

Errors in ATP Testing and Reproducibility

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Adenosine triphosphate (ATP) test results, even using pure ATP standards, can vary approximately 10 percent within a given instrument and method. In addition to this inherent test variability, real world samples have additional variability such as sample collection and homogeneity variance. Thus, when measuring a sample to determine its ATP level, the total variance in the ATP reading is due to sample variances, the ATP reagent method or test pen, and the type of instrument used. To control the sample variance, it is important to know the methodology and minimize as much variability as possible.

The ATP method is based on measuring the amount of light produced by the chemical reaction of ATP with a luciferin/luciferase chemical/enzyme mixture. The amount of ATP in the sample is related to the activity of a cell-rupturing (lysing) agent that splits open the cells and releases their ATP contents into the test solution. The absolute RLUs (Relative Light Units) measured are dependent on the concentration of the ATP in the test solution and the concentration of luciferin and luciferase added to the test system. Any changes in the concentrations of lysing agent, luciferin or luciferase in the test system will affect the RLU that is measured by a given instrument. Some instruments operate with photomultiplier tubes and others with photodiodes, which determines sensitivity and thus RLUs recorded from a given sample.

The reaction time for the cell rupturing can vary depending upon the cell type (bacterial, fungal or yeast). This lysing reaction is the source of the greatest variance in homogenous samples. Therefore, a test method should be optimized for the amount of cell rupturing agent and stability of light emission lifetime. Some units can use different test pens in which the levels of reagents in the pens may be different. This causes a variance in the results. Since ATP numbers are relative, and there is no standard or uniformity in the ATP methodology, the same test pen and instrument must be used to track changes in ATP analyses.

Erratic Results

Solution 1: Sample solution contact time

In general, if erratic results are obtained, the contact time of the sample and test pen solution must be increased for the lysing agent reaction to be optimized. It is suggested that the ATP pen or lysing agents and the test sample should be mixed and allowed to stand for 30 to 90 seconds prior to addition of luciferin/luciferase reagent from top ampoule on the pen or directly when individual reagents are used. The pen or cuvette is then added to the meter and the RLUs are measured. If pens are used as a source of reagents and the unit only takes test tubes or cuvettes, the test solution must be poured into the test tube after the test sample has been mixed and acclimated in the test pen for 30 – 90 seconds. It is critical and most important to record and report the highest reading. This is true for any test pen or reagent method.

To determine the highest or maximum RLU value, take multiple readings on the instrument until there is a decline in readings. This decline is caused by decrease in the concentration of ATP in the test solution as it is utilized by both the luciferin/luciferase reaction and other ATP utilizing reactions. The highest reading prior to this decline is the reading that needs to be reported.

Solution 2: In the real world, samples are generally not true solutions.

Any small sample of particulate or clumped material can be the source of a significant amount of ATP, and therefore varying amounts of ATP due to sample variation. It is therefore critical to work with consistent samples with maximal homogeneity.

If the sample has particulate matter, which cannot be homogeneously suspended prior to testing, it should be filtered to obtain a solution free of particulate matter. This can be accomplished in the field by pouring through a micron filter (use a filter within the range of 0.5μ to about 10μ). If a micron filter is unavailable use approximately 1-3 layers of facial tissues. An alternative procedure is to allow the particulate matter in the sample to settle. Then an aliquot from the supernatant or top liquor can be used for testing.

The key message is: Perform ATP tests on consistent and identical samples in a consistent manner of reagent addition and mixing. The ATP method is accurate, but the precision is very much a function of the sample, and technique.

More details on ATP testing can be obtained from the authors at 888-739-0377