	0.6×10^6	< 100	< 100	< 100	< 100
	0.9×10^6	< 100	< 100	< 100	< 100
36	1.4×10^6	< 100	< 100	< 100	< 100
	2.0×10^6	< 100	< 100	< 100	< 100
37	2.2×10^6	< 100	< 100	< 100	< 100
	1.6×10^6	< 100	< 100	< 100	< 100
42	4.5×10^5	< 100	< 100	< 100	< 100
	0.9×10^6	< 100	< 100	< 100	< 100
43	2.0×10^6	< 100	< 100	< 100	< 100
	1.3×10^6	< 100	< 100	< 100	< 100
44	2.2×10^6	< 100	< 100	< 100	< 100
	1.9×10^6	< 100	< 100	< 100	< 100

Table 33

Challenge Tests of Sample No. 45 to 50 with S. aureus

Sample No.	Average count per ml after incubation for				
	0 day	7 days	14 days	21 days	28 days
45	4.5×10^6	< 100	< 100	< 100	< 100
	5.0×10^6	< 100	< 100	< 100	< 100
46	3.9×10^6	< 100	< 100	< 100	< 100
	0.6×10^7	< 100	< 100	< 100	< 100
47	1.9×10^6	< 100	< 100	< 100	< 100
	4.4×10^6	< 100	< 100	< 100	< 100
48	4.4×10^6	< 100	< 100	< 100	< 100
	5.1×10^6	< 100	< 100	< 100	< 100
49	3.5×10^6	< 100	< 100	< 100	< 100
	3.9×10^6	< 100	< 100	< 100	< 100
50	3.2×10^6	< 100	< 100	< 100	< 100
	0.7×10^6	< 100	< 100	< 100	< 100

Table 34

Challenge Tests of Sample No. 45 to 50 with Ps. aeruginosa

Sample No.	Average count per ml after incubation for				
	0 day	7 days	14 days	21 days	28 days
45	3.0×10^6	< 100	< 100	< 100	< 100
	1.1×10^6	< 100	< 100	< 100	< 100
46	3.3×10^6	< 100	< 100	< 100	< 100
	3.0×10^6	< 100	< 100	< 100	< 100
47	2.9×10^6	< 100	< 100	< 100	< 100
	5.5×10^6	< 100	< 100	< 100	< 100
48	1.1×10^6	< 100	< 100	< 100	< 100
	1.6×10^6	< 100	< 100	< 100	< 100
49	5.9×10^6	< 100	< 100	< 100	< 100
	0.9×10^7	< 100.	< 100	< 100	< 100
50	1.2×10^6	< 100	< 100	< 100	< 100
	3.5×10^6	< 100	< 100	< 100	< 100

Table 35

Challenge Tests of Sample No. 45 to 50 with E. coli

Sample No.	Average count per ml after incubation for				
	0 day	7 days	14 days	21 days	28 days
45	2.9×10^6	< 100	< 100	< 100	< 100
	0.9×10^7	< 100	< 100	< 100	< 100
46	2.0×10^6	< 100	< 100	< 100	< 100
	1.6×10^6	< 100	< 100	< 100	< 100
47	2.2×10^6	< 100	< 100	< 100	< 100
	2.2×10^6	< 100	< 100	< 100	< 100
48	5.3×10^6	< 100	< 100	< 100	< 100
	4.7×10^6	< 100	< 100	< 100	< 100
49	3.1×10^6	< 100	< 100	< 100	< 100
	7.0×10^6	< 100	< 100	< 100	< 100
50	5.5×10^6	< 100	< 100	< 100	< 100
	1.0×10^6	< 100,	< 100	< 100	< 100

Table 36

Challenge Tests of Sample No. 45 to 50 with C. albicans

Sample No.	Average count per ml after incubation for				
	0 day	7 days	14 days	21 days	28 days
45	3.2×10^6	< 100	< 100	< 100	< 100
	0.7×10^6	< 100	< 100	< 100	< 100
46	2.6×10^6	< 100	< 100	< 100	< 100
	2.5×10^6	< 100	< 100	< 100	< 100
47	1.7×10^6	< 100	< 100	< 100	< 100
	3.0×10^6	< 100	< 100	< 100	< 100
48	5.9×10^5	< 100	< 100	< 100	< 100
	4.7×10^5	< 100	< 100	< 100	< 100
49	4.0×10^6	< 100	< 100	< 100	< 100
	2.8×10^6	< 100	< 100	< 100	< 100
50	1.7×10^6	< 100	< 100	< 100	< 100
	1.2×10^6	< 100	< 100	< 100	< 100

Table 37

Challenge Tests of Sample No. 45 to 50 with A. niger

Sample No.	Average count per ml after incubation for				
	0 day	7 days	14 days	21 days	28 days
45	0.8×10^6	< 100	< 100	< 100	< 100
	0.7×10^6	< 100	< 100	< 100	< 100
46	1.0×10^6	< 100	< 100	< 100	< 100
	0.9×10^6	< 100	< 100	< 100	< 100
47	1.0×10^6	< 100	< 100	< 100	< 100
	1.5×10^6	< 100	< 100	< 100	< 100
48	0.5×10^6	< 100	< 100	< 100	< 100
	3.9×10^5	< 100	< 100	< 100	< 100
49	1.4×10^6	< 100	< 100	< 100	< 100
	5.5×10^6	< 100	< 100	< 100	< 100
50	0.9×10^5	< 100	< 100	< 100	< 100
	2.1×10^6	< 100	< 100	< 100	< 100

From the results obtained, samples of eye lotions and eye drops without antimicrobial agents (sample No. 24 to 50) were found to be effectively protected by preservatives included. These samples were further diluted to 1:2 and 1:10 dilutions with sterile saline solution, using new containers untested. The results of challenge tests for these dilutions were obtained, but are not in totally shown here because most of them were no growth obtained on the seventh day of challenge test period. Only some were found to have growth after inoculation. These samples were the sample No. 35, 36 and 37. The dilutions having growth on seventh-day of the test period were challenge tests of 1:10 dilutions to Ps. aeruginosa and C. albicans , and the diluted samples tested with A. niger. These results are shown in Table 38 and 39 on page 77 and 78 respectively.



ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

Table 38

Challenge Tests of 1:10 Dilutions of Sample No.
35 to 37 with Ps. aeruginosa and C. albicans

Sample No.	Average count per ml after incubation for				
	0 day	7 days	14 days	21 days	28 days
<u>Ps. aeruginosa</u>					
35	3.0×10^6	0.5×10^2	< 100	< 100	< 100
36	1.7×10^6	0.3×10^3	< 100	< 100	< 100
37	2.5×10^6	1.0×10^3	< 100	< 100	< 100
<u>C. albicans</u>					
35	2.1×10^6	0.8×10^4	1.7×10^2	< 100	< 100
36	0.9×10^6	0.3×10^5	0.8×10^4	0.5×10^2	0.8×10^3
37	1.4×10^6	3.9×10^5	0.8×10^4	0.5×10^3	0.8×10^3

ศูนย์วิจัยจุลชีววิทยาการ
จุฬาลงกรณ์มหาวิทยาลัย



Table 39

Challenge Tests of 1:2 and 1:10 Dilutions of
Sample No. 35 to 37 with Aspergillus niger

Sample No.	Average count per ml after incubation for				
	0 day	7 days	14 days	21 days	28 days
1:2 Dilution					
35	0.8×10^6	0.5×10^2	< 100	< 100	< 100
36	0.7×10^6	1.5×10^2	< 100	< 100	< 100
37	1.0×10^6	1.0×10^2	< 100	< 100	< 100
1:10 Dilution					
35	0.5×10^6	0.7×10^4	1.0×10^2	< 100	< 100
36	1.0×10^6	0.9×10^4	0.4×10^3	< 100	< 100
37	1.2×10^6	0.3×10^4	0.7×10^3	< 100	< 100

ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย