APPLICATION NOTE



# A protocol for the use of Nalgene Analytical Filter Funnels and Cellulose Nitrate Membranes in recovery of microorganisms during environmental and/or quality testing

**Key words:** Analytical filters, water quality, environmental testing, filter funnels, cellulose nitrate membrane, Oxoid™ culture media, Remel™ culture media, protocol, ISO 7704-1985, microbial recovery

## Abstract

Analytical filters and filter funnels are routinely used to determine the microbial counts during water quality testing as well as testing liquid products in the food and beverage industry. Thermo Scientific™ Nalgene™ Analytical Filter Funnels and Nalgene cellulose nitrate membrane filters paired with Thermo Fisher™ Oxoid™ and Remel™ culture media offer a complete and efficient solution. The sterile pre-assembled analytical filter funnels eliminate time-consuming steps of sterilizing reusable funnels and assembling filter membranes.

The functionality of Thermo Scientific Nalgene Analytical Filters and Filter Funnels was assessed by microbiological recovery as described in ISO 7704-1985. Additionally, this study includes testing of flow rates of most commonly used liquids. A protocol that follows ISO 7704-1985 guidelines for membrane testing is provided for use in the laboratory.

## Introduction

Filtration is a common practice in water quality and/or food and beverage testing. The critical considerations when establishing a filtration protocol are flow rate of the liquid tested and microbial recovery rate.


**Thermo Scientific™ Nalgene™ Analytical Filter Funnel**

Pore size and viscosity will determine the flow rate of a liquid through a membrane. To determine flow rates, a known volume of liquid is added to a membrane filter unit and vacuum is applied to the filter unit. When the vacuum is applied, the liquid will flow through the membrane. The time it takes for the volume of the liquid to flow through the membrane unit is known as the flow rate.

Analytical filters in conjunction with agar containing growth media can be used to retain and count microorganisms in liquid; also know as microbial recovery rate. Using this method, the liquid of interest is filtered through a membrane, and microbes are captured on the membrane and placed onto an agar plate containing growth media.

In general, one bacterium will grow into a colony forming unit (CFU), which can be counted with the unaided eye after incubation. Knowing the volume of liquid filtered and the number of CFUs counted, a total concentration of the microorganisms in the liquid of interest can be estimated.

Prior to testing liquid samples, analytical filters must be evaluated for performance. The ISO 7704-1985 standard for water quality testing can be used as a guideline in determining the microbial retention performance of analytical filters. The guideline was followed and a protocol was developed using the Thermo Scientific Nalgene Analytical Filter Funnels and Oxoid or Remel culture media. More specifically, the ISO 7704 Requirements state that:

– 5 (or more) replicate samples are required for each membrane filter lot tested

– A minimum of 200 colonies is needed for statistical comparison with ideally between 25-100 colonies per plate

– Membranes producing counts ≥80% of the control plate counts are considered acceptable

## Methods and Results

To determine the flow rate, a variety of liquids common in laboratories and/or the food and beverage industry were chosen for flow rate measurement: corn oil, beer, grape juice, tryptic soy broth, phosphate buffered solution with Tween, phosphate buffered saline (PBS), and deionized water. Five samples of each of the Nalgene Analytical Test Filter Funnel products were tested with 250 mL of liquid. The liquid was measured and placed into 250mL volume Nalgene filter funnels, with cellulose nitrate membranes in 0.45µm and 0.2µm pore sizes. Vacuum of ≥27" Hg was applied and the amount of time (in seconds) taken for all of the liquid to pass through the membrane was recorded. Flow rate was calculated by dividing the volume of liquid by the number of seconds taken to filter the liquid (Table 1).

**Table 1:** Filter membrane characteristics and mean flow rates (mL/sec) (n=5 per unit)

| **Filter membrane characteristics** | | | |
|---|---|---|---|
| | **Nalgene Analytical Test Filter Funnels** | | |
| **Cat. No.** | **147-2045** | **145-2045** | **145-2020** |
| Membrane Diameter (mm) | 47 | 47 | 47 |
| Pore Size | 0.45 | 0.45 | 0.2 |
| **Liquids Filtered** | **Mean flow rate ± *s* (mL/sec)** | | |
| Corn Oil | 0.23 ± 0.003 | 0.23 ± 0.004 | 0.08 ± 0.001 |
| Beer | 2.3 ± 0.8 | 3.2 ± 0.1 | 0.5 ± 0.4 |
| Grape Juice | 3.7 ± 0.6 | 4.3 ± 0.9 | 1.4 ± 0.4 |
| Tryptic Soy Broth | 6.1 ± 1.1 | 6.3 ± 1.5 | 1.3 ± 0.7 |
| Phosphate Buffer with Tween® | 8.2 ± 0.2 | 8.7 ± 0.2 | 3.1 ± 0.1 |
| Phosphate Buffered Saline | 8.8 ± 0.5 | 9.5 ± 0.3 | 3.6 ± 0.1 |
| Deionized Water | 10.3 ± 0.7 | 8.4 ± 0.2 | 3.6 ± 0.2 |

To determine whether the Nalgene Analytical Filter Funnels and Membranes adhere to the ISO 7704-1985 standards, the percent microbial recovery was determined using the membrane filter method. *Escherichia coli* (ATCC # 11775), *Enterococcus faecalis* (ATCC # 19433), and *Saccharomyces cerevisiae* (ATCC # 7754) were chosen as representative microorganisms for analysis because of their relevance in water quality testing and in the food and beverage industry.

In general, the sample was dispensed into the Thermo Scientific Nalgene Analytical Filter Funnels (n=10), vacuum was applied, and the sample was pulled through the filter membrane. Any microbes contained in the sample were retained on the filter membrane. The filter membrane was removed from the funnel and incubated with the appropriate culture medium. Specifically, *Escherichia coli* (ATCC # 11775) was grown on Oxoid Plate Count Agar, *Enterococcus faecalis* (ATCC # 19433) was grown on Oxoid Trytone Soy Agar, and *Saccharomyces cerevisiae* (ATCC # 7754) was grown on Remel Sabouraud's Dextrose Agar (Table 2). After the incubation period, any CFUs that grew were counted and percent recovery was calculated.

**Table 2:** Key materials and microorganisms used in this study

| **Key Materials** | |
|---|---|
| **Description** | **Cat. No.** |
| **Microorganisms** | |
| *Escherichia coli* (ATCC 11775) | R19201 |
| *Enterococcus faecalis* (ATCC 19433) | R19077 |
| *Saccharomyces cerevisiae* (ATCC 7754) | Internal stock culture |
| **Media** | |
| Oxoid Plate Count Agar | CM0325B |
| Oxoid Trytone Soy Agar | CM0131B |
| Remel Sabouraud's Dextrose Agar | R454462 |
| **Supplies** | |
| Nunc Petri dishes | 263991 |
| Fisherbrand™ L-Shaped Cell Spreaders | 14665231 |
| Fisherbrand Disposable Inoculating Loops | 22363600 |
| Nalgene Vacuum Manifold | DS0345-0001 |
| Nalgene Reusable Filter Holder | 300-4000 |
| Nalgene Analytical Test Filter Funnels gray with black grid | 147-2045 |
| Nalgene Analytical Test Filter Funnels white with black grid | 145-2045 |
| Nalgene Analytical Test Filter Funnels white | 145-2020 |
| Nalgene Water Quality Membrane gray | DS0205-6045 |
| Nalgene Water Quality Membrane white | DS0205-4045 |

Protocol for percent microbial recovery using the membrane filter method:

1. Prepare the Oxoid and Remel dehydrated culture media according to the manufacturer's instructions. After steam sterilization, dispense the molten agar into sterile Nunc ™ Petri dishes to a minimum depth of 3 mm. Once the agar is solidified, store the Petri dishes at 4°C until the time of testing.
2. Prepare an overnight culture of the organism in the appropriate media. The next day, determine the concentration of the overnight culture and dilute to 25-100 CFUs per filter sample in 50 mL of sterile deionized water. Prepare one sample for filtration and one for the standard pour plate.
3. To prepare the pour plate, add the 50 mL sample prepared in step 2 to a sterile petri dish and cover with the appropriate media for that sample. Allow the plate to solidify, and then incubate inverted with the agar on the top at the appropriate temperature.
4. Attach the Nalgene analytical filter funnels to the vacuum manifold and turn on vacuum. Add the prepared 50 mL sample(s) from step 2 to the filter funnel.
5. Rinse the funnels with 20-30 mL of sterile deionized water to wash down any sample that may be retained on the sides of the filter unit.
6. Once the sample has been filtered, transfer the filter membrane from the filter unit onto the surface of the prepared agar using sterile forceps. Use care to avoid trapping air bubbles between the membrane and the agar surface.
7. After appropriate incubation, count the colonies on the membrane and on the pour plate. Calculate the percent recovery, R, using the following equation:

$$R = m_m/m_c \times 100$$

Where $m_m$ is the membrane filter counts; $m_c$ is the mean of the pour plate counts

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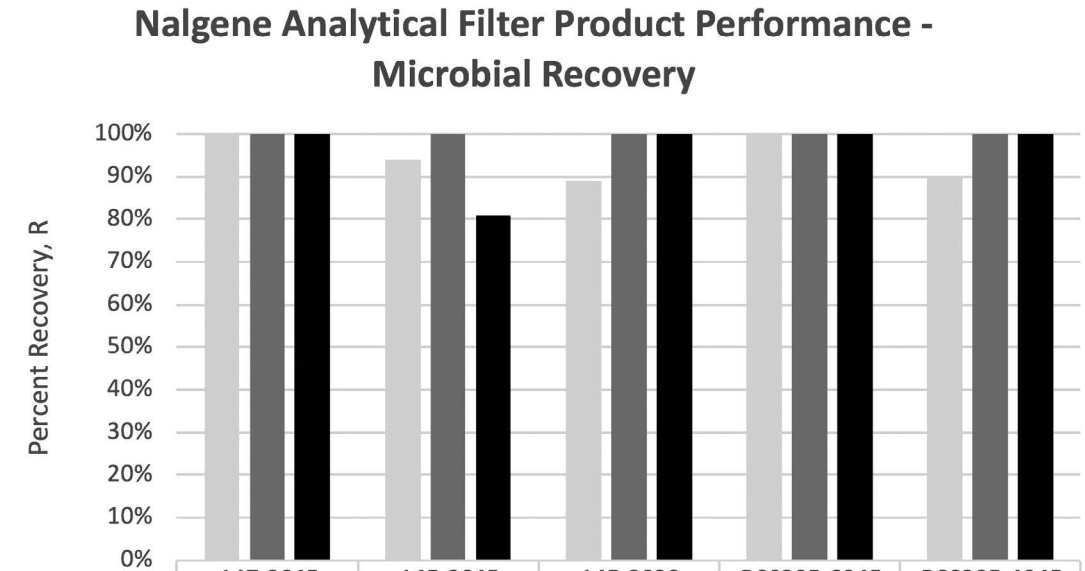
The results show that the percent recovery using Thermo Scientific Nalgene Analytical Filter Funnels and Nalgene Cellulose Nitrate Membrane filters is between 81-100% (Fig. 1). *Saccharomyces cerevisiae* had 100% recovery, whereas *Escherichia coli* ranged between 89-100% and *Enterococcus faecalis* was between 81-100% recovery depending on the filter used. The ISO 7704-1985 standards for water quality testing state that ≥80% recovery rate validates the filter membrane tested. These results demonstrate that Thermo Scientific™ Nalgene™ Analytical Filter Funnels and Membranes are suitable for water quality testing according to ISO 7704-1985 standards.

## Conclusion

The flow rates of the Nalgene™ Analytical Filter Funnels and Membranes calculated above can be used as a guideline to determine how a liquid will perform during filtration. The protocol provided using Nalgene™ Analytical Filter Funnels and Membranes and Oxoid™ or Remel™ culture media can be used to determine percent microbial recovery of a membrane. The ISO 7704-1985 standards for water quality testing were used as a guideline in determining the microbial rention performance of the Thermo Scientific™ Nalgene™ Analytical Filter Funnels and Oxoid™ or Remel™ culture media. Since greater than 80% of the colony forming units were retained by the filter, the materials used and protocol provided adhere to the standard and can be used when following ISO 7704-1985 standards for water quality testing.

The data presented are a good faith estimate of product performance at the time the test was undertaken. These data do not constitute a warranty or endorsement of suitability for any particular purpose. Thermo Fisher strongly recommends that you validate the product in your own application.




| | 147-2045 | 145-2045 | 145-2020 | DS0205-6045 | DS0205-4045 |
|---|---|---|---|---|---|
| *E. coli* | 100% | 94% | 89% | 100% | 90% |
| *S. cerevisiae* | 100% | 100% | 100% | 100% | 100% |
| *E. faecalis* | 100% | 81% | 100% | 100% | 100% |

**Figure 1: The percent recovery, R, for Nalgene analytical filter products tested**

Find out more at **thermofisher.com/analyticalfiltration**

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