The Identification of *Serratia marcescens* in a Diagnostic Contact Lens

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ABSTRACT

Background: *Serratia marcescens* is a very common organism found in contact lens systems and can cause infectious corneal ulcer. Diagnostic lenses used in office must be effectively cleaned, disinfected, and stored to prevent *Serratia sp.* from infecting your patients.

Methods: Diagnostic lenses can and should be cultured periodically in the office to assure disinfection is continued throughout the life of the lens. *Serratia* can be cultured and identified with 99% accuracy by the Vitek Jr. System.

Results: One diagnostic lens (10%) from the Michigan College of Optometry was found to harbor *Serratia marcescens* in its preserved storage solution.

Conclusion: It is very apparent in clinical practice that patients are noncompliant with their lens care regimens. Our findings show that this also occurs by clinicians and staff as well.

Key Words: *Serratia marcescens*, noncompliant, disinfection, contact lens

INTRODUCTION

The proper care for contact lenses, solutions, and care systems are very important for the prevention of microbial kerititis, and more importantly, ulcerative keratitis[1]. The incidence of ulcerative keratitis among contact lens wearing patients can be as high as 52% according to several studies. An epidemiological study from Olmsted County, Minnesota, showed that the wearing of contact lenses was the most significant risk factor for corneal ulceration[1,2,3]. However, with appropriate disinfection of lenses, the incidence of microbial activity can be reduced by as much as a 5.5 log reduction[4]. Microbial contamination of contact lenses (or their care systems) can result from such practices as failure to routinely clean lenses and storage cases, the addition of fresh fluids (including preservative-free saline and tap water) to residual fluids in storage cases, and storage of lenses for varying periods of time between disinfection and insertion[5]. There are three basic functions to the care of contact lenses which include cleaning, disinfecting, and storage[4]. Cleaning is designed to significantly reduce the level of inorganic and organic

contamination before the disinfection step. Most marketed systems accomplish this function with cleaning agents, surfactants, digital rubbing, and rinsing[4]. Cleaning and rinsing can dramatically reduce the microbial load on the lens even when presented with an initial load of 10^6 CFU/mL microorganisms. The next step, disinfection, is designed to kill microorganisms using traditional chemical disinfectants, oxidative systems, or heat[4]. The effectiveness of a disinfection procedure may be influenced by the concentration of the disinfectant, the pH of the medium, the length of exposure to the disinfectant, the ambient conditions of temperature and humidity, and the type and condition of the material to be disinfected[6]. The disinfection step should reduce the bio count by at least another 10^2-10^3 CFU, which leaves very few microorganisms on the lens following these first two steps[4]. The storage phase serves to prevent recontamination while the lens is out of the eye, which may be overnight or for a more prolonged period of time[4]. During storage, most marketed systems use sealed lens cases containing preserved or unpreserved saline solutions. (Preservatives in common marketed solutions are listed in Table 1). Proper storage of the lens allows for lens insertion without additional disinfection, rinsing or neutralization[4].

All contact lens solutions (hydrogel or rigid gas permeable) are required to be approved by the U.S. Food and Drug Administration before being sold to the general public. The F.D.A. has set guidelines for determining the efficacy of chemical disinfection and suggests the use of a U.S. Pharmacopoeia-type multi-item microbial challenge test[11]. Briefly, the test recommends inoculation of 20 lenses each with 2×10^6 CFU/mL cells in proteinaceous materials (to mimic eye secretions), and after 3 to 10 minutes of contact between the lens and the microorganism, application of the disinfection solution as

TABLE 1

Disinfectant of Commonly Marketed Systems [3,7,8,9,10]

Marketed Product	Disinfectant's Active Ingredient
Hydrogel Lenses	
Alcon Opti-Free Rinsing, Disinfecting, and Storing Solution	.001% Polyquad
Allergen Complete	.0001% PHMB
Bausch & Lomb Renu Multi-Purpose Solution	.00005% Dymed
Ciba Vision QuickCare Starting Solution	16% Isopropanol
Rigid Gas Permeable	
Alcon Opti-Free for Hard Lenses	.005% Polyquad
Alcon Soaclens	.004% Thimerosal
Allergen Wet-N-Soak	.003% BAK
Barnes Hind Gas Permeable Wetting and Soaking Solution	.005% CHG
Polymer Technology Boston Advance	.0015% PHMB
Polymer Technology Boston Advance Enhanced Comfort Formula	.0005% PHMB & .003% CHG
Ciba Vision Premus	.004% BAK
Sherman Stay-Wet	.1% Benzyl alcohol

directed for patient use. The disinfection solution passes the test if it renders all lenses negative for microorganisms. The F.D.A. recommends the use of the following organisms as their challenge organisms: *Pseudomonas aeruginosa, Staphylococus aureus, Serratia marcescens, Candida albicans, and Fusarium solani* (Table 2). This range of contaminants covers gram positive and gram negative bacteria, yeast and also molds[13]. As Table 2 demonstrates, these microbes are all American Type Culture Collection (ATCC) species, which signifies that they are laboratory grown and have not been cultured from a clinical setting. It is well documented[4] that the performance of lens care disinfectants against laboratory cultures (ATCC) are not reflective of the performance of the same disinfectants against microbial flora which are encountered in human worn lenses. One study[4], however, has revealed a relatively good correlation between the disinfection of lab microbes and clinical microbes. To support the theory that ATCC microbes are not

representative of "real" microbiological findings is the theory of biofilms, which when developed, can offer protection to a microorganism from unfavorable conditions (i.e. contact lens preservatives), and is a condition not found in ATCC organisms[12].

TABLE 2

F.D.A. Challenge Organisms for Disinfecting Regimens [13]

Pseudomonas aeruginosa	ATCC 9027
Staphylococcus aureus	ATCC 6538
Serratia marcescens	ATCC 13880
Candida albicans	ATCC 10231
Fusarium solani	ATCC 36031

Serratia marcescens is a pigmented and non-pigmented gram negative aerobic rod that belongs to the Enterobacteriaceae family[14]. It contains an endotoxin and protease that can cause a severe corneal ulcer[15], most commonly a central or paracentral ulcer, that can give rise to perforation[16]. Although historically *Serratia* species have been considered a nosocomial organism[14], the incidence of contact lens associated red eye (CLARE) secondary to *S. marcescens* been documented to be as high as 24% over a two year period[17]. This should is not too surprising, since *S. marcescens* grows well in moist environments, including soil, water, solutions, contact lens cases and the skin[17,18]. To date, of the eight *Serratia* species that have been known to be associated with human infection[16], three of these have been isolated from contact lenses, solutions, and lens cases. These include *S. liquefaciens, S. plymuthica, and S. marcescens*[2], the last of which is the most common. The treatment of *S. marcescens* corneal ulcers is well documented and requires flouroquinolones (ie. Ciloxan) with a schedule similar to that incorporated with *Pseudomonas* infection[19], although this may prove to be increasingly more difficult in the future as adaptation and resistance begin to expand.

METHODS

We examined the sterility of 80 sample solutions taken from individually sealed contact lens vials or disposable blister packs. The population that we obtained our samples from included 10 previously opened and disinfected contact lens vials from The Michigan College of Optometry stock, 10 unopened vials each from Bausch & Lomb, CIBA, and Wessley-Jessen. In addition, 10 disposable trials each from B&L, Vistakon, WJ, and CIBA were used for a total of 80 samples. Each sample was given a code denoting which vial or pack it came from and was matched with a 5% aerobic sheep blood agar plate with a corresponding code.

Contact lens vials and trial packs were opened without touching the interior of the case and a flame-sterilized loop was introduced into the solution with care as to not touch the sides of the vial or trial pack. Four loopfuls of each sample were streaked onto the 5% sheep blood plates with flame sterilization between loopfuls. Each sample was shaken before opening. Inoculation of all 80 plates were done in this fashion within a 1.5 foot radius of the Bunsen burner flame to ensure sterility of air surrounding working area. Twenty control plates were also used and each was inoculated with four loopfuls of sterilized distilled water in the same fashion as above.

Aerobic cultures were placed in an incubation chamber set at 35°C for 48 hours. Cultures were observed after 48 hours. If growth occurred on the culture medium we then recultured onto a Brain Heart Infusion agar plate (BHI) to isolate bacteria from fungi. The pH of the BHI is 7.4 which is a level at which bacteria grow easily but fungi do not. Some of our samples, by appearance, contained a fungal type contaminant and this was done to isolate the organism. Two control BHI plates were also inoculated at this time as

described earlier. Gram stains were performed on the blood agar specimens using crystal violet, Iodine, 95% Ethanol, Safranin, and sterile distilled water. The BHI plates were placed in the incubation chamber for 48 hours at 35°C.

The BHI plates were observed after 48 hours. Gram stains of the organisms as well as oxidase and catalase tests were performed. An Enterotube was used to differentiate the organism that we found as positive. The enterotube has a sterile inoculating needle made into the tube which was touched to our bacterial colony. The needle was then pulled through the tube to inoculate all the chambers. The sample was incubated for 24 hours at 35°C and the results were evaluated. Results were given for positive outcomes. These outcomes were assigned numbers which were compared to numbers contained in a catalog of organisms. A sample of the organism was then typed by the VITEK Jr. system.

The VITEK Jr. is a fully automated in vitro testing system designed for hospital specimen identification. The VITEK offers a wide range of microbiological tests, all of which may be run simultaneously. The VITEK shines light through sample preparations and measures changes in turbidity. It controls temperature and timing exactly, and scans the sample hourly with tiny photometric sensors. It then sends the measurements to a computer which produces reports of the results. A test card with tiny wells, which contain various kinds of dried media, is used to hold the sample organism. When the card is inoculated with a sample and incubated, changes occur in the media that the reader/incubator analyzes[20]. In our sample we used a gram negative identification card based on the results from our gram staining. The identification card contains the components in Table 3.

The time to differentiate our organism obtained was approximately 4 hours when we used the VITEK Jr. system. A second test was run the following day with the VITEK system and the results agreed.

Table 3

Components of VITEK Gram negative ID Card

Medium Abbrv.	Principal Component	Med. Abbrv.	Principal Component
DP3	2,4,4'-trichloro-2'-hydroxydiphenylether	RAF	Raffinose
OFG	Glucose	SOR	Sorbitol
GC	Peptone/Tryptophan	SUC	Sucrose
ACE	Acetamide	INO	Inositol
ESC	Esculin	ADO	Adonitol
PLI	Indoxyl-ß-D-Glucoside	COU	p-Coumaric
URE	Urea	H2S	Sodium Thiosulfate
CIT	Citrate	ONPG	O-nitrophenyl-ß-D-galactopyranoside
MAL	Malonate	RHA	Rhamnose
TDA	Tryptophan	ARA	L-Arabinose
PXB	Polymixin B/Peptone	GLU	Glucose
LAC	Lactose	ARG	Arginine
MLT	Maltose	LYS	Lysine Decarboxylase Base Control
MAN	Mannitol	ORN	Ornithine
XYL	Xylose		

RESULTS

Microorganisms were isolated from 46 of the 100 plates (46%). Most of the growth, however, was in areas of the plates that were never touched by the loop. These plates were thrown out of the study as contaminated. Only one of the plates had true growth within the streaked area; a sample taken from MCO trial lens stock that had already been used and disinfected. Gram staining of the sample from the sheep blood agar showed gram negative rods. Samples were taken from the BHI agar plate after another 48 hours. These plates showed no contamination of test or control plates. Gram staining of

the second sample also showed gram negative rods confirming our first stain from the sheep blood. Catalase tests showed positive results for the organism. Oxidase test showed a +/- result on two separate attempts and was inconclusive. An Enterotube was inoculated and was allowed to incubate for 24 hours at 35°C. The test revealed an Enterobacteriaceae of the *Serratia sp.*

The sample that was placed in the VITEK Jr. system following culturing on the BHI plates resulted in the findings found in Table 4.

Table 4

Results from VITEK Testing

DP3	+	OFG	+	GC	+	ACE	-	ESC	+	PLI	-
URE	-	CIT	+	MAL	-	TDA	-	PXB	+	LAC	-
MLT	+	MAN	+	XYL	-	RAF	-	SOR	+	SUC	+
INO	+	ADO	+	COU	+	H2S	-	ONP	-	RHA	-
ARA	-	GLU	+	ARG	-	LYS	+	ORN	+	OXI	-

This test data resulted in identification with 99% accuracy of *Serratia marcescens* and <1% of *Serratia liquefaciens.* The organism was tested again the following day and the same results were obtained. *Serratia marcescens*, a facultative anaerobic gram-negative rod is from the Enterobacteriaceae family and distinguished from other genera belonging to the group because of its production of three special enzymes: DNase, lipase, and gelatinase. *S. marcescens* is the most frequently isolated member of the genus, and it has been associated with a number of nosocomial outbreaks of urinary tract and wound infections, pneumonia, and septicemia[21].

DISCUSSION

Our study cultured a strain of *Serratia marcescens* in a "disinfected" diagnostic contact lens. This finding arises concerns about the efficacy and standards for the lens

care systems clinicians use to clean, disinfect, and store diagnostic contact lenses. For example, with storage, a report by Rosenthal and colleagues[22] stated that hydrogel lenses stored in polyaminopropyl biguanide (PHMB) lose their microbial kill potential as early as four hours into the storage process. This process was secondary to PHMB uptake by the lens from the solution with which it was stored in. This same study found that neither a decrease in bacteriocidal activity nor accumulation of the polyquaternium-1 (PQ-1) preservative in the lens occurred during storage with the PQ-1 solution and that microbial kill could continue even after 14 days of unmolested storage. Another report involving Rosenthal[4] shows that lenses that are stored in neutralized H_2O_2 show a steady regrowth of microorganisms as early as 24 hours following neutralization. In addition, by seven days these microbes had shown a five log increase in concentration, achieving 10^7 CFU/mL. The tremendous increase in bio count for lenses stored in H_2O_2 can be explained by their lack of a preservative, and therefore is recommended that after neutralization, these lenses be stored in the product no more than 24 hours[4]. Ciba Vision's QuickCare, uses isopropanol as its active agent, and allows soft lenses to be stored up to 30 days in their case[23], doubling the length of time of any other storage solution.

With regard to disinfecting solutions, there are many ways to quantitate and evaluate their efficacy which can include D value, safety factor, power of solution, total kill or overall log reduction at manufacturer's suggested soak times[6]. The advantages or disadvantages of these factors and their variables have been documented in the past[6]. Most frequently used, although it is not necessarily the best evaluation method, is the D value which is defined as the time required to reduce the population of viable

microorganisms by 90%[11]. With all of these methods to evaluate solution efficacy, one would think that there is one disinfectant that stands out above the rest. On the contrary however, there are many journals flooded with articles that claim a certain product as the superior disinfectant (which often coincides with the manufacturing company that the authors are associated with). For example a report by Ajello and Ajello[7] found that Ciba Vision QuickCare (Duluth, Georgia), provides overall superiority against all of the F.D.A. recommended challenge organisms and 14 of 15 different clinical isolates versus Bausch & Lomb Renu Multi-Purpose Solution (Rochester, New York) and Alcon Opti-Free Rinsing, Disinfecting, and Storage Solution (Fort Worth, TX). Another report by Keeven and colleagues[10] states that Polymer Technology (Wilmington, MA) products for rigid gas permeable lenses, Boston Advance and Boston Advance Enhanced Comfort Formula, were more effective at rapidly killing all bacteria, vegetative and yeast cells. This is in comparison to other marketed products by Allergan (Irvine, CA) Ciba Vision, Sherman(Mandebille, LA), Barnes Hind (Sunnyvale, California), and Alcon. A report by Modi, Gresh and Shih[8] states that a one-step wetting and soaking system with .005% chlorhexidine gluconate and .02% EDTA (most likely Pilkington Barnes Hind One-Step Wetting and Soaking Solution) provides better disinfection against certain F.D.A. challenge organisms versus a conditioning solution with .006% chlorhexidine gluconate and .05% EDTA. It is important to point out that often times, these studies do not include every step in the cleaning, disinfecting, and storing process. Some studies use clinically isolated microbes instead of ATCC strains. This disparity can lead to a loss of repeatability between tests. It has been found that the clinical isolates more closely resemble those confronted in clinical practice[6]. After all the pontificating, each of the

marketed products still need to meet strict F.D.A. guidelines to assure microbial kill, and depending on the test conditions, organisms used, and procedures followed, any one of these products can come out on top as the superior product. In reality, if performed according to manufacturer's directions for use, all approved disinfectants should result in a sufficient microbial reduction to aid in the prevention of microbial keratitis or ulcer. In support of this, a report comparing the disinfection ability of currently marketed lens care systems, specifically H_2O_2 versus chemical disinfection[4], has concluded that by a log reduction calculation, there is no statistically significant difference between the two methods when combating challenge organisms.

There is a new method on the horizon that not only provides hydrogel and RGP disinfection, but sterilization as well, which eliminates all viable microorganisms and spores from contact lens, solutions, and cases[24,25]. This sterilization process takes only 300 seconds to eliminate cultured *S. marcescens*, and eliminates the need for preservatives in contact lens solutions. This new method uses a modified short-wavelength ultraviolet chromatography cabinet, which incorporates radiant light, 253.7 nm, to kill organisms by cross-linking and breaking bonds between nucleic acids. Factors to consider with UV "disinfection" are still being evaluated and include the absorption by both storage solutions and lenses, softening of lens materials with repeated UV exposure, and opacification of the lenses with repeated UV exposure.

The Michigan College of Optometry Contact Lens Clinic follows the American Optometric Association recommendations for diagnostic contact lens disinfection, which calls for heat disinfection for lenses with water content less than 55%, and chemical disinfection for lenses greater than 55%. These are the recommendations also suggested

for ophthalmic lens care in the prevention of the spread of the HIV/AIDS virus[26]. There, however, was an obvious oversight in this process at MCO, as our diagnostic lens (55% water content) exhibited a strain of *S. marcescens*. This issue arises questions as to the procedures actually followed in the clinic, and displays that noncompliance is not only a problem with patients, but clinicians and staff as well.

TABLE 5[1,4,24,25]

Disinfection Systems (hydrogel & RGP)

TYPE	ADVANTAGES	DISADVANTAGES
Heat	*Kills broad spectrum of organisms	*Damages higher water content soft contact lenses
	*Easy to use	*Can decrease life of low water soft lenses
	*Inexpensive	*Can bake on deposits
	*Does not involve chemicals or preservatives	*Impossible to know if system is working properly
	*First order killing kinetics	*Inconvenient
		*Subject to electromechanical failure
Cold Chemical	*Easy to use	*Kill organisms slowly
	*Can be used with all soft lenses	*Relatively ineffective against *Acanthamoeba* and fungus
	*Continued preservation after completed disinfection	*Longer soak time required
		*Sensitivity reactions to chemical residues
Hydrogen peroxide	*Rapid antimicrobial activity	*Neutralization of chemical disinfectant required
	*Lack of irritation from preservatives	*No continued preservation after completed disinfection
	*Short term disinfection	*Peroxide irritates the eye
		*Can bleach lenses
Ultraviolet	*Sterilizes lenses and lens cases	*Lenses and solutions may absorb UV light
	*Very quick sterilization	*Lenses may soften with extended exposure
	*Can store lenses in saline solution for prolonged time	*Lenses may opacify with extended exposure

Certainly, contaminants are not going to be limited to diagnostic lenses in the office. There is still a significant rate of microbial keratitis outside the office and can be seen with just about every lens care system. Therefore, regardless of what method you recommend

to your patients for disinfection, we still feel that there needs to be constant reinforcement on lens care policies to assure that patients are cleaning, disinfecting, and storing properly. The issue of patient noncompliance while using lens care systems is well understood by

practitioners, and has been documented to be as high as 100% in a study by Lakkis and Brennan[27]. This fact gives credence to the high rate of corneal ulcers in contact lens patients[1]. Noncompliance has become so widespread that Key and Monnat[28] estimate 66% of contact lens wearers do not even clean their lenses, while Lakkis and Brennan[27] state that 95% and 96% of patients are noncompliant with cleaning and disinfecting respectively. Wilson and colleagues[5], evaluated noncompliance by studying the lens case contamination difference between asymptomatic lens wearers and those that had lens care instructions reinforced. They showed contamination rates of 54% for soft lens users and 51% for RGP users for the group that did not have instructional reinforcement. This was in contrast to 4% (SCL) and 13% (RGP) for the reinforced group. This finding correlated with studies by Kanpolat and colleagues[29] and Donzis and colleagues[30] who found contamination in 70% and 52% respectively, of lens care systems in asymptomatic rigid and soft lens patients. Is the fact that patients are asymptomatic resulting in their noncompliance? A report has not yet established the criteria that make so many contact lens users noncompliant. Rosenthal and colleagues did do a study[4] that incorporated a noncompliance survey as part of their study protocol. They evaluated different habits between chemical and hydrogen peroxide in soft lens users and found that 13% of chemical users and 6% of peroxide users do not even discard the used solution after each use. How naive are patients to try to reuse previously neutralized peroxide? This

ignorance can be explained by two reasons: 1) our patients entirely neglect the propensity for microbial infection and/or 2) practitioners are not informing (or reinforming) the patients enough about the proper use of a lens care systems and risk for microbial keratitis. We as practitioners cannot assume that a patient fully understands the use of a lens care system, even if they have previously used a specific care system. There is always a need to review techniques and instructions at every patient visit.

The clinical isolate, *Serratia marcescens*, although it is one of the F.D.A.'s challenge organisms for disinfection approval, has become an increasingly more common microbe of contact lenses, solutions, and care systems[10,17]. *S. marcescens,* which accounts for up to 10% of gram negative corneal ulcers, is the organism most frequently isolated in preserved ophthalmic solutions[10]. A study by Hume and colleagues[17] has shown up to twelve different types of the *S. marcescens* species being isolated from eyes, lenses, cases, and fingers of wearers, which has showed no evidence of a specific subset being more readily able to colonize contact lens systems over other types. These results suggest that because there are many species of *S. marcescens*, all of which can be different ribotype, serotype and biotype[17], it may become even harder in the future for contact lens disinfectants to overcome these organisms. To further complicate this issue, *S. marcescens* may acquire an even more expanded resistance, which is the result of biofilm development by the microbe. When this occurs, the bacteria may become enveloped in a matrix of hydrated exopolysaccharides, or a glycocalyx. This matrix of hydrated exopolysaccharides can lead to a concentration of nutrients and offer protection to the microorganism from conditions such as antimicrobials or preservatives, resulting in their increased resistance over other microbes[11,12]. The establishment of biofilms has already

been reported to aid in the survival of various bacteria in the presence of contact lens solutions[31]. Resistance of cultured (non ATCC) *S. marcescens* has been well documented in the past[3,6,10,12] and have showed the ineffectiveness of several commercially available products to the adapted microbe. Soft lens disinfectants by Alcon[3], and RGP disinfectants by Barnes Hines, Allergan, Ciba, and Sherman[10], and non-specified RGP solutions containing chlorhexidine based products[10,12] have all allowed adapted (non ATCC) *S. marcescens* to survive in solution.

CONCLUSION

To prevent the microbial contamination of contact lenses or care systems it is incumbent upon the wearer to clean their lenses and storage cases on a regular basis according to doctor specified regimens. With these simple techniques the rate of microbial infection can be greatly reduced. These techniques need to be repeated to the patient as many times as possible since it cannot be assumed the patient understood them the first time they were explained. Many cleaning and disinfecting solutions advertise to be superior to the rest of the market, but all need to meet strict F.D.A. guidelines to assure microbial kill. Therefore all disinfecting solutions, if used properly, will result in a sufficient microbial reduction.

Diagnostic lenses are the most likely contact lenses to get contaminated in the optometric office because of their inherent nature. Lenses are placed on a patient's eye, removed, and remain in a storage solution for a variable amount of time until a technician has the time to clean them. It is then dependent upon the technician to use adequate hygiene and have a clean working environment and the doctor to post specific guidelines

to the disinfecting of each lens type. In these controlled conditions contamination can still occur, as is shown in this study.

Despite experimental studies and corporate claims, patients are still contracting infectious keratitis and ulcers. New techniques, however, are coming to fruition and may be poised to take over as the new accepted method. Ultraviolet disinfection, using 253.7 nm radiant light from a short-wavelength ultraviolet chromatography cabinet has been shown to kill organisms in about 300 seconds. The new technique is used for hydrogel as well as rigid gas permeable lenses and may eliminate the need for contact lens solution. This new technology is still in the experimental phases and its effect on contact lenses is being evaluated.

The best way to ensure compliance for the time being is education. Patients should be warned of the potential for infection, neovascular blood vessel growth, and allergic reactions. In addition, each office visit should include a reminder about care systems and personal hygiene. The ultimate responsibility is upon us, the doctors and staff, to educate and lead by example. It is therefore imperative, that our staff, as well as ourselves, emphatically know and carry out the proper lens care procedures for each and every diagnostic lens used in office.

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