MICROBMONITOR2®

Technical Guidance

Technical Assistance for Reading Results of **MICROB**MONITOR**2**®

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Assistance for Reading Results of **MICROB**MONITOR**2**®

Usually, **Microb**Monitor**2** gives a clear visual impression of the extent of microbial contamination in a sample. The number of colonies in the test can be easily counted or, if there are too many to count, estimated using the chart provided in the instruction leaflet. Occasionally, however, the growth in the **Microb**Monitor**2** test has an unusual appearance or there may be colour interferences. This technical leaflet explains how to interpret those unusual results.

Typical Appearance of Microbial Colonies

Microbial growth in the **Microb**Monitor**2** test typically appears as purple spots (colonies) as shown in Fig. 1. Typical colonies are usually circular but may have irregular jagged edges. Different types of microbes can grow at different rates in the **Microb**Monitor**2** gel and therefore the spots may be of different sizes. The size of the colonies is not important in considering the extent of contamination. Experienced users may be able to distinguish mould colonies from those produced by yeasts and bacteria. Moulds grow more slowly but eventually produce large colonies which may have a powdery or fuzzy white, grey, green or brown surface. Colonies of bacteria and yeasts develop more quickly, usually within 1 to 2 days and often remain quite small, only a few mm across, even after prolonged incubation.

Fig. 1 **MICROB**MONITOR**2** with typical colonies

Generally, the more colonies there are in a test, the smaller they will appear. When very heavy contamination is present, there can be so many small colonies present that all the gel in the test appears to be purple. There are some incidences when it can be difficult to distinguish this heavy growth from some colour interferences.

Colour Interference

Colour interference may occur for the following reasons;

- Atypical growth of some “motile” bacteria which have ability to spread rapidly in the gel producing streaks or a patchy purple appearance
- Interference from fuel anti-oxidants which can partially react with growth indicator to give a peach, light red, pink or orange colouration in the gel.
- Interference due to exposing the test to bright light; this can produce a pink colour in the gel which is sometimes more intense in the bottle corners.
- Darkening of the test gel with prolonged storage before use; this can result in a light brown or dark pink tinge

The key to distinguishing microbial growth from possible colour interferences is to consider when and how quickly the colour appears and the intensity of the colour. Microbial growth is always purple or dark red; any other colouration is probably colour interference. Any colour which appears within a few hours of adding sample to the tests is probably not due to microbial growth. Patchy coloration is probably due to spreading of “motile” bacteria.

Each of the possible types of colour interference are described in more detail below with suggestions for interpreting results and avoiding the problem.

After incubation the test has red or purple streaks or patches in the gel. How do you interpret this?

Fig. 2 **MICROB**MONITOR**2** with spreading colonies

EXPLANATION:
Some bacteria are very motile and they can swim in the test gel to form large purple or red patches or streaks. The patches often have uneven colour intensity. Usually the problem only occurs when testing water samples or fuel samples containing suspended free water.

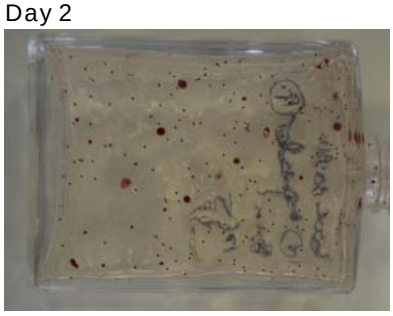
INTERPRETATION OF TESTS WITH SPREADING COLONIES (PATCHES);

- Look at the test after 1 or 2 days, before the patch has spread out; count the colonies marking them with a felt pen. If when observed again (e.g. at 4 days), a spreading patch is present, this should be ignored and only new normal colonies counted and added to the total count. Fig 3 below shows examples of a test of fuel and a test of water on successive days incubation where spreading colonies (patches) have developed by Day 4; these should be ignored.

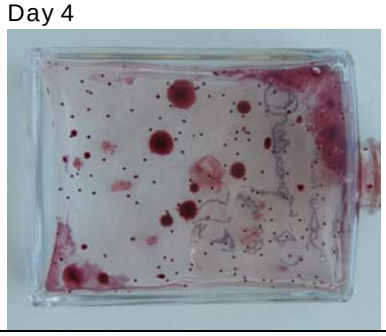
Fig. 3 **MICROB**MONITOR**2** Tests showing development of spreading colonies over time

Test of Fuel Tank Water Bottom

Day 2

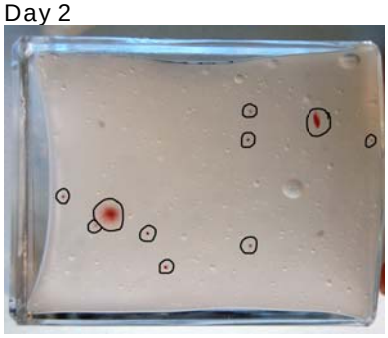


Day 4



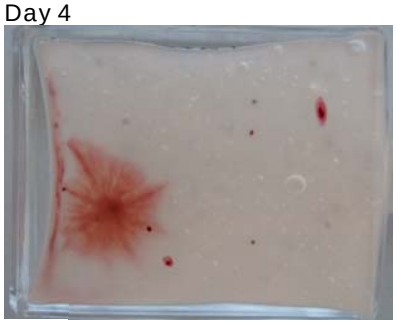
Test of Aviation Fuel

Day 2



10 Colonies counted (circled).

Day 4



Colonies have grown and one colony is beginning to spread. The total colony count is still 10.

Day 6

The spreading colony is beginning to mask other colonies making counting difficult.

- If it was not possible to observe the test before the patch spread, it is often possible to see through the patch and distinguish and count normal colonies as usual (see Fig. 4). The patch has a lighter colour intensity than normal colonies and is not clearly demarcated. Each patch should be counted as one colony and added to the total count of normal colonies. If it is difficult to determine where a patch begins and ends, record a count of "1" colony for the patch or streak and add this to the total count of normal colonies.

Fig. 4 **MICROB**MONITOR**2** with normal colonies visible through the spreading colony (patch)

- If it is not possible to distinguish and count normal colonies through the patch, count only an area of the gel which is not affected and multiply the count by an appropriate factor for the final result (*e.g.* count half of the gel area and multiply by 2).

- If using the interpretation chart to estimate the total number of colonies, try to ignore the patch and estimate only on the number of normal colonies; if necessary estimate the intensity of colonies based on an area of gel which is unaffected by the patch.

- If none of the above are possible, it may be necessary to repeat the test taking the avoidance precautions described below. You may also send a photograph of tests with unusual results to support@echamicrobiology.com and we will do our best to interpret the result for you.

AVOIDING THE PROBLEM OF SPREADING COLONIES;

- If testing fuel, allow any water to settle to the bottom of the sample before taking out the fuel for test. We recommend standardising the procedure for testing fuel system samples which contain both fuel and water. Invert the sample 3 times and then allow it to stand for a few minutes (ideally 2 minutes per cm height of fuel in the sample) so that any water settles. Fuel for test (0.5 ml for aviation fuel or 0.25 ml for other fuel types) should then be taken from half way down the fuel phase, avoiding transfer of visible interfacial particulate, water droplets or emulsion. The water phase or interface can be tested separately if required (0.01ml recommended)

- If you are testing water, you can reduce the chances of getting spreading bacteria and improve the ability to discern individual colonies by testing only 0.01 ml volumes of water using the loop dispenser provided.

- Use an incubator to keep the temperature constant and reduce condensation on the gel.

- Always shake the test properly after adding the sample. If the test is not shaken properly, heavy growth, which should give a red or purple colour indication throughout the gel, will instead appear as patchy discolouration. Streaky or patchy growth in the test is more difficult to interpret if the gel is not properly shaken and the gel remains lumpy. Always ensure the gel is properly broken up and then shaken with sample for 30 seconds. See "Tips & Tricks" in the FAQ section at www.echamicrobiology.com for further information on shaking the test.

When I use the test a pink or orange-red colour develops in all of the gel. Is this microbial growth?

Fig. 5 **MICROB**MONITOR**2** with colour interference due to anti-oxidant in B100

EXPLANATION:

Fuels and lubes, particularly those formulated with bio-ingredients, often contain anti-oxidants to improve stability. These can react chemically with the growth indicating dye in the **Microb**Monitor**2** test, resulting in a slight pink or orange-red background colour. Some biocides in fuel may increase fuel anti-oxidant colour interference. The interference is predominantly encountered in tests of B100 or automotive fuels with a very high FAME concentration (>10%) but it is occasionally encountered in some other fuels and oils.

This colour interference usually occurs within 24 hours and may become progressively darker over many days, although the colour does not develop to the extent that it interferes with the ability to read test results during the normal incubation period of the test. The colour interference does not affect the growth of microbes transferred from the sample but it can make it more difficult to distinguish microbial growth, particularly for inexperienced users. If a countable number of colonies develop, these are deep red or purple and can usually easily be distinguished from the background colour. However, if the result shows a pink, red, orange or purple discolouration throughout the gel it can be difficult to determine whether this is due to heavy contamination or colour interference.

A similar colour interference can also develop if the test is left exposed to light. This colour interference can happen within 24 hours if the test is exposed to normal daylight or room lighting, although in very bright light (e.g. a sunny window sill) the colour may start to change within an hour. The colour will not be affected whilst reading tests under normal lighting conditions but the tests should always be incubated in the dark.

INTERPRETATION OF TESTS WITH DISCOLOURATION AFTER INCUBATION;

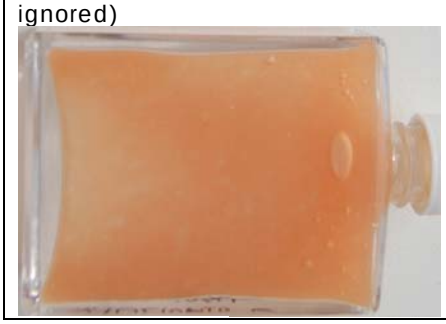
- It is usually possible to distinguish heavy contamination (10,000 or more colonies) from colour interference by the colour. Ignore a uniform, light pink or orange discolouration as this is unlikely to be microbial growth (see negative test in picture on the left below).

- If normal dark red or purple colonies are visible against the background colour, ignore the background colour and count the colonies as usual (see positive tests in pictures centre and on the right below).

Fig. 6 MICROBMONITOR**2** tests showing colour interference (with and without growth)

Negative Test (the uniform background colour should be ignored)

Positive Tests (Colonies are distinguishable and still countable; the background colour should be ignored)



- Interpret tests with a uniform **red** or **purple** colour as having 10,000 colonies or more (see test on the left in Fig 7 below).

- If tests have a **dark pink** or **orange** colour, examine them closely to see if they have a speckled appearance (see test on the right in Fig 7 below). If they have, this is probably due to a large number of very small microbial colonies (10,000 colonies or more). If the appearance is uniform and not speckled, this is probably due to strong colour interference.

Fig. 7 MICROBMONITOR2 tests showing heavy microbial growth

MicrobMonitor**2** with purple colour due to heavy microbial growth (10,000 colonies or more)	**Microb**Monitor**2** with orange colour; the speckled appearance shows this is due to heavy microbial growth (10,000 colonies or more) not colour interference.
	

- Examine the tests within 2 days; heavy contamination will usually be indicated by a **dark pink**, **red** or **purple** gel at this stage but colour interference, generally does not become dark until many days after adding sample to the test. Do not attempt to read tests after the maximum recommended incubation time (4 days if incubated at approximately 25°C or 6 - 7 days if incubated below this temperature).

- Users who are experienced in handling microbial cultures can do a simple test to determine whether a test showing colour but no discernable colonies has background interference or microbial growth. Open the test and stab a loop dispenser into the gel and then stab the loop into the gel of a new test and shake and incubate this as normal. (Discard the loop as biohazardous waste, e.g. in disinfectant). If microbial growth was present in the original test this will be transferred to the new test and will grow to give a dark red or purple colour indication (10,000 colonies or more). If no colour develops in the confirmatory test then the original test was negative. **Note**; handling open microbial cultures should only be conducted by those with relevant training; always wash hands before and after working with cultures.

- A blotchy appearance may be due to growth of spreading bacteria (see page 3)

- If after considering the above it is still not possible to distinguish whether the test has heavy contamination or colour interference, it may be necessary to repeat the test taking the avoidance precautions described below. You may also send a photograph of tests with unusual results to support@echamicrobiology.com and we will do our best to interpret the result for you.

AVOIDING THE PROBLEM OF COLOUR INTERFERENCE;

- If possible, for samples known to give interference problems, test smaller volumes of sample. For example, test 0.1 ml of fuels instead of 0.25 ml or for oils test 0.01 ml instead of 0.1 ml. This reduces the intensity of the background colours. Interpret tests using the relevant line on the interpretative chart. Bear in mind this will reduce the minimum detection limit of the test but, if the original colour was due to heavy contamination, this will not be a problem. Test minimum detection limit can be improved by conducting several tests on each sample and totalling the number of colonies in all tests.

- Never examine tests beyond the recommended incubation period.
- Incubate tests in the dark and only expose to light during use and examinations.

Before I use the test it already has a pink, orange-red or brown colour. Can I still use the test?

Fig. 8 MICROBMONITOR**2** showing discolouration due to age (exceeded shelf life).

EXPLANATION:

Some of the ingredients used in **Microb**Monitor**2** are of natural origin and some minor variations do occur and this can result in slight variations in the colour of the product. This is normal and does not affect the functionality of the tests. Over the course of the product's shelf life, especially when not refrigerated, it is normal for the gel to darken slightly. Tests nearing the end of their shelf life can have a light brown or dark pink tinge in the gel and even new tests can have a slight pink tinge. These tests can still be used. The **Microb**Monitor**2** gel is also sensitive to light, especially direct sunlight. Exposure of the test to light for several days will result in a pink colour development within the gel.

DETERMINING WHETHER DISCOLOURED TESTS ARE FIT FOR USE;

- Providing the user can distinguish microbial growth from the background colour, tests are fit for use.

Fig. 9 Darkening of **MICROB**MONITOR**2** Tests with age

MicrobMonitor**2** Tests **A**&**B** show normal variation of colour at time of production.		**Microb**Monitor**2 C** shows typical colouration towards the end of shelf life.	**Microb**Monitor**2 D** shows a **Microb**Monitor**2** test two years past its expiry date.
			
FIT FOR USE			DO NOT USE

- In Fig 9 above, tests A, B and C are fit for use. Ignore the background colour and use as normal.
- We would not recommend using a test with the appearance of that shown in D because it would be difficult to distinguish microbial colonies (although in fact this test would still grow microbes).
- If your tests looks like D and is still within its designated 1 year shelf life please contact us at support@echamicrobiology.com. (Note, as we cannot validate clients' storage temperatures, only product within the designated 1 year shelf life is covered by warranty).

Fig. 10 Discolouration of **MICROB**MONITOR**2** Tests due to light exposure	
The **Microb**Monitor**2** test in picture **E** was exposed to strong direct sunlight for 5 minutes and shows minor discolouration, particularly in the corners. 	The **Microb**Monitor**2** test in picture **F** was exposed to strong direct sunlight for two hours. 
FIT FOR USE	DO NOT USE

AVOIDING THE PROBLEM OF DISCOLOURATION BEFORE USE;

- Store **Microb**Monitor**2** in a cool dark location.
- Avoid exposing the tests to light during storage.
- Avoid exposing the tests to elevated temperatures in storage and as far practical, in transit.
- Storing tests in a refrigerator can extend the shelf life of the test by 1 year
- Do not leave the tests out of the box in direct sunlight even for a short time.

Check www.echamicrobiology.com for all our latest technical leaflets or contact support@echamicrobiology.com

- For interpretation of results of tests of aviation fuel samples from **aircraft** please see our leaflet EP096 *How to Interpret* **Microb**Monitor**2** *Test Results in Accordance with IATA Guidelines for Aircraft Drain Samples.*
- For interpretation of results of tests of **aviation** fuel distribution system samples please see our leaflet EP119 *How to Interpret* **Microb**Monitor**2** *Test Results for Aviation Fuel Distribution System Samples*
- For interpretation of results of tests of **diesel** fuel distribution system samples please see our leaflet EP132 *Routine Monitoring of Diesel Fuel Tanks and Distribution Systems with* **Microb**Monitor**2**