Sig Tests®

SIG SULPHIDE®

What is the test?

The test consists of a screw capped, glass tube, half filled with a buff coloured selective microbiological culture medium that semi-quantitatively indicates the presence of sulphide generating bacteria by the rate and extent of the development of a black colour. Sample is added to the tube, which is kept warm (incubated) for up to six days; results are examined regularly.

What is the test used for?

The Sig Sulphide test is used to detect the presence of microorganisms that can generate corrosive sulphide e.g. Sulphate Reducing Bacteria (SRB). The test can be used to test samples of bilge water, ballast water, fuel tank bottom water, metal working fluids, process water, lubricants, and environmental waters. Metal surfaces which are prone to attack by SRB can also be tested. Surfaces and corrosion pits are tested using a swab which is then thrust into the Sig Sulphide tube. Soil, mud and core samples from drills can also be tested by suspending samples in clean water and then testing this.

Background information

Metal working fluids, process waters, bilge waters, ballast waters, lubricants, paper pulp, fuels, crude oil and sea water can all contain sulphur in varying forms such as sulphate, sulphite, sulphonate, sulphurised oil and mercaptans.

These can all be reduced to sulphide by a consortia of microorganisms containing both aerobic and anaerobic bacteria. The anaerobic bacteria in the consortia are usually referred to as Sulphate Reducing Bacteria (SRB). Reduction of these sulphur compounds often leads to hydrogen sulphide gas being produced by SRB as an end product.

Hydrogen sulphide gas is not only an unpleasant smelling gas and skin irritant but it is more toxic than hydrogen cyanide and can kill. Hydrogen sulphide stains and corrodes ferrous and non-ferrous metals, stone and concrete and it discolours metal working fluids and fuel tank water bottoms. Sulphide generating bacteria cause rapid pitting corrosion of steel in contact with process water, ships' bilge water, sludges, crude oil and fuel tank water bottoms, detergent washes and various chemical solutions and slurries. Sulphide generated in the water bottom of a fuel tank can dissolve in the fuel in the tank. Sulphide production from emulsifying components (e.g. Petroleum sulphonates) leads to emulsion instability.

Test procedure

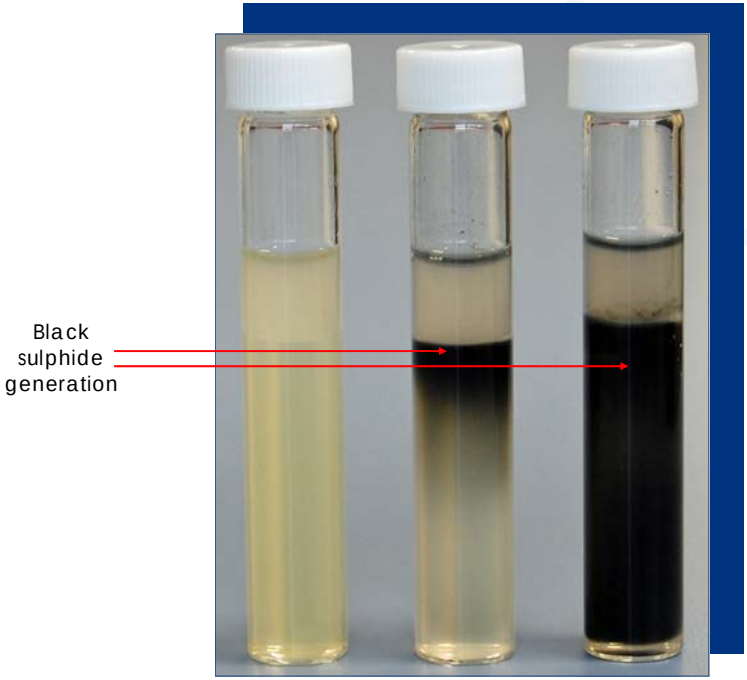
Remove the viscose seal and unscrew the cap. Add about 2ml of sample onto the surface of the gel. Some samples, particularly bilge and fuel tank samples, may contain oil. Ensure that most of the sample transferred to the gel is water phase. To assist this, small sterile pipettes can be used for the transfer and are available from **ECHA**. Re-cap and incubate the tube upright at about 30°C. When testing samples from systems where the ambient temperature is higher than 30°C, it is desirable to incubate the test at a correspondingly higher temperature. A black line at the interface after incubation is a positive result and the speed at which this spreads downwards into the gel indicates the severity of the SRB contamination. After 6 days the absence of blackening is a negative result. Examine the test daily if possible and record the percentage of the gel which has turned black e.g. 40 and 100% as in Fig.1 which shows typical results.

Corrosion pits can be tested by swabbing them with a sterile swab (**ECHA** catalogue ref. ECHA 15/SW/03, pack of 10), then thrusting the swab into the Sig Sulphide gel and breaking off the shaft. Incubate normally and look for blackening around the swab.

Note: Very high levels of sulphide already present in samples may cause an immediate positive (i.e. A black line at the gel surface). This does not necessarily mean that viable SRB are present although this is the probable source of the sulphide. If viable SRB are present the black colour will spread during incubation.

Fig. 1: Sig Sulphide test

Unused tube on left. Positive samples on right after 5 days incubation at 30°C.



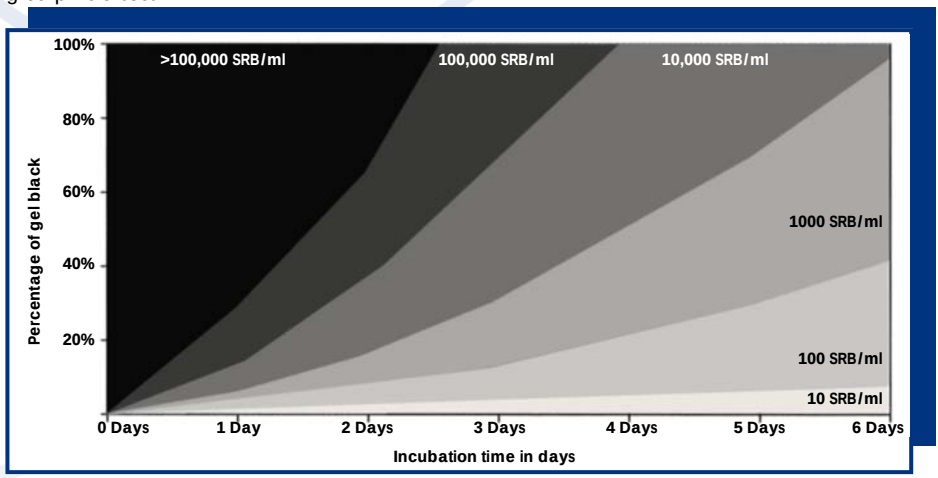
Interpretation of test results

The test has been calibrated against the Standard Test Method for SRB, NACE TM0194-94, using American Petroleum Institute (API) Medium (now called Sodium Lactate SRB Medium). The calibration graph is given in Fig.2. A variety of typical samples were used for the calibration but it must be appreciated that different samples and different sampling locations and different consortia of sulphide generating bacteria could yield differing calibration graphs. This graph is therefore only an example of possible calibration graphs but in practise it provides a useful indicator of probable microbial numbers and this has been confirmed at **ECHA** by testing many hundreds of samples with both the Sig Sulphide test and the NACE test. If it is used and results are viewed and recorded on more than one day, take the highest recorded figure on any day as the result. For most purposes, the results can be categorised as light, moderate and heavy contamination by sulphide generating bacteria. Any positive result after 1 day will always indicate a very severe contamination. Sulphide generating bacteria are generally most numerous in slimes or 'biofilms' on surfaces; therefore positive results in a fluid phase sample taken several centimetres away from a surface (e.g. The water from the bottom of a fuel storage tank) will suggest that much greater numbers will be present on the surface itself. In general any positive result is cause for concern and the urgency of remedial action will be determined by the severity of the sulphide generating contamination detected and its operational significance.

If you require further information on the use and interpretation of the Sig Sulphide test please contact **ECHA**.

Fig. 2: Sig Sulphide test calibration graph

Correlation between number of SRB per ml determined in API Medium (NACE TM0194-94) and Sig Sulphide test.



>100,000 SRB/ml	100,000 SRB/ml	10,000 SRB/ml	1,000 SRB/ml	100 SRB/ml	10 SRB/ml
Heavy contamination		Moderate contamination		Light contamination	

Disposal

Dispose of the Sig Sulphide tests after use after first immersing them (opened) in strong disinfectant overnight.

Storage and shelf life

The test can be stored for at least a year at ambient temperature. If the appearance of the test has not changed after prolonged storage it should be suitable for use.

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

It is the nature of microbiology that no single procedure will give a comprehensive indication of the numbers and nature of all the microbes present in a system. 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 Sig Sulphide 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.

Sig Tests® test kits are manufactured by:
ECHA Microbiology Ltd.,
Units 22 & 23 Willowbrook Technology Park,
Llandogo Road, St Mellons,
Cardiff, CF3 0EF, UK
Tel: +44 (0)29 2036 5930
Fax: +44 (0)29 2036 1195
E-mail: sales@echamicrobiology.com
Web: **www.echamicrobiology.com**