doubt after the animals have been carried over the most difficult period and are being fed at longer intervals they will require less attention if the apparatus is properly constructed. In the final analysis the experiment is largely a matter of mechanics.

These difficulties are mentioned not to dicourage but to explain the abberrant results reported on the first series of experiments. More recent experiments gave better results. Certainly the value of such animals in experimental investigations concerning bacteriophage, filtrable vira and life cycles warrant further investigation along these lines.

Summary

It is my purpose in this paper to point out the practical possibilities of using germ free animals in bacteriological investigation especially when the research concerns life cycles or change of bacteria. Experiments show that guinea pigs can be obtained and raised in a germ free condition and put to practical use only when the technique and apparatus is especially adapted to that end. It is believed that the apparatus shown in my laboratories simplifies the technique and adapts it to this practical end.

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# THE MECHANIZATION OF CERTAIN BACTERIOLOGICAL PROCEDURES

## A Consideration of the Factors Involved in Mechanizing Single Cell Technique and the Plating Method of Counting Viable Bacterial Cells

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This paper is concerned with a discussion of the mechanization of two important bacteriological procedures, (1) single cell isolation and culture, (2) the plating out of bacterial cells in a nutrient agar for the purpose of estimating the viable cells.

Single cell methods are important in selecting pure strains of bacteria or certain organisms from a pure strain. Its practical applications are concerned with the preparation of antigens, the diagnosis of certain diseases and its use in research bacteriology.

Plating technique as referred to in this paper is used to count viable cells in samples such as milk or broth cultures. Its practical application concerns its use in the milk industries or in problems such as the study of growth curves where a great many counts must be made.

Both procedures as they stand at the present time allow too great a personal factor to be consistent. Furthermore they do not conform close enough to mechanistic principles to permit their easiest manipulation in routine tasks. With these ideas in mind an effort has been made

to reduce the personal factor to a minimum by completely mechanizing the steps involved and making their application automatic wherever possible. As in all biological problems there are certain physiological factors concerned with the cells which cannot be mechanized but differences resulting from such factors can be decreased to a certain extent by automatically controlling their manipulation so that every step of the procedure is repeated in exactly the same way.

## MECHANIZATION OF SINGLE CELL CULTURE

This technique of selecting single bacterial cells from a culture by means of micro-pipettes of glass was invented by Barber, 1904. Since its introduction it has become an important accessory in research bacteriology and has remained, up to the present time, practically unchanged. The need for specialization and its more complete mechanization is apparent from its somewhat limited use in problems where it could be used to advantage and in many semi-mechanical modifications introduced to simplify the method. Objections to the technique as it exists and is practiced at present might be summed up as follows:

1. It is not adaptable to mass work where many hundreds of isolations must be made.
2. It is difficult to make satisfactory micro-pipettes by hand.
3. It is difficult to change pipettes and to mount them in the manipulator.
4. It is difficult to prepare a satisfactory isolation surface.
5. The isolation droplet ordinarily formed on a grease film does not allow for the best definition of the isolated cells and once made are difficult to prevent from spreading beyond the confines of the field.
6. Only a low percentage of the isolated cells will grow.
7. There is no adequate control of physical conditions of moisture, temperature and the like, under which the cells are isolated.

Overcoming these difficulties involves a strict attention to details, and the design of special apparatus which can be worked without a great deal of technical ability on the part of the operator. The method and apparatus designed to mechanize single cell methods are described in a series of articles now in the hands of the editors of the *Journal of Bacteriology*. The apparatus and methods employed consist of:

1. An automatic mechanical pipette puller which makes mechanically perfect pipettes and requires no special skill or attention on the part of the operator.
2. A new and easy way of mounting and changing micro-pipettes.
3. A new type of isolation surface and automatic isolation droplet former. The isolation droplets made by this method and with this apparatus have the advantage of being flat with mechanically limited edges so that a definite area can be searched and the best possible visual conditions are obtained. Hundreds of isolation droplets containing bacterial cells can be made at once and in a routine manner.
4. A micro-manipulator, the action of which is initiated by checked levers which require a push to operate. This apparatus possesses the added advantage of being enclosed at all times and is heated electrically

to a constant temperature thus insuring constant moisture conditions as well as temperature conditions.

5. A new type of micro suction and injection apparatus which uses no pushers other than air and which can be accurately controlled with a button.

By facilitating manipulation and decreasing the time which it takes to isolate single cells much of the objection to low percentages of growth from single isolated cells should be eliminated. With completely mechanized and specialized apparatus the use of single cell technique is not difficult and requires no special skill. It is hoped that by simplifying the method it will be used with less hesitation.

## MECHANIZATION OF PLATING TECHNIQUE AS IT APPLIES TO COUNTING VIABLE BACTERIAL CELLS IN A SAMPLE

It is generally agreed by investigators familiar with plating as a means of counting viable bacteria that the personal factor together with inadequate apparatus is responsible for the wide degree of error in successive bacterial counts from the same sample. On the other hand it is generally agreed that the plating method is the most acceptable and reliable way of estimating viable bacteria and that it is basically sound. Assuming then that plating contaminated samples on a nutrient surface is basically sound, efforts have been made to mechanize the steps of the procedure and to make them more uniform.

An examination of the source of error in the technique as it stands at present shows that it lies in these factors:

1. Making exact dilutions of the sample.
2. Delivering a certain amount of the diluted sample to the nutrient medium.
3. Bacterial cells when plated out in melted agar are trapped at all levels. Some cells will not develop as deep colonies. Those colonies which are deep are difficult to count.
4. The methods at present are too time consuming and this also allows for a certain degree of error.
5. The degree of skill and the exact care involved in the most accurate work is not possessed by the average worker nor is it always possible to exercise care in the press of routine requirements.

Many investigators have devised means of eliminating these errors. Surface plating methods have been devised, roll tubes used and various physical or chemical principles employed. These modifications have been more or less unsatisfactory because of the failure to mechanize the procedure properly and so adapt it to routine. Plating technique has been mechanized with a corresponding reduction in the personal equation and an increase in accuracy in the following way:

1. By designating an automatic dilution pipette which measures the sample automatically and allows further dilution to be made in the same pipette. It is desirable to have this dilution pipette correspond quite closely in use to the pipettes in use at present.
2. By using a motor driven turn table to turn a petri dish of hardened agar.

3. By spreading the culture with a special device which compensates for the unevenness of the plating surface and which deposits the cells separately on the isolation surface in regular formation. Every cell can be removed from the spreader permitting the use of small quantities of the diluted sample.

4. By using a new type of counting apparatus which eliminates eye strain and allows all cells on the plate to be counted with little effort.

Comparative results have justified the use of this new technique. A description of the methods and the apparatus is being prepared at this writing and will be submitted to the editors of the *Journal of Bacteriology* in the near future. The apparatus used in the mechanization of single cell culture and plating may be seen in my laboratories.

## SUMMARY

It is believed that by applying mechanical principles to certain bacteriological methods it is possible to simplify the technique and make for greater accuracy in the final results. This paper presents a consideration of the necessary factors concerned with mechanizing single cell culture and plating. Apparatus has been designed, constructed and used to obtain this mechanization. Comparative results obtained by the use of the apparatus have shown the soundness of the methods.

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