

IMPROVED METHOD OF PHAGE ASSAY BY A PLAQUE COUNT METHOD ON MICROSCOPE SLIDES IN TRAYS¹

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Generally phage assays are carried out in petri plates by the Gratia technique (Ann. inst. more reliable than the Gratia procedure with a *Staphylococcus aureus* K₁-phage P₁ system.



Figure 1. Tray modification of Jones-Krueger plaque count method; arrangement of slides and appearance of plaques after overnight incubation at 28 C, using *Staphylococcus aureus* K₁ and phage P₁.

Pasteur, 57, 652, 1936). In 1951, Jones and Krueger (J. Gen. Physiol., 34, 374, 1951) published a plaque count technique which involved placing a layer of 0.3 per cent agar medium containing phage and assay cells on microscope slides in a large petri dish, 2 slides per dish. Statistical analysis showed this method to be

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Since then, the Jones-Krueger method has been further simplified by placing a large number of slides on moistened blotters in flat trays which are then covered with glass sheets to retain moisture. Using trays 33.7 cm long, 23.5 cm wide, 1.3 cm high, the authors have routinely placed 32 slides per tray and have incubated trays in stacks of 6. This technique has proved much faster and more economical of medium and space than the Gratia technique.

The method works well with the P₁₄ and P₃₁

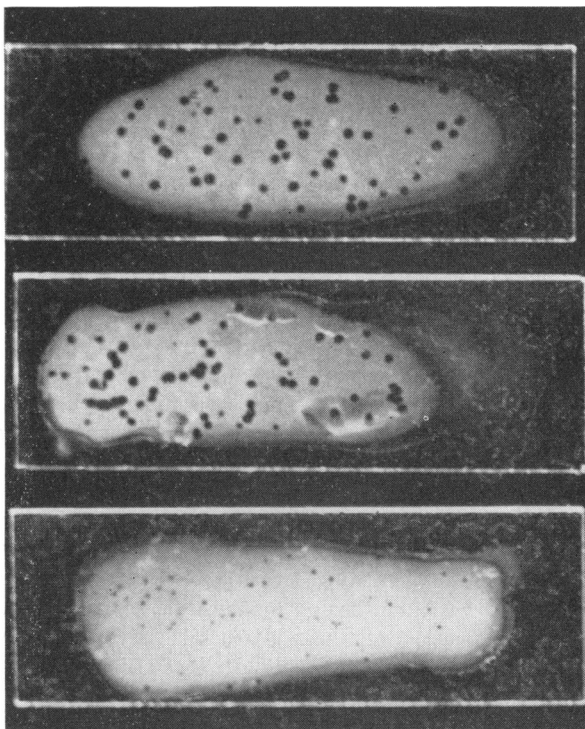


Figure 2. Effect of cell concentration on size and number of plaques in *Staphylococcus aureus* K₁-phage P₁ system at 28 C and with 0.30 per cent agar added to tryptose phosphate broth. Slides from top to bottom were layered with assay mixtures containing approximately 100 phage particles per ml and 1.0×10^7 , 3.0×10^7 , and 2.5×10^8 bacteria per ml, respectively. The inoculum cells were previously grown for 18 hr at 37 C on tryptose phosphate agar. When the plaque size is 1 to 2 mm, 50 to 250 plaques per slide give the most reliable readings.

phages on *S. aureus* strain 145; with the P₁ phage on the *S. aureus* typing strains; with T₁, T₂, and T₆ coliphages on *Escherichia coli* strain B; with T₁ and T₆ on *E. coli* strain K-12; with phage N on *Bacillus mycoides* strain N; and with a pseudomonad bacterium-bacteriophage system.

Practical points in adapting the modification to other systems are as follows: the blotters (cut to fit inside the trays) are wet with 1:1000 Roccal. The slides, after layering a 0.5-ml sample with a pipette, are air-dried for about 20 min before covering the tray with a glass sheet, the edges of which are bound with masking tape. For each system used, one should determine the optimal agar concentration, number and age of assay cells, medium, and temperature: each of these factors affects the number and size of plaques formed. Figure 1 shows the arrangement of the slides on the tray, figure 2 the effect of varying the cell concentration on the plaque size and number in a staphylococcal system.

With the staphylococcal and coliform systems, incubation at 28 C produces larger and more numerous plaques than at 37 C. If the slides are incubated at 37 C, the glass tops should be fitted carefully to the tray to prevent drying out of the agar. A slip of paper is placed under the corner of each tray with an identifying notation. A quick estimate of the plaque count can be obtained after 2 hr at 37 C, but usually trays are incubated overnight. Plaques are counted by holding each slide against another slide divided into squares with a glass-marking pencil. The blotters and slides can be used over and over again. Blotters are allowed to air-dry between titrations. Slides are washed in detergent and dried in an oven.

The method has been routinely used for 8 years and rarely have any contaminants appeared after overnight incubation, presumably because of the large inoculum of assay cells possessing short generation times.