

Results

In this experiment, five commercial rigid gas permeable (RGP) contact lens solutions with a sterile saline control were tested for antimicrobial effectiveness against *Methicillin-Resistant Staphylococcus Aureus*, a strain of *S. Aureus* that is resistant against many antibiotics (especially penicillin). There were definite variances in effectiveness; some solutions tended to perform much better than other solutions. Despite the solutions' great differences, the four different trials were very similar, usually only differing in results for each solution by a well or two. This consistency shows that the results must be reliable.

Solution 1, the sterile saline control, was not effective against staph bacteria, and the only well that the bacteria did not grow in throughout all trials was well A (90% solution). Bacteria always grew in every single of the following wells, the concentration of growth increasing with each well down (the percentage of solution decreases). Growth consisted of a dark yellow, very concentrated dot of bacterial growth in the center of the well with a "cloud" of opaque, off-white growth along the bottom of the well surrounding it (note: all growth easily visible to the eye and in the pictures were against the bottom of the wells.) The growth that occurred was large and unrestrained, and appeared to flourish. In all resazurin trials, the saline control was only able to completely stop the bacteria in well A. Solution 1, the control, was least effective in prohibiting and killing bacteria.

Solution 2, Boston Advance, was a very effective antimicrobial agent against the tested strain of *S. Aureus*. In trial 1, bacteria began to grow in well H and was only alive in well H. There was a defined "dot" in the bottommost center of the well, with a smaller cloud of growth around it than in Solution 1. In trial 2, the bacteria grew profusely in well H as well. In trial 3, bacteria grew very slightly in well F (only very, very slightly. Just a small amount of live staph resulted in a bluish-purple color in the resazurin test), and abundantly in G and H. Lastly, in trial 4, staph grew in wells G and H and survived only in those two wells.

The average percentage of solution in which staph began to grow was 1.58203125%, and all the growth in the wells were around a medium-high amount (compared to Solution 1's definite "high". Refer to Figure 23). In resazurin trial 1, bacteria was alive only in well H, the last well. In trial 2, there was a medium amount of live staph in well F, but with a lot in wells G and H. Lastly, resazurin trial 3's results showed that the bacteria was alive only in wells G and H. It was hypothesized correctly that Boston Advance would be most effective for the two-step solutions.

Solution 3, Lobob Soaking Solution, was mediocre in terms of antimicrobial power. For all trials, Lobob prohibited growth in half the wells, but the bacteria grew in the other remaining half, and the average percentage of solution in which bacteria began to grow was 5.625%. All growth tended to be either very minimal or a medium amount; there was a less concentrated central "dot" of growth and its bacterial "cloud" had a smaller diameter than the two previous solutions. It performed mediocly in the resazurin trials: In resazurin trial 1, a great amount of bacteria was alive from well F down. In the following resazurin trials, there was an issue with contamination in the first few wells; in resazurin trial 2, lots of bacteria survived in well A. There was a medium-high (light lavender hue) amount in well B, a medium growth in well C, medium-high growth in well E., and lots of live staph from well F down.

The reason staph also survived well in the first few wells in trials 3 and 4, was likely due to contamination from another means other than the solution itself. It could not have been caused by any contamination in the solution because it would not have made sense if it survived in the well with the most solution and least broth (broth is a bacteria culturing medium that contains the nutrients needed for growth), but not in the middle wells that contained less solution and more broth. Additionally, there was no growth recorded by the plate reader, so contamination must have had occurred while adding the resazurin, which explains why only the resazurin results had been affected by contamination.

Solution 4, Simplus, performed exceptionally well, killing nearly all the bacteria and averaging 0.52734375% for the percentage in which bacteria began to grow. In the wells that staph did grow in, the growth was a medium-high amount: a

concentrated golden-yellow dot in the center, with a small halo of translucent off-white bacterial growth around it. Simplus also performed very well in the resazurin trials, killing almost all bacteria; in trials 1 and 4, staph was only alive in well H, while in trial 3, it was alive in both well G and H, though just barely in well G (the resazurin color was a blue with just a hint of a purple hue). Simplus was the most effective anti-*S. Aureus* solution tested, so the hypothesis that Menicare would be the the best-performing out of the one-steps was incorrect.

Solution 5, Menicare, averaged 2.8125% for the percentage in which bacteria began to grow, performing third “best” of all solutions. In all trials, growth began in well F (2.8125% solution). Menicare was the one solution that tended to have differently shaped growth. In trial 1, there was very minimal growth with no “cloud” of staph growth. For trial 2. For trial 3, it started off dispersed, wide, and an almost opaque white, but in the wells further down, turned into a smaller, more condensed circle that was more of a creamy yellow. For resazurin trial 1, bacteria were alive in well G and H, while in trials 2 and 3, they survived in wells F through H. Staph also flourished in the first few wells, which indicated that there had been contamination at some point in a way similar to Lobob’s (see end of paragraph on Lobob on previous page). Menicare did not have stronger antimicrobial properties than the other one-step solutions, so the original hypothesis for the one-step solutions was incorrect.

Lastly, Solution 6, Opti-Free, did not eliminate or stop the growth of *S. Aureus* as well as the other solutions with preservatives (excluding the saline control). In trials 1 and 2, bacteria began growing from well D, and the growth was dark, golden yellow, a concentrated “dot” with an opaque, off-white cloud of bacterial growth surrounding it. In trial 3, bacteria grew from well D down, and in trial 4, staph began growing from well C. The growth was almost transparent in well C, but turned much more opaque in the wells with a lesser concentration of solution. The “dot” gradually turned yellower as the concentration of bacteria increased as a result of the decreased concentration of solution.

The average percentage of Opti-Free in which bacteria began to grow was 14.0625%. This placed the solution as second “worst”, after the sterile saline

control, in terms of antimicrobial efficiency against MRSA bacteria. In all resazurin trials, bacteria survived from well C all the way down to the last well, making Opti-Free both least effective in prohibiting growth and in killing bacterial growth of the solutions with preservatives added (Excluding the saline. The saline control did not contain any preservatives.).

Solution Ranking from best-performing to worst-performing	Average Percentage of Solution in Which Bacteria Began to Grow
#1) Solution 4:	0.52734375%
#2) Solution 1:	1.58203125%
#3) Solution 5:	2.8125%
#4) Solution 3:	5.625%
#5) Solution 6:	14.0625%
#6) Solution 1:	45%

One glitch that was encountered during this experiment was the solutions' susceptibility to forming bubbles while being pipetted, particularly Boston Advance and Simplus. The formation of bubbles interfered with accuracy, and, in the case of the original Trial 3, completely threw off the results (Trial 3 had to be redone). The other main problem encountered was contamination. There were many procedures done to prevent contamination -working next to a bunsen burner to avoid airborne contamination, cleaning the work surface and equipment frequently, and flaming containers before and after use to kill possible microorganisms on them- yet in resazurin trials 3 and 4, the first few wells of Solutions 3 and 5 were likely contaminated. Resazurin trial 1's negative controls suffered from contamination, and the positive controls were not as they should have been due to likely inaccurate measurements.

However, despite these problems encountered or likely encountered, the four trials' results were very similar, and in most cases, the percentage of each solution in which bacteria began to grow varied only by a single well's difference. In conclusion, the results showed that for the one-step solutions, Simplus was most effective, and for the two-step solutions, Boston Advance was most effective.

Trial 1

All solutions were still their original clear color, but some had bacterial growth at the bottom of the well. The bacteria coalesced in the bottom of the wells (because the wells were rounded at the bottom and the effect of gravity pulled them to the bottom). Wells with high growth exhibited a darker, golden-yellow dot of concentrated bacteria in the middle of the well with a “halo” of paler yellow growth surrounding it. Medium growth was opaque and a yellowish-cream color. Wells with lesser growth formed a cloudy, white spot on the bottom of the well. (Refer to Figure 23). Referring to Figure 24 throughout this paper may help as well.

Solutions:	Observations of Bacterial Growth
Solution 1 (Sterile Saline Control)	Dark yellow, concentrated dot of bacterial growth in center of well; opaque off-white growth surrounding it.
Solution 2 (Boston Advance):	Defined “dot” in center, but with a smaller cloud of growth around it than in Solution 1
Solution 3 (Lobob Soaking Solution):	Less concentrated central growth; its bacterial “cloud” has a smaller diameter than the two previous solutions.
Solution 4 (Simplus):	No growth
Solution 5 (Menicare GP):	Very minimal growth; no “cloud” of bacteria
Solution 6 (Opti-Free GP):	Lots of growth: dark golden yellow, concentrated “dot” with opaque, off-white cloud of bacterial growth surrounding it.

Results of Minimum Inhibition Concentration	(MIC- the procedure where the percentage of solution is progressively diluted in different wells.)
Solution 1:	Growth starts in well B (45% solution)
Solution 2:	Growth starts in well H (0.703125% solution)
Solution 3:	Growth starts in well E (5.625% solution)
Solution 4:	No growth
Solution 5:	Growth starts in well F (2.8125% solution)
Solution 6:	Growth starts in well D (11.25% solution)

Negative Control:	Contamination in some wells
Positive Control:	Growth in some wells, none in others (inaccurate measurements?)

Other miscellaneous observations: Solutions 2 and 4 were bubbly (because they were more viscous, the bubbles formed during pipetting.)

Other solutions did not contain bubbles.

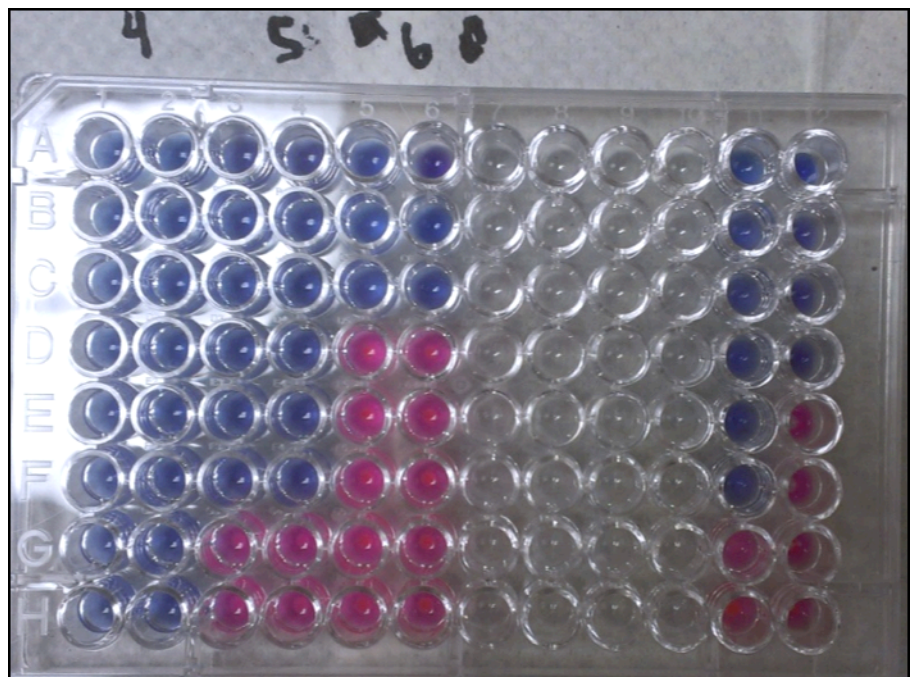
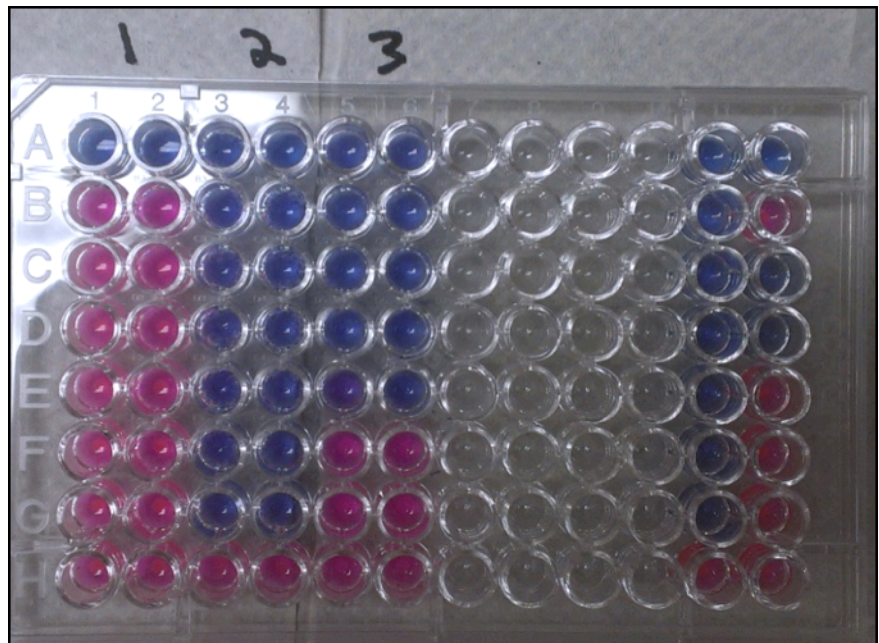
Solution:	Resazurin Results
Solution 1	Lots of bacteria still alive from well B down
Solution 2	A great amount is alive in well H
Solution 3	Small amount of bacteria alive in well E. Lots of bacteria still alive from well F down
Solution 4	All bacteria killed.
Solution 5:	Lots of bacteria alive in wells G and H.
Solution 6:	Lots of bacteria alive from well D down.

Resazurin is a blue dye that, when exposed to a decrease in oxygen, turns pink. Blue indicates no live bacteria, purple indicates a medium amount, and pink indicates a high amount.

Figure 1: Top: Resazurin results for solutions 1,2,3. Far left column is the positive control, and the column to its left is the negative control.

Figure 2: Bottom: Resazurin results for solutions 4,5,6. Far left column is the positive control, and the column to its left is the negative control.

There was contamination in the positive controls, as well as possibly inaccurate measurements in the negative controls.



Exp01

(bubbles interfering with reading)

1 2 3

Plate01											
1	2	3	4	5	6	7	8	9	10	11	12
0.038	0.038	0.043	0.040	0.039	0.040	0.052	0.048	0.048	0.048	0.039	0.038
0.084	0.169	0.042	0.043	0.042	0.041	0.048	0.048	0.048	0.049	0.041	1.381
1.058	1.130	0.110	0.046	0.048	0.040	0.049	0.048	0.048	0.052	0.063	0.040
1.270	1.276	0.403	0.863	0.040	0.041	0.088	0.048	0.048	0.069	0.041	0.040
1.449	1.450	0.667	0.041	0.847	0.336	0.156	0.048	0.049	0.048	0.041	1.543
1.475	1.467	0.195	0.042	1.277	1.223	0.158	0.049	0.049	0.048	0.041	1.513
1.414	1.387	0.045	0.043	1.266	1.278	0.140	0.050	0.048	0.066	0.043	1.468
1.377	1.390	0.786	0.713	1.311	1.375	0.091	0.048	0.048	0.048	1.469	1.463

Endpoint
Lm1 600
Automix: Off
Calibrate: On
Column Priority
C. Speed: Normal

Plate Last Read:
5:13 PM 11/20/2012

Wavelength Combination: !Lm1

Mean Temperature: 27.1

Reader: VersaMaxPLUS ROM v1.21 Aug 18 2005

Figure 3: Above: Plate readings for solutions 1,2,3
Figure 4: Below: Plate readings for solutions 4,5,6

The plates were read by a scanner that measured how much of a laser passed through the bottom of the well. Larger numbers indicate blockage of light-- growth of bacteria, while smaller numbers indicate less blockage the light-- less growth.

The negative control's (the column marked with a "-" sign) range of numbers (in here, around 0.038 to about 0.043, not including the contaminated wells) indicates no growth, so one can use that number to tell which other wells have growth and which do not.

Exp01

4
No growth

Plate01											
1	2	3	4	5	6	7	8	9	10	11	12
0.041	0.040	0.057	0.041	0.043	0.097	0.049	0.049	0.048	0.057	0.039	0.045
0.043	0.041	0.041	0.044	0.045	0.041	0.048	0.048	0.050	0.050	0.042	0.050
0.157	0.042	0.041	0.041	0.043	0.040	0.048	0.047	0.048	0.048	0.041	0.084
0.047	1.243	0.042	0.041	1.184	1.064	0.049	0.048	0.048	0.050	0.041	0.068
0.043	0.048	0.043	0.044	1.396	1.438	0.049	0.049	0.050	0.050	0.041	0.208
0.385	0.045	0.461	0.375	1.403	1.466	0.049	0.050	0.051	0.049	0.042	0.392
0.044	1.090	1.353	1.337	1.309	1.368	0.049	0.049	0.048	0.054	0.846	1.350
0.151	0.047	1.361	1.396	1.338	1.407	0.051	0.089	0.048	0.059	1.598	1.438

Endpoint
Lm1 600
Automix: Off
Calibrate: On
Column Priority
C. Speed: Normal

Plate Last Read:
5:19 PM 11/20/20

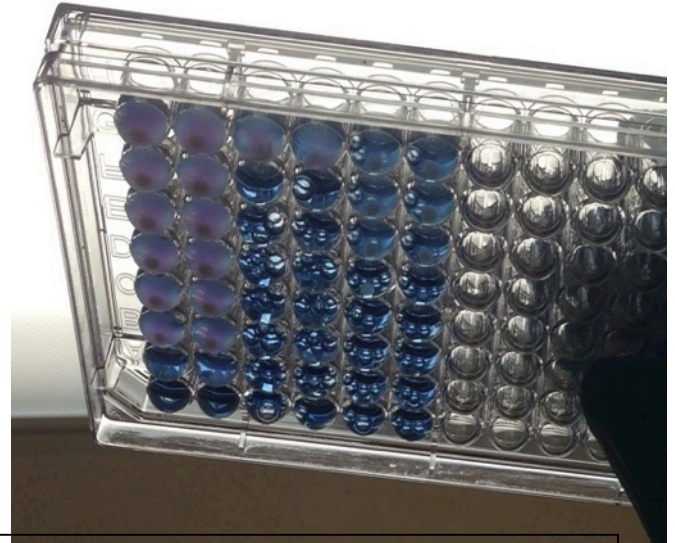
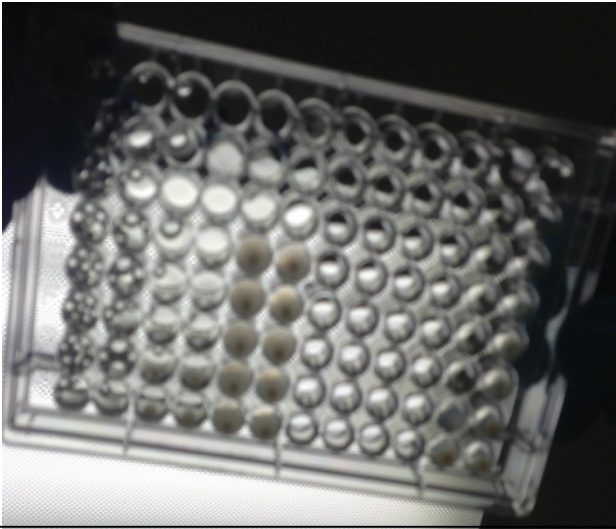
Wavelength Combination: !Lm1

Mean Temperature: 27.1

Reader: VersaMaxPLUS ROM v1.21 Aug 18 2005

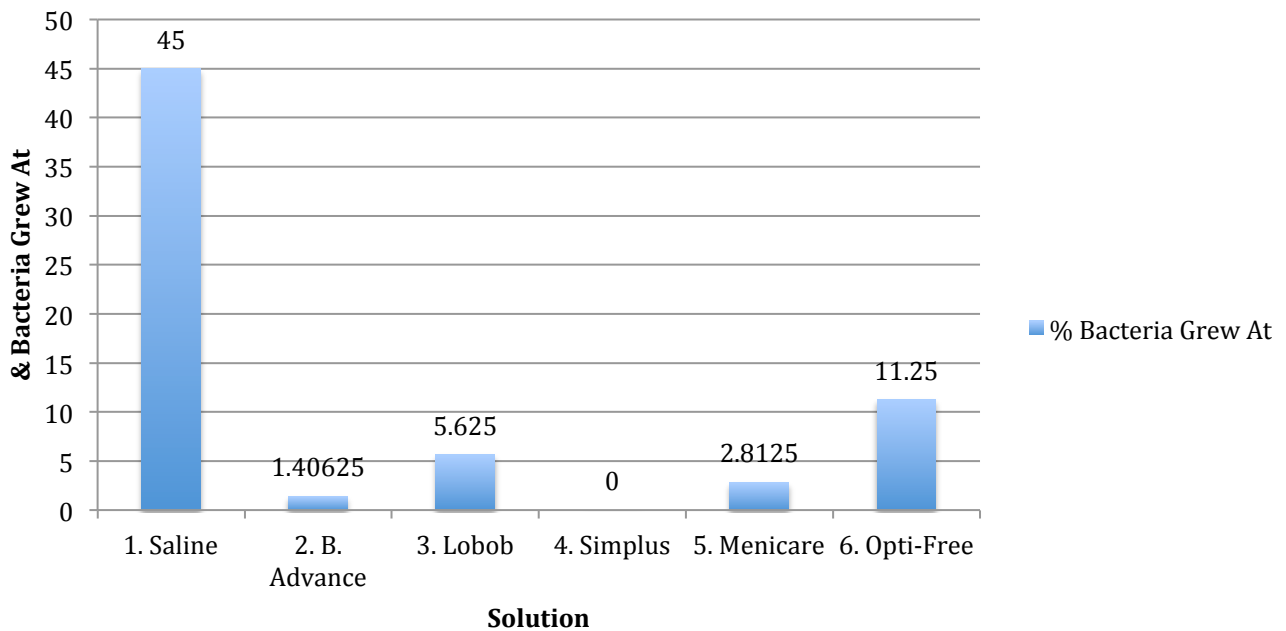
Figure 5: Left: Solutions 4,5,6 (in stated order with 2 wells each) with after incubating.

Figure 6: Right: Solutions 1,2,3 (in stated order; bottom view) after a few minutes of having resazurin added to.



Note for all graphs: Y-Axis shows the percentage of the contact solution that bacteria had begun to grow in (i.e. bacteria began to grow in the saline at a saline concentration of 45% (the other 45% being ca-mhb broth and the remaining 10% bacteria).

Trial 1: Percentage of Solution in Which Bacteria Began to Grow



Trial 2

All solutions were still their clear color (as in Trial 1). The bacteria's characteristics and growth patterns appeared the same as in Trial 1.

Results:

Results of Minimum Inhibition Concentration	(MIC- the procedure where the percentage of solution is progressively diluted in different wells.)
Solution 1:	Growth starts in well C.
Solution 2:	Growth starts in well H
Solution 3:	Growth starts in well E
Solution 4:	Growth starts in well H
Solution 5:	Growth starts in well F
Solution 6:	Growth starts in well D

Solutions	Observations of Bacterial Growth
Solution 1:	Lots of concentrated bacteria in the wells with growth.
Solution 2:	Lots of concentrated bacteria in the wells with growth.
Solution 3:	Starts off as a very pale yellow pinprick of bacteria, but gradually increases in size as the solution concentration decreases. However, even in the last well, the growth is still smaller and does not cover the entire bottom of the well.
Solution 4:	Not much growth; minimal growth in comparison with other solutions' growth, but still has a "dot".
Solution 5:	Starts off very small, and increases in size very slight as the concentration of solution decreases (like sol. 3). The last well's growth is still small compared to solutions with a lot of growth.
Solution 6:	Lots of bacteria in all wells with growth. There is a visible increase as concentration as solution decreases.

Negative Control	No growth in negative controls (which means there was no contamination in the CA-MHB broth.)
Positive Control	There was lots of growth in positive controls.

Note: The resazurin was not added for this trial.

Exp01

1 2 3

Plate01 (+) (-)

1	2	3	4	5	6	7	8	9	10	11	12
0.042	0.042	0.044	0.040	0.040	0.041	0.048	0.048	0.048	1.628	0.049	0.043
0.050	0.055	0.043	0.042	0.041	0.041	0.049	0.049	0.048	1.439	0.048	0.041
1.067	1.029	0.041	0.040	0.040	0.040	0.048	0.048	0.049	1.467	0.048	0.041
1.281	1.299	0.040	0.041	0.040	0.041	0.048	0.048	0.050	1.436	0.048	0.041
1.446	1.438	0.042	0.041	0.317	0.137	0.049	0.049	0.049	1.508	0.049	0.040
1.459	1.424	0.042	0.041	1.281	1.215	0.048	0.048	0.049	1.515	0.049	0.040
1.372	1.360	0.041	0.042	1.171	1.161	0.049	0.049	0.049	1.468	0.048	0.043
1.415	1.367	1.139	1.227	1.365	1.322	0.048	0.049	0.048	1.444	0.048	0.044

Endpoint
Lm1 600
Automix: Off
Calibrate: On
Column Priority
C. Speed: Normal

Plate Last Read
3:37 PM 11/29

Wavelength Combination: !Lm1

Mean Temperature: 24.4

Reader: VersaMaxPLUS ROM v1.21 Aug 18 2005

CMHB broth 'bacteria'

Figure 7: Above: Plate readings for solutions 1,2,3
 Figure 8: Below: Plate readings for solutions 4,5,6

Exp01

4 5 6

Plate01 (+) (-)

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.041	0.041	0.040	0.046	0.043	0.041	0.048	0.048	0.048	1.663	0.048	0.041
B	0.041	0.042	0.042	0.043	0.046	0.045	0.048	0.048	0.048	1.401	0.048	0.041
C	0.041	0.041	0.039	0.043	0.042	0.041	0.048	0.048	0.048	1.376	0.048	0.041
D	0.041	0.042	0.042	0.041	1.104	1.078	0.047	0.048	0.049	1.376	0.048	0.041
E	0.043	0.040	0.042	0.045	1.507	1.522	0.047	0.048	0.049	1.441	0.048	0.040
F	0.041	0.041	0.619	0.629	1.546	1.560	0.048	0.049	0.049	1.443	0.049	0.045
G	0.041	0.041	1.280	1.116	1.500	1.475	0.049	0.048	0.049	1.423	0.052	0.043
H	1.486	1.501	1.328	1.273	1.525	1.535	0.048	0.048	0.048	1.448	0.048	0.040

Endpoint
Lm1 600
Automix: Off
Calibrate: On
Column Priority
C. Speed: Normal

Plate Last Read:
3:39 PM 11/29/201

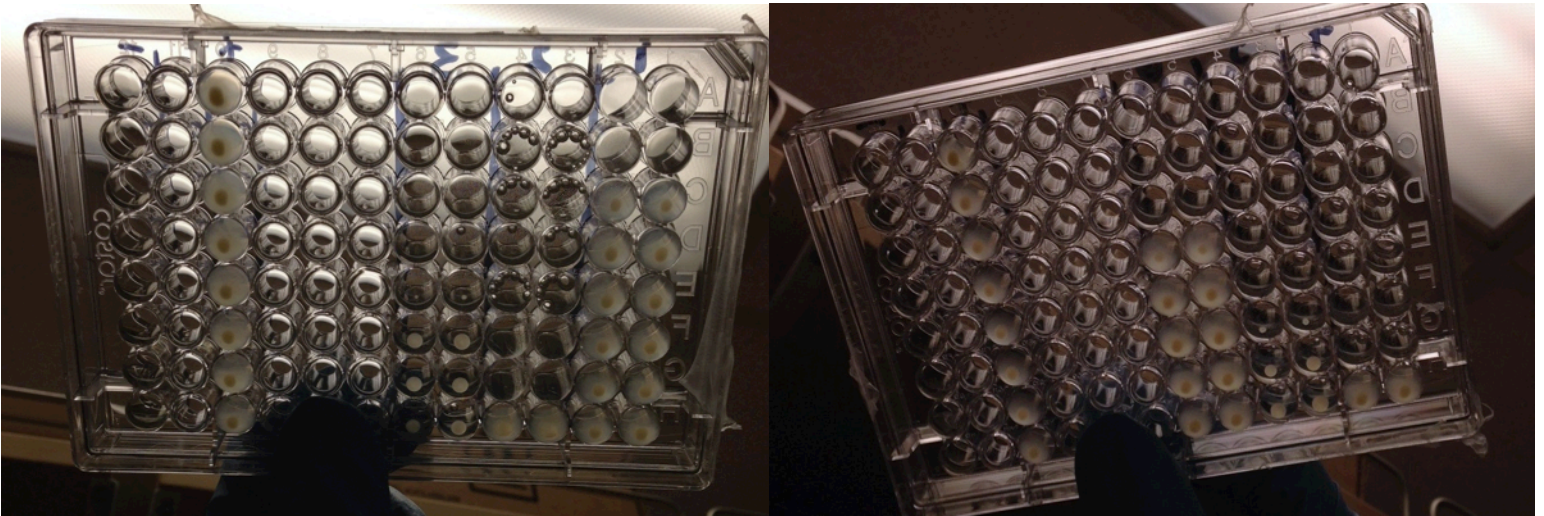
Wavelength Combination: !Lm1

Mean Temperature: 24.5

Reader: VersaMaxPLUS ROM v1.21 Aug 18 2005

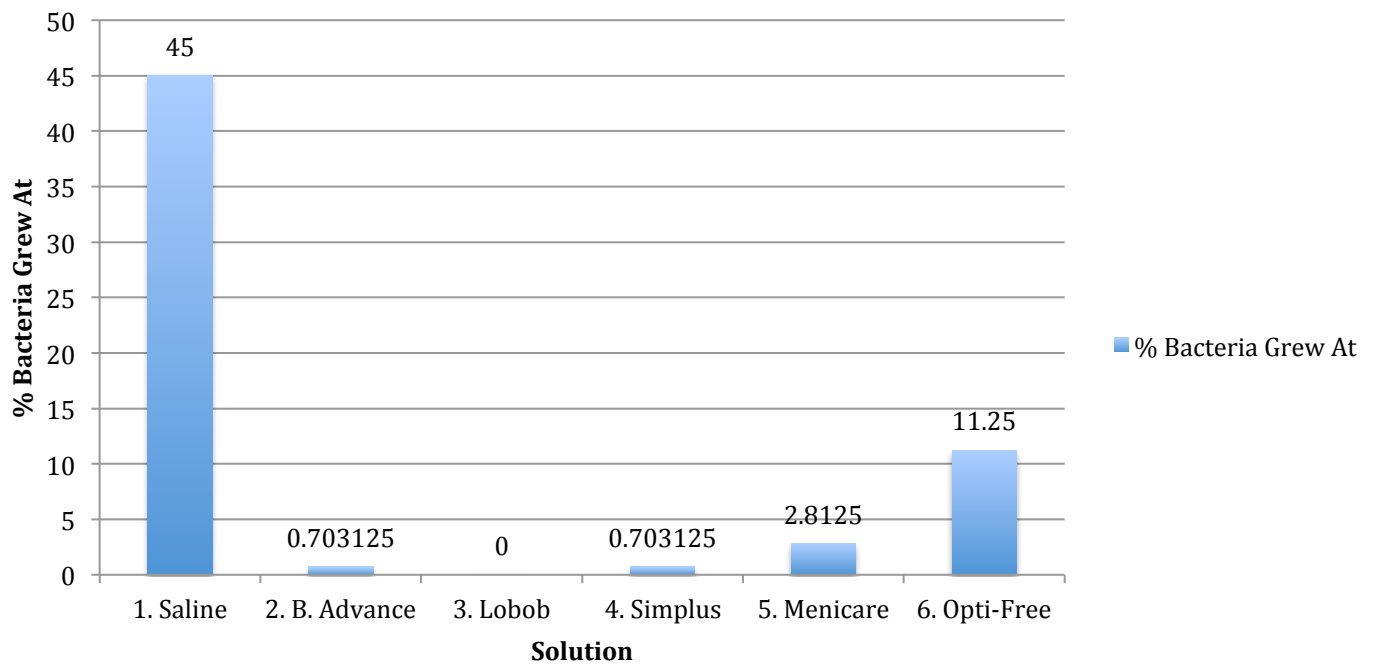
Figure 9: Left: Solutions 1,2,3 after incubation. (sol. 1 is the two far right columns, 2 is the next two columns to its left, etc.)

Figure 10: Right: Solutions 4,5,6 after incubation. (sol. 4 is the two far right columns, 5 is the next two columns to its left, etc.)



Other miscellaneous observations:
Solution 2 was bubbly
Other solutions did not contain bubbles.

Trial 2: Percentage of Solution in Which Bacteria Began to Grow



Trial 3

Note: The original Trial 3's results turned out as inaccurate and presented incorrect data that was evidently incorrect because of how far off from the previous 2 trials' results it was (there were only very slight differences in the first two trials' results). Therefore, Trial 3 was redone and, its second attempt, gave clean, accurate data.

Results of Minimum Inhibition Concentration	(MIC- the procedure where the percentage of solution is progressively diluted in different wells.)
Solution 1:	Growth starts in well B (45% solution)
Solution 2:	Growth starts in well F (2.8125% solution)
Solution 3:	Growth starts in well E (5.625% solution)
Solution 4:	Growth starts in well H (0.703125% solution)
Solution 5:	Growth starts in well F (2.8125% solution)
Solution 6:	Growth starts in well D (11.25% solution)

Solutions	Observations of Bacterial Growth
Solution 1:	Lots of concentrated, almost golden-colored bacteria dot with white "cloud" of bacterial growth.
Solution 2:	Unusually wide, yellow circular growth in center surrounded by translucent white "cloud".
Solution 3:	Off-white circles that are larger than in Solution 1, but smaller than in Solution 2. The color gradually turns into a light yellow as the concentration of solution decreases.
Solution 4:	Concentrated golden-yellow dot in center, with a small halo of translucent off-white bacterial growth around it.
Solution 5:	Starts off dispersed, wide and an almost opaque white, but turns into a smaller circle that is more of a creamy yellow.
Solution 6:	Bright yellow "dot" with cloudy, very pale yellow growth radiating from center.

Negative Control	No growth in negative controls (which means there was no contamination in the CA-MHB broth.)
Positive Control	There was lots of growth in positive controls.

Exp01

■ = with growth

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.044	0.044	0.047	0.042	0.040	0.040	0.048	0.048	0.048	1.591	0.048	0.041
B	0.059	0.067	0.043	0.045	0.043	0.042	0.048	0.048	0.048	1.428	0.048	0.041
C	1.054	1.025	0.042	0.042	0.042	0.042	0.048	0.048	0.048	1.452	0.049	0.041
D	1.283	1.268	0.042	0.041	0.044	0.041	0.047	0.048	0.048	1.448	0.047	0.041
E	1.524	1.483	0.044	0.043	1.310	1.269	0.047	0.048	0.049	1.490	0.048	0.040
F	1.538	1.512	0.042	0.066	1.187	1.163	0.048	0.049	0.048	1.530	0.049	0.041
G	1.506	1.421	1.151	1.077	1.721	1.668	0.049	0.048	0.048	1.569	0.048	0.042
H	1.485	1.500	1.063	1.129	1.484	1.481	0.048	0.047	0.048	1.592	0.049	0.042

Endpoint
 Lm1 600
 Automix: Off
 Calibrate: On
 Column Priority
 C. Speed: Normal

Plate Last Read:
 3:22 PM 12/6/2012

Wavelength Combination: !Lm1

Mean Temperature: 24.9

Reader: VersaMaxPLUS ROM v1.21 Aug 18 2005

Figure 11: Above: Plate readings for solutions 1,2,3
 Figure 12: Below: Plate readings for solutions 4,5,6

Exp01

■ = with growth

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.042	0.041	0.051	0.041	0.045	0.043	0.048	0.049	0.049	1.693	0.048	0.049
B	0.041	0.043	0.042	0.042	0.049	0.047	0.065	0.048	0.048	1.583	0.058	0.042
C	0.042	0.043	0.042	0.043	0.054	0.043	0.048	0.053	0.048	1.509	0.048	0.041
D	0.042	0.043	0.044	0.043	1.396	1.343	0.048	0.048	0.048	1.448	0.049	0.041
E	0.047	0.046	0.051	0.049	1.540	1.552	0.047	0.048	0.052	1.516	0.049	0.041
F	0.043	0.044	1.027	1.001	1.610	1.620	0.049	0.049	0.049	1.502	0.049	0.042
G	0.043	0.043	1.381	1.329	1.560	1.566	0.050	0.049	0.049	1.475	0.048	0.042
H	1.525	1.527	1.378	1.367	1.569	1.575	0.048	0.049	0.055	1.513	0.048	0.041

Endpoint
 Lm1 600
 Automix: Off
 Calibrate: On
 Column Priority
 C. Speed: Normal

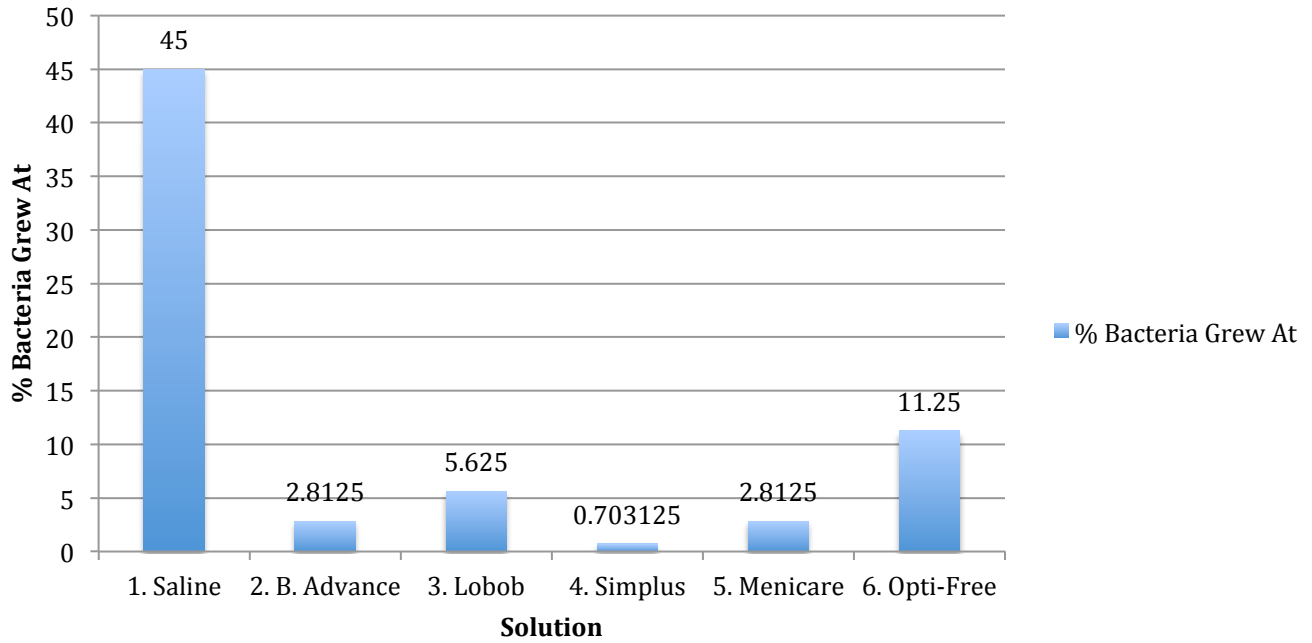
Plate Last Read:
 3:23 PM 12/6/2012

Wavelength Combination: !Lm1

Mean Temperature: 25.0

Reader: VersaMaxPLUS ROM v1.21 Aug 18 2005

Trial 3: Percentage of Solution in Which Bacteria Began to Grow

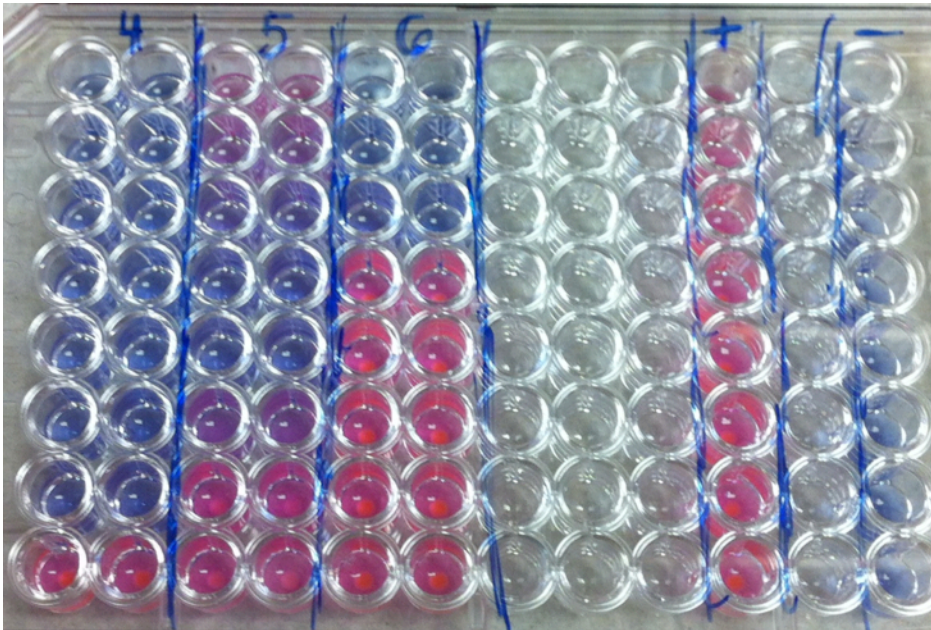
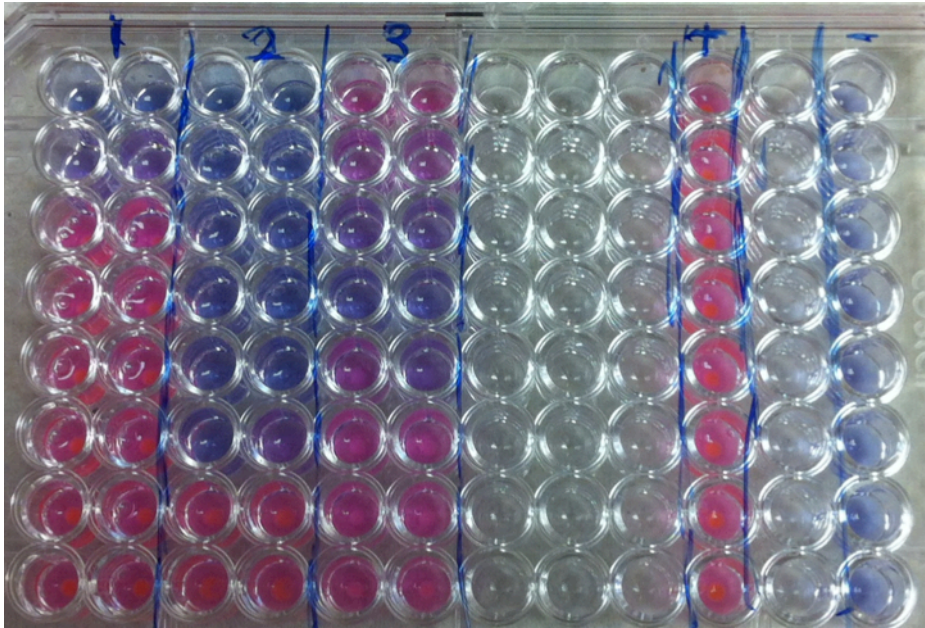
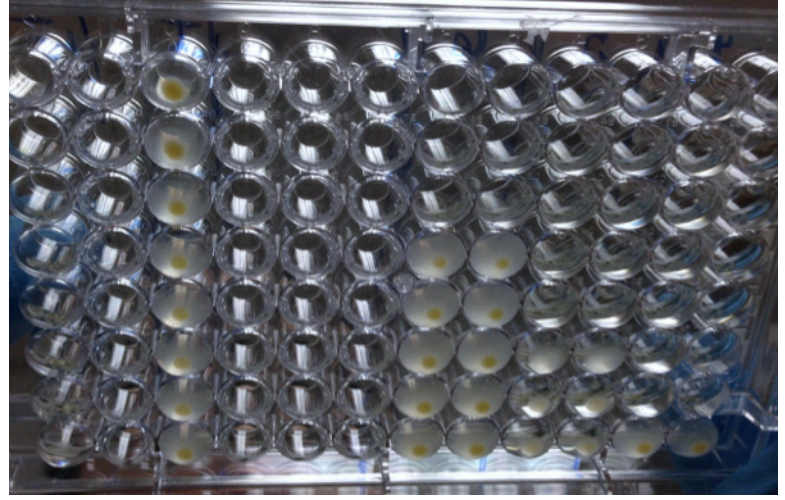
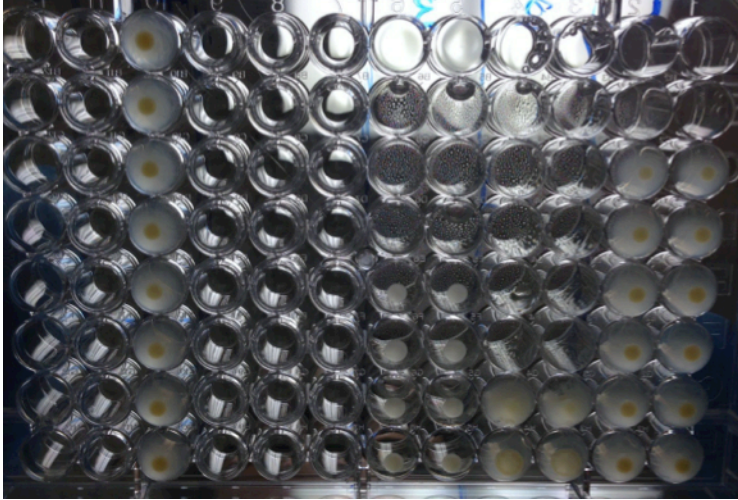


Solution	Resazurin Results:
Solution 1:	Medium amount of live staph in well B. Lots of growth from well C down.
Solution 2:	Medium amount of live bacteria in well F. Lots of growth in wells G and H.
Solution 3:	Lots of live staph in well A. Medium-high (light lavender hue) amount in well B. Medium growth in well C. Medium-high growth in well E. Lots of live staph from well F down.
Solution 4:	Lots of live staph in well H. Very slight in well G.
Solution 5:	High amount of live bacteria in well A. Medium amount in wells B and F. Lots in wells G and H.
Solution 6:	High amount of live staph from wells D down.

Below:

Figure 13: Left: Solutions 1,2,3 (1 being the two columns on the far right, with 2 on its left, etc.)

Figure 14: Right: Solutions 4,5,6 (4 being the two columns on the far right, with 5 on its left, etc.)



Resazurin Pictures:

Figure 15: Top:
Solutions 1,2,3 with
resazurin added.

Figure 16: Bottom:
Solutions 4,5,6 with
resazurin added.

Trial 4

Note: This trial was done in flat-bottomed wells, unlike the others, which were done in rounded-bottom wells, so the pictures of bacteria (as well as observations) may look different due to the fact that gravity does not pull the bacteria to the bottom anymore.

Results of Minimum Inhibition Concentration	(MIC- the procedure where the percentage of solution is progressively diluted in different wells.)
Solution 1:	Growth starts in well B (45% solution)
Solution 2:	Growth starts in well G (0.140625% solution)
Solution 3:	Growth starts in well E (5.625% solution)
Solution 4:	Growth starts in well H (0.703125% solution)
Solution 5:	Growth starts in well F (2.8125% solution)
Solution 6:	Growth starts in well C (22.5% solution)

Solutions	Observations of Bacterial Growth
Solution 1:	Cloudy and translucent. In well B, the solution is only very slightly cloudy; the growth is barely noticeable. The wells with more advanced growth starts of as a very slightly yellowish white, and then gradually turns into a yellower hue.
Solution 2:	Well G is almost transparent (more than solution 1) and is off-white. In well H, the growth is cloudier and slightly yellower.
Solution 3:	Off-white color that is less yellow than in solution 1. Translucent.
Solution 4:	Bacterial growth is a yellow hued white.
Solution 5:	Off-white, translucent. To the naked eye, the growth does not seem to increase or decrease (although the plate reader detected a difference).
Solution 6:	Growth is almost transparent in well C, but turns much more opaque in later wells. Gradually turns yellower as the concentration of solution decreases. The yellowish color of the bacterial growth (and therefore the concentration of bacteria) is greater than the other solutions.

Negative Control	No growth in negative controls (which means there was no contamination in the CA-MHB broth.)
Positive Control	There was lots of growth in positive controls.

Exp01

	Plate01												Endpoint Lm1: 600 Automix: Off Calibrate: On Column Priority C. Speed: Normal
	1	2	3	4	5	6	7	8	9	10	11	12	
A	0.043	0.042	0.041	0.040	0.040	0.040	0.046	0.045	0.045	1.035	0.046	0.039	Plate Last Read: 3:25 PM 12/6/20
B	0.067	0.059	0.042	0.042	0.041	0.041	0.046	0.045	0.046	0.940	0.046	0.039	
C	0.653	0.613	0.040	0.040	0.040	0.040	0.046	0.046	0.046	0.961	0.046	0.039	
D	0.742	0.741	0.040	0.040	0.039	0.040	0.046	0.046	0.046	1.014	0.046	0.039	
E	0.806	0.717	0.040	0.039	0.538	0.593	0.046	0.045	0.046	0.964	0.046	0.039	
F	0.890	0.883	0.039	0.039	0.671	0.662	0.046	0.045	0.046	0.994	0.046	0.039	
G	0.923	0.875	0.655	0.531	0.636	0.658	0.046	0.046	0.046	1.027	0.046	0.038	
H	0.983	1.011	0.609	0.585	0.807	0.819	0.046	0.046	0.046	1.071	0.046	0.039	

Wavelength Combination: !Lm1

Mean Temperature: 25.0

Reader: VersaMaxPLUS ROM v1.21 Aug 18 2005

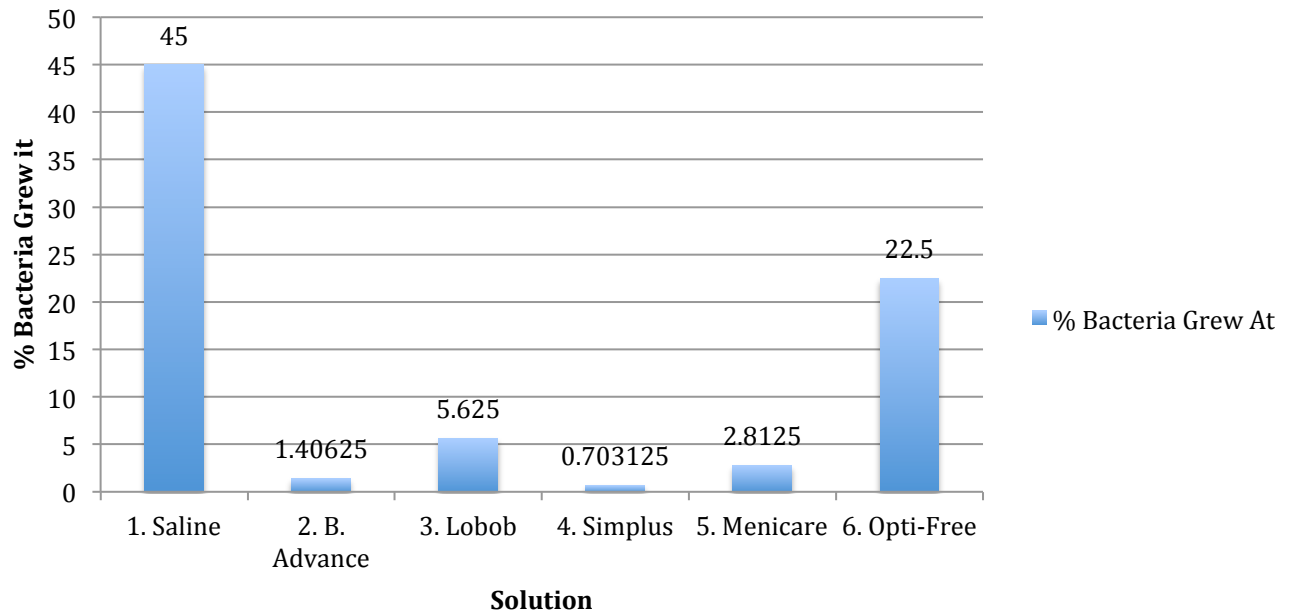
Figure 17: Above: Plate readings for solutions 1,2,3
Figure 18: Below: Plate readings for solutions 4,5,6

Exp01

	Plate01												Endpoint Lm1: 600 Automix: Off Calibrate: On Column Priority C. Speed: Normal
	1	2	3	4	5	6	7	8	9	10	11	12	
A	0.041	0.041	0.040	0.041	0.042	0.042	0.046	0.046	0.046	1.029	0.046	0.039	Plate Last Read: 3:25 PM 12/6/20
B	0.041	0.043	0.041	0.041	0.050	0.045	0.046	0.046	0.046	1.004	0.046	0.039	
C	0.040	0.040	0.039	0.041	0.161	0.058	0.046	0.046	0.046	0.964	0.046	0.039	
D	0.041	0.040	0.041	0.040	0.928	0.888	0.051	0.046	0.103	0.980	0.046	0.039	
E	0.039	0.040	0.047	0.041	1.117	1.099	0.119	0.091	0.118	0.921	0.046	0.039	
F	0.039	0.039	0.703	0.700	1.222	1.191	0.045	0.046	0.046	0.958	0.046	0.038	
G	0.041	0.039	0.660	0.635	1.131	1.161	0.046	0.046	0.046	0.953	0.047	0.038	
H	1.024	1.067	0.842	0.800	1.178	1.165	0.046	0.046	0.046	1.017	0.046	0.039	

Wavelength Combination: !Lm1

Trial 4: Percentage of Solution in Which Bacteria Began to Grow



Solution	Resazurin Results:
Solution 1:	Medium amount of live staph in well B. Lots of growth from well C down.
Solution 2:	Lots of growth in wells G and H.
Solution 3:	Medium amount of live staph in well A. Very, very slight growth in well B. Lots of live staph from well F down.
Solution 4:	Lots of live staph in well H.
Solution 5:	High amount of live bacteria in well A. Medium amount in wells B and F. Lots in wells G and H.
Solution 6:	High amount of live staph from well D down.

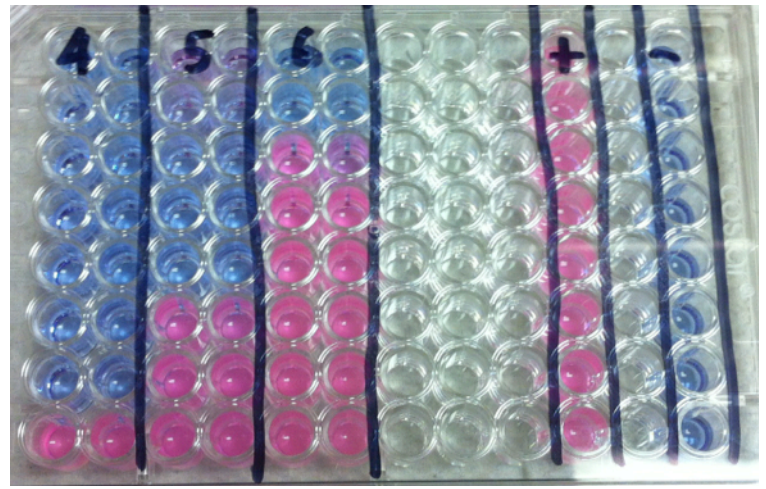
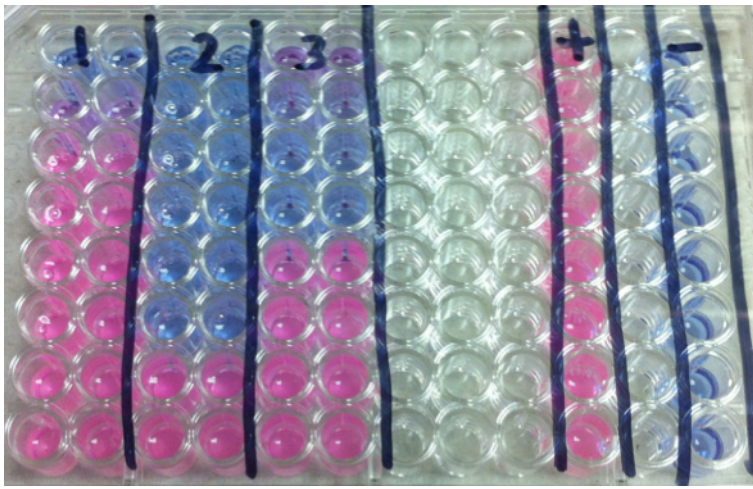


Figure 19: Above left: Solutions 1,2,3 with resazurin added.

Figure 20: Above right: Solutions 4,5,6 with resazurin added.



Figure 21: Left top: Solutions 1,2,3 (Solution 1 being the two columns on the far left, Solution 2 on its left, etc.)

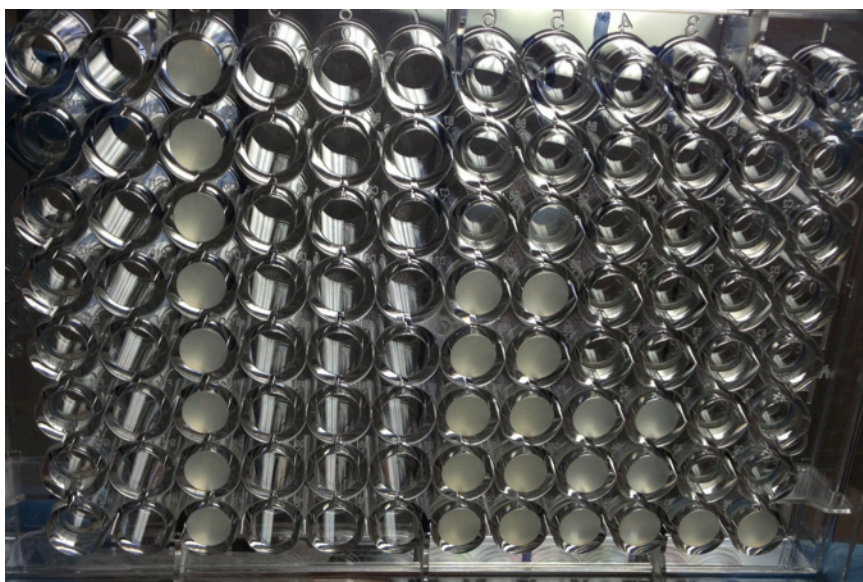


Figure 22: Left bottom: Solutions 4,5,6 (Solution 4 being the two columns on the far left, Solution 5 on its left, etc.)



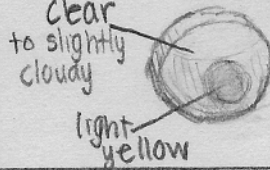

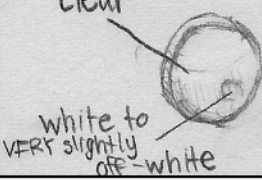

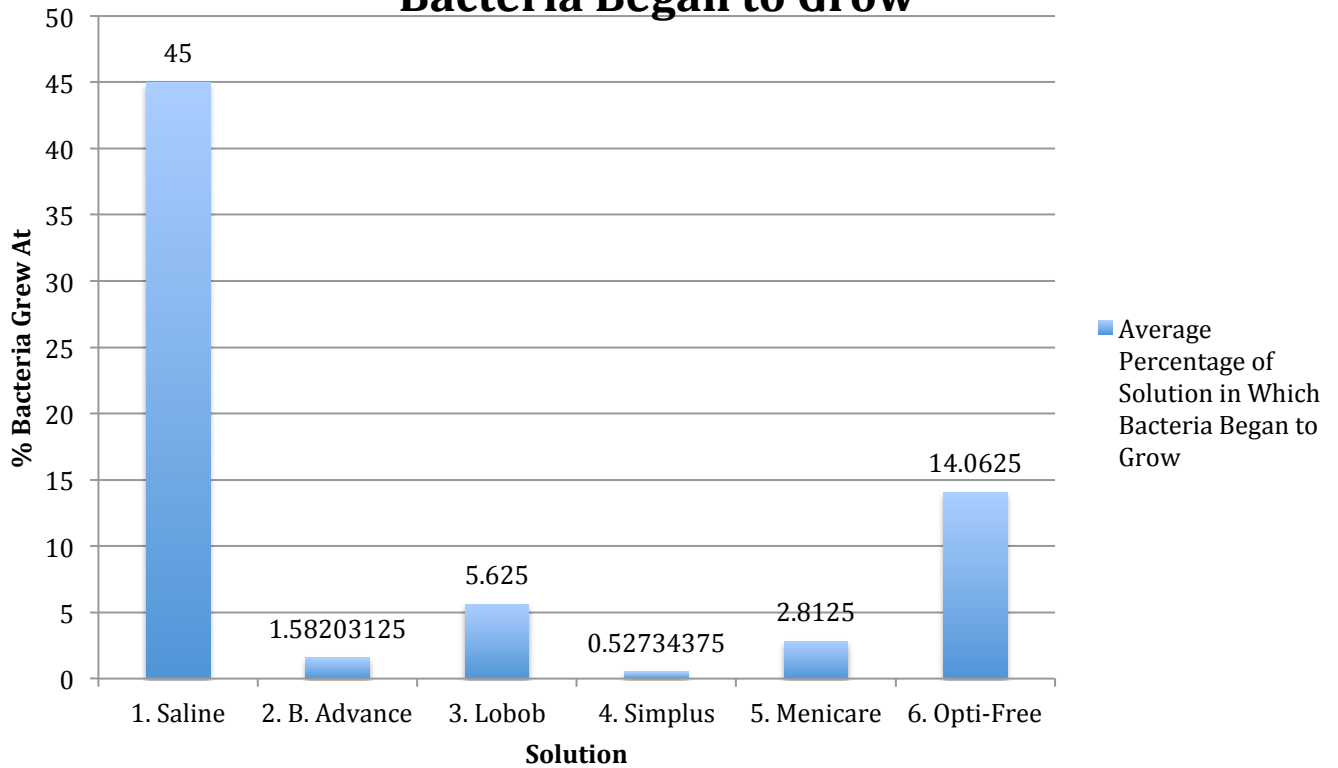
		"Stages" of Bacterial Growth (view of wells from bottom)	
		Rounded wells	Flat wells
High growth	 <p>cream-colored; cloudy golden-yellow</p> <ul style="list-style-type: none"> - concentrated "dot" in center (where bacteria collected) - foggy areas of growth radiating from the "dot" 	 <ul style="list-style-type: none"> - cloudy; creamy, yellow-hued color - almost opaque - fills entire well bottom 	
Medium growth	 <p>clear to slightly cloudy light yellow</p> <ul style="list-style-type: none"> - opaque light yellow, circular blob - solution is generally clear around it 	 <ul style="list-style-type: none"> - off-white, translucent - fills the bottom of the entire well 	
Low growth	 <p>clear white to VERY slightly off-white</p> <ul style="list-style-type: none"> - white, smaller circle growth - clear around it (the solution) 	 <ul style="list-style-type: none"> - almost transparent, just barely an off-white hue - fills entire well bottom 	

Figure 23: A chart for the "stages" of bacterial growth described throughout this paper. Refer to this chart when reading observations.

Solution:	Average Percentage of Solution in Which Bacteria Began to Grow
Solution 1:	45%
Solution 2:	1.0546875%
Solution 3:	5.625%
Solution 4:	0.52734375%
Solution 5:	2.8125%
Solution 6:	14.0625%

Average Percentage of Solution in Which Bacteria Began to Grow



Other Photos Taken During Experimentation

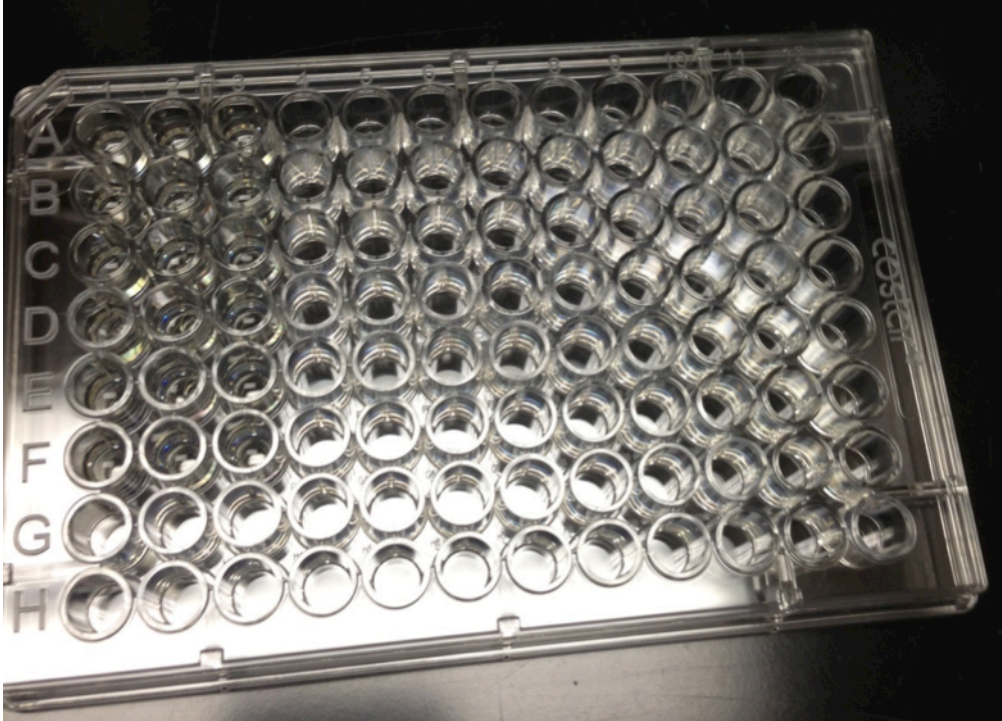


Figure 24: A fresh, blank assay plate. In this photo, the 12 columns and the rows labeled A through H are visible. In the solution dilutions, well A always contained 90% solution, 10% bacteria. Well B contained 45% solution, well C contained 22.5%, well D contained 11.25%, etc.

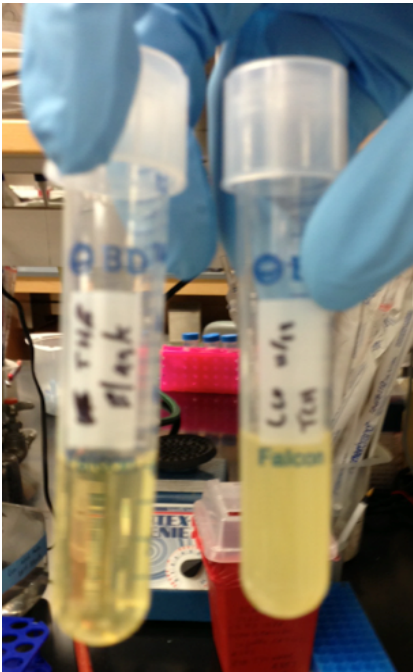


Figure 25: Right: Grown bacterial colonies on an agar plate. A single colony was used to prepare the bacteria/Todd Hewitt Broth (THB) mixture that was tested against the solutions.

Figure 26: Left: A tube with just THB broth (left) and a tube with bacteria grown in it (right) are compared. The tube with bacteria is distinctly cloudier.

