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Standard Practice for
Manual Sampling of Liquid Fuels, Associated Materials and Fuel System Components for Microbiological Testing[1]

INTRODUCTION

There are several important characteristics that distinguish microbiological parameters from other parameters for which manually collected fuel samples are tested.

Microbes, when present in fuels or fuel systems are invariably present as contaminants. Similarly to particulates, microbes are discrete entities rather than dissolved solutes in fuel, however, unlike inanimate particles; microbes can proliferate or die during the interval between sampling and testing.

An important consequence of this is that microbes introduced into the sample from sources other than the sample itself, can proliferate and potentially eclipse the population indigenous to the sample.

Although microbes can be transported in fuel, they require free-water in order to grow and proliferate. Consequently, microbes tend to form colonies that are embedded in hydrophilic matrices. These matrices are most likely to form at system interfaces, including: fuel-water, fuel-structure, bottom-water-structure and air and fuel-vapor to structure. Microbes growing within these colonies produce chemicals (metabolites and biomolecular detritus) that are deteriogenic (can degrade fuel and fuel system components) and diffuse into fuel.

These factors combine to require unique practices specific to the collection of samples that are intended for microbiological testing.

1. Scope*

1.1 This practice covers aspects of sample device preparation and sample handling that prevent samples from becoming contaminated with microorganisms not originally contained within the sample.

1.2 This practice also covers sample handling considerations that reflect the perishability of samples collected for microbiological testing.

1.3 This practice supplements Practice D4057 by providing guidance specific to the manual sampling of fuels when samples are to be tested for microbial contamination.

1.4 The values stated in SI units are to be regarded as standard. No other units of measurement are included in this standard.

1.5 *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety, health, and environmental practices and determine the applicability of regulatory limitations prior to use.*

1.6 *This international standard was developed in accordance with internationally recognized principles on standardization established in the Decision on Principles for the Development of International Standards, Guides and Recommendations issued by the World Trade Organization Technical Barriers to Trade (TBT) Committee.*

2. Referenced Documents

2.1 *ASTM Standards:*[2]

D396 Specification for Fuel Oils
D910 Specification for Leaded Aviation Gasolines
D975 Specification for Diesel Fuel
D1129 Terminology Relating to Water
D1193 Specification for Reagent Water
D1655 Specification for Aviation Turbine Fuels
D2069 Specification for Marine Fuels (Withdrawn 2003)[3]

A Summary of Changes section appears at the end of this standard

2.2 *American Petroleum Institute (API) Standard:*[4]

2.3 *Petroleum Equipment Institute (PEI) Standard:*[5]

3. Terminology

3.1 For definition of terms used in this method refer to Terminologies D1129 and D4175, Practice D4057 and Guide D6469.

3.2 *Definitions:*

3.2.1 *aseptic, adj*—sterile, free from viable microbiological contamination.

3.2.2 *scrape sample, n*—a portion of residue removed from a surface by forceful strokes of an instrument such as a spatula.

4. Summary of Practices

4.1 *Liquid Sampling:*

4.1.1 Fuel and fuel-associated bottom-water samples intended for microbiological testing are collected similarly to conventional samples as described in Practice D4057, however specific measures are added to reduce the risk of sample contamination.

4.1.2 Sampling devices are disinfected before collecting microbiological samples.

4.1.3 Sterile sample containers are used.

4.1.4 Unique chain of custody procedures are used to minimize the potential qualitative, quantitative or both types of changes in the sample between sampling and testing.

4.2 *Surface Sampling:*

4.2.1 Sterile swabs are used to collect surface samples for microbiological testing.

4.2.2 Swabbed areas are measured to facilitate test result normalization into parameter units per unit surface area (for example CFU/cm^2).

4.2.3 The post-sampling chain of custody procedures for liquid samples apply.

4.3 *Filter Media:*

4.3.1 *Canister Elements:*

4.3.1.1 Filter elements are transferred aseptically to sterile plastic bags.

4.3.1.2 The post-sampling chain of custody procedures for liquid samples apply.

4.3.2 *Depth Media:*

4.3.2.1 Media core-samples are collected aseptically and transferred to tared, sterile containers.

4.3.2.2 The post-sampling chain of custody procedures for liquid samples apply.

5. Significance and Use

5.1 Representative samples of fuel products and associated substances are required for the determination of microbial contamination in fuels and fuel systems in order to accurately assess the biodeterioration risk posed to the fuel, fuel-system components or both. Uncontrolled microbial contamination can affect fuel specification properties adversely.[6] As discussed in Guide D6469, microbes can cause a variety of operational problems, including filter plugging and microbially influenced corrosion (MIC), the latter of which causes valve failure, tank and pipeline failure.

5.2 These practices for microbiological sampling decrease the risk of contaminating samples with extraneous microbes, thereby increasing the probability that the original microbial population in the sample does not change significantly between the time of sampling and the time of testing.

5.3 The objective of sampling for microbiological testing is to obtain representative samples that are likely to reflect the degree and nature of microbial contamination in the system from which the samples are collected. Manual 47[7] addresses the rationale for and design of microbial contamination programs. Recognizing that microbiological contamination is not distributed uniformly throughout fuel systems, both the number and types of samples collected will normally be different from the samples collected per Practice D4057 in order to determine whether product meets specifications.

5.4 The physical, chemical and microbiological property tests to be performed on a sample will dictate the sampling procedures, the sample quantity required, and many of the sample handling requirements.

5.5 Fuel systems are not normally designed to facilitate optimal microbiological sampling. Consequently, the selection

[6] Passman, F. J., McFarland, B. L., and Hillyer, M. J., "Oxygenated Gasoline Biodeterioration and its Control in Laboratory Microcosms," *International Biodeterioration and Biodegradation*, Vol 47, No. 2, 2001, pp. 95-106.

[7] Hill, G., "Sampling Methods for Detecting Microbial Contamination in Fuels and Fuel Systems," in Passman, F. J., Ed., *ASTM Manual 47–Fuel and Fuel System Microbiology: Fundamentals, Diagnosis and Contamination Control*, ASTM International, West Conshohocken, PA, 2003.

of sampling device and sample source reflect compromises between accessibility and suitability for meeting the sample collection objective.

5.6 The guidance provided in Practice D4057 generally applies to this practice as well. Consequently, this practice will address only those procedures that apply uniquely to micro-biological sampling.

6. Apparatus

6.1 The general considerations provided in Practice D4057 apply here. Sample containers come in a variety of shapes, sizes and materials. To paraphrase D4057, Paragraph 6.1, in order to be able to select the right container for a given application one must ensure that there will be no interaction between the sampled material and the container which would affect the integrity of the other. For general microbiological testing, either glass or plastic containers are appropriate. However, containers should be appropriate for the specific method of analysis intended.

6.1.1 *Sample Container Cleanliness:*

6.1.1.1 Sample containers must be clean and should be sterile.

6.1.1.2 For the purposes of most microbiological testing, previously unused containers that are received in original manufacturer's packaging are sufficiently clean to substitute for sterile containers.

6.1.1.3 Practice D5245 provides details on cleaning previously used glassware, plasticware and equipment.

6.1.1.4 Method D3508 specifies the protocol for sterilizing containers and labware.

6.2 *Sampling Devices*—Sampling devices are described in detail under each of the specific sampling procedures.

6.2.1 *Sampling Device Cleanliness:*

6.2.1.1 Sampling devices shall be cleaned between use in accordance with 8.2.1, except cleaning is not necessary between repeated spot samples obtained either for the purpose of filling a single sample container or filling multiple sample containers intended to be used as replicate spot samples. Such replicates may be used to test the sample for different parameters, when the contents of a single sample container are used for a single analysis (for example Practice D6974), for obtaining replicate data in order to determine parameter variability, or both.

6.2.1.2 It can be impractical to sterilize some types of sampling devices used to obtain liquid petroleum, petroleum product or fuel-associated, free-water samples (see 8.2).

6.3 *Funnel*—20 cm to 25 cm diameter mouth; ≤1.9 cm diameter outlet (diameter small enough to fit into mouth of sample container).

6.4 *Absorbent Spill Pads.*

6.5 *Gloves; Surgical*—Used to prevent the contamination of samples with microorganisms indigenous to human skin.

NOTE 1—The use of surgical gloves may create a static electricity discharge risk that presents an explosion hazard when handling certain fuels. Additionally, polymers from which some surgical gloves are manufactured are incompatible with certain fuels, and can disintegrate on contact with such fuel, thereby creating a skin contact hazard. Where either spark, product incompatibility or both types of risk exist, use an alternative, clean, non-porous glove that has been disinfected in accordance with 8.2 in order to address the explosion hazard risk and still minimize the risk of contaminating samples with microbes associated with human skin.

6.6 *Spatula*—Stainless steel; 1.5 cm by 10 cm for collecting surface residue samples.

6.7 *Swabs*—Sterile, ATP-free.

7. Reagents

7.1 *Alcohol,* ≥70 % methanol, ethanol or isopropanol, technical grade.

7.2 *Water*—Type I Reagent Grade or better (Specification D1193; Terminology D1129).

8. Manual Sampling Considerations

8.1 The considerations detailed in Practice D4057 Section 7 apply.

8.2 *Sampling Device Disinfection:*

8.2.1 Before collecting a sample, the sampling device shall be cleaned and disinfected. Due to the risk of fire and explosion when handling liquid fuels with boiling points below 90 °C, procedures generally used to disinfect apparatus used for microbiological sampling cannot be used in the liquid fuel environment. The following procedure shall be used instead:

8.2.1.1 Clean the device, taking particular care to remove any liquid and particulate residue remaining from previous samples.

8.2.1.2 Rinse device with alcohol (7.1) by filling the device approximately ¼ to ⅓ with alcohol and shaking the closed device for 30 s.

8.2.1.3 Drain the alcohol thoroughly from the device into a suitable disposal container.

8.2.1.4 Allow all residual alcohol to evaporate from device surfaces.

9. Special Precautions

9.1 The precautions enumerated in Practice D4057 Section 8 apply to sampling for microbiological testing.

9.2 *Contamination Control*—Additional caution is required to prevent the contamination of samples with non-indigenous microbes.

9.2.1 The normal microflora of healthy skin is $>1 \times 10^3$ bacteria$/cm^2$. Precautions shall be taken to minimize the risk of contaminating samples with skin microflora. Wearing surgical gloves provides an adequate barrier between the skin, sampling devices and sample containers. Gloves should either be replaced or rinsed with 70 % alcohol (7.1) between samples. (See Note 1.)

9.2.2 *Sampling Devices* can become contaminated with residue from collected samples. The procedure described in 8.2 minimizes the risk of cross-contamination. Device disinfection should be completed just before sample collection in order to reduce the risk of contamination from airborne microbes. All surfaces with which the sample will come into contact shall be disinfected. After collecting sample and before dispensing sample into sample container, wipe any debris from the sampler's external surfaces and use alcohol to disinfect the funnel surface over which the sample will flow.

9.2.3 *Drain and Tap Samples*—Microbiological testing may also be performed on drain samples. If sampling from a fluid drain line or dispenser nozzle, clean the area around the discharge orifice and wipe the area with alcohol (7.1).

9.2.4 *Sample Containers* should remain closed until just before the sample is dispensed from the sampling device into the container, and should be re-closed immediately after the sample has been dispensed. This reduces the risk of contamination from airborne particles or during sample container handling.

9.3 *Sample Perishability*—Microbes are living organisms. Consequently, samples for microbiological testing are highly perishable. Optimally, microbiological tests are initiated within 4 h after sample collection. If testing is to be performed within 4 h, samples may be stored at ambient temperature. If the delay will be longer, store samples on ice, in insulated containers containing ice packs or under refrigeration at 5 °C to 10 °C.

NOTE 2—Be careful to avoid freezing the sample as the freeze-thaw process is likely to kill microbes.

10. Sampling Procedures (General)

10.1 *Sample Selection*—Microbes are not distributed uniformly throughout fuel systems. Consequently, the probability of detecting microbial contamination depends substantially on the type of sample and the location within the fuel system from which the sample is collected. Hill[7] provides details of the essential sample selection considerations. The most critical issues are listed here:

10.2 *Objective*—The three primary objectives of microbiological sampling are:

10.2.1 *Product Condition*—The bioburden being transported in the liquid fuel product. Spot samples of product are most appropriate to meet this objective. Tap or thief samples are appropriate. Thief samples should be taken from the bottom-third of tanks. Tap samples may be obtained from dispenser nozzles, tank drains, or sampling taps.

10.2.2 *Product Biodeterioration Potential*—the presence of a bioburden with potential to change product properties. As noted in the Introduction, microbial communities tend to develop at interfaces. For example, microbial population densities at the fuel-water interface are typically 10^3 to 10^6 times population densities in the overlying fuel. Consequently, in tanks with measurable bottom-water, fuel water interface layer, or alternatively, bottom-samples are best suited for detecting microbial contamination. Samples from the surfaces of system components, including filter media, are also useful for diagnosing product biodeterioration risk.

10.2.3 *System Component Biodeterioration*—MIC can result in tank, valve or pipeline failure. Flocs of biomass can plug fuel filters. The preferred samples for assessing component biodeterioration risk are surface samples. Removable pipeline spools, corrosion coupons, valve components and filter media provide accessible surfaces for sampling. Bottom-samples (10.2.2) are also useful for assessing system biodeterioration risk.

10.3 *Sample Handling:*

10.3.1 *Liquid Samples*—Samples should be dispensed into sample containers via a funnel or other suitable device to prevent spillage. Before transferring a sample from the sampler to the container, wipe any debris from the sampler's external surfaces and use alcohol to disinfect the funnel surface over which the sample will flow. In accordance with 8.2.1.4, allow all residual alcohol to evaporate from funnel after disinfecting it and before using it to decant sample from device to container. Label sample container in accordance with 15.1. Store sample in dark for transport to testing facility.

10.3.2 *System Components*—Aseptically remove component from system and transfer to a plastic bag for transport to testing facility. Smaller components may be stored in self-sealing storage bags. Larger components may be stored in large garbage bags. Label sample container in accordance with 15.5. Store sample in dark for transport to testing facility.

NOTE 3—If possible, do not permit component to dry completely before transferring it to storage bag. Desiccation can kill microbes that were previously viable.

NOTE 4—Before using plastic bags for component sample storage, test for compatibility with the fuel. Petroleum products differ in their aggressiveness against the polymers from which storage bags are manufactured.

11. Tank Sampling

11.1 *Core Thief Spot Sampling Procedure:*

11.1.1 *Application*—This procedure may be used for sampling liquids, including fuel grades defined under Specifications D396, D910, D975, D1655, D2069, D2880, D3699, D4814, D6227 and D6751; for free-water and sludge/sediment at the bottom of tanks containing these liquid fuel products.

11.1.2 *Apparatus:*

11.1.2.1 Innage gauging tape and plumb bob for measuring innage and bottom-water depth.

11.1.2.2 Water-detection paste appropriate for petroleum product contained in tank from which sample is to be collected.

11.1.2.3 A typical closed-core type thief is shown in Fig. 1*a-d* and a typical bailer sampler is shown in Fig. 1*e-g*. The thief shall be designed so that a sample can be obtained from the tank bottom. The size of the thief should be selected depending upon the internal diameter of the fitting or pipe through which it will be lowered and the volume of the sample required. The thief should be capable of penetrating the liquid fuel product in the tank. It should also be capable of penetrating any membranous interface-layer that may have developed between the fuel and bottom-water. The thief shall include the following features:

(a) Uniform cross-section and bottom closure, and

(b) Innage gauging tape so that sample can be taken at any depth in the vertical cross-section of the tank.
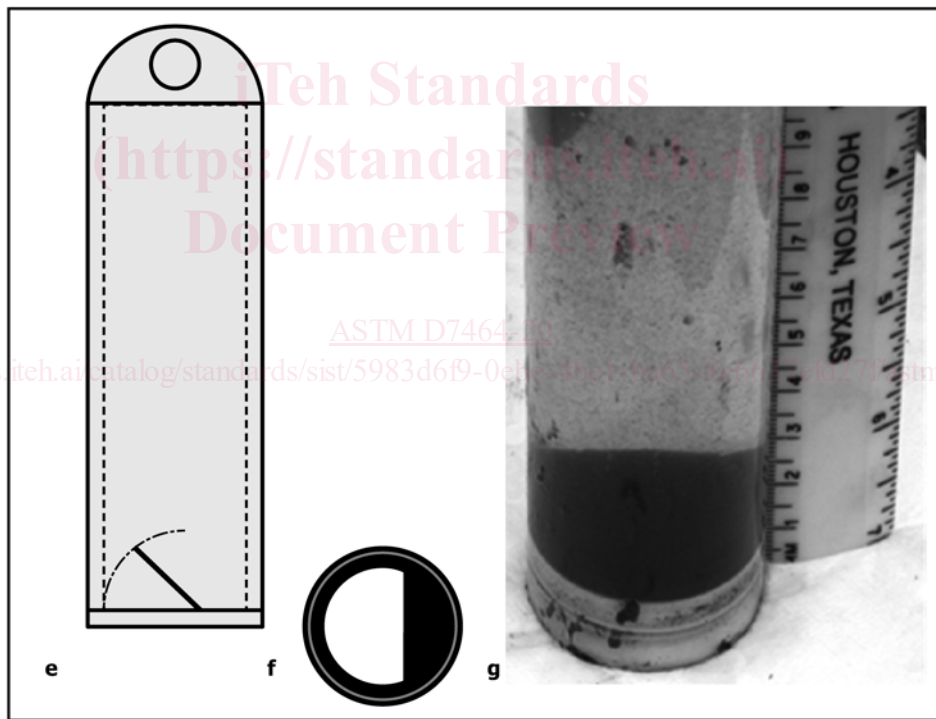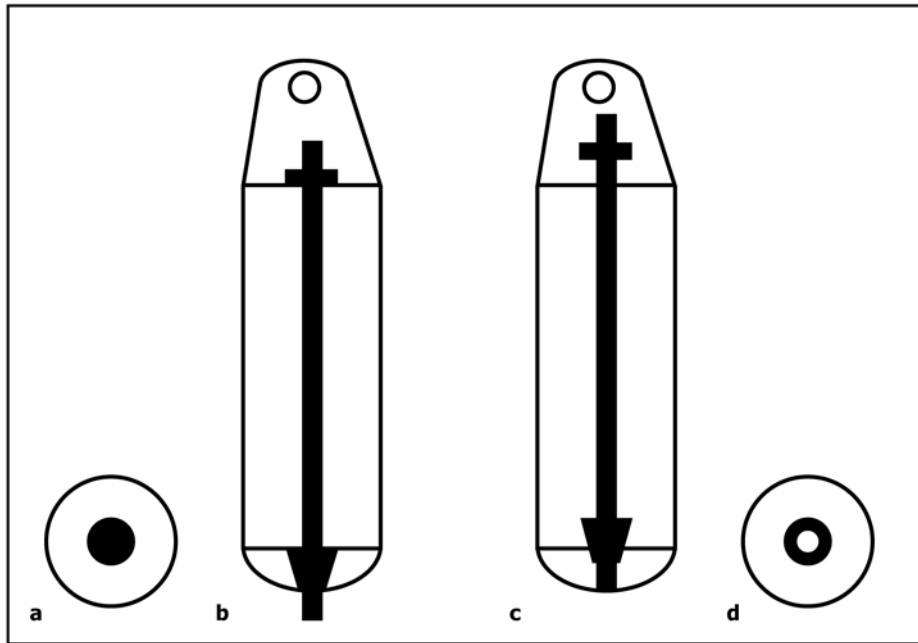
11.1.2.4 A device for draining the thief's contents into a sample bottle. For a closed-core thief, this is typically a wire or O-ring fitted through an orifice in the top of the thief's plunger. For a bailer sampler, this is typically a hook-shaped devise used to open the bailer's bottom-valve.

11.1.2.5 Funnel (6.3).

11.1.3 *Procedure:*

11.1.3.1 Determine innage and bottom-water heights in accordance with API Manual of Petroleum Measurement Standards, Chapter 3 and PEI 900-08.

(1) Attach plumb bob to innage tape.

(a) closed-thief sampler; bottom-view; closed-position
(b) closed-thief sampler; side-view; closed-position
(c) closed-thief sampler; side-view; open-position
(d) closed-thief sampler; bottom-view; open-position
(e) bailer; side-view; check-valve open
(f) bailer; bottom-view; check-valve open
(g) photograph: bailer with fuel and bottom-water sample

FIG. 1 Closed-Thief and Bailer-Type Sampling Devices

(2) Apply water-detecting paste to length of plumb. Paste should form uniform strip approximately 1.5 cm wide from tip to top of the bob.

(3) Lower the plumb bob to the bottom of the tank. Be careful to stop paying-out innage-tape as soon as the bob touches the bottom of the tank, so that bob remains vertical.