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Rapid Plate Test for Evaluating Phage Induction Capacity

V. W. MAYER, M. G. GABRIDGE, AND E. J. OSWALD

Cell Biology Branch, Division of Nutrition, Food and Drug Administration, Department of Health, Education, and Welfare, Washington, D.C. 20204

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An agar plate test is presented as a screening test for phage induction capacity of various chemicals.

Screening tests which detect phage induction capacity (2, 4, 9) are valuable because of the correlation between carcinogenicity, mutagenicity, phage induction, and carcinostatic activity (7). A compound which can induce the production of infective phage from a lysogenic bacterium thus has a potential for carcinogenic, mutagenic, or carcinostatic action (2, 9).

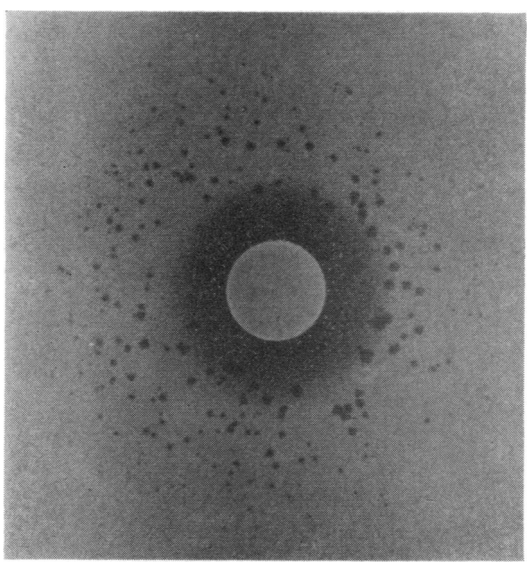

FIG. 1. *Phage induction plate test of 5 μg per disc of mitomycin C. Note the zone of toxicity immediately around the disc, followed by the zone of induction. Small colonies appear within the toxic zone which represent either phage resistant or mitomycin C resistant cells.* × *3.3.*

To determine phage induction, lysogenic cells are exposed to the test compound in a broth medium, and are then diluted, plated, and incubated. The surviving bacteria and the induced phage are then enumerated. These tests are rather inefficient for mass screening, however, because of limitations on time, equipment, and personnel. Although refinements have been introduced (3, 5), they have not been substantially documented and fail to match the speed and efficiency of the agar plate test (10) used in mutagenicity testing.

TABLE 1. *Comparison of phage induction results from plate and broth tests*

Agent	Plate test	Broth test[a]
Mitomycin C	+	+ (8)
Streptozotocin	+	+ (6)
Streptonigrin	+	+ (6)
N-methyl-*N'*-nitro-*N*-nitrosoguanidine	+	+, − (1, 4)
β-Propiolactone	+	+, − (9, 4)
Hydrogen peroxide	+	+ (4)
Hydroxyurea	+	− (4)
Ethyl carbamate	−	− (4)
5-Bromodeoxyuridine	+	+, − (9, 4)
Triethylenemelamine	+	+ (4)
Azaserine	+	+ (6)
2-Aminopurine	+	
Formaldehyde	+	
Urea	−	
Diethyl sulfate	+	
Cyclohexylamine	−	
N-(OH)-cyclohexylamine	−	
Maleic hydrazide	+	
Sodium chloride	+	
Hydrochloric acid (concn)	+	
Sodium hydroxide (5 N)	+	
N-nitrosodimethylamine	−	

[a] References for sources of results are given in parentheses.

We have recently modified the phage induction assay so that it incorporates most of the advantages of the existing tests (3, 5) and is analogous to the mutagenicity plate test (10). *Escher-*

ichia coli, strain P4X6 (lysogenic for lambda phage), and *E. coli*, strain W1485 (lambda sensitive), were incubated for 18 hr at 37 C in 1% tryptone broth. Cell concentrations were determined with a Bausch and Lomb Spectronic-model 20 spectrophotometer (660 nm), and bacteria were diluted into molten 0.6% nutrient agar overlay (0.8% nutrient broth; 0.6% agar; 0.5% sodium chloride) to yield 1×10^4 lysogenic and 1×10^7 sensitive cells per ml. A 2-ml amount of seeded overlay was spread over a base plate containing 20 ml of tryptone agar. This permitted limited growth, and the ratio of the two strains essentially eliminated background plaques.

Compounds were added to the surface of these plates as crystals or saturated antibiotic assay discs. Plates were incubated overnight at 36 C and observed for plaques in the area adjacent to the sample or its toxicity zone. Compounds were tested on duplicate or triplicate plates and the tests were repeated several times. Spontaneous plaques were determined by examining untreated plates, and positive controls, e.g., mitomycin C (Fig. 1), were included to check the efficiency of the system.

Table 1 summarizes our findings on 21 of the chemicals tested with this plate technique. Our results show agreement with seven of eight compounds previously tested in broth by other workers. In three cases there were conflicting reports in the literature. Of 11 previously unreported chemicals, 6 induced lambda phage and 5 were negative.

The phage induction test as described here is rapid, reliable, and efficient, and is well adapted to mass testing.

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LITERATURE CITED

1. Allan, R. K., and D. R. McCalla. 1966. Prophage induction by *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine. Can. J. Microbiol. 12:202–204.
2. Endo, H., M. Ishizawa, T. Kamiya, and S. Sonoda. 1963. Relation between tumouricidal and prophage-inducing action. Nature 198:258–260.
3. Gado, I., G. Savtchenko, and I. Horváth. 1965–1966. Agar-diffusion method for the screening of anticancer substances by phage induction. Acta Microbiol. Acad. Sci. Hung. 12:363–365.
4. Heinemann, B., and A. J. Howard. 1964. Induction of lambda-bacteriophage in *Escherichia coli* as a screening test for potential antitumor agents. Appl. Microbiol. 12:234–239.
5. Ikeda, Y., and T. Iijima. 1965. Simplified methods for selecting mutagenic and phage-inducing agents from microbial products. J. Gen. Appl. Microbiol. (Tokyo) 11:129–135.
6. Lein, J., B. Heinemann, and A. Gourevitch. 1962. Induction of lysogenic bacteria as a method of detecting potential antitumour agents. Nature 196:783–784.
7. Lwoff, A. 1953. Lysogeny. Bacteriol. Rev. 17:269–337.
8. Otsuji, N., M. Sekiguchi, T. Iijima, and Y. Takagi. 1959. Induction of phage formation in the lysogenic *Escherichia coli* K-12 by mitomycin C. Nature 184:1079–1080.
9. Price, K. E., R. E. Buck, and J. Lein. 1965. Incidence of antineoplastic activity among antibiotics found to be inducers of lysogenic bacteria. Antimicrob. Agents Chemother. —1964, p. 505–517.
10. Szybalski, W. 1958. Special microbiological systems. II. Observations on chemical mutagenesis in micro-organisms. Ann. N.Y. Acad. Sci. 76:475–489.