March 1971

Brief 71-10051

NASA TECH BRIEF

Goddard Space Flight Center

Bacterial Adenosine Triphosphate as a Measure of Urinary Tract Infection

A rapid, routine procedure detects and counts the bacteria present in urine samples. The bacterial level indicates the extent of urinary tract infection. The procedure depends on the presence and measurement of adenosine triphosphate (ATP), a nucleotide present in all known living matter. A quantitative determination is made by measuring the light emitted in the bioluminescent reaction of ATP with the enzyme luciferase.

To use the ATP assay for the detection of bacteria in urine, non-bacterial ATP must first be removed. Sources of urinary non-bacterial ATP are free, soluble ATP and ATP in red and white blood cells, the latter sometimes being present in urine. Removal of non-bacterial ATP is accomplished by first rupturing the red and white blood cells of the urine sample, thereby releasing the ATP in a free, soluble state. All the free, soluble non-bacterial ATP is then hydrolyzed by the addition of an ATPase enzyme. The above steps do not affect any bacterial cells present.

After removing the non-bacterial ATP from the urine sample, the ATPase is inactivated and the bacterial cells ruptured (ATP released) by the addition of an acid. The acid is then neutralized by the addition of a buffer at a pH necessary for optimal luciferase activity. The urine sample is combined with a luciferase-luciferin mixture, and the light emitted from the resultant bioluminescent reaction is detected and recorded by a photometer system. If all of the reaction components are maintained in a concentration in excess of the ATP, the light emission is directly proportional to the amount of ATP introduced. A direct relation can then be drawn between the magnitude of the light emission and the ATP concentration using a standard calibration curve for comparison. The number of bacteria equivalent to the concentration in the urine sample can be obtained by dividing the ATP concentration by $3 \times 10^{-10} \mu g$, the average ATP content of a bacterial cell.

The method developed is adaptable to determining bacterial levels in other aqueous body fluids such as lymph fluid, plasma, blood, spinal fluid, saliva, and mucous. Further, it is particularly applicable to measuring bacterial levels in aqueous body fluids which have, in addition to the bacteria, both free, soluble ATP and non-bacterial cells containing ATP.

Note:

Requests for further information may be directed to:

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Patent status:

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