

# CONSENSUS STANDARDS RELATING TO BIODETERIORATION ASSESSMENT AND PREVENTION

## ASTM STANDARDS

The following standards include industrial process fluid condition monitoring protocols used to diagnose biodeterioration, microbiological test methods, and protocols for evaluating microbicide performance. ASTM Standards can be purchased as PDF files from [www.astm.org](http://www.astm.org).

### *Fuel and Lubricant Biodeterioration*

#### Microbiology Testing

##### **D6469 Guide for Microbial Contamination in Fuels and Fuel Systems**

This guide provides personnel who have a limited microbiological background with an understanding of the symptoms, occurrence, and consequences of chronic microbial contamination. The guide also suggests means for detection and control of microbial contamination in fuels and fuel systems. This guide applies primarily to gasoline, aviation, boiler, industrial gas turbine, diesel, marine, furnace fuels and blend stocks (see Specifications D396, D910, D975, D1655, D2069, D2880, D3699, D4814, D6227, and D6751), and fuel systems. However, the principles discussed herein also apply generally to crude oil and all liquid petroleum fuels. ASTM Manual 47 provides a more detailed treatment of the concepts introduced in this guide; it also provides a compilation of all of the standards referenced herein that are not found in the Annual Book of ASTM Standards, Section Five on Petroleum Products and Lubricants.

##### **D6974 Practice for Enumeration of Viable Bacteria and Fungi in Liquid Fuels—Filtration and Culture Procedures**

This practice covers a membrane filter (MF) procedure for the detection and enumeration of Heterotrophic bacteria (HPC) and fungi in liquid fuels with kinematic viscosities  $\leq 24 \text{ mm}^2 \cdot \text{s}^{-1}$  at ambient temperature.

##### **D7464 Practice for Manual Sampling of Liquid Fuels, Associated Materials and Fuel System Components for Microbiological Testing**

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.

##### **D7847 Guide for Interlaboratory Studies for Microbiological Test Methods**

1.1 Microbiological test methods present challenges that are unique relative to chemical or physical parameters, because microbes proliferate, die off and continue to be metabolically active in samples after those samples have been drawn from their source.

1.1.1 Microbial activity depends on the presence of available water. Consequently, the detection and quantification of microbial contamination in fuels and lubricants is made more complicated by the general absence of available water from these fluids.

1.1.2 Detectability depends on the physiological state and taxonomic profile of microbes in samples. These two parameters are affected by various factors that are discussed in this guide, and contribute to microbial data variability.

1.2 This guide addresses the unique considerations that must be accounted for in the design and execution of interlaboratory studies intended to determine the precision of microbiological test methods designed to quantify microbial contamination in fuels, lubricants and similar low water-content (water activity <0.8) fluids.

#### **D7978 Test Method for Determination of the Viable Aerobic Microbial Content of Fuels and Associated Water—Thixotropic Gel Culture Method**

This test method is intended to provide a tool for assessing whether fuel storage and distribution facilities or end user fuel tanks are subject to microbial growth and alert fuel suppliers or users to the potential for fuel quality or operational problems and/or the requirement for preventative or remedial measures.

#### **D8070 Test Method for Screening of Fuels and Fuel Associated Aqueous Specimens for Microbial Contamination by Lateral Flow Immunoassay**

This test method is intended to provide a tool for assessing whether fuel storage and distribution facilities, or end user fuel tanks, are subject to microbial growth, and to alert fuel suppliers or users to the potential for fuel quality or operational problems or the requirement for preventative or remedial measures, or both.

#### **E979 Practice for Evaluation of Antimicrobial Agents as Preservatives for Invert Emulsion and Other Water Containing Hydraulic Fluids**

This laboratory practice is designed to evaluate the utility and effectiveness of antimicrobial agents intended to control microbial growth in invert emulsions and other water containing hydraulic fluids.

#### **E1259 Practice for Evaluation of Antimicrobials in Liquid Fuels Boiling Below 390°C**

This practice is designed to evaluate antimicrobial agents for the prevention of microbially influenced deterioration of liquid fuels (as defined by Specification D396, D910, D975, D1655, D2069, D2880, D3699, D4814, D6227, D6751, and D7467), system deterioration, or both.

#### **E1326 Guide for Evaluating Nonconventional Microbiological Tests Used for Enumerating Bacteria**

The purpose of this guide is to assist users and producers of non-culture microbiological tests in determining the applicability of the test for processing different types of samples and evaluating the accuracy of the results. Culture test procedures such as the Heterotrophic (Standard) Plate Count, the Most Probable Number (MPN) method and the Spread Plate Count are widely cited and accepted for the enumeration of microorganisms. However, these methods have their limitations, such as performance time. Moreover, any given culture test method typically recovers only a portion of the total viable microbes present in a sample. It is these limitations that have recently led to the marketing of a variety of non-culture procedures, test kits and instruments.

#### **E2169 Practice for Selecting Antimicrobial Pesticides for Use in Water-Miscible Metalworking Fluids**

This practice provides recommendations for selecting antimicrobial pesticides (microbicides) for use in water-miscible metalworking fluids (MWF). It presents information regarding regulatory requirements, as well as technical factors including target microbes, efficacy, and chemical compatibility.

### **E2275 Practice for Evaluating Water-Miscible Metalworking Fluid Bioresistance and Antimicrobial Pesticide Performance**

This practice addresses the evaluation of the relative inherent bioresistance of water-miscible metalworking fluids, the bioresistance attributable to augmentation with antimicrobial pesticides or both. It replaces Methods D3946 and E686.

#### **Physical & Chemical Testing**

### **D130 Test Method for Corrosiveness to Copper from Petroleum Products by Copper Strip Test**

This test method covers the determination of the corrosiveness to copper of aviation gasoline, aviation turbine fuel, automotive gasoline, cleaners (Stoddard) solvent, kerosine, diesel fuel, distillate fuel oil, lubricating oil, and natural gasoline or other hydrocarbons having a vapor pressure no greater than 124 kPa (18 psi) at 37.8 °C.

### **D445 Test Method for Kinematic Viscosity of Transparent and Opaque Liquids (and Calculation of Dynamic Viscosity)**

This test method specifies a procedure for the determination of the kinematic viscosity,  $\nu$ , of liquid petroleum products, both transparent and opaque, by measuring the time for a volume of liquid to flow under gravity through a calibrated glass capillary viscometer. The dynamic viscosity,  $\eta$ , can be obtained by multiplying the kinematic viscosity,  $\nu$ , by the density,  $\rho$ , of the liquid.

### **D664 Test Method for Acid Number of Petroleum Products by Potentiometric Titration**

This test method covers procedures for the determination of acidic constituents in petroleum products, lubricants, biodiesel, and blends of biodiesel. .1Test Method A—For petroleum products and lubricants soluble or nearly soluble in mixtures of toluene and propan-2-ol. It is applicable for the determination of acids whose dissociation constants in water are larger than  $10^{-9}$ ; extremely weak acids whose dissociation constants are smaller than  $10^{-9}$  do not interfere. Salts react if their hydrolysis constants are larger than  $10^{-9}$ . The range of acid numbers included in the precision statement is 0.1 mg/g KOH to 150 mg/g KOH. .2Test Method B—Developed specifically for biodiesel and biodiesel blends with low acidity and slightly different solubility. This test method requires the use of an automatic titrator with automatic endpoint-seeking capability. Note 1:In new and used oils, the constituents that may be considered to have acidic characteristics include organic and inorganic acids, esters, phenolic compounds, lactones, resins, salts of heavy metals, salts of ammonia and other weak bases, acid salts of polybasic acids, and addition agents such as inhibitors and detergents

### **D974 Test Method for Acid and Base Number by Color-Indicator Titration**

This test method covers the determination of acidic or basic constituents (Note 1) in petroleum products<sup>2</sup> and lubricants soluble or nearly soluble in mixtures of toluene and isopropyl alcohol. It is applicable for the determination of acids or bases whose dissociation constants in water are larger than

10<sup>-9</sup>; extremely weak acids or bases whose dissociation constants are smaller than 10<sup>-9</sup> do not interfere. Salts react if their hydrolysis constants are larger than 10<sup>-9</sup>.

#### **D1298 Test Method for Density, Relative Density, or API Gravity of Crude Petroleum and Liquid Petroleum Products by Hydrometer Method**

This test method covers the laboratory determination using a glass hydrometer in conjunction with a series of calculations, of the density, relative density, or API gravity of crude petroleum, petroleum products, or mixtures of petroleum and nonpetroleum products normally handled as liquids, and having a Reid vapor pressure of 101.325 kPa (14.696 psi) or less. Values are determined at existing temperatures and corrected to 15 °C or 60 °F by means of a series of calculations and international standard tables

#### **D1331 Test Methods for Surface and Interfacial Tension of Solutions of Paints, Solvents, Solutions of Surface-Active Agents, and Related Materials**

These test methods cover the determination of surface tension and interfacial tension of a variety of liquid materials, including but not restricted to paints, solvents, and solutions of surface-active agents, as defined in Terminology D459. Four methods are covered as follows: Method A—Surface Tension by du Noüy ring. Method B—Interfacial Tension by du Noüy ring. Method C—Surface Tension by Wilhelmy plate. Method D—Interfacial Tension by Wilhelmy plate.

#### **D1744 Test Method for Determination of Water in Liquid Petroleum Products by Karl Fischer Reagent**

#### **D2068 Test Method for Determining Filter Blocking Tendency**

This test method covers three procedures for the determination of the filter blocking tendency (FBT) and filterability of middle distillate fuel oils and liquid fuels such as biodiesel and biodiesel blends. The three procedures and associated filter types are applicable to fuels within the viscosity range of 1.3 mm<sup>2</sup> to 6.0 mm<sup>2</sup>/s at 40 °C. Note 1: ASTM specification fuels falling within the scope of this test method are: Specification D396 Grades No 1 and 2; Specification D975 Grades 1-D, low sulfur 1-D and 2-D; Specification D2880 Grades 1-GT and 2-GT; Specification D6751.

#### **D2274 Test Method for Oxidation Stability of Distillate Fuel Oil (Accelerated Method)**

This test method covers the measurement of the inherent stability of middle distillate petroleum fuels under specified oxidizing conditions at 95 °C. Note 1: Fuels used in establishing the precision measures for this test method were described as gas oil, diesel fuel, No. 2 heating oil, and DFM, a Navy distillate fuel suitable for diesels, boilers, and gas turbines. (The term DFM is no longer used when referring to fuel meeting MIL-F-16884 requirements; rather it is called F76 as it conforms to NATO F76 requirements.) While the test method may be used for fuels outside the range of these fuels, the precision measures may not apply.

#### **D2276 Test Method for Particulate Contaminant in Aviation Fuel by Line Sampling**

This test method covers the determination of particulate contaminant in aviation turbine fuel using a field monitor.

#### **D3240 Test Method for Undissolved Water In Aviation Turbine Fuels**

This test method covers the measurement of undissolved water in aviation turbine fuels in flowing fuel streams without exposing the fuel sample to the atmosphere or to a sample container. The usual range of test readings covers from 1 to 60 ppm of free water. This test method does not detect water dissolved in the fuel, and thus test results for comparable fuel streams can vary with fuel temperature and the degree of water solubility in the fuel.

#### **D3241 Test Method for Thermal Oxidation Stability of Aviation Turbine Fuels**

This test method covers the procedure for rating the tendencies of gas turbine fuels to deposit decomposition products within the fuel system.

#### **D3242 Test Method for Acidity in Aviation Turbine Fuel**

This test method covers the determination of the acidity in aviation turbine fuel in the range from 0.000 mg/g to 0.100 mg/g KOH.

#### **D4176 Test Method for Free Water and Particulate Contamination in Distillate Fuels (Visual Inspection Procedures)**

This test method covers two procedures for estimating the presence of suspended free water and solid particulate contamination in distillate fuels having distillation end points below 400 °C and an ASTM color of 5 or less. .1Both procedures can be used as field tests at storage temperatures, or as laboratory tests at controlled temperatures. .2Procedure 1 provides a rapid pass/fail method for contamination. Procedure 2 provides a gross numerical rating of haze appearance.

#### **D4860 Test Method for Free Water and Particulate Contamination in Middle Distillate Fuels (Clear and Bright Numerical Rating)**

This test method covers a rapid, portable means for field and laboratory use to inspect visually for particulate matter and numerically rate free water in aviation turbine and distillate fuels.

#### **D4952 Test Method for Qualitative Analysis for Active Sulfur Species in Fuels and Solvents (Doctor Test)**

This test method covers and is intended primarily for the detection of mercaptans in motor fuel, kerosine, and similar petroleum products. This method may also provide information on hydrogen sulfide and elemental sulfur that may be present in these sample types.

#### **D5304 Test Method for Assessing Middle Distillate Fuel Storage Stability by Oxygen Overpressure**

This test method covers a procedure for assessing the potential storage stability of middle distillate fuels such as Grade No. 1D and Grade No. 2D diesel fuels, in accordance with Specification D975.

#### **D5452 Test Method for Particulate Contamination in Aviation Fuels by Laboratory Filtration**

This test method covers the gravimetric determination by filtration of particulate contaminant in a sample of aviation turbine fuel delivered to a laboratory. The sample is filtered through a test membrane and a control membrane using vacuum. The mass change difference identifies the contaminant level per unit volume.

#### **D6217 Test Method for Particulate Contamination in Middle Distillate Fuels by Laboratory Filtration**

This test method covers the determination of the mass of particulate contamination in a middle distillate fuel by filtration. This test method is suitable for all No. 1 and No. 2 grades in Specifications D396, D975, D2880 and D3699 and for grades DMA and DMB in Specification D2069.

#### **D6224 Practice for In-Service Monitoring of Lubricating Oil for Auxiliary Power Plant Equipment**

This practice covers the requirements for the effective monitoring of mineral oil and phosphate ester fluid lubricating oils in service auxiliary (non-turbine) equipment used for power generation. Auxiliary equipment covered includes gears, hydraulic systems, diesel engines, pumps, compressors, and electrohydraulic control (EHC) systems. It includes sampling and testing schedules and recommended action steps, as well as information on how oils degrade.

#### **D6304 Test Method for Determination of Water in Petroleum Products, Lubricating Oils, and Additives by Coulometric Karl Fischer Titration**

This test method covers the direct determination of water in the range of 10 mg/kg to 25 000 mg/kg entrained water in petroleum products and hydrocarbons using automated instrumentation. This test method also covers the indirect analysis of water thermally removed from samples and swept with dry inert gas into the Karl Fischer titration cell. Mercaptan, sulfide (S<sup>-</sup> or H<sub>2</sub>S), sulfur, and other compounds are known to interfere with this test method.

#### **D6426 Test Method for Determining Filterability of Middle Distillate Fuel Oils**

This test method covers a procedure for determining the filterability of distillate fuel oils within the viscosity range from 1.70 mm<sup>2</sup>/s to 6.20 mm<sup>2</sup>/s (cSt) at 40 °C.

#### ***Water System Biodeterioration***

##### **Microbiology**

#### **D3862 Test Method for Retention Characteristics of 0.2-µm Membrane Filters Used in Routine Filtration Procedures for the Evaluation of Microbiological Water Quality**

This test method covers a procedure to test membrane filters for their ability to retain bacteria whose diameter is equal to or slightly larger than the 0.2-µm pore size of the membrane filter.

#### **D4012 Test Method for Adenosine Triphosphate (ATP) Content of Microorganisms in Water**

This test method covers a protocol for capturing, extracting and quantifying the cellular adenosine triphosphate (cATP) content associated with microorganisms normally found in laboratory cultures, waters, wastewaters, and in plankton and periphyton samples from waters.

#### **D4412 Test Methods for Sulfate-Reducing Bacteria in Water and Water-Formed Deposits**

These test methods cover the procedure for the detection and enumeration by the most probable number (MPN) technique of sulfate-reducing bacteria in water or water-formed deposits.

#### **D4454 Test Method for Simultaneous Enumeration of Total and Respiring Bacteria in Aquatic Systems by Microscopy**

This test method covers the detection and enumeration of aquatic bacteria by the use of an acridine-orange epifluorescence direct-microscopic counting procedure. This test method is applicable to environmental waters and potable waters. **Note:** This standard was withdrawn in 2015 because it had not been reviewed since 2007. Historical versions of D4454 remain available at [www.astm.org](http://www.astm.org).

#### **D5246 Test Method for Isolation and Enumeration of Pseudomonas aeruginosa from Water**

The test method covers the isolation and enumeration of Pseudomonas aeruginosa. Testing was performed on spiked samples using reagent grade water as the diluent from surface waters; recreational waters; ground water, water supplies; especially rural nonchlorinated sources; waste water; and saline waters. The detection limit of this test method is one microorganism per 100 mL.

#### **D6530 Test Method for Total Active Biomass in Cooling Tower Waters (Kool Kount Assay; KKA)**

This test method covers the determination of viable active biomass in cooling tower water in the range from  $10^2$  to  $10^8$  cfu/mL. It is a semiquantitative test method.

#### **D8243 Test Method for Determination of APS Reductase to Estimate Sulfate Reducing Bacterial Bioburdens in Water – Enzyme-Linked Immunosorbent Assay Method**

This test method provides a protocol for using enzyme-linked immunosorbent assay (ELISA) technology to test water samples for the enzyme adenosine 5'-phosphosulfate reductase (APSr) concentration.

#### **E1326 Guide for Evaluating Non-culture Microbiological Tests**

The purpose of this guide is to assist users and producers of non-culture microbiological tests in determining the applicability of the test for processing different types of samples and evaluating the accuracy of the results. Culture test procedures such as the Heterotrophic (Standard) Plate Count, the Most Probable Number (MPN) method and the Spread Plate Count are widely cited and accepted for the enumeration of microorganisms. However, these methods have their limitations, such as performance time. Moreover, any given culture test method typically recovers only a portion of the total viable microbes present in a sample. It is these limitations that have recently led to the marketing of a variety of non-culture procedures, test kits and instruments.

#### **F1094 Test Methods for Microbiological Monitoring of Water Used for Processing Electron and Microelectronic Devices by Direct Pressure Tap Sampling Valve and by the Presterilized Plastic Bag Method**

These test methods cover sampling and analysis of high purity water from water purification systems and water transmission systems by the direct sampling tap and filtration of the sample collected in the bag. These test methods cover both the sampling of water lines and the subsequent microbiological analysis of the sample by the culture technique. The microorganisms recovered from the water samples and counted on the filters include both aerobes and facultative anaerobes.

### **Physical and Chemical Testing**

#### **D888 Test Methods for Dissolved Oxygen in Water**

These test methods cover the determination of dissolved oxygen in water. Three test methods are given as follows:

	Range, mg/L	Sections
Test Method A—Titrimetric Procedure—High Level	>1.0	8 – 15
Test Method B—Instrumental Probe Procedure— Electrochemical	0.05 to 20	16 – 25
Test Method C—Instrumental Probe Procedure— Luminescence-Based Sensor	0.05 to 20	26 – 31

### D932 Practice for Filamentous Iron Bacteria in Water and Water-Formed Deposits

This practice covers the determination of filamentous iron bacteria (FIB) by microscopic examination. This practice provides for the identification of the following genera of bacteria found in water and water-formed deposits: *Siderocapsa*, *Gallionella (Dioymohelix)*, *Sphaerotilus*, *Crenothrix*, *Leptothrix*, and *Clonothrix*.

### D1067 Test Methods for Acidity or Alkalinity of Water

These test methods<sup>2</sup> cover the determination of acidity or alkalinity of all types of water. Three test methods are given as follows:

	Sections
Test Method A (Electrometric Titration)	7 to 15
Test Method B (Electrometric or Color-Change Titration)	16 to 24
Test Method C (Color-Change Titration After Hydrogen Peroxide Oxidation and Boiling)	25 to 33

### D1126 Test Method for Hardness in Water

This test method covers the determination of hardness in water by titration. This test method is applicable to waters that are clear in appearance and free of chemicals that will complex calcium or magnesium. The lower detection limit of this test method is approximately 2 to 5 mg/L as CaCO<sub>3</sub>; the upper limit can be extended to all concentrations by sample dilution. It is possible to differentiate between hardness due to calcium ions and that due to magnesium ions by this test method.

### D1293 Test Methods for pH of Water

These test methods cover the determination of pH by electrometric measurement using the glass electrode as the sensor. Two test methods are given as follows:

	Sections
Test Method A—Precise Laboratory Measurement	8 to 15
Test Method B—Routine or Continuous Measurement	16 to 24

### D1426 Test Methods for Ammonia Nitrogen In Water



These test methods cover the determination of ammonia nitrogen, exclusive of organic nitrogen, in water. Two test methods are included as follows:

	Sections
Test Method A—Direct Nesslerization	7 – 16
Test Method B—Ion Selective Electrode	17 – 24

### **D3867 Test Methods for Nitrite-Nitrate in Water**

These test methods cover the determination of nitrite nitrogen, nitrate nitrogen, and combined nitrite-nitrate nitrogen in water and wastewater in the range from 0.05 to 1.0 mg/L nitrogen. Two test methods<sup>2</sup> are given as follows:

	Sections
Test Method A—Automated Cadmium Reduction	9 to 18
Test Method B—Manual Cadmium Reduction	19 to 28

### ***Microbicide Performance***

#### **E645 Practice for Evaluation of Microbicides Used in Cooling Water Systems**

This practice outlines a procedure for evaluating the efficacy of microbicides (algicides, bactericides, and fungicides) that will be used for controlling microbial growth in cooling water systems. The microbicides will be evaluated using simulated or real cooling tower water against (1) microbes from cooling water, (2) microbes in microbiological deposits (biofilms) from operating cooling systems, or (3) microorganisms known to contaminate cooling water systems, or a combination thereof. This practice should be performed by individuals familiar with microbiological techniques.

#### **E1428 Test Method for Evaluating the Performance of Antimicrobials in or on Polymeric Solids Against Staining by *Streptomyces* species (A Pink Stain Organism)**

This test method is intended to assess susceptibility of flat two dimensional vinyl films and other solid polymer products as well as products that may directly contact vinyl to pink-staining by the actinomycete bacteria *Streptomyces* species. This test method may not be suitable for highly textured or porous substrates.

#### **E1891 Guide for Determination of a Survival Curve for Antimicrobial Agents Against Selected Microorganisms and Calculation of a D-Value and Concentration Coefficient**

This guide covers the methods for determining the death rate kinetics expressed as D-values. These values can be derived from the construction of a kill curve (or survivor curve) or by using other procedures for determining the number of survivors after exposure to antimicrobial chemicals or formulations. Options for calculations will be presented as well as the method for calculation of a concentration coefficient. The test methods are designed to evaluate antimicrobial agents in formulations to define a survivor curve and to subsequently calculate a D-value. The tests are designed

to produce data and calculate values that provide basic information of the rate-of-kill of antimicrobial formulations tested against single, selected microorganisms. In addition, calculated D-values from survivor curves from exposure at different dilutions of antimicrobial can be used to show the effect of dilution by calculation of the concentration exponent,  $\eta$  (2). D-value determination assumes the ideal of first-order killing reactions that are reflected in a straight-line reduction in count where a count-versus-time plot is done. The goal here is not to determine the time at which no survivors are found, but to determine a standard value that can be used in processing and exposure determinations or used to estimate dilutions. As an example of potential use of kill curve data, the published FDA, OTC Tentative Final Monograph for Health-Care Antiseptic Drug Products, Proposed Rule, June 17, 1994 has suggested the testing of topically applied antimicrobial products using survival curve (or kill curve) calculations. The methods described in this guide are applicable to these products, but adjustments such as the use of antifoaming agents when the reaction mixture is stirred may be necessary to counteract the presence of detergents in many formulations. Frequently the sampling for these tests is done after very short intervals of exposure to the form.

#### **E2111 Quantitative Carrier Test Method to Evaluate the Bactericidal, Fungicidal, Mycobactericidal, and Sporocidal Potencies of Liquid Chemicals**

This test method is designed for use in product development and for the generation of product potency data. This test method permits the loading of each carrier with a known volume of the test organism. The incorporation of controls can also determine the initial load of colony forming units (CFU) of organisms on the test carriers and any loss in CFU after the mandatory drying of the inoculum.

#### **E2149 Test Method for Determining the Antimicrobial Activity of Antimicrobial Agents Under Dynamic Contact Conditions**

This test method is designed to evaluate the antimicrobial activity of non-leaching, antimicrobial-treated specimens under dynamic contact conditions. This dynamic shake flask test was developed for routine quality control and screening tests in order to overcome difficulties in using classical antimicrobial test methods to evaluate substrate-bound antimicrobials. These difficulties include ensuring contact of inoculum to treated surface (as in AATCC 100), flexibility of retrieval at different contact times, use of inappropriately applied static conditions (as in AATCC 147), sensitivity, and reproducibility.

#### **E2180 Test Method for Determining the Activity of Incorporated Antimicrobial Agent(s) In Polymeric or Hydrophobic Materials**

This test method is designed to evaluate (quantitatively) the antimicrobial effectiveness of agents incorporated or bound into or onto mainly flat (two dimensional) hydrophobic or polymeric surfaces. The method focuses primarily on assessing antibacterial activity; however, other microorganisms such as yeast and fungal conidia may be tested using this method.

#### **E2197 Quantitative Disk Carrier Test Method for Determining Bactericidal, Virucidal, Fungicidal, Mycobactericidal, and Sporocidal Activities of Chemicals**

This test method is designed to evaluate the ability of test substances to inactivate vegetative bacteria, viruses, fungi, mycobacteria, and bacterial spores (1-7) on disk carriers of brushed stainless steel that represent hard, nonporous environmental surfaces and medical devices. It is also designed to have survivors that can be compared to the mean of no less than three control carriers to determine if the

performance standard has been met. For proper statistical evaluation of the results, the number of viable organisms in the test inoculum should be sufficiently high to take into account both the performance standard and the experimental variations in the results.

#### **E2315 Guide for Assessment of Antimicrobial Activity Using a Time-Kill Procedure**

This guide covers an example of a method that measures the changes in a population of aerobic microorganisms within a specified sampling time when antimicrobial test materials are present. Several options for organism selection and growth, inoculum preparation, sampling times and temperatures are provided. When the technique is performed as a specific test method, it is critical that the above mentioned variables have been standardized. Antimicrobial activity of specific materials, as measured by this technique, may vary significantly depending on variables selected. It is important to understand the limitations of in vitro tests, especially comparisons of results from tests performed with different parameters. As an example, test results of microorganisms requiring growth supplements or special incubation conditions may not be directly comparable to organisms evaluated without those stated conditions.

#### **E2783 Test Method for Assessment of Antimicrobial Activity for Water Miscible Compounds Using a Time-Kill Procedure**

This test method measures the changes of a population of aerobic and anaerobic microorganisms within a specific sampling time when tested against antimicrobial test materials in vitro. The organisms used are standardized as to growth requirements and inoculum preparation and must grow under the conditions of the test. The primary purpose of this test method is to provide a set of standardized conditions and test organisms to facilitate comparative assessments of antimicrobial materials miscible in aqueous system

#### **E3152 Guide for Standard Test Methods and Practices Available for Determining Antifungal Activity on Natural or Synthetic Substrates Treated with Antimicrobial Agents**

This guide provides information on various test methods currently available to assess antifungal activity on natural or synthetic substrates.

#### **E3160 Test Method for Quantitative Evaluation of the Antibacterial Properties of Porous Antibacterial Treated Articles**

To determine the bactericidal or bacteriostatic properties of porous articles treated with an active biocidal agent, samples of porous treated materials, such as textiles or paper, are inoculated with a defined suspension of microorganisms and then incubated. The changes in numbers of the bacterial populations on the treated article are compared with untreated articles either over designated time or they are compared to the initial bacterial population at “zero time” for the treated article to measure antibacterial properties.

#### **F1885 Guide for Irradiation of Dried Spices, Herbs, and Vegetable Seasonings to Control Pathogens and Other Microorganisms**

This guide covers procedures for irradiation of dried spices, herbs, and vegetable seasonings for microbiological control. Generally, these items have moisture content of 4.5 to 12 % and are available in

whole, ground, chopped, or other finely divided forms, or as blends. The blends may contain sodium chloride and minor amounts of dry food materials ordinarily used in such blends.

### ***Biofilm Testing***

#### **E2196 Test Method for Quantification of *Pseudomonas aeruginosa* Biofilm Grown with Medium Shear and Continuous Flow Using Rotating Disk Reactor**

This test method is used for growing a reproducible *Pseudomonas aeruginosa* biofilm in a continuously stirred tank reactor (CSTR) under medium shear conditions. In addition, the test method describes how to sample and analyze biofilm for viable cells.

#### **E2562 Test Method for Quantification of *Pseudomonas aeruginosa* Biofilm Grown with High Shear and Continuous Flow using CDC Biofilm Reactor**

This test method specifies the operational parameters required to grow a reproducible *Pseudomonas aeruginosa* ATCC 700888 biofilm under high shear. The resulting biofilm is representative of generalized situations where biofilm exists under high shear rather than being representative of one particular environment.

#### **E2647 Test Method for Quantification of *Pseudomonas aeruginosa* Biofilm Grown Using Drip Flow Biofilm Reactor with Low Shear and Continuous Flow**

This test method specifies the operational parameters required to grow a repeatable *Pseudomonas aeruginosa* biofilm close to the air/liquid interface in a reactor with a continuous flow of nutrients under low fluid shear conditions. The resulting biofilm is representative of generalized situations where biofilm exists at the air/liquid interface under low fluid shear rather than representative of one particular environment.

#### **E2799 Test Method for Testing Disinfectant Efficacy against *Pseudomonas aeruginosa* Biofilm using the MBEC Assay**

This test method specifies the operational parameters required to grow and treat a *Pseudomonas aeruginosa* biofilm in a high throughput screening assay known as the MBEC (Minimum Biofilm Eradication Concentration) Physiology and Genetics Assay. The assay device consists of a plastic lid with ninety-six (96) pegs and a corresponding receiver plate with ninety-six (96) individual wells that have a maximum 200  $\mu$ L working volume. Biofilm is established on the pegs under batch conditions (that is, no flow of nutrients into or out of an individual well) with gentle mixing. The established biofilm is transferred to a new receiver plate for disinfectant efficacy testing. The reactor design allows for the simultaneous testing of multiple disinfectants or one disinfectant with multiple concentrations, and replicate samples, making the assay an efficient screening tool.

#### **E2871 Test Method for Determining Disinfectant Efficacy Against Biofilm Grown in the CDC Biofilm Reactor Using the Single Tube Method**

This test method specifies the operational parameters required to perform a quantitative liquid disinfectant efficacy test against bacterial biofilm.

#### **E3180 Test Method for Quantification of a *Bacillus subtilis* Biofilm Comprised of Vegetative Cells and Spores Grown Using the Colony Biofilm Model**

This test method specifies the operational parameters required to grow and quantify a *Bacillus subtilis* biofilm comprised of vegetative cells and endospores (spores) using the colony biofilm method (CBM). The resulting biofilm is representative of static environments that can develop a sporulating biofilm rather than being representative of one particular environment.

### ***Mycology and Indoor Air Quality***

#### **D6329 Guide for Developing Methodology for Evaluating the Ability of Indoor Materials to Support Microbial Growth Using Static Environmental Chambers**

Many different types of microorganisms (for example, bacteria, fungi, viruses, algae) can occupy indoor spaces. Materials that support microbial growth are potential indoor sources of biocontaminants (for example, spores and toxins) that can become airborne indoor biopollutants. This guide describes a simple, relatively cost effective approach to evaluating the ability of a variety of materials to support microbial growth using a small chamber method.

#### **D7338 Guide for Assessment Of Fungal Growth in Buildings**

This guide provides a compendium of information and a menu of options for assessment of fungal growth in buildings, but does not recommend a specific course of action. Due to the wide variety of fungal problems affecting buildings and their occupants, and the wide variety of buildings, it is not possible to describe a set of uniform steps that will always be performed during an assessment (that is, a standard practice); therefore the user of this guide must decide which steps are appropriate for a given situation or building.

#### **D7391 Test Method for Categorization and Quantification of Airborne Fungal Structures in an Inertial Impaction Sample by Optical Microscopy**

This test method is a procedure that uses direct microscopy to analyze the deposit on an inertial impaction sample. This test method describes procedures for categorizing and enumerating fungal structures by morphological type. Typically, categories may be as small as genus (for example, *Cladosporium*) or as large as phylum (for example, basidiospores).

#### **D7658 Test Method for Direct Microscopy of Fungal Structures from Tape**

This test method uses optical microscopy for the detection, semi-quantification, and identification of fungal structures in tape lift preparations

#### **D7788 Practice for Collection of Total Airborne Fungal Structures via Inertial Impaction Methodology**

The purpose of this practice is to describe procedures for the collection of airborne fungal spores or fragments, or both, using inertial impaction sampling techniques.

#### **D7789 Practice for Collection of Fungal Material from Surfaces by Swab**

The purpose of this standard practice is to describe the procedures for collection of surface samples using sterile swabs.

#### **D7855/D7855M Test Method for Determination of Mold Growth on Coated Building Products Designed for Interior Applications Using an Environmental Chamber and Indirect Inoculation**

This test method covers an environmental chamber and the conditions of operation to evaluate in a 4-week period the relative resistance to mold growth and microbial surface defacement on coated building products designed for interior application using an indirect inoculation method. The apparatus is designed so it can be easily built or obtained by any interested party.

#### **D7819 Test Method for Enumeration of Yeast and Mold on Fresh (Uncured) Hides and Skins**

This test method covers the enumeration of yeast and mold on fresh (uncured) hides and skins. This test method is applicable to uncured hides and skins.

#### **D7910 Practice for Collection of Fungal Material From Surfaces by Tape Lift**

This practice describes the protocols for collection of surface samples using tape lifts and their delivery to the laboratory.

#### **D8068 Practice for Collection of Culturable Airborne Fungi or Bacteria on Agar Plates by Inertial Impaction Systems**

The purpose of this practice is to describe procedures for the collection of culturable airborne fungal spores or fragments or bacteria on agar plates using inertial impaction sampling techniques.

#### **E884 Practice for Sampling Airborne Microorganisms at Municipal Solid-Waste Processing Facilities**

This practice covers sampling of airborne microorganisms at municipal solid-waste processing facilities, hereafter referred to as facilities. Investigators should consult Practice D1357 for the general principles of conducting an air-sampling program.

#### **G21 Practice for Determining Resistance of Synthetic Polymeric Materials to Fungi**

This practice covers determination of the effect of fungi on the properties of synthetic polymeric materials in the form of molded and fabricated articles, tubes, rods, sheets, and film materials. Changes in optical, mechanical, and electrical properties may be determined by the applicable ASTM methods.

#### ***Materials Biodeterioration & Biodegradation***

#### **C1894 Guide for Microbially Induced Corrosion of Concrete Products**

This guide discusses microbially induced corrosion (MIC) of concrete products and laboratory test methods for determining the resistance of concrete to MIC. Although the guide is intended for concrete products, it also covers cementitious mortar and paste that are used in specialized applications or laboratory investigations.

#### **D3274 Test Method for Evaluating Degree of Surface Disfigurement of Paint Films by Fungal or Algal Growth, or Soil and Dirt Accumulation**

Fungal growth, frequently referred to as mildew in the paint industry, causes defacement of paint film exposed outdoors. The visual rating of paint surface disfigurement due to fungal or algal attack is required in order to compare the performance of different coatings.

#### **D5511 Test Method for Determining Anaerobic Biodegradation of Plastic Materials Under High-Solids Anaerobic-Digestion Conditions**

This test method covers the determination of the degree and rate of anaerobic biodegradation of plastic materials in high-solids anaerobic conditions. The test materials are exposed to a methanogenic inoculum derived from anaerobic digesters operating only on pretreated household waste. The anaerobic decomposition takes place under high-solids (more than 30 % total solids) and static non-mixed conditions.

#### **D5526 Test Method for Determining Anaerobic Biodegradation of Plastic Materials Under Accelerated Landfill Conditions**

This test method covers determination of the degree and rate of anaerobic biodegradation of plastic materials in an accelerated-landfill test environment. This test method is also designed to produce mixtures of household waste and plastic materials after different degrees of decomposition under conditions that resemble landfill conditions. The test materials are mixed with pretreated household waste and exposed to a methanogenic inoculum derived from anaerobic digesters operating only on pretreated household waste. The anaerobic decomposition occurs under dry (more than 30 % total solids) and static nonmixed conditions. The mixtures obtained after this test method can be used to assess the environmental and health risks of plastic materials that are degraded in a landfill.

#### **D5590 Test Method for Determining the Resistance of Paint Films and Related Coatings to Fungal Defacement by Accelerated Four-Week Agar Plate Assay**

Defacement of paint and coating films by fungal growth (mold, mildew) is a common phenomenon, and defacement by algal growth can also occur under certain conditions. It is generally known that differences in the environment, lighting, temperature, humidity, substrate pH, and other factors in addition to the coating composition affect the susceptibility of a given painted surface. This test method attempts to provide a means to comparatively evaluate different coating formulations for their relative performance under a given set of conditions. It does not imply that a coating that resists growth under these conditions will necessarily resist growth in the actual application. The method is not intended to simulate or replace indoor or outdoor exposure of paint films or related coatings.

#### **D6139 Test Method for Determining the Aerobic Aquatic Biodegradation of Lubricants or Their Components Using the Gledhill Shake Flask**

This test method covers the determination of the degree of aerobic aquatic biodegradation of fully formulated lubricants or their components on exposure to an inoculum under controlled laboratory conditions. This test method is an ultimate biodegradation test that measures carbon dioxide (CO<sub>2</sub>) evolution.

#### **D7475 Test Method for Determining the Aerobic Degradation and Anaerobic Biodegradation of Plastic Materials under Accelerated Bioreactor Landfill Conditions**

This test method is used to determine the degree and rate of aerobic degradation (as indicated by loss of tensile strength, molecular weight, possibly resulting in disintegration and fragmentation) and anaerobic biodegradation of plastic materials in an accelerated aerobic-anaerobic bioreactor landfill test environment. It can simulate the change from aerobic to anaerobic environments over time as landfill depth increases. In Tier 1, the test plastic material is mixed with household waste, then pretreated and

stabilized aerobically in the presence of air, in a sealed vessel in a temperature range that is consistent with the average temperature range of those recorded for landfills. The tier is an accelerated simulation of degradation with concomitant oxygen consumption and depletion with time as if oxidative degradation proceeds. In Tier 2 samples of the plastic materials pretreated aerobically as described in Tier 1, are exposed to a methanogenic inoculum derived from anaerobic digesters operating only on pretreated household waste. The anaerobic decomposition and biodegradation occur under dry (more than 30 % total solids) and static non-mixed conditions.

#### **D7817 Test Method for Enumeration of Yeast and Mold in Raceway Brine, Brine-Cured Hides and Skins**

This test method covers the enumeration of yeast and mold. This test method is applicable to raceway brine, brine-cured hides and skins, and pre-charge raceway liquor.

#### **D7818 Test Method for Enumeration of Proteolytic Bacteria in Fresh (Uncured) Hides and Skins**

This test method covers the enumeration of bacteria that can hydrolyze protein/collagen in fresh (uncured) hides and skins. This test method is applicable to uncured hides and skins.

#### **D7816 Test Method for Enumeration of Halophilic and Proteolytic Bacteria in Raceway Brine, Brine-Cured Hides and Skins**

This test method covers the enumeration of bacteria that can tolerate high salt concentrations or can hydrolyze protein/collagen, or both. This test method is applicable to raceway brine, brine-cured hides and skins, and pre-charge raceway liquor.

#### **E1943 Guide for Remediation of Ground Water by Natural Attenuation at Petroleum Release Sites**

This is a guide for determining the appropriateness of remediation by natural attenuation and implementing remediation by natural attenuation at a given petroleum release site, either as a stand alone remedial action or in combination with other remedial actions.

#### **F2998 Guide for Using Fluorescence Microscopy to Quantify the Spread Area of Fixed Cells**

This guide describes several measurement and technical issues involved in quantifying the spread area of fixed cells. Cell spreading and the distribution of cell spread areas of a population of cells are the result of a biological response that is dependent on intracellular signaling mechanisms and the characteristics of cell adhesion to a surface. Cell spread area is a morphological feature that can be responsive to alteration in the metabolic state or the state of stress of the cells. Changes in cell spread area can also indicate an alteration in the adhesion substrate that may be due to differences in manufacturing of the substrate material or be in response to extracellular matrix secretions. High quality measurement of cell spread area can serve as a useful metric for benchmarking and detecting changes cell behavior under experimental conditions.

#### ***Terminology Standards***

##### **D1129 Terminology Relating to Water**

##### **D1356 Terminology Relating to Sampling and Analysis of Atmospheres**



This terminology is a collective vocabulary relating to sampling and analysis of atmospheres. As a convenience to general interest, it contains most of the standard terms, definitions, and nomenclature under the jurisdiction of Committee D22.

#### **D4175 Terminology Relating to Petroleum Products, Liquid Fuels, and Lubricants**

This terminology standard covers the compilation of terminology developed by Committee D02 on Petroleum Products, Liquid Fuels, and Lubricants, except that it does not include terms/definitions specific only to the standards in which they appear. The terminology, mostly definitions, is unique to petroleum, petroleum products, lubricants, and certain products from biomass and chemical synthesis. Meanings of the same terms outside of applications to petroleum, petroleum products, and lubricants can be found in other compilations and in dictionaries of general usage.

#### **E2523 Terminology for Metalworking Fluids and Operations**

Personnel from a wide range of disciplines contribute to metalworking fluid management and plant environment health and safety management. Consequently, terms familiar to some stakeholders will be unfamiliar to others. This terminology standard provides, in a single document, a compilation of definitions used by personnel involved with both metalworking environment health and safety and fluid management.

#### **E2756 Terminology Relating to Antimicrobial and Antiviral Agents**

The purpose of this terminology standard is to establish uniformity in terms used in the field of antimicrobial and antiviral agent testing. Terms are adapted from related fields such as regulatory terms defined by law and definitions as supported by test requirements.

#### ***ASTM Monographs and Manuals***

Manual 1 MNL1 - 9<sup>TH</sup> Significance of Tests for Petroleum Products: 9<sup>th</sup> Edition

Spectral analysis holds significant promise for determining values of bulk properties of spark ignition fuel and as a significant tool for the blending process. Spectral analysis includes various types of infrared and Raman technologies. Properties predicted are octane, volatility, distillation and certain specific chemical groups of interest. Research into spectral analysis of spark ignition fuels started earnestly in the early 1990s, resulting in early adoption, confusion about the technology, and later enhancements to the technology. The main mathematical method is multivariate, including partial least squares and principle component analysis. There are different approaches to predict nonlinear property values. Lean six-sigma measurement studies and control charting allow for establishment and maintenance of confidence in the test method for process control and, ultimately for product certification. Changes in the octane specification from that of a neat subgrade to “as if” specifications for 10 % ethanol blends are addressed, especially in light of the current FTC octane rule. Spectral analysis can be a very useful tool to proactively enhance refinery fuels blending by providing insight into the specific linear contribution of each blend component for each property around a given blend recipe by lean six-sigma design of experiment. This results in more precise blending control and better confidence that a blend is on-spec with minimal property giveaway.

Sample collection is the first step in petroleum, petroleum product, liquid fuel, and lubricant testing. This chapter discusses universal sampling considerations: sample heterogeneity, the respective roles of representative and nonrepresentative, diagnostic samples; the collection of replicate samples, sample chain of custody and handling; and sample container selection. The significance of each ASTM standard pertaining to petroleum and petroleum products—including alternative (nonpetroleum) fuel and lubricant products, such as vegetable oils, fatty acid methyl esters, alcohols, and so on—is then reviewed. The chapter includes a glossary compiled from all of the standards reviewed in the section “Sampling Practices.”

### **Manual 37 MNL37 - 2<sup>ND</sup> Fuels and Lubricants Handbook: Technology, Properties, Performance, and Testing, 2<sup>nd</sup> Edition**

No other book provides the extensive, in-depth coverage of fluid properties and test methodologies together.

Manual 37 is a comprehensive, in-depth, well-referenced handbook that provides a detailed overview of all of the important ASTM and non-ASTM fuels and lubricants test procedures. Readers will get a thorough overview of the application-related properties being tested and an extensive discussion of the principles behind the tests and their relationship to the properties themselves.

This newly revised edition, updated from the 2003 publication, includes new breadth and depth of discussion on important petroleum industry technology. It is a must-have for anyone in the global industry involved in the formulation, use, and specification of fuels and lubricants.

You'll get 49 chapters on general material production; fuels; lubricants; non-petroleum process fluids and ionic fluids; and testing.

#### *Chapter 35 | Biodeterioration*

Biodeterioration includes all of the direct and indirect processes by which organisms adversely affect systems and products. This chapter opens with an introduction to fundamental microbiological topics, understanding of which is essential to the subsequent discussion of biodeterioration processes and their detection and control. Although fuels, metalworking fluids, and other lubricants share many similarities, each presents unique challenges to biodeterioration detection and control. Consequently, the chapter subdivides the primary topics into subsections based on fluid and fluid-system type. This chapter provides a broad but relatively shallow introduction into the subject. The bibliography directs the reader to more in-depth coverage of the major topics covered.

### **Manual 47 MNL47 Fuel and Fuel System Microbiology: Fundamentals, Diagnosis, and Contamination Control**

This new ASTM manual brings together the various test procedures that technicians need to diagnose the contamination in fuels and fuel systems. It also suggests the means for detection and control of microbial contamination. As a complement to ASTM D6469 Standard Guide for Microbial Contamination in Fuels and Fuel Systems, this new publication provides an overview of:

- Microbiological principles underlying fuel and fuel system biodeterioration
- Sample collection and handling

- Recommendations for disinfecting and removing microbial contamination from fuels and fuel systems
- A variety of diagnostic tests

It applies to furnace, aviation gasoline, diesel, aviation turbine, marine, industrial gas turbine, kerosene, gasoline, fuel systems, crude oil, and all liquid petroleum fuels.

### **Microbiology of Petroleum Reservoirs**

Subsurface oil reservoirs are complex ecosystems comprising physiologically diverse indigenous microbial communities. Those populations have been the subject of intense investigation in recent years. Their study and characterization is of great relevance to understanding the activities and roles of indigenous microorganisms in oil reservoirs, as well as the genesis and transformations of petroleum hydrocarbons. Furthermore, their study can contribute to predict or avoid detrimental effects caused by deleterious microorganisms as well as to promote the development of new and innovative technologies to enhance oil productivity and recovery efficiency from poorly performing and depleted oil fields. As microorganisms inhabiting oil reservoirs are adapted to extreme environmental conditions, they usually harbor enzymes or metabolic functions that might be of great interest for scientific and industrial applications. The current knowledge of the microbial ecology of oil fields, however, is still largely insufficient and further studies are required. This review provides a microbiological perspective of oil reservoirs, focused on the application of microorganisms to increase oil recovery from mature reservoirs as an alternative to the traditional chemical-enhanced oil-recovery methods.

## EI (ENERGY INSTITUTE) STANDARDS

EI standards can be purchased as PDF files from <https://publishing.energyinst.org/ip-test-methods/full-list-of-ip-test-methods-publications>.

### ***Microbiology Testing***

#### **IP 385: Determination of the viable aerobic microbial content of fuels and fuel components boiling below 390°C - Filtration and culture method** {technically equivalent to ASTM D6974}

This standard describes two procedures, A and B, for the determination of the viable aerobic microbial content of middle distillate fuels and

associated waters. Viable aerobic bacteria and viable aerobic fungi (yeasts and moulds) are evaluated by this method.

Procedure A should be used for the enumeration of viable aerobic microbial units in middle distillate fuels and is suitable for enumeration of viable aerobic mould colony forming units up to 50,000 per litre, and viable aerobic yeast and bacterial colony forming units up to 100,000 per litre separately.

Procedure B should be used for the enumeration of viable aerobic microbial units in fuel associated water and is suitable for enumeration of viable aerobic mould colony forming units up to  $5 \times 10^8$  per millilitre, and viable aerobic yeast and bacterial colony forming units up to  $1 \times 10^9$  per millilitre separately.

#### **IP 472: Determination of fungal fragment content of fuels boiling below 390°C**

This standard describes a method for the collection of fungal fragments contained in a fuel sample and an estimation of their number.

Care must be taken to only count fragments of fungal origin and not any other fibres of non-microbiological origin; representative micrographs are provided in annex A to inform the operator on distinguishing between fungal fragments and other non-microbial fibres.

#### **IP 613: Determination of the viable aerobic microbial content of fuels and associated water - Thixotropic Gel Culture Method**

This standard describes a procedure for use in the field or in a laboratory to quantify viable aerobic microorganisms present as contaminants in middle distillate fuels, gasolines, biofuel blends and residual fuels and associated water. The procedure quantitatively assess the viable aerobic microbial content as microbial colony forming units (cfu) and determines whether the microbial contamination in samples drawn from fuel tanks and systems is absent or present at light, moderate and heavy levels.

### ***Petroleum Product Physical and Chemical Testing***

#### **IP 1: Determination of acidity, neutralization value - Colour indicator titration method**

This standard describes methods for the determination of the acidity of unused lubricating oil, fuel oil, and petrolatum.

Two alternative methods, A and B, are given for the determination of acidity. Method A is more suitable than method B for dark coloured oils and oils of viscosity higher than 120 mm<sup>2</sup>/s (cSt) at 40 °C and petrolatum. Method B is often preferred for pale coloured, low viscosity oils such as transformer oil. Method C is used to calculate organic acidity.

#### **IP 125: Determination of cast iron corrosion characteristics of petroleum products**

This standard specifies a procedure for the assessment of the corrosion characteristics of water-mix metal working fluids, in the form of aqueous dilutions, when in contact with cast iron. It is applicable to pre-mixed aqueous dilutions or to supplied concentrates which are diluted in the manner described in IP 263. Metal working fluids should not permit the corrosion of metals with which they come into contact, and cast iron is chosen as a typical sensitive example of such metals.

#### **IP 139: Petroleum products and lubricants - Determination of acid or base number - Colour-indicator titration method**

This International Standard specifies a colour-indicator titration method for the determination of acid or basic constituents in petroleum products and lubricants soluble in mixtures of toluene and propan-2-ol. It is applicable for the determination of acids or bases whose dissociation constants in water are greater than 10<sup>-9</sup>; extremely weak acids or bases whose dissociation constants are less than 10<sup>-9</sup> do not interfere. Salts react if their hydrolysis constants are greater than 10<sup>-9</sup>.

#### **IP 146: Determination of foaming characteristics of lubricating oils**

This document specifies a test method for determining the foaming characteristics of lubricating oils at specified temperatures. It is applicable to lubricants which may or may not contain additives to modify or suppress the tendency to form stable foam.

#### **IP 157: Determination of the oxidation stability of inhibited mineral oils (the TOST test)**

This standard specifies a method of evaluating the oxidation stability of inhibited turbine oils, hydraulic oils and circulating oils having a density less than that of water and containing rust and oxidation inhibitors, in the presence of oxygen, water and copper and iron metals, at an elevated temperature.

#### **IP 196: Petroleum products - Determination of colour (ASTM scale)**

This International Standard specifies a method for the visual determination of the colour of a variety of petroleum products, such as lubricating oils, heating fuels, diesel fuels and petroleum waxes. It is limited to products that do not contain artificial dyes.

#### **IP 226: Petroleum products - Calculation of viscosity index from kinematic viscosity**

This International Standard specifies two procedures for calculating the viscosity index (VI) of petroleum products and related materials, such as lubricating oils, from their kinematic viscosities at 40 °C and 100 °C.

Procedure A is applicable to petroleum products of viscosity index up to and including 100;

Procedure B is applicable to petroleum products of viscosity index 100 or greater.

#### **IP 229: Determination of the relative oxidation stability by rotating bomb of mineral turbine oil copy**

This standard specifies a method for the estimation of the oxidation stability of unused turbine oils having the same composition (base stock and additives) and for controlling the continuity of this property from batch to batch.

**IP 378: Determination of storage stability at 43 °C of distillate fuel**

This method covers one method for an evaluation of the inherent storage stability of distillate fuels having flash points above 38 °C and 90% recovered points below 340 °C.

**IP 386: Crude petroleum - Determination of water - Coulometric Karl Fischer titration method**

This International Standard specifies a coulometric Karl Fischer titration method for the direct determination of water in crude petroleum. It covers the range 0,050 % (m/m) to 5,00 % (m/m) water in crude petroleum containing less than 0,05 % (m/m) but more than 0,005 % (m/m) of either mercaptan sulfur or sulfide ion sulfur or both. It covers the range 0,020 % (m/m) to 5,00 % (m/m) water in crude petroleum containing less than 0,005 % (m/m) of either mercaptan sulfur or sulfide ion sulfur or both.

**IP 412: Petroleum products - Determination of water separability of petroleum oils and synthetic fluids**

This International Standard specifies a method for measuring the ability of petroleum oils or synthetic fluids to separate from water at a specified temperature.

**IP 415: Determination of particulate content of middle distillate fuels - Laboratory filtration method**

This Standard describes a method for the determination of the particulate content of middle distillate fuels with a closed flash point of 38 °C or higher.

It is not applicable to light distillate fuels (gasolines) or to aviation fuels, or to marine diesel distillates.

This standard is applicable to middle distillate fuels having a particulate content of up to 25g/m<sup>3</sup> or, when expressed on a mass/mass basis, up to 25mg/kg.

**IP 423: Determination of particulate contaminant in aviation turbine fuels by laboratory filtration**

This standard specifies a method for the gravimetric determination of particulate contaminant in aviation turbine fuel by laboratory filtration. It should be recognised that, due to the difficulty associated with obtaining reliable results, a single one-off determination by this method is of limited value. The prime applicability of this method is to indicate significant trends or changes in particulate levels at specific locations where typical values have been established. This information may be used to monitor the impact of operational changes.

**IP 431: Petroleum products - Determination of acid number - Semi-micro colour-indicator titration method**

This International Standard specifies a semi-micro colour-indicator method for the determination of acidic constituents in new or used petroleum products and lubricants soluble in mixtures of toluene and propan-2-01, or capable of existing as suspensions in such mixtures, and where the suspended material is sufficiently dissociated that its acidic components can be titrated.

The method is especially intended for applications in which the amount of sample available to be analysed is too small to allow accurate analysis by ISO 6619 or ISO 6618. It is applicable to the determination of acids having dissociation constants in water larger than  $10^{-9}$ . Extremely weak acids having dissociation constants smaller than  $10^{-9}$  do not interfere. Salts titrate if their hydrolysis contents are larger than  $10^{-9}$ .

#### **IP 438: Petroleum products - Determination of water - Coulometric Karl Fischer titration method**

This International Standard specifies a method for the direct determination of water in petroleum products boiling below 390 °C. It covers the mass fraction range 0,003 % (m/m) to 0,100 % (m/m). It is not applicable to products containing ketones or to residual fuel oils.

#### **IP 439: Petroleum products - Determination of water - Potentiometric Karl Fischer titration method**

This International Standard specifies a method for the direct determination of water in petroleum products boiling below 390 °C. It covers the mass fraction range 0,003 % (m/m) to 0, 100 % (m/m).

#### **IP 440: Liquid petroleum products - Determination of contamination in middle distillates**

This European Standard specifies a method for the determination of the content of undissolved substances, referred to as total contamination, in middle distillates, in diesel fuels containing up to 30 % (V/V) fatty acid methylesters (FAME), and in neat FAME. The working range is from 12 mg/kg to 30 mg/kg and it was established in an interlaboratory study by applying EN ISO 4259.

This European Standard in general applies to products having a kinematic viscosity not exceeding 8 mm<sup>2</sup>/s at 20 °C, or 5 mm<sup>2</sup>/s at 40 °C, e.g. diesel fuel as specified in EN 590 and FAME as in EN 14214.

This test method may be used for diesel fuels containing more than 30 % (V/V) FAME and for petroleum products having a kinematic viscosity exceeding 8 mm<sup>2</sup>/s at 20 °C, or 5 mm<sup>2</sup>/s at 40 °C, however, in such cases the precision of the test method has not been defined.

#### **IP 449: Petroleum products and lubricants - Determination of acid number - Non-aqueous potentiometric titration method**

This European Standard specifies a method for determination of acid number by potentiometric titration, of lubricating oils and additives soluble in mixtures of propan-2-01, dimethylsulfoxide and toluene. It is applicable in the range 0,1 mg KOH/g to 250 mg KOH/g, and to unused and used lubricating oils and additives.

In the case of engine oils, used engine oils and oils with a high polymer content, e.g. viscosity index improvers, precision will be poor as the electrode performance will be impaired, and the application of this method is not recommended. Precision data are only valid for industrial oils and related products.

#### **IP 453: Determination of the high temperature foaming characteristics of lubricating oils**

This standard specifies a method for the determination of the foaming characteristics of automotive lubricants and transmission fluids at 150 °C. The standard is used to assess the tendency of oils to foam at high temperature in applications such as high-speed gearing, high volume pumping and splash lubrication, where inadequate lubrication, cavitation, and loss of lubricant due to overflow, may lead to mechanical failure.

#### **IP 467: Determination of the high temperature stability of middle distillate fuels**

This standard specifies a procedure for the assessment of the relative stability of middle distillate fuels under high temperature ageing conditions with limited air exposure. The test method applies to kerosines and gas oils, excluding aviation kerosine, having a flash point determined using a closed cup tester exceeding 38 °C. It is not applicable to fuels containing residues from petroleum processing.

This test method provides an indication of the thermal oxidative stability of middle distillate fuels when heated to high temperatures that may occur in engine or burner systems, and can be used for investigations of operational problems related to fuel thermal stability. It has not been substantially correlated to engine or burner operation. For storage stability, longer term storage tests, such as IP 378, or intermediate temperature tests, such as IP 388, are likely to give better predictions. No quantitative relationship exists between the reflectance ratings obtained as a result of this procedure and the mass of insolubles formed during the ageing process.

#### **IP 471: Determination of the water content of distillate petroleum products - Potentiometric Karl Fischer titration method**

This method specifies a method for the direct determination of water in fuel oil using a potentiometric Karl Fischer titration procedure over the range 0,05% (m/m) to 2,00% (m/m) in fuel oil containing less than 0,05% (m/m) of either mercaptan sulfur or sulfide ion sulfur or both. It covers the range 0,02% (m/m) to 2,00% (m/m) water in fuel oil containing less than 0,005% (m/m) of either mercaptan sulfur or sulfide sulfur or both.

#### **IP 564: Determination of the level of cleanliness of aviation turbine fuel - Laboratory automatic particle counter method**

This standard describes a method for determining the level of dispersed particles in aviation turbine fuel, specifically dirt particles and water droplets in the range from 4 µm to 30 µm, up to a maximum of 40 000 cumulative counts per ml.

#### **IP 565: Determination of the level of cleanliness of aviation turbine fuel - Portable automatic particle counter method**

This standard describes a method for determining the level of dispersed particles in aviation turbine fuel, specifically dirt particles and water droplets in the range from 4 :m(C) to 30 :m(C), up to a maximum of 60 000 cumulative counts per ml.

#### **IP 582: Petroleum products - Determination of boiling range distribution - Gas chromatography method (ISO 3924:2016)**

This International Standard specifies a method for the determination of the boiling range distribution of petroleum products. The method is applicable to petroleum products and fractions with a final boiling point of 538 °C or lower at atmospheric pressure as determined by this International Standard. This International Standard is not applicable to gasoline samples or gasoline components. The method is limited to products having a boiling range greater than 55 °C and having a vapour pressure sufficiently low to permit sampling at ambient temperature.



The method has successfully been applied to samples containing fatty acid methyl esters (FAME) up to 10 % (V/V).

**IP 596: Petroleum products - Determination of distillation characteristics of petroleum products - Micro distillation method**

This test method specifies a procedure for determination of the distillation characteristics of petroleum products having boiling range between 20 °C to 400 °C at atmospheric pressure using an automatic micro distillation apparatus. The measurement is made on a small test portion.

This test method is applicable to such products as: light and middle distillates; automotive spark ignition engine fuels; automotive spark-ignition engine fuels containing up to 20 % ethanol; aviation gasolines; aviation turbine fuels; regular and low sulfur diesel fuels; FAME (B100) biodiesel blends up to 30 % biodiesel; special petroleum spirits; naphtha's; white spirits; kerosene's; burner fuels, and marine fuels.

The test method is also applicable to hydrocarbons with a narrow boiling range, such as organic solvents or oxygenated compounds.

The test method is not applicable to products containing appreciable quantities of residual material.

***EI Monographs and Manuals***

**Guidelines for the management of microbiologically influenced corrosion in oil and gas production**

Published: November 2017

REF/ISBN: 9780852939796

Edition: 1<sup>st</sup>

Microbiological activity can accelerate corrosion in oil and gas production; if left uncontrolled, it can give rise to material failures, production loss, and economic and environmental implications.

These guidelines present salient microbiological information and are intended for use by corrosion engineering staff on operational oil and gas production facilities. Consequently, a working knowledge of corrosion science and technology has been assumed, but no prior knowledge of microbiological matters is expected.

**A practical evaluation of 21st century microbiological techniques for the upstream oil and gas industry**

Published: November 2012

REF/ISBN: 9780852936382

Edition: 1<sup>st</sup>

In the past the oil and gas industry relied upon the development of microbiological monitoring and identification techniques intended for other commercial sectors, adapting these for its own needs when necessary. An increased dependence on molecular microbiological methods (MMM) in other sectors is gradually being embraced by the oil and gas industry. One of the reasons for this is a lack of knowledge

on the strengths and weaknesses of MMM in relation to microbiological problems in oilfield, refinery and pipeline situations.

This report aims to compare traditional oil industry microbiological techniques with MMM. Ten samples were collected and analysed from three oil production platforms in the North Sea: water from a seawater system; produced water from oil separators; pigging debris, and surface solids from an oil export spool section. Each sample was analysed using traditional microbiological techniques and MMM. An assessment was then made on the strengths and weaknesses of each technique in relation to oil industry requirements. By discussing the results from the various techniques in terms of the underlying science, the reader will be better equipped to specify the appropriate technique for a particular problem and interpret the results from the chosen techniques.

This publication will be of particular interest to microbiologists working in the oil and gas industry in relation to oilfield, refinery and pipeline situations.

### **Guidelines for the investigation of the microbial content of liquid fuels and for the implementation of avoidance and remedial strategies**

Published: November 2019

REF/ISBN: 9781787251151

Edition: 3<sup>rd</sup>

This publication provides an overview of the factors which cause and exacerbate microbial growth and the problems caused, offering detailed procedures for good housekeeping to avoid microbial growth, and describes the indicators of contamination and appropriate

analytical methods for investigation. It outlines suitable plans for routine condition monitoring including procedures for sampling and use of on-site tests, and emphasises that the primary mechanism for control of microbial growth is through regular and rigorous attention to good housekeeping and system maintenance.

This third edition is intended as an information resource, providing practical advice for controlling, monitoring, investigating and remediating microbial contamination. It is targeted at operational staff and product quality managers responsible for all liquid fuels, including those used in aviation, marine, ground transportation, power generation and heating. It addresses all stages of fuel production, distribution and use, including considerations for refineries, supply and distribution facilities, pipelines, local suppliers, retail outlets, bunker supplies and major fuel users such as ships, offshore platforms, aircraft operators, power stations, and road and rail fleet operators.

This document provides the practical basis to ensure that attention to best practice is maintained. It is intended to provide the fuels industry with the knowledge to fully understand the risks and successfully maintain and implement procedures to meet the existing and future challenges presented by microbial growth in fuel tanks and systems.

### **Guidelines on detecting, controlling and mitigating microbial growth in oils and fuels used at power generation facilities**

Published: June 2020

REF/ISBN: 9781787251885

Edition: 1<sup>st</sup>

This guidance document focuses on addressing operational problems from microbial growth in turbine, lubricating, transformer and other oils and diesel fuels at power stations. The consequences of microbial growth in oils include additive depletion, tank and pipe sludging, filter plugging, gauging problems, corrosion, increased acidity, attack on paint surfaces, viscosity changes and smell and discolouration.

This guidance document provides sufficient background information to the power generation industry stakeholders who are unfamiliar with microbiology as a tool to empower them to recognise and effectively control microbial contamination in fuel and turbine oil systems. It offers information about both turbine oil and fuel product and system biodeterioration. Much of the fuel-related material derives from Energy Institute (EI) Guidelines for the Investigation of the Microbial Content of Liquid Fuels and for the Implementation of Avoidance and Remedial Strategies (EI Fuel Guidelines).

## **NACE STANDARDS**

The following standards include industrial process fluid condition monitoring protocols used to diagnose biodeterioration, microbiological test methods, and protocols for evaluating microbicide performance. NACE Standards can be purchased as PDF files from <https://store.nace.org/standards>.

### **TM0106-2016 Detection, Testing and Evaluation of Microbiologically Influenced Corrosion (MIC) on External Surfaces of Buried Pipelines**

Types of microorganisms and mechanisms by which MIC occurs on external surfaces of buried ferrous-based metal pipelines. Testing for the presence of bacteria, research results and interpretation.

### **TM0194-2014, Field Monitoring of Bacterial Growth in Oil and Gas Systems**

Intended for use by technical field and service personnel. Describes field methods for estimating bacterial populations found in oilfield systems. Sampling methods and media for enumerating bacteria are described.

### **TM0212-2018 Detection, Testing, and Evaluation of Microbiologically Influenced Corrosion on Internal Surfaces of Pipelines**

Standard test method for microbiologically influenced corrosion (MIC) on internal surfaces of pipelines. Types of microorganisms, MIC mechanisms, sampling and testing. Research results and interpretation of test.

### ***NACE Monographs and Manuals***

#### **Pipeline CICS&#151; Microbiologically Influenced Corrosion of Pipelines**

Product Number: 37246

ISBN: PIP-Microbiolog

This MIC book is a compilation of technical papers from NACE topical symposia and conferences, articles from Materials Performance magazine, CORROSION Journal book proceedings, and technical committee reports. 2001 by NACE, 8-1/2" x 11", softbound, 15 papers.

#### **Water and Wastewater CICS&#151; Microbiological Influenced Corrosion in Water Systems**

Product Number: 37221

ISBN: WAT-Microbiolog

Corrosion Information Compilation Series (CICS) are compilations of papers and articles from NACE topical symposia and conferences, Materials Performance magazine, CORROSION, book proceedings, and technical committee reports. 2001 by NACE, 8-1/2" x 11", softbound, 18 papers.

#### **Microbiology in a Geothermal Operation**

Product Number: 51319-12818-SG

Author: Sabine Doddema

Publication Date: 2019

A geothermic system faced serious injection obstruction problems two-and-a-half months after start-up. The obstruction was so severe that the operation was suspended and research was done in order to determine the cause. To solve the obstruction problem the system was treated downhole using acid and biocide. During this treatment dangerous amounts of H<sub>2</sub>S were released. This study was initiated to understand the reason of this problematic and give options for treatment and prevention. The results show high amount of bacteria in the obstruction material indicating that microorganisms caused the obstruction. The detected species were typical thermophilic species with optimal growth temperature at 40-60 °C. The change in temperature in combination with the used oxygen scavenger containing nutrients necessary for microbial growth and activity is suspected to have caused the obstruction. The H<sub>2</sub>S formation was likely caused by a combination of biological FeS formation and the release of H<sub>2</sub>S by the addition of acid during cleaning. To prevent this type of issues biocide treatment is recommended not only during but also before operation is started. Alternatives for biocides have been investigated as well however more research is needed to understand the practical feasibility of those alternatives.

### **Microbiologically Influenced Corrosion in the Upstream Oil and Gas Industry**

Product Number: 38622

ISBN: 9781498726566

Author: Skovhus, Enning, and Lee

Publication Date: 2017

Microorganisms are ubiquitously present in petroleum reservoirs and the facilities that produce them. Pipelines, vessels, and other equipment used in upstream oil and gas operations provide a vast and predominantly anoxic environment for microorganisms to thrive. The biggest technical challenge resulting from microbial activity in these engineered environments is the impact on materials integrity. Oilfield microorganisms can affect materials integrity profoundly through a multitude of elusive (bio)chemical mechanisms, collectively referred to as microbiologically influenced corrosion (MIC). MIC is estimated to account for 20 to 30% of all corrosion-related costs in the oil and gas industry.

This book is intended as a comprehensive reference for integrity engineers, production chemists, oilfield microbiologists, and scientists working in the field of petroleum microbiology or corrosion. Exhaustively researched by leaders from both industry and academia, this book discusses the latest technological and scientific advances as well as relevant case studies to convey to readers an understanding of MIC and its effective management.