Routine Monitoring of Marine Distillate Fuels on Ships and Offshore Installations with **Microb**Monitor²

Technical Guidance

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BACKGROUND

MicrobMonitor2 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).*

Microbial growth may occur wherever any water accumulates in diesel and gas oil fuel tanks and systems. Only very small quantities of water are required; films of water less than 1mm thick are sufficient to support microbial growth. It is very difficult to keep fuel tanks completely free of water in the normal marine operating environment. Water can enter through tank vents with humid air. This water and also water dissolved in fuel will condense out when it contacts cold tank surfaces (tank sweating), particularly where the vessel hull forms part of the tank. Water may also be introduced during bunkering, if the fuel supplier does not have adequate fuel handling and quality control procedures. Fuel bunkered from barges is particularly prone to water contamination.

When the presence of water allows heavy microbial growth to occur, fuel quality can be affected. Microbial "biomass" becomes suspended in the fuel and can then plug fuel filters, block fuel lines and foul fuel injectors. Microbial growth can also cause pitting corrosion of fuel tanks and fuel system components. There is a possibility for serious operational problems which can impact on the safe and reliable operation of the vessel or platform. Modern diesel fuels supplied for marine use may be particularly susceptible to microbial growth due to the presence of biodiesel (Fatty Acid Methyl Esters or FAME); some specifications allow blending of biodiesel at up to 7% but even fuels which do not intentionally contain FAME may have trace levels. For example the current ISO 8217:2010 DMA specification allows a *de minimis* FAME concentration of 0.1%. There is also some evidence that low sulphur fuels may be more susceptible to microbial growth.

Regular removal of water from tank sump drains is an important procedure in preventing microbial growth. However, whilst this is advisable for Settling and Service Tanks it is not possible for ship's bunker tanks, such as double bottom tanks. Effective use of on-board fuel purifiers can assist in ensuring that any contamination and water is removed from fuel as it is transferred from bunker / storage tanks. Because, these measures cannot keep fuel tanks completely free of water and microbial growth, microbiological monitoring is recommended to ensure systems remain free of contamination. Microbiological monitoring is particularly recommended where there is a known risk that fuel bunkered may contain FAME. Microbiological monitoring can be easily accomplished with simple on-board tests like the **Microb**Monitor2 test. When test results indicate significant contamination is present then preventative and remedial measures can be implemented, such as additional filtration or purification of fuel, biocide treatment or in severe cases tank cleaning. This Technical Guidance document provides information on using the **Microb**Monitor2 test kit to monitor for microbial contamination in diesel and gas oil fuel tanks on ships and offshore installations;

- When, where and how to take samples
- How to conduct the test for marine fuel samples and associated water
- How to interpret test results
- Appropriate actions when contamination is detected.

SAMPLING - WHEN AND WHERE

Usually, most microbial contamination will be present in tank bottoms and system low points, particularly in any free water and at the fuel water interface; growth will normally be detected here first before it spreads into the fuel and affects bulk fuel quality. Therefore for routine monitoring, it is best to test low point, dead bottom or drain line samples as these will provide the earliest and most consistent indication of contamination. If fuel has recently been delivered into the tank allow the tank contents to settle before sampling.

For routine monitoring, the frequency of sampling and testing should be based on the perceived risk and/or any previous experience of microbial growth problems.

Consider a fuel tank to have a "**high risk**" of microbial growth if heavy microbial contamination has been detected on more than one occasion and/or significant microbial growth has been observed during internal inspection or if there have been incidents of filter plugging, pitting corrosion or other operational problems related to microbial contamination. We recommend tanks at high risk should be tested monthly.

Consider a fuel tank to have a "**moderate risk**" of microbial growth if there has been a single incident of heavy microbial contamination detected in the previous 2 years and/or if the facility operates under conditions which may be conducive to microbial growth (e.g. facilities in hot, humid environments, facilities where water or dirt is known to ingress or accumulate in tanks) or there is a history of microbial contamination in the bunkers supplied. We recommend tanks at moderate risk should be tested every 3 - 6 months.

Fuels tanks on vessels, platforms or other facilities which do not operate under conditions specifically conducive to microbial growth can be considered "**low risk**" if no samples have shown heavy contamination and there have been no other indications of microbial growth in the previous 2 years. Some limited sampling and testing of these facilities (e.g. annually) might be advisable.

If heavy contamination is detected or suspected in tank drain / dead bottom samples it may be appropriate to test additional samples to investigate quality of fuel (e.g. fuel lines to and from day / service tanks and retained bunker samples. (N.B. tests of retained samples may underestimate contamination if samples are old).

HOW TO SAMPLE

It is important to be consistent in the procedure used for sampling and testing. Appropriate sampling procedures are described in the Energy Institute *Guidelines for the investigation of the microbial content of petroleum fuels and for the implementation of avoidance and remedial strategies* (Energy Institute, London) and ASTM D 7464 *Standard Practice for Manual Sampling of Liquid Fuels, Associated Material, and Fuel System Components for Microbiological Testing* (ASTM International, PA, USA).

Sampling equipment and sampling valves should be clean and, if possible, decontaminated by rinsing or wiping with a 70% alcohol solution (ensure all residues of alcohol evaporate before taking the sample or it will affect the test result). Particular attention should be paid to the cleanliness of any containers or tools used to collect samples and to rubber or plastic hoses attached to the ends of sampling points as these can harbour dirt and microbial growth (and consequently cause false positive test results). Ideally, sterile sampling containers should be used but in practice it is usually sufficient to use new, clean (NOT previously used) containers. The **Microb**Monitor Sampling kit, available from ECHA Microbiology includes a special sterile 1 litre sample bottle and also an alcohol wipe for cleaning sample points and equipment. It is a good idea to rinse sampling equipment (e.g. bottom samplers, bacon bombs and all level samplers) with fuel from the tank or system to be sampled before taking the sample for test.

When sampling from drain lines, take the sample as soon as the contents of the drain line have been flushed away (i.e. sample the first product/water to come from the tank or filter vessel).

To improve the representation of the system sampled it is preferable to take samples of about 1 litre in volume; this will enable easier visual observation of the sample for water, dirt, particulates and suspected microbial growth. However, only a very small volume of sample is actually required for the **Microb**Monitor2 test.

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 samples are to be returned to a laboratory or other facility for testing then ideally the test should be conducted within 48 hours. Samples will give increasingly less reliable results as they get older. Alternatively, part or all of the **Microb**Monitor2 test procedure can be conducted at the sampling location as described below (see **Testing on-site**).

TESTING

For full details on the procedure for conducting a **Microb**Monitor2 test refer to the Instruction for use provided with the test. A quick reference guide to the test procedure for diesel and gas oil samples is shown on page 5 of this leaflet. An aliquot of 0.25 ml of fuel or 0.01 ml of water from the sample is added to the gel in the **Microb**Monitor2 test bottle; the bottle is shaken to disperse the sample and the gel is then tapped into a flat layer. The bottle is incubated (usually for 4 days at 25°C) and then the number of microbial colonies which grow is counted or alternatively estimated using a reference chart (see page 7).

The **Microb**Monitor2 test can be used to test fuel phase and/or water phase in samples. However, because water phase may not always be recovered in samples, for the purposes of consistency in routine monitoring, we recommend that fuel phase from above any water phase or interface present is always tested, after allowing water to settle out. In some circumstances, for example when investigating contamination sources or assessing the extent of microbial growth in a tank or system, it may also be informative to assess the microbial content in any free water present in the sample. Water phase will usually contain substantially higher numbers of microbial cfu than fuel phase and a different interpretation of results is required.

Testing Fuel: Shake the sample by hand for approximately 30 seconds and then allow it to stand for 12 min ± 1 min. If the depth of the fuel phase in the sample is less than 6 cm then allow a settling time of 2 min per cm. Using the syringe provided, draw 0.25 ml of fuel from approximately 3 cm below the surface of the fuel phase of the sample and transfer to the **Microb**Monitor2 and complete the test as described in the Instructions for Use. If there is less than 6 cm depth of fuel, draw sample from approximately halfway down the fuel phase. The transfer of visible interfacial particulate, water droplets or emulsion in the aliquot to be tested shall be avoided. If the sample is not in a container that enables the use of the syringe to remove an aliquot for test from 3 cm below the surface of the fuel then it should be transferred to a suitable, sterile container, avoiding transfer of visible interfacial particulate, water droplets or emulsion.

Testing Associated Water: If an assessment of microbial content of free water phase is required, after completion of the fuel phase analysis, allow the sample to stand until water phase has settled to the bottom of the sample. To enable ready access to water phase, it may be necessary to first decant off some fuel phase from the sample. Use the syringe to remove water from the bottom of the sample and transfer to a separate small, sterile container. Avoid transferring any of the fuel with the water. Once water has been removed from any fuel, invert the container containing the water three times to homogenise the water prior to sampling. Immediately after inverting the container transfer 0.01ml water to the M**icrob**Monitor2 test bottle using the sterile "loop" provided.

Note on volume of fuel tested for various fuel types

For most marine distillate fuels we recommend the volume of fuel tested is 0.25 ml. This test volume gives an appropriate detection level. However, if it is desired to make the test more sensitive, 0.5 ml can be tested providing the fuel sample does not produce an unacceptable level of background colour interference (usually a uniform orange or pale pink colour). The performance of the test using 0.5 ml has only been validated for NATO specification F-76 fuel and aviation fuels. Some fuels (particularly those containing FAME), can cause a colour interference in the **Microb**Monitor2 test gel which makes the results more difficult to read if a volume larger than 0.25 ml is tested.

The **Microb**Monitor2 test can also be used to test heavy and residual marine fuels. For this application it is recommended 0.01 ml is tested using the loop dispenser provided with the test kit. Although, heavy and residual marine fuels may be heavily contaminated with microorganisms, microbial growth generally has less effect on their fitness for use. The interpretation chart on page 7 is not applicable for heavy and residual fuels (contact ECHA Microbiology for advice on interpretation). It may be advisable to test samples from heavy and residual fuel tanks for Sulphate Reducing Bacteria (SRB) (see other useful tests below) as these fuels are particularly susceptible to SRB growth.

Testing on site

To avoid delays in testing, the first stages of the **Microb**Monitor2 test procedure can be conducted on-site at the sampling location in one of two ways;

- Add 0.25 ml of fuel sample (or 0.01 ml water) to the **Microb**Monitor2 test bottle on-site. Then return the test bottle to a laboratory or other suitable facility and complete the remaining part of the test procedure (shake the gel, tap into a flat layer then incubate). If using this procedure, it does not matter whether the test bottles are agitated during transport and it is not necessary to keep them flat. However, the test should be returned to the test facility and shaken and incubated within 6 hours of the sample being added to the test bottle. Transport time can be extended if tests are kept cool (2 to 8°C).

OR

- Add 0.25 ml of fuel sample (or 0.01 ml water) to the **Microb**Monitor2 test bottle and then shake and make the gel form a flat layer, as per test instructions. Then return the test bottle to a laboratory or other suitable facility for incubation. If using this procedure, the test bottle should be kept flat during transport and should not be agitated excessively. Providing the gel is not agitated, it does not matter how long it takes to return the test bottle to the incubation facility. However, if the temperature during transportation is lower than the recommended incubation temperature (25°C) then it may take longer for colonies to develop and the incubation time should therefore be extended by a time equivalent to the transportation time.

Other useful tests

Visual examination of drain line and bottom samples can provide indication that heavy microbial growth is occurring. Presence of discoloured water (brown or black), a translucent slimy layer at the interface between the fuel and water layers or soft, grey, brown sludge or debris in the fuel or water layer are all indications of likely microbiological activity. Soft brown or black sludge or spotting in tanks or a thin, translucent, slimy coating on tank surfaces also indicates microbial growth.

Another useful and simple test is to measure the pH of any water in tanks using indicator papers. Significant acidity (e.g. pH 5 or lower) is a possible indicator of microbial activity.

If there is perceived to be a risk of Microbially Influenced Corrosion in the facility, then an occasional test for Sulphate Reducing Bacteria (SRB) in water in tanks is advised. Use the **Microb**Monitor **Sig Sulphide Test** (contact ECHA Microbiology for details).

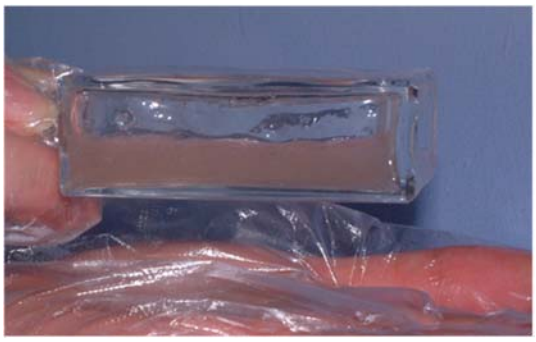
How to Test **Marine Distillate** Fuel with **Microb**Monitor²

1. Break and discard the plastic seal. Remove the bottle cap and place on a clean surface with the inside face upwards. Use the syringe to add 0.25 ml fuel to the M**icrob**Monitor2 bottle (or 0.01 ml water using the sterile loop). Re-cap and label the bottle.

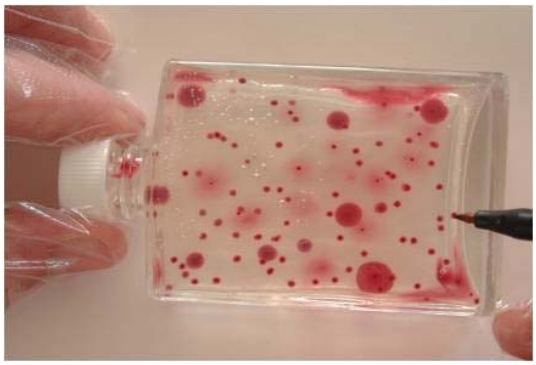
2. Tap bottle to break up gel.

3. Shake vigorously for 30 seconds.

4. Flick gel into bottom of test bottle.



5. Tap bottle to make a flat layer of gel. Allow to set. Keep bottle in a warm dark place at 25˚C for 4 days. Examine daily. Try not to disturb the gel.



6. To examine test, hold bottle against a light background and count all of the red / purple colonies, marking them off on the bottle with a felt tip pen. Re-incubate and examine if necessary for up to 4 days (see Notes). If numbers are high an estimate can be made by comparison to the chart provided. Record all results and sample information.

Disposal of tests showing microbial growth can best be achieved by incinerating, or by 24 hour disinfection using **two** of the chlorine release disinfectant tablets provided ("Aquatabs"). Tests should then be disposed of in accordance with local waste disposal regulations.

Notes

Ignore any uniform pale pink/peach colouration of the gel which can be caused by fuel additives such as anti-oxidants.

Ignore air bubbles which may form in the gel.

Try not to disturb the gel during incubation and always leave bottle flat to prevent the gel sliding.

If incubation is only possible at lower temperatures, the results will take longer to appear.

Storage: Shelf life is 12 months from production date when stored between 2 to 22°C in the dark, but this may be prolonged by refrigeration (2 to 8°C). Do not store frozen. Avoid exposure of tests to light during storage and use.

INTERPRETATION OF TEST RESULTS

A general interpretation chart is provided in **Microb**Monitor2 test kit instructions but the chart on the page 7 can be used to specifically interpret test results of samples of marine distillate fuel from Storage, Supply & Distribution Facilities and End User Tanks. According to test results, this chart defines contamination as Acceptable, Moderate or Heavy for each of two categories of sample;

- **Category A**. Bulk fuel samples; e.g. suction level or other layer samples of fuel from storage tanks, representative bunkering samples (e.g. drip sample), samples from manifolds, samples from fuel lines delivering to engines.

- **Category B**. Drain, bottom or low point samples; e.g. samples from storage tanks and filters in supply & distribution facilities, drain samples from end user settling and service tanks. This category is appropriate for drain/bottom samples taken for routine monitoring of tanks.

Levels of microbial contamination in water phase will usually be much higher than in fuel phase which is why separate guidance is given for water phase and fuel phase in samples from drain lines or tank bottoms (Category B). In accordance with industry convention, water phase results are expressed per millilitre whilst fuel phase results are expressed per litre.

There are no universally accepted standards or specification limits for microbial contamination in marine fuel and the values shown are recommendations of ECHA Microbiology; they are generally consistent with other industry guidance specific to fuel storage and supply. All values are for guidance only and variation to these limits may be appropriate in consideration of sampling location, operating practice and experience and the perceived risk; in some cases more stringent standards may be appropriate for fuel in long term storage or for fuel used in gas turbines.

Bottom, drain and low point samples will indicate worst case and will not necessarily reflect the status of bulk fuel delivered from the tank. Heavy contamination in the tank bottom indicates a potential for contaminating bulk fuel; when fuel is received into a tank it is likely to disturb any contamination on the tank bottom into the bulk fuel. Contamination in bottoms of ship's day tanks and bunker tanks may also be disturbed into bulk fuel if the vessel operates in rough seas. Thus, heavy contamination in the tank bottom indicates a potential for operational problems such as plugging of filters, fuel lines and fuel system orifices.

Increasing trends of contamination may be as important as absolute limit values. It is recommended to retest a fresh sample if moderate or heavy contamination is detected, to confirm the result before taking corrective action. In some cases contamination can be transient and corrective action is not necessary but persistent indications of moderate or heavy contamination should instigate remedial measures (seek expert advice where appropriate).

Testing bulk fuel layer samples, tank suction level samples and representative bunker samples can provide indication of status of fuel delivered from the tank and provide assurances about fuel quality. However, it should be appreciated that results of tests of samples of bulk fuel and fuel at point of delivery will be applicable only to the time of sampling; microbial contamination in bulk fuel may be unevenly distributed and levels of contamination detected will vary with time. Contamination levels in bulk fuel in storage tanks will generally decrease with product settling and increase if tank bottoms are disturbed.

When testing retained bunker samples it is important to consider that if samples are several weeks or even months old, the test is likely to underestimate the extent of contamination present. Nevertheless, testing such samples may still be useful in some cases as it can highlight that unacceptable levels of contamination were present in the bunkered fuel; a negative result for an old sample would not, however, exclude the possibility that contamination was present in the original bunkering.

Numbers of cfu/litre cannot be used alone to indicate whether fuel is fit for purpose and consideration of other test data and operating circumstances will be appropriate. Where heavy contamination is indicated in bulk fuel, further investigation by a competent laboratory is recommended.

The diagram on page 8 provides a summary of a typical microbiological monitoring programme and recommended actions in response to various levels of contamination detected.

This leaflet is appropriate for samples of marine distillate fuel. Other technical leaflets are available at www.microbmonitor.com

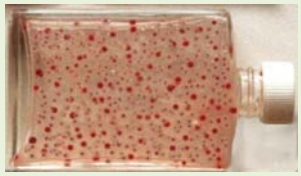
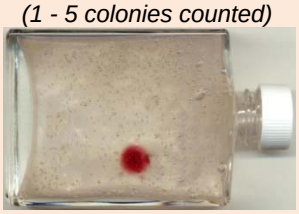
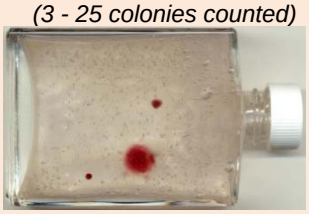
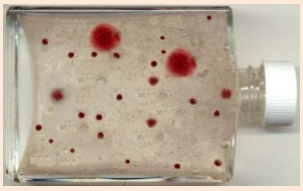
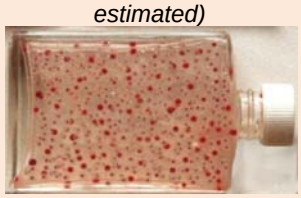
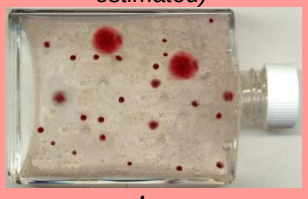
- For interpretation of results of tests of **aviation fuel samples from aircraft** please see our leaflet EP096 *How to Routine Monitoring of Aircraft Fuel Tanks with **Microb**Monitor² in Accordance with IATA Guidance.*

- For interpretation of results of tests of **aviation fuel distribution and supply systems** please see our leaflet EP119 *Routine Monitoring of Aviation Fuels in Supply and Distribution Facilities, Airport Depots and Into-plane Operations with **Microb**Monitor².*

- For interpretation of results of tests of samples from **diesel fuel storage tanks** please see our leaflet and EP132 *Routine Monitoring of Diesel Fuel Tanks and Distribution Systems with **Microb**Monitor².*

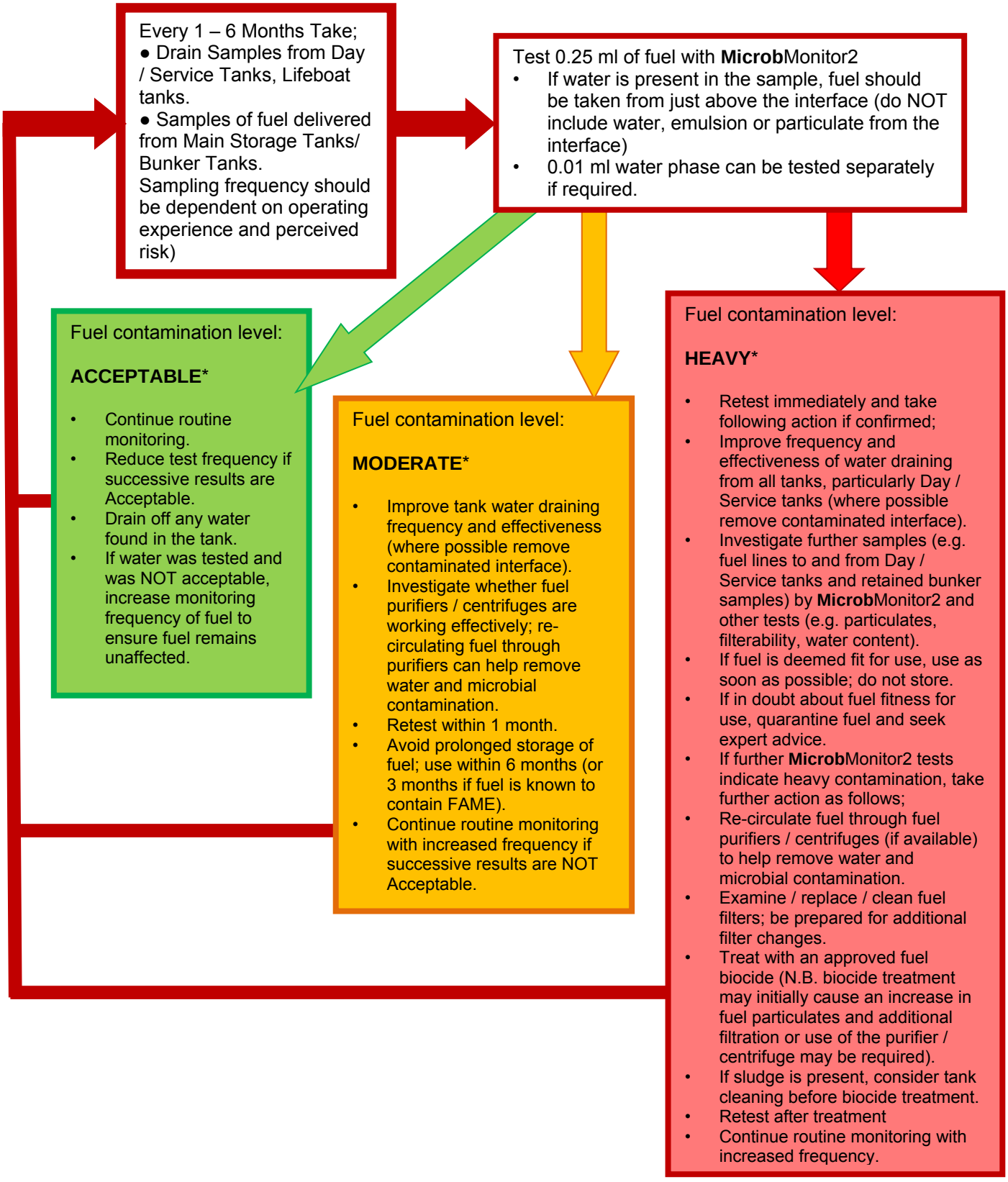
The advice in this Technical Guidance is offered in good faith and is based on our best technical interpretation of information available to us. However, the recommendations may not be applicable in all circumstances and there may be factors of which we are unaware which could influence the appropriateness and validity of the recommendations made. ECHA Microbiology Ltd. does not accept any liability for any decision or action taken as a consequence of the results obtained by MicrobMonitor2 or recommendations in this document. Please see the Instructions for Use for full conditions of use of MicrobMonitor².

How to Interpret **Microb**Monitor² Test Results For **Marine Distillate** Fuel

Interpretation	SAMPLE TYPE		
	Category A. • Representative fuel sample; e.g. bunker tank, bunkering line. • Critical tanks (e.g. lifeboat & emergency generator tanks) • Fuel in long term storage (0.25 ml tested)	**Category B.** Drains, bottom or low point samples; e.g. from storage tanks, service/day tanks and settling tanks.	
		Fuel phase (0.25 ml tested)	**Water phase** (0.01 ml tested)
Acceptable	**<4,000 cfu/litre** *(No colonies)* 	**< 10,000 cfu/litre** *(<3 colonies counted)*  **to** 	**<100,000 cfu/ml** *(<1000 colonies estimated)*  **to** 
Moderate	**4,000 – 20,000 cfu/litre** *(1 - 5 colonies counted)*  **to** 	**10,000 - 100,000 cfu/litre** *(3 - 25 colonies counted)*  **to** 	**100,000 - 1,000,000 cfu/ml** *(1000 – 10,000 colonies estimated)*  **to** 
Heavy	**>20,000 cfu/litre** *(>5 colonies counted or estimated)*  **to** 	**>100,000 cfu/litre** *(>25 colonies counted or estimated)*  **to** 	**>1,000,000 cfu/ml** *(>10,000 colonies estimated)* 

The pictures shown are typical results for MicrobMonitor². The size and shape of the colonies may vary but it is the number which is important. See notes on page 5 for further notes on reading tests. For interpreting tests where colonies show unusual appearance or are not distinct see our leaflet Technical Assistance for Reading Results of MicrobMonitor2 (EP157).

Routine Monitoring of Marine Distillate Fuel with **Microb**Monitor²



* See interpretation chart on page 7 for definition of acceptable, moderate and heavy contamination levels for various samples.