The University *of* Western Ontario
BIOLOGICAL AGENTS REGISTRY FORM
Approved Biohazards Subcommittee: October 14, 2011
Biosafety Website: www.uwo.ca/humanresources/biosafety/

This form must be completed by each Principal Investigator holding a grant administered by the University of Western Ontario (UWO) or in charge of a laboratory/facility where the use of Level 1, 2 or 3 biological agents is described in the laboratory or animal work proposed. The form must also be completed if any work is proposed involving animals carrying zoonotic agents infectious to humans or involving plants, fungi, or insects that require Public Health Agency of Canada (PHAC) or Canadian Food Inspection Agency (CFIA) permits.

This form must be updated at least every 3 years or when there are changes to the biological agents being used.

Containment Levels will be established in accordance with Laboratory Biosafety Guidelines, 3rd edition, 2004, Public Health Agency of Canada (PHAC) or Containment Standards for Veterinary Facilities, 1st edition 1996, Canadian Food Inspection Agency (CFIA).

Electronically completed forms are to be submitted to Occupational Health and Safety, (OHS), (Support Services Building, Room 4190 or to jstanle2@uwo.ca) for distribution to the Biohazards Subcommittee. For questions regarding this form, please contact the Biosafety Officer at extension 81135 or biosafety@uwo.ca. If there are changes to the information on this form (excluding grant title and funding agencies), contact Occupational Health and Safety for a modification form. See website: www.uwo.ca/humanresources/biosafety.

Please ensure that all questions are fully and clearly answered. Failure to do so will lead to the form being returned, which will cause delays in your approval and frustration for you and your colleagues on the Committee.

If you are re-submitting this form as requested by the Biohazards Subcommittee, please make modifications to the form in bold print, highlighted in yellow. Please re-submit forms electronically.

PRINCIPAL INVESTIGATOR:	**Madhumita Ray**
DEPARTMENT:	**Chemical and Biochemical Engineering**
ADDRESS:	**TEB 443**
PHONE NUMBER:	**519-661-2111 ext 81273**
EMERGENCY PHONE NUMBER(S):	**519-859-7578**
EMAIL:	**mray@eng.uwo.ca**

Location of experimental work to be carried out :

Building : **TEB** Room(s): **313**

Building : Room(s):

Building : Room(s):

***For work being performed at Institutions affiliated with the University of Western Ontario, the Safety Officer for the Institution where experiments will take place must sign the form prior to its being sent to the University of Western Ontario Biosafety Officer (See Section 15.0, Approvals).**

FUNDING AGENCY/AGENCIES: **NSERC/CRD**

GRANT TITLE(S): **Micropollutant and pathogen reduction in shudge due to advanced oxidation**

UNDERGRADUATE COURSE NAME(IF APPLICABLE):

List all personnel working under Principal Investigators supervision in this location:

Name	UWO E-mail Address	Date of Biosafety Training
Sura Ali	**sali327@uwo.ca**	**Jun 1, 2011**

Please explain how the biological agents are used in your project and how they are stored and disposed of. The BARF without this description will not be reviewed.

1) -We work with the effluent samples from waste water plant
-They will be stored separately in a closed bag after sealing each sample with parafilm. They will be disposed by autoclaving at 121 C for 20 min.

2)We work also with the Muta-chromoplateTM, the kit contains two positive controls which are mutagenic and suspected to be carcinogenic agents. We handle the kit and the tested samples as potentially hazardous materials by using biohazard bag.
-The kit will be stored in the refrigerator
-Salmonella strains will be kept in the freezer at -20 C and -80 C (S. Typhimurium TA 100, TA 98, TA 97a, TA 102 and TA 1535) they are deficient mutants.

Changes to this page

Please include a ONE page research summary or teaching protocol in lay terms.
Forms with summaries more than one page will not be reviewed.

Salmonella samples will be disposed by autoclaving at 121 C for 20 mins.
Experimental protocols
1 - Direct plating of wastewater sample on m-endo agar LES, FC agar and m-enterococcus agar for enumeration of indicator bacteria, which indicate the presence of pathogenic bacteria.
2 - MPN (most probable number) of the wastewater sample to detect also the indicator bacteria in sewage which are total coliform, fecal coliform and fecal streptococci.
3 - AMES test: we are working with the Muta-chromo plate TM to detect the mutagens of the environmental samples by using Salmonella strains and different mutagens and different concentrations according to the strain subtype
4- YES assay for detection of the estrogenity activity in the wastewater which can cause the destructive disorder

Changes to this page.

1.0 Microorganisms

1.1 Does your work involve the use of biological agents? ☒ YES ☐ NO
(non-pathogenic and pathogenic biological agents including but not limited to bacteria and other microorganisms, viruses, prions, parasites or pathogens of plant or animal origin)? If no, please proceed to Section 2.0

Do you use microorganisms that require a permit from the CFIA? ☐ YES ☒ NO
If YES, please give the name of the species _____
What is the origin of the microorganism(s)? _____

Please describe the risk (if any) of escape and how this will be mitigated:

Please attach the CFIA permit.

Please describe any CFIA permit conditions:

1.2 Please complete the table below:

Full Scientific Name of Biological Agent(s)* (Be specific)	Is it known to be a human pathogen? YES/NO	Is it known to be an animal pathogen? YES/NO	Is it known to be a zoonotic agent? YES/NO	Maximum quantity to be cultured at one time? (in Litres)	Source/ Supplier	PHAC or CFIA Containment Level
Salmonella Typhimurium TA100	☐ Yes ☒ No	☐ Yes ☒ No	☐ Yes ☒ No	**0.0001**	**ebpi**	☐ 1 ☒ 2 ☐ 2+ ☐ 3
S.Typhimurium TA98	☐ Yes ☒ No	☐ Yes ☒ No	☐ Yes ☒ No	**0.0001**	**ebpi**	☐ 1 ☒ 2 ☐ 2+ ☐ 3
S.Typhimurium TA 97a	☐ Yes ☒ No	☐ Yes ☒ No	☐ Yes ☒ No	**0.0001**	**ebpi**	☐ 1 ☒ 2 ☐ 2+ ☐ 3
S.Typhimuirum TA 97b	☐ Yes ☒ No	☐ Yes ☒ No	☐ Yes ☒ No	**0.0001**	**ebpi**	☐ 1 ☒ 2 ☐ 2+ ☐ 3
S.Typhimurium TA102, TA 1535	☐ Yes ☒ No	☐ Yes ☒ No	☐ Yes ☒ No	**0.0001**	**ebpi**	☐ 1 ☒ 2 ☐ 2+ ☐ 3
	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No			☐ 1 ☐ 2 ☐ 2+ ☐ 3
	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No			☐ 1 ☐ 2 ☐ 2+ ☐ 3
	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No			☐ 1 ☐ 2 ☐ 2+ ☐ 3

Please attach a Material Safety Data Sheet or equivalent from the supplier if the bacterium used is not on this link:
http://www.uwo.ca/humanresources/docandform/docs/ohs/CFIA_Ecoli_list.pdf

Additional Comments: _____

Changes made to 1.2

2.0 Cell Culture

2.1 Does your work involve the use of cell cultures? ☐ YES ☒ NO
(If NO, please proceed to Section 3.0)

2.2 Please indicate the type of primary cells (i.e. derived from fresh tissue) that will be grown in culture:

Cell Type	Is this cell type used in your work?	Source of Primary Cell Culture Tissue	AUS Protocol Number
Human	☐ Yes ☐ No		Not applicable
Rodent	☐ Yes ☐ No		
Non-human primate	☐ Yes ☐ No		
Other (specify)	☐ Yes ☐ No		

2.3 Please indicate the type of established cells that will be grown in culture in:

Cell Type	Is this cell type used in your work?	Specific cell line(s)*	Containment Level of each cell line	Supplier / Source of cell line(s)
Human	☐ Yes ☐ No			
Rodent	☐ Yes ☐ No			
Non-human primate	☐ Yes ☐ No			
Other (specify)	☐ Yes ☐ No			

Please attach a Material Safety Data Sheet or equivalent from the supplier. (For more information, see www.atcc.org)

2.4 For above named cell types(s) indicate PHAC or CFIA containment level required ☐ 1 ☐ 2 ☐ 2+ ☐ 3

Additional Comments: _____

3.0 Use of Human Source Materials

3.1 Does your work involve the use of human source materials? ☐ YES ☒ NO
If no, please proceed to Section 4.0

3.2 Indicate in the table below the Human Source Material to be used.

Human Source Material	**Source/Supplier /Company Name**	**Is Human Source Material Infected With An Infectious Agent? YES/UNKNOWN**	**Name of Infectious Agent (If applicable)**	**PHAC or CFIA Containment Level (Select one)**
Human Blood (whole) or other Body Fluid		☐ Yes ☐ Unknown		☐1 ☐2 ☐2+ ☐3
Human Blood (fraction) or other Body Fluid		☐ Yes ☐ Unknown		☐1 ☐2 ☐2+ ☐3
Human Organs or Tissues (unpreserved)		☐ Yes ☐ Unknown		☐1 ☐2 ☐2+ ☐3
Human Organs or Tissues (preserved)		Not Applicable		Not Applicable

Additional Comments: _____

4.0 Genetically Modified Organisms and Cell lines

4.1 Will genetic modifications be made to the microorganisms, biological agents, or cells described in Sections 1.0 and 2.0? ☐ YES ☒ NO If **NO**, please proceed to Section 5.0

4.2 Will genetic modification(s) involving plasmids be done? ☐ YES, complete table below ☐ NO

Bacteria Used for Cloning *	Plasmid(s) **	Source of Plasmid	Gene Transformed or Transfected	Will there be a change due to transformation of the bacteria?	Will there be a change in the pathogenicity of the bacteria after the genetic modification?	What are the consequences due to the transformation of the bacteria?

**** Please attach a Material Safety Data Sheet or equivalent if available.***
***** Please attach a plasmid map.***
******No Material Safety Data Sheet is required for the following strains of E. coli:***
http://www.uwo.ca/humanresources/docandform/docs/ohs/CFIA_Ecoli_list.pdf

4.3 Will genetic modification(s) of bacteria and/or cells involving viral vectors be made?
☐ YES, complete table below ☐ NO

Virus Used for Vector Construction	Vector(s) *	Source of Vector	Gene(s) Transduced	Describe the change that results from transduction

**** Please attach a Material Safety Data Sheet or equivalent.***

4.3.1 Will virus be replication defective? ☐ YES ☐ NO

4.3.2 Will virus be infectious to humans or animals? ☐ YES ☐ NO

4.3.3 Will this be expected to increase the containment level required? ☐ YES ☐ NO

5.0 Will genetic sequences from the following be involved?

- HIV ☒ NO ☐ YES, specify
- HTLV 1 or 2 or genes from any Level 1 or Level 2 pathogens ☒ NO ☐ YES, specify
- SV 40 Large T antigen ☒ NO ☐ YES
- E1A oncogene ☒ NO ☐ YES
- Known oncogenes ☒ NO ☐ YES, specify
- Other human or animal pathogen and or their toxins ☒ NO ☐ YES, specify

5.1 Is any work being conducted with prions or prion sequences? ☒ NO ☐ YES

Additional Comments: _____

6.0 Human Gene Therapy Trials

6.1 Will human clinical trials be conducted involving a biological agent? ☐ YES ☒ NO
(including but not limited to microorganisms, viruses, prions, parasites or pathogens of plant or animal origin)
If no, please proceed to Section 7.0

6.2 If YES, please specify which biological agent will be used:
Please attach a full description of the biological agent.

6.3 Will the biological agent be able to replicate in the host? ☐ YES ☐ NO

6.4 How will the biological agent be administered?

6.5 Please give the Health Care Facility where the clinical trial will be conducted:

6.6 Has human ethics approval been obtained? ☐ YES, number: ☐ NO ☐ PENDING

7.0 Animal Experiments

7.1 Will live animals be used? ☐ YES ☒ NO If **NO**, please proceed to section 8.0

7.2 Name of animal species to be used

7.3 AUS protocol #

7.4 List the location(s) for the animal experimentation and housing.

7.5 Will any of the agents listed in section 4.0 be used in live animals
☐ NO ☐ YES, specify:

7.6 Will the agent(s) be shed by the animal:
☐ YES ☐ NO, please justify:

8.0 Use of Animal species with Zoonotic Hazards

8.1 Will any animals with zoonotic hazards or their organs, tissues, lavages or other body fluids including blood be used (see list below)? ☐ YES ☒ NO - If **NO**, please proceed to section 9.0

8.2 Will live animals be used? ☐ YES ☐ NO

8.3 If **YES**, please specify the animal(s) used:

♦ Pound source dogs	☐ YES	☐ NO
♦ Pound source cats	☐ YES	☐ NO
♦ Cattle, sheep or goats	☐ YES, species	☐ NO
♦ Non-human primates	☐ YES, species	☐ NO
♦ Wild caught animals	☐ YES, species & colony #	☐ NO
♦ Birds	☐ YES, species	☐ NO
♦ Others (wild or domestic)	☐ YES, specify	☐ NO

8.4 If no live animals are used, please specify the source of the specimens:

9.0 Biological Toxins and Hormones

9.1 Will toxins or hormones of biological origin be used? ☐ YES ☒ NO If **NO**, please proceed to Section 10.0

9.2 If YES, please name the toxin(s) or hormones(s)
Please attach information, such as a Material Safety Data Sheet, for the toxin(s) used.

9.3 What is the LD_{50} (specify species) of the toxin or hormone

9.4 How much of the toxin or hormone is handled at one time*?

9.5 How much of the toxin or hormone is stored*?

9.6 Will any biological toxins or hormones be used in live animals? ☐ YES ☐ NO
If **YES**, Please provide details:

*For information on biosecurity requirements, please see:
http://www.uwo.ca/humanresources/docandform/docs/healthandsafety/biosafety/Biosecurity_Requirements.pdf

Additional Comments: _____

10.0 Insects

10.1 Do you use insects? ☐ YES ☒ NO - If **NO**, please proceed to Section 11.0

10.2 If YES, please give the name of the species.

10.3 What is the origin of the insect?

10.4 What is the life stage of the insect?

10.5 What is your intention? ☐ Initiate and maintain colony, give location:
☐ "One-time" use, give location:

10.6 Please describe the risk (if any) of escape and how this will be mitigated:

10.7 Do you use insects that require a permit from the CFIA permit? ☐ YES ☐ NO
If **YES**, Please attach the CFIA permit & describe any CFIA permit conditions:

11.0 Plants

11.1 Do you use plants? ☐ YES ☒ NO - If **NO**, please proceed to Section 12.0

11.2 If YES, please give the name of the species.

11.3 What is the origin of the plant?

11.4 What is the form of the plant (seed, seedling, plant, tree...)?

11.5 What is your intention? ☐ Grow and maintain a crop ☐ "One-time" use

11.6 Do you do any modifications to the plant? ☐ YES ☐ NO
If yes, please describe:

11.7 Please describe the risk (if any) of loss of the material from the lab and how this will be mitigated:

11.8 Is the CFIA permit attached? ☐ YES ☐ NO
If **YES**, Please attach the CFIA permit & describe any CFIA permit conditions:

12.0 Import Requirements

12.1 Will any of the above agents be imported? ☐ YES, country of origin ☒ NO
If **NO**, please proceed to Section 13.0

12.2 Has an Import Permit been obtained from HC for human pathogens? ☐ YES ☐ NO

12.3 Has an import permit been obtained from CFIA for animal or plant pathogens? ☐ YES ☐ NO

12.4 Has the import permit been sent to OHS? ☐ YES, please provide permit # ☐ NO

13.0 Training Requirements for Personnel Named on Form

All personnel named on the above form who will be using any of the above named agents are required to attend the following training courses given by OHS:

- Biosafety
- Laboratory and Environmental/Waste Management Safety
- WHMIS (Western or equivalent)
- Employee Health and Safety Orientation

As the Principal Investigator, I have ensured that all of the personnel named on the form who will be using any of the biological agents in Sections 1.0 to 9.0 have been trained.

An X in the check box indicates you agree with the above statement... ☒
Enter Your Name MR ***Date:*** 25 April, 2012
Madhumita Ray

14.0 Containment Levels

14.1 For the work described in sections 1.0 to 9.0, please indicate the highest HC or CFIA Containment Level required. ☒ 1 ☐ 2 ☐ 2+ ☐ 3

14.2 Has the facility been certified by OHS for this level of containment?
☐ YES, location and date of most recent biosafety inspection:
☐ NO, please certify
☒ NOT REQUIRED for Level 1 containment

14.3 Please indicate permit number (not applicable for first time applicants):

15.0 Procedures to be Followed

15.1 Are additional risk reduction measures necessary beyond containment level 1, 2, 2+ or 3 measures that are unique to these agents? ☒ YES ☐ NO

If **YES** please describe:

Make sure that to work in the safety cabinet wearing safety glasses, mask, gloves, lab coat, and clean with ethanol , bleach and disinfectant before and after working carefully and autoclave all the waste.

15.2 Please outline what will be done if there is an exposure to the biological agents listed such as a needlestick injury or an accidental splash:
Tell the supervisor, go to the nearest health care clinic with the MSDS of the biological agent or any information help.

15.3 As the Principal Investigator, I will ensure that this project will follow the Western Biosafety Guidelines and Procedures Manual for Containment Level 1 & 2 Laboratories (and the Level 3 Facilities Manual for Level 3 projects). I will ensure that UWO faculty, staff and students working in my laboratory have an up-to-date Hazard Communication Form, found at http://www.shs.uwo.ca/workplace/workplacehealth.html

An X in the check box indicates you agree with the above statement... ☑
Enter Your Name Mr. Madhumita Ray ***Date:*** 25 April, 2012

15.4 Additional Comments: _____

16.0 Approvals

1) UWO Biohazards Subcommittee:
SIGNATURE: _____
Date: _____

2) Safety Officer for the University of Western Ontario
SIGNATURE: _____
Date: _____

3) Safety Officer for Institution where experiments will take place (if not UWO):
SIGNATURE: _____
Date: _____

Approval Number: _____ Expiry Date (3 years from Approval): _____

Special Conditions of Approval:

-------- Original Message --------
Subject:RE: Biological Agents Registry Form (Dr. M. Ray) - expired
Date: Thu, 31 May 2012 14:08:22 -0400
From: Mita Ray <mray@eng.uwo.ca>
To: Jennifer Stanley <jstanle2@uwo.ca>
CC: Sura Ali <sali327@uwo.ca>, Fate Hashemi <fhashem4@uwo.ca>, Lars Rehmann <rehmann@eng.uwo.ca>

Dear Jennifer,
Here is the corrected biosafety form. In addition, please see the following responses from the queries posed by the inspector.

1. Typing/spelling errors are taken care of.
2. Disposal procedures for *Salmonella Typhimurium* is addressed as: "Salmonella samples will be disposed by autoclaving at 121 C for 20 mins".
3. Although, initially planned, We are not working with Clostridium, and hence it is not listed.
4. The researchers are mostly working with effluents of wastewater which has lower pathogen content.
5. Sura has been vaccinated with Hepatitis A (two times, last on May 7, 2012), Tetanus, Diphtheria, and Pertussis.

Salmonella requires Containment level of 2 and Dr. Rehmann's lab (TEB 313) (where the work is being conducted) is certified to level 2.
Hope this helps. Let me know if anything else is required to get the funds released.
Best regards

Mita

Madhumita B. Ray, Ph.D., P. Eng
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Canada
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New Info

Risk Group Classification for Infectious Agents

Leave feedback about this database (questions, comments or suggestions) ›

‹ Main Riskgroup Page and Definitions

SEARCH: Bacteria › Viruses › Fungi › Parasites ›

Bacteria Search Results

Genus: Salmonella **Species:** typhimurium		
	Risk Group Level	**Notes**
Australia/New Zealand 2002:		
Belgium 2004:	2	
Switzerland 2003:	2	Exceptions are derivates of strain LT2 with stable mutations in the genes aroA, galE oder cya und crp (e.g. strains for Ames Test TA 98, TA 100, TA 1535, TA1530, TA 2631)
United Kingdom 2004:	2	
Germany 2001:	2	AR
NIH 2002	2	
European Community 2000:	2	
Singapore 2004:	2	Singapore Schedule:
Japan:		
Human Pathogen: Yes **Animal Pathogen:** Yes **Plant Pathogen:** No		**Select Agent CDC:** No **Select Agent USDA:** No
MSDS:		

American Biological Safety Association, 1200 Allanson Road, Mundelein, IL 60060-3808
Phone: 1-866-425-1385 (toll free), 847-949-1517 **Fax:** 847-566-4580 **E-mail:** info@absa.org

471
Adopted:
21st July 1997

OECD GUIDELINE FOR TESTING OF CHEMICALS

Bacterial Reverse Mutation Test

INTRODUCTION

1. The bacterial reverse mutation test uses amino-acid requiring strains of *Salmonella typhimurium* and *Escherichia coli* to detect point mutations, which involve substitution, addition or deletion of one or a few DNA base pairs (1)(2)(3). The principle of this bacterial reverse mutation test is that it detects mutations which revert mutations present in the test strains and restore the functional capability of the bacteria to synthesize an essential amino acid. The revertant bacteria are detected by their ability to grow in the absence of the amino acid required by the parent test strain.

2. Point mutations are the cause of many human genetic diseases and there is substantial evidence that point mutations in oncogenes and tumour suppressor genes of somatic cells are involved in tumour formation in humans and experimental animals. The bacterial reverse mutation test is rapid, inexpensive and relatively easy to perform. Many of the test strains have several features that make them more sensitive for the detection of mutations, including responsive DNA sequences at the reversion sites, increased cell permeability to large molecules and elimination of DNA repair systems or enhancement of error-prone DNA repair processes. The specificity of the test strains can provide some useful information on the types of mutations that are induced by genotoxic agents. A very large data base of results for a wide variety of structures is available for bacterial reverse mutation tests and well-established methodologies have been developed for testing chemicals with different physico-chemical properties, including volatile compounds.

3. Definitions used are set out in the Annex.

INITIAL CONSIDERATIONS

4. The bacterial reverse mutation test utilises prokaryotic cells, which differ from mammalian cells in such factors as uptake, metabolism, chromosome structure and DNA repair processes. Tests conducted *in vitro* generally require the use of an exogenous source of metabolic activation. *In vitro* metabolic activation systems cannot mimic entirely the mammalian *in vivo* conditions. The test therefore does not provide direct information on the mutagenic and carcinogenic potency of a substance in mammals.

5. The bacterial reverse mutation test is commonly employed as an initial screen for genotoxic activity and, in particular, for point mutation-inducing activity. An extensive data base has demonstrated that many chemicals that are positive in this test also exhibit mutagenic activity in other tests. There are examples of mutagenic agents which are not detected by this test; reasons for these shortcomings can be ascribed to the specific nature of the endpoint detected, differences in metabolic activation, or differences in bioavailability. On the other hand, factors which enhance the sensitivity of the bacterial reverse mutation test can lead to an overestimation of mutagenic activity.

6. The bacterial reverse mutation test may not be appropriate for the evaluation of certain classes of chemicals, for example highly bactericidal compounds (e.g. certain antibiotics) and those which are thought (or known) to interfere specifically with the mammalian cell replication system (e.g. some topoisomerase inhibitors and some nucleoside analogues). In such cases, mammalian mutation tests may be more appropriate.

7. Although many compounds that are positive in this test are mammalian carcinogens, the correlation is not absolute. It is dependent on chemical class and there are carcinogens that are not detected by this test because they act through other, non-genotoxic mechanisms or mechanisms absent in bacterial cells.

PRINCIPLE OF THE TEST METHOD

8. Suspensions of bacterial cells are exposed to the test substance in the presence and in the absence of an exogenous metabolic activation system. In the plate incorporation method, these suspensions are mixed with an overlay agar and plated immediately onto minimal medium. In the preincubation method, the treatment mixture is incubated and then mixed with an overlay agar before plating onto minimal medium. For both techniques, after two or three days of incubation, revertant colonies are counted and compared to the number of spontaneous revertant colonies on solvent control plates.

9. Several procedures for performing the bacterial reverse mutation test have been described. Among those commonly used are the plate incorporation method (1)(2)(3)(4), the preincubation method (2)(3)(5)(6)(7)(8), the fluctuation method (9)(10), and the suspension method (11). Modifications for the testing of gases or vapours have been described (12).

10. The procedures described in this guideline pertain primarily to the plate incorporation and preincubation methods. Either of them is acceptable for conducting experiments both with and without metabolic activation. Some compounds may be detected more efficiently using the preincubation method. These compounds belong to chemical classes that include short chain aliphatic nitrosamines, divalent metals, aldehydes, azo-dyes and diazo compounds, pyrollizidine alkaloids, allyl compounds and nitro compounds (3). It is also recognised that certain classes of mutagens are not always detected using standard procedures such as the plate incorporation method or preincubation method. These should be regarded as "special cases" and it is strongly recommended that alternative procedures should be used for their detection. The following "special cases" could be identified (together with examples of procedures that could be used for their detection): azo-dyes and diazo compounds (3)(5)(6)(13), gases and volatile chemicals (12)(14)(15)(16), and glycosides (17)(18). A deviation from the standard procedure needs to be scientifically justified.

DESCRIPTION OF THE METHOD

Preparations

Bacteria

11. Fresh cultures of bacteria should be grown up to the late exponential or early stationary phase of growth (approximately 10^9 cells per ml). Cultures in late stationary phase should not be used. It is essential that the cultures used in the experiment contain a high titre of viable bacteria. The titre may be demonstrated either from historical control data on growth curves, or in each assay through the determination of viable cell numbers by a plating experiment.

12. The recommended culture temperature is 37°C.

13. At least five strains of bacteria should be used. These should include four strains of *S. typhimurium* (TA1535; TA1537 or TA97a or TA97; TA98; and TA100) that have been shown to be reliable and reproducibly responsive between laboratories. These four *S. typhimurium* strains have GC base pairs at the primary reversion site and it is known that they may not detect certain oxidising mutagens, cross-linking agents and hydrazines. Such substances may be detected by *E.coli* WP2 strains or *S. typhimurium* TA102 (19) which have an AT base pair at the primary reversion site. Therefore the recommended combination of strains is:

1. *S. typhimurium* TA1535, and
2. *S. typhimurium* TA1537 or TA97 or TA97a, and
3. *S.* typhimurium TA98, and
4. *S.* typhimurium TA100, and
5. *E. coli* WP2 uvrA, or *E. coli* WP2 uvrA (pKM101), or *S. typhimurium* TA102.

In order to detect cross-linking mutagens it may be preferable to include TA102 or to add a DNA repair-proficient strain of *E.coli* [e.g. *E.coli* WP2 or *E.coli* WP2 (pKM101).]

14. Established procedures for stock culture preparation, marker verification and storage should be used. The amino-acid requirement for growth should be demonstrated for each frozen stock culture preparation (histidine for *S. typhimurium* strains, and tryptophan for *E. coli* strains). Other phenotypic characteristics should be similarly checked, namely: the presence or absence of R-factor plasmids where appropriate [i.e. ampicillin resistance in strains TA98, TA100 and TA97a or TA97, WP2 uvrA and WP2 uvrA (pKM101), and ampicillin + tetracycline resistance in strain TA102]; the presence of characteristic mutations (i.e. rfa mutation in *S. typhimurium* through sensitivity to crystal violet, and uvrA mutation in *E. coli* or uvrB mutation in *S. typhimurium*, through sensitivity to ultra-violet light) (2)(3). The strains should also yield spontaneous revertant colony plate counts within the frequency ranges expected from the laboratory's historical control data and preferably within the range reported in the literature.

Medium

15. An appropriate minimal agar (e.g. containing Vogel-Bonner minimal medium E and glucose) and an overlay agar containing histidine and biotin or tryptophan, to allow for a few cell divisions, is used (1)(2)(9).

Metabolic activation

16. Bacteria should be exposed to the test substance both in the presence and absence of an appropriate metabolic activation system. The most commonly used system is a cofactor-supplemented post-mitochondrial fraction (S9) prepared from the livers of rodents treated with enzyme-inducing agents such as Aroclor 1254 (1)(2) or a combination of phenobarbitone and ß-naphthoflavone (18)(20)(21). The post-mitochondrial fraction is usually used at concentrations in the range from 5 to 30% v/v in the S9-mix. The choice and condition of a metabolic activation system may depend upon the class of chemical being tested. In some cases it may be appropriate to utilize more than one concentration of post-mitochondrial fraction. For azo-dyes and diazo-compounds, using a reductive metabolic activation system may be more appropriate (6)(13).

Test substance/Preparation

17. Solid test substances should be dissolved or suspended in appropriate solvents or vehicles and diluted if appropriate prior to treatment of the bacteria. Liquid test substances may be added directly to the test systems and/or diluted prior to treatment. Fresh preparations should be employed unless stability data demonstrate the acceptability of storage.

Test conditions

Solvent/vehicle

18. The solvent/vehicle should not be suspected of chemical reaction with the test substance and should be compatible with the survival of the bacteria and the S9 activity (22). If other than well-known solvent/vehicles are used, their inclusion should be supported by data indicating their compatibility. It is recommended that wherever possible, the use of an aqueous solvent/vehicle be considered first. When testing water-unstable substances, the organic solvents used should be free of water.

Exposure concentrations

19. Amongst the criteria to be taken into consideration when determining the highest amount of test substance to be used are cytotoxicity and solubility in the final treatment mixture. It may be useful to determine toxicity and insolubility in a preliminary experiment. Cytotoxicity may be detected by a reduction in the number of revertant colonies, a clearing or diminution of the background lawn, or the degree of survival of treated cultures. The cytotoxicity of a substance may be altered in the presence of metabolic activation systems. Insolubility should be assessed as precipitation in the final mixture under the actual test conditions and evident to the unaided eye. The recommended maximum test concentration for soluble non-cytotoxic substances is 5 mg/plate or 5 µl/plate. For non-cytotoxic substances that are not soluble at 5 mg/plate or 5 µl/plate, one or more concentrations tested should be insoluble in the final treatment mixture. Test substances that are cytotoxic already below 5 mg/plate or 5 µl/plate should be tested up to a cytotoxic concentration. The precipitate should not interfere with the scoring.

20. At least five different analysable concentrations of the test substance should be used with approximately half log (i.e. $\sqrt{10}$) intervals between test points for an initial experiment. Smaller intervals may be appropriate when a concentration-response is being investigated.

21. Testing above the concentration of 5 mg/plate or 5 µl/plate may be considered when evaluating substances containing substantial amounts of potentially mutagenic impurities.

Controls

22. Concurrent strain-specific positive and negative (solvent or vehicle) controls, both with and without metabolic activation, should be included in each assay. Positive control concentrations that demonstrate the effective performance of each assay should be selected.

23. For assays employing a metabolic activation system, the positive control reference substance(s) should be selected on the basis of the type of bacteria strains used. The following chemicals are examples of suitable positive controls for assays with metabolic activation:

Specializing in Biomolecular Testing kits for Toxicity, Mutagenicity, and Genotoxicity.

96 Well Reverse Mutation Ames Test

Ames Reverse Mutation

The Muta-ChromoPlate kit is a rapid cost effective reverse mutation 96 well Ames Test which can be conducted within 3-5 days.

- Frame-Shift Mutation
- Insertion Mutation
- Missense Mutation
- Nonsense Mutation
- Deletion Mutation
- Duplication Mutation

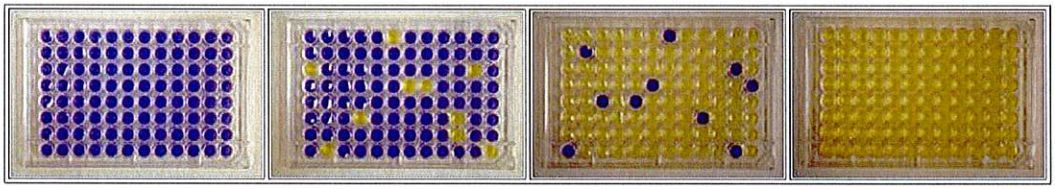
MUTA-CHROMOPLATE™

5051 - Basic Kit

B5051 - Bacterial Strain Kit

The Muta-ChromoPlate kit is a 96 well microplate version of the salmonella typhimurium Ames Test, used for detection of mutagenic activity (Follows the OECD 471 Guidelines for Testing Of Chemicals - Bacterial Reverse Mutation Test).

Developed to test mutagenic materials in water soluble extracts of , sediment, air, chemicals, food components, cosmetics, waste waters, potable waters and any other material that can be solubilized or placed into micro suspension in water such that the material being tested can be taken up by the test strain. The Muta-ChromoPlate provides a clear colour endpoint. Reagents, cultures and other consumable components are supplied ready-to-use in a non-specialized laboratory. Te



The Muta-ChromoPlate provides a clear colour endpoint. Reagents, cultures and other consumable components are supplied ready-to-use in a non-specialized laboratory. The Muta-ChromoPlate kit is based on the most generally used and bacterial reverse mutation test, known as the Ames Test (Ames et al., 1975 Mutation Research 31:347)

The test employs a mutant strain, or several strains, of Salmonella typhimurium, carrying mutation(s) in the operon coding for histidine biosynthesis. When these bacteria are exposed to mutagenic agents, under certain conditions, reverse mutation from amino acid (histidine) auxotrophy to prototrophy occurs.

Traditionally, reverse-mutation assays have been performed using agar plates. An alternate assay performed entirely in liquid culture is the 'Fluctuation Test' based on multiple yes/no colour endpoints (Hubbard, S.A *et al.*, 1994, pp. 141-160, in Kilbey *et al.* (Eds.), Handbook of Mutagenicity Testing (2nd Ed,.) Elsevier Sciences, NY. This test principle is being applied in the Muta-ChromoPlate test kit.

The Muta-ChromoPlate Kit is generally more sensitive (up to 10 times) than the pour-plate assay, because it allows testing of higher concentrations of sample (up to 75% v/v). The assay procedure is simple and requires minimal training. Consumable components are provided with ready-to-use and step-by-step instructions. "Instructions for Use" are provided with the basic kit. The only equipment required are a 37 degree Celsius incubator and a single and a multi-channel micropipettor.

S9 Activation Enzymes

S9 is a crude liver enzyme extract that can, under certain conditions, convert materials without any genotoxic activity to active genotoxic entities. The chemical process involved is probably different for different materials. In addition, the lifetime of the activated moieties is

extremely variable: some may be extremely short-lived. This is the reason for incubating the S-9 with the bacteria and the tested material at the same time.

Applications:

Testing of pharmaceuticals for mutagenic activity.

Testing of industrial effluents for presence of possible mutagenic compounds.

Screening of municipal discharges for possible routine presence or spills of mutagenic compounds.

Screening of surface and ground water for mutagenic residues.

Screening of potable water supplies for the presence of chemicals with mutagenic potential.

Screening of water soluble air pollutants for mutagenic agents.

Evaluation of pure or complexed raw mixtures for potential mutagenicity.

A convenient and easy to use teaching look for university and college laboratories.

The Muta-ChromoPlate kit (Ames Test Kit) is designed to be user friendly by removing potential contamination concerns.

Strains Currently Available to be shipped are: TA97a, TA98, TA100, TA102 and the TA1535.

Reverse Mutations in Various Bacterial Strains

Base-Pair Substitutions TA100; TA1535; TA102 (Site A-T)	**What is a Base Pair Substitution?** Insertion Mutation Missense Mutation Nonsense Mutation Deletion Mutation Duplication Mutation
Frame Shift Mutations TA98; TA97a	**What is a Frame shift Mutation?** Frame Shift Mutation

Click Here for a visual understanding on how the Muta-ChromoPlate kit is preformed

2011 Pricing

Chemical and CAS No.
9,10-Dimethylanthracene [CAS no. 781-43-1]
7,12-Dimethylbenzanthracene [CAS no. 57-97-6]
Congo Red [CAS no. 573-58-0] (for the reductive metabolic activation method)
Benzo(a)pyrene [CAS no. 50-32-8]
Cyclophosphamide (monohydrate) [CAS no. 50-18-0 (CAS no. 6055-19-2)]
2-Aminoanthracene [CAS no. 613-13-8]

2-Aminoanthracene should not be used as the sole indicator of the efficacy of the S9-mix. If 2-aminoanthracene is used, each batch of S9 should also be characterised with a mutagen that requires metabolic activation by microsomal enzymes, e.g., benzo(a)pyrene, dimethylbenzanthracene.

24. For assays performed without metabolic activation system, examples of strain-specific positive controls are:

	Chemical and CAS No.	Strain
(a)	Sodium azide [CAS no. 26628-22-8]	TA1535 and TA100
(b)	2-Nitrofluorene [CAS no. 607-57-8]	TA98
(c)	9-Aminoacridine [CAS no. 90-45-9] or ICR191 [CAS no. 17070-45-0]	TA1537, TA97 and TA97a
(d)	Cumene hydroperoxide [CAS no. 80-15-9]	TA102
(e)	Mitomycin C [CAS no. 50-07-7]	WP2 uvrA and TA102
(f)	N-Ethyl-N-nitro-N-nitrosoguanidine [CAS no. 70-25-7] or 4-nitroquinoline 1-oxide [CAS no. 56-57-5]	WP2, WP2 uvrA and WP2 uvrA (pKM101)
(g)	Furylfuramide (AF-2) [CAS no. 3688-53-7]	plasmid-containing strains

25. Other appropriate positive control reference substances may be used. The use of chemical class-related positive control chemicals may be considered, when available.

26. Negative controls, consisting of solvent or vehicle alone, without test substance, and otherwise treated in the same way as the treatment groups, should be included. In addition, untreated controls should also be used unless there are historical control data demonstrating that no deleterious or mutagenic effects are induced by the chosen solvent.

PROCEDURE

Treatment with test substance

27. For the plate incorporation method (1)(2)(3)(4), without metabolic activation, usually 0.05 ml or 0.1 ml of the test solutions, 0.1 ml of fresh bacterial culture (containing approximately 10^8 viable cells) and 0.5 ml of sterile buffer are mixed with 2.0 ml of overlay agar. For the assay with metabolic activation, usually 0.5 ml of metabolic activation mixture containing an adequate amount of post-mitochondrial fraction (in the range from 5 to 30% v/v in the metabolic activation mixture) are mixed with the overlay agar (2.0 ml), together with the bacteria and test substance/test solution. The contents of each tube are mixed and poured over the surface of a minimal agar plate. The overlay agar is allowed to solidify before incubation.

28. For the preincubation method (2)(3)(5)(6) the test substance/test solution is preincubated with the test strain (containing approximately 10^8 viable cells) and sterile buffer or the metabolic activation system (0.5 ml) usually for 20 min. or more at 30°-37°C prior to mixing with the overlay agar and pouring onto the surface of a minimal agar plate. Usually, 0.05 or 0.1 ml of test substance/test solution, 0.1 ml of bacteria, and 0.5 ml of S9-mix or sterile buffer, are mixed with 2.0 ml of overlay agar. Tubes should be aerated during pre-incubation by using a shaker.

29. For an adequate estimate of variation, triplicate plating should be used at each dose level. The use of duplicate plating is acceptable when scientifically justified. The occasional loss of a plate does not necessarily invalidate the assay.

30. Gaseous or volatile substances should be tested by appropriate methods, such as in sealed vessels (12)(14)(15)(16).

Incubation

31. All plates in a given assay should be incubated at 37°C for 48-72 hours. After the incubation period, the number of revertant colonies per plate is counted.

DATA AND REPORTING

Treatment of results

32. Data should be presented as the number of revertant colonies per plate. The number of revertant colonies on both negative (solvent control, and untreated control if used) and positive control plates should also be given.

33. Individual plate counts, the mean number of revertant colonies per plate and the standard deviation should be presented for the test substance and positive and negative (untreated and/or solvent) controls.

34. There is no requirement for verification of a clear positive response. Equivocal results should be clarified by further testing preferably using a modification of experimental conditions. Negative results need to be confirmed on a case-by-case basis. In those cases where confirmation of negative results is not considered necessary, justification should be provided. Modification of study parameters to extend the range of conditions assessed should be considered in follow-up experiments. Study parameters that might be modified include the concentration spacing, the method of treatment (plate incorporation or liquid preincubation), and metabolic activation conditions.

Evaluation and interpretation of results

35. There are several criteria for determining a positive result, such as a concentration-related increase over the range tested and/or a reproducible increase at one or more concentrations in the number of revertant colonies per plate in at least one strain with or without metabolic activation system (23). Biological relevance of the results should be considered first. Statistical methods may be used as an aid in evaluating the test results (24). However, statistical significance should not be the only determining factor for a positive response.

36. A test substance for which the results do not meet the above criteria is considered non-mutagenic in this test

37. Although most experiments will give clearly positive or negative results, in rare cases the data set will preclude making a definite judgement about the activity of the test substance. Results may remain equivocal or questionable regardless of the number of times the experiment is repeated.

38. Positive results from the bacterial reverse mutation test indicate that a substance induces point mutations by base substitutions or frameshifts in the genome of either *Salmonella typhimurium* and/or *Escherichia coli*. Negative results indicate that under the test conditions, the test substance is not mutagenic in the tested species.

Test report

39. The test report must include the following information:

Test substance:

- identification data and CAS no., if known;
- physical nature and purity;
- physicochemical properties relevant to the conduct of the study;
- stability of the test substance, if known.

Solvent/Vehicle:

- justification for choice of solvent/vehicle;
- solubility and stability of the test substance in solvent/vehicle, if known.

Strains:

- strains used;
- number of cells per culture;
- strain characteristics.

Test conditions:

- amount of test substance per plate (mg/plate or μg/plate) with rationale for selection of dose and number of plates per concentration;
- media used;
- type and composition of metabolic activation system, including acceptability criteria;
- treatment procedures.

Results:

- signs of toxicity;
- signs of precipitation;
- individual plate counts;
- the mean number of revertant colonies per plate and standard deviation;
- dose-response relationship, where possible;
- statistical analyses, if any;
- concurrent negative (solvent/vehicle) and positive control data, with ranges, means and standard deviations;
- historical negative (solvent/vehicle) and positive control data, with e.g. ranges, means and standard deviations.

Discussion of the results.

Conclusion.

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ANNEX

DEFINITIONS

A reverse mutation test in either *Salmonella typhimurium* or *Escherichia coli* detects mutation in an amino-acid requiring strain (histidine or tryptophan, respectively) to produce a strain independent of an outside supply of amino-acid.

Base pair substitution mutagens are agents that cause a base change in DNA. In a reversion test this change may occur at the site of the original mutation, or at a second site in the bacterial genome.

Frameshift mutagens are agents that cause the addition or deletion of one or more base pairs in the DNA, thus changing the reading frame in the RNA