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**API
RECOMMENDED PRACTICE
For
BIOLOGICAL ANALYSIS OF SUBSURFACE
INJECTION WATERS**

OFFICIAL PUBLICATION



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NOTE

The second edition, December 1965, superseded the first edition, May 1959, titled *Recommended Practices for Biological Analysis of Water-flood Injection Waters*. This third edition supersedes the second edition.

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API RECOMMENDED PRACTICE FOR BIOLOGICAL ANALYSIS OF SUBSURFACE INJECTION WATERS

FOREWORD

a. This recommended practice has been developed by the Subcommittee on Biological Analysis of Water to be Used for Water-flood Purposes, of the Committees on Drilling and Production Practice, in cooperation with research laboratories of operating and chemical companies and university life sciences departments. Membership of the committee is listed on the preceding page.

b. The objective of the study committee was to set up standard methods of tests for determination of the effectiveness of chemicals for treating injection waters to control the growth of microorganisms and to develop procedures for the biological analysis of injection waters. It is believed that these methods will help eliminate some of the confusion concerning biological analysis of injection waters. The use of the standard procedures will allow comparison of data from various water floods.

SECTION I EXAMINATION FOR MICROORGANISMS

Scope

1. A knowledge of the microbial population of an injection water is necessary to determine whether a biological problem exists. This knowledge is also necessary to evaluate the effectiveness of a chemical treatment.

2. These procedures are intended to reveal the number and types of microorganisms present in a particular water sample.

Purity of Reagents

3. Unless otherwise indicated, all chemical reagents shall meet American Chemical Society* specifications. Prepared bacteriological media can be obtained from biological supply houses.

Sampling Methods

4. The following sampling techniques should be adhered to:

- a. A clean sterile bottle will be used.
- b. The tap should be allowed to flow at least 3 min at the sampling rate prior to taking the sample.
- c. The sample should be taken in such a manner as to preclude contamination from external sources.
- d. The time, date, temperature, and appearance of the water should be recorded at the time of sampling, and this information should be included with the sample. If possible, the total solids and/or mineral content of the water should be included.
- e. The sample should be handled in such a manner as to avoid radical changes in temperature between time of sampling and time for examination of the sample.
- f. The maximum time between sampling and examination should not exceed 24 hours. The sample should be cultured as soon as possible. If an examination cannot be initiated within the 24-hour period, the following statement should be included in the report: "These results do not necessarily represent

microbial content of the water at the time of sampling."

g. Slime masses and other deposits should be sampled separately.

Microscopic Examination of Water

5. Microscopic examination makes possible the detection of microorganisms which are not easily cultivated on laboratory media. In addition, it may permit an estimate of the microbial population. This information may be used as a guide in making plate counts.

6. The best method of examination is by the use of the phase contact microscope. With this microscope, one can more easily view the microorganisms and avoid the distorting tendencies of some of the staining techniques.

7. The examination should include identification of microorganisms as follows:

- a. Algae and protozoa
 - (1) Flagellates
 - (2) Ciliates
 - (3) Diatoms
 - (4) Filamentous
- b. Bacteria
 - (1) Iron bacteria—describe the type, i.e., sheath or stalk
 - (2) Sulfur bacteria for the presence of sulfur granules
 - (3) Bacterial slime
 - (4) Total direct microscopic counts
- c. Fungi

8. With the exception of 7.b. (4), the microscopic estimates of each type shall be reported as none, present, present in large numbers.

9. The membrane filter is very useful in the detection and enumeration of iron bacteria, algae, and protozoa. A measured quantity of the water under examination can be filtered through the membrane filter. The filter should be dried, cut into small sections, and the sections placed on microscope slides. The filter sections can be rendered transparent by a drop of immersion oil, then subjected to the usual microbiological examination.

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