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NASA TECH BRIEF

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Rapid Method for Determination of Antimicrobial Susceptibilities Pattern of Urinary Bacteria

A quick method has been developed for detecting bacterial sensitivity to antimicrobial agents by measuring the level of adenosine triphosphate (ATP) remaining in the bacteria. The amount of ATP in a sample is determined by measuring the light emitted during the reaction of the sample with a mixture of luciferase and luciferin. This method enables data to be obtained within 2-1/2 hours after treatment with an antibiotic, as compared to the 18 hours required using conventional agar diffusion, broth dilution, and agar dilution techniques.

A bacterial sample is cultured and assayed for its adenosine triphosphate content by first destroying all nonbacterial ATP in solution. The bacterial cells are ruptured to release ATP; the remaining ATP is measured via bioluminescence. After the initial assay, the culture is subjected to an antibiotic for a growth period and then is remeasured for bacterial ATP, using the same luciferase-luciferin technique. Data obtained from each assay, before and after exposure of the bacterium to the antibiotic, are then used to calculate an ATP index, which is a measure of bacterial sensitivity to a specific antibiotic.

When adapting this technique to detect bacteria in urine, a method first had to be developed to separate nonbacterial sources of ATP from the bacteria. Typical sources include free soluble ATP and the ATP present in red and white blood cells. To separate, the urine sample is treated with a nonionic detergent which ruptures any red and white blood cells present, releasing ATP into solution in a freely soluble form. The free nonbacterial ATP is hydrolyzed with an ATP hydrolyzing enzyme, or ATPase; the ATPase then is denatured via a chemical or heat.

Bacterial ATP is released by rupturing the bacterial cells with ar acid. The solution is neutralized; the pH and ionic strength of the sample are adjusted by a buffer for optimum luciferase activity. The solution is then treated with the luciferase-luciferin mixture. If ATP is present, light is emitted from the bioluminescent reaction and is detected and recorded.

Note:

Requests for further information may be directed to:

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Patent status:

This invention is owned by NASA, and a patent application has been filed. Inquiries concerning nonexclusive or exclusive license for its commercial development should be addressed to:

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