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**Committee D02 on Petroleum Products, Liquid Fuels, and Lubricants
Subcommittee D02.14 on Stability and Cleanliness of Liquid Fuels**

Research Report: D02-1854

**Interlaboratory Study to Establish Precision Statements for ASTM
D7687-17, Test Method for Measurement of Cellular Adenosine
Triphosphate in Fuel, Fuel/Water Mixtures, and Fuel-Associated Water
with Sample Concentration by Filtration**

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1. Introduction:

1.1 Interlaboratory Study 1259 was conducted to establish a precision statement for D7687, Test Method for Measurement of Cellular Adenosine Triphosphate in Fuel, Fuel/Water Mixtures, and Fuel-Associated Water with Sample Concentration by Filtration. Previously, Research Report D02-1720 established the method's preliminary precision.

1.2 The test plan design for this ILS was developed in consultation with Alex Lau, David Lawrey and the ASTM ILS support team.

1.3 Primary considerations driving test matrix design:

1.3.1 ASTM Practice D6300 Practice for Determination of Precision and Bias Data for Use in Test Methods for Petroleum Products and Lubricants:

≥6 participating labs

≥2 replicate specimens of each sample type

Low, medium and high bioburdens

1.3.2 ASTM Guide D7847 Guide for Interlaboratory Studies for Microbiological Test Methods

Conduct ILS in single facility in order to minimize individual or interactive contributions of sample history (delay between subsample dispensing and testing, temperature history between dispensing and testing, conditions under which testing was performed),

Use uncharacterized, natural challenge population recovered from contaminated retail site fuel tank (see 5.1),

Acclimate challenge population by growing in primary and secondary microcosms (see 6.1 and 6.2).

1.3.3 Research Report D02-1720 and data generated in response to negatives posted to WK23762 (Work Item covering D7687 development) demonstrated:

a) luminometer model did not significantly impact test results (95% confidence level); and

b) sample matrix (fuel grade, fuel blend stock; water) did not impact test results (90% confidence level).

Consequently, in consultation with Alex Lau, it was decided to include three fuel grades and one bottoms-water type (see Table 2) and to use a single luminometer model that met Method D7687 §6.4 specifications. The latter decision was further reinforced by Research Report E34-1002 in which data from three different luminometer models were compared and it was determined that 2% of the total variability was attributable to luminometer model and that overall, variability due to luminometer model was not significant at the 95% confidence level.

2. Test Method:

The Test Method used for this ILS is D7687-11. To obtain a copy of D7687, go to ASTM's website, www.astm.org, or contact ASTM Customer Service by phone at **610-832-9585** (8:30 a.m. - 4:30 p.m. Eastern U.S. Standard Time, Monday through Friday) or by email at service@astm.org.

3. Preliminary Robustness Study:

In 2009, ILS #0367 was completed (RR D02-1720) to evaluate D7687 repeatability and preliminary (two-operator) reproducibility. In that study, replicate specimens were drawn from a single container. Consequently, biomass variability was not a source of variation. The preliminary ILS demonstrated that neither blend stock nor fuel grade were statistically significant sources of variation.

In preparation for the current ILS, a preliminary study was performed to determine sample perishability – biomass change as a function of sample age.

The results, shown in Table 1 indicated that except for bottoms-water samples, [cATP] remained stable for at least 2-days and that bottoms-water [cATP] remained stable for 1-day.

Table 1. [cATP] (Log₁₀ pg/mL) versus time.

Sample Type	Date			AVG	s
	25-Nov-16	26-Nov-16	27-Nov-16		
Jet	2.63	2.48	2.04	2.4	0.31
Diesel	4.38	5.00	4.18	4.5	0.43
GMO	3.52	4.08	3.43	3.7	0.35
Petrol	1.00	1.30	1.73	1.3	0.37
Water	5.87	6.79	>9		

Based on these results, the ILS test plan called for dispensing all subsamples the day (15h to 18h) before the ILS.

4. Participating Laboratories:

The following laboratories participated in this interlaboratory study:

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