COUNTING PROCEDURE FOR PHASE CONTRAST MICROSCOPY

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The bacterial culture to be investigated is mixed at 45 C with an equal amount of liquid culture medium containing 1 to 2 per cent agar and a small amount of India ink. About 5 μL of this mixture is rapidly spread on a slide kept at 45 C. A cover slip is immediately placed on top of the film and the preparation is left to cool. Within a few minutes, a solid, bacteria-containing layer, 10 to 20 μ thick, is formed between slide and cover slip. The preparation is studied under the microscope using an oil immersion objective. The bacteria in a field of view are counted by focusing gradually from the slide to the cover slip. The thickness of the agar layer is measured using the scale of the fine adjustment of the microscope. This can be done with a precision of about 0.5 μ by focusing on some India ink particles immediately below the cover slip and immediately above the slide. The position of the slide on the stage is then changed and new areas of the agar layer are investigated in the manner just described. The diameter of the field of view is measured with a stage micrometer, and the average number of bacteria per ml of the original bacterial suspension is calculated.

In an experiment of this kind, performed with a Leitz Ortholux microscope, 14 fields, containing together 243 bacteria (*Proteus vulgaris*), were investigated. The thickness of the agar layer varied between 7 and 24 μ. From the data obtained it was calculated that the original bacterial suspension contained $(1.92 \pm 0.39) \times 10^8$ bacteria (99 per cent fiducial limits). Counts performed with a conventional counting chamber having a depth of 0.1 mm gave the figure $(2.19 \pm 0.30) \times 10^8$.

The procedure described above would be especially useful for investigations concerning material that is not easily stained and thus can be most suitably studied with phase contrast microscopy, e. g., L-forms and pleuropneumonia-like organisms.

It is the author's experience that this method gives more reproducible results than counts performed with a conventional counting chamber having a depth of only 0.01 mm.

SENSITIVITY *IN VITRO* OF EIGHTEEN STRAINS OF *PASTEURELLA TULARENSIS* TO ERYTHROMYCIN

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It has been demonstrated that chloramphenicol (Parker *et al.*, J. Am. Med. Assoc., **143,** 7, 1950) and chlortetracycline (Woodward *et al.*, J. Am. Med. Assoc., **139,** 830, 1949) produce a satisfactory remission of the clinical symptoms of tularemia but that streptomycin and dihydrostreptomycin remain the drugs of choice in the treatment of infections caused by *Pasteurella tularensis* (*Bacterium tularense*). This report deals with the sensitivity *in vitro* to varying concentrations of erythromycin of 18 strains of this agent which ranged from avirulence to maximal virulence for mice. One strain of *Pasteurella novicida* was also tested.

The organisms were grown on glucose-cysteine-blood agar slants (Downs *et al.*, J. Bacteriol., **53,** 89, 1947) for 48 hr at 37 C. Growth from each slant was harvested in 1 ml of physiological saline. The suspension was divided equally between duplicate plates of glucose-cysteine-blood agar, spread evenly over the surface with sterile glass rods, and allowed to dry. Three sets of