



PIPELINE FLUIDS BACTERIA KIT
Fluids-Microbiology Survey
USER INSTRUCTIONS

SPECIAL NOTE: Before using this Fluids-Microbiology Survey kit READ ALL INSTRUCTIONS.
This kit only contains enough materials for one (1) test.

MICROBIOLOGY AND GENERAL CHEMISTRY

This kit will test for the following parameters:

- APBs - Acid Producing Bacteria (Red Cap)
- SRBs - Sulfate Reducing Bacteria (Green Cap)

MICROBIOLOGY AND GENERAL CHEMISTRY TESTING

The testing is performed by the bottles and syringes contained in this kit. The following instructions provide the procedures for the testing of pipeline fluids.

INSTRUCTIONS

- Step 1. The fluid to be tested is to be drained from the collected fluid sample into the first 30 ml sample cup.
- Step 2. Immerse the water finding test paper strip into the fluid in the sample cup.
- Step 3. If the water finding test paper strip DOES NOT change color, then the fluids are hydrocarbons. Record this information on the enclosed Microbiology Survey Reporting Form. No further testing can be performed upon this fluid sample.
- Step 4. If the water finding test paper strip DOES change immediately to a lavender color upon immersion in the fluid, then the fluid is to be tested for pH and the bacteria bottles are to be inoculated, per the following steps.
- Step 5. **pH Test.** Remove the pH strip from the plastic bag and fold the strip in half. Place the folded edge only of the pH strip into the fluid, making sure to wet only the tip of this folded area (will be the wide, non-numbered, yellow color bar of the pH strip). Remove the pH strip from the fluid and compare the color in the fold of the strip to the color chart on the pH strip. If there is not an exact color match with this scale, then estimate an intermediate value. Record the pH reading on the enclosed Microbiology Survey Reporting Form. Upon completion of this task, the used water finding test paper strip, pH strip, and first 30 ml sample cup may be discarded.
- Step 6. The fluid to be tested is to be drained from the collected fluid sample into the second 30 ml sample cup, keeping the fluid in the cup as clean as possible. Prevent fingers and foreign materials (leaves, dirt, etc.) from contaminating the fluid

Microbiology Testing

Inoculation of APBs Bottles

- Step 7. Number the **red-cap** bottles 1 through 5. (Located in the foam block).
- Step 8. Remove the metal tabs from the centers of all **red-cap** bottles. Do not remove the entire metal seal.
- Step 9. Using one of the enclosed alcohol swabs, wipe the exposed rubber part of the cap on each of the **red-cap** bottles.
- Step 10. Unwrap one of the four syringes. Draw one (1) ml of fluid from the second 30 ml sample cup into the first syringe.
- Step 11. Insert the syringe needle into the top of the first **red-cap** bottle and inject the sample into the bottle (**red-cap** bottle 1). Move the syringe plunger up and down a couple of times to mix the sample in the bottle. Empty the syringe into this bottle prior to removing the syringe.
- Step 12. Remove the empty syringe and set it aside. *You will not use it again.*
- Step 13. Unwrap the second of the four syringes. Insert it into the first **red-cap** bottle.
- Step 14. Now pull up on the syringe plunger to draw up one (1) ml of sample from the first **red-cap** bottle into the syringe.
- Step 15. Pull the syringe out of **red-cap** bottle 1, insert the needle into **red-cap** bottle 2, and inject the liquid into bottle 2. Once again, move the syringe plunger up and down to mix the sample, draw up one (1) ml of liquid, and inject it into **red-cap** bottle 3.
- Step 16. Repeat the above step until all of the **red-cap** bottles have been injected. Note: you do not have to draw any liquid out of the last **red-cap** bottle (bottle 5).
- Step 17. Remove the empty syringe from the last **red-cap** bottle and set the syringe aside. *You will not use it again.*

Inoculation of SRBs Bottles

- Step 18. Number the **green-cap** bottles 1 through 5. (Located in the foam block).
- Step 19. Remove the metal tabs from the centers of all **green-cap** bottles. Do not remove the entire metal seal.
- Step 20. Using one of the enclosed alcohol swabs, wipe the exposed rubber part of the cap on each of the **green-cap** bottles.
- Step 21. Unwrap the third of the four (4) syringes. Draw one (1) ml of fluid from the second 30 ml sample cup into the syringe.
- Step 22. Insert the syringe needle into the top of the first **green-cap** bottle and inject the sample into the bottle (**green-cap** bottle 1). Move the syringe plunger up and down a couple of times to mix the sample in the bottle. Empty the syringe into this bottle prior to removing the syringe.
- Step 23. Remove the empty syringe and set it aside. *You will not use it again.*
- Step 24. Unwrap the fourth syringe. Insert it into the first **green-cap** bottle.
- Step 25. Now pull up on the syringe plunger to draw up one (1) ml of sample from the first **green-cap** bottle into the syringe.
- Step 26. Pull the syringe out of **green-cap** bottle 1, insert the needle into **green-cap** bottle 2, inject the liquid into bottle 2. Once again, move the syringe plunger up and down to mix the sample, draw up one (1) ml of liquid, and inject into **green-cap** bottle 3.
- Step 27. Repeat the above step until all of the **green-cap** bottles have been injected. Note: you do not have to draw any liquid out of the last **green-cap** bottle (bottle 5).
- Step 28. Remove the empty syringe from the last **green-cap** bottle and set the syringe aside. *You will not use it again.*

- Step 29. Upon completion of Step 28, the second sample cup, syringes, etc. may be discarded. The syringes are to be discarded per the section entitled "Syringe Disposal". The sample cups, alcohol swabs, etc. can be disposed of in the trash.

Recording the Microbiology Survey Results

- Step 30. The carton in which the Microbiology Survey APB and SRB bottles, syringes, etc. were originally shipped can be used as an incubator for the completion of the Microbiology Survey. After inoculating all the bacteria bottles, place the foam block containing the inoculated SRB and APB bottles back into the box, and close the lid. All other materials can then be discarded. The Microbiology Survey record found as a label on the lid of the box can be completed, for ease of reference.
- Step 31. Keep the bottles out of direct sunlight and at room temperature for one month (30 days).
- Step 32. Ten (10) days after inoculation, check the APB (**red-cap**) bottles for any color changes. If the liquid has changed from red to yellow, count that bottle as having changed (positive). Record the number of APB (**red-cap**) bottles, starting with Bottle 1, that have changed on the enclosed Microbiology Survey Reporting Form. After the ten (10) days, no further observations are needed for the APB (**red-cap**) bottles.
- Step 33. Twenty-eight (28) to thirty (30) days after inoculation, check the SRB (**green-cap**) bottles for any color changes. If the liquid has changed from clear to black, count that bottle as having changed (positive). Record the number of SRB (**green-cap**) bottles, starting with Bottle 1, that have changed on the enclosed Microbiology Survey Reporting Form. After the thirty (30) days, no further observations are needed for the SRB (**green-cap**) bottles.
- Step 34. After the results have been recorded on the Microbiology Survey Reporting Form, the APB and SRB bottles can be discarded in the trash. No special handling or disposal requirements are applicable for their disposal.

Syringe Disposal

Needles and syringe barrels are to be destroyed and disposed in accordance with Federal and Local laws. At a minimum, the needles must be destroyed before discarding by cutting the tips off the needle or by bending back the needle tips. Syringes must be destroyed by breaking or shattering the barrel.

(Company)

(Location)

MICROBIOLOGY SURVEY REPORTING FORM

Storage Field	Line Number	
Bell Hole Number	Well Number	Drip Name and Number
Township/District	County	State
Comments		
Sample Location	Sample Number	
Sample Type <input type="checkbox"/> - Soil <input type="checkbox"/> - Scale <input type="checkbox"/> - Solids <input type="checkbox"/> - Sludge/Gunk <input type="checkbox"/> - Mud <input type="checkbox"/> - Slime <input type="checkbox"/> - MIC <input type="checkbox"/> - Pipeline Dust <input type="checkbox"/> - Other _____		
Comments		
Sample Collection Date	Sample Collection Time <input type="checkbox"/> - AM <input type="checkbox"/> - PM	Sampler
SAMPLE DESCRIPTION Color _____ Odor _____ Texture _____		
pH MEASUREMENT pH _____	WEATHER CONDITIONS Air Temperature _____ Conditions _____	
MICROBIOLOGY SURVEY RESULTS		
Positive Culture Bottles Bottle Number (Red Cap) Number of Days	APBs <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> (1) (2) (3) (4) (5)	_____ Bacteria Colonies/ml
	_____ <input checked="" type="checkbox"/> Record color change from red to yellow after 10 days	
Positive Culture Bottles Bottle Number (Green Cap) Number of Days	SRBs <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> (1) (2) (3) (4) (5)	_____ Bacteria Colonies/ml
	_____ <input checked="" type="checkbox"/> Record color change from clear to black after 28-30 days	
TESTED BY	DATE	
COMMENTS		

MICROBIOLOGY SURVEY

INTERPRETATION OF RESULTS

1. POSITIVE RESULTS ARE AS FOLLOWS:

<u>BACTERIA</u>	<u>MEDIA</u>	<u>POSITIVE</u>
APBs	Red	Media turns yellow
SRBs	Clear With Nail	Media turns dark gray to black

2. The number of bottles that show positive results in the interval time (10 days APBs and 28 to 30 days SRBs) can be used to calculate the bacteria level in the original sample by the following table:

<u>NUMBER OF POSITIVE BOTTLES</u>	<u>BACTERIA COLONIES/ML</u>	<u>REPORTING VALUE</u>
0	<1 - 10	<10
1	1 - 10	10
2	10 - 100	100
3	100 - 1,000	1,000
4	1,000 - 10,000	10,000
5	≥10,000 - 100,000	100,000

3. When a sample has elevated H₂S levels, the sulfate reducer (SRBs) nutrient bottle no. 1 will often turn “positive” (black) within 15 - 60 seconds of inoculation. This occurrence should be considered “no growth”, if only this bottle is positive after 28 days. If SRB bottle no. 2 turns black immediately, a new sample should be obtained and the H₂S purged from the sample before inoculation. If SRB bottle no. 2 turns black hours or days after inoculation, the bottle should be marked “positive” and the results recorded, per the table in Item 2.