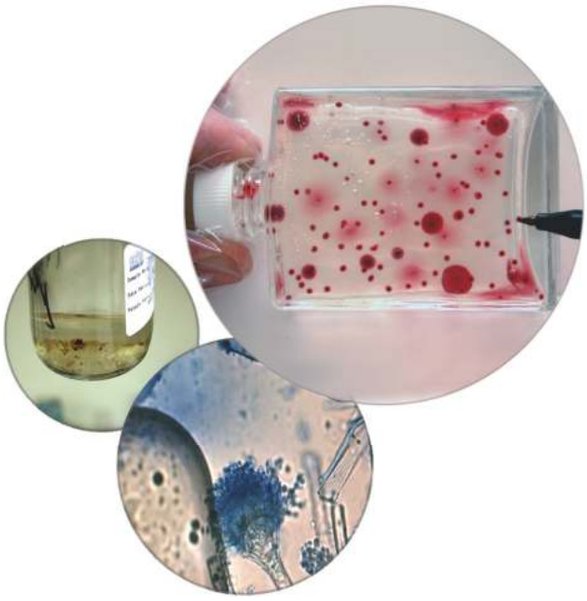
MICROBMONITOR® 2

For Detection and Enumeration of Contaminating Microbes in Fuels, Lubricants and Water

Instructions for Use



Important Notice

Please read the Instructions for Use carefully before testing samples.

The test kit should only be used as part of an investigatory process into contamination in petroleum products and associated water. The test kit must be used strictly in accordance with these instructions and/or Standard Test Method IP613 or ASTM D7978 or other instructions authorised by ECHA Microbiology Ltd. The results only relate to the portion of sample tested and not necessarily to other petroleum product in the system. Although guidance may be sought by the user on sampling and the interpretation of results, the responsibility for carrying out the sampling and test procedures correctly is that of the end-user and not ECHA Microbiology Ltd. The test kit is designed to detect a recognized group of micro-organisms of industrial significance, but it is in the nature of microbiology that there may be micro-organisms present which are not detected by the test procedure. ECHA Microbiology Ltd does not accept any liability for any decision or assessment taken or made as a consequence of the results obtained.

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WHAT IS THE MICROBMONITOR®2 TEST?

MicrobMonitor2 is a simple and easy to use test kit which enables the quantitative assessment of the viable microbial content in petroleum products including liquid fuels, oils and associated water. **Microb**Monitor2 enables on-site or laboratory testing in accordance with ASTM and IP Standard Methods ASTM D7978 and IP613 - *Determination of the viable aerobic microbial content of fuels and associated water - Thixotropic Gel Culture Method.* **Microb**Monitor2 is recommended in the *IATA Guidance Material on Microbiological Contamination in Aircraft Fuel Tanks* for monitoring microbial contamination in aircraft fuel tanks and is listed in the Aircraft Maintenance Manuals of Airbus, Boeing, BAE Systems and other leading aircraft manufacturers. The test is used by a wide range of military services for monitoring contamination in aviation, marine and ground fuels and is NATO Codified (6640-99-834-3573).

MicrobMonitor2 provides a total count of microbial colony forming units (CFU). CFUs are the conventional unit of measure for microbial contamination used in a wide range of industries. **Microb**Monitor2 detects the important bacteria, yeasts and moulds which can contaminate petroleum products including *Hormoconis resinae, Aspergillus, Candida and Pseudomonas* species.

The **Microb**Monitor2 test consists of a screw-capped bottle containing a thixotropic nutrient gel. A sample is added to the gel using a sterile plastic loop or syringe (provided). The bottle is shaken and the gel liquefies and the sample, and any microorganisms in it are dispersed. The gel is re-set into a flat layer and the bottle is incubated in the dark. The gel contains components which sustain the growth of viable microorganisms and the sample itself contributes additional nutrients. Viable microorganisms in the sample grow into visible spots known as "colonies", and a reactive compound changes the colour of these colonies to red or purple so that they can be easily seen.

The number of colonies formed is a direct estimate of the number of viable microbial particles (CFU) present in the sample. The actual number of colonies is counted or is estimated by reference to the Test Results Chart (page 9). The volume tested can be 0.01 ml (using a sterile loop dispenser) or between 0.1 ml and 0.5 ml (using a sterile syringe). The number of colonies formed is considered in relation to the volume of sample added to the test and expressed as CFU/litre of fuel, or CFU/ml of oil or water associated with fuel.

MicrobMonitor2 is available in packs of 5 and 50 tests, each containing everything that is required to perform the test:

- **Microb**Monitor2 test bottles
- Sterile syringes*
- Sterile loop dispensers*
- Bottle labels
- This instruction leaflet.

* The 50 pack is available with or without sterile syringes and loop dispensers.

MicrobMonitor2 has been developed and patented by ECHA Microbiology Ltd. and it is marketed worldwide by ECHA and its distributors.

WHAT IS THE MICROBMONITOR®2 TEST USED FOR?

The **Microb**Monitor2 is used to test for microbial contamination in fuel and oil products, and to monitor microbial growth in storage tanks and supply facilities. **Microb**Monitor2 can be used as a routine monitoring tool, for providing assurances about product quality, for investigation of incidents and for confirming effectiveness of measures taken to prevent or remediate microbial growth and contamination.

This instruction leaflet provides general instructions for using **Microb**Monitor2. Further technical instruction on the use of **Microb**Monitor2 in specific applications is provided in the technical application leaflets listed below (free to download from **www.echamicrobiology.com**). These leaflets include advice on sampling, sample preparation, interpretation of test results and appropriate actions when contamination is detected:

- EP096 *How to Interpret* **Microb**Monitor2 Test *Results in Accordance with IATA Guidelines for Aircraft Drain Samples.*
- EP119 *Routine Monitoring of Aviation Fuels in Supply and Distribution Facilities, Airport Depots and Into-plane Operations with* ***Microb****Monitor2.*
- EP132 *Routine Monitoring of Diesel Fuel Tanks and Distribution Systems with* ***Microb****Monitor2.*
- EP166 *Routine Monitoring of Marine Diesel on Ships and Offshore Installations with* ***Microb****Monitor2* **.**
- EP157 *Technical Assistance Reading Results of* ***Microb****Monitor2.*

PREPARATION FOR TESTING

Sampling considerations

Sampling should be conducted in a clean and consistent manner which prevents introduction of background contamination into the sample. Once fuel samples have been taken, any microbes present will tend to slowly die and it is important to test samples as soon as possible. If the test is carried out more than 24 h after sampling, it is possible that the results will not reflect the viable microbial content present at the time of sampling. To avoid this, part or all of the **Microb**Monitor2 test procedure can be conducted at the sampling location.

Sample type and recommended test volumes

MicrobMonitor2 can be used to test a wide variety of petroleum products and associated water. The volume of sample which should be tested will depend on the type of sample. Refer to the table below to determine the appropriate volume of sample to test and which measuring device to use.

Table 1

Sample	Recommended test volume	Measuring device
Aviation kerosene	0.5 ml	Syringe
Other middle distillate fuels (e.g. diesel, marine gas oil, heating oil), biofuels & gasoline	0.25 ml	
Water phase from aircraft fuel tank drains	0.1 ml	
Heavy and residual fuels	0.01 ml	Loop
Lubricating, hydraulic and other oils	0.01 ml	
Water phase from fuel storage tanks	0.01 ml	

The volume of sample tested can be changed in order to increase or decrease the detection level of the test. However, testing volumes greater than those recommended above may in some circumstances lead to underestimation of microbial numbers.

Determining whether to test fuel/oil or water phase

Depending on the system sampled and the sampling location, samples may contain fuel/oil and/or free water phase. **Microb**Monitor2 can be used to test either fuel/oil or associated water. Water phase will usually contain far more microorganisms than are present in fuel or oil phase and therefore a smaller volume is usually tested. Because free water phase may not always be recovered in samples, to ensure consistency when conducting regular microbiological monitoring, it is usually recommended that fuel or oil phase from above any free water is tested. However, in some cases it is recommended to additionally test water phase (e.g. samples from aircraft fuel tanks, and when investigating suspected microbial growth in facilities). Further information is provided in the relevant technical guidance documents listed on page 2.

To determine if the sample contains free water hold it up to the light and visually examine it. It may help to gently swirl the sample to create a vortex. In order to enable access to the sample with the measuring devices provided, it may be necessary to transfer some sample, after shaking, to a smaller clean container.

Preparing the MICROBMONITOR®2 test bottles

If the **Microb**Monitor2 test bottles have been stored refrigerated, allow them to equilibrate to ambient temperature before they are used. Remove the seal from the cap of the bottle before using each **Microb**Monitor2 test. Avoid prolonged exposure of the **Microb**Monitor2 test bottles to direct sunlight or other bright light during preparation, incubation and examination of tests.

Cleanliness during testing

MicrobMonitor2 tests can be conducted on-site, in an office or in a laboratory. However, precautions should be taken to ensure testing is conducted in a reasonably clean area to avoid contamination of the sample and the test by microorganisms other than those in the test sample. Wash hands before and after testing. During testing wear clean, nitrile, vinyl or polythene gloves. Avoid touching areas of the syringe and loop dispenser which come into direct contact with the sample and avoid touching the inside of the **Microb**Monitor2 test bottle cap and neck when dispensing sample.

TEST PROCEDURE

A. Add sample to the MICROBMONITOR®2 test bottle.

Follow the appropriate steps below to add distillate fuel or biofuel (A.1), heavy/residual fuel or oil (A.2), water (A.3) or surfaces deposits (A.4) to the **Microb**Monitor2 test bottle.

A.1 ***Aviation kerosene, other middle distillate fuels and gasoline.***

A.1.1 Immediately prior to testing, shake the sample for 30 seconds. If fuel samples contain visible free water, the sample should be allowed to stand for 12 minutes to enable water to settle from the sample to be tested. For consistency, it may be advisable to apply this settling time for all fuel samples even if free water is not observed.

A.1.2 Remove the cap of the **Microb**Monitor2 test bottle taking care not to touch the inside and place it on a clean surface.

A.1.3 Open the peel pack containing a syringe at the handle end and remove the syringe taking care not to touch the lower barrel and nozzle.

A.1.4 Using the syringe, draw fuel from approximately 3 cm below the surface of the sample. If there is less than 6 cm depth of fuel phase, draw sample from approximately halfway down the fuel phase. Avoid the transfer of visible interfacial particulate, water droplets or emulsion in the aliquot to be tested.

A.1.5 Draw fuel into the syringe until it is more than half full and then, with the syringe nozzle uppermost, press the plunger to expel air. Expel surplus fuel until the end of the plunger aligns with the mark for the required volume of sample (see table 1).

A.1.6 Insert the tip of the syringe into the neck of the **Microb**Monitor2 test bottle and dispense the sample by fully depressing the syringe plunger.

A.1.7 Replace the cap on the **Microb**Monitor2 bottle and tighten. Record the sample details and test date on the label provided and stick it on a narrow side of the bottle. Proceed to step B.

A.2 ***Heavy and residual fuels and oils.***

A.2.1 Immediately prior to testing, shake the sample for 30 seconds.

A.2.2 Remove the cap of the **Microb**Monitor2 test bottle and place it on a clean surface.

A.2.3 Open the peel pack containing a loop dispenser and remove it taking care not to touch the loop end and the lower part of the handle.

A.2.4 Immerse the loop end into the fuel/oil to approximately 3 cm below the surface of the sample or, if there is less than 6 cm depth of fuel/oil phase, to halfway down the fuel/oil phase. Remove the loop from the sample and allow surplus fuel/oil to drain off; ensure a film of fuel/oil is trapped within the loop.

A.2.5 Insert the loop into the neck of the **Microb**Monitor2 test bottle and stab it into the gel and agitate it briefly to transfer the sample.

A.2.6 Replace the cap on the **Microb**Monitor2 bottle and tighten. Record the sample details and test date on the label provided and stick it on a narrow side of the bottle. Proceed to step B.

A.3 ***Water phase associated with fuel/oil.***

A.3.1 If a test of water phase is required, allow the sample to stand until free water has settled to the bottom of the sample. It is recommended the water phase test is conducted after any fuel/oil phase test. The test procedure is essentially the same as described above but an appropriate technique for separating/removing water from the sample needs to be used.

A.3.2 As appropriate for the volume of sample being tested (see table 1) use the loop dispenser or syringe to measure the required volume of water.

a) To test 0.1 ml of water using the syringe;

The water phase can be drawn directly from the bottom of the sample and added to the **Microb**Monitor2 test bottle. To enable easy access to the water it may be necessary to first decant off some fuel from the sample and/or transfer the water phase to a separate small, sterile container using a syringe or a long sterile pipette (supplied separately). Mix gently by swirling but avoid mixing the fuel and water layers together.

Open the peel pack containing a syringe at the handle end and remove the syringe taking care not to touch the lower barrel and nozzle. Insert the barrel of the syringe into the sample so that the nozzle is in the water phase. Draw water into the syringe, remove from the sample and then, with the syringe nozzle uppermost, expel air. Expel surplus water until the end of the plunger aligns with the 0.1 ml mark. Insert the tip of the syringe into the neck of the **Microb**Monitor2 test bottle and dispense the sample by fully depressing the syringe plunger. Replace the cap on the **Microb**Monitor2 bottle and tighten. Record the sample details and test date on the label provided and stick it on a narrow side of the bottle. Proceed to step B.

b) To test 0.01 ml of water using the loop dispenser;

A syringe can be used to draw water from the bottom of the sample and then a drop of water placed into the loop and added to the **Microb**Monitor2 test bottle. Alternatively, transfer the water phase to a separate small, sterile container using a syringe or a long sterile pipette (supplied separately). Avoid transfer of fuel. Invert the container with the separated water three times to mix.

Remove the cap of the **Microb**Monitor2 test bottle and place it on a clean surface. Open the peel pack containing a loop dispenser and remove it taking care not to touch the loop end and the lower part of the handle. Immerse the loop end into the separated water or fill the loop with a drop of water using a syringe. Allow surplus water to drain away but ensure the circle of the loop remains filled with water; note, if a residue of fuel is present this can impair the ability of the loop to fill with water. Insert the loop into the neck of the **Microb**Monitor2 test bottle and stab it into the gel and agitate it briefly to transfer the sample. Replace the cap on the **Microb**Monitor2 bottle and tighten. Record the sample details and test date on the label provided and stick it on a narrow side of the bottle. Proceed to step B.

Note The **Microb**Monitor Sampling Kit (supplied separately) is a sample bottle which has a side siphon and water collection chamber to enable easy separation of water from fuel samples.

A.4 Testing surfaces.

A.4.1 **Microb**Monitor2 can be used to test for microbial contamination on surfaces, e.g. to test deposits on tank surfaces or filters. Sterile swabs (available separately) should be used to remove surface contamination and transfer this to a **Microb**Monitor2 test bottle.

A.4.2 Open the peel pack containing a swab at the handle end and remove the swab, taking care not to touch the cotton end of the swab and the handle near the cotton end.

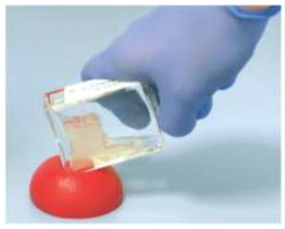
A.4.3 Rub the surface to be tested using the cotton end of the swab, rotating the swab handle so that all parts of the cotton end contact the surface. If possible swab a known area of surface.

A.4.4 Insert the cotton end of the swab into the neck of the **Microb**Monitor2 test bottle and stab it into the gel and agitate for about 15 seconds and then remove and discard.

A.4.5 Replace the cap on the **Microb**Monitor2 bottle and tighten. Record the sample details and test date on the label provided and stick it on a narrow side of the bottle. Proceed to step B.

B Shake to disperse sample in the **MICROB**MONITOR®2 gel.

B.1 Loosen and break up the gel in the **Microb**Monitor2 bottle containing the dispensed sample, by tapping the bottle firmly in the palm of your hand or on a rubber bung or **Microb**Monitor "Bumper" (available separately).



B.2 Shake the bottle vigorously for approximately 30 seconds to liquefy the gel and disperse the sample. The test bottle can be tapped repeatedly for 30 seconds on a **Microb**Monitor "Bumper" to assist with shaking. After shaking, the gel should be slightly viscous but free of lumps and have a uniform consistency and clarity. The presence of bubbles in the gel is normal and will not affect the test.

B.3 Stop shaking the test bottle abruptly so that the gel collects in the bottom of the bottle. Proceed immediately to B4.

B.4 Tap the bottle in the palm of the hand until the gel forms a flat layer on one of the larger flat sides. Ensure that a uniform layer reaching all corners is obtained.

Notes on transport of tests

It is preferable to conduct testing on-site to avoid errors due to changes in the microbial content of samples during transport. If there are likely to be delays of more than 24 hours in transporting samples to a testing facility, these errors can be avoided by conducting the first stages of the **Microb**Monitor2 test procedure (up to and including step B) on-site at the sampling location and then returning the **Microb**Monitor2 test to the testing facility to complete the incubation (step C). The test bottle should be kept flat during transport, should not be agitated excessively and should be returned to the testing facility within 4 days. See note on incubation of tests (step C) concerning appropriate adjustment of the incubation time.

Alternatively, **Microb**Monitor2 test bottles can be transported to the test facility after sample has been added (step A) before shaking; the test can then be shaken (step B) at the test facility. Incubation (step C) should be commenced within 6 hours of the sample being added to the test bottle (or within 2 days if tests are kept cool (2 to 8°C)). If tests are transported to a test facility before shaking, it is not necessary to keep them flat during transport and moderate agitation will not affect the test result.

C Incubate the MICROBMONITOR®2 test.

Transfer the **Microb**Monitor2 test bottle to a warm, dark location or incubator to maintain a nominal temperature of 25°C ± 3°C. In normal circumstances the gel should be incubated for 4 days. Avoid exposure to light during incubation.

The gel will set firmly after a few hours. Keep the gel on the lower surface of the bottle and avoid excessive agitation or prolonged tilting of the bottle during incubation, examination and any transportation.

Notes on incubation of tests.

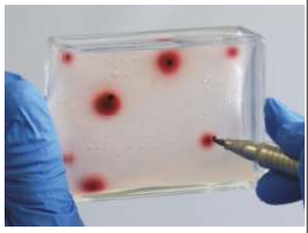
Occasional temperature fluctuations (e.g. overnight) below the specified temperature range should not affect the number of colonies which develop and will not critically affect the test result, but microbial colonies may take longer to become visible and thus an extension of the specified incubation time should be applied. If the temperature falls below the specified range during incubation, the incubation time should be extended by a time equivalent to the total time the temperature is estimated to have fallen below the specified range. If the incubation temperature falls below the specified range for a period or periods totaling 4 days or more, microbial contamination will be underestimated and the result should be considered invalid. The temperature should not be allowed to drop below 4°C during incubation.

Incubation of the culture medium above the specified temperature range may prejudice the ability to detect some types of microorganism. However, where microbial contamination occurs in tanks or systems where the contents have a higher temperature than 25°C, the contaminating microorganisms will often have a preference for growth at higher temperatures; in such cases it may be appropriate to incubate the test at a temperature similar to the system sampled.

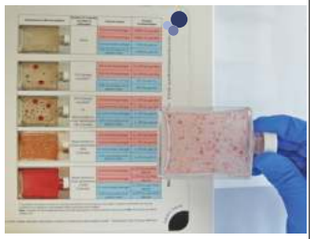
When using **Microb**Monitor2 to test for compliance with a specific industry standard (e.g. the IATA Contamination Limits shown in table 2 on page 8), if the test result indicates contamination is in the highest category (e.g. Heavy) before 4 days, it is generally acceptable to read and report the result without further incubation.

D Examine the MICROBMONITOR®2 test.

D.1 If possible examine the test daily during incubation. As a minimum, examine it on at least one occasion in the first 3 days and again on the final day of incubation. To examine the test, hold it against a light background and count the number of all visible purple colonies. A hand magnifying lens may help you to identify and count small colonies. All purple colonies in all parts of the test bottle should be counted, including any which are in gel which is not part of the flat layer. Once a colony is counted do not count it again even if it grows larger; it is the number of colonies that is important, not their size. It is recommended that colonies are marked with a marker pen on the bottle to ensure that they are not counted twice. Ignore any air bubbles which may form in the gel.



D.2 It is usually possible to count up to about 250 colonies. If the number of colonies is too numerous to count, visually compare the test to the Test Results Chart (see page 9) holding the test against a white background. The chart provides an estimate of the colony count.



Notes on examining tests

Colonies are usually circular but may have irregular edges. Different types of microorganisms can grow at different rates in the gel culture medium and therefore the colonies may be of different sizes. At the recommended incubation temperature, colonies of bacteria and yeasts usually develop within 1 to 2 days and remain quite small. Moulds develop more slowly but eventually produce large colonies which may have a powdery or "fuzzy" appearance. Generally, the more colonies there are in the test bottle, the smaller the colonies will be. Ignore any microbial colonies which develop after the specified incubation period is complete (allowing for any extension applied due to temperature falling below the specified range). The test may change appearance on prolonged incubation but this should be disregarded. Colonies will tend to become visible more quickly in samples with a higher viable microbial content.

Occasionally, some antioxidant additives in fuels may interfere with the growth indicating compound in the test and produce a uniform light peach or orange colour in the gel (usually within 12 hours). This colour change will not interfere with the growth of any microorganisms and in most cases microbial colonies can be counted or estimated ignoring the background colour. In exceptional cases the interference may be so strong that users may find it difficult to distinguish colour interference from an estimated count of 10,000 colonies. In such cases the fuel should be retested using a smaller test volume (e.g. 0.01 ml) so that the interference effect is diluted out; if the original result was genuinely due to heavy microbial contamination it would normally be expected that the retest will show a discernible number of red/purple colonies and the background colour will be less intense.

Some bacteria can, on prolonged incubation, spread through the gel producing a large irregularly shaped colony, streak or patch of red or purple colour in the gel. These bacteria usually grow quickly and thus if tests are examined while the colonies are still small (e.g. after 1 or 2 days incubation) they are more easily counted. The centre of each streak or patch should be counted as a single colony. Continue incubation and count any new colonies which develop.

See technical leaflet EP157 *Technical Assistance Reading Results of* ***Microb****Monitor2* for further information on reading and interpreting tests with unusual or atypical appearance.

E Calculate the number of microbial CFU in the sample.

- For tests of fuel, numbers of microbes are conventionally expressed as number of CFU per **litre**.
- For tests of water phase or oil, numbers of microbes are conventionally expressed as number of CFU per **ml**.

a) Results of test of fuel phase sample:

If 0.5 ml of fuel has been tested, multiply the number of colonies counted or estimated by 2000 to give the number of microbial CFU per litre of fuel. If there are no colonies after the specified incubation time there are less than 2000 microbial CFU per litre of fuel.

If 0.25 ml of fuel has been tested, multiply the number of colonies counted or estimated by 4000 to give the number of microbial CFU per litre of fuel. If there are no colonies after the specified incubation time there are less than 4000 microbial CFU per litre of fuel.

If a different volume of fuel has been tested, then a calculation can be made as follows:

$$\text{Number of microbial CFU per litre} = \frac{\text{Number of colonies counted or estimated} \times 1000}{\text{Volume of fuel tested (ml)}}$$

b) Results of test of water phase or oil:

If 0.1 ml of water has been tested, multiply the number of colonies counted or estimated by 10 to give the number of microbial CFU per ml of water. If there are no colonies after the specified incubation time there are less than 10 microbial CFU per ml of water.

If 0.01 ml of water or oil has been tested, multiply the number of colonies counted or estimated by 100 to give the number of microbial CFU per ml of water or oil. If there are no colonies after the specified incubation time there are less than 100 microbial CFU per ml of water or oil.

If a different volume of water or oil has been tested, then a calculation can be made as follows:

$$\text{Number of microbial CFU per ml} = \frac{\text{Number of colonies counted or estimated}}{\text{Volume of water or oil tested (ml)}}$$

Note

If the number of colonies is too numerous to count and a sample volume of 0.5 ml or 0.25 ml of fuel or 0.1 ml or 0.01 ml of water or oil was tested, the Test Results Chart can be used directly to determine the approximate number of microbial CFU per litre of fuel or per ml of water or oil.

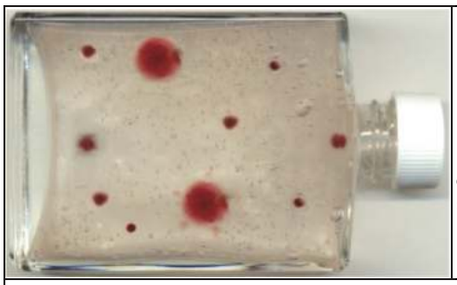
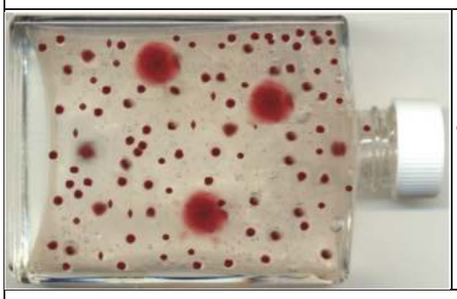
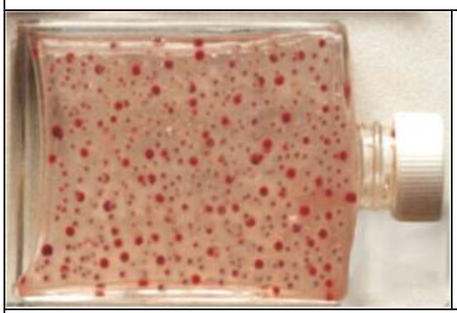
INTERPRETATION OF TEST RESULTS

There are no universally accepted limits or specifications for microbiological contamination in fuels or oils. The number of microbial CFU which define moderate and heavy contamination levels will depend on a number of factors including the fuel type and intended use, the sampling location, whether the sample is taken from supply and distribution facilities or from point of end use, the intended fuel storage time and specific operational circumstances. Some guidance limits are shown in Table 2 below. These limits are intended to provide early indication that microbial growth is occurring in the facility sampled. Higher levels of contamination will often be required before the onset of operational problems or any detrimental effect on fuel fitness for use. For further information see the technical guidance leaflets listed on page 2.

Table 2

Sample	Moderate	Heavy	Comment
Fuel from aircraft fuel tank drain	4000 to 20,000 CFU / litre	>20,000 CFU / litre	IATA Contamination Limits
Water from aircraft fuel tank drain	1000 to 10,000 CFU / ml	>10,000 CFU / ml	
Bulk representative fuel sample from supply & distribution	4000 to 20,000 CFU / litre	>20,000 CFU / ml	e.g. Aviation fuel, diesel, marine gas oil
Fuel from bottom / drain of supply & distribution tank / filter	10,000 to 100,000 CFU / litre	>100,000 CFU / litre	
Water phase from bottom / drain of supply & distribution fuel tank / filter	100,000 to 1,000,000 CFU / ml	>1,000,000 CFU / ml	
Lubricating / hydraulic oil in circulation	100 to 10,000 CFU / ml	>10,000 CFU / ml	
Lubricating / hydraulic oil sump	1000 to 10,000 CFU / ml	>10,000 CFU / ml	

MICROBMONITOR®**2** Test Results Chart

Appearance after incubation	Number of colonies in test bottle (counted or estimated)	Volume tested	Microbial Content of Sample
	None	0.5 ml of fuel (syringe)	<2000 CFU per litre
		0.25 ml of fuel (syringe)	<4000 CFU per litre
		0.1 ml of water (syringe)	<10 CFU per ml
		0.01 ml of water or oil (loop dispenser)	<100 CFU per ml
	10 Colonies If possible, count the exact number of colonies and calculate the actual number of CFU present in the sample	0.5 ml of fuel (syringe)	2 x 10^4 CFU per litre
		0.25 ml of fuel (syringe)	4 x 10^4 CFU per litre
		0.1 ml of water (syringe)	100 CFU per ml
		0.01 ml of water or oil (loop dispenser)	1000 CFU per ml
	100 Colonies If possible, count the exact number of colonies and calculate the actual number of CFU present in the sample or Estimate 100 colonies if result is similar to picture	0.5 ml of fuel (syringe)	c. 10^5 CFU per litre
		0.25 ml of fuel (syringe)	c. 10^5 CFU per litre
		0.1 ml of water (syringe)	c. 10^3 CFU per ml
		0.01 ml of water or oil (loop dispenser)	c. 10^4 CFU per ml
	Estimate 1000 colonies if result is similar to picture	0.5 ml of fuel (syringe)	c. 10^6 CFU per litre
		0.25 ml of fuel (syringe)	c. 10^6 CFU per litre
		0.1 ml of water (syringe)	c.10^4 CFU per ml
		0.01 ml of water or oil (loop dispenser)	c.10^5 CFU per ml
	Estimate 10,000 or more colonies if result similar to picture	0.5 ml of fuel (syringe)	c.10^7 CFU per litre or above
		0.25 ml of fuel (syringe)	c.10^7 CFU per litre or above
		0.1 ml of water (syringe)	c.10^5 CFU per ml or above
		0.01 ml of water or oil (loop dispenser)	c.10^6 CFU per ml or above

DISPOSAL

Wear gloves and avoid touching the inside of the bottle or cap and wash hands after handling any **Microb**Monitor2 test bottles showing microbial growth. Before disposal, disinfect all **Microb**Monitor2 test bottles which show growth by immersion of the opened bottles in a strong disinfectant solution (e.g. household bleach) overnight or by incineration or by using chlorine release tablets (available separately).

Decontaminated tests, unused tests or those showing no microbial growth can be disposed of as normal waste in accordance with local waste regulations.

STORAGE AND SHELF LIFE

Store **Microb**Monitor2 tests between 2 to 22°C, in the dark. Do not expose to strong light during storage or use. Do not store **Microb**Monitor2 frozen. Temporary exposure to freezing temperatures during transport will not adversely affect the product. A slight pink discoloration in the **Microb**Monitor2 gel may develop over time during storage but this will not affect the performance of the test.

Expiry dates are printed on the product batch label. An extended expiry date applies to unopened product stored between (2 - 8°C). Expiry dates apply strictly to product stored as stipulated.

ALSO AVAILABLE FROM ECHA

For use with MICROBMONITOR®2

The following items can assist in performing the **Microb**Monitor2 test procedure:

- **Microb**Monitor Sampling Kit (ECHA16/SB) - A sterile, polypropylene bottle for sampling fuel systems and which enables easy separation of water phase from fuel phase; includes alcohol wipes for decontaminating sample points.
- Incubator (ECHA14/IN) - enables optimal, consistent incubation temperature of **Microb**Monitor2 tests; holds 12 **Microb**Monitor2 test bottles; available battery or mains (110/230 Volt) operated.
- **Microb**Monitor Bumpers (ECHA16/TB) - soft, rubber-like, hemispheres which can be stuck to a table top and enable easy breaking and shaking of **Microb**Monitor2 gel.
- Chlorine Release tablets (ECHA21/CP) - to disinfect **MicrobM**onitor2 test bottles showing microbial growth for easy disposal.
- Sterile swabs (ECHA15/SW) - swabs for sampling surface contamination (e.g. tank surfaces, filter cartridges etc.).
- Long, sterile bulb pipettes (ECHA07/PP/EL23) - to enable water to be removed from the bottom of samples.

Other Test Kits & Ancillary Items

- SigTests®: Sig Sulphide (ECHA02/SS) — simple test kit for Sulphate Reducing Bacteria (SRB) which can cause serious corrosion and sulphide spoilage in fuel, oil and water systems. Can be used on-site or in the laboratory.
- Biocide Rapide (ECHA01/BR) — simple rapid test kit for assessing presence and approximate concentration of commonly used fuel and oil biocides.
- Any level and bottom sampling devices (ECHA23) — for safe sampling from fuel and oil storage tanks in compliance with industry standards.
- For our full range of test kits and ancillaries see www.echamicrobiology.com.

Technical support and services

ECHA Microbiology Ltd. provides a full technical service to support our product range. ECHA also offers laboratory testing, training courses, consultancy and on-site attendance for audit and investigation of microbial contamination and corrosion associated with petroleum products and petroleum, marine, aviation and other industrial facilities.

Sample & test details		**MICROB**MONITOR®**2** test results					
		Colonies counted Day 1	Colonies counted Day 2	Colonies Counted Day 3	Colonies Counted Day 4	Colonies Counted Day ______	Microbial Contamination in sample CFU/ litre or CFU/ ml
Sample Ref.							
Date tested							
Type							
Location							
Volume tested (ml)	0.5/0.25/0.1/0.01/other__						
Incubation Temp. (°C)							
Sample Ref.							
Date tested							
Type							
Location							
Volume tested (ml)	0.5/0.25/0.1/0.01/other__						
Incubation Temp. (°C)							
Sample Ref.							
Date tested							
Type							
Location							
Volume tested (ml)	0.5/0.25/0.1/0.01/other__						
Incubation Temp. (°C)							
Sample Ref.							
Date tested							
Type							
Location							
Volume tested (ml)	0.5/0.25/0.1/0.01/other__						
Incubation Temp. (°C)							
Sample Ref.							
Date tested							
Type							
Location							
Volume tested (ml)	0.5/0.25/0.1/0.01/other__						
Incubation Temp. (°C)							