



ENVIROTEK LABORATORIES, INC.

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ANTIBACTERIAL TEST REPORT

Report #: 14-206
Date: May 14, 2007
Standard Performed: Antibacterial Test
Method Performed: m-ColiBlue-24
By: Jaime A. Young
Customer: Biotite, Co.

EXECUTIVE SUMMARY

Biotite Mineral solution manufactured by Biotite, Co. was tested for antibacterial properties. Four control batches were prepared with four different types of Coliform bacteria in sterilized water and tested for Total Coliform/E. coli using the m-ColiBlue-24 method. Four similar Coliform batches were prepared and treated with the Biotite Mineral solution in a 1:1000 proportion and tested again using the same Coliform method. Coliform bacteria were present in the control batches but absent in the treated batches.

INTRODUCTION

Biotite Mineral solution manufactured by Biotite, Co. was tested for antibacterial properties. Four control batches were prepared with four different types of Coliform bacteria in sterilized water and tested for Total Coliform/e. coli using the m-ColiBlue-24 method. The four type of bacteria used were Escherichia coli, Enterobacter cloacae, Proteus mirabilis, and Pseudomonas aeruginosa. Four similar Coliform batches were prepared and treated with the Biotite Mineral solution in a 1:1000 proportion and tested again using the same Coliform method. Coliform bacteria were present in the control batches but absent in the treated batches.

MATERIALS

Biotite Mineral concentrated Solution
PotableWater Coliform ERA Catalog # 694 containing Escherichia coli, Enterobacter cloacae, Proteus mirabilis, and Pseudomonas aeruginosa at 500 colonies forming units/mL (CFU/mL)
Sterilized water
Incubator
Reagents and chemicals necessary to perform EPA approved methods for drinking water analysis.

PROCEDURE

1. Remove the vials from the refrigerator and allow them to warm to room temperature.
2. Place the sterilized water containers in the incubator at 35 to 37 °C for approximately 30 minutes.
3. After the 30-minute warming period, remove one sterilized water container from the incubator.
4. Carefully open the sterilized water container.
5. Open the appropriate bacteria sample vial and aseptically transfer the gelatin tablet into the sterilized water container and labeled accordingly.
6. Properly dispose the empty glass vial and pouch.
7. Reseal the sterilized water container that now contains the bacteria sample.
8. Prepared all the samples as above.
9. Place the inoculated samples into the incubator at 35 to 37 °C for an additional 30 minutes to dissolve the samples.

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10. After the 30-minute dissolution period, gently shake each inoculated sample to ensure that the gelatin tablet has completely dissolved.
11. Prepare the filter units and filter the first four samples as per method mColiBlue-24 (these are the control samples).
12. Place the filter membrane in the Petri dish and incubate for 24 hours at 35 to 37 °C.
13. Add 0.1 mL of the Biotite Mineral solution to the next four 100-mL sample prepared with the Coliform bacteria and repeat steps 11 and 12 (these are the treated samples).

RESULTS

| Parameter | Control Samples | Biotite Mineral Solution | EPA MCL |
|------------------------|-----------------|--------------------------|----------|
| Escherichia coli | 500 CFU/mL | 0 CFU/mL | 0 CFU/mL |
| Enterobacter cloacae | 500 CFU/mL | 0 CFU/mL | 0 CFU/mL |
| Proteus mirabilis | 500 CFU/mL | 0 CFU/mL | 0 CFU/mL |
| Pseudomonas aeruginosa | 500 CFU/mL | 0 CFU/mL | 0 CFU/mL |

CONCLUSION

The Biotite Mineral solution prepared at 1:1000 effectively kills the four types of bacteria present in the challenge water at 500 CFU/mL.

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