TEMPERATURE INSTABILITY OF ReNu with MoistureLoc: A NEW THEORY TO EXPLAIN THE WORLDWIDE *FUSARIUM* KERATITIS EPIDEMIC OF 2004-2006

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ABSTRACT

Purpose: A 2006 US Food and Drug Administration (FDA) inspection of Bausch & Lomb's (B&L's) Greenville, South Carolina, manufacturing site indicated that B&L failed to regulate storage and transport temperatures of their products. The present study investigated the effect of storage temperature on the ability of contact lens solutions to inhibit growth of *Fusarium* species.

Methods: Six contact lens solutions were studied: ReNu with MoistureLoc (ReNu ML), ReNu MultiPlus, Complete Moistureplus, AQuify, Clear Care, and OPTI-FREE RepleniSH. Two bottles of each solution were separately stored at room temperature and 60°C for 4 weeks, serially diluted, then tested for their ability to inhibit growth of 11 *Fusarium* isolates (7 of which were associated with the keratitis epidemic).

Results: ReNu ML demonstrated the greatest decline in efficacy after 60°C storage. Clear Care and ReNu MultiPlus performed the best. Regarding the keratitis epidemic isolates only, the ReNu ML bottle stored at room temperature allowed growth in 27 of 84 combinations vs 67 of 84 combinations with the 60°C stored bottle.

Conclusions: When exposed to prolonged temperature elevation, ReNu ML loses its in vitro fungistatic activity to a much greater extent than other products. Improper temperature control of ReNu ML may have contributed to the *Fusarium* keratitis epidemic of 2004-2006.

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INTRODUCTION

In August 2004 Bausch & Lomb ([B&L], Rochester, New York) introduced a new multipurpose soft contact lens solution, ReNu with MoistureLoc Multi-Purpose Solution (ReNu ML), containing 2 ingredients not found in other soft contact lens solutions currently on the market: the bisbiguanide antimicrobial agent alexidine (dihydrochloride) and a moisture-retaining polysaccharide that holds water close to the contact lens surface, polyquaternium-10. ReNu ML also contained poloxamer 407, a surfactant that inhibits protein and debris from attaching to the contact lens surface.[1]

In July 2005 the Hong Kong Department of Health became aware of an increased incidence of *Fusarium* keratitis, which they reported to B&L.[2] On February 21, 2006, a news bulletin from Singapore was posted on the Internet reporting a total of 35 cases of ReNu-related *Fusarium* keratitis.[3] Later, a case-control study from Singapore, which included 61 patients with contact lens–associated *Fusarium* keratitis, noted markedly significantly elevated odds ratios for exposure to two B&L products: ReNu ML (99.3) and ReNu MultiPlus (21.5).[4] However, Chang and colleagues,[1] using a multivariable model of single-solution users, and after controlling for the practice of reusing old solution, identified only the use of ReNu ML in the month before symptom onset as significantly associated with having *Fusarium* keratitis (adjusted odds ratio, 22.3), and not the use of ReNu MultiPlus (adjusted odds ratio, 2.4).

On March 8, 2006 (3 cases from Newark, New Jersey) and on March 10 (2 cases from Dayton, Ohio, by 2 of the present authors [J.D.B., R.E.W.]), both the US Food and Drug Administration (FDA) and the Centers for Disease Control and Prevention (CDC) received the first US reports of ReNu-related *Fusarium* keratitis. As of June 30, 2006, a total of 154 confirmed cases in soft contact lens wearers from 33 states and one territory were identified in the US epidemic.[1]

Numerous researchers have since attempted to explain the etiology of this epidemic. B&L investigators have acknowledged that all original cases appear to be related to the ReNu ML produced in the Greenville, South Carolina, manufacturing plant.[5] Chang and colleagues [1] reported that tests by the CDC found no fungal contamination of unopened bottles produced by that plant, including bottles with the same lot numbers as those which were used by affected patients in the epidemic, and that there was multilocus genotyping of the clinical isolates from affected patients, essentially excluding the possibility of a single-point source contamination of the solution itself. They therefore concluded that this epidemic was due to a failure of ReNu ML to disinfect adequately after point-of-use contamination rather than from intrinsic contamination with *Fusarium*.[1,6] Factors hypothesized to have contributed to the epidemic include direct uptake of alexidine by the contact lenses (thereby reducing its antimicrobial efficacy),[7] reduced antimicrobial activity of evaporated ReNu ML,[5] enhanced growth of *Fusarium* spp on ReNu ML biofilms on contact lens cases,[8-10] direct penetration of *Fusarium* spp into soft contact lenses,[11,12] and patient noncompliance.[5] However, none of these factors, either alone or in combination, would explain why only the ReNu ML produced in the Greenville, South Carolina, plant (as opposed to the other ReNu ML manufacturing sites in Milan, Italy; Beijing, China; and Bhiwadi, India) had been implicated.

While the B&L researchers Levy and colleagues [5] stated that "product and plant inspections had failed to show any remarkable anomalies," the following should be noted. From March 22 to May 15, 2006, investigators from the FDA inspected B&L's Greenville, South Carolina, facility, which had manufactured the suspect ReNu ML supplied to Hong Kong, Singapore, and the United States. They subsequently released their findings in FDA Form 483, which noted, among many other deviations from FDA quality system regulations, that[13,14]:

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1. "…the firm (B&L) does not monitor the temperature of the storage warehouse at the distribution center, although product labeling specifies that products should be stored at room temperature."
2. "…the trucking company does not have a climate control system in the trailer to monitor temperature conditions."
3. "Temperature conditions within the aseptic processing area are not being documented to ensure conditions are consistently within established specifications…"
4. "There are no procedures indicating the amount of time finished products are allowed to remain stored in trailers before finding a location in the warehouse for storage."
5. "The firm (B&L) does not have a test method to evaluate the degradation of alexidine in the ReNu w/MoistureLoc Multipurpose Solution."

From the above official FDA statements, which, to our knowledge, have not been published previously in the scientific literature, it is possible that the ReNu ML contact lens solution produced in the Greenville, South Carolina, plant was stored and transported in non–temperature controlled environments for unknown periods of time and at unknown temperatures, contrary to product labeling specification. The authors have no way of knowing whether or not B&L monitored the temperature of the storage warehouses and/or transporting vehicles at any of the manufacturing sites in Italy, China, and India.

Because of the above facts, and in order to investigate further the cause of this worldwide epidemic, we undertook a study to determine the effects of elevated temperature on the antifungal properties of ReNu ML and other contact lens solutions.

METHODS

The following 6 multipurpose soft contact lens solutions (listed with their respective antimicrobial agent[s]) were used: ReNu with MoistureLoc (ReNu ML), containing alexidine 0.00045% (B&L, Rochester, New York); ReNu MultiPlus, containing polyaminopropyl biguanide 0.0001% (B&L, Rochester, New York); Complete Moistureplus, containing polyhexamethylene biguanide 0.0001% (Advanced Medical Optics, Santa Ana, California); AQuify, containing polyhexanide 0.0001% (CIBA Vision, Duluth, Georgia); Clear Care, containing hydrogen peroxide 3% (CIBA Vision, Duluth, Georgia); and OPTI-FREE RepleniSH, containing myristamidopropyl dimethylamine 0.0005% and polyquaternium-1 0.001% (Alcon Laboratories, Fort Worth, Texas). All of the bottles were procured in the Dayton, Ohio, area; the 2 B&L products were labeled as "Made in USA," presumably at the Greenville, South Carolina, plant, their only US manufacturing facility for ReNu products.

Eleven isolates of *Fusarium* species were obtained from the following institutions: the CDC, Atlanta, Georgia (7 isolates); Stanford University Medical Center, Palo Alto, California (2 isolates); and CompuNet Clinical Laboratories, Dayton, Ohio (2 isolates). Isolates from the CDC represented 3 different species complexes and multiple genotypes from the *Fusarium* keratitis epidemic of 2004-2006.[1] Isolates from Stanford University and CompuNet Clinical Laboratories were not involved in the epidemic (Table 1).

TABLE 1. SUMMARY OF *FUSARIUM* ISOLATES

ISOLATE*	*FUSARIUM* SPECIES	GEOGRAPHIC ORIGIN	CLINICAL SOURCE
CDC B6902	*F solani*	New Jersey	Cornea
CDC B6947	*F oxysporum*	Ohio	Contact lens case
CDC B6980	*F cf incarnatum*	Ohio	Cornea
CDC B6984	*F solani*	Tennessee	Cornea
CDC B7029	*F solani*	Kentucky	Contact lens case
CDC B7090	*F solani*	Ohio	Contact lens case
CDC B7092	*F solani*	South Dakota	Contact lens
Stanford 1	Not determined	California	Leg wound
Stanford 2	Not determined	California	Skin biopsy
CCL1	*F oxysporum*	Ohio	Unknown
CCL2	*F dimerum*	Ohio	Unknown

*CDC *Fusarium* isolates are from the Centers for Disease Control and Prevention, Atlanta, Georgia, and were involved in the *Fusarium* keratitis epidemic of 2004-2006. Stanford 1 and 2 are *Fusarium* isolates from Stanford University Medical Center, Palo Alto, California, and CCL1 and 2 are *Fusarium* isolates from CompuNet Clinical Laboratories, Dayton, Ohio, and none of those 4 isolates were involved in the *Fusarium* keratitis epidemic of 2004-2006.

PILOT STUDY

One sealed, unopened, unexpired bottle of each of the 6 contact lens solutions was maintained at room temperature (23°C [73.4°F], as monitored by a standard indoor thermostat in a strictly temperature-controlled laboratory), and a second sealed, unopened, unexpired bottle was maintained in a water bath at 60°C (140°F) for 4 weeks and then allowed to return to room temperature. At the end of the 4-week period, a 10-mL aliquot was removed from the bottle that had been stored at room temperature and was boiled in a glass tube for 10 minutes and then allowed to return to room temperature. Serial dilutions of the 3 samples (room temperature, 60°C, and boiled) of each contact lens solution were then made in sterile plastic tubes using Sabouraud dextrose broth (SDB). Because the 2 *Fusarium* keratitis epidemic isolates obtained by the present authors (J.D.B., R.E.W.) had been previously sent to the CDC at their request, 2 different clinical isolates of *Fusarium* spp from CompuNet Clinical Laboratories in Dayton, Ohio (CCL1 and CCL2), not associated with the *Fusarium* keratitis epidemic of 2004-2006, were used. These isolates were viable and available in our laboratory; we do not know the exact clinical sources of these isolates, but both specimens were stored in the laboratory either by freezing at −70°C (CCL1) or by subculturing (CCL2). Each isolate was plated on Sabouraud dextrose agar (SDA) and incubated for approximately 7 days or until growth and sporulation were present. Testing was performed by adapting the recommendations of the National Committee for Clinical Laboratory Standards on susceptibility testing of filamentous fungi.[15] Suspensions of each isolate were prepared in SDB to a McFarland 0.5 standard density equivalent. The suspension was then diluted 1:50 in SDB. A 0.5-mL aliquot of the 1:50 dilution of each *Fusarium* suspension was added to 0.5 mL of each serial dilution of the contact lens solution subjected to the 3 temperatures (room temperature, 60°C, and boiled) in sterile plastic tubes for final dilutions of 1:2, 1:4, 1:8, and 1:16 for each contact lens solution subjected to the various temperatures. A single sample for each dilution was performed. The tubes were incubated at 35°C for 1 week and then observed for fungal growth by visual inspection. A growth control consisting of 0.5 mL of the 1:50 diluted *Fusarium* suspension and 0.5 mL of SDB was performed for both isolates.

EXTENDED STUDY

Based on the results of the pilot study, the following 4 contact lens solutions were used in the extended study: ReNu with MoistureLoc (ReNu ML), ReNu MultiPlus, Clear Care, and OPTI-FREE RepleniSH. One sealed, unopened bottle of each of the 4 contact lens solutions was maintained at room temperature (23°C [73.4°F], as monitored by a standard indoor thermostat in a strictly temperature-controlled laboratory), and a second sealed, unopened bottle was maintained in a water bath at 60°C (140°F) for 4 weeks. With the exception of one ReNu ML bottle, all of the other bottles were unexpired, and each of the 2 bottles for the other (non-ReNu ML) product brands had the same lot numbers. Because of limited availability due to the worldwide recall, the 2 ReNu ML bottles had different lot numbers and expiration dates. The bottle stored at room temperature had an expiration date approximately 6 weeks prior to the inoculation of the samples, and the bottle stored at 60°C had an expiration date approximately 6 weeks after the inoculation of the samples. Thus, the ReNu ML bottle stored at room temperature was slightly past its expiration date, but the ReNu ML bottle stored at 60°C was within its expiration date at the time of inoculation of the samples of contact lens solutions. For all solutions, the bottles stored at 60°C were then stored at room temperature for approximately 2 weeks prior to the next phase of the experiment. Serial dilutions of the 2 samples (room temperature and 60°C) of each contact lens solution were then made in sterile 48-well tissue culture plates using Hanks Balanced Salt Solution. *Fusarium* isolates were plated on SDA and incubated for approximately 7 days or until growth and sporulation were present. Testing was again performed by adapting the recommendations of the National Committee for Clinical Laboratory Standards on susceptibility testing of filamentous fungi.[15] Suspensions of each isolate were prepared in sterile saline to a McFarland 0.5 standard density equivalent. A 1:50 dilution of the suspension was made in both Sabouraud dextrose broth (SDB) and RPMI-1640 medium with glutamine (RPMI [the recommended medium[15]]). This suspension was plated on SDA and trypticase soy agar with 5% sheep's blood (SBA) to later verify purity after growth for 72 hours at 30°C. A 0.5-mL aliquot of the 1:50 dilution of each *Fusarium* suspension was added to 0.5 mL of each serial dilution of the contact lens solutions subjected to the 2 temperatures (room temperature and 60°C) in sterile 48-well tissue culture plates for final dilutions of 1:2, 1:4, and 1:8 for each contact lens solution subjected to the 2 temperatures. Testing was performed in duplicate for the CDC and Stanford University isolates and in quintuplicate for the Compunet Clinical Laboratory isolates in each medium (SDB and RPMI). The plates were incubated at 35°C for 48 hours and then observed for fungal growth by visual inspection. Positive wells were plated on SBA and incubated for 48 hours to verify purity of *Fusarium* growth. A growth control consisting of 0.5 mL of the 1:50 diluted *Fusarium* suspension and 0.5 mL of SDB or RPMI was performed for all isolates. The inhibitory titer was defined as the highest dilution of contact lens solution that was able to inhibit fungal growth in both duplicate or in at least 3 of 5 quintuplicate samples tested. The increase in inhibitory titer is the difference in inhibitory titer between contact lens solutions stored at 23°C vs 60°C (Table 2).

RESULTS

PILOT STUDY

With regard to *Fusarium* isolate CCL2, no contact lens solution allowed growth of the organism at 1:2 or 1:4 dilutions under any temperature conditions, and only one solution (AQuify) demonstrated a one-dilution decline in inhibition of CCL2 growth after incubation at 60°C or boiling (data not shown). *Fusarium* isolate CCL1 proved to be more resistant to inhibition by the contact lens solutions (Table 3). At room temperature, OPTI-FREE RepleniSH and Complete Moistureplus were the least efficacious in inhibiting CCL1; both allowed growth at dilutions of 1:4 and higher. However, after storage at 60°C for 4 weeks, OPTI-FREE RepleniSH and ReNu ML were the only products to allow growth of CCL1 at all dilutions, and ReNu ML was the only solution that demonstrated a 2-dilution (≥4-fold concentration) decline in efficacy relative to room temperature. After boiling, OPTI-FREE RepleniSH was the only

product to allow growth of CCL1 at all dilutions and was the only product to show a decline in efficacy relative to room temperature. The solutions that were the most efficacious in inhibiting *Fusarium* growth were Clear Care and ReNu MultiPlus. Clear Care, containing hydrogen peroxide 3%, did not allow growth of either *Fusarium* isolate under any conditions, while ReNu MultiPlus allowed minimal growth in only one sample. Fungal growth was observed in the control tube for each of the 2 isolates.

TABLE 2A. DEFINITION OF INHIBITORY TITER*

DILUTION OF CONTACT LENS SOLUTION	*FUSARIUM* GROWTH PATTERN: FOUR POSSIBLE OUTCOMES IN EXTENDED STUDY			
1:2	0	0	0	+
1:4	0	0	+	+
1:8	0	+	+	+
Inhibitory Titer	**≥1:8**	**1:4**	**1:2**	**<1:2**

0, no visible fungal growth; +, visible fungal growth.

*The inhibitory titer was defined as the highest dilution of contact lens solution that was able to inhibit fungal growth in both duplicate or in at least 3 of 5 quintuplicate samples tested.

TABLE 2B. DEFINITION OF INCREASE IN INHIBITORY TITER†

IF THE INHIBITORY TITER WITH THE CONTACT LENS SOLUTION STORED AT 23°C WAS:	AND, IF THE INHIBITORY TITER WITH THE CONTACT LENS SOLUTION STORED AT 60°C WAS:	THEN, THE INCREASE IN INHIBITORY TITER FROM 23°C TO 60°C STORAGE WOULD BE:
1:2	<1:2	2
1:4	1:2	2
≥1:8	1:4	≥2
1:4	<1:2	4
≥1:8	1:2	≥4
≥1:8	<1:2	≥8

†The increase in inhibitory titer is the difference in inhibitory titer between contact lens solutions stored at 23°C (73.4°F) vs 60°C (140°F).

EXTENDED STUDY

Results are summarized in Tables 4 and 5. A total of 336 combinations were tested for each of the 4 contact lens solutions: duplicate (2) combinations for each of the 7 CDC and 2 Stanford *Fusarium* isolates, quintuplicate (5) combinations for each of the 2 Compunet Clinical Laboratory *Fusarium* isolates, all performed in 2 different media (RPMI and SDB), at 3 different dilutions (1:2, 1:4, and 1:8), and at 2 different storage temperatures (room temperature and 60°C). In general, SDB supported fungal growth better than RPMI, although inhibitory patterns were similar in both media. Clear Care and ReNu MultiPlus inhibited fungal growth completely under all conditions tested for each solution (all 11 isolates, all 3 dilutions, in both media, and after incubation at room temperature and 60°C [data not shown]).

In RPMI, OPTI-FREE RepleniSH stored at room temperature completely inhibited growth of all 11 isolates; after storage at 60°C, only one isolate (CCL1) was able to grow (but only at a contact lens solution dilution of 1:8). In SDB, OPTI-FREE RepleniSH stored at room temperature allowed growth of 6 of the 11 isolates. After storage at 60°C, 3 of those isolates (CDC B6947, CDC B6980, and CDC B7029) were able to grow reproducibly at lower dilutions (ie, higher contact lens solution concentrations) than with the OPTI-FREE RepleniSH stored at room temperature. One other isolate (CCL1) also was able to grow at lower dilutions after 60°C storage,

but only in 2 of the 5 quintuplicate samples.

ReNu ML was less successful at inhibiting fungal growth than the other 3 products, and again demonstrated the greatest decline in efficacy after storage at 60°C. In RPMI, ReNu ML completely inhibited growth of 4 isolates (CDC B6984, Stanford 1 and 2, and CCL2) at both room temperature and 60°C, but allowed growth of the other 7 isolates at both temperatures. Of those 7 isolates that grew in RPMI after room temperature storage, 6 were able to grow at lower dilutions after 60°C storage. The increase in inhibitory

TABLE 3. PILOT STUDY: CONTACT LENS SOLUTION INHIBITION OF *FUSARIUM* GROWTH OF CCL1 ISOLATE AFTER STORAGE AT 23°C, 60°C, OR BOILING

STORAGE TEMPERATURE*	PRODUCT	DILUTION			
		1:2	1:4	1:8	1:16
23°C	ReNu ML	0	0	+	+
23°C	ReNu MP	0	0	+	0
23°C	Complete	0	+	+	+
23°C	AQuify	0	0	+	+
23°C	Clear Care	0	0	0	0
23°C	OPTI-FREE	0	+	+	+
60°C	ReNu ML	+	+	+	+
60°C	ReNu MP	0	0	0	0
60°C	Complete	0	+	+	+
60°C	AQuify	0	0	+	+
60°C	Clear Care	0	0	0	0
60°C	OPTI-FREE	+	+	+	+
Boiled	ReNu ML	0	0	+	+
Boiled	ReNu MP	0	0	0	0
Boiled	Complete	0	0	+	+
Boiled	AQuify	0	0	+	+
Boiled	Clear Care	0	0	0	0
Boiled	OPTI-FREE	+	+	+	+

AQuify, AQuify (CIBA Vision, Duluth, Georgia); CCL1, CompuNet Clinical Laboratory isolate 1, *Fusarium oxysporum* (see Table 1); Clear Care, Clear Care (CIBA Vision, Duluth, Georgia); Complete, Complete Moistureplus (Advanced Medical Optics, Santa Ana, California); OPTI-FREE, OPTI-FREE RepleniSH (Alcon Laboratories, Fort Worth, Texas); ReNu ML, ReNu with MoistureLoc (Bausch & Lomb, Rochester, New York); ReNu MP, ReNu MultiPlus (Bausch & Lomb, Rochester, New York); 0, no visible growth; +, visible fungal growth.

*23°C: contact lens solution stored at room temperature (23°C [73.4°F]); 60°C: contact lens solution stored in a 60°C (140°F) water bath for 4 weeks; Boiling: contact lens solution boiled for 10 minutes.

titer between room temperature and 60°C storage in RPMI was 4 or ≥4 for 5 of the 6 isolates, and ≥2 for the remaining isolate. In SDB, ReNu ML (both room temperature and 60°C) completely inhibited growth of 3 isolates (CDC B6984 and Stanford 1 and 2) but allowed growth of 7 of the other 8 isolates after room temperature storage, and 8 of 8 after 60°C storage. Of those 8 isolates that grew in SDB after 60°C storage, 7 were able to grow at lower dilutions than with the ReNu ML that had been stored at room temperature. The increase in inhibitory titer between room temperature and 60°C storage in SDB was 4 for one of the isolates, ≥2 for one of the isolates, and 2 for the remaining 5 isolates. The difference in increase in inhibitory titer (Table 5) was highly significant between ReNu MoistureLoc and OPTI-FREE ($P = .0075$), and between ReNu MoistureLoc and ReNu MultiPlus or Clear Care ($P = .00002$). However, the difference was not significant between OPTI-FREE and ReNu MultiPlus or Clear Care ($P = .1078$). The P values for homogeneity of distributions were calculated using the Fisher exact test.

Fungal growth was observed in the control wells for all isolates.

TABLE 4. EXTENDED STUDY: CONTACT LENS SOLUTION INHIBITION OF *FUSARIUM* GROWTH AFTER STORAGE AT 23°C OR 60°C IN RPMI AND SDB CULTURE MEDIUM*

FUSARIUM ISOLATE†	OPTI-FREE INHIBITORY TITER‡			RENU MOISTURELOC INHIBITORY TITER‡		
	23°C	60°C	Δ	23°C	60°C	Δ
RPMI culture medium						
CDC B6902	≥1:8	≥1:8	0	≥1:8	1:2	≥4
CDC B6947	≥1:8	≥1:8	0	1:4	<1:2	4
CDC B6980	≥1:8	≥1:8	0	1:4	<1:2	4
CDC B6984	≥1:8	≥1:8	0	≥1:8	≥1:8	0
CDC B7029	≥1:8	≥1:8	0	≥1:8	1:4	≥2
CDC B7090	≥1:8	≥1:8	0	1:4	<1:2	4
CDC B7092	≥1:8	≥1:8	0	1:4	1:4	0
Stanford 1	≥1:8	≥1:8	0	≥1:8	≥1:8	0
Stanford 2	≥1:8	≥1:8	0	≥1:8	≥1:8	0
CCL1	≥1:8	1:4	≥2	1:4	<1:2	4
CCL2	≥1:8	≥1:8	0	≥1:8	≥1:8	0
SDB culture medium						
CDC B6902	1:4	1:4	0	1:4	1:2	2
CDC B6947	1:2	<1:2	2	1:2	<1:2	2
CDC B6980	1:4	1:2	2	1:4	1:2	2
CDC B6984	≥1:8	≥1:8	0	≥1:8	≥1:8	0
CDC B7029	1:2	<1:2	2	1:2	1:2	0
CDC B7090	≥1:8	≥1:8	0	1:4	1:2	2
CDC B7092	1:4	1:4	0	1:4	1:2	2
Stanford 1	≥1:8	≥1:8	0	≥1:8	≥1:8	0
Stanford 2	≥1:8	≥1:8	0	≥1:8	≥1:8	0
CCL1	<1:2	<1:2	0	1:4	<1:2	4
CCL2	≥1:8	≥1:8	0	≥1:8	1:4	≥2

RPMI, RPMI-1640 medium with glutamine; SDB, Sabouraud dextrose broth.

*Testing was performed in duplicate for each medium and temperature tested for the CDC and Stanford isolates and in quintuplicate for the CCL isolates.

†*Fusarium* isolates are described in detail in Table 1.

‡Inhibitory titer of OPTI-FREE RepleniSH (Alcon Laboratories, Fort Worth, Texas) and ReNu with MoistureLoc (Bausch & Lomb, Rochester, New York) represents the highest dilution of the contact lens solution tested (1:2, 1:4, or 1:8) that inhibited fungal growth in both duplicate or in at least 3 of 5 quintuplicate samples tested after storage at room temperature (23°C [73.4°F]) or after storage in a 60°C (140°F) water bath for 4 weeks. Delta (Δ) represents the increase in inhibitory titer required to inhibit fungal growth between solutions stored at 23°C and 60°C. Inhibitory titer and increase in inhibitory titer are defined and explained in detail in Table 2. ReNu MultiPlus (Bausch & Lomb, Rochester, New York) and Clear Care (CIBA Vision, Duluth, Georgia) inhibited fungal growth completely in all combinations of isolates, dilutions, media, and temperatures tested (data not shown).

TABLE 5. INCREASE IN *FUSARIUM* INHIBITORY TITER OF CONTACT LENS SOLUTIONS AFTER STORAGE AT ROOM TEMPERATURE VS 60°C*

CONTACT LENS SOLUTION†	INCREASE		
	0	**2 to ≥2**	**4 to ≥8**
ReNu MoistureLoc	9	7	6
OPTI-FREE	18	4	0
ReNu MultiPlus	22	0	0
Clear Care	22	0	0

*Inhibitory titer represents the highest dilution of the contact lens solution tested (1:2, 1:4, or 1:8) in both duplicate or in at least 3 of 5 quintuplicate samples tested that inhibited fungal growth after storage at room temperature (23°C [73.4°F]) or after storage in a 60°C (140°F) water bath for 4 weeks. Increase in inhibitory titer represents the increase in titer required to inhibit fungal growth between contact lens solutions stored at 23°C vs 60°C. Zero (0) indicates no change in inhibitory titer. Inhibitory titer and increase in inhibitory titer are defined and explained in detail in Table 2. The difference in increase in inhibitory titer was highly significant between ReNu MoistureLoc and OPTI-FREE (P = .0075), and between ReNu MoistureLoc and ReNu MultiPlus or Clear Care (P = .00002), whereas, the difference was not significant between OPTI-FREE and ReNu MultiPlus or Clear Care (P = .1078). The P values for homogeneity of distributions were calculated using the Fisher exact test.
†Each contact lens solution (ReNu with MoistureLoc [Bausch & Lomb, Rochester, New York], OPTI-FREE RepleniSH [Alcon Laboratories, Fort Worth, Texas], ReNu MultiPlus [Bausch & Lomb, Rochester, New York], and Clear Care [CIBA Vision, Duluth, Georgia]) was tested with 11 different *Fusarium* isolates in 2 different culture media (total 22) in either duplicates or quintuplicates.

DISCUSSION

The Arrhenius equation, $k = Ae^{-Ea/RT}$, is a mathematical expression of the dependence of the rate of a chemical reaction, k, on temperature, T, in Kelvins (K).[16] As a rule, this rate will approximately double for every 10 K (or 10°C) rise in temperature. (A is a term that includes factors like the frequency of molecular collision and molecular orientation; E_a is the minimum activation energy needed for a chemical reaction to occur; e is Euler's constant, the base of Naperian (natural) logarithms, having a value of 2.71828; and R is the so-called "gas constant" in the familiar equation $pV=nRT$.)

The Arrhenius equation is the basis for one of the FDA's 1997 guidelines concerning contact lens care products, in which they noted that "generally every 10°C increase for tested temperature will enhance the expiration date by a factor of 2 compared to the normal storage temperature."[17] Thus, the approximate theoretical relative fractional shelf life equals $1/2^{\Delta t/10}$, where Δt is the storage temperature elevation, above room temperature, in °C. For example, if a product were stored at 53°C (30°C above room temperature of 23°C), the theoretical shelf life would be: $1/2^{30/10} = 1/2^3 = 1/8$ of the shelf life if stored at room temperature. A thermally induced chemical alteration resulting in a decrease in antimicrobial concentration may be critical, as Cohen[18] noted that a 2-fold reduction in antimicrobial concentration in contact lens solutions was associated with a 10-fold decrease in reduction of fungal contamination.

Leung and coworkers [19] studied the effect of storage temperature and time on the efficacy of 4 multipurpose solutions for soft contact lenses, including ReNu MultiPlus (but not ReNu ML, as the product had not been released at the time of their study). The investigators noted that the antimicrobial activity of ReNu MultiPlus toward *Pseudomonas aeruginosa* dropped below the FDA guideline when stored at 30°C (86°F) for 2 months. They also noted decreased activity of ReNu MultiPlus and Complete Multi-Purpose (containing polyhexamethylene biguanide 0.0001% [Allergan, Irvine, California]) toward *P aeruginosa* when the solutions were stored at 4°C (39°F). They concluded that multipurpose contact lens solution stability may be adversely affected by higher temperatures, lower temperatures, and fluctuating temperatures, as well as by prolonged use of the same bottle and by the presence of air within the bottle.

All of the products tested in the present study have a manufacturer's recommendation of room temperature storage. Therefore, one would assume and expect that immediately after bottling, the manufacturers would ensure that the products are stored in warehouses and shipped in trucks or cargo ships under temperature-controlled environments until delivered to the retailer. Once the product is purchased, it is the responsibility of the consumer to maintain the solution at room temperature. Lack of temperature control anywhere along the way may jeopardize the product's antimicrobial efficacy.

Previous investigators have measured the temperature rise within enclosed vehicles relative to ambient temperature. King and coworkers[20] found that with an ambient temperature of 36.8°C (98.2°F), the temperature within the vehicle reached up to 67°C (153°F) within 15 minutes. McLaren and coworkers[21] found up to a 27°C (49°F) temperature rise within enclosed vehicles relative to ambient

temperatures of 22°C to 36°C (72°F to 96°F) after 60 minutes.

Morgan and coworkers,[22] while studying the impact of temperature on the stability of latanoprost (Xalatan; Pfizer Ophthalmics, Pharmacia, and Upjohn, Kalamazoo, Michigan), noted that in the southern United States during the summer months, temperatures within enclosed spaces such as vehicles can reach 75°C (167°F). In 2007 in Dayton, Ohio, a dark-colored automobile parked in direct midsummer sunlight from 9:30 AM to 12:30 PM, when the ambient temperature measured 33°C (91°F), was noted to have an internal temperature of 74°C (166°F). Non–temperature controlled warehouses can easily reach 40°C (104°F) in summer months.[23] Maximum temperatures in the Greenville, South Carolina, area in June 2005 were typically above 32°C (90°F).[24] Thus, it is apparent that the logistics of storing, transporting, and delivering liquid pharmaceuticals and contact lens solutions in a temperature-controlled environment is an extremely important issue.

Our study demonstrated an in vitro loss of antimicrobial activity of ReNu ML with regard to multiple clinical isolates of *Fusarium* when the product was exposed to a prolonged (4-week) temperature of 60°C (140°F). The 5 other multipurpose soft contact lens solutions tested with ReNu ML in the pilot study were selected because they were the more commonly used products at the time of the study. Based on the results of the pilot study, in our extended study we chose to investigate further the products that demonstrated the most (Clear Care) and least (OPTI-FREE RepleniSH) fungistatic efficacy, as well as the other product manufactured by B&L in their Greenville, South Carolina, plant (ReNu MultiPlus). The results of the extended study verified and confirmed the results of the pilot study. A water bath in our laboratory is maintained at 60°C for other purposes. This temperature and water bath were used in order to simulate conditions to which some of the manufacturer's bottles may have been exposed during storage and transport, or even, perhaps, after purchase. Future studies using multiple temperatures with fungal as well as bacterial organisms may be beneficial. Interestingly, in our pilot study, ReNu ML that was boiled inhibited *Fusarium* growth of 2 isolates (CCL1 and 2) equally as well as the ReNu ML stored at room temperature. This suggests that alexidine inactivation is both time- and temperature-dependent. While the B&L investigators Levy and colleagues[5] report performing "extended storage studies" as well as "elevated temperature studies, including impact on biocidal efficacy versus *F. solani*," they do not specify the experimental conditions, the temperature(s) used, or the duration of exposure, and they neglected to report the results of those specific studies.

The *Fusarium* spp tested in our extended study included 7 clinical isolates obtained from the CDC that were directly involved in the *Fusarium* keratitis epidemic of 2004-2006, as well as 4 separate clinical isolates from 2 different sources that were not associated with the epidemic. The differences in product efficacies with the individual isolates varied and likely represent differing susceptibilities between isolates to the various antimicrobial agents in the different contact lens solutions. As noted by Aziz, Bullock, and colleagues[25,26] in their study of *Aspergillus fumigatus* endogenous endophthalmitis and Imamura and coworkers[10] in their study of fungal biofilm formation on soft contact lenses, growth and virulence phenotypes of laboratory strains typically weaken with multiple subculturing. This is consistent with the findings of our study, in which the laboratory isolate that had been maintained by subculturing (CCL2) showed minimal to no growth in the numerous samples among all soft contact lens solutions tested, whereas the laboratory isolate that had been maintained by freezing (CCL1) was more resistant. In contrast, ReNu ML only completely inhibited growth in 1 of the 7 CDC epidemic isolates. With regard to the other 6 CDC epidemic isolates, ReNu ML stored at 60°C (in an unexpired bottle) allowed growth in 67 of 72 combinations vs growth in only 27 of 72 combinations using ReNu ML stored at room temperature (in a bottle just past its expiration date).

While B&L products other than ReNu ML manufactured in and distributed out of the Greenville, South Carolina, plant would likely have been subjected to the same temperature conditions, our findings that ReNu MultiPlus completely inhibited fungal growth in all combinations tested at all temperatures would be compatible with the lack of association of ReNu MultiPlus with this epidemic, as suggested by Chang and associates,[1] and in contrast to the findings of an elevated odds ratio for ReNu MultiPlus noted by Saw and colleagues.[4] Furthermore, Levy[27] has suggested that the elevated odds ratio for ReNu MultiPlus found by Saw and colleagues [4] may have been a result of recall bias (of which solution was used) and/or multiple solution use by affected patients, particularly because after the time of the worldwide recall of ReNu ML, there has been an abrupt decline in reports of *Fusarium* keratitis despite the continued availability and use of ReNu MultiPlus.[6] While our findings that OPTI-FREE RepleniSH also demonstrated some decline in fungistatic efficacy after 60°C storage, we are not aware of any association between that product and *Fusarium* keratitis. However, ongoing storage temperature control of that product is also clearly very important.

The precise temperature, duration of exposure to elevated temperature, and extent of temperature fluctuation that may diminish the antimicrobial activity of a particular contact lens solution are not known, and thus additional studies may be warranted. However, our findings, coupled with the FDA reports of B&L's failure to regulate the storage and transport temperatures in and beyond the Greenville, South Carolina, plant, may be significant. Previously cited studies have suggested that ReNu ML has characteristics making it vulnerable to *Fusarium* infection.[5,7] Our study demonstrated loss of fungistatic activity of ReNu ML upon prolonged (4-week) exposure to high temperature (60°C [140°F]). These factors, together with the FDA findings of temperature control issues in and beyond the Greenville, South Carolina, plant, may have potentiated a set of circumstances that led to the epidemic of ReNu ML–associated *Fusarium* keratitis in 2004-2006. Knowledge of the potential loss of antimicrobial activity of contact lens solutions and other pharmaceutical products when exposed to higher temperatures and the risk of such exposure when storing and transporting those products may help prevent such epidemics in the future.

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PEER DISCUSSION

DR. JAMES CHODOSH: Filamentous fungal infections of the cornea are characteristically vision threatening, can be very difficult to treat, and overall carry a poorer prognosis than most other microbial causes of corneal infection. In a 2007 review of fungal keratitis, Tuli and coworkers suggested that the incidence of fungal keratitis, in particular those cases associated with contact lens use, was rising, and that the rise had begun well before the now well known ReNu MoistureLoc® *Fusarium* epidemic of 2004 to 2006.[1] In their article, the authors cite several possible reasons for increasing rates of fungal keratitis in contact lens users, including a shift away from thermal disinfection and peroxide based solutions toward multipurpose solutions for storage and cleaning, a trend toward no-rub solutions, and perhaps, worsening lens care by contact lens wearers – although no evidence was provided to support the latter idea.

The MoistureLoc® epidemic of 2004-2006 certainly captured the attention of physicians and the public. In places like Gainesville and Oklahoma City, where contact lens related fungal keratitis is not uncommon, the increased cases barely registered. However, in cities such as Singapore, New York, and San Francisco, a few treating physicians experienced a staggering change – some eye care providers who had never seen a single case of contact lens related *Fusarium* keratitis saw two, three or more in a short time interval.[2] Thankfully, the association between *Fusarium* keratitis and ReNu MoistureLoc® solution was made in due order.

The cause of *Fusarium* keratitis in patients using the solution has not been simple to resolve. The easy answers to the epidemic were not confirmed – *Fusarium* organisms among the MoistureLoc® cases were phylogenetically diverse rather than from a single clone,[3] and no organisms were recovered from the factory, warehouse, or solution filtrate. Subsequent attempts to explain the MoistureLoc® cases focused on less satisfying causes, for example, the loss of antifungal activity by MoistureLoc® solution when subjected to evaporation,[4,5] and the tendency of clinical isolates of *Fusarium* to form MoistureLoc® resistant biofilms.[6]

In the present report, Bullock and coworkers present evidence to support an alternate theory for the MoistureLoc® *Fusarium* keratitis epidemic. They note that all the *Fusarium* cases associated with ReNu MoistureLoc® solution used product manufactured in Bausch & Lomb's Greenville, SC manufacturing plant, including those cases occurring in Hong Kong, Singapore, and the continental U.S. They further note that the Food & Drug Administration inspection of the Greenville plant found an absence of any temperature monitoring or climate control in the storage warehouse and trailer trucks where product would have been stored and transported. Therefore, Bullock and colleagues initiated a study of the effect of prolonged elevated temperature during solution storage on the capacity of commonly used multipurpose contact lens solutions to inhibit growth of eleven *Fusarium* isolates from various sources, including 7 from the MoistureLoc® epidemic. The results were dramatic: increased temperature was associated with reduction in the capacity of MoistureLoc®, but not other solutions tested, to inhibit growth of *Fusarium*. That said, the authors provide no information on how long contact lens solutions from the Greenville plant might in reality have been stored, and nothing beyond anecdote to determine what temperatures the product might have been exposed to. For this study, they chose a single temperature – 60°C – because a water bath at that temperature was readily available. Determination of a dose response relationship between temperature, time, and antifungal activity would be necessary to fully test their hypothesis.

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DR. RICHARD L. LINDSTROM: I consult for Bausch & Lomb, AMO, and Alcon and they all make contact lens solutions, although I consult on the surgical side, not on the contact lens side. An awkward comment, but I believe that this study was funded directly or indirectly by a group of trial lawyers. There is an active case right now with ongoing litigation in this particular area. If it is true, then my question is about whether or not a study funded by a group of trial lawyers has ever been presented by our meeting. I am not sure

that presenting it at our society meeting and publishing it in the peer reviewed literature is a good idea while the case is active. The second question I have for Dr. Bullock and Dr. Warwar relates to this issue. If this study was funded, directly or indirectly by a group of trial lawyers and either of you are a consultant to the attorney, then I do not understand your comment that you have no conflict of interest. So I would like you to speak to that. Thank you.

DR. EVELYN A. PAYSSE: I have no conflict of interest. Opti-Free® was also found to be inferior to the other solutions tested, and it is still on the market. Are there any actions or recommendations from the CDC for Opti-Free® at this point? Are there any controls being implemented for thermal protection of these solutions?

DR. JOHN D. BULLOCK: We appreciate the kind comments of Dr. Chodosh and his interesting discussion. In future studies, we will take his excellent suggestion and use varying temperatures.

I would now like to respond to Dr. Lindstrom's remarks. This study was not funded by attorneys. This study was performed in response to the report which I found on the internet concerning the FDA's inspection of Bausch & Lomb's Greenville, South Carolina plant (FDA Form 483), which showed a lack of temperature monitoring systems, among many other deficiencies. Compunet Clinical Laboratory provided all of the laboratory paraphernalia and solutions for free. All of the personnel involved in this study worked for free, receiving no compensation, whatsoever. All of the other authors, including Dr. Warwar, had no conflicts of interest. I have consulted with three different law firms in this matter: two brief consultations initially, before any laboratory work was contemplated. My contact with the third firm began very recently, after completion of our laboratory study. The fees that I received were all donated to Wright State University and I have not served as an expert witness in this matter nor have I provided any type of expert report or affidavit. Thus, Dr. Lindstrom, you are totally incorrect in stating that this study was "funded by a group of trial lawyers."

DR. RONALD E. WARWAR: I do not have any financial disclosures, and I have had no contact with any plaintiff attorneys. This study was not funded by anyone but by ourselves and Compunet Clinical Laboratory. Furthermore, the conception, implementation, interpretation, preparation, and funding of the experiments and the manuscript itself was performed entirely and solely by the authors of this manuscript, without any input from any attorneys, whatsoever.

Concerning Dr. Paysse's question about Opti-Free®, it did show some decline in efficacy after heating to 60ºC, but it was not statistically significant, unlike the highly significant decline seen with ReNu® with MoistureLoc®. The other contact lens solutions showed no decline in efficacy. Thus, one take-home message from this study is that it is important to monitor the temperatures of all contact lens solutions. Perhaps there have not been any outbreaks associated with OPTI-FREE® because it has been under reasonable temperature control. The importance of storage temperature control applies to all contact lens solutions.