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What is the ames test

The Ames test, named after its creator Dr. Bruce N. Ames, is a bacterial assay designed to identify substances capable of inducing mutations in DNA. By employing specific strains of bacteria, this test provides a rapid and cost-effective means of assessing the mutagenic potential of chemicals. It holds particular significance in the field of toxicology, aiding in the identification of potential carcinogens and the determination of genotoxicity for various compounds.Select ServicesBacterial Strains: The test begins by selecting specific bacterial strains that lack the ability to synthesize histidine. These strains typically have mutations in the genes responsible for histidine biosynthesis, rendering them histidine-dependent for growth.Test Substance Preparation: The chemical substance under evaluation is prepared in various concentrations or doses. It is important to choose a suitable solvent or vehicle for the substance to ensure proper solubility and minimize any potential interference with the bacteria.Metabolic Activation System: The Ames test includes the addition of a metabolic activation system derived from mammalian liver extracts. This system is used to simulate the metabolic processes that occur in living organisms, as some chemicals require metabolic activation to exert their mutagenic effects.Plate Incorporation: Petri dishes containing agar medium lacking histidine are prepared. These dishes are divided into different sectors or plates for different concentrations of the test substance, including positive and negative controls. The negative control consists of a substance known to be non-mutagenic, while the positive control contains a substance with known mutagenic properties.Pre-Incubation: A small volume of each bacterial strain is added to tubes containing the test substance, metabolic activation system, and appropriate growth medium. These tubes are incubated at a suitable temperature for a specified period, allowing the chemicals to interact with the bacteria and any mutagenic effects to manifest.Plate Spreading: After the pre-incubation period, the contents of each tube are spread onto the respective Petri dishes. The bacteria are evenly distributed over the agar surface using a sterile spreader.Incubation: The plates are then incubated under suitable conditions to allow bacterial growth. Incubation times may vary depending on the bacterial strains used and the mutagenic potential of the test substance.Colony Counting: Following incubation, the plates are examined, and the resulting colonies are counted. The number of colonies corresponds to the surviving bacteria that have undergone mutations and regained the ability to grow on a histidine-deficient medium.Data Analysis: The number of colonies on each plate is recorded and compared to the control plates. Statistical analysis is performed to determine the significance of the results and to assess the mutagenic potential of the test substance.A flowchart for Ames test procedure (Afifi et al., 2016).Interpreting Ames Test ResultsInterpreting the results of the Ames test involves analyzing the colony growth on the selective media and comparing it to the control plates. Here is a detailed explanation of how to interpret the Ames test results:Colony Counting: After the plates have been incubated, the colonies that have grown on the selective media are counted. Each colony represents a bacterial cell that has undergone a mutation and regained the ability to grow in the absence of histidine.Comparing Test Plates to Control Plates: The number of colonies on the test plates (exposed to the test substance) is compared to the number of colonies on the control plates (positive and negative controls). This comparison helps determine the mutagenic potential of the test substance.Positive Control: The positive control consists of a substance with known mutagenic properties. It serves as a reference point to validate the test's sensitivity and the reliability of the experimental setup. If the positive control produces a significantly higher number of colonies compared to the negative control, it confirms that the assay is functioning properly.Negative Control: The negative control contains a substance known to be non-mutagenic. It provides a baseline to assess the background level of spontaneous mutations that may occur during the test. The number of colonies on the negative control plates should be relatively low.Test Substance Evaluation: The mutagenic potential of the test substance is evaluated by comparing the number of colonies on the test plates with those on the control plates. If the test substance causes a significant increase in colony formation compared to the negative control, it suggests a mutagenic effect.Statistical Analysis: Statistical analysis is often applied to determine the significance of the results. Various statistical methods, such as the t-test or chi-square test, may be used to assess the significance of the differences in colony counts between the test and control groups.Dose-Response Relationship: In some cases, the Ames test may be conducted using different concentrations or doses of the test substance. This allows for the evaluation of a dose-response relationship, where the mutagenic potential is assessed at various levels of exposure. A higher number of colonies at higher concentrations may indicate a stronger mutagenic effect.It is important to note that the Ames test provides an indication of the mutagenic potential of a substance but does not directly predict its carcinogenicity or toxicity in humans. Further testing, such as in vivo studies and epidemiological research, is often required to make comprehensive conclusions about the safety and potential risks associated with a particular chemical.By carefully evaluating the colony growth and comparing it to the control plates, researchers can draw meaningful conclusions regarding the mutagenic potential of the test substance, supporting subsequent decision-making processes and regulatory assessments.Significance and Applications of the Ames TestChemical Safety AssessmentThe primary significance of the Ames test lies in its ability to assess the genotoxic potential of chemical substances. The test serves as a rapid and cost-effective screening tool to identify potential mutagens, particularly those that can induce DNA mutations. This information is crucial for evaluating the safety of chemicals and making informed decisions regarding their usage, regulation, and potential hazards to human health and the environment.Identification of Potential CarcinogensThe Ames test plays a key role in identifying potential carcinogens. Carcinogens are substances that can cause cancer by initiating or promoting genetic mutations. By screening chemicals for mutagenicity, the test helps identify compounds that have the potential to induce cancer and aids in prioritizing further testing and evaluation of potentially hazardous substances.Regulatory ComplianceRegulatory agencies worldwide, including the U.S. Environmental Protection Agency (EPA) and the European Chemicals Agency (ECHA), recognize the value of the Ames test and rely on its results in regulatory decision-making processes. The test data are utilized in assessing the safety and potential risks associated with chemicals, determining permissible exposure limits, and implementing appropriate regulatory measures to protect human health and the environment.Pharmaceutical DevelopmentThe Ames test finds extensive application in the pharmaceutical industry. It helps evaluate the mutagenic potential of drug candidates during preclinical development. By identifying compounds with mutagenic activity, pharmaceutical companies can modify or eliminate potentially harmful substances from further development, ensuring the safety of pharmaceutical products.Chemical ScreeningThe Ames test is widely employed in the screening of various chemical substances, including pesticides, industrial chemicals, food additives, and environmental pollutants. By testing large numbers of compounds, researchers can identify potentially hazardous substances early in the development or manufacturing process. This allows for the adoption of preventive measures, alternative compound selection, or the development of safer alternatives.Structure-Activity Relationship (SAR) StudiesThe Ames test data contribute to the development of structure-activity relationship models. By correlating the structural characteristics of chemicals with their mutagenic potential, researchers can predict the mutagenicity of new compounds based on their similarity to known mutagens. This approach aids in the early identification and elimination of potentially hazardous chemicals from further development.Scientific Research and Knowledge AdvancementThe Ames test has helped us understand genotoxicity and mutagenicity. It has contributed to the development of various genotoxicity assays and provided vital insights into the processes of mutagenesis. The Ames test knowledge has improved our ability to estimate the potential dangers connected with mutagenic chemical exposure and has advanced the area of toxicology in general.ReferenceAfifi, M., Eid, I., Zaher, R., Abd El-Karem, H., Karim, A. A. E., & El-Nagdy, M. (2016). Identification of Radiation Effects on Carcinogenic Food Estimated by Ames Test. Arab Journal of Nuclear Science and Applications, 49(4), 23-30. * For Research Use Only. Not for use in diagnostic procedures. Illustration of Ames Test protocol (created in BioRender). The Ames test is a widely used assay for evaluating the mutagenicity of a chemical substance. It uses several strains of auxotrophic Salmonella typhimurium, known to carry a mutation that prevents histidine biosynthesis, an essential amino acid for their growth. As a result, histidine must be supplemented in the culture media. Otherwise, his- S. typhimurium cannot grow. In the Ames test, mutagenicity is determined by exposing his- S. typhimurium to varying concentrations of the suspected substance and then selecting for reversion events. To facilitate selection, bacteria must be inoculated on HIS-selective media. This allows only bacteria that have undergone reverse mutations and reverted to their prototrophic state to survive and grow. A positive test, illustrated by the formation of visible colonies, indicates the chemical is mutagenic. This protocol includes three S. typhimurium strains - TA 98, TA 100, and TA 102 - (table 1) that are offshoots of the original strains used by Ames. In addition to lacking proper histidine biosynthesis, they contain the R-factor, which has mutations that impair their ability to repair DNA and to properly form the lipopolysaccharide cell wall. The impaired DNA repair enhances the mutation rate, and the abnormal cell wall formation allows the entry of compounds that would normally be unable to enter the intracellular space. As a result, these strains have a higher basal mutation rate and are more accessible to possible mutagens. The three strains also include plasmids to detect what type of mutation occurred. Further adaptions to the Ames test include rodent liver extract (S9). Bacteria lack the metabolic capabilities of higher-ordered organisms, and the inclusion of liver extract enables additional biochemical reactions to occur. In organisms with hepatic systems, enzymes like P450 catalyze many xenobiotics. The metabolized product may become mutagenic, so including liver extract makes it possible to discover mutagenic metabolites that wouldn't be detected with just the bacteria. TA 98FrameshifthisD3052uvrB-, rfa-+RCGCGCGCGpKM101TA 100Base-pair substitutionhisG46uvrB-, rfa-+RGGGpKM101TA 102Transition/transversionhisG428uvrB-, rfa-+RTAApKM101, pAQ1TA 1538FrameshifthisD3052uvrB-, rfa--RCGCGCGCGNoneTA 1535Base-pair substitutionhisG46uvrB-, rfa--RGGGNoneTA 1537FrameshifthisC3076uvrB-, rfa-+RTAANoneTA 1537FrameshifthisC3076uvrB-, rfa-+RTAANoneE. coli WP2uvrABase-pair substitutiontrpE65uvrA- A:TNoneE. coli WP2uvrA[pKM101]Base-pair substitutiontrpE65uvrA- A:TpKM101 Each experiment requires the following conditions for each of the three strains. The appropriate positive control depends on whether the S9 mix is present. Strains TA 98, TA 100, and TA 102 that have the S9 mix present require 2-anthramine as a positive control (2 µg/mL). If there is no S9 mix present, then use the following as a positive control: sodium azide (1 µg/mL), 2-nitrofluorine (1 µg/mL), and mitomycin C (125 ng/mL). The negative control is distilled water (dH_2O) for samples with or without the S9 mix. Test SampleYesYesNoNoNoNoS9 MixYesNoYesYesNoNoPositive ControlNoNoYesNoYesNoNegative ControlNoNoNoYesNoYes Materials Petri plates, sterile. 90 mm diameter, 15 mm depth Sodium azide 2-aminofluorene Mitomycin C 200 proof/100% ethanol, laboratory-grade Magnesium sulphate heptahydrate Ammonium sodium phosphate dibasic tetrahydrate Potassium chloride Oxoid nutrient broth #2 Citric acid monohydrate Potassium phosphate anhydrous, dibasic L-histidine hydrochloride D-biotin NADP sodium salt Magnesium chloride hexahydrate Sodium hydroxide Hydrochloric acid (for adjusting the pH of sodium phosphate buffer) Disodium hydrogen phosphate Glucose-6-phosphate (G6P) monosodium Agar Set up new colonies for each strain. Each S. typhimurium strain should be incubated overnight in Oxoid nutrient broth #2 at 37°C on a shaker set to 120 rpm. On the day of the experiment, prepare stock solutions of the positive control mutagens for tubes containing S9 and those without. Label 10 mL Falcon tubes appropriately for each strain. See Table 2 for the necessary conditions. Prepare minimal glucose plates. Aliquot 20 mL into each 90mm x 15 mm Petri plate. Label all minimal glucose agar plates. Prepare heated agar solution. Keep around 45°C in a water bath. Aliquot 5 mL of the heated agar into each 10 mL Falcon tube. Add the following to each tube containing heated agar: Vortex each 10 mL tube quickly and distribute evenly onto minimal glucose plates. Let the mixture cool for 2-3 mins. Cover plates with aluminum foil to protect them from light. Incubate for 48 hours at 37°C. Examine for colony formation. The colonies should be distributed evenly. For proper statistical analysis and comparison, refer to the: Statistical analysis of the Ames Salmonella/microsome test - PubMed. Recipes Heat 125 mL dH_2O to 45°C Dissolve 15.45 mg D-biotin and 12 mg L-histidine hydrochloride in the prewarmed dH_2O Autoclave the solution around 120°C for 20 mins and store at 4°C Vogel-Bonner Medium E (VBME) 50X stock solution (return to protocol) Heat 335 mL of dH_2O to 45°C Dissolve the following into the prewarmed dH_2O: 5 g magnesium sulfate heptahydrate 50 g citric acid monohydrate 250 g potassium phosphate anhydrous, dibasic 87.5 g sodium ammonium phosphate Keep the solution on a hot plate Once everything has dissolved, transfer the solution into a new bottle. Autoclave the solution around 120°C for 20 mins Allow time for the solution to cool off Seal the bottle and store it at 4°C 0.2 M sodium phosphate buffer (return to protocol) Dissolve 6.9 g sodium dihydrogen phosphate monohydrate into 250 mL dH_2O In a separate container, dissolve 7.1 g disodium hydrogen phosphate into 250 mL dH_2O In a sterile third container, aliquot 30 mL of the solution in step 4a and then add 220 mL of the solution from step 4b to a total of 250 mL Measure pH and adjust to 7.4 Autoclave the combined solution around 120°C for 20 mins Minimum glucose mixture (return to protocol) Dissolve 7.5 g fresh agar in 465 mL dH_2O Autoclave the solution around 120°C for 20 mins. Allow time for the solution to cool Aliquot 25 mL 40% glucose Aliquot 10 mL 50X VBME stock solution from recipe 2 1 M NADP solution (return to protocol) Dissolve 191.5 mg NADP (sodium salt) into 2.5 mL dH_2O Vortex the mixture, and once mixed, keep it in an ice bath Otherwise, store at 4°C for up to 6 months 1 M G6P solution (return to protocol) Dissolve 1.41 g G6P in 5 mL dH_2O Vortex the mixture, and once mixed, keep it in an ice bath Otherwise, store at 4°C for up to 6 months Potassium magnesium solution (return to protocol) Dissolve 30.75 g potassium chloride and 20.35 g magnesium chloride (hexahydrate) into 250 mL dH_2O Autoclave the solution around 120°C for 20 mins and store at 4°C S9 mix (return to protocol) Measure out 9.86 mL dH_2O and add the following solutions in order 12.5 mL sodium phosphate buffer (recipe 3) 1 mL NADP solution (recipe 6) 0.125 mL 1 M G6P solution (recipe 7) 0.5 mL magnesium chloride/potassium chloride solution (recipe 8) b. Do not freeze Positive control mutagen stock solutions (return to protocol) Dissolve 10 µg of the compound in 990 µL dH_2O Sodium azide 2-nitrofluorine 2-antramine Mitomycin References Langer, P. J., Shanabruch, W. G., & Walker, G. C. (1981). Functional organization of plasmid pKM101. Journal of bacteriology, 145(3), 1310–1316. Margolin, B. H., Kaplan, N., & Zeiger, E. (1981). Statistical analysis of the Ames Salmonella/microsome test. Proceedings of the National Academy of Sciences of the United States of America, 78(6), 3779–3783. Rodríguez, E., Piccini, C., Sosa, V., & Zunino, P. (2012). The use of the ames test as a tool for addressing problem-based learning in the microbiology lab. Journal of microbiology & biology education, 13(2), 175–177. Vijay, U., Gupta, S., Mathur, P., Suravajhala, P., & Bhatnagar, P. (2018). Microbial Mutagenicity Assay: Ames Test. Bio-protocol, 8(6), e2763. Original created on November 16, 2022, last updated on November 21, 2022Tagged under: Ames Test, Reverse Mutation Test, Mutagen, Ames test for mutagenicity As a library, NLM provides access to scientific literature. Inclusion in an NLM database does not imply endorsement of, or agreement with, the contents by NLM or the National Institutes of Health. Learn more: PMC Disclaimer | PMC Copyright Notice . 2018 Mar 20;8(6):e2763. doi: 10.21769/BioProtoc.2763 The Microbial mutagenicity Ames test is a bacterial bioassay accomplished in vitro to evaluate the mutagenicity of various environmental carcinogens and toxins. While Ames test is used to identify the revert mutations which are present in strains, it can also be used to detect the mutagenicity of environmental samples such as drugs, dyes, reagents, cosmetics, waste water, pesticides and other substances which are easily solubilized in a liquid suspension. We present the protocol for conducting Ames test in the laboratory. Keywords: Mutagenicity, Carcinogenicity, Salmonella strains , Gene mutation, Revertants The Microbial Ames test is a simple, rapid and robust bacterial assay consisting of different strains and applications of Salmonella typhimurium/E. coli, used for ascertaining the mutagenic potential (Levin et al., 1982 ; Gupta et al., 2009). In 1975, Ames and his followers standardized the traditional Ames assay protocol and reappraised in 1980's (Maron and Ames, 1983). Induction of new mutations replacing existing mutations allows restoring of gene function. The newly formed mutant cells are allowed to grow in the absence of histidine and form colonies, hence this test is also called as 'Reversion assay' (Ames, 1971). While traditional Ames test is quite laborious and time consuming for initial monitoring of mutagenic compounds, miniaturization of liquid suspension significantly impacted the usability by making it more convenient. The standard doses (2 µl, 5 µl, 10 µl, 50 µl and 100 µl) were set to evaluate the mutagenicity from lower to higher concentration (Hayes, 1982). Mice liver has been used as a tissue for preparing homogenate 9,000 × g (S9 hepatic fraction) whereas in S9 mix, hepatocytes are used to minimize the mammalian metabolic activation formed in the mice liver. In Ames bioassay, the sensitivity of a compound for mutagenicity is based on the knowledge that a substance which is mutagenic in the presence of liver enzymes metabolizing compound might be a carcinogen (Mathur et al., 2005). Genetic Approach: The Salmonella/E. coli tester strains: Several strains of Salmonella typhimurium have been used in Ames assay which requires histidine synthesis to assess the mutagenicity. In the histidine operon, each tester strain contains a different mutation. In addition to the histidine mutation, the standard tester strain of Salmonella typhimurium contains other mutations that greatly enhance their ability to detect the mutations (Figure 1). One of the mutations (rfa) causes partial loss of the lipopolysaccharides barrier that coats the surface of the bacteria and increases permeability to large molecules such as benzo[a]pyrene allowing not to penetrate in the normal cell wall (Mortelman and Zeiger, 2000). The mutagens present in the tested samples give rise to induced revertants on a minimal medium (absence of histidine). They are further used to observe revertants in previously mutated strains (that are not able to grow in a medium without histidine). The other mutation (uvrB) is a deletion mutation in which deletion of a gene, coding for the DNA excision repair system, causing gradually increased sensitivity in detecting many mutagens (Ames et al., 1973a). The reason behind this mutation is the deletion excising the uvrB gene emulsifying these bacteria requiring biotin for growth. The standard strains such as TA 97, TA 98, TA 100 and TA 102 contain the R-factor plasmid, pKM101. These R-factor strains are reverted by a number of mutagens that are detected weakly or not at all with the non R-factor parent strains (Ames et al., 1975a). (modified from Many studies (Ames et al., 1975b ; Levin et al., 1982) revealed that development of plasmid pKM101 in TA 1535 and TA 1538 strains leads to complement other isogenic strains such as TA 98, TA 100, TA 104 and TA 102. The his G46 mutation in TA 100 and TA 1535 codes for the first enzyme of histidine biosynthesis (hisG) (Ames et al., 1975b). This mutation, determined by DNA sequence analysis, substitutes proline (-GGG-) for leucine (-GAG-) in the wild type organism (Barnes et al., 1982). The tester strains TA 1535 and its R-factor derivative present in TA 100, detect mutagens which causes base-pair substitutions generally at one of these G-C pairs. The hisD3052 mutation in TA 1538 and TA 98 is in the hisD gene coding for histodinol dehydrogenase. TA 1538 and its R-factor derivative TA 98 detect various frameshift mutagens in repetitive sequences as 'hot spots' resulting in a frame shift mutation (Walker and Dobson, 1979; Shanabruch and Walker, 1980) (Table 1). Strain Reversion event Histidine mutation LPS defect, markers R-factor Target DNA Plasmid References TA 98 Frame shift D3052 rfa, uvrB +R CGCGCGCG pKM101 Simmon et al., 1977 TA 1538 Frame shift D3052 rfa, uvrB -R CGCGCGCG - Ames et al., 1975b TA 100 Base pair substitution G46 rfa, uvrB +R GGG pKM101 Maron and Ames, 1981 and 1983 TA 1535 Base pair substitution G46 rfa, uvrB -R GGG - Maron and Ames, 1983 TA 1537 Frame shift C3076 rfa, uvrB - CCC - Zeiger et al., 1985 TA 102 Transition/Transversion G428 rfa, uvrB +R TAA pKM101, pAQ1 Venitt and Boswoth, 1983 E. coli WP2 uvrA Base pair substitution - uvrA - - - Brusick et al., 1980 Levin et al. (1982) described a standard strain Salmonella typhimurium bacterium called TA 102 which was used to evaluate the effect of some compounds reacting with nucleotides AT. Tester strain TA102 containing nucleotides AT, present in hisG gene carrying plasmid pAQ1. There are certain mutagenic agents which are detected by TA 102 but not by TA 1535, TA 1537, TA 1538, TA 98 and TA 100 (Wilcox et al., 1990). Before performing experiment, a new set of fresh strains are prepared; and the genotypes are assessed (R-factor, His, rfa and uvrB mutations). For these, we refer readers to many excellent reviews (Walker, 1979; Czyz et al., 2002 ; Fluckiger- Isler et al., 2004). Certain carcinogens present in active forms in biological reaction are easily catalyzed by cytochrome-P450. Metabolic activation system is absent in Salmonella, and in order to improve the potentiality of bacterial test systems, liver extracts of Swiss albino mice are used. This serves as a rich source in converting carcinogens to electrophilic chemicals that are incorporated to detect in vivo mutagens and carcinogens (Garner et al., 1972 ; Ames et al., 1973a). The crude liver homogenate as 9,000 × g S9 fraction contains free endoplasmic reticulum, microsomes, soluble enzymes and some cofactors set with S9 concentration to 10% (Franz and Malling, 1975). The oxygenase requires the reduced form of Nicotinamide Adenine Dinucleotide Phosphate (NADP) which is generally in situ by the action of glucose-6-phosphate dehydrogenase and reducing NADP both work as cofactors in assay (Prival et al., 1984 ; Henderson et al., 2000). While water is considered as a negative control, sodium azide, 2-nitrofluorine and mitomycin for TA 98, TA 100 and TA 102 without S9 metabolic activation and 2-anthramine with S9 hepatic fraction are used as positive controls for conducting the test (Table 2). Before performing the experiment, fresh solutions must be prepared. Positive controls With S9 metabolic activation Without S9 metabolic activation 2-Aminofluorene 2-Nitrofluorene 1,8-Dihydroxyanthraquinone Sodium azide 2-Aminoanthracene Mitomycin C Cyclophosphamide Methyl methanesulfonate 2-Anthramine 9-Aminoacridine Spontaneous Reversion Control: Each strain of Salmonella contains a specific mutant range. Selection of solvents shows the effect on the frequency range of spontaneous mutant (Maron and Ames, 1983) (Table 3). The range of revertants varies in research laboratories. The spontaneous revertants are visible through unaided eyes (Figure 2). (Mortelmans and Stocker, 1979) Strain Spontaneous Revertants With S9 Without S9 TA 98 20-50 20-50 TA 100 75-200 75-200 TA 102 100-300 200-400 TA 104 200-300 300-400 TA 1535 5-20 5-20 TA1537 5-20 5-20 (Vijay, 2014) Materials Tips (1,000 µl, 200 µl, 10 µl) (Tarsons) Sterile Petri plates (HiMedia Laboratories, catalog number: PW001) Erlenmeyer flask and beaker (SchottDuran,10 ml, 250 ml, 500 ml) Eppendorf tubes (Tarsons,1.5 ml, 2.0 ml) Metal loop holder (metal loop Ch-2, HiMedia Laboratories, catalog number: LA012) L shaped spreader(HiMedia Laboratories, catalog number: PW1085) Mutagens Sodium azide (HiMedia Laboratories, catalog number: GRM1038) 4-Nitroquinoline N-oxide (Sigma-Aldrich, catalog number: N8141) 2-Aminofluorene (Sigma-Aldrich, catalog number: A55500) Benzo(a)pyrene (Sigma-Aldrich, catalog number: B1760) Mitomycin C (Roche Diagnostics, catalog number: 10107409001) 2,4,7-Trinitro-9-fluorenone (Accustandard, catalog number: R-033S) 4-Nitro-o-phenylenediamine (Sigma-Aldrich, catalog number: 108898) Reagents Oxoid nutrient broth No. 2 (Sigma-Aldrich, catalog number: 70123) Note: This product has been discontinued. 70% ethanol Magnesium sulphate heptahydrate ($MgSO_4 \cdot 7H_2O$) (HiMedia Laboratories, catalog number: RM683) Citric acid monohydrate (HiMedia Laboratories, catalog number: GRM1008) Potassium phosphate, dibasic (K_2HPO_4) (anhydrous) (Merck, catalog number: 61788005001730) Sodium ammonium phosphate tetrahydrate ($NaNH_4HPO_4 \cdot 4H_2O$) (Sigma-Aldrich, catalog number: S9506) D-biotin (HiMedia Laboratories, catalog number: TC096) L-histidine (HiMedia Laboratories, catalog number: TC076) Hydrochloric acid (HCl) (HiMedia Laboratories, catalog number: AS003) Potassium chloride (KCl) (Merck, catalog number: 61753305001730) Magnesium chloride hexahydrate ($MgCl_2 \cdot 6H_2O$) (HiMedia Laboratories, catalog number: MB040) Sodium dihydrogen phosphate monohydrate ($NaH_2PO_4 \cdot H_2O$) (Merck, catalog number: 1063700050) Disodium hydrogen phosphate (Na_2HPO_4) (HiMedia Laboratories, catalog number: TC051) NADP (sodium salt) (HiMedia Laboratories, catalog number: RM392) D-glucose-6-phosphate (monosodium salt) (Sigma-Aldrich, catalog number: G7879) Ampicillin trihydrate (Sigma-Aldrich, catalog number: A6140) Sodium hydroxide (NaOH) (Merck, catalog number: 106462) Crystal violet (Sigma-Aldrich, catalog number: C6158) Agar-Agar (Himedia Laboratories, catalog number: RM026) Nutrient broth (HiMedia Laboratories, catalog number: M002) Tetracycline (Sigma-Aldrich, catalog number: 87128) Dimethylsulfoxide (HiMedia Laboratories, catalog number: TC185) Vogel-Bonner medium E (50x) (see Recipes) 0.5 mM histidine/biotin solution (see Recipes) Salt solution (1.65 M KCl + 0.4 M $MgCl_2$) (see Recipes) 0.2 M sodium phosphate buffer, pH 7.4 (see Recipes) 1 M Nicotinamide Adenine Dinucleotide Phosphate (NADP) solution (see Recipes) 1 M glucose-6-phosphate (see Recipes) Ampicillin solution (4 mg/ml) (see Recipes) Crystal violet solution (0.1%) (see Recipes) Minimal glucose plates (see Recipes) Histidine/Biotin plates (see Recipes) Ampicillin and tetracycline* plates (see Recipes) Nutrient agar plates (see Recipes) S9 mix (Rat Liver Microsomal Enzymes + Cofactors) (see Recipes) Sodium azide (see Recipes) Mitomycin (see Recipes) 2-Anthramine (see Recipes) Orbital shaking incubator (Remi, model: RIS-24(BL)) Laminar Flow hood (Bio safety cabinet) (Deepak Meditech Pvt Ltd., Steri clean) Pipettes (Eppendorf, model: Research® plus, catalog number: 3120000062, 1,000 µl; catalog number: 3120000046, 200 µl; catalog number: 3120000020, 10 µl) Vortex mixer (Labnet International, catalog number: S0100) Hot water bath (Daiki Sciences, catalog number: KBLee2001) Autoclave (TSC) Automatic Colony counter (Sonar) Refrigerator centrifuge (Thermo Fisher Scientific, Thermo ScientificTM, model: Heraeus Biofuge Primo R) pH meter (Labindia Analytical Instruments, model: PICO pH Meter, catalog number: PC13330101) Tissue tearor (Bio Spec Products, catalog number: 985370-04) Before performing the experiment, inoculate a single fresh colony of standard strains of S. typhimurium TA 98, 100 and 102, in oxoid nutrient broth-2 and incubate for 10-12 h at 37 °C in an incubator shaker at 120 rpm to ensure sufficient aeration for 1 x 109 bacterial cells. Each strain of S. typhimurium is grown separately in Erlenmeyer flasks (10 ml). Prepare fresh mutagen for each experiment (see Recipes). Negative control: Autoclaved distilled water Positive controls for TA 98, TA 100 and TA 102 without S9 metabolic activation (S9 mix): sodium azide (1 µg/ml) 2-nitrofluorine (1 µg/ml) and mitomycin (0.125 µg/ml) For TA 98, TA 100 and TA 102 with S9 metabolic activation (S9 mix): 2-Anthramine (2 µg/ml) Preparation of minimal glucose agar (MGA) plates: Mix the medium of minimal glucose agar plates (Recipe 9) and pour 25 ml into each Petri dish. Prepare the plates freshly before use. Label all minimal glucose agar plates and Eppendorf tubes prior to experiment. To the 2 ml sterile Eppendorf tubes, add the following each: 0.1 ml fresh culture of Salmonella strains 0.2 ml of His/Bio solution 0.5 ml sodium phosphate buffer (absence of S9 mix) or 0.5 ml S9 (presence of S9 mix) 0.1 ml of test sample or 0.1 ml of positive or negative control Make up to 1 ml with autoclaved distilled water. Mix the contents of Eppendorf tubes and pour onto Petri plates and spread using L-shaped spreader on the surface of MGA plates. Cover the Petri plates with sterile aluminum foil to protect the testing sample from photo reactive substances. After incubation of 48 h at 37 °C, spontaneous revertants colonies appear and are clearly visible with unaided eyes. All plates are run in triplicates. Revertants form a uniform lawn of auxotrophic bacteria on the surface the background of medium. Non-statistical analysis The most widely used method for non-statistical analysis of result in Ames test is 'two-fold rule' described by Mortelmans and Zeiger (2000) and Morino-Caniello and Piegorsch (1996). On the basis that the increase in the number of revertant colonies, the concentration of the tested sample goes up (dose-dependent manner), mutagenicity ratio (MR) is calculated first by counting the number of revertant colonies per plate and then calculating the MR as described by Maron and Ames (1983) using the formula below (see Sample data below for results): M.R=S.R+I.RS.RNegative control M.R = Mutagenicity Ratio S.R = Spontaneous Revertants I.R = Induced Revertants Sample data Medical liquid waste was collected from different health care premises of Jaipur city. Salmonella mutagenicity test was performed on all the samples in their crude natural state using the plate incorporation procedure described by Maron and Ames, 1983. The results of Salmonella mutagenicity assay was analyzed through Mutagenicity Ratio method and shown in Table 4. Health centers Concentrations (µl) Mutagenicity Ratio TA 98 Mutagenicity Ratio TA 100 Mutagenicity Ratio TA 102 -S9 +S9 -S9 +S9 -S9 +S9 Government Hospital (Untreated) (GH) 2 + + + + + + 5 + + + + + + 10 + + + + + + 50 + + + + + + 100 + + + + + + Private Hospital (Untreated) (PH) 2 + + + + + + 5 + + + + + + 10 + + + + + + 50 + + + + + + 100 + + + + + + Private Hospital (Treated) (PH) 2 - - - - - - 5 - - - - - - 10 - - - - - - 50 - - + - - - 100 - - - - - - +Mutagenicity Ratio > 2.0 imply mutagenic, -Ratio < 2.0 imply non-mutagenic Conclusion The Ames test is a widely accepted bacterial assay to detect the mutagenicity in pathogenic bacteria. In this protocol, although we have shown the step wise methodology to perform Ames assay applicable for three strains, this method can be used for studying all compounds to infer mutagenicity. Whereas the Ames assay experiments involve sterile measures, care must be taken in ensuring the sample/plasmid is not contaminated. The improved methods to detect the genotoxicity of compounds help us troubleshoot methods for studying the compounds tested in clinical trials. Sterilization (safety considerations while working with Salmonella) As S. typhimurium is a pathogenic bacterium, it is prudent to use precautionary measures every time and apply standard biosafety guidelines such as using plugged pipettes, proper sterilization by 70% ethanol and autoclaving all contaminated material. Handling of chemicals and strains should be done in biosafety cabinet. Before and after the use, cabinet must be sterilized using 70% ethanol and exposed to 15 min UV. Care must be taken to protect from chemical exposure by wearing gowns, eye glasses and gloves. Before discarding, all contaminated material (e.g., test tubes, pipettes and pipette tips, gowns and gloves) should be properly autoclaved. Limitations Ames assay consists of Salmonella typhimurium strains and so it is not a perfect model for human. Mice liver S9 hepatic fraction is used to minimize the mammalian metabolic activations formed in the hepatic system so that the mutagenicity of metabolites can be assessed. There are several differences between human and mice metabolism which can affect the mutagenicity of testing substances. Major disadvantages of fluctuation test is slower and slightly more laborious than Ames protocol. The test is primarily used for testing aqueous samples containing low levels of mutagen and therefore, this test is well adapted for evaluating the mutagenicity of wastewater samples. Vogel-Bonner medium E (50x) For Minimal agar (Recipe 9) Ingredients Per 500 ml Warm distilled H_2O (45 °C) 335 ml Magnesium sulfate ($MgSO_4 \cdot 7H_2O$) 5 mg Citric acid monohydrate 50 mg Potassium phosphate, dibasic (anhydrous) (K_2HPO_4) 250 mg Sodium ammonium phosphate ($NaNH_4HPO_4 \cdot 4H_2O$) 87.5 mg Salts are added to the warm water in a flask. Place the flask on a hot plate After each salt dissolves entirely, transfer the solution into glass bottles and autoclave for 20 min at 121 °C When the solution gets cool, cap the bottle tightly Store the solution at 4 °C 0.5 mM histidine/biotin solution For mutagenic bioassay Ingredients Per 125 ml D-Biotin (F.W. 247.3) 15.45 mg L-Histidine·HCl (F.W. 191.7) 12.0 mg Distilled H_2O 125 ml Dissolve the biotin in hot distilled water. The solution is autoclaved for 20 min, at 121 °C and then stored at 4 °C Salt solution (1.65 M KCl + 0.4 M $MgCl_2$) For S9 hepatic fraction Ingredients Per 250 ml Potassium chloride (KCl) 30.75 mg Magnesium chloride ($MgCl_2 \cdot 6H_2O$) 20.35 mg Distilled H_2O to final concentration of 250 ml All the components are dissolved in water. The solution is autoclaved for 20 min, at 121 °C and then stored at 4 °C 0.2 M sodium phosphate buffer, pH 7.4 For S9 hepatic fraction Ingredients Per 250 ml 0.2 M sodium dihydrogen phosphate ($NaH_2PO_4 \cdot H_2O$) 30 ml (6.9 mg/250 ml) 0.2 M disodium hydrogen phosphate (Na_2HPO_4) 220 ml (7.1 mg/250 ml) Adjust pH to 7.4. Sterilize the buffer by autoclaving for 20 min at 121 °C 1 M nicotinamide adenine dinucleotide phosphate (NADP) solution For S9 hepatic fraction Ingredients Per 2.5 ml NADP 191.5 mg Sterile distilled H_2O 2.5 ml NADP is dissolved in the distilled water and mixed by vortexing. Tubes are placed in an ice bath. The solution can be used for up to six months 1 M glucose-6-phosphate For S9 hepatic fraction Ingredients Per 5 ml Glucose-6-phosphate (G-6-P) 1.41 mg Sterile distilled H_2O 5 ml Glucose-6-phosphate is dissolved in the 5 ml distilled water and mixed by vortexing. Tubes are placed in an ice bath. The solution can be used for up to six months Ampicillin solution (4 mg/ml) Used in tests of ampicillin resistance Master plates for R-factor strains Ingredients Per 500 ml Ampicillin trihydrate 0.4 mg Sodium hydroxide (0.02 N) 50 ml Ampicillin trihydrate is dissolved in the 50 ml of NaOH (0.02 N) and mixed by vortexing. Tubes are placed in an ice bath Crystal violet solution (0.1%) Used in tests for crystal violet sensitivity (to confirm rfa mutation) Ingredients Per 500 ml Crystal violet 0.05 mg Distilled H_2O 50 ml Minimal glucose plates Used in Mutagenic bioassay Ingredients Per 500 ml Agar 7.5 mg Distilled H_2O 465 ml 50x VB salts (Recipe 1) 10 ml 40% glucose 25 ml Add agar in 465 ml of distilled water and autoclave for 20 min, at 121 °C. After cooling, add the salts and glucose gently Histidine/Biotin plates (Master plates for non R-factor strains) Used in tests for histidine requirement Ingredients Per 500 ml Agar 7.5 mg Distilled H_2O 457 ml 50x VB salts 10 ml 40% glucose 25 ml Sterile histidine (2 g per 400 ml H_2O) 5 ml Sterile 0.5 mM biotin 3 ml Dissolve agar in the given concentration in distilled water. Autoclave each solution separately for 20 min. After cooling of solution, add each salt gently Ampicillin and tetracycline* plates Master plates for the cultivation of strains containing the plasmids pKM101 and pAQ1* Ingredients Per 500 ml Agar 7.5 mg Distilled H_2O 405 ml 50x VB salts 10 ml 40% glucose 25 ml Sterile histidine (2 g per 400 ml H_2O) 5 ml Sterile 0.5 mM biotin 3 ml Sterile ampicillin solution (8 mg/ml 0.02 N NaOH) 1.58 ml *Sterile tetracycline solution (8 mg/ml 0.02 N HCl) 0.125 ml Dissolve agar in the given concentration in distilled water. Autoclave each solution separately for 20 min. After cooling of solution, add each salt gently *Note: TA 102 is resistant to tetracycline. The shelf life of the plates is two weeks at 4 °C. Nutrient agar plates Used in tests for genotypes [Crystal violet sensitivity (rfa) and UV sensitivity (AuvrB)] and viability of bacteria Ingredients Per 500 ml Nutrient agar 7.5 mg Distilled H_2O 500 ml Dissolve agar in the given concentration in distilled water. Autoclave separately for 20 min. Pour the cooled solution into the Petri plates S9 mix (Rat Liver Microsomal Enzymes + Cofactors) Ingredients Standard S9 mix Per 25 ml Mice liver 1.0 ml (2%) $MgCl_2$-KCl salts 0.5 ml 1 M glucose-6-phosphate 0.125 ml 0.1 M NADP 1.0 ml 0.2 M phosphate buffer, pH 7.4 12.5 ml Sterile distilled H_2O 9.86 ml Note: Add each ingredient in the reverse order listed above (First water, and then phosphate buffer...). Avoid refreezing the S9 mix. Sodium azide Used in Mutagenicity assay Ingredients Per ml Sodium azide 10 µg Autoclave distilled H_2O 990 µl (to make a total volume of 1 ml) Working concentrations are prepared by taking 1, 2, 4 µl of 10 mg/ml 2-Nitrofluorine Used in Mutagenicity assay Ingredients Per ml 2-Nitrofluroine 10 µg Autoclave distilled H_2O 990 µl (to make a total volume of 1 ml) Working concentrations are prepared by taking 1, 2, 4 µl of 10 mg/ml Mitomycin Used in Mutagenicity assay Ingredients Per ml Mitomycin 10 µg Autoclave distilled H_2O 990 µl (to make a total volume of 1 ml) Working concentrations are prepared by taking 1, 2, 4 µl of 10 mg/ml 2-Anthramine Used in Mutagenicity assay Ingredients Per ml 2-Anthramine 10 µg Autoclave distilled H_2O 990 µl (to make a total volume of 1 ml) Working concentrations are prepared by taking 1, 2, 4 µl of 10 mg/ml

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Ames test it is a biological assay to assess the mutagenic potential of chemical compounds. It utilizes bacteria to test whether a given chemical can cause mutations in the DNA of the test organism. The test was developed by Bruce N. Ames in 1970s to determine if a chemical at hand is a mutagen.To determine the mutagenic activity of chemicals by observing whether they cause mutations in sample bacteria.Ames test uses several strains of bacteria (Salmonella, E.coli) that carry a particular mutation.Point mutations are made in the histidine (Salmonella typhimurium) or the tryptophan (Escherichia coli) operon, rendering the bacteria incapable of producing the corresponding amino acid.These mutations result in his- or trp- organisms that cannot grow unless histidine or tryptophan is supplied.But culturing His- Salmonella is in a media containing certain chemicals, causes mutation in histidine encoding gene, such that they regain the ability to synthesize histidine (His+). This is to say that when a mutagenic event occurs, base substitutions or frameshifts within the gene can cause a reversion to amino acid prototrophy. This is the reverse mutation.These reverted bacteria will then grow in histidine- or tryptophan-deficient media, respectively.A sample's mutagenic potential is assessed by exposing amino acid-requiring organisms to varying concentrations of chemical and selecting for the reversion event. Media lacking the specific amino acid are used for this selection which allow only those cells that have undergone the reversion to histidine / tryptophan prototrophy to survive and grow. If the test sample causes this reversion, it is a mutagen.I) Isolate an auxotrophic strain of Salmonella Typhimurium for histidine. (ie. His-ve)II) Prepare a test suspension of His-ve Salmonella Typhimurium in a plain buffer with test chemical (eg. 2-aminofluorene). Also add a small amount of histidine.Note: small amount of histidine is required so bacteria starts growing. Once histidine is depleted only those bacteria mutated to gain the ability to synthesize histidine form colonies.III) Also prepare a control suspension of His-ve Salmonella Typhimurium but without test chemicals.IV) Incubate the suspensions at 37°C for 20 minutesV) Prepare the two agar plate and spread the suspension on agar plate.VI) Incubate the plates at 37°C for 48 hours.VII) After48 hours count the number of colonies in each plate.The mutagenicity of chemicals is proportional to number of colonies observed.If there is a large number of colonies on the test plate in comparison to control, then such chemical are said to be mutagens.Very few numbers of colonies can be seen on control plate also. This may be due to spontaneous point mutation on hisidine encoding gene.While Ames test is used to identify the revert mutations which are present in strains, it can also be used to detect the mutagenicity of environmental samples such as drugs, dyes, reagents, cosmetics, waste water, pesticides and other substances which are easily solubilized in a liquid suspension.Simple, rapid and robust bacterial assay.Ease and low cost of the test make it invaluable for screening substances in our environment for possible carcinogenicity.Ames test can detects suitable mutants in large population of bacteria with high sensitivity.Some substances that cause cancer in laboratory animals (dioxin, for example) do not give a positive Ames test (and vice-versa)Ames assay consists of Salmonella typhimurium strains and so it is not a perfect model for human. 20Dateien/Short%20Protocol/Ames/Ames%20MPF%20Penta1%20Short%20Protocol_2.0.pdfwww.biology-pages.info/A/AmesTest.htmlwww.geneticsga.org/education/pdf/GSA_DeStasio_Ames_Student_Resources.pdf Posts:Salmonella Shigella (SS) Agar- Composition, Principle, Uses, Preparation and Result InterpretationWidal Test- Introduction, Principle, Procedure, Interpretation and LimitationIndole Test- Principle, Reagents, Procedure, Result Interpretation and LimitationsXylose Lysine Deoxycholate (XLD) Agar- Principle, Uses, Composition, Preparation and Colony Characteristics