

What is the test?

The test consists of a screw capped, glass tube, half filled with a pale orange coloured selective microbiological culture medium that indicates the presence of nitrite reducing bacteria (NRB) in an aqueous sample by changing colour from pale orange to pink and also by producing gas bubbles. Sample is added to the tube, which is kept warm (incubated) for up to five days; results are examined regularly. The test is also a good indicator that microbial nitrate reduction is taking place, as microbes first degrade nitrate to nitrite and this is then reduced by NRB.

What is the test used for?

The Sig Nitrite test is used to detect the presence of nitrite reducing bacteria (NRB) for example in cooling waters, metal working fluids, effluents and waters which contain nitrite corrosion inhibitor. The Sig Nitrite test can also be used to confirm that in systems where nitrite is added to inhibit microbial reduction of sulphate, a suitable microbial NRB population is present. Sig Nitrite tests can also be used to confirm that de-nitrification is taking place in effluents, sewage etc.

Background information

Some microbes, which normally use atmospheric oxygen, have the ability to switch to nitrite as an oxygen source. The nitrite is reduced to ammonia, nitrogen gas and other gases, and the fluid rapidly becomes corrosive. The presence of nitrite reducing microorganisms can result in rapid depletion of nitrite corrosion inhibitors. Nitrate can be used to inhibit sulphide generation by Sulphate Reducing Bacteria (SRB) in some systems. In such cases the presence of nitrite/nitrate reducing bacteria is desirable.

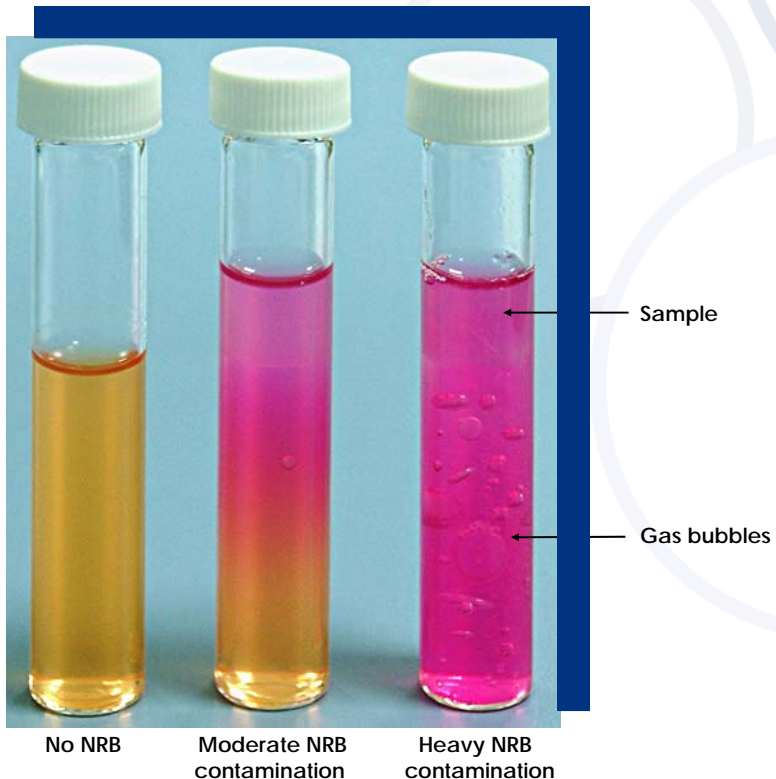
Test procedure

Remove the viscose seal and unscrew the cap. Pour about 2ml of sample onto the gel; re-cap and incubate the tube upright at about 30°C for up to 5 days. When testing samples from systems where the ambient temperature is higher than 30°C, the test should ideally be incubated at a correspondingly higher temperature. Examine daily if possible and note the result. Daily examination is particularly important in the first three days of incubation to determine the level of NRB contamination present.

A positive result is indicated by a bright pink colouration (alkalinity due to the presence of ammonia) of the gel and/or bubbles of gas in the gel. The rate at which changes occur in the gel is a semi-quantitative indication of the number of NRB in the sample. A positive result after 1 day indicates a heavy NRB contamination ($>10^6$ nitrite reducing bacteria per ml). A positive result first detected after 2 days suggests moderate NRB contamination (10^3 - 10^5 per ml) and a positive first detected after 3 days indicates about 10-100 NRB per ml. No change after 5 days is negative. Typical results are shown below in Fig. 1.

Note: Samples which are themselves very alkaline may produce an immediate pink colour in the gel. Samples which are very acidic will produce an immediate yellow colour in the gel and there may be so much acid present that any ammonia produced is neutralised and cannot turn the gel pink. In both cases a significant result is given by gas formation in the gel and not by colour change.

Fig. 1



Interpretation of test results

The test has been calibrated by testing aqueous suspensions containing know numbers of a nitrite reducing *Pseudomonas sp.* of bacteria as determined by standard Total Viable Count (TVC). The calibration chart is given in Fig. 2. It must be appreciated that different samples and different species or consortia of nitrite reducing bacteria would probably yield slightly different results. This chart is therefore only an example of possible calibration. For most purposes, the results can be categorised as light, moderate and heavy contamination by nitrite reducing bacteria: different situations and different sampling points will influence this categorisation. Any positive result after 1 day will always indicate a very severe NRB contamination.

Fig. 2: Sig Nitrite test calibration graph

Correlation between the numbers of nitrite reducing *Pseudomonas* per ml determined by the standard Total Viable Count method and by Sig Nitrite test.

Reaction after each day's incubation at 30°C					Number of cfu/ml
Day 1	Day 2	Day 3	Day4	Day5	
•••••	•••••	•••••	•••••	•••••	10 ⁹
••	•••••	•••••	•••••	•••••	10 ⁸
•	•••••	•••••	•••••	•••••	10 ⁷
•	•••	•••••	•••••	•••••	10 ⁶
-	•	•••	•••••	•••••	10 ³ -10 ⁵
-	-	•••	•••••	•••••	10-100

Key

- 100% pink colouration and copious gas bubbles
- 75-99% pink colouration and copious gas bubbles
- 50-74% pink colouration and gas bubbles
- 25-49% pink colouration and gas bubbles
- 0-24% pink colouration and gas bubbles
- No pink colour and no gas bubbles

Disposal

Dispose of the Sig Nitrite test after use after first immersing it (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 (see www.echamicrobiology.com).

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 Nitrite 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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