MEMORANDUM

BRISTOL LABORATORIES

UNIT OF BRISTOL-MYERS COMPANY

FROM A. Gourevitch **DATE** December 5, 1955

TO J. Lederberg **SUBJECT** New Antibiotics Screening Program - Consultantship Arrangement

C.C. J. Lein

Dear Joshua:

Joe suggested I send you the message at this time so that I can go into some procedural details of our screening for phage induction.

Your suggestions were very helpful, and we modified our procedure to take them into consideration. Let me first describe the procedure as we use it now and then discuss some of the points of divergence with the one you suggested, and also the difficulties we encounter.

We use a 12 to 16 hour old, nutrient broth culture of K-12, which we inoculate into M-9 medium at the rate of 10,000 cells per ml. We place 1 ml of this suspension in 3/4" test tubes, and add 0.2 ml fermented broth. For control, 0.2 ml of non-fermented broth is added. The broths are agitated for 2 hours on a Khan shaker. The tubes are placed on the shaker with the tube axis slanted in the direction of shaking, in order to increase aeration. After 2 hours agitation, 10 ml nutrient broth (+ 0.5% NaCl) is added to each tube, and they are agitated 2 more hours. At that time platings are done, without dilution. We use now plates containing nutrient agar + 0.5% NaCl + 50 µg/ml streptomycin. For plating, we prepare soft (0.5% agar) nutrient agar + 0.5% NaCl and dispense in Khan tubes. The agar is melted and cooled to 45°C. Prior to plating, 2 drops of a 24 hour culture of W-3001 is added to each tube, followed by 1 ml of treated broth. The contents of the tubes are mixed by repeated inversion, and poured over a plate. Duplicate plates are used for each broth. The plates are left right side up for about 45 minutes, after which they are incubated at 37°C upside down.

Under these conditions the non-fermented control will have anywhere between 10 and 100 plaques, and a large increase in plaque count over the control can be observed visually. The presumptive positives can be then rerun with dilutions.

One thing I should mention immediately, and that is the age of the K-12 culture. We realize that 16 hours is too old. I am getting myself a little thermostatically controlled bath to take home, so that I can inoculate cultures later at night and use a younger culture. We noted that our controls come out much lower if a 10 hour culture is used instead of a 16 hour one.

Before choosing this procedure we compared it with our old procedure (4 hour incubation in stationary tubes) and with the procedure in which all incubation was done on the shaker but omitting the addition of nutrient broth. The results with azaserine were as follows:

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	Stationary incubation	4 hour incubation on shaker in M-9	2 hour incubation on shaker in M-9 followed by 2 hours in nutrient broth
Control	200	200	400
+ 1 γ/ml azaserine	1800	4500	620,000

We decided to stick with M-9 because with the addition of fermentation broth the difference between M-9 and your synthetic medium is negligible. The fermentation broth contains yeast and cottonseed meal proteins, so that we really deal with supplemented medium in the first place. We usually do get higher plaque yields with the control containing the non-fermented broth than all synthetic medium control.

One of the big differences between the procedure outlined and the one you suggest lies in the cell density during the induction. You suggest 10^8 cells per ml and we use 10^4. My main consideration in choosing the lower figure is due to the fact that under our conditions no dilution is necessary. We usually can tell at a glance a 10 fold increase in plaque counts, and consider these cultures as presumptive positives which are then studied a little closer. This of course is only possible because we do not get too many presumptive positive broths coming through; this could not be assumed a priori. If you know of any reason for our not using 10^4 cells, I would appreciate learning of it. I actually can see some virtue in our method. The fact that we do not dilute not only saves time but also reduces the experimental error due to the dilution and the fluctuation from tube to tube.

Now let me turn to some problems we encounter. Concerning the plaque size, we find that the dryness of the plate surface is very critical. When we use old plates, the plaques come out very small, whereas fresh plates yield large and irregular plaques.

One of the things plaguing us is an occasional lack of replication. In most cases, replication is not too bad (we run 2 plates for each experiment), but sometimes it is atrocious. Also there is quite often a discrepancy between different dilutions. We have cases where non-diluted broth gives a count of 800 plaques per ml and the same broth diluted 100 times -- no plaques at all on both plates. As diluent we use Soerensen pH 7.0 buffer. Maybe we should use a different diluent?

A. Gourevitch

AG:jlg