HANDBOOK OF MUTAGENICITY TEST PROCEDURES

SECOND EDITION

HANDBOOK OF MUTAGENICITY TEST PROCEDURES

SECOND EDITION

EDITED BY

B.J. Kilbey

Department of Genetics, University of Edinburgh, Scotland

M. Legator

Department of Medicine, University of Texas, Galveston, TX, USA

W. Nichols

Institute for Medical Research, Camden, NJ, USA

C. Ramel

Wallenberg Laboratory, University of Stockholm, Sweden

ELSEVIER

1984

AMSTERDAM · NEW YORK · OXFORD

ISBN 0-444-80519-2

Published by:
Elsevier Science Publishers BV
PO Box 211
1000 AE Amsterdam
The Netherlands

Sole distributors for the USA and Canada:
Elsevier Science Publishing Company, Inc.
52 Vanderbilt Avenue
New York, NY 10017
USA

Library of Congress Cataloging in Publication Data
Main entry under title:

Handbook of mutagenicity test procedures.

Includes bibliographical references and index.
1. Mutagenicity testing--Handbooks, manuals, etc.
I. Kilbey, B. J. [DNLM: 1. Mutagenicity tests--Methods.
QH 465.A1 H236]
QH465.A1H36 1984 616'.042 83-25293
ISBN 0-444-80519-2 (U.S.)

Printed in the Netherlands

PREFACE TO THE FIRST EDITION

AT A MEETING ON THE BIOLOGICAL ACTIVITY OF FLUORESCENT WHITENING AGENTS which was held in Stockholm in 1973, a number of us became concerned over variations in procedures adopted by different laboratories using nominally the same test for mutagenesis. In view of the wide variety of compounds subjected to these routines, some degree of variation in method cannot, of course, be avoided. However, to facilitate comparisons, it seemed extremely important to keep procedural variation to a minimum. With this in mind, Marvin Legator, Claes Ramel, Warren Nichols and I invited some 25 people with special experience in particular techniques of mutagen testing to set these out in a form which could provide anyone attempting a specific test with all the necessary background information on materials, experimental design, pitfalls and difficulties needed to perform the test in an acceptable way with a minimal need for additional expert help. By combining this material in one volume we hoped to provide an 'on the bench' methodological text for most of the common assays for genetic damage apparently in use.

The present volume is the result of this attempt. Some articles are far more extensive than we had originally envisaged but this may well be to the advantage of the user. Furthermore, in addition to our first list of tests, we have obtained contributions on the use of data from population records, and the handling and safety aspects of mutagens and carcinogens. We have also included a chapter which discusses some of the basic statistical concepts which must be borne in mind when mutation experiments are designed. A negative response of a population to a suspected mutagen may well result from lack of mutagenic activity but it may also be an artifact resulting from the exposure of too small a population in the first place. In few areas of research is it so important to attach confidence limits to negative results.

Our choice of material may, and probably will, be criticised. The methods included here have been sufficiently used and tested to make us reasonably confident of their reliability. We were, of course, aware of a number of exciting developments which are taking place but which are still at the very

important proving stages. Because of this, we have regretfully excluded them but we hope that we will be able to include them in a later, updated version of the book if, and when, it is prepared. This is also true of developments currently taking place in the methods used to measure the reaction mutagens with cellular components. Obviously these techniques will be of great significance for problems of risk evaluation.

The object of the book was not to suggest to industrial laboratories or any other section of the Scientific community which tests are the most satisfactory ones. We have, of course, attempted to persuade the authors to contribute information concerning the manpower and material requirements, the damage assayed and the level of expertise necessary for each test. However, when the experimenter is faced with making a choice between tests we would strongly advise him to seek the help and advice of both his National Regulatory Bodies and of those bodies which are able to provide a reservoir of scientific help and information such as the Environmental Mutagen Societies of America, Europe, India and Japan. It is also well worth reminding possible users of these tests that the Environmental Mutagen Information Center at the Biology Division of the Oak Ridge National Laboratory in Tennessee is an important source of documentation on much of the existing experimental data.

Finally, we would like to thank all those who have contributed encouragement or helpful advice and suggestions. Most of all, however, we must thank every contributor to what we hope will be a useful aid to those concerned with tests of potential mutagens.

BRIAN KILBEY

EDINBURGH 1977

PREFACE TO THE SECOND EDITION

THE MOTIVATION FOR PREPARING A SECOND EDITION OF THE *Handbook of Mutagenicity Test Procedures* has not changed. We still wish to provide a bench guide, which is as comprehensive as possible, to the techniques in use for the detection of mutagenic chemicals. In response to the suggestions from several colleagues, however, we have tried to include not only methods which are known to be reliable from their repeated usage but also some methods which are not widely used at present but which might eventually prove to be most useful in screening for mutagenic effects. Once again we do not recommend specific tests but we have tried to present a broad spectrum of approaches, hopefully in sufficient detail to allow the would be experimenter to avoid the more obvious pitfalls when he or she decides to use an unfamiliar technique.

B.J. KILBEY
EDINBURGH 1983

List of Contributors

Adler, I.D.	Institut für Genetik, Gesellschaft für Strahlen- und Umweltforschung, Neuherberg bei München, FRG
Albertini, R.J.	College of Medicine, University of Vermont, Burlington, VT 05405, USA
Ames, B.N.	Biochemistry Department, University of California, Berkeley, CA 94720, USA
Andersson, H.C.	Department of Genetics, University of Uppsala, Box 7003, S-750 07 Uppsala, Sweden
Bartsch, H.	Programme of Environmental Carcinogens and Host Factors, Division of Chemical and Biological Carcinogenesis, International Agency for Research on Cancer, 150, Cours Albert-Thomas, F-69372 Lyon Cedex 08, France
Basler, A.	Institut für Humangenetik und Anthropologie der Universität Düsseldorf, Universitätsstrasse 1, Gebäude 23.12, D-4000 Düsseldorf, FRG
Bateman, A.J.	Paterson Laboratories, Christie Hospital and Holt Radium Institute, Manchester M20 9BX, UK
Batson, A.G.	Burroughs Wellcome Co., Research Triangle Park, NC 27709, USA
Beije, B.	Wallenberg Laboratory, Environmental Toxicology Unit, Division of Cellular Toxicology, Stockholm University, S-106 91 Stockholm, Sweden
Bond, D.J.	Department of Genetics, University of Edinburgh, Edinburgh EH9 3JN, UK
Bradley, M.O.	Merck Institute for Therapeutic Research, West Point, PA 19486, USA
Bradt, C.I.	Institute for Medical Research, Camden, NJ 08103, USA
Bridges, J.W.	Robens Institute of Industrial and Environmental Health and Safety, University of Surrey, Guildford, Surrey, UK
Cleaver, J.E.	Laboratory of Radiobiology, University of California, San Francisco, CA 94143, USA

CLIVE, D.	Burroughs Wellcome Co., Research Triangle Park, NC 27709, USA
CONNOR, T.H.	University of Texas Health Science Center at Houston School of Public Health, P.O. Box 20186, Houston, TX 77025, USA
DAY, III, R.S.	37/3C23, Nucleic Acids Section, LMC, DCCP, NCI, NIH, Bethesda, MD 20205, USA
DEMARS, R.	Laboratory of Genetics, University of Wisconsin, Madison, WI 53706, USA
EHRENBERG, L.	Wallenberg Laboratory, Stockholm University, S-106 91 Stockholm, Sweden
ELLENBERGER, J.	Department of Experimental Pathology and Toxicology, Boehringer Ingelheim KG, Ingelheim, FRG
EVANS, H.J.	Medical Research Council, Clinical and Population Cytogenetics Unit, Western General Hospital, Edinburgh, UK
GAMON, L.	Department of Microbiology, Institute for Medical Research, Copewood Street, Camden, NJ 08103, USA
GATEHOUSE, D.	Genetic Toxicology Section, Histology Department, Pathology Division, Glaxo Research Ltd., Ware, Hertfordshire, UK
GORDON, L.	Lawrence Livermore National Laboratory, Biomedical Sciences Division, University of California, P.O. Box 5507, L-452, Livermore, CA 94550, USA
GREEN, M.H.L.	MRC Cell Mutation Unit, University of Sussex, Falmer, Brighton BN1 9RR, UK
GUALANDI, G.	Istituto Superiore di Sanità, Via R. Elena 299, Rome, Italy
GUPTA, R.S.	Department of Biochemistry, McMaster University, 1200 Main Street W, Hamilton, Ontario L8N 3Z5, Canada
HEDDLE, J.A.	Ludwig Institute for Cancer Research, 9, Earl Street, Toronto, Ontario M4Y 1M4, Canada
HUBBARD, S.A.	Robens Institute of Industrial and Environmental Health and Safety, University of Surrey, Guildford, Surrey, UK
JACOBS, L.	Laboratory of Genetics, University of Wisconsin, Madison, WI 53706, USA
JENSSEN, D.	Division of Toxicological Genetics, Environmental Toxicology Unit, Wallenberg Laboratory, University of Stockholm, S-106 91 Stockholm, Sweden
KADA, T.	Department of Induced Mutation, National Institute of Genetics, Mishima, Shizuoka-ken 411, Osaka 532, Japan
KERKLAAN, P.	Department of Radiation Genetics and Chemical Mutagenesis, The State University of Leiden, Leiden, The Netherlands

KIHLMAN, B.A.	Department of Genetics, University of Uppsala, Box 7003, S-750 07 Uppsala, Sweden
LEGATOR, M.S.	Department of Preventive Medicine and Community Health, Division of Genetic Toxicology, University of Texas Medical Branch, Galveston, TX 77550, USA
LÉONARD, A.	Laboratory of Mammalian Genetics, Department of Radiobiology, C.E.N.-S.C.K. B-2400 Mol, Belgium
MALAVEILLE, C.	Programme of Environmental Carcinogens and Host Factors, Division of Chemical and Biological Carcinogenesis, International Agency for Research on Cancer, 150, Cours Albert-Thomas, F-69372 Lyon Cedex 08, France
MARON, D.M.	Biochemistry Department, University of California, Berkeley, CA 94720, USA
MCGARRITY, G.J.	Department of Microbiology, Institute for Medical Research, Copewood Street, Camden, NJ 08103, USA
MILLER, J.R.	Central Research Division, Takeda Chemical Ltd., Osaka, Japan
MILLER, R.C.	Pennsylvania Hospital, Philadelphia, PA, USA
MOHN, G.	Department of Radiation Genetics and Chemical Mutagenesis, The State University of Leiden, Leiden, The Netherlands
MORPURGO, G.	Orto Botanico di Roma, L. go C. di Svezia 24, Rome, Italy
MULVIHILL, J.J.	Clinical Epidemiology Branch, National Cancer Institute, Bethesda, MD 20205, USA
NICHOLS, W.W.	Institute for Medical Research, Camden, NJ 08103, USA
NYGREN, J.	Department of Genetics, University of Umeå, S-901 87 Umeå, Sweden
PARRY, E.M.	Department of Genetics, University College of Swansea, Swansea SA2 8PP, West Glamorgan, UK
PARRY, J.M.	Department of Genetics, University College of Swansea, Swansea SA2 8PP, West Glamorgan, UK
PERRY, P.E.	MRC Clinical and Population Cytogenetics Unit, Western General Hospital, Edinburgh, UK
RASMUSON, Å.	Department of Genetics, University of Umeå, S-901 87 Umeå, Sweden
RASMUSON, B.	Department of Genetics, University of Umeå, S-901 87 Umeå, Sweden
RÖHRBORN, G.	Institut für Humangenetik und Anthropologie der Universität Düsseldorf, Universitätsstrasse 1, Gebäude 23.12, D-4000 Düsseldorf, FRG
ROSENKRANZ, H.S.	Center for the Environmental Health Sciences, Department of Epidemiology and Community Health, Case Western Reserve University, School of Medicine, Cleveland, OH 44106, USA

RUSSELL, L.B.	Biology Division, Oak Ridge National Laboratory, Oak Ridge, TN 37830, USA
SADAIE, Y.	Department of Induced Mutation, National Institute of Genetics, Mishima, Shizuoka-ken 411, Osaka 532, Japan
SAKAMOTO, Y.	Central Research Institute, Takeda Chemical Industries Ltd., Osaka 532, Japan
SALAMONE, M.F.	Ontario Ministry of the Environment, Biohazard Unit, P.O. Box 2131, Resources Road, Rexdale, Ontario M9W 5L1, Canada
SEARLE, A.G.	Medical Research Council, Radiobiology Unit, Harwell, Oxfordshire OX11 0RD, UK
SINA, J.F.	Merck Institute for Therapeutic Research, West Point, PA 19486, USA
SOBELS, F.H.	Department of Radiation Genetics and Chemical Mutagenesis, University of Leiden, Leiden, The Netherlands
STEINER, T.	Department of Microbiology, Institute for Medical Research, Copewood Street, Camden, NJ 08103, USA
STUART, E.	Ludwig Institute for Cancer Research, 9 Earl Street, Toronto, Ontario M4Y 1M4, Canada
SYLWESTER, D.L.	Department of Mathematics, University of Vermont, Burlington, VT 05405, USA
THOMSON, E.J.	MRC Clinical and Population Cytogenetics Unit, Western General Hospital, Edinburgh, UK
TURNER, N.T.	Burroughs Wellcome Co., Research Triangle Park, NC 27709, USA
VOGEL, E.	Department of Radiation Genetics and Chemical Mutagenesis, University of Leiden, Leiden, The Netherlands
WACHTMEISTER, C.A.	Wallenberg Laboratory, Stockholm University, S-106 91 Stockholm, Sweden
WATCHMAKER, G.	Lawrence Livermore National Laboratory, Biomedical Sciences Division, University of California, P.O. Box 5507, L-452, Livermore, CA 94550, USA
WÜRGLER, F.E.	Institute of Toxicology, Swiss Federal Institute of Technology and University of Zurich, CH-8603 Schwerzenbach near Zürich, Switzerland
WYROBEK, A.J.	Lawrence Livermore National Laboratory, Biomedical Sciences Division, University of California, P.O. Box 5507, L-452, Livermore, CA 94550, USA
ZIMMERMANN, F.K.	Genetik, Fachbereich Biologie, Technische Hochschule, Schnittspahnstrasse 10, D-6100 Darmstadt, FRG
ZIOLKOWSKI, C.H.J.	37/3C23, Nucleic Acids Section, LMC, DCCP, NCI, NIH, Bethesda, MD 20205, USA

CONTENTS

HANDBOOK OF MUTAGENICITY TEST PROCEDURES
second edition
edited by B.J. Kilbey, M. Legator, W. Nichols and C. Ramel

1

NEWER METHODS FOR DETERMINING GENOTOXICITY USING DNA REPAIR-DEFICIENT AND REPAIR-PROFICIENT *ESCHERICHIA COLI*

Herbert S. Rosenkranz

Center for the Environmental Health Sciences and Department of Epidemiology and Community Health, Case Western Reserve University, School of Medicine, Cleveland, OH 44106, USA

SINCE THEIR ORIGINAL DESCRIPTION [11], MICROBIAL ASSAYS USING pairs of DNA repair-deficient and DNA repair-proficient strains of microorganisms for identifying chemicals possessing genotoxic potential have been widely used as part of screening protocols for the determination and identification of potential carcinogens. Although the initial report dealt with DNA polymerase-deficient and -proficient *E. coli*, since then procedures using other microorganisms (*Salmonella*, *Proteus mirabilis*, *Bacillus subtilis*) have been described [7]. Ultimately the method was extended to eukaryotic cells such as DNA repair-proficient and -deficient Chinese hamster cells (CHO) and human cells derived from patients with xeroderma pigmentosum.

A survey of the literature [7] listed 55 pairs of DNA repair-proficient and deficient tester microorganisms. Basically, however, these assay procedures consist of one of two experimental modes: agar diffusion, which measures zones of growth inhibition, and procedures based on differential lethality.

Ideally, the assay should be performed with pairs of growing cultures exposed to a series of concentrations of test chemicals for various intervals and the number of surviving microorganisms determined by serial dilutions and plating. This, however, is impractical for screening purposes. Accordingly, a number of simplified procedures have been used with good results. A critical evaluation of the various methods used has revealed that the assay is useful for the detection of potential carcinogens [7], and accordingly it has been included in a number of screening protocols.

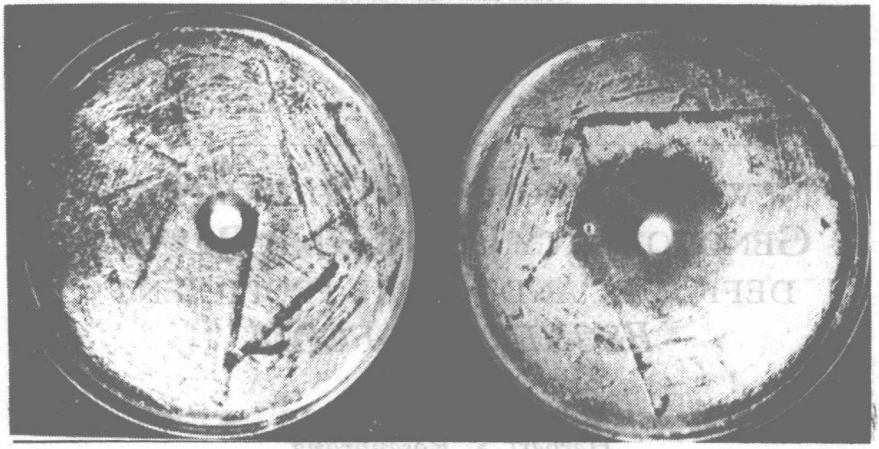

Fig. 1.1 Disc diffusion assay: Effect of polyvinylpyrrolidone-iodine (PVP-I) on the growth of *E. coli pol*A$^+$ (left) and *E. coli pol*A1$^-$ (right). In this modification of the disc diffusion assay, the bacteria were spread on the surface of the agar and discs impregnated with 1 µl PVP-I were placed on the surface of the agar. (This procedure results in non-uniform growth and uneven edges of the zones of growth inhibitor. Larger zones and even growth are obtained when the bacteria are incorporated into the agar overlay.)

The original disc diffusion procedure (Fig. 1.1) has been discussed in detail elsewhere [10] and will, therefore, not be described here. This chapter deals mainly with newer developments which have claimed to improve and broaden the applicability of the assay.

It should be noted that although the source and/or trade names of growth media are given, there is no evidence that the use of a particular medium is crucial to the success of the procedure.

A simplified differential lethality assay

A simplified differential lethality assay ('treat and plate') using improved DNA repair-deficient and -proficient strains has been described by Tweats et al. [13]. Although the assay was developed using the newly constructed *uvr*A*lex*A*rec*A derivative of *E. coli* WP2, the procedure can also be used with other DNA repair-deficient and -proficient strains. Basically, the test consists of exposing cells (approximately 2000 per 0.5 ml of buffer) to three dilutions of the test chemicals for two hours at 37°C, whereupon triplicate 10-µl amounts of the treated and untreated cultures are dropped onto the surface of nutrient agar plates. Up to twelve different 10-µl spots can be placed on a

single plate treated as well as untreated cultures can be deposited on the same plate. Surviving cells are counted after overnight incubation at 37°C. Results are transformed into percent survival and the chemical is classified as positive if the survival of the DNA repair-deficient microorganism is appreciably lower (see below) than that of its isogenic repair-proficient parent strain.

Experimental procedures

Media: Oxoid Nutrient Broth No. 2 is the growth medium. For the determination of bacterial survival, portions of cultures are deposited onto Oxoid Nutrient Broth supplemented with Davis New Zealand agar (15 g/l) or onto Oxoid Nutrient Agar plates.

Cultures: *E. coli* WP2, WP67 *uvr*A*pol*A and CM871 *uvr*A*rec*A*lex*A were used in the original procedure. It would appear that other pairs of isogenic strains could also be used. For long-term storage, bacteria growing in Oxoid Nutrient Broth No. 2 are supplemented with glycerol and stored in liquid nitrogen. For short-term cultures, the strains are kept on Oxoid Nutrient Agar slants or as single colonies on Oxoid Nutrient Agar No. 2 plates at 4°C for up to four weeks.

Activation mixtures: S9 from the livers of Aroclor 1254-induced rats (Sprague-Dawley) are prepared as described [1]. S9 preparations are stored at −70°C until used. The S9 mix consists of 1 ml S9; 1 ml NADP, 40 mM; 1 ml glucose 6-phosphate, 40 mM; 1 ml $MgSO_4$, 70 mM; 5 ml 0.1 M phosphate buffer (pH 7.4); and 1 ml water. These mixtures are filtered through Millipore membranes (0.45 μm), kept at 0°C, and 2.5 ml amounts added to 10 ml of diluted bacterial cultures, which are then dispersed as described below.

Chemicals: Chemicals are freshly dissolved in either distilled water or in dimethylsulfoxide (DMSO). It was found, however, that final DMSO concentrations of 5% and higher were bactericidal and inactivated S9 as well. Care must be taken, therefore, to maintain the final DMSO concentration at 2% or less.

Differential killing: Cultures (10 ml nutrient broths) derived from single colonies are prepared by overnight growth with agitation. The cultures are diluted with buffer (0.1 M phosphate, pH 7.0) to a final bacterial density of approximately 2×10^3 bacteria per ml. This corresponds to a dilution of approximately 2.5×10^{-5} for WP67 and CM871 and 5×10^{-5} for WP2.