D12 A Forensic Investigation of an Epidemic of Blindness

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The goal of this presentation is to review the details of the blinding worldwide *Fusarium keratitis* epidemic of 2004-2006 and present laboratory proof that defective plastic bottles and improper temperature control resulted in fungistatic failure of the contact lens solution.

This presentation will impact the forensic science community by discussing why the previous theory to explain the *Fusarium keratitis* epidemic of 2004-2006 was based on a false premise; namely, that the components of the contact lens solution itself interacted in such a way that Fusarium growth was facilitated. If this were correct, then cases of *Fusarium keratitis* should have been traced to all four worldwide factories where the solution had been manufactured. In fact, the cases could be traced to only one factory, in Greenville, South Carolina. The attendees will learn the details of the epidemic and the laboratory investigation which showed that defective plastic bottles and improper temperature control resulted in fungistatic failure. The attendees should learn not to accept superficial answers to difficult questions. As the Nobel Laureate Albert Szent-Gyorgyi said: "Research is to see what everybody has seen and think what nobody has thought."

Background: In August 2004, Bausch & Lomb (B&L) introduced a new contact lens solution, ReNu with MoistureLoc (ReNuML), containing the antimicrobial agent alexidine; an agent not found in other contact lens solutions. In July 2005, an increased incidence of *Fusarium keratitis* was noted in Hong Kong and in February 2006, the first 35 of 62 cases of ReNu-related *Fusarium keratitis* were reported from the Republic of Singapore. In early March 2006, the first U.S. reports of ReNu-related *Fusarium keratitis* were reported to both the Food and Drug Administration (FDA) and the Centers for Disease Control and Prevention (CDC) from Newark, New Jersey and (by JDB) Dayton, Ohio. A total of 154 confirmed cases were ultimately identified in the U.S. outbreak and the use of ReNuML was significantly associated with having *Fusarium keratitis* (adjusted odds ratio, 22.3). In mid-May 2006, the product was finally withdrawn from the world market. At the termination of the epidemic, hundreds of cases of *Fusarium keratitis* had occurred worldwide with many resulting in permanent blindness. Numerous researchers have since attempted to explain the etiology of this epidemic. B&L investigators acknowledged that all of the cases were related to the ReNuML solution produced only in their Greenville, South Carolina plant (as opposed to the other manufacturing sites in Italy, China, and India). The CDC found no fungal contamination of unopened bottles produced by that plant (including bottles with the same lot numbers as those that were used by affected patients) and noted multilocus genotyping of clinical isolates from affected patients, essentially excluding the possibility of a single-point source contamination of the solution itself. They concluded that this epidemic was due to a failure of ReNuML to disinfect adequately after point-of- use contamination rather than from intrinsic contamination with *Fusarium*. Factors hypothesized to have contributed to this epidemic include direct uptake of alexidine by contact lenses, reduced antimicrobial activity of evaporated ReNuML, enhanced growth of *Fusarium* on ReNuML biofilms on contact lens cases, direct penetration of *Fusarium* into soft contact lenses, and patient noncompliance. However, none of these factors, either alone or in combination, would explain why only the ReNuML produced in South Carolina had been implicated. A recently published study (Bullock et al, *Arch Ophthalmol* 2008;126[11]:1493-1498) reported that a May 2006 FDA inspection of B&L's Greenville, South Carolina manufacturing site affirmed that B&L had failed to regulate storage and transport temperatures of their products, even though the label on the bottle clearly stated: "Store at room temperature." Historic climatological and other data revealed that the solution, when stored and transported without temperature controls, could have been exposed to temperatures as high as 75^{O}C (167^{O}F). Six different contact lens solutions were then studied for temperature stability. Two bottles of each solution were separately stored at room temperature and 60°C (140^{O}F) for 4 weeks, serially diluted, and then tested for their ability to inhibit growth in two different fungal media of 11 *Fusarium* isolates (7 of which were associated with the *Fusarium keratitis* epidemic). ReNuML demonstrated the greatest decline in efficacy after 60°C storage. Regarding the *Fusarium keratitis* epidemic isolates only, the ReNu with MoistureLoc bottle stored at room temperature allowed growth in 27 of 84 combinations vs. 67 of 84 combinations with the 60°C-stored bottle ($P<0.0001$). Thus, when exposed to prolonged temperature elevation, ReNuML lost its *in vitro* fungistatic activity to a much greater extent than other commercial products. That study concluded that improper temperature control of ReNuML may have contributed to the *Fusarium keratitis* epidemic of 2004-2006. Bullock et al also demonstrated that boiling (~100^{O}C/212^{O}F) the solution for ten minutes in a glass tube did not degrade its fungistatic capability, suggesting that the plastic container, in combination with prolonged heat exposure, could have been the cause of the observed fungistatic failure.

Purpose: To demonstrate the effects of container properties and storage temperatures on the ability of the ReNuML contact lens solution, previously implicated in the *Fusarium keratitis* epidemic of 2004-2006, to

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inhibit growth of *Fusarium* species.

Methods: The solution was divided into six aliquots and stored separately for four weeks at room temperature (RT), 42^{o}C (108^{o}F), and 60^{o}C, in both their original plastic bottles and similarly-sized glass containers, then tested in triplicate for their ability to inhibit the growth of seven *Fusarium* isolates previously associated with the *Fusarium keratitis* epidemic of 2004-2006.

Results: When stored in glass containers, the solution demonstrated no fungistatic deterioration at all three temperature levels. However, when the solution was stored in its original plastic container at 60^{o}C, a highly statistically significant fungistatic deterioration of the solution was noted compared to those stored in plastic at either RT ($P = 4.0 \times 10^{-7}$), 42^{o}C ($P = 2.10 \times 10^{-6}$), or in a glass container at 60^{o}C ($P = 1.29 \times 10^{-6}$).

Conclusions: When stored in its original plastic (as opposed to a glass) container and exposed to prolonged temperature elevation (60^{o}C for four weeks), the contact lens solution implicated in the *Fusarium keratitis* epidemic of 2004-2006 loses its *in vitro* fungistatic capability. The temperature required for fungistatic failure is >42^{o}C and ≤60^{o}C. Thus, a lengthy and highly detailed forensic investigation revealed that this epidemic was associated with a combination of defective plastic bottles and improper storage temperatures. In the interest of preventing future epidemics, since the exact type of plastic containers used at each of the various manufacturing sites is presently undivulged, this information should be revealed.

Blindness, Epidemic, Fusarium

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