MICROBMONITOR®2

Technical Guidance

Routine Monitoring of Aircraft Fuel Tanks with MICROBMONITOR®2 in Accordance with IATA 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 aviation 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 prevent water forming in aircraft fuel tanks. Dissolved water in fuel will condense out as an aircraft ascends and the fuel becomes cooler, and water in humid air will condense out as it enters cold fuel tanks during aircraft descent. When heavy microbial growth occurs, fuel quality is affected and fuel may become off specification. Microbial "biomass" can clog engine fuel filters causing filter bypass alarms. It may also foul fuel quantity indicator probes in the fuel tank causing erratic, degraded or erroneous fuel quantity readings. 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 operation of the aircraft.

Prevention of microbial growth is a key safety requirement for aircraft operators. Fuel tanks are usually fitted with scavenge systems which collect water and pass it to the engine. However, some water may still accumulate and operators should routinely remove water from tanks by employing a regular tank sump draining procedure. The aircraft manufacturer can advise on the best procedure and which drain valves to use. Because water is often frozen when an aircraft lands, it may be necessary to schedule tank sump draining so that it occurs after aircraft ground time or when the aircraft is located in a warmer geographical region. It is also important to check the operation of the water scavenge system during maintenance checks and ensure pipes are not blocked. Further information is provided in the IATA *Guidance Material on Microbiological Contamination in Aircraft Fuel Tanks* and in section 28-10-00 of Aircraft Maintenance Manuals.

Although regular tank sump draining goes a long way to preventing accumulation of water and microbial growth, because it is not possible to keep aircraft tanks completely free of water, IATA recommend microbiological monitoring to ensure systems remain free of contamination. The **Microb**Monitor2 test is recommended by IATA for monitoring aircraft fuel tanks. This recommendation has been adopted by all leading aircraft manufacturers (including Boeing and Airbus). This Technical Guidance document provides information on using the **Microb**Monitor2 test kit to monitor for microbial contamination in aircraft fuel tanks;

- When, where and how to take samples
- How to conduct the test for aviation fuel samples and associated water
- How to interpret test results
- Appropriate actions when contamination is detected (tank cleaning and/or biocide treatment).

SAMPLING

Guidance on where and when to take samples is given in Aircraft Maintenance Manuals (AMM). In aircraft fuel tanks contamination is often unevenly distributed around the tank but most will be present in any free water and on surfaces prone to condensation. Usually sampling will be done at the same time as the routine tank sump draining task using tank sumping tools. Samples from tank sump drain points will provide the earliest and most consistent indication of tank contamination. Where possible, sample drain points which enable water to be recovered in the sample. At least one drain point for each tank should be sampled; the aircraft manufacturer should be able to advise which drain points are most appropriate. It should be ensured that water in the tank is not frozen at time of taking samples.

It is important when testing routinely to be consistent in the procedure for sampling and testing, so that results over time can be compared. If water is present in the tank, every effort should be made to recover this. 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; any dirt present can contaminate samples 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.

IATA recommends that a 1 litre sample is taken; 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.

IATA recommends that each aircraft tank is tested yearly but the frequency of sampling and testing should be based on the perceived risk and/or any previous experience of microbial growth problems.

Consider an aircraft operation to be at "**high risk**" if heavy microbial contamination has been detected in fuel tanks on more than one occasion and/or significant microbial growth has been observed during inspection of tanks during maintenance or if there have been incidents of filter by-pass or fuel quantity indicator system malfunction. We recommend aircraft at high risk should be tested monthly.

Consider an aircraft to be at "**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 aircraft operates under conditions which may be conducive to microbial growth; e.g. short haul or low altitude flight operations, operations in or to hot humid regions, aircraft in storage, intermittently utilised aircraft (e.g. corporate operations)and aircraft fuelling at facilities where there is a history of contaminated fuel supply. We recommend aircraft at moderate risk should be tested every 3 - 6 months.

Aircraft / operations which have no history of microbiological contamination and which do not operate under conditions specifically conducive to microbial growth can be considered "**low risk**". We recommend aircraft at low risk should be tested every year or at each C-check.

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 (IATA actually recommends within 24 hours but this is not always practical). 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 the apron or in the hangar**).

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 aviation fuels is shown on the following page. An aliquot of 0.5 ml of fuel or 0.1 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 estimated.

IATA Guidelines recommend that every effort is made to recover and test water in tank sump drain samples but this is sometimes not possible, particularly if the tank sump draining task is conducted regularly. Even when water in a sample is tested, it may be useful to also test fuel phase from above the water to enable comparison of results with tests of other fuel samples which did not contain any free water phase. The **Microb**Monitor2 test can be used to test fuel phase and/or water phase in samples.

Testing Fuel: If testing fuel phase, 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 0.5 ml 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 water is present in aircraft drain samples, IATA recommend this is always tested. If testing both fuel phase and water phase, the water phase test should be tested after drawing the fuel phase for test from the sample, as described above. 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 0.1ml water to the M**icrob**Monitor2 test bottle using the sterile "syringe" provided. For easy testing, use the special **Microb**Monitor Sampling kit (available from ECHA Microbiology) which can separate fuel and water.

Note the volume of water tested when assessing aircraft fuel tank samples is larger (0.1 ml) than the volume recommend for testing when assessing water in fuel supply facilities (0.01 ml); this is because water in aircraft tanks should be clean condensate water and a higher detection level is generally needed. Water in storage tanks in the fuel supply chain may originate from environmental contamination (e.g. sea water, tank wash water etc.) and have a higher "background" microbial count. The guideline limits for water phase from aircraft fuel tank drain samples may be reviewed based on operator experience. If "Moderate" or "Heavy" counts in water phase are obtained routinely but fuel phase tests on the same samples nearly always show "Light" contamination and tank inspections show no indications of microbial growth, then there is justification in relaxing the limit values for water phase tests (e.g. "Light" could be <10,000 cfu/ml, "Moderate" 10,000 to 100,000 cfu/ml and "Heavy" >100,000 cfu/ml). If so modify the test protocol so that 0.01 ml of water phase is tested using the loop dispenser provided; this will enable better discernment between "Moderate" and "Heavy" contamination.

TESTING -on the apron or in the hangar

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 0.5 ml of fuel sample or 0.1 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 0.5 ml of fuel sample or 0.1 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.

Visual examination of fuel tank drain 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.

INTERPRETATION OF TEST RESULTS

A general interpretation chart is provided in **Microb**Monitor2 test kit instructions but the chart on page 6 can be used to specifically interpret test results of samples from aircraft fuel tanks. According to test results, this chart defines contamination as Acceptable, Moderate or Heavy in accordance with the limits stated in the IATA *Guidance Material on Microbiological Contamination in Aircraft Fuel 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. In accordance with industry convention, water phase results are expressed per millilitre whilst fuel phase results are expressed per litre.

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 aircraft in long term storage.

Drain point samples will not necessarily reflect the status of bulk fuel in the aircraft tank. 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. When fuel is received into a tank or when the aircraft is in flight, any contamination in the tank is likely to be disturbed and suspended into the fuel and increase particulate levels. Thus, heavy contamination in the tank drain indicates a potential risk to safe operation.

Increasing trends of contamination may be as important as absolute limit values. It is recommended to retest a fresh sample within 10 days 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.

BIOCIDE TREATMENT

Where moderate contamination is confirmed, biocide treatment is recommended. If heavy contamination is confirmed tank cleaning and then biocide treatment is recommended. Some aircraft manufacturers permit an initial biocide treatment of a partially filled tank; full tank treatment is required if subsequent testing indicates the initial treatment was not effective. Always follow the relevant AMM; only use approved biocides and always apply the recommended concentration for the correct soak time. Note that a number of factors will affect the success of biocide treatment including the extent and type of microbial contamination present, whether slimes (biofilms) are present, the amount of water in the tank and also the temperature in the tank when the treatment is conducted. Biocides take longer to kill microbes at colder temperatures; flight time should never be included as part of the biocide soak time and if treating outside in cold winter conditions it may be necessary to extend the biocide soak time beyond that recommended by the biocide manufacturer.

Biocide treatment may cause break up and release of dead microbial biomass into the fuel and consequently engine and APU fuel filters should be changed 50 - 75 hours after treatment.

It is important to confirm biocide treatment is successful by retesting samples from the tank after the treatment has been applied. Some tests suffer interference and incorrect readings if they are used to test fuel containing biocide. IATA recommend allowing 5 flights before retesting after biocide treatment to ensure all biocide residues in fuel have disappeared. **Microb**Monitor2 does not suffer from interference from biocides and if required can be used to confirm efficacy of treatment immediately at the end of the recommended biocide soak time. However, we recommend at least one re-fuelling is conducted before re-testing to confirm biocide treatment.

The diagram on page 7 provides a summary of a typical microbiological monitoring programme and recommended actions in response to various levels of contamination detected. This corresponds to the recommendations in IATA Guidelines and the AMM's of leading aircraft manufacturers.

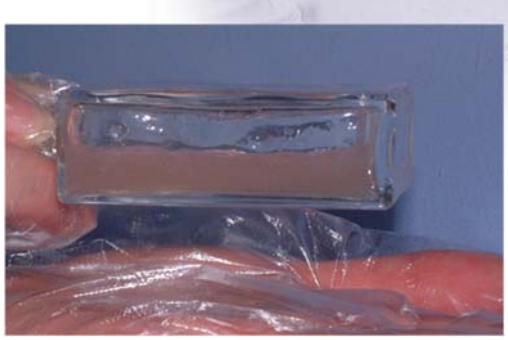
How to Test **Aviation** Fuel from Aircraft Fuel Tanks with **MICROB**MONITOR®2

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 0.5 ml fuel or 0.1 ml water to the M**icrob**Monitor2 bottle. Re-cap and label the bottle.

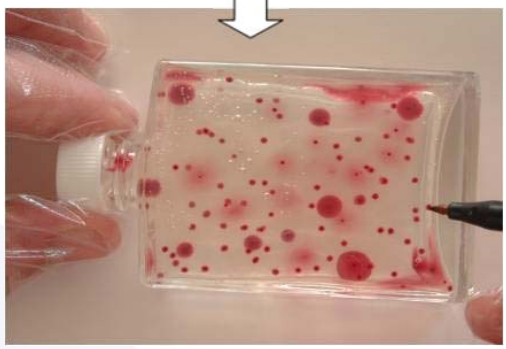
2. Tap bottle to break up gel.

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.

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 the chlorine release tablets (available separately). Decontaminated tests, unused tests or tests showing no microbial growth can be disposed of as normal waste 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.

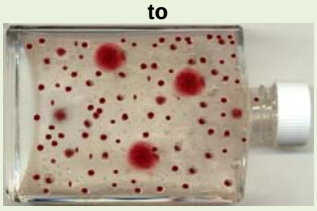
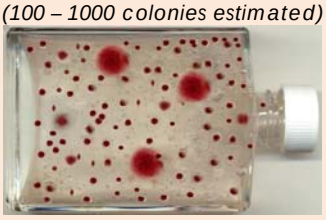
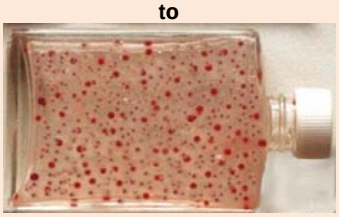
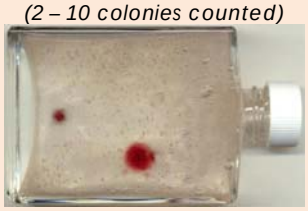
Ignore air bubbles which may form in the gel.

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.

How to Interpret MICROBMONITOR®2 Test Results For Aircraft Fuel Tank Drain Samples in Accordance with IATA Guidance

Interpretation	**Water phase (if present and tested)** (0.1 ml tested)	**Fuel phase** (0.5 ml tested)
NEGLIGIBLE **(Acceptable)**	**<1000 cfu/ml** *(<100 colonies estimated)*  **to** 	**< 4,000 cfu/litre** *(<2 colonies counted)*  **to** 
MODERATE	**1000 – 10,000 cfu/ml** *(100 – 1000 colonies estimated)*  **to** 	**4000 – 20,000 cfu/litre** *(2 – 10 colonies counted)*  **to** 
HEAVY	**>10,000 cfu/ml** *(>1000 colonies estimated)*  **To** 	**>20,000 cfu/litre** *(>10 colonies counted or estimated)*  **to** 

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 **Microb**Monitor2 (EP157).

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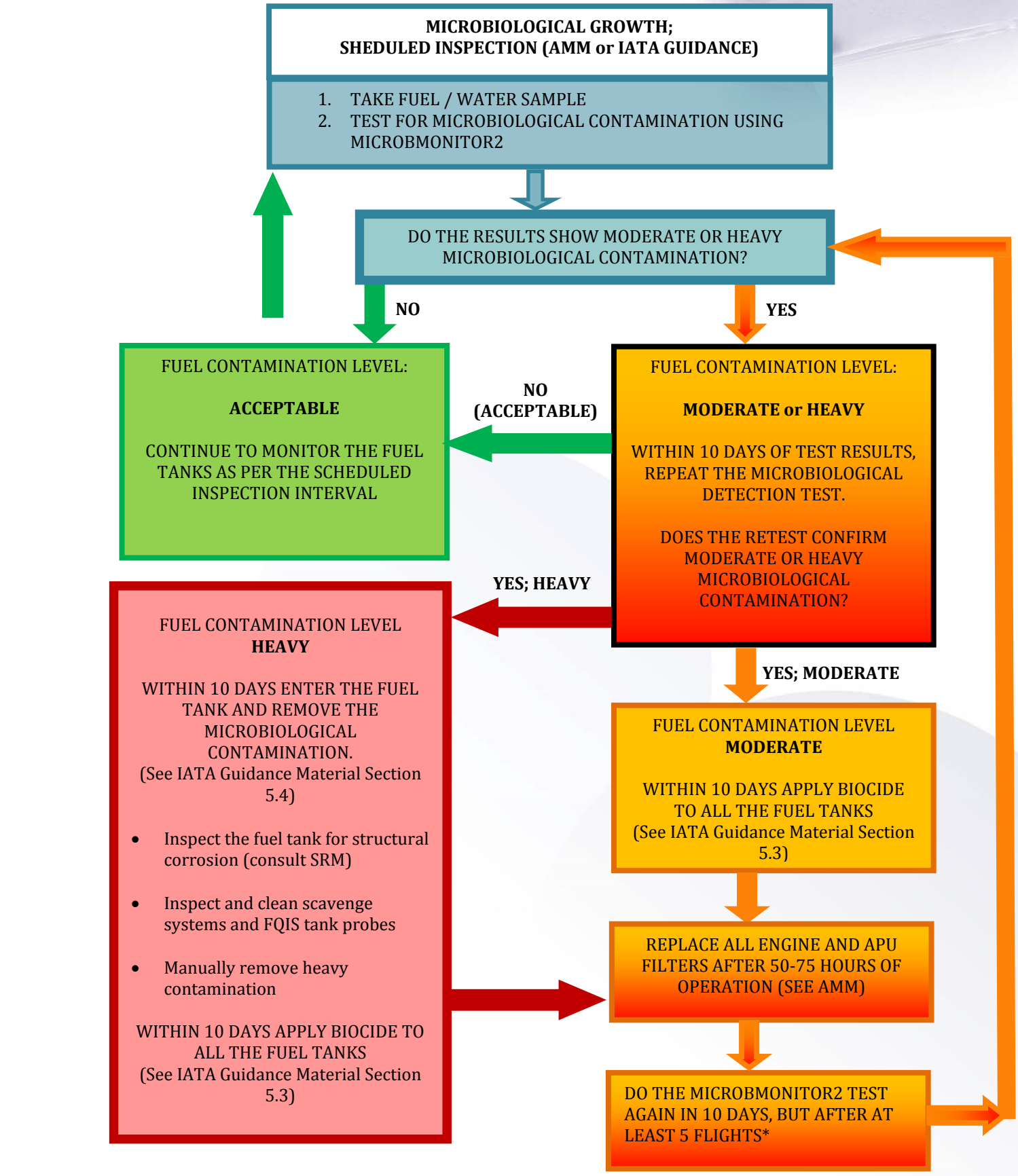

* IATA Guidelines recommend allowing 5 flights before re-testing after biocide treatment because some test kits give false results in the presence of biocide. However, if using the **Microb**Monitor2 test this repeat test can be conducted sooner.

Routine Monitoring of Aircraft Fuel Tanks with MICROBMONITOR®2 in Accordance with IATA Guidance

This leaflet is appropriate only for drain samples of aviation fuel from aircraft fuel tanks. Other technical leaflets are available at www.microbmonitor.com

- 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.*
- For interpretation of results of tests of samples from **marine diesel end user tanks** please see our leaflet and EP166 *Routine Monitoring of Marine Diesel on Ships and Offshore Installations 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 **Microb**Monitor2 or recommendations in this document. Please see the Instructions for Use for full conditions of use of **Microb**Monitor2.

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