Designation: D7464 – 08

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

This standard is issued under the fixed designation D7464; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

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 and health practices and determine the applicability of regulatory limitations prior to use.*

2. Referenced Documents

2.1 *ASTM Standards:*[2]

D396 Specification for Fuel Oils
D910 Specification for Aviation Gasolines
D975 Specification for Diesel Fuel Oils
D1129 Terminology Relating to Water
D1193 Specification for Reagent Water
D1655 Specification for Aviation Turbine Fuels
D2069 Specification for Marine Fuels[3]
D2880 Specification for Gas Turbine Fuel Oils
D3508 Method for Evaluating Water Testing Membrane Filters for Fecal Coliform Recovery (Discontinued 1994)[3]
D3699 Specification for Kerosine
D4057 Practice for Manual Sampling of Petroleum and Petroleum Products
D4814 Specification for Automotive Spark-Ignition Engine Fuel
D5245 Practice for Cleaning Laboratory Glassware, Plasticware, and Equipment Used in Microbiological Analyses

[1] This practice is under the jurisdiction of ASTM Committee D02 on Petroleum Products and Lubricants and is the direct responsibility of Subcommittee D02.14 on Stability and Cleanliness of Liquid Fuels.

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[2] For referenced ASTM standards, visit the ASTM website, www.astm.org, or contact ASTM Customer Service at service@astm.org. For *Annual Book of ASTM Standards* volume information, refer to the standard's Document Summary page on the ASTM website.

[3] Withdrawn. The last approved version of this historical standard is referenced on www.astm.org.

D6227 Specification for Grade 82 Unleaded Aviation Gasoline
D6469 Guide for Microbial Contamination in Fuels and Fuel Systems
D6751 Specification for Biodiesel Fuel Blend Stock (B100) for Middle Distillate Fuels
D6974 Practice for Enumeration of Viable Bacteria and Fungi in Liquid Fuels—Filtration and Culture Procedures

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

Manual of Petroleum Measurement Standards Chapter 3—Tank Gauging, section 1A—Standard Practice for the Manual Gauging of Petroleum and Petroleum Products

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

900-08 Recommended Practices for the Inspection and Maintenance of UST Systems

3. Terminology

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

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

3.1.2 *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 a representative sample that is likely to reflect the degree and nature of microbial contamination in the system from which the sample is collected. Manual 47[7] addresses the rational for and design of microbial contamination programs.

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 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 microbiological 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*:

[4] Available from American Petroleum Institute (API), 1220 L. St., NW, Washington, DC 20005-4070, http://www.api.org.

[5] Available from Petroleum Equipment Institute website, www.pei.org.

[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.

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 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 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). Follow the guidance provide in Practice D4057, Paragraph 13.6.

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 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.

(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.

(4) Follow water-detection paste manufacturer's recommendations for exposure interval and then reel in innage tape; observing tape for wet-dry interface.

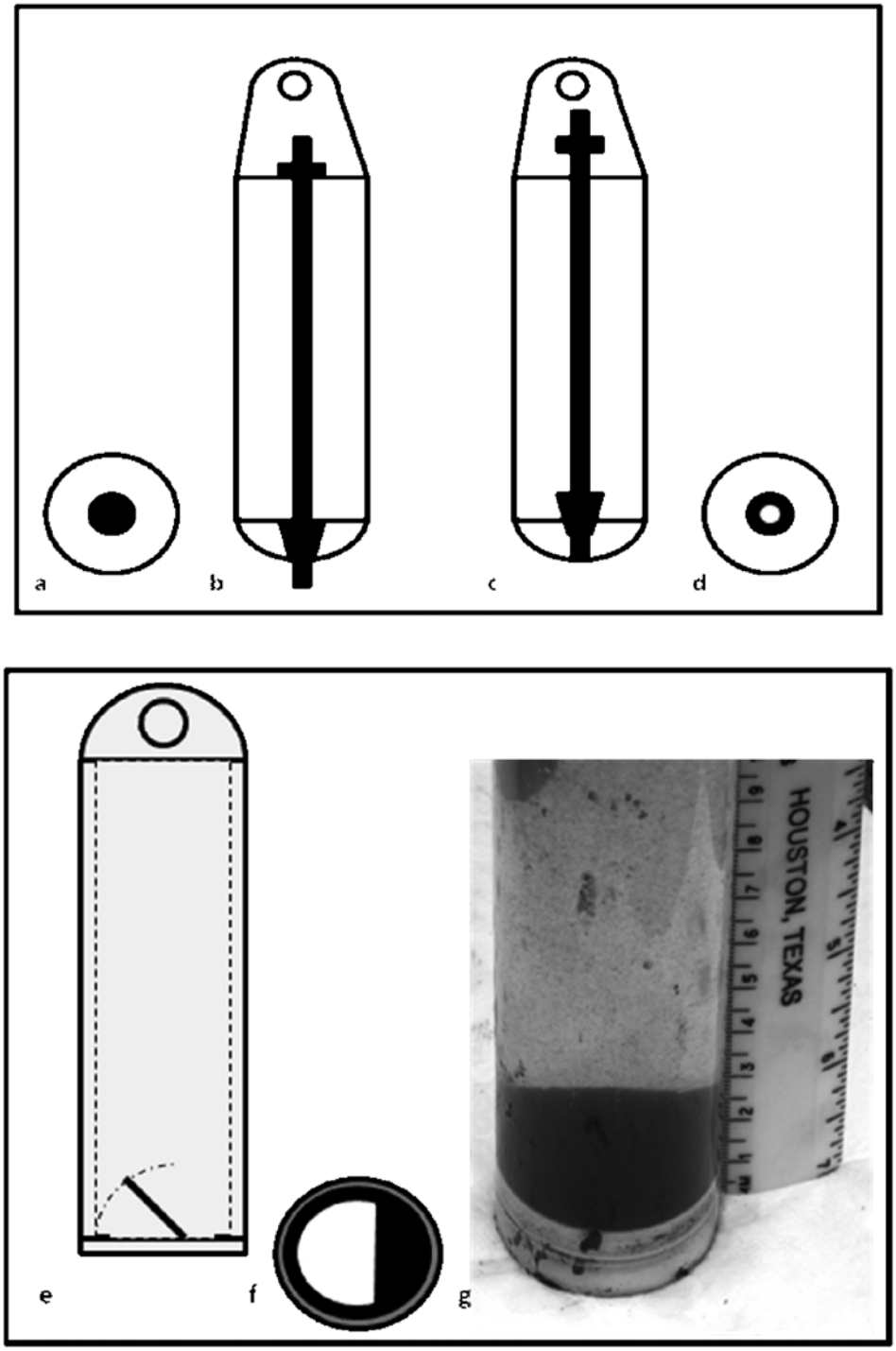
(5) Record total innage (dry-wet interface on innage tape).

(6) Inspect water-detection paste on bob. Report height of bottom-water layer.

11.1.3.2 Place spill pads underneath the sample container and on the surface of the ground/tank top between the access port from which the sample will be taken and the sample container.

11.1.3.3 Disinfect the sampling device and funnel in accordance with 8.2.1.

11.1.3.4 Lower the clean, dry thief or bailer through the access port (this may be a tank hatch, gauge-well port, fill-pipe, electronic gauge-well or other tank-top fitting of sufficient



(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

diameter to permit the lowering of the sampling device through the liquid to the tank-bottom) until it strikes bottom, or reaches the desired depth through the liquid column.

NOTE 5—Closed-core sampling thieves fill as the hydrostatic head of the liquid column forces liquid (and sediment) through the thief's opening. Consequently, the thief will draw in liquid/substance at the level of its

opening. If the sampling objective is to obtain a vertical core of the tank column between the bottom and top of the sampling device, a bailer-type sampler is more suitable. This is particularly important if it is desired to observe the bottom-water, invert-emulsion and overlying petroleum product interfaces.

11.1.3.5 When full, remove the thief or bailer and transfer the contents to the sample container (6.2) via a funnel (6.3).

11.1.3.6 Depending on the tests to be performed (see Guide D6469), microbiological testing of bottom-water can require 200 to 250 mL of water-phase fluid. If sufficient water is not recovered from a single spot sample, a composite sample may be prepared by combining two or more samples. The composite sample fuel phase may be decanted as necessary to provide sufficient capacity to collect additional samples in the sample bottle. If insufficient water is obtained with the first spot sample, the volume of water and invert emulsion should be recorded before continuing to prepare a composite sample. If no water is present in a spot sample, do not continue the composite collection process. Collect all sub-samples contributing to the composite from a single location. Unless sludge or other debris interferes with device closure, the sampling device need not be rinsed between successive collections for a single composite sample.

11.1.3.7 Immediately close and label the sample container (15.1).

11.1.3.8 When the sampling operation is complete, clean and disassemble the sampler components.

11.2 *Tap Sampling*:

11.2.1 *Application*—The tap sampling procedure is applicable when sampling liquid fuels from tank drain-lines, sampling taps or dispenser nozzles. Samples obtained from taps can be used to compare non-bottom samples with bottom-samples or as surrogates when it is impractical to obtain bottom-samples in accordance with 11.1. Tank drain samples—drawn from drain-lines installed at the tank's bottom, dead-center—are more likely to be representative of tank contents than are other types of tap samples.

11.2.2 *Apparatus*:

11.2.2.1 *Absorbent Spill Pads* (6.4).

11.2.2.2 *Funnel* (6.3).

11.2.3 *Procedure*:

11.2.3.1 Wipe surface of tap, drain-line or nozzle to remove dirt and debris from surfaces that can contaminate sample.

11.2.3.2 Rinse surface of tap, drain-line or nozzle with alcohol (8.2.1).

11.2.3.3 Rinse funnel (6.3) with alcohol (8.2.1).

11.2.3.4 Open sample container and place discharge tip of funnel into container. Take precautions to avoid contaminating the container lid.

11.2.3.5 Open tap, drain-line or nozzle and fill container, no more than 90 %. The first liquid dispensed is liquid that has been resident in the line, not liquid from the tank. If the intent is to sample tank contents, then drain one-volume of the drain line into an appropriate waste container before dispensing sample into the sample container.

11.2.3.6 Close tap, drain-line or nozzle and immediately replace the cover onto the container.

11.2.3.7 Label the container in accordance with 15.1.

11.2.3.8 When the sampling operation is complete, clean the funnel.

12. Surface Sampling

12.1 *Application*—Because most biomass is found adhering to system surfaces (inner surfaces of tanks, piping, covers, vents, valves, filter-housings, etc.), samples from accessible surfaces can yield important information regarding the presence of biodeteriogenic microbial communities in fuel systems.

12.1.1 Surface samples can be collected as swab or scrape samples.

12.1.2 Swab samples are more appropriate when the surface from which the sample will be obtained has no visible biomass or residue present.

12.1.3 Scrape samples are more appropriate when the surface from which the sample will be obtained is covered either partially or completely with visible residue, encrustation or both.

12.2 *Apparatus*:

12.2.1 *Swab Sample*:

12.2.1.1 *Swab*, sterile cotton or synthetic fiber; individually wrapped (6.7).

NOTE 6—For specific microbiological tests, such as ATP or Endotoxin, synthetic fiber swabs, certified to be free of the molecules to be tested, are required. For culture testing, sterile cotton swabs are adequate.

12.2.1.2 *Container*, culture tube, self-sealing sleeve; may contain storage medium.

12.2.2 *Scrape Sample*:

12.2.2.1 *Spatula* (6.6).

12.2.2.2 *Container*, sterile, wide mouth glass or HDPE jar, or self-sealing plastic bag. The size and composition of the container will depend on the sample volume and composition. Ensure that the container composition is compatible with the sample (6.2).

12.3 *Procedure*:

12.3.1 *Swab Sample*:

12.3.1.1 Remove sterile swab from package

12.3.1.2 Sweep swab back and forth across dimension of area being sampled. Rotate swab during process and repeat process four times as illustrated in Fig. 2: right-left; up-down; diagonal, right-top to left-bottom; and diagonal left-top to right bottom. This four-fold area coverage provides quantitative removal of biomass adhering to the surface.

12.3.1.3 Place swab in storage container for transport to testing facility.

12.3.1.4 Label sample container in accordance with 15.2.

12.3.1.5 For quantitative results (data reported as parameter per cm^2 surface area), measure the dimensions of the area sampled and compute the area in cm^2.

12.3.2 *Scrape Sample*:

12.3.2.1 For quantitative results, tare sample container.

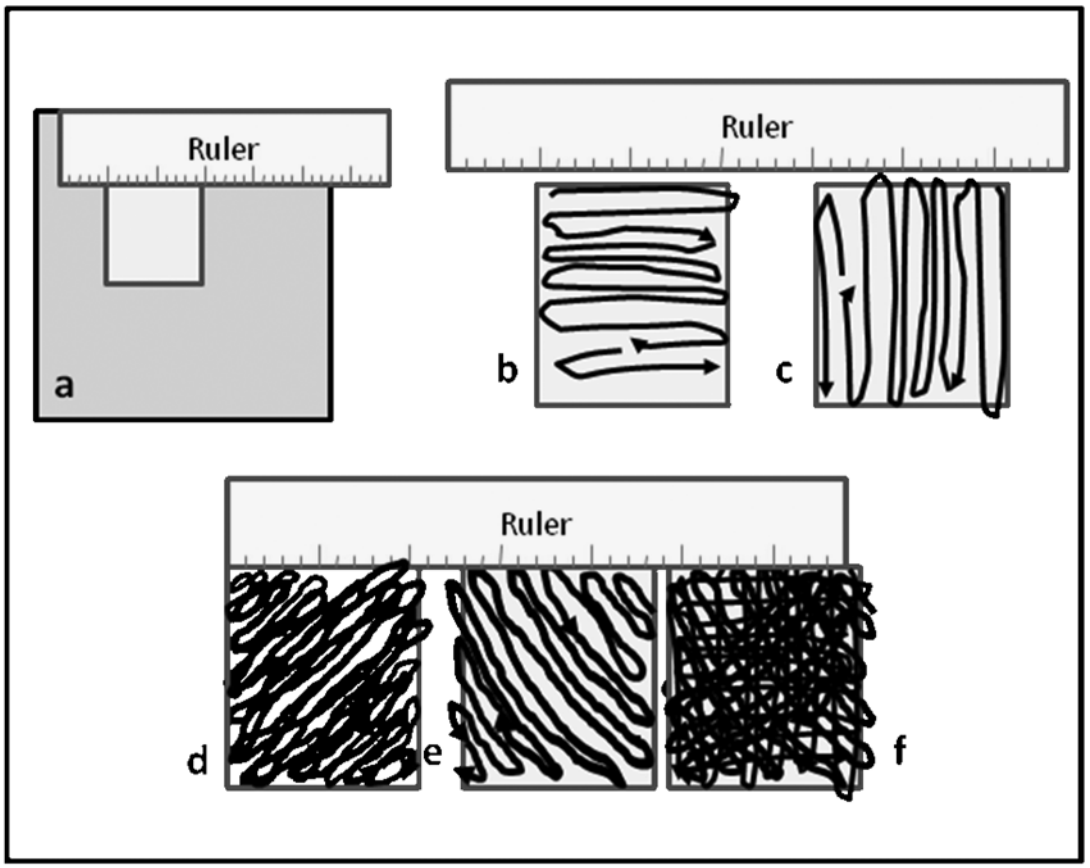
12.3.2.2 Disinfect spatula in accordance with 8.2.1.

12.3.2.3 Scrape residue from sampled surface into sample container.

12.3.2.4 For quantitative results, weigh sample container.

12.3.2.5 Label sample container in accordance with 15.2.

12.3.2.6 When the sampling operation is complete, clean the spatula.



(a) measure area to be swabbed
(b) first-pass: move swab back and forth across sampled area
(c) second-pass: move swab back and forth across area; swab pattern axis at 90° angle from first-pass
(d) third-pass; swab pattern axis at 45° from third-pass
(e) fourth-pass; swab pattern axis at 90° from third-pass
(f) net surface-coverage effect of first through fourth passes

FIG. 2 Surface Sampling; Swab-Sample

13. Filter Media Sampling

13.1 *Canister Filters*:

13.1.1 *Application*—Flocs of biomass abraded or sheered from system surfaces are transported to and trapped onto filter media. Consequently, filter media are good sources of microbial contamination data. Canister filters include filters that are individually encased in a container and paper-like media cartridges that are installed either individually or on multiple filter housings.

13.1.2 *Procedure*:

13.1.2.1 Label sample container (10.3.2) in accordance with 15.3.

13.1.2.2 Wipe dirt and debris from surface of canister/filter housing.

13.1.2.3 Remove canister/cartridge from its mounting.

13.1.2.4 Partially drain canister/cartridge (approximately 90 %) to minimize fluid carry-over but maintain media wetting.

13.1.2.5 Place canister/cartridge into pre-labeled sample container and seal the container.

13.2 *Depth Media*:

13.2.1 *Application*—Some filter media are comprised of particles (for example, activated carbon granules, kaolinite clay, and diatomaceous earth) that is loaded in bulk into filter housings. As with canister filters, flocs of biomass swept from upstream, are entrained on and among depth filter particles. The heaviest accumulations of biomass are most likely to be found on the upstream side of depth filters. Core-samples from these media are useful for diagnosing upstream biomass that may otherwise be undetectable.

13.2.2 *Apparatus*:

13.2.2.1 *Cork-borer*—A 1 cm or 2 cm diameter cork borer is suitable for collecting depth filter samples.

13.2.2.2 *Sample Container*—A wide-mouthed jar or self-sealing plastic bag with sufficient capacity and material compatibility with the sample chemistry.

13.2.3 *Procedure*:

13.2.3.1 For quantitative data, tare sample container. If the core is to be subdivided into multiple samples (for example each 1 cm-length of core to be retained as a separate sample), prepare an appropriate number of tared containers.

13.2.3.2 Disinfect cork-borer in accordance with 8.2.1.

13.2.3.3 Insert cork-borer vertically into depth medium.

13.2.3.4 Carefully remove cork-borer from medium.

13.2.3.5 Dispense either entire core into a single container or segments of core into successive containers.

13.2.3.6 Label container(s) in accordance with 15.4.

13.2.3.7 Upon completion of sampling, clean the sampling device.

14. Component Samples

14.1 *Application*—Components such as valves, screens, pumps, etc. have surfaces exposed to non-laminar flow. These surfaces can sustain microbial communities that can cause product and system biodeterioration. Laboratory examination of system components can reveal biocontamination that might otherwise go undetected.

14.2 *Apparatus*—Tools required to remove components from their installed locations.

14.3 *Procedure*:

14.3.1 Clean the exposed component surfaces.

14.3.2 Clean the surfaces of the tool(s) to be used to remove the component.

14.3.3 In accordance with 8.2.1, disinfect the tool(s) and exposed component surfaces.

14.3.4 Pre-label sample container (10.3.2) in accordance with 15.5.

14.3.5 Remove component, taking precautions to prevent contaminating component's internal surfaces with dirt, debris or human contact.

14.3.6 Place component into sample container and seal the container.

15. Sample Labeling

15.1 *Liquid Samples*—In accordance with D4057, Paragraph 12.4, sample containers should be labeled immediately after a sample is obtained. Use water proof and oil-proof ink or a pencil sufficiently hard to dent the tag. Include the following information on the label:

15.1.1 Date and time,

15.1.2 Sampler type,

15.1.3 Sample source (gauge-well, fill-pipe, water-drain, bottom, mid-column, 1 m above bottom, surface, etc.),

15.1.4 Name, number and owner of the vessel, car, tank or container,

15.1.5 Grade of material,

15.1.6 Reference symbol or identification number, and

15.1.7 If sample is a composite of multiple drops (see 11.1.3.6), number of sub-samples (drops) from which composite is comprised.

15.2 *Surface Samples*—Surface sample labels should contain the same information as listed in 15.1.1 through 15.1.6. Additionally, surface sample labels should include:

15.2.1 Location of sampled area (for example, internal surface of filter housing #1; turbine riser, 10 to 20 cm above turbine adapter; etc.).

15.2.2 Dimensions of sampled surface area, either in cm^2 or X cm by Y cm.

15.2.3 For quantitative testing, tare weight of sample container.

15.3 *Filter Media; Canister and Cartridge Filters*—Labels for canister and cartridge should provide the same information as 15.1.1 through 15.1.6. Additionally, filter labels should list:

15.3.1 Filter manufacturer name and filter part number,

15.3.2 Filter rating (nominal pore-size),

15.3.3 Filter performance characteristics (particulate, water-absorbing, etc.),

15.3.4 Filter installation date, and

15.3.5 Volume of fuel filtered since installation (estimated or actual).

15.4 *Filter Media; Depth*—Labels for loose media should provide the same information as 15.1.1 through 15.1.6. Additionally, sample labels should include:

15.4.1 Type of material (for example, activated carbon),

15.4.2 Location of core relative to reference point on filter housing (for example, 3:00 position, 4 cm from shell),

15.4.3 For quantitative testing, tare weight of sample container,

15.4.4 Last back-flush date,

15.4.5 Date media installed/re-charged, and

15.4.6 Volume of fuel filtered (estimate or actual).

15.5 *System Components*—System component labels should contain the same information as liquid samples (15.1.1 through 15.1.6). Additionally, labels should list:

15.5.1 *Part Identification*:

15.5.1.1 Part name,

15.5.1.2 Part manufacturer,

15.5.1.3 Manufacturer's part number, and

15.5.1.4 Part location in system—reference system schematic or describe location (for example, fuel gauge; right wing-tank).

16. Sample Documentation

16.1 *Sample Inventory*—Records of all sample collection should be maintained. Inventory sheets should be designed to capture all information that is provided on sample labels (Section 15).

16.2 *Chain of Custody*—Samples on which non-local testing is to be performed should have chain of custody forms. Chain of custody forms should include all label information. Additionally forms should include:

16.2.1 *Shipping Information*—Date and time shipped, carrier identity and point of contact information, parcel tracking number, identity and signature of person who prepared sample for shipment.

16.2.2 *Laboratory Information*—Date and time of receipt by laboratory, identity and signature of person receiving sample, sample disposition (storage conditions, assignment of laboratory identification number, sample splitting for processing by different analysts or laboratory sections, etc.).

16.2.3 *Testing Information*—Lists of tests requested (name and method number), name and signature of analyst, date and time testing initiated and completed, date and time report completed.

17. Keywords

17.1 bailer sampling; bottom-water; component sampling; core sampling; filtration media sampling; fuel; microbiology; sample chain of custody; sample handling; sample labeling; sampling; tap sampling; thief sampling