February 20, 1947.

Dear Professor Lederberg,

Thank you for your letter of the 9th.

I am afraid I have no methods of the type you suggest. My own ideas are along the lines of modification of the "layer plate" technique, either by use of cellophane membranes or by allowing active substances to diffuse upwards into the inoculated agar. With fast spreading methods, these techniques have manifest advantages, and though in theory they sound satisfactory enough, I have not had time to experiment much with them. On the basis of my experience I would suggest (for Prototroch or Aga mediterranea agar) the growth of the plate of agar until symptoms first appear. The removal either by heat (at 60°C point 30) of the fresh colonies and then the resubmitting of the plate by incubation of the skim Medlarstil gene in a filter paper to allow liquid containing the factor to be run underneath, or alternatively the whole agar layer could be soaked...
I have, as published, used the filtration technique of Fries. This appears to depend on the power of germination of the organism in the absence of the deficient factor. Similarly, the spores will be able to germinate on their reserves of sufficient extent to make them "partial cisp," considered from the point of view of filtration, comparable with that of the normal. Again one would expect their heat or alcohol-susceptibility to be much the same.

I have tried no mass-cultivation, nor mass spore-suspension-miscule, in presence of the substance whose deficient mutants are under study. This appears to me to have possibilities, in a work of bacterial study, but with regard the difficulty in distinguishing identical mutants from similar mutants (as members of the same a deficient clone) would render any study of mutation frequencies liable to error. However this might prove a means of obtaining large numbers of mutants of the mass spore technique was accompanied by heavy exposure to mutagens.

As my work is more concerned with the chemical synthetic mechanism (a paper will shortly appear in Biochimica Biophysica), I am engaged now upon quantitative work stemming from the original findings. So far these appear to be confirmed although the picture may not be quite so simple as then represented. I will let you have the reprint, if I manage to get any.

Thanking you for your very kind invitation and hoping that my observations will be of little use to you.

Yours sincerely,

Donald Henderson.