



To determine the presence of acid-producing bacteria in water samples

Used in oil, gas and petrochemical industries, air industries, food industries, water and waste water, and other industries.

The risk of corrosion of metal facilities is one of the main issues in the management of water systems as well as oil and gas systems. Corrosion has doubled the importance of this issue due to its irreversible nature as well as the high compensation cost. Over the past years, the cause of corrosion has changed from a complex electrochemical process to a common process driven by microorganisms. Two groups of bacteria play an important role in corrosion. These two groups include sulfate reducing bacteria (SRB) and acid producing bacteria (APB). Of course, the role of thiosulfate reducing bacteria (TRB) cannot be ignored either.

APB (Acid-Producing Bacteria) are a diverse group of heterotrophic bacteria whose common feature is the production of acidic organic substances as a result of growth in reducing (anaerobic) conditions. As a result of the activity of APB bacteria, the pH of the culture medium decreases significantly and reaches 3.5 to 5.5. These acidic conditions can be very corrosive. APB bacteria are considered a good companion for SRB bacteria in the corrosion processes caused by metals due to their acid production activity that takes place in the absence of oxygen. Identifying and estimating the population and aggression level of APB bacteria plays a vital role in timely identification and prevention of microbial corrosion in industries.

APB bacteria are very important due to the creation of reduction conditions, the creation of biocoating (a special form of growth on surfaces) and the creation of sufficient amounts of organic materials capable of breaking down into fatty acids.

MicrobCheck™ APB test kit is designed as a 50 ml falcon containing culture medium and floating ball.

Manufacturer's Recommendation

Avoid contact with the inner wall of the falcon. Perform the test under sterile conditions.

After opening the Falcon, place the door upside down on a clean surface with its bottom facing the ground.

Do not shake or rotate the falcon after the sample is added. Let the ball float on the surface of the culture medium.

In many cases, APB bacteria grow deep in biofilms and not directly in running water. Make sure you get the sample from the right place.



Test Method

Preparation

Collect at least 25 ml of sample.

Pour 19 ml of the sample into the falcon and close it.

Write down the date and name of the sample on the falcon.

Sampling of acidic waters

If the pH of the examined sample is less than 6, it can lead to a false positive result. Therefore, it is necessary to adjust the pH of such samples to 6.9 - 7.2 using sterile KOH. Due to the shock caused by adding base to the sample, it is necessary to subtract 2 days from the first day of observing the reaction to estimate the population of APB bacteria. For example, if the first reaction was observed on day 5, it is necessary to consider this number as 3.

Sampling of waters with high salt concentration

If the amount of salt in the tested water is more than 6%, it will cause a false negative response. Therefore, it is necessary to dilute the examined sample using sterile water to the extent that its salt content reaches less than 6%.

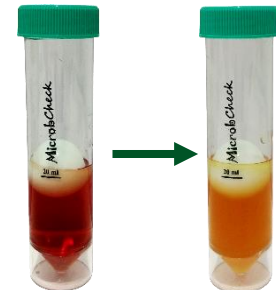
Incubation

Keep the Falcon at room temperature and away from sunlight.

View the sample daily for 8 days. Note the date of the first observed reaction.

Presence / Absence

A positive reaction in the MicrobCheck™ APB test is indicated by a change in the color of the culture medium. At the start of the test, the color of the culture medium is red. A positive test is indicated by a change in color to yellow or orange. This color change can be accompanied by turbidity or can be created after it. Usually, this reaction occurs at the bottom of the falcon and spreads slowly along the length of the falcon.



Estimation of Population and Aggression Level

If the test result is positive, estimate the bacterial population and its aggression level according to the table below. The larger the bacterial population, the faster the color change reaction occurs and the more aggressive it is. For example, if a color change occurs inside the tube 1 day after incubation, it means that the aggressiveness of the bacterial population is very high.

Note that the first time when the color change occurs is considered as a reference for estimating the bacterial population.

Some APB bacteria are from *coliform* group. For this reason, if ABP bacterial populations with high or relatively high aggression level are detected in the sample, it is necessary to test the presence/absence of *coliform* bacteria.



Aggression Level	Time Lag (day)	Population (cfu/ml)
Very aggressive	1	500,000
Very aggressive	2	100,000
Very aggressive	3	20,000
Moderately Aggressive	4	5,000
Moderately Aggressive	5	500
Moderately Aggressive	6	100
Normal Background	7	10
Normal Background	8	≤ 2

Quality Control of the MicrobCheck™ APB Test Kit

To confirm the quality and performance of MicrobCheck™ APB kit, the specified strains can be cultured and the specified reaction patterns can be checked. After adding the bacterial dilution, wait until the suspension enters the culture medium and avoid shaking the falcon. Store the kit at room temperature and observe the reactions for at least 8 days.

Organism (ATCC)	Pattern
<i>Citrobacter freundii</i> (8090)	Turbidity and color change to yellow
<i>Escherichia coli</i> (25922)	Turbidity and color change to yellow
<i>Enterobacter aerogenes</i> (13048)	Turbidity and color change to yellow
<i>Salmonella Typhimurium</i> (14028)	Turbidity and color change to yellow

Best Time to Use

The expiration date of the kits is 6 months and it is necessary to store them in the refrigerator (4-8°C). It is recommended to avoid frequent temperature changes and storage in the freezer.

Disposal

Test kits are completely contaminated after use and bacterial growth. As a result, they need to be autoclaved or burn in a furnace. If this is not possible, open the falcons under the laboratory hood and fill it with bleach liquid with a concentration of 5 to 10%. Let it sit overnight and then throw it away.

