



ATP (RLU) vs. Dip Slides/ Plate Counts/ Petri Film (CFU/mL)

	ATP (RLU)	Dip Slides/ Plate Counts/ Petri Film (CFU/mL)
Method Summary	ATP (adenosine triphosphate) is a chemical inside all living cells. The ATP method releases the ATP from the cells by lysing the cells. The concentration of ATP released to solution is then measured via an enzymatic process. This process results in light output that is proportional to the concentration of ATP in a solution at time of testing. This light output is measured in RLU's (Relative Light Units). Results are obtained in minutes after sampling. RLU of organisms in solutions or on surfaces can be measured.	These methods measure the number of colonies (CFU (Colony Forming Units)) that are visually detectable on a growth media after a given incubation period. Solutions are diluted, plated onto growth media and incubated for 24 – 72 hours. To be seen with the naked eye these cells need to grow and multiply on the media to greater than 10^6 cells. Some tests use metabolic dyes to help increase visualization of cells.
Quantitation	All living cells contain the same relative concentration of ATP at a given level of metabolism. The ATP method will detect ATP from all cells whether in clumps or single cells and the measured RLU is proportional to their total cell mass. When Cells die, they no longer produce ATP and all available ATP is utilized by remaining metabolic processes.	1 CFU can arise from 1 cell or a clump of cells placed on media surface. Thus unless cells are dispersed into single cells prior to plating, the CFU determined only relates to number of clumps of cells rather than an individual cell number.
What is measured	Measures instantaneous level of cellular metabolism at sampling time. 100% of ATP released from cells can be detected.	Measures number of colonies that grow/recover after 24 – 72 hour incubation time. Typically only 10% of cells are ever detected.
Does source of cells matter?	Easily adaptable to solution and surface evaluations. Most environmental and industrial problems are associated with organisms on surfaces.	Usually only used for determination of cell number in solution. Most problems are associated with organisms on surfaces.
Does size of cell matter	The larger a cell is the greater it's mass and thus the greater amount of ATP in that cell. A clump of cells (10 individual cells) will have 10 X the amount of ATP in it as a single similar sized cell. <i>(See slides 1 and 2 below)</i>	1 cell or clump of cells will register as 1 cfu no matter what the cell size is. <i>(See slides 1 and 2 below)</i> Depending on degree of agitation during a serial dilution, clumps can lead to significant errors in reported cfu/mL.
Numbers vs. Biofouling	Large cell clumps with a high metabolic rate can cause fouling at a faster rate than individual single cells. This rate of potential fouling is directly proportional to RLU.	Rate of fouling from 1 CFU that arises from single non-clumped cell will be much slower than that from 1 CFU that comes from a clump of cells. Thus CFU is Not proportional to biofouling.

Factors that can effect Number Obtained	During testing ATP is released to solution by breaking the cell walls Rate and extent of cell breakage depends on: - Type of cells - Type of lysing reagents - Concentration of lysing reagents Some chemicals can affect the reagent enzymes in the ATP method and thus have an effect on the RLU reported by the ATP Photometer.	Solutions need to be diluted to point that CFU's are separated on surface and countable. Diluents can affect survival of organisms.
Numbers related to:	RLU detected dependent on: - Sample size (use standard volume or specific surface area) - Mass of the cells in sample rather than number of cells. - Metabolic state of the cells immediately prior to sampling - Stability of ATP in solution after released from cells - Type of detector - Photomultiplier - Photo diode - Sensitivity settings of instrument - Stability of ATP in reagents - Type and concentration of reagents used	Number of CFU detected dependent on: - Media - Different media support growth of different organisms (Bacteria; Fungi; Yeast; Algae); Typically only 10% of cells will grow on a given media - Accuracy of Dilution series and volume plated - Organism type - Time and Temperature of Incubation - Ability of cells to recover from Treatment - Organism reacting with metabolic dyes

Free VS Total ATP - Some reagent manufactures provide reagents systems that differentiate Total from Free ATP. The reagent systems for Free ATP do not include a material to lyse cells and release ATP from inside intact cells to solution where it can be measured. Theoretically any ATP measured has come from cells that were killed/ lysed by some mechanism and thus released into solution prior to running the assay. In many solutions ATP itself is very labile and will degrade and thus theoretically no free ATP should be found unless a cell has just been lysed immediately prior to running the assay.

AMSA Inc believes it is best to rely on Total ATP numbers rather then determining Free ATP and subtracting from the Total to get a number that theoretically corresponds to viable cells that have not been lysed



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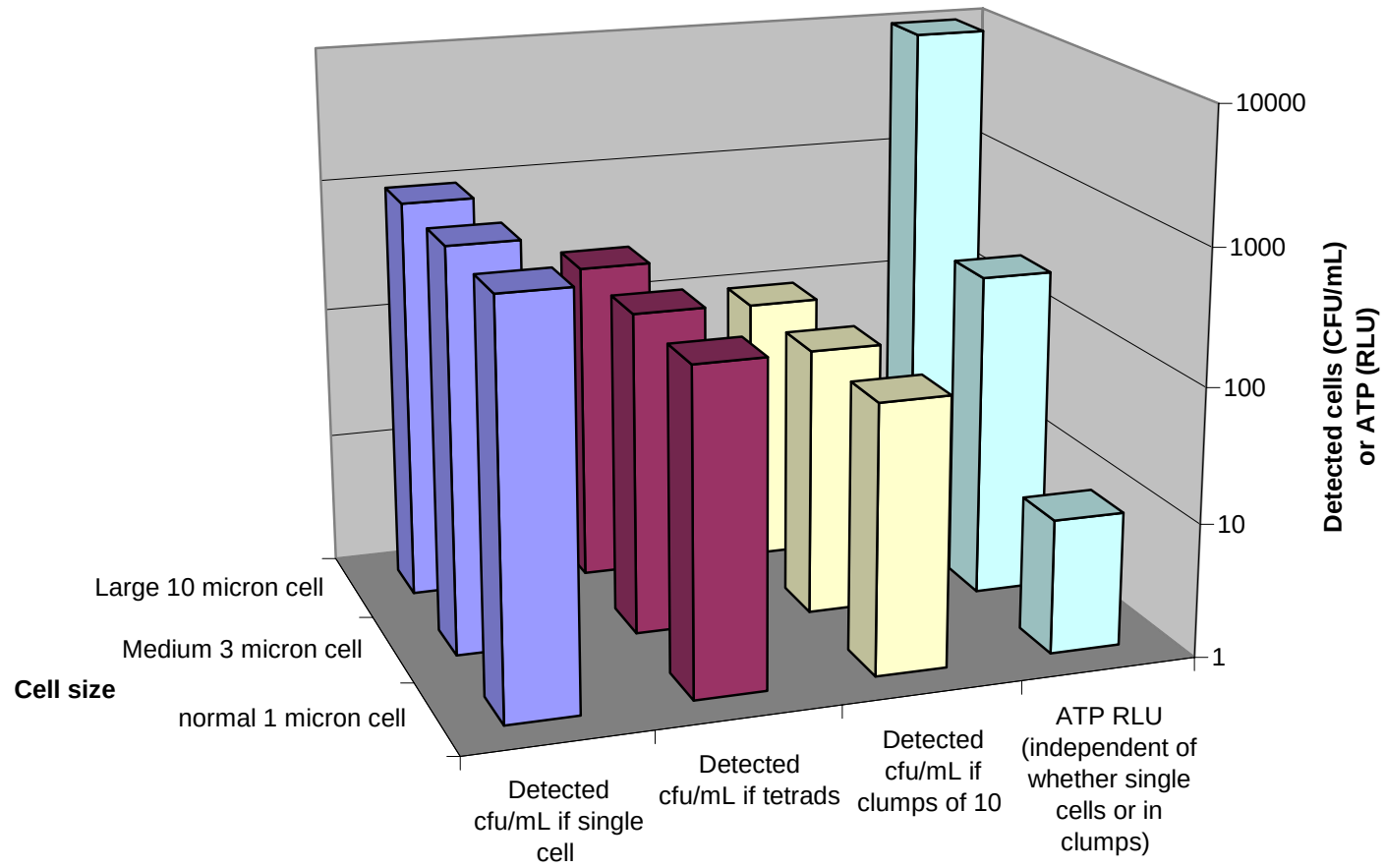
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Slide 1

Theoretical 1000 Healthy Cells/mL - 100% detection on Growth Media Effect of Cell Size and Potential Clumping of Cells



Slide 2

**Possible detected CFU/mL vs
ATP measured for 1000 different size cells/mL**

