

What is the test used for?

The Sig Rapid WB Test enables rapid (1 hour), semi-quantitative detection of microbial proliferation in water associated with distillate fuel. The Sig Rapid WB test can be conducted on-site and is sufficiently rapid to enable testing immediately prior to transfer of location or custody of the product. It can be used for testing water in:

Cargoes/Bulk Fuel:

- Product in shore tanks before loading on vessels
- Compartment bottoms in a loaded tanker prior to discharge
- Stored product in tank farms
- Slops/slime in empty vessels or shore tanks prior to loading Fuel Deliveries and End-user Fuel:
- Road and rail delivery tankers
- Aviation fuel delivery pipeline low points and filter and coalescer sump drains
- Garage forecourt automotive diesel tank bottoms
- Private diesel, gas oil, heating oil tanks
- Ships bunkers (storage tanks, day/service tank drains, water separator drains)

The test should be performed on water in the sample. The test cannot be used for direct testing of fuel phase although traces of fuel will not affect the test.

What is the test?

The Test contains:

- 10 Screw-capped glass tubes (red spot on cap) each containing a powder reagent.
- 1 Screw-capped glass tube (blue spot on cap) containing 10 developer tablets.
- Interpretative colour chart.
- Labels.
- 10 Sterile transfer pipettes.

The Sig Rapid WB reagent powder produces a yellow colouration when it reacts with enzymes produced by microorganisms which proliferate in water associated with fuel. The reaction proceeds at an optimum rate when incubated at 32 - 38°C. Room temperature can be used but the reaction is significantly slower. The yellow colour is most strongly expressed after the addition of the colour developer tablet following incubation. The yellow colour developed in the test can be estimated by comparison with the colour chart supplied.

Background information

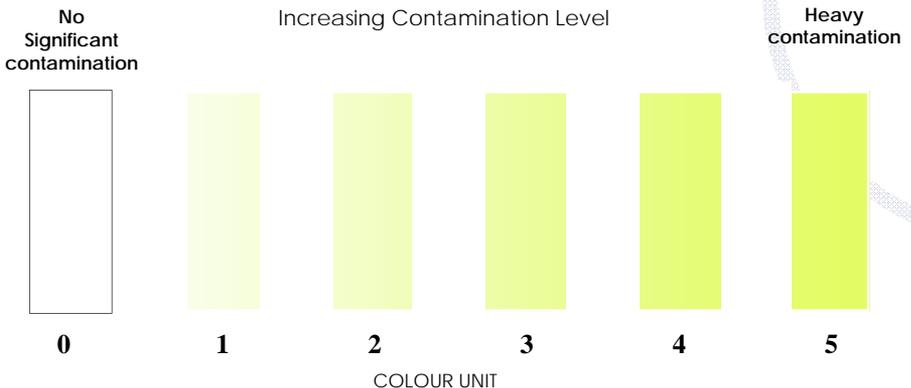
Microbial proliferation in fuel cannot occur without the presence of some free water. The microbes first develop in this water and later spread into the fuel. Few fuel systems with water associated with them are completely free of microorganisms but the level of sensitivity of the Sig Rapid WB test is such that normal and acceptable levels of microbial contamination are not detected. A positive result in the test indicates an abnormal level of microbial contamination in the water tested and by implication strongly suggests unacceptable microbial proliferation in some of the fuel/water system sampled. Conversely a negative result in the test strongly suggests that the fuel/water system sampled will be unlikely to be operationally affected by microbial spoilage. Microbes in large volumes of fuel tend to settle towards the bottom and upper fuel may not be heavily infected. However, movements of fuel will probably disturb and redistribute bottom contamination.

No guarantees are however implied by a positive or negative result and any other relevant evidence should be taken into consideration.

Test procedure

- Swirl the aqueous sample to disperse the microorganisms through the water phase.
- Using a sterile bulb pipette, transfer c. 5 ml of aqueous sample to the test reagent tube (with red spot marker on cap); this should now be about a third full.
- Replace the cap and shake vigorously (it is not necessary to completely dissolve the reagent).
- Note any initial yellow colour in the tube by comparison to the colour chart (e.g. Chart Colour 1); any initial yellow colour should not exceed Chart Colour 1.
- Incubate in a warm place at 32-38°C for 1 hour. Alternatively incubate at warm room temperature for 2 hours.
- After incubation add one developer tablet (tube labelled with blue spot marker on cap) to the reagent tube and shake.
- Note the final yellow colour in the tube by comparison to the colour chart (e.g. Chart Colour 4).
- Only an increase in colour intensity is significant; if there was an initial yellow colouration prior to incubation, subtract this from the colour unit recorded after incubation. For example if initial colour was 1 and the final colour was 4, then a result of 3 should be recorded.

Colour Chart - Test incubated at 35°C for 1 hour.



Additional Information

- If less than 5ml of water/sludge is present in the sample, the test can still be performed by estimating the approximate volume present and then adding tap water which has been boiled and then cooled to make a total volume of 5ml. When interpreting the results allow for the dilution made
- The sensitivity of the test can be increased by prolonging the incubation time to 2 - 3 hours. Any yellow colour then assessed will correspond to fewer microorganisms in the samples than indicated by the chart.
- The yellow colour intensity will continue to develop with increasing incubation time and therefore a consistent incubation period should be used.

Interpretation of test results

The Sig Rapid WB detects enzymes associated with microbial proliferation in fuel water phase; generally higher levels of enzyme will be associated with higher numbers of microbes. The test is sensitive to the activity of all types of micro-organisms which cause fuel spoilage, but not necessarily to the same extent. The colour chart provided (opposite) gives an indication of the level of contamination present in the sample. The table below shows suggested interpretations based on testing typical kerosene fuel samples.

Heavily contaminated samples may start to turn yellow after 30 minutes at 32-38°C thus providing an early indication of contamination.

Colour Unit	
0 - 1	Water associated with fuel is not significantly infected at this time. Fuel is likely to be free of significant contamination.
1 - 2	Water associated with fuel is moderately infected. Fuel may have light or moderate infection.
3 - 5	Water associated with fuel is significantly infected. Fuel may also be significantly contaminated and there may be adverse operational implications.

Disposal

No harmful chemicals are used in the test and no additional growth of microbes occurs. The hazard posed from the test is thus no greater than the hazard posed by the sample material. Used tests can therefore be disposed of, with due regard for the sample material, in accordance with appropriate local and/or national regulations.

Storage and shelf life

In order of preference keep test kits in a freezer, a refrigerator or a cool, dark cupboard. In a freezer, shelf life is 12 months. In a refrigerator or cool place, shelf life is likely to be six months depending on temperature; it can be checked at any time by testing a sample of fresh distilled water. No more than a tinge of yellow colour is acceptable.

Other products and services

ECHA aims to provide all of the products and services needed for solving microbiological problems in industry. ECHA supply a wide range of test kits and ancillary products such as swabs, incubators and sample bottles. ECHA also offer comprehensive microbiological analytical services, consultancy and training and can advise on the choice and application methods of biocides (see www.echamicrobiology.co.uk).

Disclaimer

The integrity of the sample (with absence of extraneous contamination) may affect the test results obtained. Factors such as the relationship of the sampling point to the system as a whole may affect the interpretation of results. ECHA therefore accepts no liability for any decision or assessment taken or made as a consequence of the information provided, the Test Kit results obtained or the use of the test kits as described. The procedures recommended and the opinions expressed within this instruction leaflet, are given by ECHA Microbiology Ltd., the designers and manufacturers of the **MicrobMonitor** Sig Rapid WB test, in good faith and are based on ECHA Microbiology Ltd.'s many years experience of sampling, testing, remediation and prevention of microbiological contamination and corrosion in industry.

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