

# A Critical Evaluation of the Multi-Item Microbial Challenge Test in Ophthalmic Disinfectant Testing

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The Multi-item Microbial Challenge Test (MIMCT) is a stringent, carrier assay of the disinfecting efficacy of a contact lens care regimen. The test involves four steps: 1) *in vitro* contamination of contact lenses in the presence of organic load; 2) cleansing the contact lens by mechanical rubbing and subsequent rinsing; 3) disinfection of the cleansed lens; and 4) assaying the contact lens and the disinfecting solution for viable microorganisms. The variability of the individual steps was evaluated in this study by the Contributions of Elements protocol. The efficacy of the cleansing step, while reputed to be highly variable, was found to be reproducible and dependent upon the formulation under test. A contact lens disinfecting regimen must reduce the level of contaminants to approximately  $10^{-4}$  CFU/lens to enjoy a 95% chance of passing the MIMCT. This reflects a 10-log unit reduction from the initial inoculum of approximately  $10^6$  CFU/lens. The stringency of the MIMCT depends on the ability of the recovery system to allow the growth of low numbers of residual challenge microorganisms in the presence of the disinfecting solution. Neutralizers included in the recovery system to inhibit the disinfectant are therefore critically important to the stringency of the assay. The design of experiments to evaluate neutralizers specifically for the MIMCT is discussed with suggestions for an improved procedure.

## Introduction

Passing the Multi-item Microbial Challenge Test (MIMCT) is accepted as evidence of a contact lens chemical disinfection regimen's efficacy. This test examines the ability of a specific regimen to disinfect a contact lens in the presence of organic load. The test consists of several sequential steps designed to emulate standard contact lens cleaning (rub and rinse) and disinfection. The lens, with an organic load, is inoculated with high concentrations of microorganism. The contaminated lens is then subjected to a regimen that can consist of manual rubbing (cleaning), rinsing, chemical disinfection, and, if necessary, a final rinse.

The initial cleaning removes particulate debris from the surface of the lens and may significantly reduce the number of microorganisms. Rinsing the contact lens has also been shown to significantly reduce the level of microbial contamination.<sup>1,2</sup>

Chemical disinfection is then performed. After chemical disinfection, the contact lenses and aliquots of the disinfecting solution are incubated separately in neutralizing/growth medium (recovery medium) to assay for surviving microorganisms. A chemical disinfection regimen will pass the MIMCT if no viable microorganisms can be found on the lenses or in any of the solution samples.

The test is reputed to be highly variable and operator-dependent.<sup>3</sup> This putative variability can be analyzed by examining how the individual elements of the regimen contribute to its overall efficacy. The Contribution of Elements test separately examines the rubbing and the rinsing steps of the MIMCT.<sup>4,5</sup> As these manipulations are the ones most likely to be operator-dependent, a measure of the variability of these steps will provide an estimate of the variability of the assay.

The Contribution of Elements test uses two challenge

organisms, a prokaryote and a eukaryote, and one lens type. The MIMCT employs 20 contact lenses from FDA group I (low water, non-ionic) and group IV (high water, ionic) for each of six test organisms. These organisms include *Staphylococcus hominis* (reclassified from *Staphylococcus epidermidis*<sup>6</sup>), *Pseudomonas aeruginosa*, *Serratia marcescens*, *Candida albicans*, *Aspergillus fumigatus*, and the herpes simplex virus. Following disinfection, the contact lenses and aliquots of the disinfecting solutions are assayed for viable microorganisms. The disinfecting regimen fails the test if microbial growth is shown in any one of the approximately 720 samples of lenses and solutions.

Disinfection can be defined as a probability function. For the purposes of this investigation, disinfection is the reduction of vegetative microbial contaminant level to approximately  $10^{-3}$  CFU/mL. This definition is consistent with the FDA soak time requirement of 9D-values (representing a reduction to  $10^{-3}$  from a  $10^6$  CFU/mL inoculum) for disinfecting solutions. We used a probability model based upon the Poisson distribution to study the suitability of the MIMCT to measure this level of disinfection. This probability analysis assumes that the survival of a single CFU following disinfection would result in growth.

The MIMCT registers disinfecting failures as growth in a liquid neutralization/recovery medium. If an inadequate neutralizer is utilized, the residual disinfecting solution could inhibit the growth of surviving microorganisms.<sup>7,8</sup> The nutritional quality of the medium is also a concern—if the broth used is a poor recovery medium, the surviving organisms will not grow. Either of these events would prevent recognition of a failure. The current Neutralizer Efficacy protocol is discussed as a control experiment to the MIMCT.

The purpose of this study was to evaluate the measured variability of the rubbing and rinsing steps of the MIMCT through data derived from assays of the Contribution of Elements. A probability model is presented to estimate the level of remaining microorganisms for consistent passing of the MIMCT. In addition, an improved protocol is suggested for the evaluation of neutralizers utilized in this test.

### Materials and methods

Test organisms used include *S. hominis* (ATCC 17917), *C. albicans* (ATCC 10231), and *Saccharomyces cerevisiae* (used to prepare the organic load).<sup>5</sup> *S. hominis* was grown to confluence on nutrient agar slants of trypticase soy agar (TSA) (Difco Laboratories, Detroit, MI) and harvested into phosphate buffered saline (PBS), pH 7.2. *C. albicans* and *S. cerevisiae* were grown to confluence on slants of Sabouraud's dextrose agar (SDA) (Difco). Samples were washed twice in PBS and then standardized to  $10^8$  colony forming units (CFU) per milliliter by turbidity.

A 3-day culture of *S. cerevisiae* (Wards 85W5000) on SDA slants was harvested in TPBS (PBS with 0.1% Tween 80) and washed in PBS. The suspension (final volume 8 mL) was adjusted to approximately  $10^8$  CFU/mL by turbidity. This suspension was held in a boiling water bath for 30 minutes. The boiled tubes were allowed to cool, then the particulate matter was separated by centrifugation. The particulate matter was

resuspended in 7 mL of heat-inactivated horse serum.

Testing was performed in accordance with the current guidelines,<sup>5</sup> as detailed below for the Contributions of Elements test. Each assay was performed twice with 20 lenses over a 2-day period. This procedure resulted in a total of 40 lenses per assay configuration.

1. Two sets of 20 new polymacon (Soflens®) (Bausch & Lomb, Rochester, NY) contact lenses were individually inoculated with  $10^6$  CFU of challenge organism in organic load. Lenses were incubated at room temperature for 3–10 minutes.
2. The contaminated contact lenses were then cleaned by rubbing between the thumb and forefinger while wearing sterile gloves. The lenses were rubbed for approximately 30 seconds. The lenses were then rinsed for approximately 30 seconds with fresh solution by a second technician wearing sterile gloves.
3. Following treatment, the lenses were transferred to 10 mL of Dey-Engley Broth (DEB) (Difco) and vortexed.
4. The contact lens and two 1 mL aliquots of the broth media were then plated in Dey-Engley Agar (DEA) (Difco) for determination of remaining viable microorganisms.

Five Contribution of Elements assays were performed over a 5-year period. The tests involved at least seven different operators following the protocol detailed above. The different regimens assayed and solutions used are described below:

#### Solutions used:

- Trial 1:* Both cleaning and rinsing were assayed using a solution containing sodium borate, sorbic acid, EDTA, boric acid, NaCl, and tetric 1107 with a pH of 6.7–6.9.
- Trial 2:* Both cleaning and rinsing assayed with a solution containing NaCl, EDTA, NaPO<sub>4</sub>, sorbic acid, tetric 1107, and a disinfectant with a pH of 6.5–6.6.
- Trial 3:* Cleaning was performed using a solution containing hydroxypropylmethylcellulose (HPMC), sodium borate, sorbic acid, EDTA, NaCl, and tetric 1107 with a pH of 6.7–6.9. Rinsing was performed using a solution containing sodium borate, boric acid, EDTA, NaCl, tetric 1107, and PABP with a pH of 7.0–7.4.
- Trial 4:* Both cleaning and rinsing assayed using a solution containing sodium borate, boric acid, EDTA, NaCl, tetric 1107, and PABP with a pH of 7.0–7.4.
- Trial 5:* Cleaning was performed using a solution containing HPMC, sodium borate, sorbic acid, EDTA, NaCl, and tetric 1107 with a pH of 6.7–6.9. Rinsing was performed using a solution containing sodium thiosulfate, EDTA, sodium borate, boric acid, NaCl, tetric 1107, and PABP with a pH of 7.0–7.4.

Several statistical tests used were valid only if the data were normally distributed. The counts of surviving microorganisms (CFU) were transformed to their  $\log_{10}$  values for these statistical analyses. Use of  $\log_{10}$  values requires some approximation in the special case where the original value equals 0 CFU. We have chosen to assign the value of 0.5 CFU in this case. This value lies midway between the lowest measurable value, (1

**TABLE I** Probability of contact lens disinfection as a function of remaining microorganisms

Microorganisms remaining (CFU/lens)	Probability of no recoverable organisms remaining		
	1 lens	20 lenses	Passage
10	0.0001	<0.0001	<0.0001
5	0.0067	<0.0001	<0.0001
1	0.3679	<0.0001	<0.0001
0.5	0.6065	0.0001	<0.0001
0.1	0.9048	0.1353	<0.0001
0.05	0.9512	0.3679	<0.0001
0.01	0.9901	0.8187	0.0907
0.005	0.9950	0.9048	0.3012
0.001	0.9990	0.9802	0.7866
0.0005	0.9995	0.9901	0.8869
0.0001	0.9999	0.9980	0.9763

These values were derived by use of the Poisson distribution (see text for details).

CFU) and the lowest possible value (0 CFU). The data in Table I did not use the  $\log_{10}$  transformation for the determination of probabilities as the analysis utilized the Poisson distribution. If the concentration of microorganisms remaining on the contact lens after disinfection is denoted  $\lambda$ , then  $e^{-\lambda}$  is equal to P, the probability of the absence of recoverable microorganisms on any single lens in that treatment population. The probability of twenty lenses without microbial growth is  $P^{20}$ . Therefore the probability of passing the MIMCT (i.e., 20 lenses for six organisms using two soft contact lens types) is  $(P^{20})^{12}$ .

## Results

Data are presented in Table II from five Contribution of Elements assays. The tests involved different solutions and were all performed on polyacon (FDA group I) lenses. Rinsing was found to be far more efficient at removing microorganisms from the surface of the lenses than was cleaning. Figure 1 presents the data as a ratio of rinsing:cleaning efficacy. As shown in Table II, each step exhibits a degree of variation. The low variance of these populations is remarkable, and contrary to the popular belief of the test's imprecision. The probability of sterility for any lens can be determined from the Poisson distribution if the average number of CFU per lens remaining after cleansing is known. Given the probability of a single sterile lens, the probability of multiple sterile test articles,  $n$ , is merely the individual probability raised to the  $n$ th power. Table I provides probabilities for 20 lenses to be devoid of recoverable microorganisms, and for passing the MIMCT at specific concentrations of microorganisms. Passing the test is defined as no recoverable microorganisms in 20 sampling events for each of two contact lens types for six organisms assayed individually. Acceptable risk for passing the test (at least 95% probability) is achieved with an average concentration of  $10^{-4}$  CFU/lens for 20 lenses.

The effects of reducing the number of lenses employed in the MIMCT are calculated as described above and presented in Figure 2. Reducing the number of lenses utilized in the assay

from the recommended 20 has a significant impact on the stringency of the test. The use of fewer lenses increases the probability of passing by less effective disinfecting solutions.

## Discussion

There are stringent requirements for demonstrating the safety and efficacy of new contact lens care solutions. The primary means of demonstrating disinfecting efficacy are a suspension test and the MIMCT, a carrier test. These two tests examine different aspects of contact lens disinfection. The suspension test is performed by directly inoculating the disinfecting solution with challenge microorganism and, over time, assaying the suspension for survivors. These data are used to generate an approximate D-value, defined as the amount of time required to kill 90% of the challenge microorganisms, assuming linear kill kinetics. This measure is intended to quantify the inherent disinfecting efficacy of the solution. However, this determination is limited as an objective measure of chemical disinfection because it assumes first order "kill kinetics," a situation rarely seen with chemical disinfectants.<sup>9,10</sup> However, the data generated from the suspension assay are of great value in demonstrating the kinetics of disinfection if not incorrectly reported as D-values. The data from suspension assays can indicate the inherent antimicrobial capacity of the disinfecting solution in the absence of the lens care regimen.

The MIMCT is a carrier disinfection test, using a contact lens as the carrier of the challenge microorganism. This test is designed to assess the efficacy of a complete regimen of contact lens disinfection, which includes rubbing and rinsing as well as chemical disinfection. This assay incorporates the possible

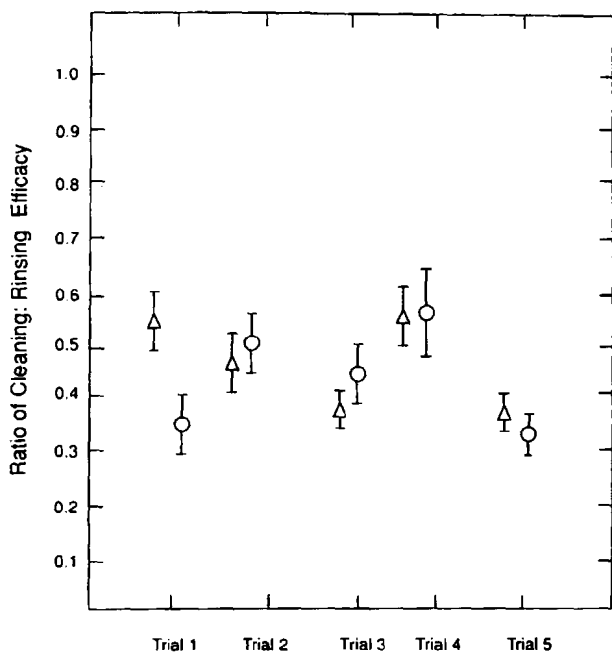
**TABLE II** Determination of cleaning and rinsing efficacy

	<i>S. hominis</i>		<i>C. albicans</i>	
	Clean	Rinse	Clean	Rinse
<b>Trial 1</b>				
ALR	1.54	2.82	1.04	2.89
s <sup>2</sup>	0.11	0.06	0.11	0.21
<b>Trial 2</b>				
ALR	1.38	3.01	1.50	3.01
s <sup>2</sup>	0.11	0.46	0.04	0.71
<b>Trial 3</b>				
ALR	1.67	4.34	1.53	3.58
s <sup>2</sup>	0.03	0.38	0.04	1.23
<b>Trial 4</b>				
ALR	2.33	4.42	1.92	3.58
s <sup>2</sup>	0.16	0.43	0.14	1.23
<b>Trial 5</b>				
ALR	1.68	4.56	1.53	4.90
s <sup>2</sup>	0.03	0.20	0.05	0.06

ALR = average log reduction.

s<sup>2</sup> = pooled sample variance.

Each trial represents average log reductions ( $\log_{10}$ ) for 40 lenses tested in a Contribution of Elements test as specified in the Draft FDA Guidelines.<sup>5</sup> Each set of 20 lenses was tested in duplicate and the pooled sample variance (s<sup>2</sup>) determined with 38 degrees of freedom. These trials were performed with different cleaning and rinsing solutions.



**Figure 1** Ratio of rinsing to cleaning efficacy. The reduction ( $\log_{10}$ ) from a  $10^6$  CFU/lens inoculum accomplished separately by rinsing and by cleaning (data from Table II) for *S. hominis* ( $\Delta$ ) and *C. albicans* ( $\circ$ ). The ratio of the two values with the respective 95% confidence interval (determined by Fieller's Theorem<sup>10</sup>) is indicative of the efficacy and variability of the steps.

effects of an organic load, an important consideration, as proteins and lipids adhering to a patient's lens may affect a disinfectant's activity.<sup>11</sup>

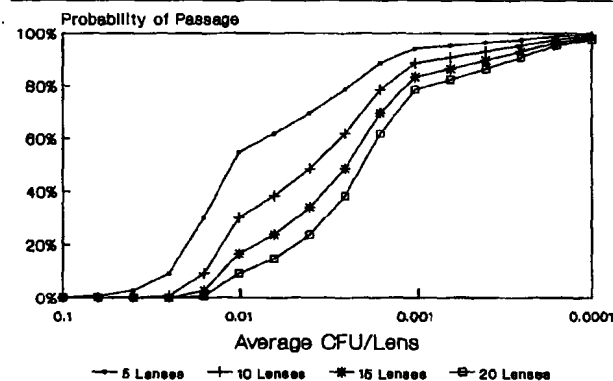
The MIMCT is subject to operator-related variability in the cleaning and rinsing steps, as shown in Table II and Figure 1. It is worth noting that the amount of variance in these populations is small, implying a high degree of reproducibility. These assays were all performed over the course of 2 days, thereby reflecting normal day-to-day variation as well as operator variability. From these data we conclude that the cleaning and rinsing steps are not unduly variable when performed by skilled technicians. The sample variance for the tests shows the rinsing step to be the more variable of the two treatments. The ratio of cleaning to rinsing demonstrates the relative efficacies of the two, as well as providing a pooled-measure of the variability of both cleansing components. This variability (displayed in Figure 1 as 95% confidence intervals) is not significant to the outcome of the test.

We have formulated a probability model designed to estimate the likelihood of passing the MIMCT, assuming a specific number of survivors. This model is a useful means for evaluating the risks associated both with assaying different disinfecting solutions and in modifying the test from the guideline procedures. The overall concentration of all microorganisms must be reduced to approximately 0.0001 ( $10^{-4}$ ) CFU/lens before a disinfecting regimen has a 98% probability of passing the MIMCT.

In absolute numbers, most of the initial  $10^6$  CFU microbial inoculum in this test is removed by the rubbing step, with the rinsing step being indispensable and responsible for most of the mechanical removal of microorganisms. However, previous reports in the literature<sup>1-3</sup> state that 10-100 CFU/lens remain. Successful chemical disinfectants must remove this load. Therefore, we conclude that the MIMCT is indeed a stringent carrier assay of disinfecting efficacy.

The level of reduction in microbial count accomplished by the cleansing of the contact lens underscores the need for patient compliance. However, a thousand-fold reduction is not sufficient to consistently pass the carrier disinfection test as currently practiced. It is reasonable to suppose that the healthy eye is robust to challenge by pathogenic microorganisms, but little is currently known about the infectious dose of specific pathogens for the onset of keratitis in the uncompromised eye. Many risk factors may contribute to the infectious dose of a pathogen. These include age, sex, diet, general hygiene, and underlying ocular health (reviewed by Ross-Degnan<sup>12</sup>). Furthermore, the infectious dose cannot be assumed to be as low as one microorganism. Quite simply, we do not know what a safe level of microbial contamination on a contact lens may be, or even if there is a safe level. This test provides a significant margin of safety to the consumer when performed as prescribed in the FDA guidelines. Modification of this test to include fewer contact lenses per assay significantly reduces its stringency.

Current guidelines<sup>5</sup> require the use of 20 contact lenses per assay. Given two lens types and six challenge organisms, this is a total of 240 lenses per test. The MIMCT is an extremely large test and expensive to perform. Reducing the number of lenses examined would lower the cost of the test but would also affect its stringency. We explored the relationship between the number of lenses tested and the likelihood of passing using the probability model presented in Figure 2. Marginal disinfecting solutions stand a progressively better chance of passing the MIMCT as the number of lenses assayed decreases.



**Figure 2** Effect of different sample sizes. Lowering the number of contact lenses examined for sterility dramatically increases the probability of a marginal disinfecting solution passing the MIMCT. This calculation was performed (as described in the Materials and methods), using the formula  $(p^n)^2$ , where "n" is defined as the number of contact lenses tested (5, 10, 15, or 20).

Finally, a detailed evaluation of neutralizers is critical to any discussion of disinfecting efficacy.<sup>7,8,13</sup> An assumption made throughout this analysis is that the recovery medium allows for complete growth of all surviving microorganisms at the end of the disinfection period. The final determination of efficacy is based upon the absence of growth in liquid culture. Therefore, the quality and properties of the recovery medium are of critical importance to the test. The neutralizing/recovery medium must possess two properties. First, it must adequately neutralize the disinfecting agent to allow unrestrained microbial growth. This characteristic, neutralizer efficacy [NE], is important for the accuracy of a biocidal assay. Disinfectants are neutralized either by dilution or by inclusion of specific reagents that inhibit their activity. A second necessary characteristic of the neutralizing/recovery medium is its growth promotion properties. Several chemicals known to inhibit specific disinfectants may be toxic to the test organisms.<sup>14-17</sup> These two activities, NE and growth promotion (or neutralizer toxicity [NT]), are equally important in accurate determination of the efficacy of a contact lens disinfecting solution.

The currently accepted NE protocol<sup>18</sup> measures these properties in a manner designed to study the neutralization of biostatic agents, not the currently marketed biocidal agents. NT is not measured in liquid, as the test is performed. Rather, after a maximum time of 60 minutes, a  $10^5$  CFU/mL inoculum in the neutralizing/recovery broth medium is plated for viable cells on a rich agar medium. No attempt is made to show growth in the neutralizing/recovery broth, merely survival of a large inoculum for 1, 10, 30, or 60 minutes. This medium is to be used to allow small numbers of survivors to grow to the point of turbidity in the MIMCT. Measuring survival of a large inoculum is not relevant to this critical requirement.

NE is measured in an analogous manner. Challenge organisms at  $10^6$  CFU/mL are inoculated directly into the disinfecting solution for 3, 10, or 30 minutes and then diluted 1:100 into a neutralizing/recovery broth. This broth is then incubated, and periodic plate counts are taken. The procedure may yield some information on recovery of injured organisms,<sup>15,16</sup> but these data are difficult to interpret and are not directly applicable to the stated objectives of the test.

The major weakness in this method is that it does not examine the characteristics of the neutralizing/recovery broth medium under the conditions of the MIMCT. A valid method to examine neutralizer toxicity and efficacy for the MIMCT would measure recovery of a small inoculum (10–100 CFU) in each of three different media configurations for each test organism. Comparisons of growth between pairs of the different media configurations would provide an appropriate measure of NE and NT.<sup>13</sup>

The first configuration would consist of inoculation of a rich broth medium, establishing the viability and growth characteristics of the particular challenge organism. The second configuration would be inoculation of the neutralizing/recovery broth without the disinfectant. Comparison between growth of each challenge organism in the rich broth and growth in the neutralizing/recovery broth medium provides the basis for a

determination of the neutralizer toxicity. It is of obvious importance that the neutralizing/recovery broth promote growth of the challenge organisms.

The final configuration would be the neutralizing/recovery broth in the presence of the disinfectant. The ratio of broth:disinfectant in this control experiment must be the same as that in the actual test. The MIMCT assays for growth in liquid neutralizing/recovery medium after disinfection. This portion of the NE-NT assay addresses the ability of the neutralizing/recovery broth to neutralize levels of disinfectant likely to be seen in final assay. The comparison of growth between the neutralizing medium in the presence and the absence of the disinfectant would allow for determination of neutralizer efficacy. It is of critical importance that these control experiments be conducted in a manner identical to that in which the test is performed.

## Conclusions

Contact lens disinfection solutions are tested in full regimen as part of the currently accepted practices for carrier disinfectant testing. The level of reduction of microorganisms on a contact lens by rubbing and by rinsing is significant and is indeed subject to some variation. This variability, however, is not significant to the outcome of the test. Probability modeling demonstrates the stringency of this method as a test of the efficacy of a chemical disinfectant regimen. It requires an average of a  $10^{10}$  reduction of the bacterial and fungal challenges for passing when performed with 20 lenses. Reduction of this number of lenses lowers the stringency of the assay for marginally effective systems. The NE protocol is not rigorously linked to the MIMCT protocol, and a proposal for an improved Neutralizer Evaluation method is presented.

## Acknowledgments

Robert Franco, Mary Mowrey-McKee, Elaine Keller-Petryk, and O.W. Lever for their review and suggestions.

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 Accepted for publication January 30, 1992.