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**IMPORTANT TECHNICAL CONSIDERATIONS WHEN CONTEMPLATING  
BIOLUMINESCENCE AS AN ANALYTICAL TOOL FOR THE DETERMINATION  
OF MICROBIAL CONTAMINATION OF PETROLEUM FUELS AND  
OTHER MATRICES**

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Bioluminescence has become a very versatile analytical tool used for the assay of microbial ATP. With the availability of high purity and commercially pure prepared reagents, highly sensitive photomultiplier tubes (PMT), software, miniaturization of portable power supply and equipment, this analytical technique has moved from the research laboratories and universities and into the field where technicians and laypersons with a minimum amount of training can quantify the microbial load of fuel samples from pipelines, storage tanks, vehicles and aircraft on a nearly real time basis. As such this technique has earned its rightful place as a valid analytical tool used to quantify the microbial load present in petroleum fuels, some biofuels and their respective fuel associated bottom water.

Since 1947 when William D. McElroy isolated and purified the heat-stable luciferin and labile enzyme luciferase from the firefly, many analytical challenges have been identified and resolved in order that bioluminescence could become a reputable and reliable analytical tool. For example, in order to ensure optimal light emissions proportional to the ATP concentration the chemicals and solutions used to duplicate reactions found in nature has to be commercially available; in order to ensure the maximum optical output of photons from the cuvette to the detector, the cuvette material has to be of a certain minimum optically quality; temperature compensation and computer algorithms has to be available to ensure results are corrected to the optimal temperature; and the detector has to be of a specific design capable of sensing low light / photon output in the spectrum of 460nm to 580nm from the bioluminescence reaction over an interval of several seconds as well as managing background noise levels.

This paper will address the more technically important considerations when contemplating bioluminescence as a valid analytical test method for the determination of microbial contamination of petroleum fuels and other matrices.