

AUTOMATED DETECTION OF BACTERIA IN URINE

**Albert J. Fleig
Grace L. Picciolo
Emmett W. Chappelle
Burton N. Kelbaugh**

We have developed a method for detecting the presence of bacteria in urine which may have a substantial impact on present health care procedures. The incidence of urinary tract infections is second only to that of infections of the respiratory system. A large hospital laboratory typically examines up to 4,000 urine specimens a month. The present method of detection is to dip out a drop of urine and spread it on an agar plate (Figure 1). The plate is then capped and incubated for from 1 to 4 days. Then the plate is visually examined (Figure 2) for indication of bacterial growth, and an estimate of the quantity of bacteria present in the original specimen is made. At Johns Hopkins Hospital from four to six medical technicians are occupied full time in performing these assays. It is expensive in terms of trained personnel, space, and time required for an assay. In addition, it is a tedious, repetitive, subjective task subject to much human error.

Our method is derived from work on extraterrestrial life detection done by the former Space Biology Branch. The method utilizes the bioluminescent reaction of adenosine triphosphate (ATP) with luciferin and luciferase derived from the tails of fireflies. All bacteria contain ATP; thus, all bacterially contaminated urine will contain ATP. However, urine samples also contain red and white blood cells and skin cells which also contain ATP. Thus, it is necessary to eliminate all nonbacterial ATP before performing the bioluminescent assay.

A nonionic detergent is added to the urine sample. This detergent lyses or ruptures the cell walls of all the nonbacterial cells but does not affect the bacteria. Then potato apyrase is added, which hydrolyzes or destroys all of the ATP, which has been released. Then perchloric acid is added, which inhibits the apyrase and lyses any bacterial cells present in the urine. If (and

only if) there were bacterial cells, free ATP would be present in the specimen, and the injection of luciferase and luciferin will produce a flash of light.

We have developed a device (Figure 3) which completely automates this process and are conducting preliminary trials of it at Johns Hopkins Hospital. An entire assay takes 15 minutes, and the device processes samples at this rate of one per minute. Preliminary test results have been very encouraging. In a run of several hundred specimens, we have correctly detected *every* specimen which was later found positive by the present laboratory procedure. In addition, we found indication of bacterial infection in roughly 20 percent of specimens considered negative by present methods and typically found much higher levels of infection than the present methods do, even when both approaches indicated the presence of infection.

There are several possible explanations for the difference in results from the two approaches. A fundamental difference is that our ATP assay detects bacteria in the specimen as obtained, while culture methods detect only those bacteria which will grow in the particular culture environment being used. Thus, bacteria which do not reproduce on agar, at the pH being used, in contact with oxygen, or for any of a number of other reasons will not normally be found by present hospital methods but would be with our ATP-based assay. Urine may contain bacteriostatic agents, either naturally produced (as might be the case with a low level, long term infection) or as a result of drug therapy. These bacteriostatic agents may preclude detection of the bacteria via routine culture methods but would not hinder the ATP assay. A second basic difference is that we are inferring the presence of bacteria based upon the existence of ATP in the processed urine. Thus, an erroneous positive reading would result if there are sources of ATP which are not deleted by our processing procedure. However, our studies to date indicate that this is not the case.

There are several important potential implications of the ATP assay for patient health care. First, it will be possible to reduce the number of urine specimens which must be cultured by roughly 40 percent by eliminating all specimens with negative ATP assay results, thus, reducing the personnel and space (and ultimately the cost) required for the assay. And it will be possible to return the negative result 1 day sooner which may alter medical treatment, e.g., administration of antibiotics. The device may also make it

possible to screen large numbers of patients in classes with above-normal incidence of bacterial infection, such as teenage girls, pregnant women, and diabetics. The only requirement for the above is that a thorough clinical trial should confirm that the ATP assay does not miss any cases of infection detected by present methods.

The ATP assay also provides information not presently available, and if further trials establish the correctness of our results, this may be even more important. In particular, the ATP assay may correctly detect bacteria which would be missed (or underestimated) by present methods. The ATP assay provides an accurate, replicable quantitative result in place of a subjective estimate. Although the impact of this change is impossible to predict, Lord Kelvin's observation, ". . . But nearly all the grandest discoveries of science have been but the rewards of accurate measurement and patient long continued labor in the minute sifting of numerical results," may apply.

There are still several steps to be completed before this technology is successfully transferred to the medical community. A thorough clinical trial is required to establish the accuracy and reliability of the assay and to resolve the discrepancies in results between the ATP assay and present standard cultural techniques. Commercial development and marketing of the device will then complete the process. As a result of discussions with the National Institute of General Medical Sciences, NIH, we have prepared a proposal for a clinical trial involving Johns Hopkins Hospital, the NIH Clinical Center, and GSFC. Several commercial firms have expressed interest in marketing the device if the clinical trials are successful.



Figure 1



Figure 2

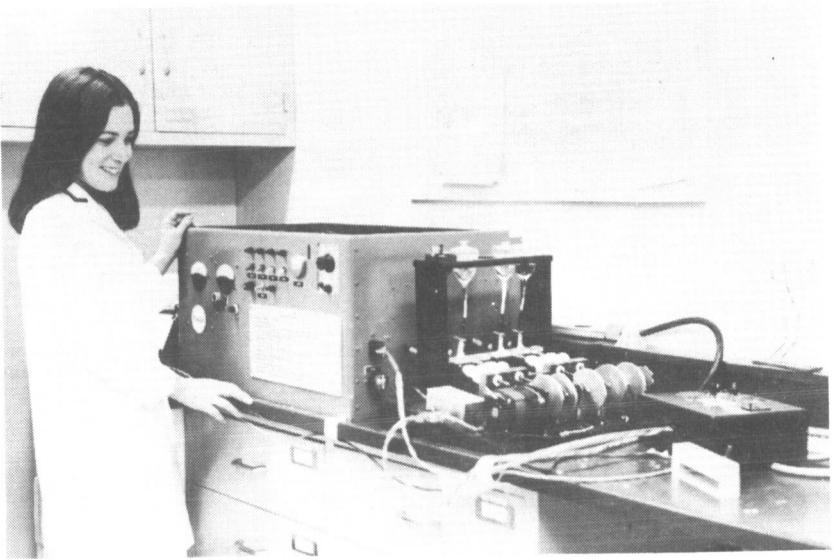


Figure 3

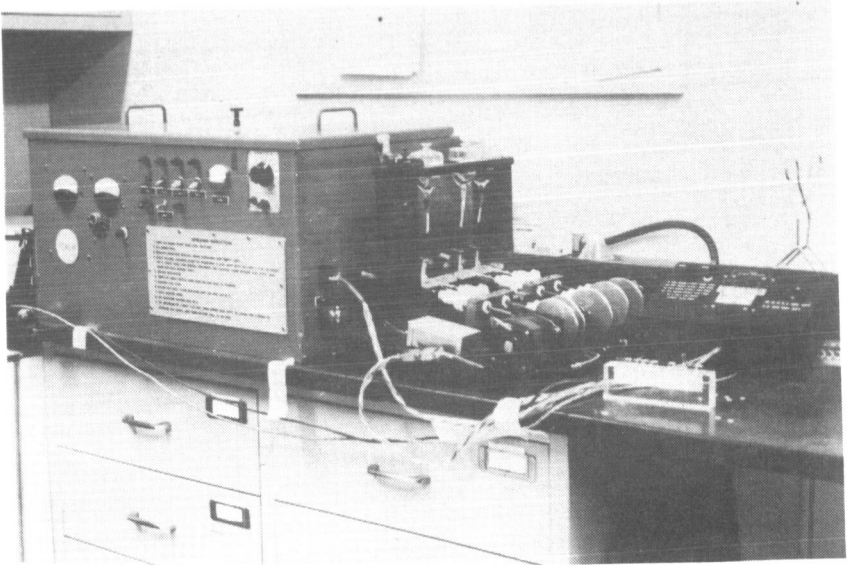


Figure 4