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Official publication of the American College of Chest Physicians



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Dis Chest 1950;17:202-205
DOI 10.1378/chest.17.2.202

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A M E R I C A N C O L L E G E O F



P H Y S I C I A N S[®]

A Slide Culture Method for Streptomycin Sensitivity Testing*

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Ever since Koch first cultivated the tubercle bacillus, methods have been sought to hasten its growth (in vitro). In his original communication Koch¹ described a technique of examining cultures of tubercle bacilli grown in coagulated blood serum placed in watch glasses or hollow glass slides. He examined these preparations by low-power magnification and was able to detect growth in one week. Pryce² in 1941, stimulated interest in a slide-culture method for cultivating tubercle bacilli in sputum. He made slide cells by placing a glass ring over a micro-slide to which a liquid medium was added. With this method colony formation was visible after only three or four days. Rosenberg³ modified this technique by decontaminating the sputum smears with acid and entirely immersing the slides in tubes of liquid medium. Muller⁴ also described a modification of Pryce's technique which he used to estimate the bacteriostatic power of chemicals on the tubercle bacillus. Berry and Lowry⁵ reported early growth of tubercle bacilli from pathological material with a similar technique and gave an excellent review of the slide culture literature.

Because the existing methods⁶ of performing streptomycin sensitivity tests are laborious and time-consuming and indeed are often too slow to be of clinical value, it was suggested by two groups of workers that the slide culture method could be adapted to rapid streptomycin sensitivity testing. Giammalvo and associates⁷ employed a laked-blood medium whereas Cummings and Drummond⁸ suggested the use of Tween-albumin medium.⁹

This study concerns itself with a comparison of the slide-culture methods with the solid culture technique used routinely by most laboratories.

*Investigation begun at the Tuberculosis Evaluation Laboratory, Communicable Disease Center, Public Health Service, Atlanta, Georgia, and completed at the Tuberculosis Research Laboratory, Lawson Veterans Administration Hospital and Department of Medicine, Emory University School of Medicine, Atlanta, Georgia.

**Sponsored by the Veterans Administration and published with the approval of the Chief Medical Director. The statements and conclusions published by the authors are the result of their own study and do not necessarily reflect the opinion or policy of the Veterans Administration.

Method

Sputum specimens were obtained from patients prior to, during, and after streptomycin therapy. The specimens were divided; one part was treated by the classical NaOH method of concentration⁹ and seeded on tubes of Lowenstein-Jensen medium containing graded amounts of streptomycin (0, 1, 10 and 100 micrograms per ml. of medium). These were incubated at 37 degrees C. and examined at weekly intervals for a total of five weeks. The remaining portion of the sputum was smeared over a series of five micro-slides (3 x 1/2 inches) which were decontaminated by immersion in Petri dishes containing 5 per cent H₂SO₄. After 10 minutes the slides were transferred to Petri dishes containing sterile distilled water and left for two minutes. The slides were then removed with sterile forceps and placed in tubes of Dubos medium containing the same concentrations of streptomycin; two slides placed in medium without streptomycin serve as controls. Oval culture tubes (23 x 11.5 mm.; length 152 mm.) were employed for the purpose of allowing direct microscopic examination of the slide through the flat surface of the tube. The slide was introduced in contact with the flat wall of the tube to which it was held by a film of medium drawn up by capillary attraction. The tubes were incubated at 37 degrees C. and examined at the end of five days. If colonies with serpentine cords were noted, the slides were removed and stained by the Ziehl-Neelsen method. If no growth was noted the cultures were reincubated for a total of 14 days at which time all slides were removed and stained. Growth was nearly always detectable by the seventh day.

A record was kept of the time of appearance, number, and size of colonies appearing by both culture methods. The final reading was determined by the least concentration of streptomycin which completely inhibited growth.

Results

In this series of 176 sputum specimens it will be seen (Table 1) that positive cultures were obtained by both methods in 38 per cent of cases. Both cultures were negative in 39.2 per cent of the tests, giving a total agreement of 77.2 per cent for the two methods. The routine culture revealed 34 positive (19.3 per cent) which were missed by the slide-culture method, whereas only six positives (3.5 per cent) obtained by slide-cultures were missed by the routine culture method.

Of the 67 cultures which were positive by both methods, there were disagreements in sensitivity readings in only eight tests. Table 2 reveals that in most of these instances a higher degree

of resistance of tubercle bacilli to streptomycin was obtained by the routine culture method.

Discussion

The slide cultures were examined and could be interpreted two weeks before the routine cultures were positive. This was of great advantage clinically since any change in therapy could be instituted more rapidly. However, in those cases in which the sputum contained only small numbers of tubercle bacilli as evidenced by direct smear examination, the slide culture at times showed no growth. The inability to detect small numbers of tubercle bacilli appears to us to be the greatest limitation to the use of the slide culture method. This is probably due to the small amount of sputum which can be examined by the slide-culture method in comparison to the large amount of material which can be seeded on routine cultures. When pure cultures are employed the slide cul-

TABLE 1
Comparison of Growth on Slide Culture and Routine Culture
Streptomycin Sensitivity Tests Performed Directly
from Sputum Specimens

	Number	Per cent
Slide culture +, Routine culture +	67	38.0
Slide culture -, Routine culture -	69	39.2
Slide culture +, Routine culture -	6	3.5
Slide culture -, Routine culture +	34	19.3
<i>Totals</i>	176	100.0

TABLE 2
Comparison of Streptomycin Sensitivity Readings on Tubercle Bacilli
Obtained by Slide and Routine Culture from 67 Specimens
in Which Both Methods Yielded Growth.

	Number	Per cent
Slide culture sensitive to 10 mcg./ml. Routine culture resistant to 10 mcg./ml.	6	9.0
Slide culture resistant to 10 mcg./ml. Routine culture sensitive to 10 mcg./ml.	2	3.0
<i>Total disagreements</i>	8	12.0
Slide and routine readings same <i>Total agreements</i>	59	88.0
<i>Total tests</i>	67	100.0

ture technique supports the growth of small numbers of tubercle bacilli.

It is interesting to note that from a study of the size of the micro-colonies one can estimate "partially resistant strains" by comparing colony size on the control slide with the size of the colonies on the slides grown in streptomycin containing medium. Another advantage of the slide culture method is that the relatively short period of incubation does not allow for the development of resistance of the tubercle bacilli *in vitro*, a possibility which as yet has not been ruled out in the routine culture method which requires prolonged incubation.

SUMMARY

- 1) A rapid slide culture method for performing streptomycin sensitivity tests is described.
- 2) A comparison of this technique with the routine streptomycin sensitivity method is made on 176 sputum specimens.
- 3) The slide-culture technique has the advantages of simplicity and rapidity but at times failed to show growth when small numbers of tubercle bacilli were present in the specimen.

RESUMEN

- 1) Se describe un método rápido de porta-cultura para controlar la sensibilidad a la streptomycina.
- 2) Se compara esta técnica, con la técnica ordinaria para la sensibilidad a la streptomycina en 176 esputos.
- 3) La técnica del porta-cultura, tiene la ventaja de la rapidez y simplicidad, pero a veces no muestra crecimiento, cuando el número de bacilos tuberculosos es pequeño.

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