Important Technical Considerations When Evaluating Bioluminescence As A Field Test For The Determination of Microbial Contamination of Petroleum Fuels and Other Matrices In The Field.

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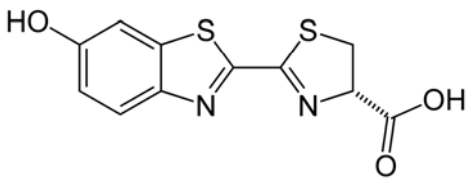
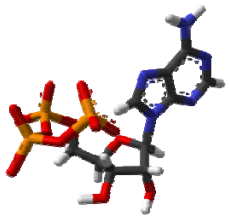
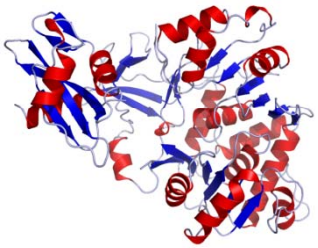
Howard Chesneau, President

Fuel Quality Services, Inc.

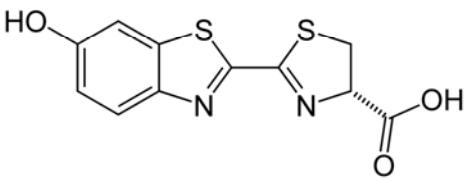


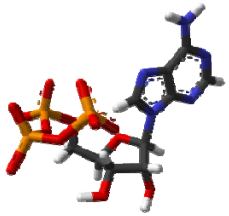


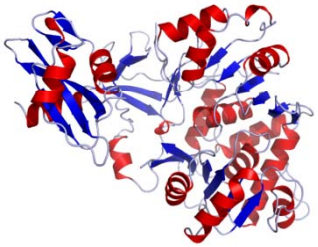


Introduction

- **Fuel Quality Services, Inc., (FQS) is a service company based in the U.S. that offers technical services and products to the fuel industry from producers to end users,**
- **In the United States, clients grappling with increasing microbial issues in fuel systems desire a detection system that is rapid, robust and reliable.**

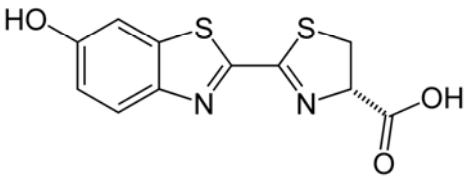


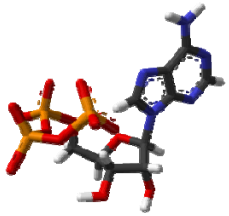


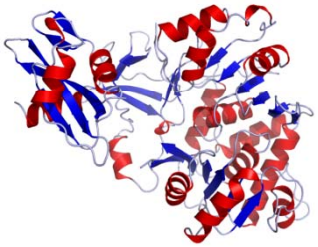


Introduction

- **An ideal testing platform should be:**
  - **user friendly,**
  - **robust,**
  - **reliable**
  - **providing rapid test results in various environmental conditions.**

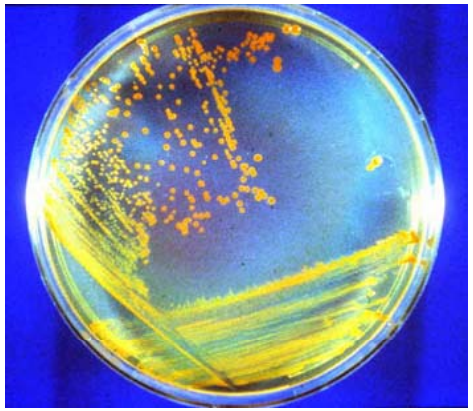


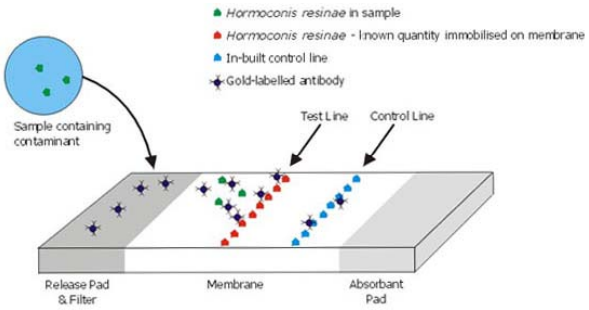




Limitations Association with Current Microbial Testing

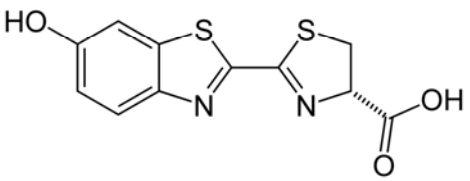
- **In spite of new advances,**
  - **Growth based methods still require an incubation period,**
  - **immunoassay tends to be species specific and may therefore fail to detect contamination by other species**

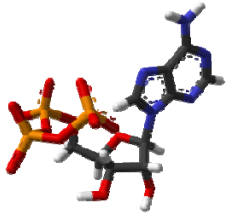


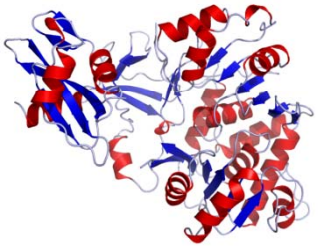


Limitations Association with Current Microbial Testing

- **Bioluminescence testing limitations:**
  - **detector type, stray light and noise**
  - **detector sensitivity optimized for signal wavelength,**
  - **test material selection (i.e., reaction tubes)**
  - **Assay chemistry optimization,**
  - **user interface, number of procedural steps, and external contamination issues.**







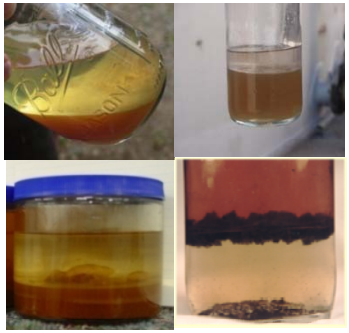
Limitations Association with Current Microbial Testing

- **Successful test development associated with a bioluminescence testing depends on:**
  - **knowledge of bioluminescence,**
  - **knowledge of matrix effects from fuels and fuel systems,**

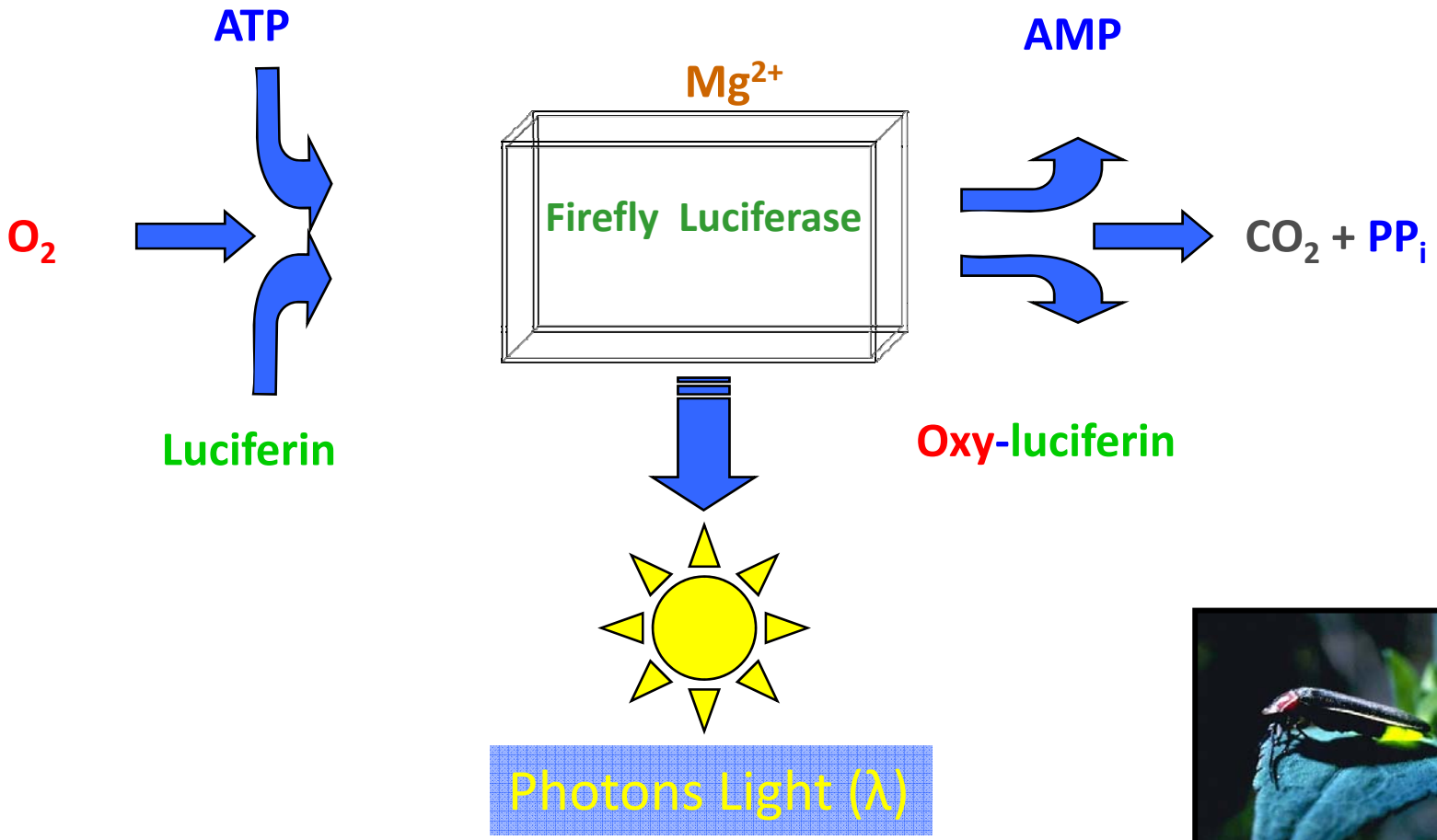
E. Newton Harvey

W. D. McElroy

Osamu Shimomura



Overview of Firefly Bioluminescence



Bioluminescence Consideration

- **Bioluminescence is found in variety organisms, one of the most common examples is the firefly,**
- **Over the millennia, nature has perfected the bioluminescence reaction so that it is optimized within the organism,**

Bioluminescence Consideration

- **However, replicating conditions present in nature in test platforms is critical to optimize the sample signal response sensitivity while reducing sources of systematic error,**

Overview of Bioluminescence Test

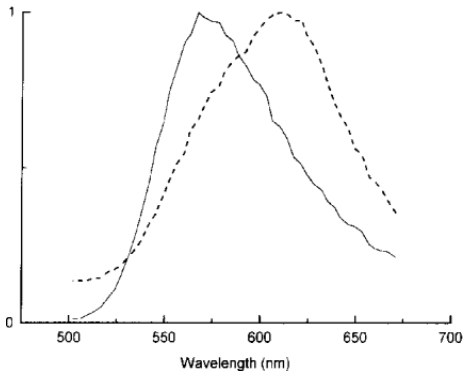
Light Intensity α [ATP]

ATP
ATP ATP
ATP
ATP ATP
ATP

Bacteria/Fungi

Acquisition of ATP

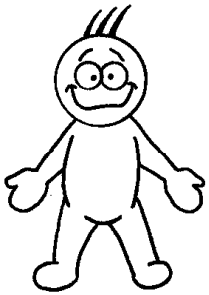
In Vitro Bioluminescence Reaction



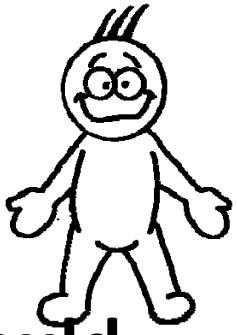
Photons Light (λ)

Bioluminescence Consideration

- **Several elements should be considered when evaluating a bioluminescence test platform for field testing of fuels and fuel associated water samples:**
  - **User knowledge/skill level,**
  - **Test Instrument and integrated features,**
  - **Test Materials and Reagents.**

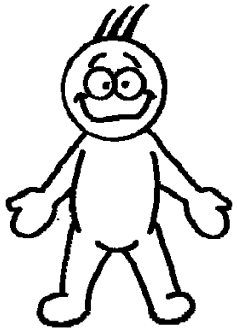


User Requirements



- **In depth knowledge of bioluminescence should not be a requirement,**
- **The test must be easily learned and retained,**
- **The test should “self contained” to eliminate many of the external and internal variables that lead to test errors,**
- **The test can be done in the field or laboratory in varying conditions,**

User Requirements



- **The test should provide rapid test results,**
- **The test should be quantitative and objective,**
- **For immediate action, the test should need no data processing**
- **Test protocols and action limits should apply for all types of fuel system samples,**
- **Waste products should not result in hazardous or biological waste.**

Test Instrument and Features

- **Due to varying test conditions and locations, the instrument platform should be:**
  - **of reasonable size, compactness and weight for portability,**
  - **powered by both main and battery power,**

Test Instrument and Features

- **robust to withstand movement, minor impacts, and changing temperatures,**
- **standardized to eliminate possible sources of random events and systematic error contributed by the equipment or user that could significantly bias results.**

Test Instrument and Features

- **The test platform should offer:**
  - **a standardized detector system that optimizes detection sensitivity and linearity performance over the entire range of detection,**

Test Instrument and Features

- **Additional useful features**
  - **integrated internal self-checks confirming that instrument setting meet factory specifications and disabling of system for specific error codes (Fail-safe operation).**
  - **integrated features like temperature compensation to allow testing over a wide temperature range to still report values normalized to 22 ^{0}C,**

Test Instrument and Features

- **Signal detection is optimized by:**
  - **Detector type photomultiplier vs. photodiode**
  - **No stray light and low level, stable background**
  - **Count time and signal integration,**
  - **Temperature detection / compensation**
  - **Cuvettes / well plates that do not auto-luminesce or phosphoresce**

Test Materials and Reagents

- **The chemical reaction can be replicated and optimized following a strict set of conditions that control sample:**
  - **Chemistry (co-factors, oxygen, neutralizers …)**
  - **Buffer conditions: pH, ionic strength …**
  - **Temperature (temperature compensation or temperature control)**

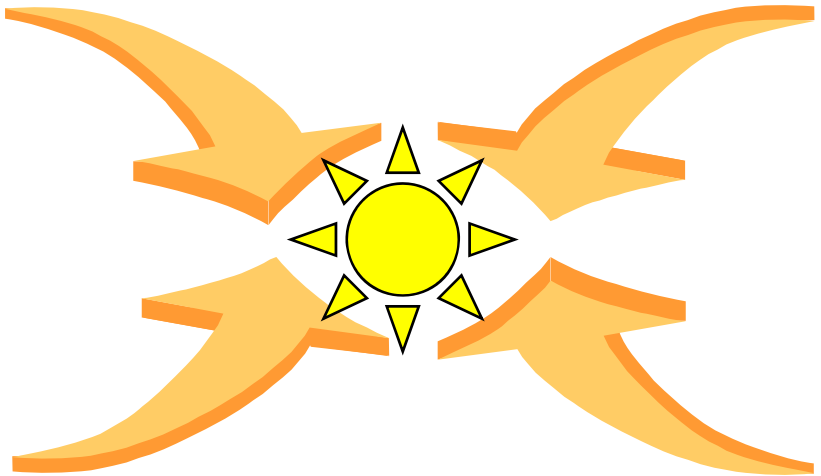
Test Materials and Reagents

- **The associated test material should:**
  - **minimize handling of reagents and samples by the user, which could introduce cross contamination resulting in false positives,**

Test Materials and Reagents

- **be constructed of materials that are compatible with the detection equipment, test reagents, test chemicals and do not auto-luminesce or phosphoresce, potentially creating a false positives.**
- **free of ATP background contamination**

Factors affecting light signal



Temperature

optimum 22°C

Inhibitors

Cl^- ; PO_4^{-2} metal ions, detergents

pH

optimum 7.8

Time delay

Stabilised kinetics

decay 5-10% / min

Strategies to Overcome inhibition

- **Neutralisation:**
  buffering; EDTA; Na-thiosulphate; chemical neutralisers
- **Dilution:**
  pre-dilution of sample; large buffer volume in assay
- **Separation from matrix**
  Centrifugation / filtration / extraction

Selecting the strategy

- **Chemical neutralization**
  - **Ionic strength in itself causes inhibition**
  - **neutralizers demands knowledge of type of interfering compounds**
- **Dilution**
  - **Reduces [ATP] in assay**
- **Filtration / centrifugation**
  - **Removes extra-cellular ATP. May cause cell stress leading to increased leakage of extracellular ATP**
- **Extraction (from fuel matrix)**
  - **Can help remove fuel soluble interfering compounds. Concentrates ATP / micro-organisms / dispersed water but will not remove water soluble interferences**

Testing for Inhibition: Internal Standard Method

% signal (with sample) **=** $\frac{100 \times (SA - S)}{CA - C}\%$

% inhibition **=** $\frac{100 \times [1 - (SA - S)]}{CA - C}\%$

- **Measure signal from sample = S**
- **Add known amount of ATP (= internal standard)**
- **Measure signal from sample + ATP = SA**
- **Measure signal from control (PF water) = C**
- **Add same amount of ATP (=internal standard)**
- **Measure signal from control + ATP = CA**

Testing for Inhibition: Dilution Method

- **Mix sample in question with sufficient ATP to obtain**
- **a HIGH signal**
- **Prepare a dilution series of sample + ATP**
- **Test (several repeats) of each dilution**
- **Calculate mean signal for each dilution**
- **Compare S_0 / S_1, S_1 / S_2 ….S_i / (S_{i+1}) with dilution factor**
- **The signal reduction should be a constant factor,**
- **approximately equal to the dilution factor**

Testing for Inhibition Dilution Method

Dilution	$\overline{S_i}$	$\frac{\overline{S_i}}{\overline{S_{i-1}}}$	$\log(S_i)$	$\log(S_i) - \log(S_{i-1})$
w.s. + ATP:	10600	3.93	4.0263	0.5949
10^{-1}	2700	9.31	3.4314	0.9690
10^{-2}	290	5.50	2.4624	0.7406
10^{-3}	52.7		1.7218	

water+ATP:	40667	9.92	4.6092	0.9964
10^{-1}	4100	10	3.6128	1.0000
10^{-2}	410	8.67	2.6128	0.9379
10^{-3}	47.3		1.6749	

Questions and Answers