A COMPARISON OF TWO MEMBRANE FILTER TECHNIQUES FOR THE ENUMERATION OF TOTAL COLIFORMS IN POTABLE WATER

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I. INTRODUCTION

Potable water is that which contains no impurities that offend the sense of smell, sight, or taste and lacks any chemical or biological contaminants that could possibly endanger the public health. The terms "potable water" and "drinking water" are often used as synonyms.

The U. S. Public Health Service established the first drinking water standards in 1914 to protect persons travelling in the U. S. and to aid in the enforcement of Interstate Quarantine Regulations (1). Since then, the standards have been changed and updated several times to become a basis for more rigid enforcement of drinking water quality. The Safe Drinking Water Act of 1974 delegated power to the Environmental Protection Agency to set and enforce maximum limits on contaminants permitted in drinking water (1). This has resulted in rigid operational and quality controls that water treatment plants must maintain.

To test the bacteriological quality of drinking water, samples of treated water must be collected from the distribution system each month. The number of samples to be examined during the month is dependent on the population served by the system (1). Problem areas such as dead-end mains, remote areas, and reservoirs in the distribution system must be tested also. Bacteriological standards are based on testing for members of the coliform group. They are defined as all aerobic and facultative anaerobic, non-spore forming,

Gram-negative rods that ferment lactose with gas production within 48 hours of incubation at 35°C (2). The 14th Edition of Standard Methods for the Examination of Water and Wastewater describes two acceptable methods for enumeration of the coliform group: the Multiple-Tube Fermentation Technique (or Most Probable Number [MPN]) and the Membrane Filter (MF) Technique.

The MF technique may be performed with or without an enrichment step that consists of incubating the bacteria-laden filter on a rich, non-selective media before transferring it to a selective medium for the final incubation period. Standard Methods (2) states that enrichment is not usually a necessary step when sewage or nonpotable water is examined. However, this has been disputed in many instances and an enrichment period has been proven to result in higher bacterial counts when stressed bacteria from such water samples are enumerated (3, 4, 5, 6). For potable waters, Standard Methods suggests the elimination of the enrichment step after repeated testing has revealed that sufficient results are obtained using the single-step (non-enrichment) technique.

The study presented in this thesis was based on the premise that any coliforms that have been exposed to natural or man-made stress (such as chlorination) require an enrichment step to allow repair of the cell's damaged physiological systems and improve the recovery of individual cells on selective media. The objective of this laboratory study was to compare two membrane filter techniques

presented in Standard Methods (2) for the enumeration of total coliforms in chlorinated potable waters. Coliform recoveries were determined by a two-step enrichment procedure and a single-step procedure without enrichment.

II. LITERATURE REVIEW

Coliforms as Pollution Indicators

In 1914, the United States Public Health Service established our nation's first bacteriological drinking water standard based on the presence of the microbe Bacterium coli (Escherichia coli) in public water supplies (7). The adoption of this standard occurred approximately 30 years after Theodor Escherich identified E. coli as an indicator of fecal pollution (7). Even today, the coliform group, of which E. coli is a member, remains the preferred group of indicator organisms of sewage pollution after eleven revisions of Standard Methods. The methods used for detection of coliforms, however, have undergone dramatic changes. For example, the Public Health Service Drinking Water Standards of 1974 specified the planting of five, 10-ml water samples in fermentation tubes containing at least 30 ml of lactose peptone broth and the observation of gas formation with 48 hours of incubation at 37°C. Positive tubes were then plated on either lactose litmus agar on Endo's medium. Typical colonies formed on the plates were returned to lactose peptone broth for confirmation as aerobic, lactose-fermenting organisms, i.e., coliforms (7). To date, research is being conducted into rapid coliform enumeration methods using such techniques as colorimetric enzyme assays for fecal coliforms. At the present time, however, water and sewage treatment plant operators use the multiple tube fermentation and membrane filter techniques that have developed over the past six decades for the bacteriological analysis of their water source and/or finished product.

The coliform group is identified by its ability to ferment lactose with gas production within 48 hours at 35°C (2). Fecal coliforms originate in the animal gut and their presence in a water supply increases the probability that enteric pathogens are also present. Because they do not persist for long periods outside their warm-blooded hosts, fecal coliforms suggest a recent sewage contamination (8). Non-fecal coliforms are generally associated with soil runoff, but some subgroups may also be found in fecal discharges (9).

Neither fecal coliforms nor non-fecal coliforms are acceptable in a treated potable water, therefore, drinking water standards are based on total coliform enumeration (8). A positive total coliform count in a treated water or in the distribution system indicates inadequate treatment procedures caused by such factors as poor plant design, improper operating procedures, poor sanitary practices in plant operation, or faults or aftergrowth in the distribution system (8).

A general bacterial population as estimated by the "total plate count" (2), has been found to be significant in potable water quality also because it is able to suppress coliform growth in test media if the numbers are high enough (10). The maintenance of a 0.1 to 0.3 mg/l chlorine residual in the distribution system controls the general bacterial population to below the 500 cells/ml level (10). Geldreich (10) stated that this level is sufficient to prevent significant interference with total coliform determinations.

The Stressed Coliform

For years, bacteriological researchers have been aware that various forms of stress may play an important role in the success of enumeration of bacteria on prepared media. Stress is considered to be any adverse environmental condition that a microbe may encounter that causes it to multiply slowly or not at all when standard bacteriological testing is conducted (11). Organisms can experience physiological damage that inhibits their ability to divide. Thus, they go undetected during testing and cause the analyst to incorrectly assume that the sample is of higher sanitary quality that it actually is. This could be dangerous because even injured pathogens may retain their virulence (11).

Earlier studies on stress were more oriented toward bacteriological research to determine the effects of various laboratory methods on growing or stored cultures. In 1929, Stark and Stark (12) studied the effects of increased temperatures on young and mature cells. Five years later, Sherman and Cameron (13) varied environmental factors such as temperature, pH, and osmotic pressure of growing cells to determine lethal limits within a cell's natural range of growth. Studies have also been done using cultures that have been stored on agar slants at lowered temperatures for months before use (14). These earlier microbiologists had become aware that they were losing viable cells during such lab procedures as transferring bacteria between media of different temperatures or osmotic pressures, using old stock slants from cold storage, or using very

young cells in investigations where they may encounter stress. This viable cell loss prompted researchers to take a closer look at the sensitivity of bacterial cells.

More recently, microbial stress has become a concern in a wide variety of areas. Food industries that are required to maintain uncontaminated products must worry about stress to indicator organisms by freezing and thawing, pasteurization, sanitizers, salt concentration, and many other conditions that occur during food preparation that may inhibit the accuracy of bacteriological quality control tests. Sewage treatment plant operators must deal with stressed indicator organisms in chlorinated effluents while water treatment plant operators encounter organisms that have been stressed by environmental conditions in the raw water source and those stressed by chlorine in the finished water. If they survive the treatment process without detection, stressed organisms may pass through the potable water distribution system where they could find more favorable conditions for growth. Of even greater significance is the possibility that virulent virus particles could pass unheeded into the distribution system. This occurrence is probable because of the greater resistance of viruses to chlorination and the lack of a suitable enumeration technique for viruses at the present time.

The concern over stress to indicator organisms (coliforms) has greatly increased the amount of research being done in this area. In the 1950's, many studies were undertaken to discover the con-

sequences of stress by chlorination (15, 16, 17, 18) and heat (16, 17). All investigations proved that bacteria can indeed be injured by stress, but the survivors can be "reactivated" if favorable conditions for their repair and growth are provided. The likelihood that bacteria might be reactivated in dilution media such as phosphate buffer or peptone water, was questioned and found to be true also (16, 17, 19).

The past few years have been the most active in terms of stressed coliform research. Most studies though have investigated the recovery capabilities of selective versus non-selective media involving bacteria exposed to various stresses. For instance, Maxcy (20) exposed E. coli and Enterobacter aerogenes to stress by heat, chlorine, NaCl, and freezing and thawing before testing the recovery efficiency of brilliant green lactose bile broth, desoxycholate lactose agar, violet red bile agar, and standard plate count agar. He found that the selective media could provide counts as low as 10 per cent of the count obtained by plating samples on the non-selective, standard plate count agar. Similar results were demonstrated by Braswell and Hoadley (21) using Trypticase soy agar (TSA) and M-FC medium with chlorinated secondary sewage. Scheusner et al. (22) used several selective agars versus TSA for recovery of organisms stressed by exposure to quaternary ammonium compound sanitizer. Similar studies have been conducted by Hoadley and Chen (23) who compared

the recoveries of several different indicator organisms on TSA and several types of selective media. The bacteria were recovered from six commonly used diluents ranging from sterile stream water to phosphate buffer. Again, the non-selective media gave the highest enumerations, indicating better recuperation of the bacteria from stressed conditions.

Most recent investigations have centered around stressed organisms in the natural aquatic environment. Bissonnette et al. (24) compared the enumeration capabilities of deoxycholate lactose agar, KF streptococcus agar, and TSA (supplemented with 0.3 per cent yeast extract and 0.5 per cent glucose) on two strains of E. coli and two strains of Streptococcus faecalis that had been exposed to eight different types of stream environments. The cells were brought to the lab from the stream and enriched for up to 6 hours in Trypticase soy broth (TSB) while transfers to TSA and the selective agars took place every 30 minutes. Their findings showed similar trends. The degree of cell recovery varied considerably among the samples from different stream environments, but the cells that were allowed longer periods of exposure to TSB before being transferred to a selective agar grew much better.

After three hours of enrichment in TSB, the cells responded equally as well on TSA or the selective media. The study suggests that bacteria are stressed by natural environments but they can be retrieved on selective media if allowed a two to three hour enrichment period on a non-selective media.

Klein and Wu (25) suggested that organisms that have been stressed become hypersensitive to secondary stresses. They compared the recoveries of the standard pour plate method, that creates a warming stress, to a spread plate technique. A bacterial sample from an aquatic environment was utilized in the investigation. The authors assumed the bacteria had been exposed to the primary stress in the aquatic environment. The secondary stress was introduced by warm agar in the pour plate method. Higher recoveries were obtained by the spread plate technique.

As evidenced by the previous studies mentioned, numerous attempts have been made to find a medium suitable for accurately enumerating coliforms from various environments. Therefore, the importance of a reliable technique and an appropriate medium is apparent.

Development of the Membrane Filter for Coliform Enumeration

For years before the membrane filter was brought to the United States, German and Russian scientists endeavored to perfect the use of cellulose nitrate and cellulose acetate to construct a membrane suitable for bacterial enumeration (26). In the late 1940's, Alexander Goetz travelled to Germany to study the techniques used in membrane filtration. From his efforts during this time, which included three subsequent years of research at the California Institute of Technology, Pasadena, and joint work at the Environmental Health Center, Cincinnati, Ohio, the membrane filter technique was introduced in the United States as a bacteriological

method with the potential of someday becoming a standard method. In 1951, Goetz and Tsuneishi (26) published information on the application of membrane filters in the bacterial analysis of water including a physical description of the filters, the equipment needed for operation, the techniques required, and the efficiency of the membranes in bacteriological analyses. The research at the Environmental Health Center was conducted by Clark et al. (27) who supplemented the work of Goetz by comparing the bacterial recoveries on membrane filters using various types of media. He also initiated investigation of coliform enumeration on membrane filters with the development of a modified Endo broth (Environmental Health Center, E. H. C. Modification) that was more selective for coliform organisms than any media developed previously. Clark achieved the best results through using a two hour enrichment of the filter on Albimi M lactose medium before transferring it to the E. H. C. broth for an additional 14 hours. He postulated that the increased recovery of coliforms was due to the initiation of growth on a rich, nonspecific medium that reduced the organism's susceptibility to inhibiting substances present in the more selective medium (the fuchsin-sulfite dye system of the Endo medium) (27).

Shortly after the membrane filter technique was introduced in the United States, numerous studies were undertaken to evaluate the potential of the technique as a standard method for examining water quality. At the time, the Most Probable Number (MPN) technique was in use all over the country in accordance with procedures

stipulated in Standard Methods (9th Edition).

Hajna and Damon (28) believed that the two-step enrichment technique developed by Clark et al. (27) could be made simple for water and wastewater treatment plant operators by reducing the analytical procedures to a single step. Consequently, they developed a new medium composed of desoxycholate lactose broth, with a sulfite-fuchsin indicator system (M-HD Endo broth) to be used in the membrane filter technique. The sulfite-fuchsin indicator had been shown previously to cause coliforms to form a typical metallic sheen that made them easily identifiable. They showed that many of the false positives of the lactose broth tests in the MPN procedure could be eliminated by use of the new medium and the membrane filter technique.

Comparative studies of the membrane filter and MPN methods were also carried out by Kabler et al. (29) shortly after the work of Hajna and Damon was completed. They compared coliform recoveries with three different membrane filter media agars to recoveries by the presumptive and confirmed MPN procedures. The various membrane filter media consisted of the Albimi M enrichment and Endo broth described by Clark et al. (27), a modified Endo, and the modified Endo with brilliant green fuchsin and ethyl alcohol added. The MPN procedure involved a five-tube, three-dilution test with lactose broth, followed by confirmation in brilliant green bile broth. A variation was observed between the two techniques but the reported results did not indicate which method gave better recoveries. Kabler

suggested that the two procedures did not measure precisely the same group of organisms and the sanitary significance of the differences in the test should be determined. He also indicated the need for information on the possible effects of seasonal variations on the analyses.

Three years later, and one year after the publication of the 10th edition of Standard Methods for the Examination of Water and Wastewater in which the membrane filter technique appeared as a Tentative Method, Thomas et al. (30) investigated the advantages and limitations of the technique. A discussion of the statistical basis of the MPN procedure was presented, and its positive bias proven. They proposed a bias correction factor for the MPN test that was later used by many researchers in the correlation of their data. Thomas et al. also recommended coliform standards for the adoption of the membrane filter technique as an alternate standard method.

The advantages and limitations of the membrane filter technique were restated by Clark et al. (31) in 1957. Advantages included: 1) shortened testing time (24 hours) as opposed to MPN procedures (48 hours or more); 2) reductions in required materials, space, equipment, labor and preparation for the test; 3) an increase in the volume of water that may be processed; 4) increased preciseness of results; and 5) adaptability to the development of field testing. (A delay in incubating filtered samples is permitted). Many limitations in the technique centered around laboratory errors such as 1) inhibited growth when there is low humidity during incubation;

2) the possible presence of air bubbles between the membrane and the absorbent pad; 3) elimination of an enrichment period which is provided in the presumptive MPN test; 4) possible flooding of the membrane with media; 5) the appearance of excessive numbers of colonies on the membrane; and 7) the inability of untrained individuals to recognize the characteristic sheen of the coliform organisms. Other limitations are not so easily controlled. These include growth inhibition by zinc or copper compounds, iron, algae, or suspended matter that can become entrapped on the filter, as well as the presence in the sample of non-coliform organisms able to produce a typical sheen, or high background bacteria numbers able to grow on Endo-type medium. Clark et al. (31) stated that the latter two conditions were not frequent. The membrane-filter technique had also been shown to give lower enumerations than the MPN method, but these were probably due partly to the positive bias of the MPN test and the inhibiting action of some types of media.

In 1958, Fifield and Schaufus (32) developed a new medium that was found to give good recovery and sheen development for coliform organisms. Their study was prompted by the decided disadvantages inherent in the two types of media commonly in use at the time for membrane filtration: the E. H. C. - Endo broth of Clark et al. (27) and the M-HD Endo broth of Hajna and Damon (28). The two-step E. H. C.-Endo broth procedure, they said, was time consuming, and the M-HD Endo medium lacked clear-cut sheen differentiation for the intermediate coliform organisms. The new MF-Endo medium developed

by Fifield and Schaufus was demonstrated to give excellent sheen characteristics and approximately 92 per cent confirmation as coliform organisms.

In the same year, McKee et al. (33) studied the recovery of coliforms stressed by physical and chemical disinfection. The recovery techniques included the MPN and membrane filter methods. Both procedures gave comparable coliform recoveries when settled sewage was disinfected by physical means but the MPN procedure provided a much higher count when chemical disinfection by chlorination was employed. This discovery led to the hypothesis that the monochloramine, formed by the reaction of chlorine and ammonia in sewage, is the most predominant bactericidal agent in chlorinated sewages but is a weaker oxidant than free chlorine. Therefore, it provides only a bacteriostatic action that may be reversed if the cell is exposed to a favorable environment where it can repair its damaged enzyme systems. McKee et al. (33) also explained that free chlorine is able to completely oxidize the sulfhydryl radical of certain enzymes important in the utilization of glucose and thereby insures death of the cell. Monochloramine, however, only partially oxidizes the sulfhydryl radical to form disulfide linkages between enzymes. This process is reversible if the cell comes in contact with an aqueous medium rich in organic matter and amino acids that can restore the damaged enzyme systems. McKee suggested that the monochloramine molecule would be more likely to diffuse from the cell in an aqueous medium (such as the lactose broth of the MPN test)

rather than on the surface of a membrane exposed to the air and limited nutrients supplied solely by capillary action. These hypotheses were used often by later investigators to explain their results.

A large step forward was achieved in membrane filter studies by McCarthy and Delaney (34) in the late 1950's and early 1960's. They attempted to find the reasons for the deficiencies and variations in membrane filter results and to develop a suitable medium that would compensate for these variabilities. It was found that slow lactose-fermenting coliform strains (predominately non-fecal types) accounted for the majority of loss in sheen formation on membrane filter media. The degradation of lactose by coliforms caused the formation of aldehyde that was believed to be responsible for the production of the metallic sheen typical of coliform colonies. Through several experiments with the addition of various substances to the basic Endo broth medium, McCarthy and Delaney (35) formulated the LES-Endo agar medium still used today as total coliform differentiation medium. Their work was the first attempt to use an agar-based medium in membrane filter studies, and the increased recoveries as compared to a broth medium were encouraging to bacteriological researchers. In addition, sheen development on the agar after enrichment was found to be more pronounced and longer lasting than it had been on other media.

McCarthy et al. (36) also compared the reliability of plate count procedures to MPN and membrane filter procedures. They con-

cluded that the membrane filter technique provided the most precise coliform count and enabled the analyst to enumerate all strains of coliform organisms.

The membrane filter technique was adopted as a standard procedure in the 11th edition of Standard Methods in 1960. Subsequent testing with the technique usually involved more specific applications of the filters. In 1962, Delaney et al. (37) devised an agar to be used with membrane filters in the enumeration of fecal coliforms only. The tryptone, bile salt, agar medium had one disadvantage. After a 20-24 hour incubation at 44.5°C, the growing colonies were stained to indicate the indole-positive colonies, i.e., fecal coliforms. The stain, however, being bactericidal to the colonies, prohibited further processing (e.g. the completed coliform test) that may be desired. Another medium, the still popular M-FC medium was developed by Geldreich et al. (38) three years later for fecal coliform analysis by membrane filtration. Analysis with this medium did not require an enrichment period and provided fecal coliform recoveries comparable to the MPN method.

A delayed-incubation, membrane-filter test for fecal coliforms was prepared by Taylor et al. (39) that made possible the sampling of fresh waters far from the analytical laboratories. The results obtained by this new method closely approximated those of the direct tests outlined by the 13th edition of Standard Methods.

During the early 1970's, there was increased interest in stressed coliform analyses. Most research that has been conducted

in the past few years involving membrane filter techniques has centered around the evaluation of procedures and materials used in the technique and the development of new methods or materials that would increase coliform recoveries.

One of the principle procedures practiced in the United States in water and wastewater treatment plants is disinfection by chlorination. Many bacteria, including indicator organisms, may escape death during the treatment process but become severely stressed. Studies have shown that these organisms are extremely succeptible to a secondary stress such as placing them in contact with a selective media (3, 4, 5, 21, 40). If allowed a period of recovery on a rich, non-selective medium, such as lactose or lauryl tryptose, coliforms are able to repair their damaged enzyme systems and will be able to grow when they are placed on the selective medium.

Lin (40) compared coliform recoveries from chlorinated secondary effluents on membrane filters using the M-FC broth developed by Geldreich (38) and the enrichment and LES-Endo media developed by McCarthy (35). He compared both types of media to the standard MPN method. The results showed that the M-FC counts were well below MPN estimates of fecal coliform densities, but recoveries by the "enrichment and agar" (E & A) technique were similar to those obtained by the MPN method for total coliforms. Braswell and Hoadley (21) confirmed that lower estimates of actual fecal coliform densities in chlorinated secondary sewage are obtained when the M-FC medium is used. The studies raised serious questions about the

reliability of the standard M-FC medium. As a result, Hufham (41) conducted studies to determine the influence of different media, filters, and incubation temperatures on coliform recoveries. He found the large amount of error in fecal coliform recoveries was dependent on these variables. He tested Millipore type HAWG filters and Gelman type GA-6 filters. His media included M-plate count broth (Difco) and M-coliform broth (BBL) in addition to the M-FC medium, at either 35°C or 44.5°C. Optimum growth was not observed for a single set of conditions but seemed to vary with membrane type when different media and incubation temperatures were used. The results of the study were presented at the Environmental Protection Agency and American Society for Testing and Material "Symposium on the Recovery of Indicator Organisms Employing Membrane Filters" in September 1977 (42).

Other studies presented at the symposium also gave comparisons between membrane filters made by Sartorius, Gelman, Millipore, Nuclepore, and Johns-Manville (43, 44, 45). Results indicated better recoveries with the Gelman filters for fecal coliforms at elevated temperatures and with Millipore, Gelman and Johns-Manville filters for total coliforms at 35°C. Glantz (46) suggested at this time that membrane filter methods must be standardized in regard to temperature, media, and the strains recovered to make the results meaningful.

The realization that the use of selective media alone was not acceptable for the enumeration of indicator organisms exposed to

stress, brought about new possibilities for more accurate results in membrane filtration.

Rose et al. (4) made a slight modification in the standard M-FC broth that resulted in higher recoveries of fecal coliforms from raw and chlorinated wastewaters and waters from reservoirs, rivers, and oceans. Their medium consisted of a two layer agar; the bottom layer was standard M-FC broth with 1.5 per cent agar added. The top layer was lactose also with a 1.5 per cent agar base. Membrane filters placed on the lactose layer were allowed a two-hour incubation period at 35°C for cell repair before transfer to 44.5°C for an additional 22-24 hours. After several hours of contact between the lactose and M-FC layers, nutrients from the M-FC layer diffuse through the lactose to contact the growing cells on the membrane filter with the substances that cause selectivity for coliforms.

A similar technique was developed by Lin (40) for fecal coliforms that gave comparable results to those obtained by Rose et al. (4). Lin's procedure involved enrichment of the membrane filter on phenol red lactose broth at 35°C for four hours before transfer to M-FC agar and incubation at 44.5°C for another 18-20 hours. Tests using chlorinated sewage effluents showed the two-step membrane filter method to be equivalent to the MPN procedure in fecal coliform recovery efficiency.

The standard procedure involving M-FC medium was modified and evaluated also by Green et al. (5) who conducted laboratory and

field evaluations of coliform recoveries from chlorinated primary and secondary effluents. They incubated samples at 35°C for five hours, then at 44.5°C for 18-19 hours. The only medium used was M-FC agar. The two-step temperature procedure resulted in higher fecal coliform counts than those incubated at only 44.5°C, but they were lower than population estimates by the MPN procedure by a ratio of 1:1.5. This was a decided improvement, however, over recoveries with the standard M-FC procedure which were seven times lower than those used by the MPN technique.

Stress to indicator organisms in the aquatic environment has recently been evaluated using the membrane filter technique by Bissonnette et al. (47) and Stuart et al. (48). The studies conducted by Bissonnette and associates showed recoveries of fecal coliforms from pure cultures and raw sewage that had been exposed to stream environments for several days. They evaluated membrane filter procedures which had both enriched and non-enriched steps and used M-Endo agar and M-FC medium as well as TSY agar. Tests with M-FC medium without prior enrichment yielded the lowest coliform counts. The highest counts were obtained when TSY agar and M-Endo agar were used following a two hour enrichment at 35°C in TSY broth. Another important factor in coliform recovery was noted by Bissonnette: the size of the filter surface pores may contribute significantly to the success of recovery. Their studies had employed Millipore type HAWG filters with a mean pore size of 0.45μm. They recognized the possibility that new filters being

designed with a surface opening diameter of 2.4μm and a retention pore size of 0.7μm could dramatically increase the coliform counts they obtained (49).

Stuart et al. (48) developed a membrane filter procedure, which they named the "injury-mitigating membrane-filter" (IM-MF) technique, for recovering stressed organisms. The medium used to recover coliforms from chlorinated sewage effluent was standard M-FC agar to which glycerol and acetate (metabolic intermediates) and chlorine reducing agents were added. The metabolic intermediates were believed to aid the bacteria in repairing their injured systems, while the reducing agents (sodium thiosulfate, thioglycolate, and glutathione) aided in reduction of chlorine and repair of cellular components oxidized by the exposure to chlorine. These workers also evaluated a procedure which involved rinsing the filter with a rich, resusitation medium after filtration and incubating the plates first at 22 to 25°C (room temperature) for 1.5 hours, then at 35°C in an incubator for 4.5 hours, and finally in a water bath at 44.5°C for 18 hours. This technique was compared to the standare M-FC and MPN procedures. Coliform recoveries by their technique were equal to or better than those by MPN procedures in half the trials and were consistently better than those obtained by the standard M-FC method.

Previous work in our laboratory (50, 51, 52) demonstrated the value of a modified membrane-filter (M-MF) technique over the standard (S-MF) techniques for quantification of fecal coliforms. The modified technique employed a lactose agar overlay of M-FC agar

and an enrichment period of five hours at 35°C. Final incubation was at 44.5°C for 18 hours. The three studies included enumeration of fecal coliforms from urban runoff and chlorinated sewage effluents, and those exposed to heavy metals. The M-MF technique gave consistently higher recoveries than the S-MF and comparable recoveries to the MPN procedure in urban runoff and chlorinated sewage effluents.

Some New Inovations in Coliform Recovery Technique

Since 1975, research has been initiated to enumerate the bacteriological quality of water employing methods other than those that have been in use for years. The ultimate goal of such research would be the replacement of the more conventional methods with rapid and reliable tests that are simple and inexpensive. Two such examples are given here, although many more possibilities have been and are being examined. The Limulus lysate assay developed by Levin and Bang (52) was investigated as an alternative to conventional methods of bacteriological water quality analysis by Evans and associates (53). The technique detects the endotoxin (lipopolysaccaride) found in cell envelopes of Gram-negative bacteria. The endotoxin will coagulate the Limulus lysate solution. Gram-positive bacteria do not have this endotoxin. The bacteria detected by this test include coliforms and enteric and heterotrophic bacteria. An alternate method of measuring the endotoxin levels is the Spectrophotometric determination of turbidity in the test tube. Simultaneous plate counts performed on the samples showed a comparable

correlation between the lysate assay and the plate counts. The author stated that commercial kits that employ this method of enumeration are already available to bacteriologists. The value of this method in potable water quality determinations seems questionable because of the non-specificity of the test for coliforms (established pollution indicators).

Another method developed by Warren et al. (54) involves a colorimetric procedure for the observance of β-galactosidase (enzyme) activity in fecal coliforms. A water sample is incubated in standard EC medium at 44.5°C for fecal coliform selectivity, and the hydrolysis of o-nitrophenyl-β-O-galactosidase (ONPG) is measured colorimetrically at varying time intervals. The theoretical basis of the test relates the level of ONPG hydrolysis to fecal coliform numbers in the medium. Other non-E. coli strains should give little interference since their growth is inhibited by the temperature and media specifications of the test conditions. In cases where stressed fecal coliforms may be encountered, the medium may be modified by withholding the bile salts until after the preincubation period.

III. MATERIALS AND METHODS

Laboratory studies were conducted between June 19, 1978, and August 21, 1978, in the Sanitary Engineering Laboratory at Virginia Polytechnic Institute and State University.

Four prepared cultures of coliform bacteria were inoculated into a synthetic water sample (prepared in the laboratory to simulate a finished water) containing known free and total chlorine residuals. Samples were removed from the test water and analyzed by the enrichment-step technique and the single-step technique (2) for total coliforms, to be described. Procedures and aseptic techniques were followed according to Standard Methods for the Examination of Water and Wastewater 14th Edition (2) at all times unless otherwise indicated. Sterilization was obtained by autoclaving at 121°C for 15 minutes.

Preparation of the Experimental Culture

The work of Clark and Pagel (55) concerning bacteria in municipal sources of raw and finished drinking water was used as the guide for determining the types and relative ratios of coliforms to be used in this study. They found that four coliform genera were most prevalent in raw waters in the following approximate ratios:

Enterobacter, 45 per cent; Escherichia, 20 per cent; Citrobacter, 15 per cent; and Klebsiella, 20 per cent.

Single strains of three coliforms: Enterobacter aerogenes (ATCC 13048), Escherichia coli (K12), and Klebsiella pneumoniae (ATCC 13883) were obtained from the microbiology department at Virginia Polytechnic Institute and State University. The fourth coliform, Citrobacter freundii (strain unknown), was procured from Carolina Biological Supply of Burlington, North Carolina. The strains were stored on nutrient agar (Difco) slants at approximately 4°C. They were kept viable by streaking a new slant from the stored slant every week and incubating the subculture overnight at 35°C.

Changes in population densities with time of growth were determined by inoculating 50 mls of nutrient broth (Difco) from the stored slant, incubating the broth at 25°C, and making plate counts on nutrient agar at intervals of every hour for eight hours. A final plate count was made at twelve hours. Cultures were incubated at 25°C because laboratory experiments, to be described, were carried out at 20°C, and the elimination of a possible thermal shock to the coliforms was desirable. The results of these growth curves are shown in Figure 1. From this procedure, it was found that eight hour cultures yielded the following populations: Enterobacter, 2×10^8 cells/ml; Escherichia, 2×10^7 cells/ml; Citrobacter, 2×10^7 cells/ml; and Klebsiella, 2×10^7 cells/ml. The cells were in mid-log growth phase at this time.

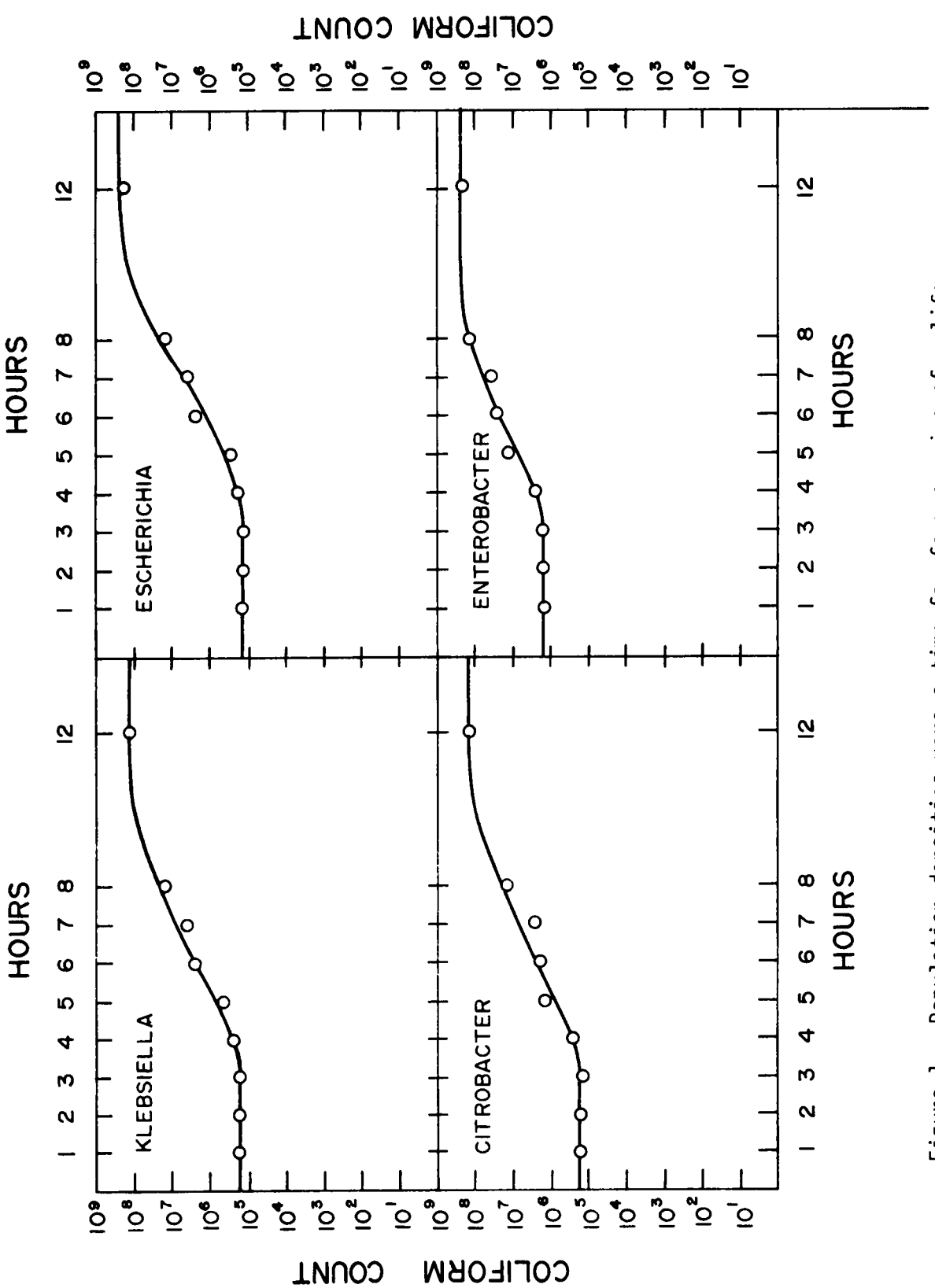

Figure 1. Population densities versus time for four species of coliforms grown in nutrient broth.

The mixture of bacteria used in these experiments consisted of 45 per cent Enterobacter, 20 per cent Escherichia, 15 per cent Citrobacter, and 20 per cent Klebsiella and was prepared by delivering 0.05 mls, 0.20 mls, 0.15 mls, and 0.20 mls of the above eight hour cultures, respectively, into 100 ml of sterile phosphate buffer. A 10-ml portion of this bacterial suspension was centrifuged at 8000 rpm in a clinical centrifuge (Fisher Scientific Model 28158H) for 30 minutes. The cells were then resuspended in 10 ml sterile phosphate buffer and recentrifuged. This washing procedure was repeated three times.

A plate count of the washed cells was done on nutrient agar (Difco) to determine the approximate size of the test inoculum.

Preparation of the Synthetic Test Water

All testing was carried out in a defined water sample. The water was prepared 24 hours ahead of time in large glass bottles that had been allowed to stand overnight containing a 10 mg/l chlorine solution to render them chlorine demand-free. The bottles were then rinsed 3 times with glass-distilled water before the addition of two liters of the glass-distilled water to each bottle. Calcium chloride and sodium bicarbonate (40 mg/l) were added to provide hardness and alkalinity. Before each experiment, the test water was cooled to approximately 20°C.

A chlorine residual was established in the prepared water sample by adding enough of a stock chlorine solution (2000 mg/l) to provide the desired residuals. The chlorine stock solution was prepared

using high-test hypochlorite (HTH, 65 per cent available chlorine). Cell viability at three chlorine residuals were evaluated: "high", approximately 0.5 mg/l; "medium", approximately 0.3 mg/l; and "low", approximately 0.1 mg/l free chlorine. A single test at 1.0 mg/l free chlorine was conducted in preliminary tests, but the concentration proved to be lethal to all organisms in a few seconds. Total chlorine was also measured immediately following free chlorine determinations. All chlorine analyses were according to Standard Methods (2) Section 411C using the Fischer and Porter Amperometric Titrator, Model 17T1010. The pH was adjusted to approximately 6.5 to 7.5 by the addition of sulfuric acid (six to eight drops of one normal). Measurements were made with a pH meter (Fisher Scientific Accumet, Model 230).

Chlorine Stress Procedure

The washed cell suspension (0.12 ml yielding approximately 3×10^3 cells/100 ml) was inoculated into a chlorine demand-free beaker (prepared in the same manner as the water bottles) containing 1200 ml of the prepared water. The free and total chlorine residuals were determined just prior to inoculation. Within the first five seconds after inoculation, 100 ml of the test water was poured into a milk dilution bottle containing one ml of sterile, one per cent sodium thiosulfate to neutralize the chlorine action. From this bottle, dilutions ranging from 10^{-1} to 10^{-2} were made into sterile, 90-ml phosphate buffer blanks and set aside. At 5 and 15 minutes, only one dilution (10^{-1}) was made. Dilutions were found to

be unnecessary for medium and high residual tests. Phosphate buffer with magnesium sulfate was used because it has been found to improve the recovery of organisms with metabolic injury (56).

During the same timed sequence, 200 ml of water were removed from the test beaker after 2, 5, 10, and 15 minutes for measurement of free and total chlorine residuals.

Immediately following the chlorine exposure, the serial dilutions were analyzed for surviving coliforms by the membrane filter technique.

Total Coliform Enumerations

The single-step coliform recovery technique (2) involved the filtration of 10-ml portions of three replicate samples from each prepared dilution. Samples were filtered through factory-delivered, presterilized membrane filters (Millipore Corporation, type HC, surface opening size 2.4μm and retention pore size 0.7μm) using a multiple-funnel, vacuum-filtration apparatus (see Figure 2). The apparatus included six Gelman magnetic filter funnels (47 mm) attached to a plexiglass support panel. The funnels were mounted with valves between each funnel so that they could either be closed off separately from the Gelman pressure/vacuum pump (Model 13152) or be used simulateously. For filtration, approximately 20 ml sterile phosphate buffer was added to the filter funnel to wet the membrane filter and allow even dispersion of the bacteria on the filter upon addition of the 10-ml sample to the funnel (56). After filtration, each funnel was rinsed well with sterile phosphate buffer

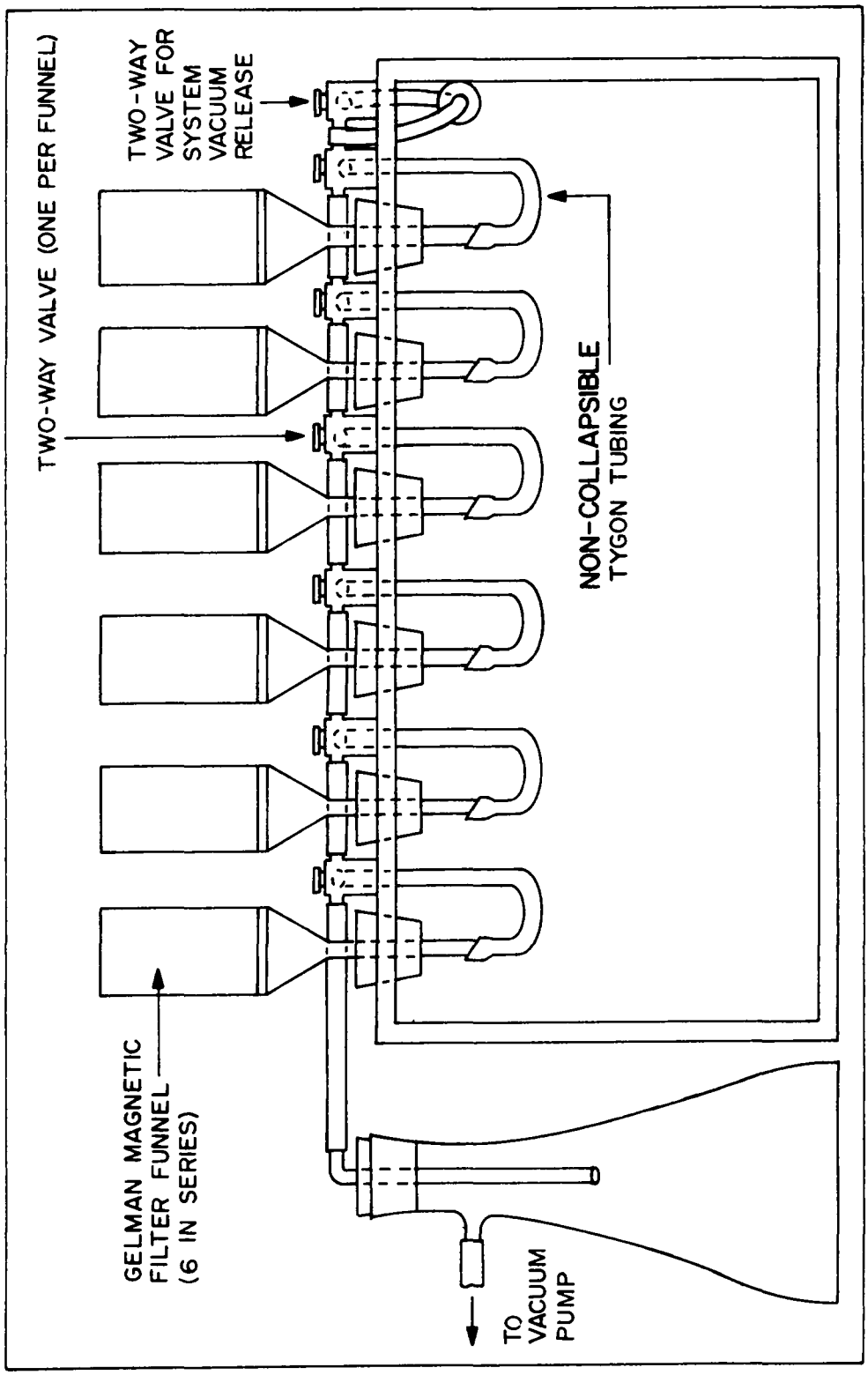

Figure 2. Membrane filter apparatus--after Brown (50).

before removal of the membrane filter. Between each filtration, the funnel was again rinsed with sterile, glass-distilled water. Possible filter contamination was occasionally tested by control filters taken from the final funnel wash. The filtration funnels and support screens were sterilized between runs by exposure to ultraviolet light for 1.5 hours.

The samples analyzed by the enrichment step technique were filtered in the same manner. Since three replicate samples of each dilution were analyzed for both recovery procedures, all six Gelman funnels could be utilized simultaneously to filter one dilution: three funnels for the single-step procedure and three for the enrichment-step procedure (see Figure 3).

After filtration was completed, the filters used in the single-step test were placed in 50 mm Gelman Petri dishes containing 4 ml of M-Endo Agar LES (BBL M-Endo Agar LES plus 0.02% ethanol) and incubated for 24 hours at 35°C. Filtered samples analyzed by the procedure involving enrichment were incubated for 2 hours at 35°C on 4 ml of lauryl tryptose agar prepared by the addition of Difco granulated agar (1.5 per cent) to lauryl tryptose broth (Difco). The filters were then transferred to M-Endo Agar LES and incubated an additional 22 hours at 35°C. Control filters were incubated along with the other samples.

Plate counts were facilitated by the use of a Bausch and Lomb dissecting microscope (magnification 15 x) and a fluorescent light source positioned at a 60° to 80° angle to the microscope stage to

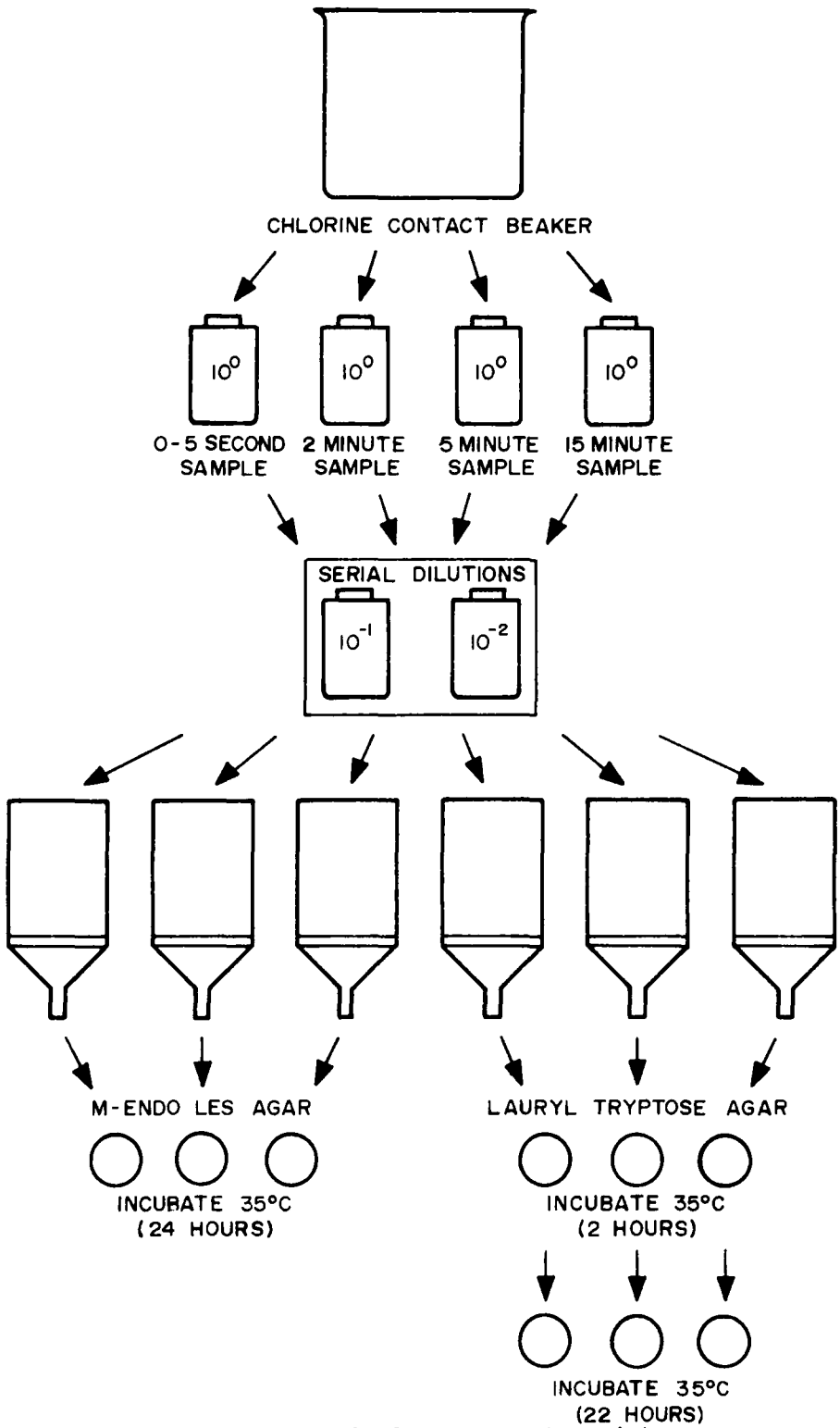

Figure 3. Protocol for single-step and enrichment-step membrane filter techniques.

permit optimum reflection of the metallic sheen of the coliform colonies (56). Colonies failing to produce a characteristic sheen were confirmed as coliforms in brilliant green bile broth (2).

For potable water sources, Standard Methods (2) recommends counting only those plates with 20 to 80 coliform colonies and a background bacteria count of no greater than 200 colonies for best statistical results. A high non-coliform count will inhibit the sheen-forming ability of any coliforms that may be present in the sample. In this study, care was taken to insure that the prepared cultures of coliforms would be the only bacteria to be enumerated by the plate counts. Therefore, since there was no interference with a background bacteria level, the plates from the highest dilution in which the colonies remained well defined were enumerated.

IV. RESULTS OF STUDY

Overview

During a two month period, nineteen tests were completed in which a population of coliforms was exposed to varying chlorine residuals and contact times. Subsequent enumerations of the stressed population of coliforms were made by two methods. The results of these tests are presented in this section. A comparison of the coliform recovery capabilities was made between:

1. The enrichment-step membrane filter method (E-MF).
2. The single-step (non-enrichment) membrane filter method (NE-MF).

The pH and temperature, and changes in free and combined chlorine residuals were closely monitored during the testing. These data are included also.

Chlorine Stress Determination

Chlorine residuals. The concentrations (mg/l) of free and combined chlorine usually varied only slightly during the timed tests. Data from each trial, showing the initial free and combined chlorine residuals and again 15 minutes after inoculation of the test waters are presented in Appendix Table A-I for the low chlorine residual tests, and in Appendix Tables A-II and A-III for the medium and high residual tests, respectively. In some tests, as is noted

in each table, there was a relatively large chlorine demand. This was found to be caused by impurities in the deionized, glass-distilled water which most likely were contributed by a contaminated organic-removal column on the distillation apparatus. Use of the water from the particular apparatus was discontinued and the chlorine demand decreased significantly in later tests. It was assumed that the demand observed in the later tests was caused by the bacterial inoculum.

Total coliform enumerations. An overview of the coliform enumerations by the enrichment step and non-enrichment membrane filter procedures is presented in Figure 4 for all chlorine residual levels and contact times. Total coliform recoveries were slightly higher by the E-MF procedure after five seconds of chlorine contact but no improvement in coliform recoveries was observed by the E-MF at contact times between 2 and 15 minutes. Prior to inoculation of the test water with coliforms, a plate count was made on nutrient agar by the spread plate procedure to determine the size of the inoculum. The results of these plate counts are presented in Appendix Tables A-I, A-II, and A-III for all trials. The same tables include the averages of triplicate plate counts by the E-MF and NE-MF procedures for coliforms in the test waters at all chlorine residuals and contact times tested. Individual membrane filter plate counts for each test are presented in Appendix Tables A-V through A-VII. Colonies that failed to produce a characteristic sheen were submitted to confirmation in brilliant green broth in the pre-

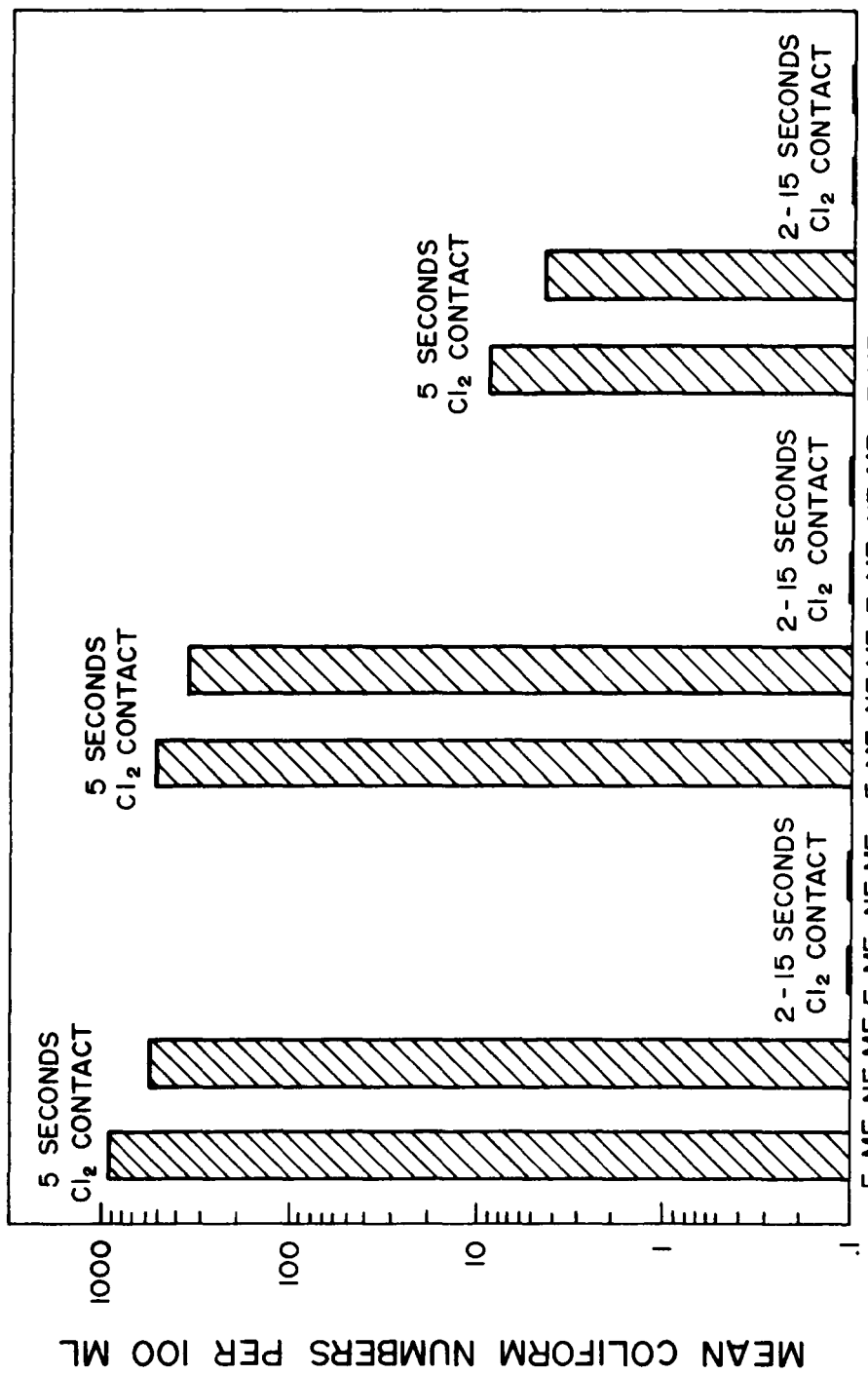

Figure 4. Mean coliform numbers for both enrichment-step and non-enrichment membrane filter plate counts from chlorine demand-free synthetic waters containing low, medium, and high free chlorine residuals.

liminary testing. All colonies tested gave positive confirmation as coliforms.

Results are reported as total coliforms per 100 ml. In 15 out of 19 trials, the plate counts by the E-MF procedure exceeded those obtained by the NE-MF by an average of 1.6:1 (the two large ratios, 13:1 [Trial 1, Appendix Table A-II] and 79:1 [Trial 4, Appendix Table A-III] were not included in the averages because they were regarded to be anomalous values). Mean ratios of the E-MF to the NE-MF are presented for each chlorine residual tested in Appendix Table A-I through A-III. In three trials (2, 4, and 5; Appendix Table A-I) the plate counts (pour plate procedure) used for estimating the size of the inoculum were less than those which were made by membrane filter techniques to enumerate the coliforms in the test water soon after inoculation. However, these anomalies were few. Possible reasons for this problem will be considered in the next chapter.

Measurement of physical conditions. The pH was maintained between 6.5 and 7.5 during all trials to insure that the bacteria were not stressed by acidic or alkaline conditions. Temperatures were kept near 20°C. Temperature and pH values, as well as those for free and combined chlorine, and the mean ratios of counts obtained by the two membrane procedures (E-MF and NE-MF) for each test are presented in Appendix Table A-IV.

Graphical comparison of the data. Nutrient agar and membrane filter plate counts were used to construct the coliform die-away

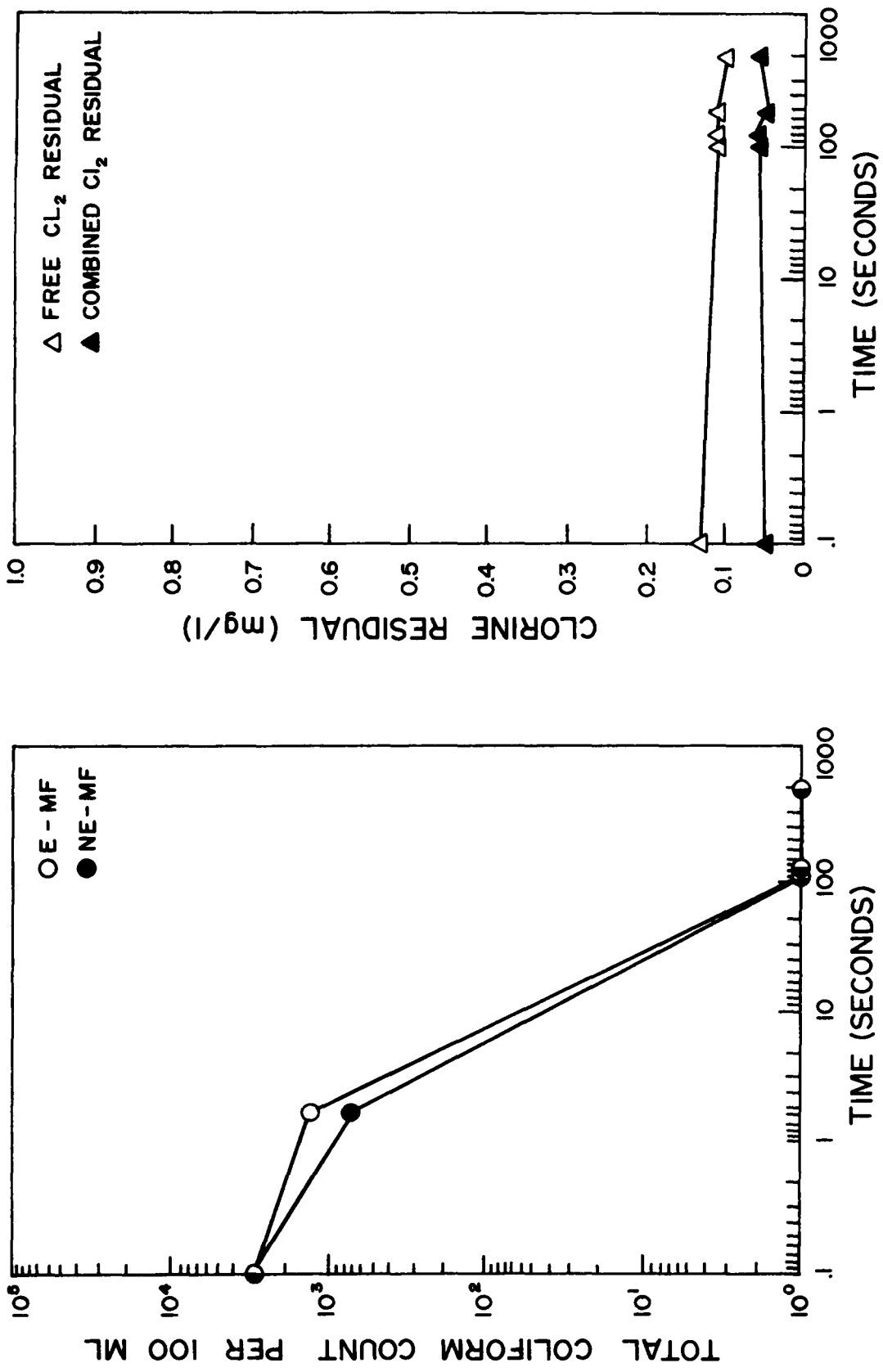

Figure 5. Changes in free and combined chlorine residuals and in coliform numbers as enumerated by an enrichment-step versus a non-enrichment membrane filter method during a low chlorine residual test, Trial # 3.

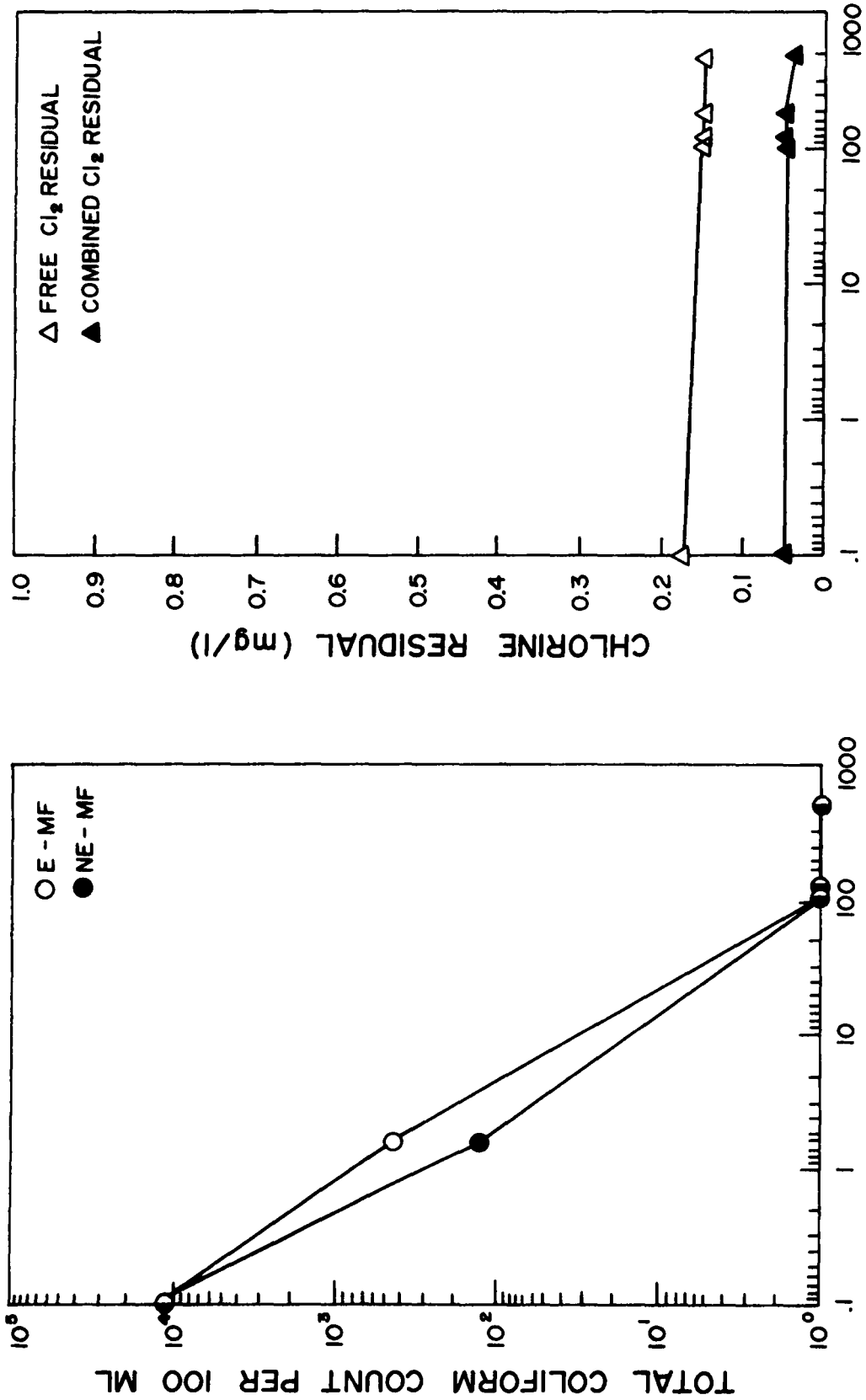

Figure 6. Changes in free and combined chlorine residuals and in coliform numbers as enumerated by an enrichment-step versus a non-enrichment membrane filter method during a low chlorine residual test, Trial # 6.

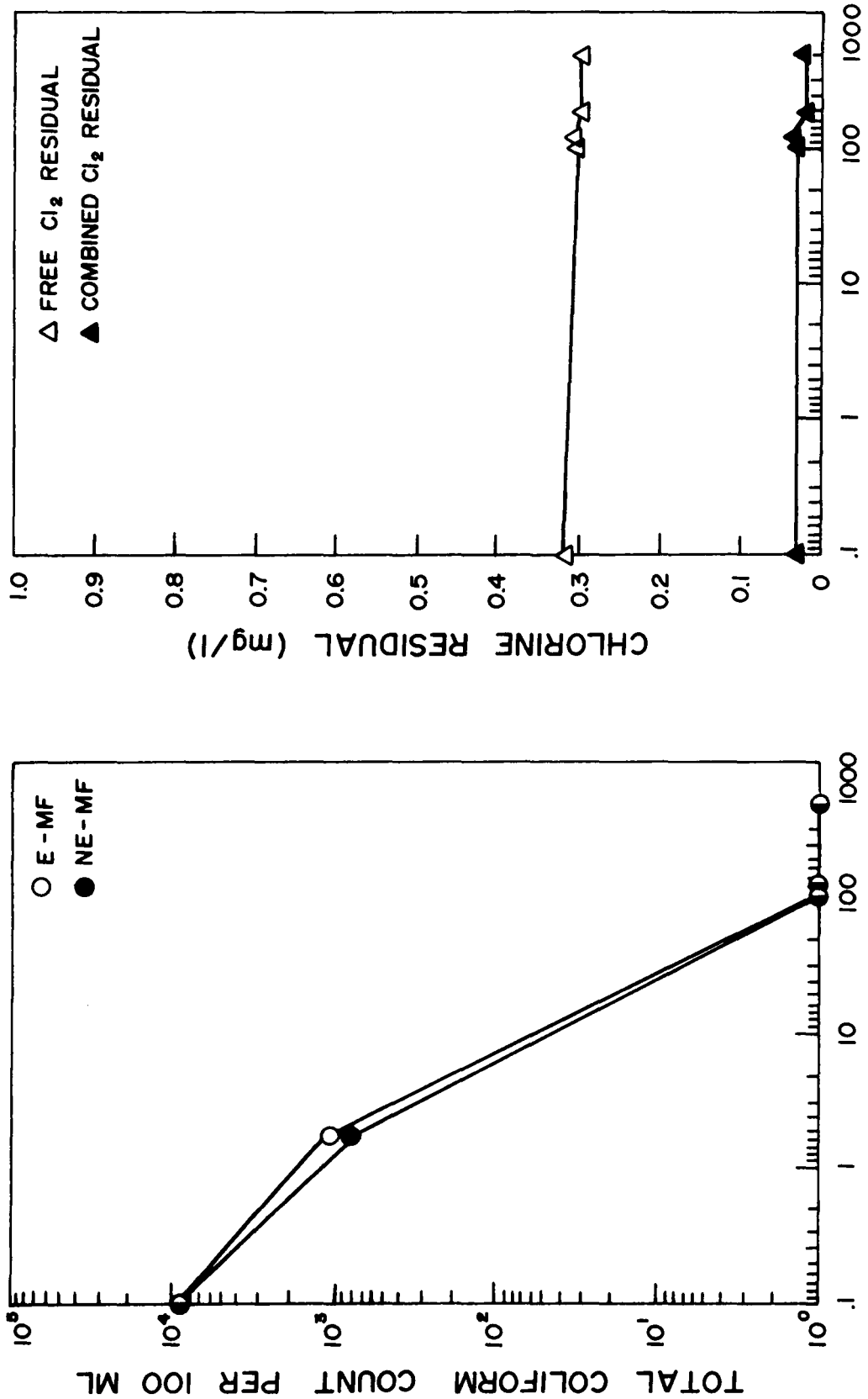

Figure 7. Changes in free and combined chlorine residuals and in coliform numbers as enumerated by an enrichment-step versus a non-enrichment membrane filter method during a medium chlorine residual test, Trial # 4.

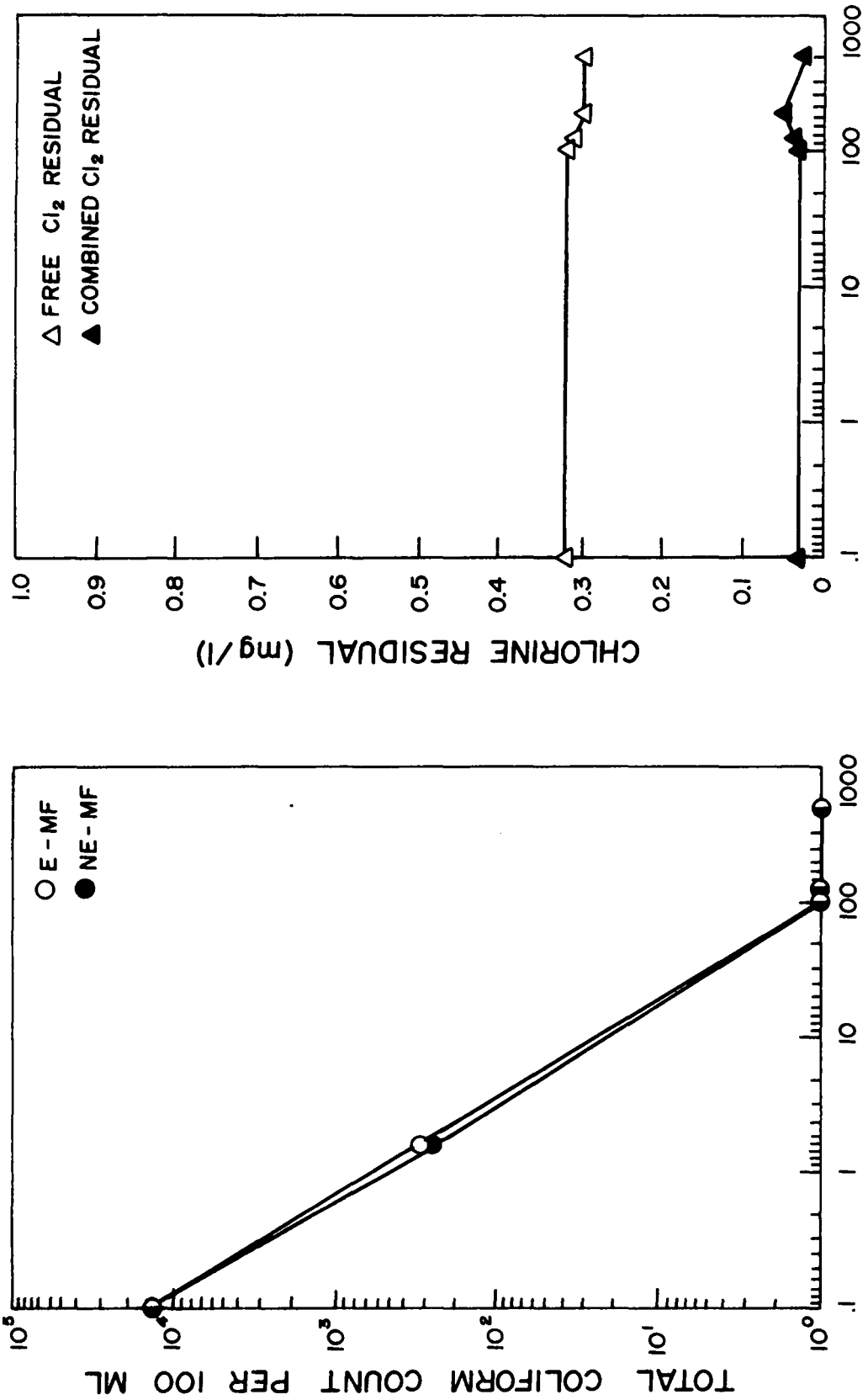

Figure 8. Changes in free and combined chlorine residuals and in coliform numbers as enumerated by an enrichment-step versus a non-enrichment membrane filter method during a medium chlorine residual test, Trial # 3.

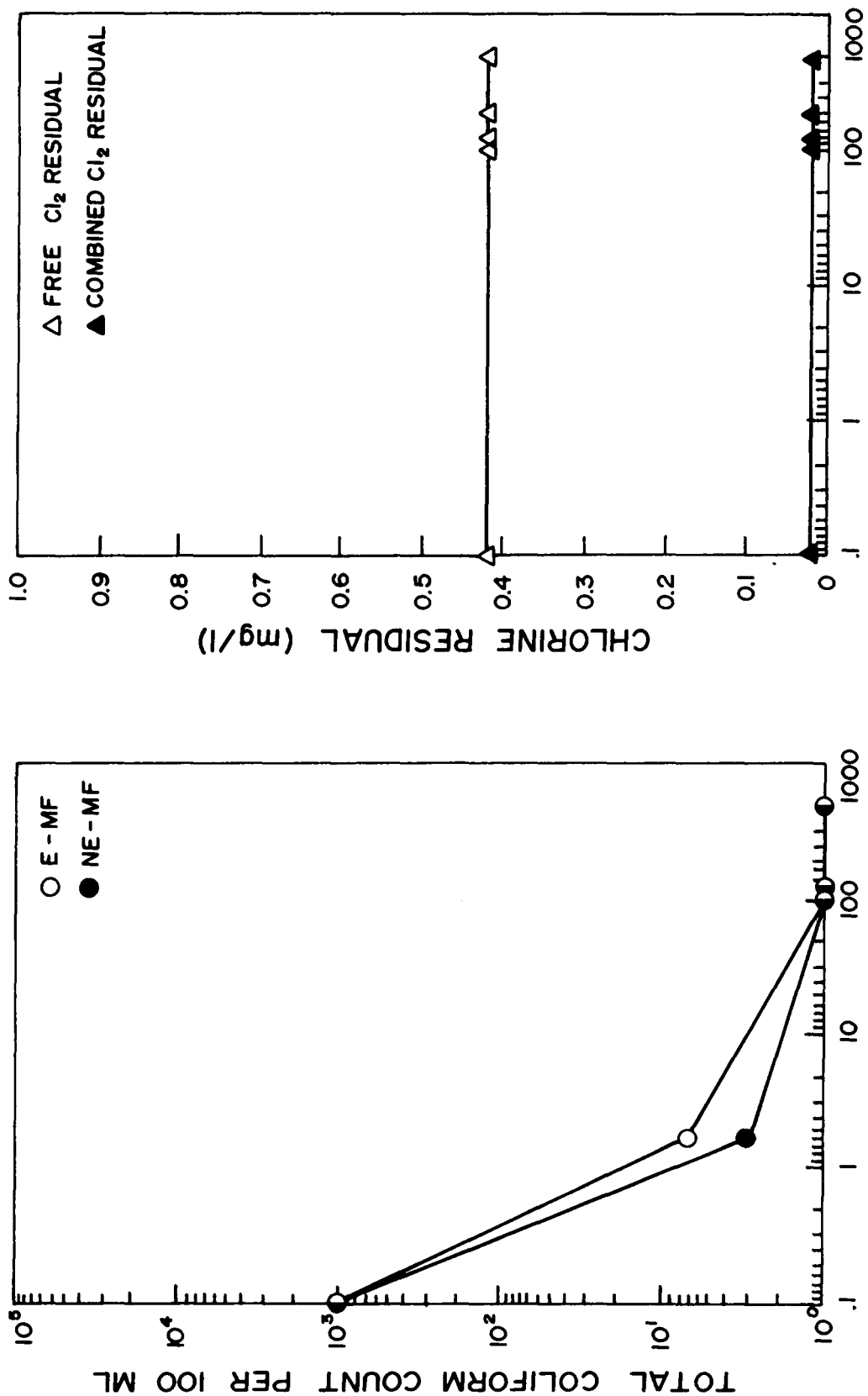

Figure 9. Changes in free and combined chlorine residuals and in coliform numbers as enumerated by an enrichment-step versus a non-enrichment membrane filter method during a high chlorine residual test, Trial # 1.

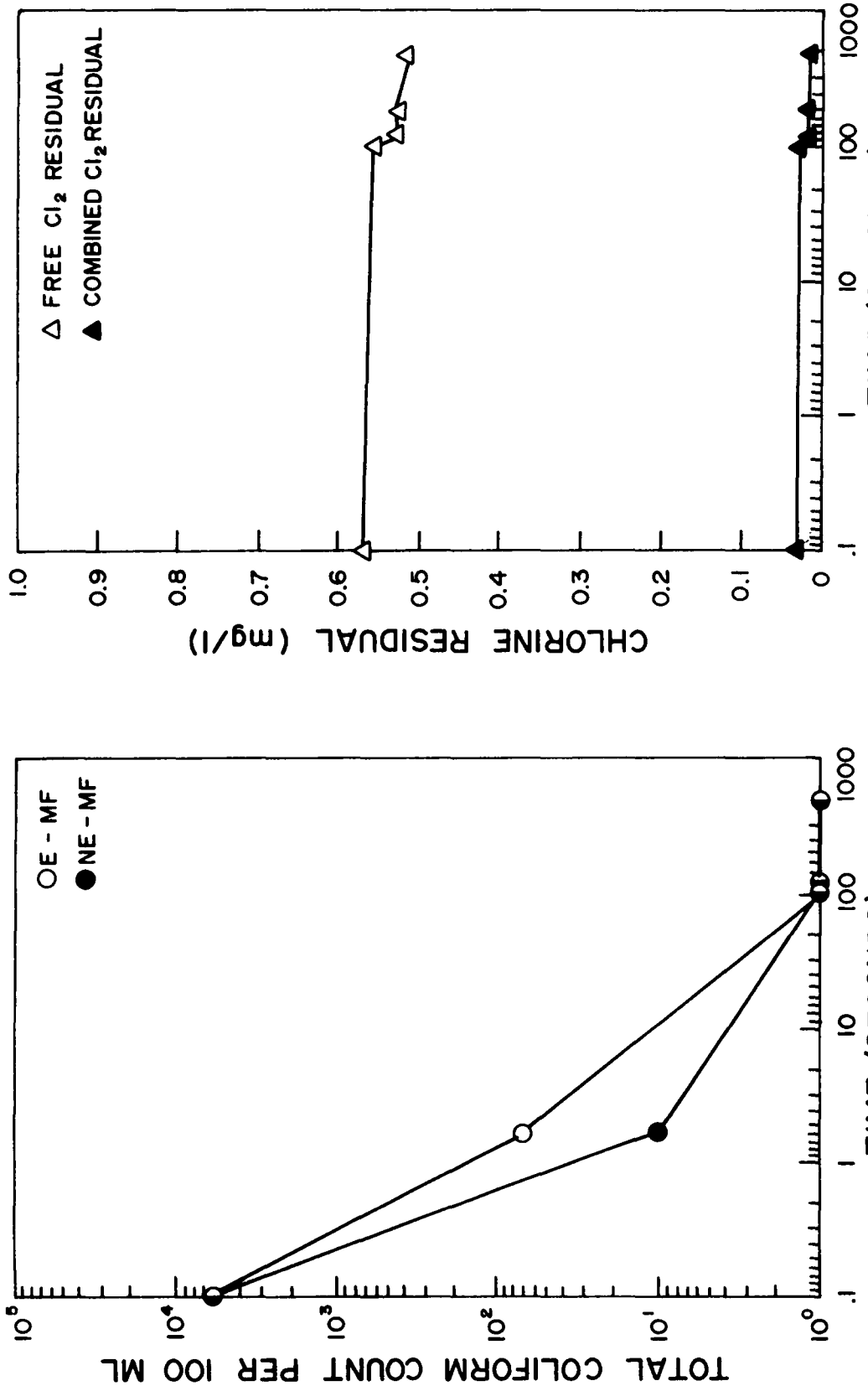

Figure 10. Changes in free and combined chlorine residuals and in coliform numbers as enumerated by an enrichment-step versus a non-enrichment membrane filter method during a high chlorine residual test, Trial # 6.

curve encountered in each chlorine stress test. These curves and the corresponding chlorine residuals for all contact times are presented for each of the nineteen trials. Two representative trials from the low, medium, and high chlorine residual tests are shown in Figures 5 through 10. Data derived from all other trials appear in Appendix Figures B-I through B-XIII. The results of testing in a synthetic water containing a chlorine demand and an initial free chlorine residual of 0.11 mg/l are presented in Figure 5. Figures 6-10, presented in the results section, represent testing carried out using mid-log growth phase coliforms in chlorine demand-free synthetic water containing free chlorine residuals ranging from 0.13-0.57 mg/l.

V. DISCUSSION OF RESULTS

The purpose of this study was to compare the coliform recoveries by two methods involving membrane filtration. Any discussion concerning the superiority of one method over the other should include considerations of the physical and chemical conditions that prevailed during the tests. The discussion of results obtained during this study has been divided into three sections.

In the first, consideration is given to the individual effects of the major experimental variables on disinfection. In the second, some of the variables are considered together in the effects they may have on the disinfection process. Finally, the experimental data are discussed in terms of their applicability to actual water treatment situations.

The Effects of Individual Experimental Conditions on Coliform Recoveries

The effects of varying chlorine residuals. Total coliform counts varied considerably from test-to-test, even within a single chlorine residual range. An attempt was made to keep the initial inoculum within a certain boundary (10^3 - 10^4 cells per 100 ml), but even so, large fluctuations resulted in the plate counts.

Though the plate counts varied from test-to-test, the ratio of coliforms enumerated by the E-MF method to those enumerated by the NE-MF method remained relatively constant (the average ratio of

E-MF: NE-MF being 1.6:1). Statistical analyses were not performed on the data to determine the significance of the ratio, but there were consistently higher recoveries with the E-MF procedure as compared to the NE-MF procedure. In one case, the NE-MF procedure gave higher plate counts than the E-MF method [NE-MF: E-NF was 1.5:1 (Trial 2, Appendix Table A-III)]. While the explanation for these results is not readily apparent, it may be associated with several physical factors which will be discussed later.

The predominant factor to be noted from this study is the rapid decrease in recoverable coliforms in the first two minutes of exposure to chlorine at all residuals. The trials in which the two lowest residuals were present, 0.11 mg/l and 0.12 mg/l free chlorine (initial concentration), were the only ones in which coliforms were recovered at each of the three contact times during the total exposure period of 15 minutes (Appendix Table A-I). The uninoculated test water used in these particular trials had a chlorine demand, however, and this demand may have diminished the disinfectant concentration rapidly enough to permit some cells to survive. This explanation seems likely because another test in which the free chlorine residual was 0.13 mg/l and the inoculum size was the same resulted in the rapid die-away that was representative of all subsequent tests.

The possibility also exists that under the conditions of this study, free chlorine residuals between 0.12 and 0.13 mg/l caused an

increased cell response to the disinfecting action of chlorine, but this would be difficult to prove due to the inability of present methods to accurately detect such small changes in chlorine residual. The pH of the reacting medium is known to affect the equilibrium concentrations of the chlorine species to such an extent that slight changes can dramatically alter the disinfecting effectiveness of a given free chlorine residual. The effects of the pH on chlorine residuals are discussed in more detail in a later section of the chapter.

Free and combined chlorine residuals remained relatively constant throughout the entire chlorine-contact period (15 minutes) except in those trials where the test water had a chlorine demand. The demand was probably due to the presence of organics, as was previously mentioned in the Results chapter. Combined chlorine residuals remained consistently well below the 0.1 mg/l concentration in all tests except those in which the test water contained a chlorine demand. When a chlorine demand was present, combined residuals increased steadily during the test, at times exceeding the free chlorine residuals. This increase in combined residual is normally due to the free chlorine combining with either organic compounds or ammonia (or both) in the water. When little or no chlorine demand was present in the test water, the combined chlorine residuals remained in the 0.02 to 0.06 mg/l range.

The changes in the chlorine residuals during testing with demand-free water, however small, may have been due to two factors: 1) the inoculum created a chlorine demand or 2) the method of analysis (amperometric titration) was inaccurate. The inoculum used during this study varied from 1,000 to 67,000 cells per 100 ml. Comparisons of the changes in chlorine residuals when two such widely variable inoculum sizes were used show that inoculum size could affect the change in chlorine concentration observed during the exposure period. For instance, this inoculum in trial number six (Appendix Table A-II) was 40,000 cells/100 ml. The free chlorine residual decreased from 0.33 mg/l to 0.28 mg/l during the 15-minute test period. In trial number one (Appendix Table A-III), 1,000 cells/100 ml were inoculated into the test water which contained a free chlorine residual of 0.42 mg/l. After 15 minutes, the free chlorine concentration remained 0.42 mg/l. Both tests were carried out at a pH of 7.0. Similarities were evident in the other trials.

The results that seemed the most questionable were the differences that were observed between the numbers of cells inoculated, and the numbers recovered within five seconds by the membrane filter techniques. In some cases, neither the differences in initial chlorine concentrations nor the rates of chlorine demand exertion seemed sufficiently great to cause the amount of variation observed in the plate counts between trials. For example, during trial number four (Appendix Table A-II), the initial

free chlorine concentration was 0.32 mg/l and the combined residual was 0.03 mg/l. The inoculum size was 9,000 cells/100 ml. The inoculum during trial number five (Appendix Table A-II) was 8,000 cells/100 ml and the initial chlorine residuals were 0.32 mg/l free and 0.06 mg/l combined chlorine. After five seconds, only one per cent of the initial inoculum of 8,000 cells/100 ml during trial five remained, but during trial four, 20 per cent of the original 9,000 cells/100 ml survived. The tests were conducted at pH 7.0 and pH 7.1 for trials five and four, respectively. The counts obtained by the membrane filter procedures showed a 1.2:1 ratio (E-MF: NE-MF) for trial four and a 3.7:1 ratio for trial five. Similar results are evident when results from other trials are compared.

In some of the tests where low chlorine residuals were present, the initial population was smaller than the subsequent plate counts obtained by the membrane filter technique. When results of the plate counts by membrane filtration were compared independently of the initial population density in the test media, the ratios of coliforms recovered by the E-MF and NE-MF procedures remained reasonably constant. This fact indicates that the results obtained by the spread plate technique were probably spurious for reasons that will be explained: Since only 0.1 ml of inoculum is placed on the agar during the spread-plate procedure, even small differences in volume can cause large discrepancies in the final

plate counts. The use of the membrane filter technique permitted larger volumes of sample (10-ml portions) to be used, therefore reducing the probability of obtaining unrepresentative plate counts. Other possible sources of variation between the plate counts obtained by the two procedures may be 1) inadequate mixing of the cells. Improper mixing of the inoculum and test water could result in an uneven bacterial distribution and, hence, either a higher or lower coliform estimate of the number of cells than actually was present. Clumping of the cells during the spread plate procedure could result in plate counts that are too low, whereas clumping during the chlorine stress procedure could tend to diminish the chlorine effectiveness and be reflected by an increase in plate counts. Of all these possible explanations, the more plausible one is that the spread-plate techniques was the source of error and, thus, the estimates of the initial cell population were incorrect.

As was noted previously, in the cases where the test water had a chlorine demand, the disinfection action of the chlorine could have been diminished by reacting with trace quantities of organics present in the water (organics other than those associated with the bacteria). This was evident in the tests involving low chlorine residuals (Appendix Table A-I). At the higher residuals, the disinfecting power of chlorine was not affected (Appendix Table A-III) because sufficient excess apparently was available

to meet the demand and kill the cells as well.

It should be noted that the discussions concerning coliform enumerations after chlorine stress are based on the plate counts made after only five seconds of chlorine contact. At all other contact times with free chlorine residuals in excess of 0.12 mg/l, there were no surviving cells on any of the plates, indicating that all the coliforms were injured sufficiently that they could not recover, even with enrichment, at some period between five seconds and two minutes after the initial exposure to chlorine.

The rapid coliform die-away exhibited in all tests indicates the effectiveness of disinfection at very low, free chlorine residuals (0.10 - 0.15 mg/l). The tests were conducted in water that was relatively chlorine demand-free in order to standardize the experimental conditions. It is believed tha the rapid die-away would be observed in any potable water containing free residuals of at least 0.10 - 0.15 mg/l. One caution that must be observed is the fact that the membrane filter plate count data for the low chlorine residual tests represent only 1:10 dilutions. It is possible that coliforms were present that would have been detected without a dilution (up to 9 cells/100 ml could have gone undetected in these tests).

Effects of pH. Most bacteria thrive in a relatively neutral environment insofar as pH is concerned. Both highly alkaline

and acidic media can stress the microbes sufficiently that they die. For these reasons, the pH of the test conditions in this study were maintained between 6.5 and 7.5. Under these conditions, no direct correlations could be drawn with confidence between the results of the coliform enumerations and the pH alone.

Effects of temperature. As with pH, temperature changes can stress organisms, even if the extremes are within their natural range of tolerance, if the transition from one temperature to the next is too severe and too sudden (13). In this study, care was taken to insure that the temperature transitions were not severe for the coliforms between the nutrient broth cultures, phosphate buffers, and test water. The range was 20° - 30°C (room temperature) in all cases. The phosphate buffer used to wash the cells was found to warm lightly when centrifuged, but the cells were allowed to cool to room temperature before testing was begun. Sherman and Cameron (13) discovered that cells are not affected by the transfer to a lower temperature as they are to a higher one, so it was assumed that the difference between room temperature and the test temperature was not sufficient to introduce a stress to the coliforms.

Summary of coliform enumerations. No generalizations can be drawn regarding the relationships between specific coliform recoveries at specific chlorine residuals because there are many factors that must also be considered. It was demonstrated, however,

that when no other demand was present in the chlorinated water, except that caused by the coliforms, chlorine residuals of 0.13 mg/l were sufficient to cause irreparable damage to all coliforms present after five seconds contact. The E-MF method provided better coliform recoveries than the NE-MF method in 79 per cent of the tests, but this figure is based on the data obtained after a chlorine contact of only five seconds in 17 of the 19 trials. The other two trials yielded viable coliforms at all contact times.

Few potable water sources exist that do not have some chlorine demand. The results of this study indicate that those waters which contain a high chlorine demand in addition to a high coliform population (i.e., more polluted waters) may require an enrichment-step in coliform enumeration after chlorination, if only low chlorine residuals are maintained in the distribution system. When viable cells persisted throughout the test period, the E-MF procedure appeared to provide higher recoveries of coliforms than the NE-MF method.

The Effects of Interactions of Experimental Variables on Coliform Recoveries

The effects of pH on chlorine residuals. The source of chlorine used in this study was calcium hypochlorite (HTH, $Ca(OCl)_2$]. When added to demand-free water, HTH will rapidly dissociate to establish an equilibrium (57):

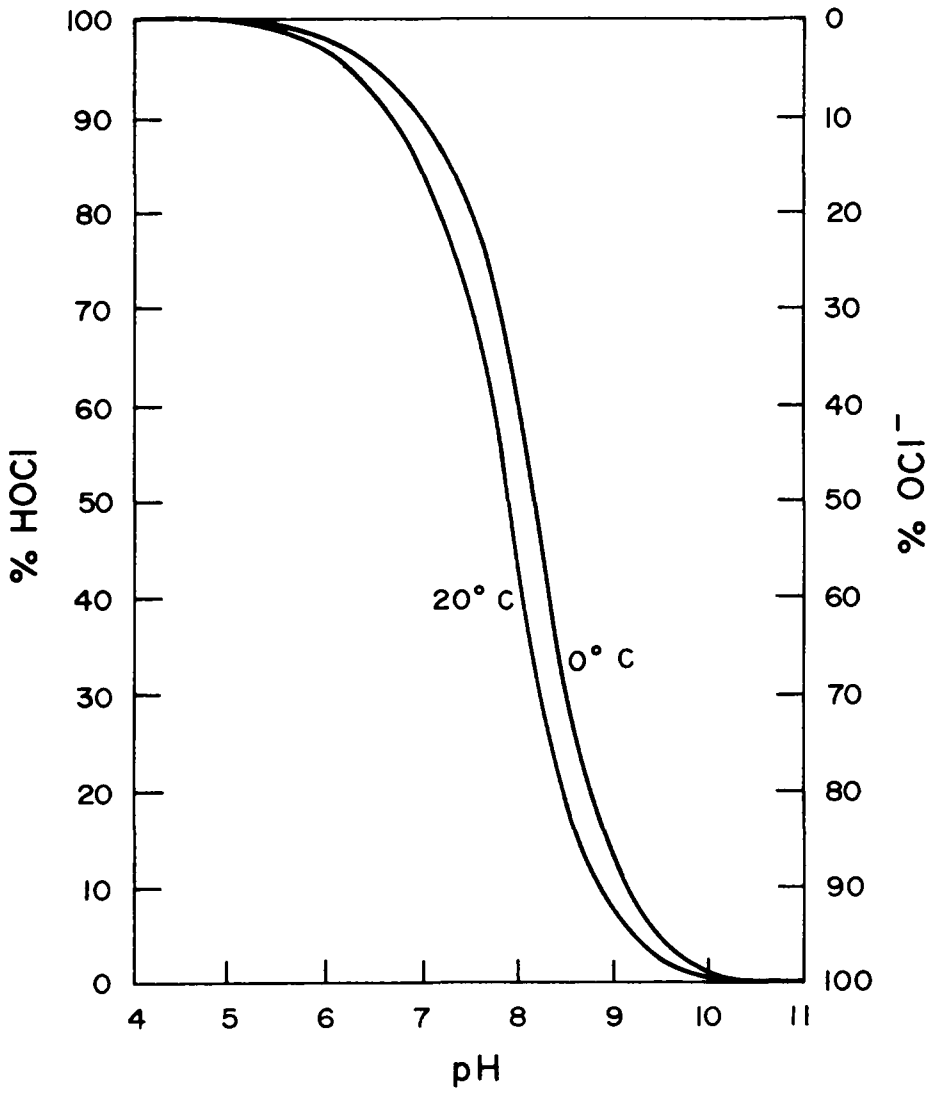

Figure 11. Effect of pH on the distribution of hypochlorous acid and hypochlorite ion in water (57).

$$HOCl \rightleftarrows H^+ + OCl^-$$

The degree of disinfection is influenced by the relative concentrations of HOCl and OCl^- at equilibrium, and pH plays an important role in the dissociation of HOCl (see Figure 11). With an increase in pH from 6 to 9, the percentage of hypochlorite ion (OCl^-) in the water increases from near 0 to near 100 per cent as hypochlorous acid (HOCl) dissociates to maintain the equilibrium. Since HOCl is the stronger disinfectant, an increase in pH results in a decrease in disinfecting effectiveness. From Figure 11, it can be seen that at pH 7.5 and 20°C, only 45 per cent of the HOCl remains intact. At pH 6.5, nearly 95 per cent of the chlorine present is in the undissociated form.

During the testing conducted in this study, the pH was maintained between 6.5 and 7.5 to prevent an alkaline or acidic stress to the coliforms. As described in the previous paragraph, however, this is a critical range that greatly influences the form of the disinfecting agent present in the water. HTH will increase the pH of a water by adding hydroxide ions (OH^-) to the water (57):

$$Ca(OCl)_2 + H_2O \rightleftarrows Ca^{++} + HOCl + OH^-$$

but the pH was decreased to the test range by the addition of sulfuric acid to the water after chlorination and prior to inoculation with coliforms.

From the previous discussion it can be concluded that as the pH decreased within the test range (7.5 - 6.5) increasing amounts of the stronger disinfecting agent (HOCl) were formed. Even differences of 0.1 pH unit may drastically alter the disinfection efficiency of the chlorine. Lower coliform recoveries would be expected from such conditions. Two trials from the high residual testing can be used to demonstrate this phenomenon because they represented the widest spread in pH values, and the inoculum size was the same for both trials. Trial three (Table A-II and A-IV) was conducted at pH 6.5 and trial four (Table A-III and A-IV) at pH 7.3. The inoculum was 7,000 cells/100 ml. Even though the free chlorine residual was greater in trial four by 0.05 mg/l, four per cent of the cells in the inoculum remained after five seconds. Only 0.04 per cent of the cells remained after the same period in trial three. It should also be noted that trial four had the highest pH value and highest coliform recoveries of all trials using high chlorine residuals.

The effects of temperature on chlorine residuals. Temperature has an effect on the percentages of HOCl and OCl^- in chlorinated water (see Figure 11), but it is only slight. Temperatures were maintained to within 2°C of 20°C. Therefore, the HOCl and OCl^- concentrations would have varied little as a result of temperature variations. Differences in coliform recoveries could not be explained by the interaction of these two factors.

Accuracy of the chlorine-analysis technique. Chlorine residual data reported in this study were measured by the Amperometric Titration Method (2). The method involves the titration of the chlorinated water sample with phenylarsine oxide solution at the appropriate pH (pH 7 for free chlorine and pH 4 for combined chlorine determinations) until the chlorine residual is dissipated. The end-point of the titration is detected by special electrodes connected to a meter that become polarized with a decrease in chlorine concentration. The amount of phenylarsine oxide titrated is equal to the chlorine residual in mg/l when a 200-ml sample is tested. Thus, it is apparent that when small chlorine residuals are measured, only small amounts of phenylarsine oxide are required for titration. The volume of each drop of phenylarsine oxide dispensed by the pipette attached to the amperometric titrator was 0.02 - 0.03 ml. Therefore, the addition of only one drop could change the apparent chlorine residual by 0.02-0.03 mg/l. These results would appear to be most significant in the determinations of combined chlorine that remained consistly near these levels throughout most of the testing.

Another factor that should be considered is the reliability of the pH measurement made during the study. On some occasions, the needle on the pH meter drifted considerably before coming to rest. A possible cause could be an equilibrium becoming established between atmospheric CO_2 and dissolved CO_2 in the sample during pH

measurements. The accuracy of the measurements themselves may be questioned also. As has been discussed, even slight differences in the pH would cause significant changes in hypochlorous acid and hypochlorite ion concentrations, and, therefore, changes in the disinfecting potential of the chlorine residuals.

General Applications to Water Treatment

The results of this study indicate that an enrichment-step procedure is not sufficient to recover coliforms exposed to free chlorine residuals of greater than 0.13 mg/l for at least two minutes. Therefore, the standard practice of maintaining a 0.1 to 0.3 mg/l free chlorine residual in a treated water distribution system should be adequate to inhibit bacterial growth. This is true, though, only for systems in good repair and where the chlorine residual is constant throughout the system. It is possible that dead-ends of the mains and remote areas of the system may suffer from decreased chlorine residuals and aftergrowth of previously stressed bacteria. During routine sampling in these areas, the application of an enrichment-step procedure for total coliforms could increase coliform recoveries in the event that aftergrowth is occurring. In systems that are in disrepair, areas of rust and corrosion could harbor bacteria from the disinfecting action of the residual chlorine and cause periodic contamination of the lines. For these reasons, a field study of the two membrane filter methods compared in this study seems warranted.

Another cause for concern determined by this study involves the fact that many colonies that appeared on the LES-Endo agar of both the E-MF and NE-MF procedures failed to produce the typical metallic sheen characteristic of coliforms. All colonies submitted to confirmation in brilliant green bile broth produced gas within 48 hours, therefore, verifying as coliforms. Since the initial inoculum contained only coliforms, all colonies appearing on the incubated membrane filters were expected to be coliforms. This failure to produce a sheen could be the result of phenotypic or genotypic manifestations. Chlorination stress may be selective for those coliforms that produce a sheen, therefore, strains that are non-sheen-forming would be the dominant colony type upon plating. On the other hand, a genotypic change may occur during chlorination that would prevent sheen formation in succeeding generations of coliforms, therefore resulting in non-sheen colonies during membrane filtration procedures.

The absence of the typical sheen on total coliform colonies has great significance in water quality monitoring because the failure to enumerate these indicators of pollution in bacteriological testing could result in an oversight of more dangerous pathogens.

VI. SUMMARY AND CONCLUSIONS

A laboratory study was conducted to compare the coliform recovery capabilities of two membrane filter procedures on coliforms exposed to varying chlorine residuals for varying periods. The degree of disinfection by various chlorine residuals was also observed. As a result of this study, the following conclusions may be made concerning coliforms exposed to different chlorine residuals and contact times.

1. An enrichment step in the membrane filter technique does not improve the recovery of mid-log growth phase coliforms in a simulated finished water where a free chlorine residual of greater than 0.13 mg/l has been maintained for at least two minutes. If these conditions are indicative of finished waters, the implementation of an enrichment membrane filter procedure in bacteriological monitoring would not increase coliform recoveries.

2. During both enrichment-step and non-enrichment membrane filtration testing, non-sheen coliform colonies were observed following various periods of chlorination. It is not known if one or a combination of all strains displayed this phenomenon.

3. None of the four coliform strains used in this study (Enterobacter aerogenes, Eshcerichia coli, Citrobacter freundii, or Klebsiella pneumoniae) were recovered from chlorine demand-free test waters containing a free chlorine residual greater than 0.13 mg/l at contact times between 2 and 15 minutes.

VII. RECOMMENDATIONS

A comparative study of the E-MF and NE-MF procedure should be made on treated waters from dead-ends and remote areas of the distribution system to determine if stressed coliforms may be recovered where chlorine residuals are diminished.

Studies should be initiated to determine the reason why some coliform colonies do not produce a typical metallic sheen on the selective LES-Endo agar following various chlorination periods.

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APPENDICES

APPENDIX TABLE A-I

Chlorine Residuals, Initial Inoculum, and MF Determinations for Low Chlorine Residual Testing

Trial Number	Free Cl_2 (mg/l) Time 0-15 min	Combined Cl_2 (mg/l) Time 0-15 min	Inoculum (TC/100 ml)[a]	Concentrations (TC/100 ml) 5 sec E-MF[b]	5 sec NE-MF[c]	2 min E-MF	2 min NE-MF	5 min E-MF	5 min NE-MF	15 min E-MF	15 min NE-MF	Mean Ratio[d] E-MF:NE-MF
1*	0.11-0.00	0.04-0.02	7,500	7,067	6,800	8,200	6,867	6,633	5,700	>300	>300	1.1:1
2*	0.12-0.08	0.00-0.00	3,300	4,733	5,167	3,033	2,877	2,500	2,000	1,933	1,833	1.03:1
3	0.13-0.10	0.05-0.06	3,000	1,467	867	<1	<1	<1	<1	<1	<1	1.7:1
4*	0.15-0.09	0.00-0.01	2,500	4,547	1,967	<1	<1	<1	<1	<1	<1	2.3:1
5*	0.15-0.04	0.06-0.16	9,200	16,333	11,667	17	7	<1	<1	<1	<1	1.4:1
6	0.17-0.15	0.05-0.04	12,000	430	230	<1	<1	<1	<1	<1	<1	1.9:1

*test water contained a chlorine demand prior to inoculation with cells

a. Total Coliforms per 100 ml as determined by nutrient agar spread plate counts.

b. Enrichment on lauryl tryptose plus 1.5 percent agar for 2 hr at 35°C followed by incubation on LES-Endo agar for 22 hr at 35°C.

c. No enrichment. Incubation on LES-Endo agar for 24 hr at 35°C.

d. Ratio of the averaged plate counts by the enrichment-step method to the averaged plate counts by the non-enrichment method.

APPENDIX TABLE A-II

Chlorine Residuals, Initial Inoculum, and MF Determinations for Medium Chlorine Residual Testing

Trial Number	Free Cl_2 (mg/l) Time 0-15 min	Combined Cl_2 (mg/l) Time 0-15 min	Inoculum (TC/100 ml)[a]	Concentrations (TC/100 ml) 5 sec E-MF[b]	5 sec NE-MF[c]	2 min E-MF	2 min NE-MF	5 min E-MF	5 min NE-MF	15 min E-MF	15 min NE-MF	Mean Ratio[d] E-MF:NE-MF
1	0.30-0.28	0.03-0.04	2,000	13	<1	<1	<1	<1	<1	<1	<1	13:1
2	0.30-0.30	0.03-0.03	7,000	650	540	<1	<1	<1	<1	<1	<1	1.2:1
3	0.32-0.30	0.03-0.03	15,000	307	257	<1	<1	<1	<1	<1	<1	1.2:1
4	0.32-0.30	0.03-0.02	9,000	1,207	837	<1	<1	<1	<1	<1	<1	1.2:1
5	0.32-0.32	0.06-0.03	8,000	73	20	<1	<1	<1	<1	<1	<1	3.7:1
6	0.33-0.28	0.04-0.04	40,000	333	230	<1	<1	<1	<1	<1	<1	1.5:1

a. Total coliforms per 100 ml as determined by nutrient agar spread plate counts.

b. Enrichment on lauryl tryptose plus 1.5 percent agar for 2 hr at 35°C followed by incubation on LES-Endo agar for 22 hr at 35°C.

c. No enrichment. Incubation on LES-Endo agar for 24 hr at 35°C.

d. Ratio of the averaged plate counts by the enrichment-step method to the averaged plate counts by the non-enrichment method.

APPENDIX TABLE A-III

Chlorine Residuals, Initial Inoculum, and MF Determinations for High and Very High Chlorine Residual Testing

Trial Number	Free Cl_2 (mg/l) Time 0-15 min	Combined Cl_2 (mg/l) Time 0-15 min	Inoculum (TC/100 ml)[a]	Concentrations (TC/100 ml) 5 sec E-MF[b]	5 sec NE-MF[c]	2 min E-MF	2 min NE-MF	5 min E-MF	5 min NE-MF	15 min E-MF	15 min NE-MF	Mean Ratio[d] E-MF:NE-MF
1	0.42-0.42	0.02-0.02	1,000	7	3	<1	<1	<1	<1	<1	<1	2.3:1
2*	0.45-0.17	0.10-0.11	2,000	50	77	<1	<1	<1	<1	<1	<1	1:1.5
3	0.48-0.45	0.03-0.06	7,000	3	<1	<1	<1	<1	<1	<1	<1	3:1
4	0.53-0.51	0.03-0.03	7,000	237	30	<1	<1	<1	<1	<1	<1	79:1
5*	0.55-0.18	0.09-0.26	58,000	<1	<1	<1	<1	<1	<1	<1	<1	1:1
6	0.57-0.52	0.03-0.02	6,000	17	10	<1	<1	<1	<1	<1	<1	1.7:1
7*	1.00-0.64	UTD[e]-0.40	67,000	<1	<1	<1	<1	<1	<1	<1	<1	1:1

*test water contained a chlorine demand prior to inoculation with cells.

a. Total Coliforms per 100 ml as determined by nutrient agar spread plate counts.

b. Enrichment on lauryl tryptose plus 1.5 percent agar for 2 hr at 35°C followed by incubation on LES-Endo agar for 22 hr at 35°C.

c. No enrichment. Incubation on LES-Endo agar for 24 hr at 35°C.

d. Ratio of the average plate counts by the enrichment-step method to the averaged plate counts by the non-enrichment method.

e. Unable to determine.

APPENDIX A-IV

Temperature, pH, Chlorine Residuals, and
Mean Ratio of the Enrichment-step Method to the
Non-enrichment Method Plate Counts for all Trial Runs

Trial Number	Free Cl_2 (mg/l) Time 0-15 min	Combined Cl_2 (mg/l) Time 0-15 min	pH	Temperature (degrees C)	Mean Ratio[a] E-MF:NE-MF
1*	0.11-0.00	0.04-0.02	7.4	20	1.1:1
2*	0.12-0.08	0.00-0.00	7.1	22	1.03:1
3	0.13-0.10	0.05-0.06	7.0	20	1.7:1
4*	0.15-0.09	0.00-0.01	6.9	21	2.3:1
5*	0.15-0.04	0.06-0.16	6.7	19	1.4:1
6*	0.17-0.15	0.05-0.04	7.0	19	1.9:1
1	0.30-0.28	0.03-0.04	7.1	18	13:1
2	0.30-0.30	0.03-0.03	7.0	21	1.2:1
3	0.32-0.30	0.03-0.03	7.1	19	1.2:1
4	0.32-0.30	0.03-0.02	7.1	18	1.2:1
5	0.32-0.32	0.06-0.03	7.0	20	3.7:1
6	0.33-0.28	0.04-0.04	7.0	19	1.5:1
1	0.42-0.42	0.02-0.02	7.0	18	2.3:1
2*	0.45-0.17	0.10-0.11	6.8	19	1:1.5
3	0.48-0.45	0.03-0.06	6.5	20	3:1
4	0.53-0.51	0.03-0.03	7.3	21	79:1
5*	0.55-0.18	0.09-0.26	6.9	20	1:1
6	0.57-0.52	0.03-0.02	6.8	19	1.7:1
7*	1.00-0.64	UTD[b]-0.40	6.8	21.5	1:1

*test water contained a chlorine demand prior to inoculation with cells.

a. Ratio of the averaged plate counts by the enrichment-step method to the averaged plate counts by the non-enrichment method.

b. Unable to determine.

APPENDIX TABLE A-V

Plate Counts by the Enrichment-step and Non-enrichment Methods for Low Chlorine Residual Testing

Trial Number	Dilution	Plate Counts (colonies/10-ml portion)							
		5 sec		2 min		5 min		15 min	
		E-MF[a]	NE-MF[b]	E-MF	NE-MF	E-MF	NE-MF	E-MF	NE-MF
1	10^{-1}	74,70,68	78,67,59	104,77,65	70,70,66	74,65,60	60,61,50	TNTC(3)	TNTC(3)
2	10^{-1}	52,48,42	60,54,41	41,27,23	30,26,19	30,26,19	23,21,16	24,12,13	22,17,16
3	10^{-1}	18,13,13	15,6,5	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0
4	10^{-1}	34,33,18	23,20,16	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0
5	10^{-2}	18,17,14	14,11,10						
	10^{-1}			4,1,0	2,0,0	0,0,0	0,0,0	0,0,0	0,0,0
6	10^{-1}	6,5,2	4,2,1	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0

a. Enrichment on lauryl tryptose plus 1.5 percent agar for 2 hr at 35°C followed by incubation on LES-Endo agar for 22 hr at 35°C.

b. No enrichment. Incubation on LES-Endo agar for 24 hr at 35°C.

APPENDIX TABLE A-VI

Plate Counts by the Enrichment-step and Non-enrichment Methods for Medium Chlorine Residual Testing

Trial Number	Dilution	Plate Counts (colonies/10-ml portion)							
		5 sec		2 min		5 min		15 min	
		E-MF[a]	NE-MF[b]	E-MF	NE-MF	E-MF	NE-MF	E-MF	NE-MF
1	10^0	3,1,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0
2	10^0	77,64,54	57,55,50	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0
3	10^0	35,29,28	28,25,24	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0
4	10^0	108,101,97	90,88,73	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0
5	10^0	9,7,6	2,2,2	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0
6	10^0	37,33,30	29,20,20	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0

a. Enrichment on lauryl tryptose plus 1.5 percent agar for 2 hr at 35°C followed by incubation on LES-Endo agar for 22 hr at 35°C.

b. No enrichment. Incubation on LES-Endo agar for 24 hr at 35°C.

APPENDIX TABLE A-VII

Plate Counts for the Enrichment-step and Non-enrichment Methods for High and Very High Chlorine Residual Testing

Trial Number	Dilution	Plate Counts (colonies/10-ml portion)							
		5 sec		2 min		5 min		15 min	
		E-MF[a]	NE-MF[b]	E-MF	NE-MF	E-MF	NE-MF	E-MF	NE-MF
1	10^0	1,1,0	1,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0
2	10^0	7,4,4	9,9,5	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0
3	10^0	1,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0
4	10^0	63,6,2	5,2,2	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0
5	10^0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0
6	10^0	3,2,0	3,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0
7	10^0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0	0,0,0

a. Enrichment on lauryl tryptose plus 1.5 percent agar for 2 hr at 35°C followed by incubation on LES-Endo agar for 22 hr at 35°C.

b. No enrichment. Incubation on LES-Endo agar for 24 hr at 35°C.

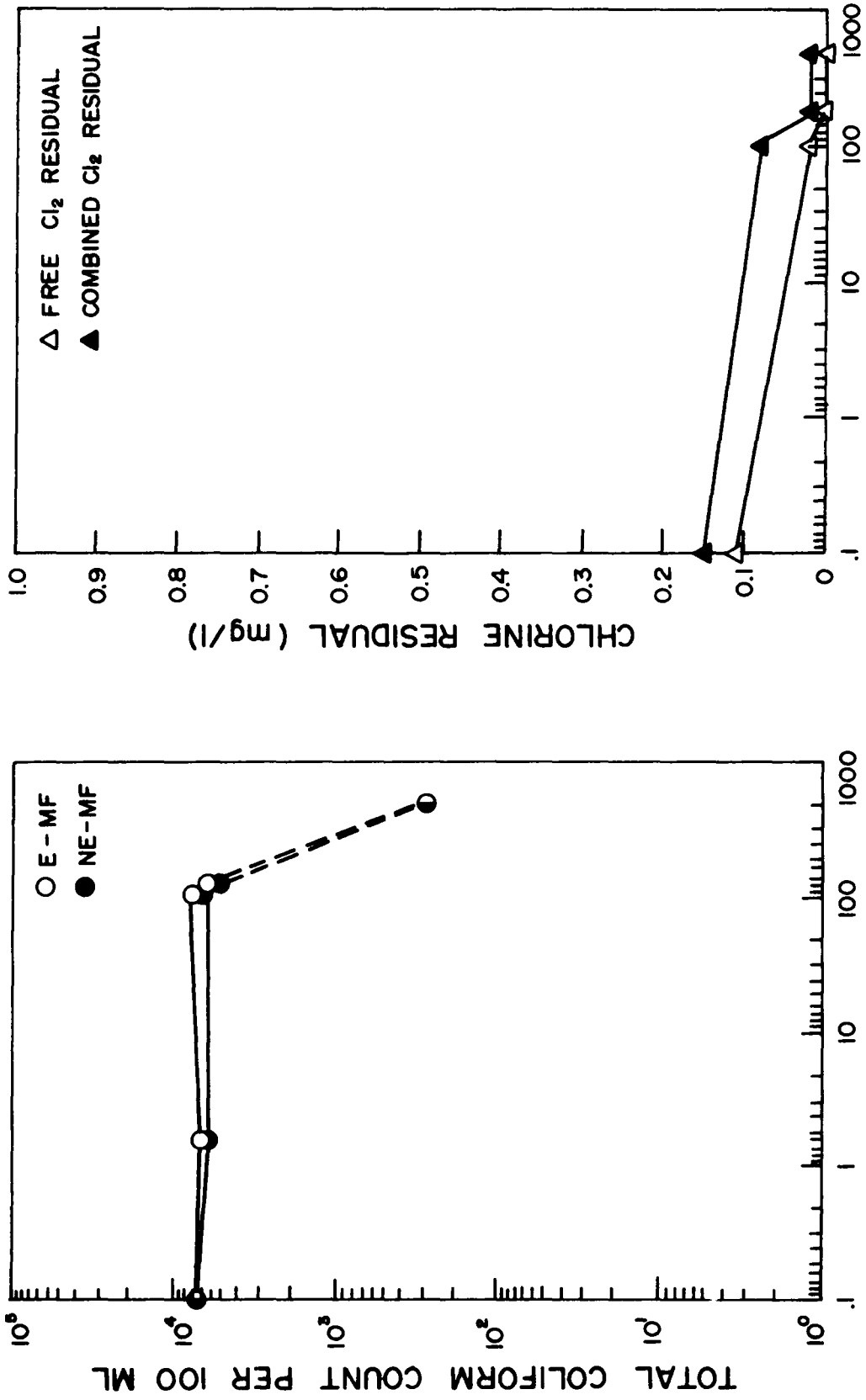

Figure B-I. Changes in free and combined chlorine residuals and in coliform numbers as enumerated by an enrichment-step versus a non-enrichment membrane filter method during a low chlorine residual test, Trial # 1.

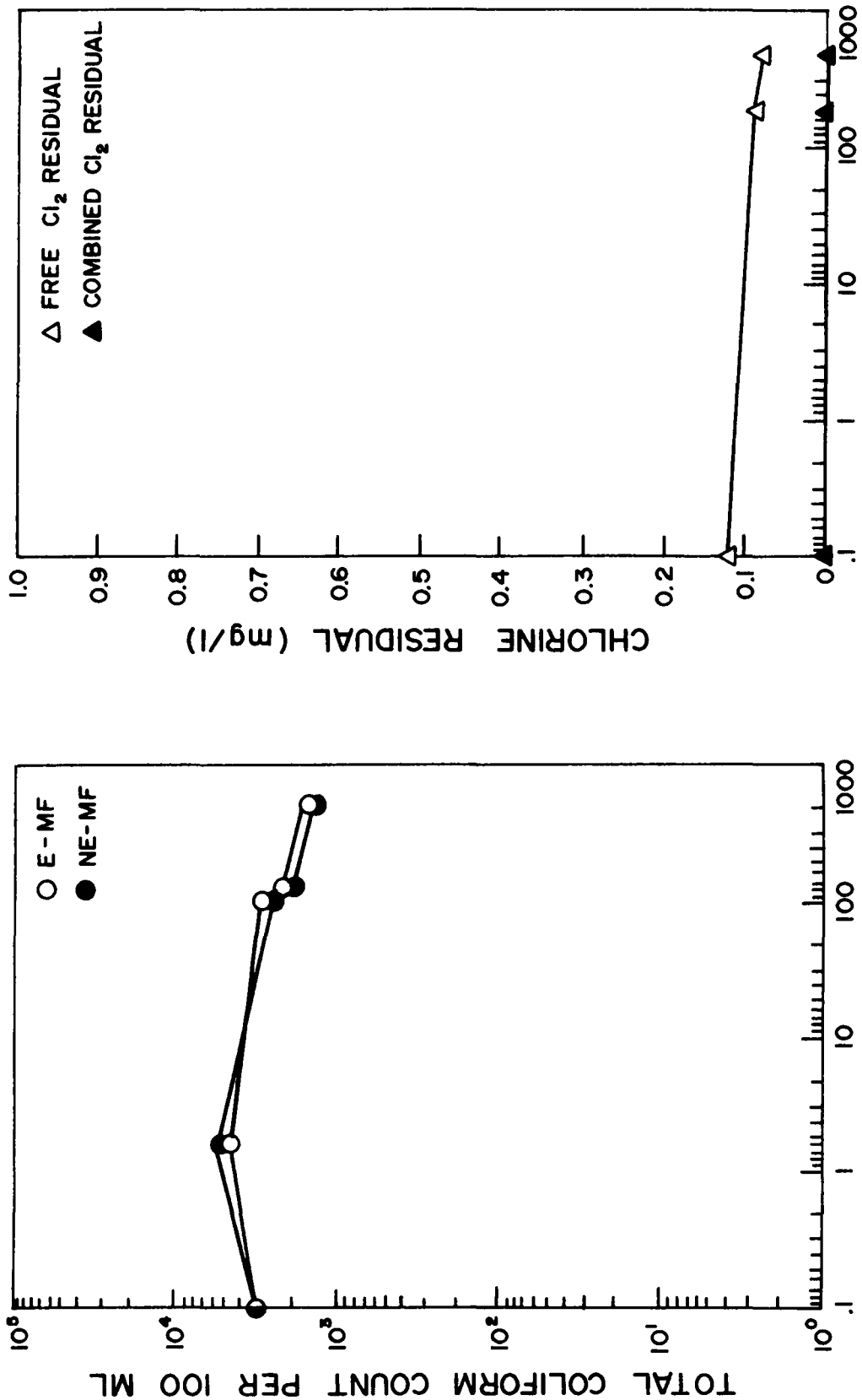

Figure B-II. Changes in free and combined chlorine residuals and in coliform numbers as enumerated by an enrichment-step versus a non-enrichment membrane filter method during a low chlorine residual test, Trial # 2.

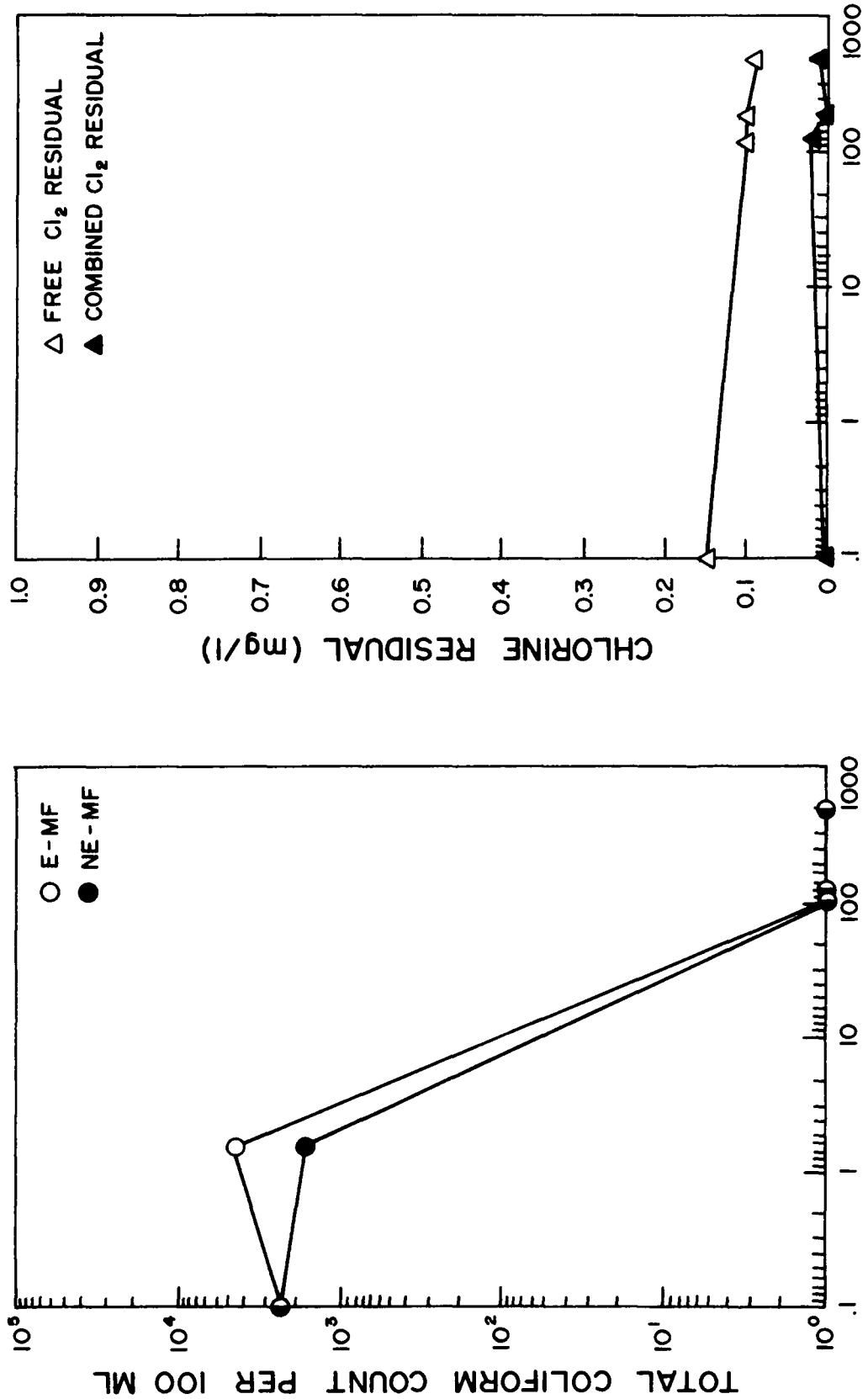

Figure B-III. Changes in free and combined chlorine residuals and in coliform numbers as enumerated by an enrichment-step versus a non-enrichment membrane filter method during a low chlorine residual test, Trial # 4.

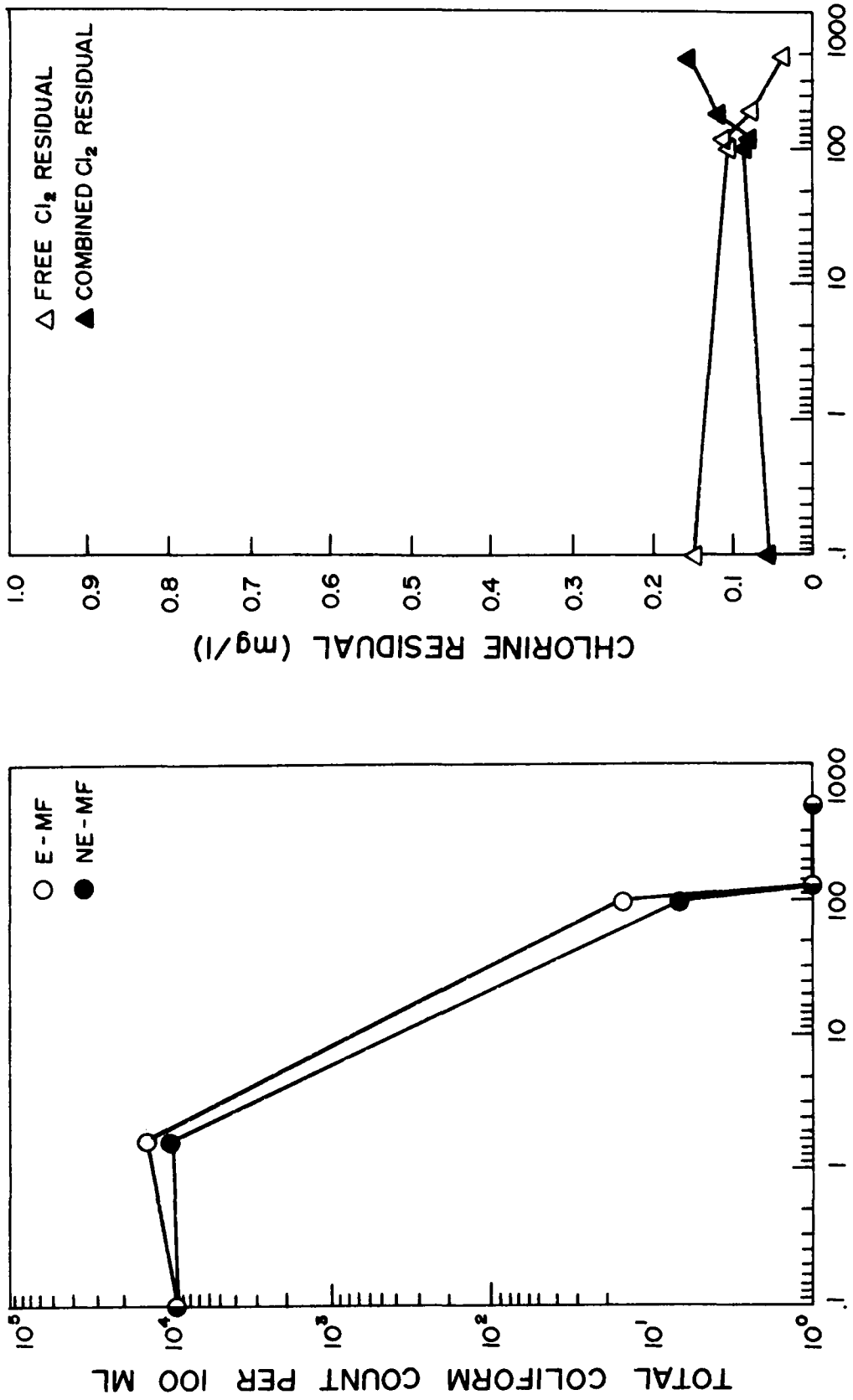

Figure B-IV. Changes in free and combined chlorine residuals and in coliform numbers as enumerated by an enrichment-step versus non-enrichment membrane filter method during a low chlorine residual test, Trial # 5.

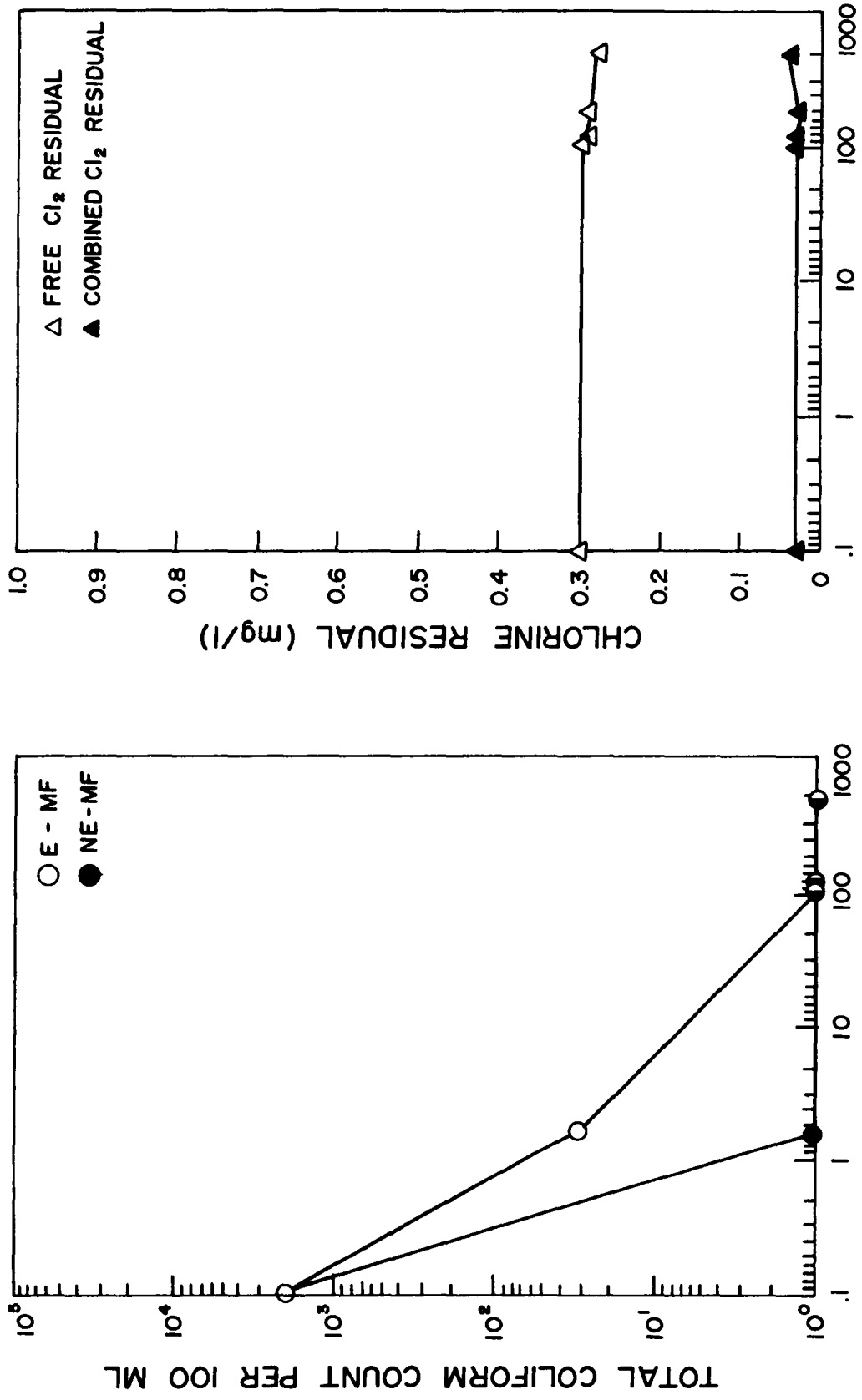

Figure B-V. Changes in free and combined chlorine residuals and in coliform numbers as enumerated by an enrichment-step versus a non-enrichment membrane filter method during a medium chlorine residual test, Trial # 1.

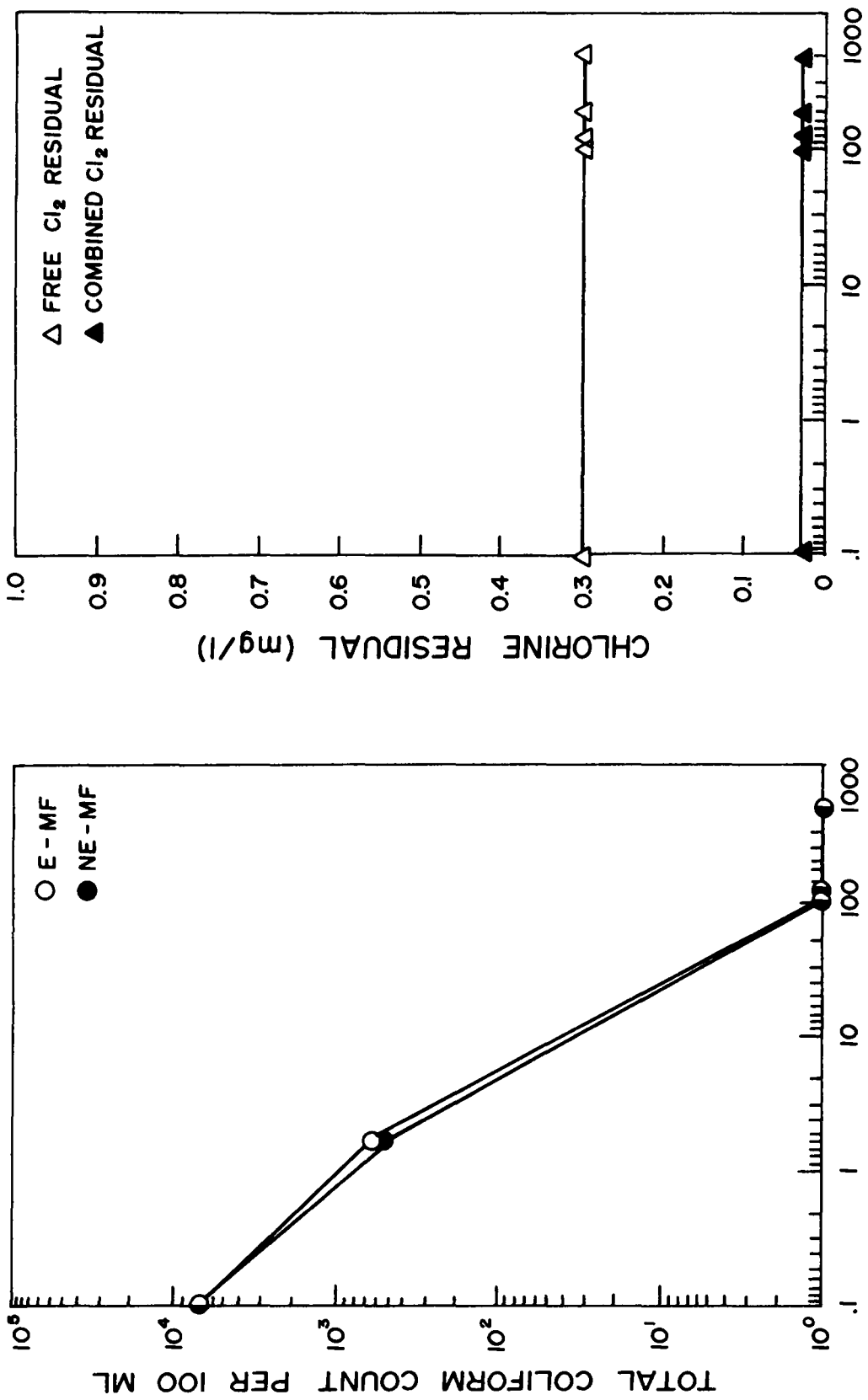

Figure B-VI. Changes in free and combined chlorine residuals and in coliform numbers as enumerated by an enrichment-step versus a non-enrichment membrane filter method during a medium chlorine residual test, Trial # 2.

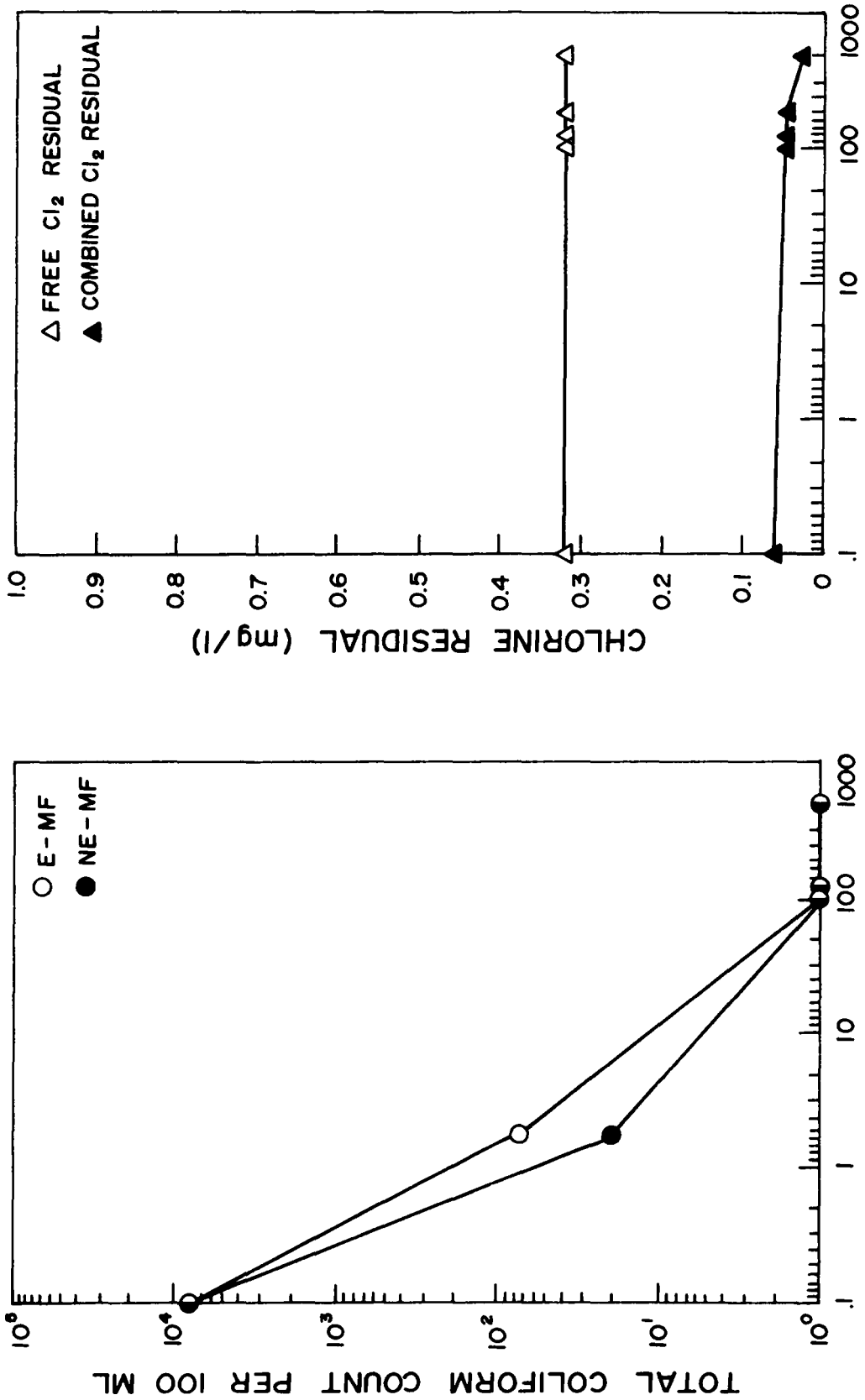

Figure B-VII. Changes in free and combined chlorine residuals and in coliform numbers as enumerated by an enrichment-step versus a non-enrichment membrane filter method during a medium chlorine residual test, Trial # 5.

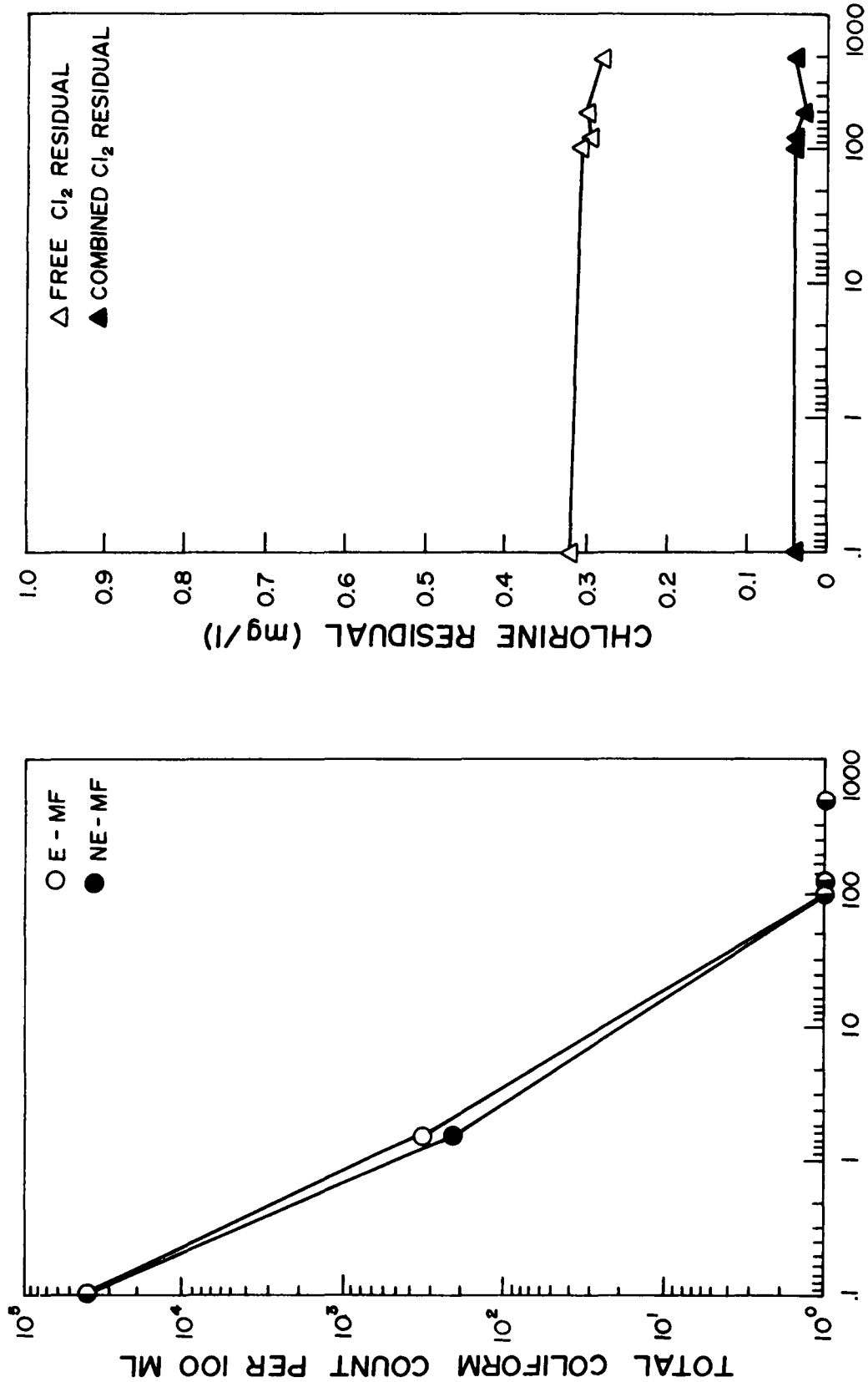

Figure B-VIII. Changes in free and combined chlorine residuals and in coliform numbers as enumerated by an enrichment-step versus an non-enrichment membrane filter method during a medium chlorine residual test, Trial # 6.

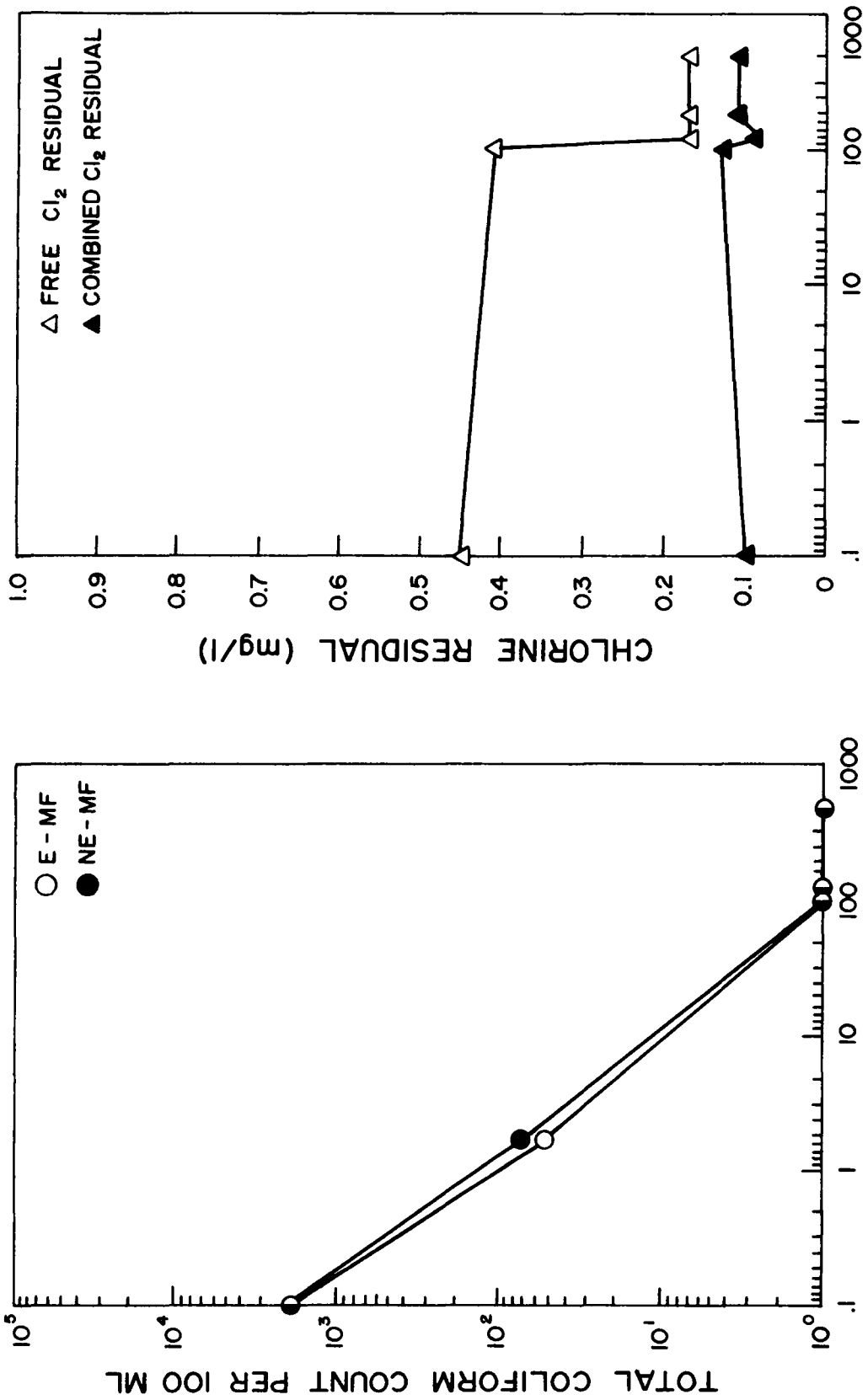

Figure B-IX. Changes in free and combined chlorine residuals and in coliform numbers as enumerated by an enrichment-step versus a non-enrichment membrane filter method during a high chlorine residual test, Trial # 2.

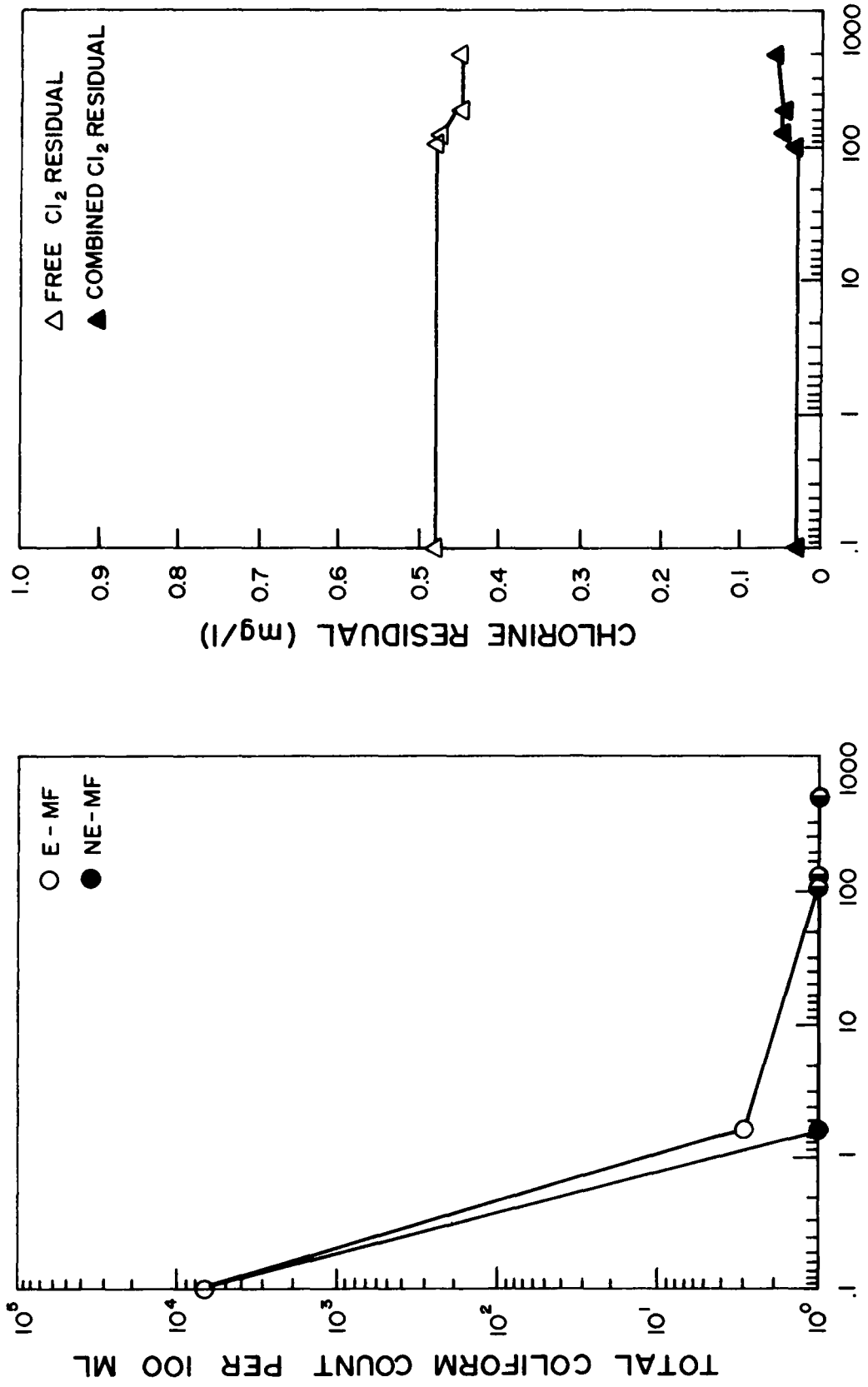

Figure B-X. Changes in free and combined chlorine residuals and in coliform numbers as enumerated by an enrichment-step versus a non-enrichment membrane filter method during a high chlorine residual test, Trial # 3.

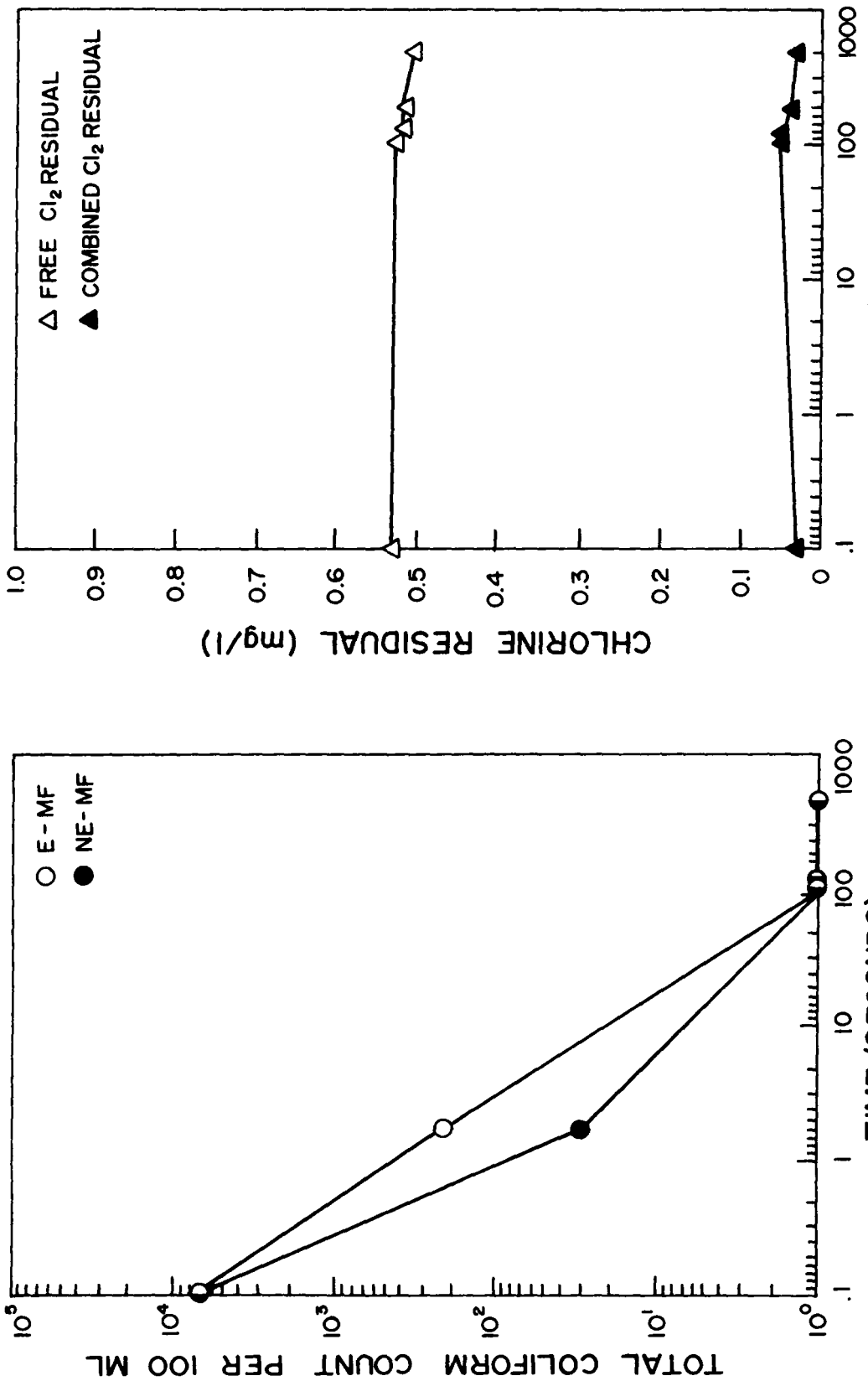

Figure B-XI. Changes in free and combined chlorine residuals and in coliform numbers as enumerated by an enrichment-step versus a non-enrichment membrane filter method during a high chlorine residual test, Trial # 4.

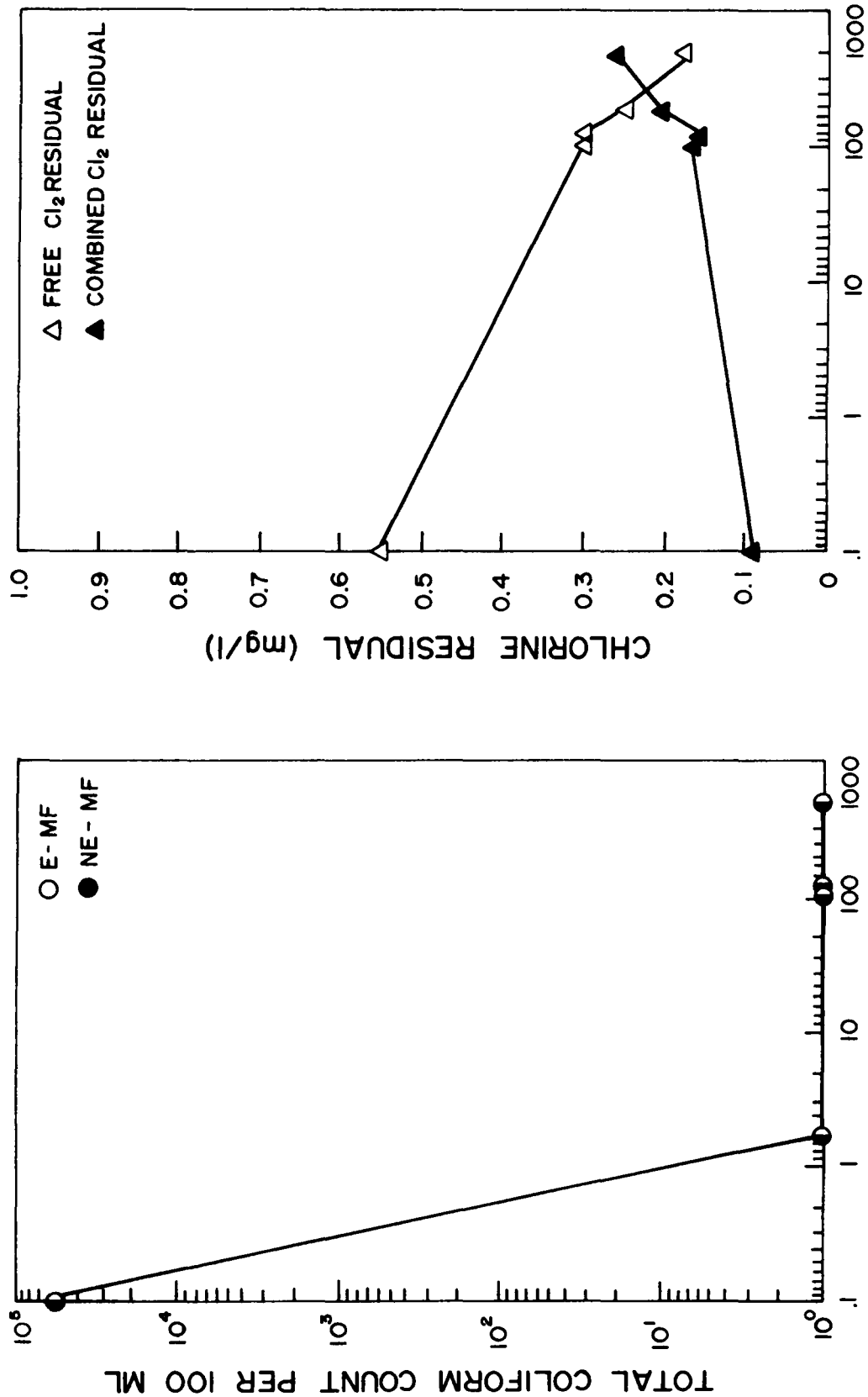

Figure B-XII. Changes in free and combined chlorine residuals and in coliform numbers as enumerated by an enrichment-step versus a non-enrichment membrane filter method during a high chlorine residual test, Trial # 5.

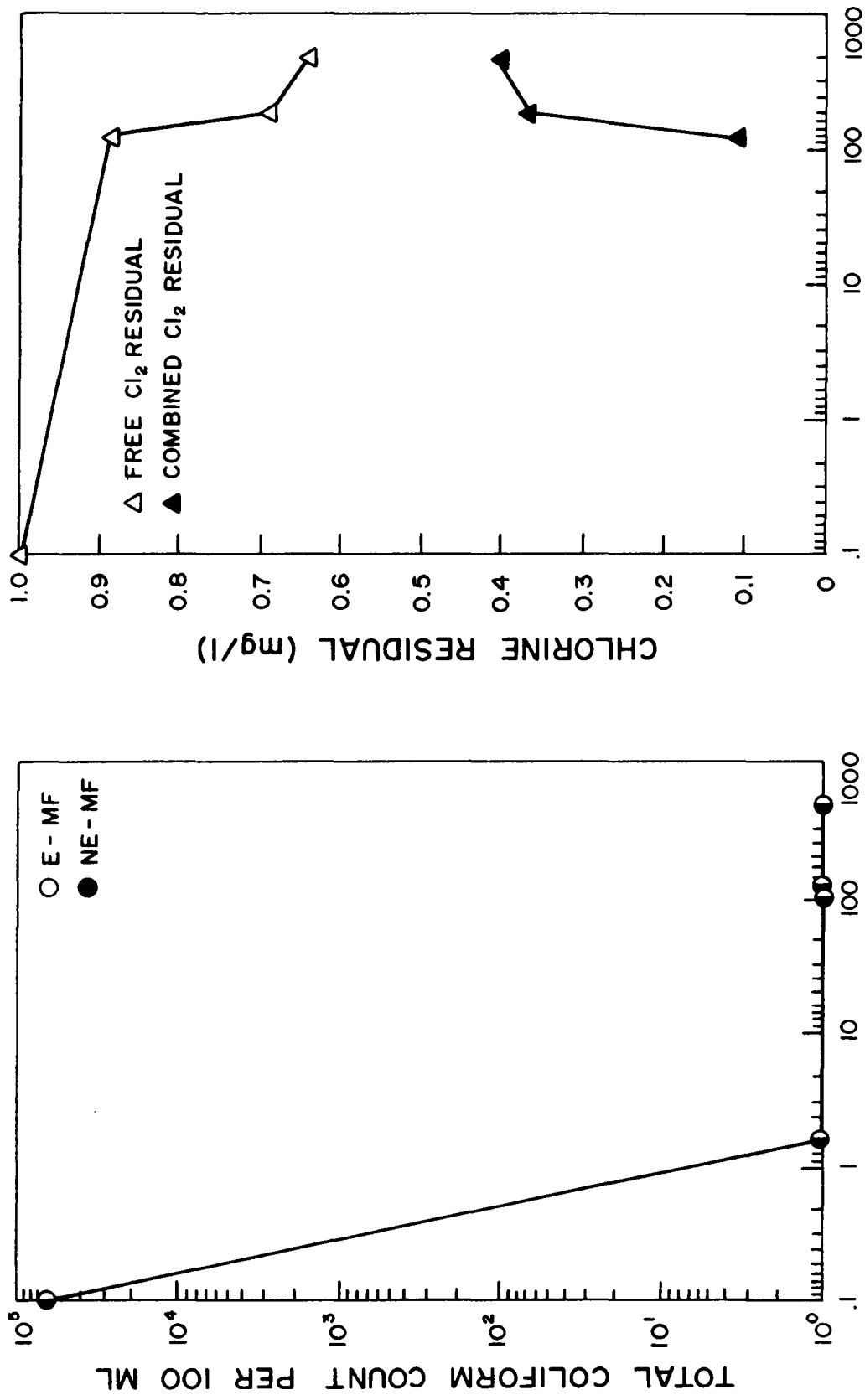

Figure B-XIII. Changes in free and combined chlorine residuals and in coliform numbers as enumerated by an enrichment-step versus a non-enrichment membrane filter method during a very high chlorine residual test, Trial # 7.

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A COMPARISON OF TWO MEMBRANE FILTER TECHNIQUES FOR THE ENUMERATION OF TOTAL COLIFORMS IN POTABLE WATERS

By

Sandra G. Shelton

(ABSTRACT)

A laboratory study was conducted to compare the coliform recovery capabilities of two membrane filter techniques on coliforms exposed to varying chlorine residuals and contact times.

An enrichment-step membrane-filter method (E-MF) involved the incubation of a filtered water sample on lauryl tryptose plus 1.5 per cent agar for two hours at 35°C prior to transfer of the filter to LES-Endo agar and a final incubation for 22 hours at 35°C. A single incubation of the membrane filter for 24 hours at 35°C was employed in a non-enrichment membrane filter method (NE-MF). A free chlorine residual was established in a synthetic test water at a neutral pH and constant temperature prior to inoculation of the water from a suspension of bacteria comprised of four coliform genera commonly found in natural waters. Timed samples from 5 seconds to 15 minutes, analyzed for coliform recovery by the E-MF and NE-MF methods revealed an average increase in recovery of the E-MF to NE-MF method of 1.6:1. When the initial inoculum was kept below 1×10^4 cells/100 ml, and the test water was chlorine demand-

free no coliforms were recovered by either method after two minutes of contact time with free chlorine residuals greater than 0.12 mg/l. Many of the recovered colonies failed to produce a characteristic sheen but were verified as coliforms.

It was concluded that all coliforms were effectively killed at chlorine contact times greater than two minutes and free chlorine residuals greater than 0.13 mg/l, in chlorine demand-free water. Free chlorine residuals of 1.0 mg/l effectively killed all coliforms within five seconds.