ENHANCED AMES TEST FOR NITROSAMINE IMPURITIES

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Jay is a growing scientist having 3 years of experience in the Mutagenicity Section. He has experience of conducting Ames Test/ Enhanced Ames Test and ***in vivo*** micronucleus test. In a short period of time, he has gained expertise in handling various mammalian cell lines, reconstructed 3D RhE skin tissues and human skin tissues. He is also handling ***In vitro*** sensitization assays – HCLAT assay using Flow Cytometer. Jay was instrumental in developing and establishing Pig-a gene mutation assay in GLP area.

Nitrosamines are compounds containing a nitroso group bonded to an amine group. This nitrosamine comes in different forms. Almost all of them are human carcinogens. These compounds are not only found in tobacco products but also in some processed foods, personal care products and rubber and latex products. Nitrosamine impurities formation in drinking water after disinfection with chlorine or ozonisation is also recorded. They are also found during drug formulations and during active pharmaceutical ingredients synthesis. The nitrosamine impurities in drugs that are used for the treatment of hypertension (valsartan, quinapril) and type 2 diabetes (metformin), led to recall of these drugs.
These nitrosamines include for example NDMA (N-Nitrosodimethylamine), NDEA (N-Nitrosodiethylamine), NMBA (N-NitrosoN-Methyl-4-aminobutyric acid), which are proven genotoxins. Nitrosamines require metabolic activation to show mutagenic potency. Activation is usually done by cytochrome P-450 dependent enzymes e.g., CYP2E1 and CYP3A4.

To determine the mutagenic potential of a chemical, the bacterial reverse mutation test or Ames test has been used widely used, as it is more cost efficient, provides quick results and has proved to be efficient when it comes to detecting number of mutagens. Although, for nitrosamines, recent publication suggests that protocol type, strain selection, methodology (i.e., plate incorporation or pre-incubation), type of solvent had little to no effect on the outcome of the test. However, as there is little availability of data for assay variables, a new guidance has been recommended by regulatory authorities to assess the mutagenic potential of test compounds. The so called “Enhanced Ames test conditions for N-Nitrosamines” include use of ***Salmonella typhimurium*** tester strains TA1537, TA1535, TA98, TA100 and ***Escherichia coli*** tester strains WP2 uvrA pKM101 by using pre-incubation method, with pre-incubation time for 30 minutes. The assay should be conducted in the absence of metabolic activation system and in the presence of 30% v/v rat and hamster S9 metabolic activation system. The rat and hamster S9 fractions should be prepared by treating the rodent with a combination of phenobarbital and β-Naphthoflavone.

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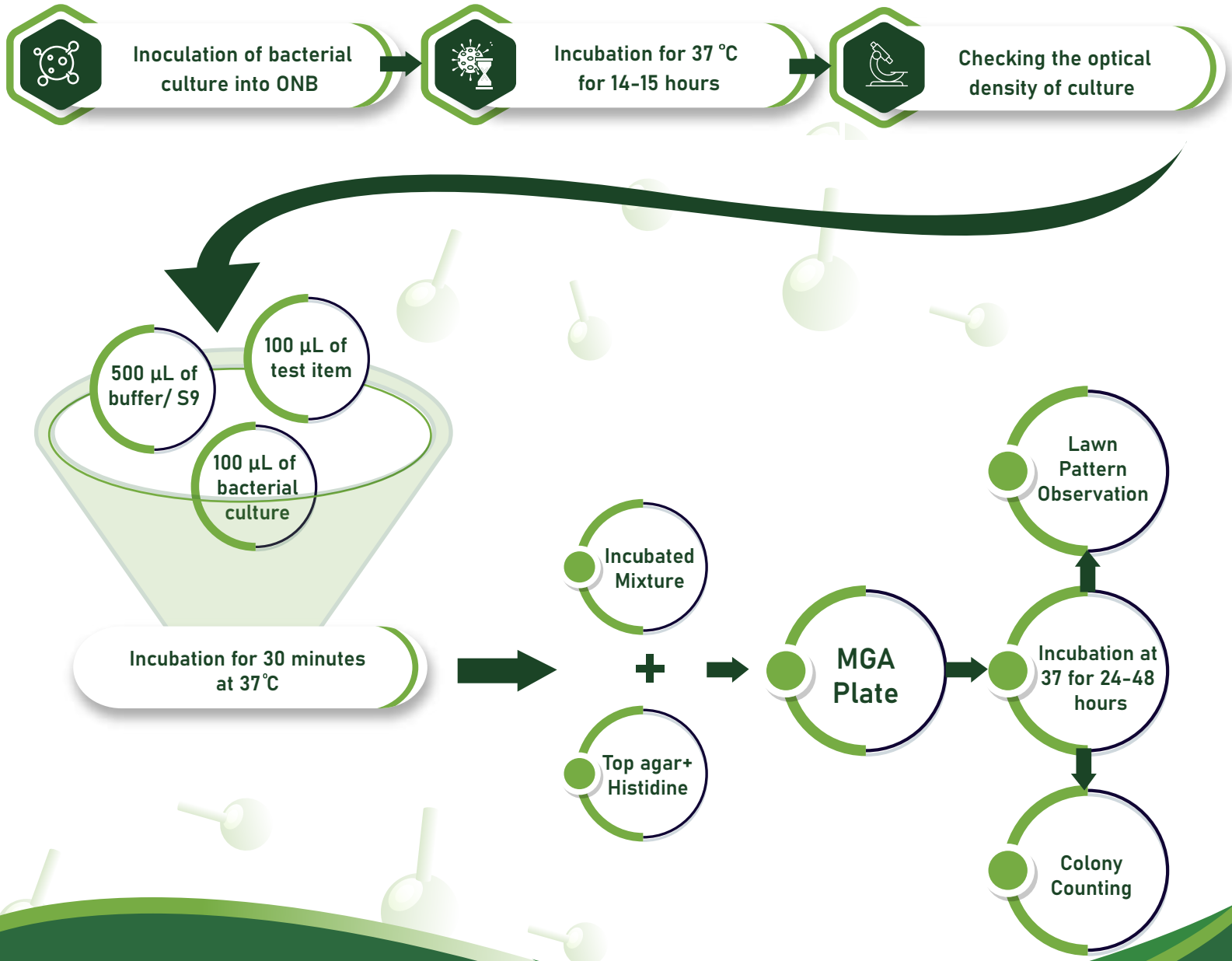
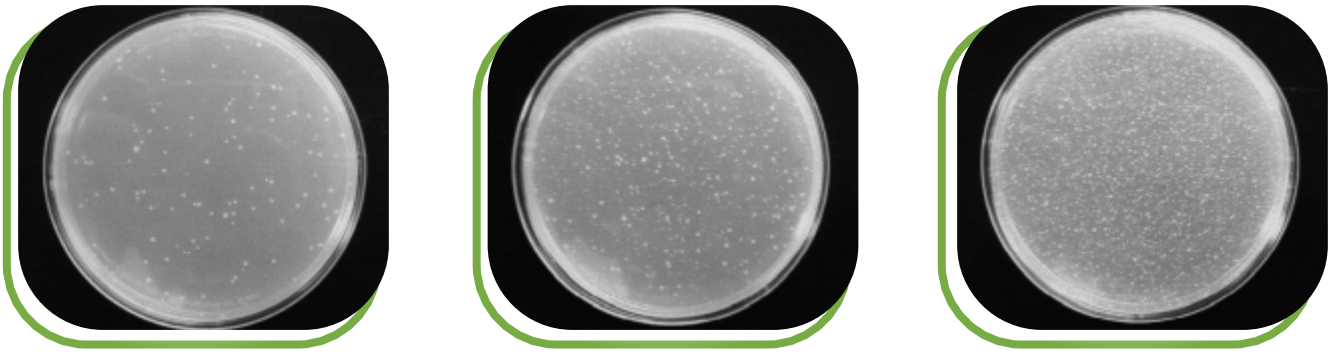
PRINCIPLE

The *Salmonella typhimurium* and *E.coli* mutants containing mutation in the histidine and tryptophan operon, respectively, are not able to grow in the absence of required amino acids. If the test item to be tested is a mutagen, it will lead to mutation in the bacterial strains, reversing the already existing mutation. This reverse mutation in the bacterial strains allows them to grow in the absence of amino acids. Which is observed by the increase in the bacterial revertant counts as compared to the negative control. The chemical to be tested might be a pro-mutagen, which requires metabolic activation to be converted into a active mutagen.
As bacteria lacks the necessary enzymes to convert pro-mutagens into active mutagenic metabolites, additional liver homogenate of rats or hamsters containing the required enzymes supplemented with co-factors are added during the treatment.

METHODOLOGY





Dose Related Increase In Revertant Colonies

Positive Controls in the absence of metabolic activation system

Concurrent strain specific positive control should be used as per OECD test guideline 471.

Positive Controls in the presence of metabolic activation system

Two known mutagenic N-Nitrosamines should be used as positive controls

Evaluation of results

Cytotoxicity will be determined by the diminishing of the bacterial background lawn pattern or decrease in the number of bacterial colonies.

The data sets for the tester strains TA1537 and TA1535 will be judged positive, if the increase in the number of bacterial revertants is more than 3-fold.

The data sets for the tester strains TA98, TA100 and E.coli pKM101 will be judged positive, if the increase in the number of bacterial revertants is more than 2-fold.

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