Disinfection of Eyelid Specula With Chlorhexidine Gluconate (Hibiclens) After Examinations for Retinopathy of Prematurity

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Background: The preferred method of cleaning eyelid specula between examinations for retinopathy of prematurity is unknown. A previous study showed that disinfection with 70% isopropyl alcohol swabs fails to eliminate viruses and bacteria from the specula.

Objective: To determine if alternative sterilization procedures would allow multiple use of a single speculum without risking nosocomial infection.

Methods: In phase 1, 40 autoclave-sterilized eyelid specula were randomized into either “cleaned” or “patient control” groups after being used for routine retinopathy of prematurity examinations performed in the outpatient setting. Specula in the cleaned group were cleaned with chlorhexidine gluconate (Hibiclens). Specula in the patient control group were not cleaned after use. All study specula were placed into enriched culture media from which bacterial and fungal cultures were obtained. In phase 2, 20 autoclave-sterilized eyelid specula were inoculated with a clinically relevant dilution of adenovirus serovar 5 or herpes simplex type 2. Specula were randomized into either a cleaned or a control group, and cell cultures and immunofluorescence assays were used to document and confirm, respectively, viral growth.

Results: In phase 1, all 20 cultures from the patient control group grew bacteria compared with 0 (0%) of 20 cultures from the cleaned group and 0 (0%) of 5 from the cleaned control group. No fungi were isolated from any group. In phase 2, all 10 cultures from specula inoculated with adenovirus serovar 5 grew virus. None of the cultures from the 5 cleaned specula inoculated with herpes simplex type 2 grew virus. In contrast, all 5 cultures in the control group were positive for growth of herpes simplex type 2.

Conclusions: Autoclave sterilization is the ideal method of sterilization of eyelid specula between neonate examinations. When an alternative disinfection technique is required, washing the speculum with chlorhexidine gluconate and tap water is preferred over wiping with a 70% isopropyl alcohol swab.

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**PATIENTS AND METHODS**

**PHASE 1**

Forty autoclave-sterilized pediatric Cook eyelid specula (Katena Products, Inc, Denville, NJ) were randomized into a cleaned or control group after being used during ROP examinations carried out in the outpatient setting. After the administration of topical proparacaine hydrochloride and using sterile gloves, the examiner (A.K.H.) placed a sterile speculum between the eyelids of a neonate and performed indirect ophthalmoscopy with scleral depression in the usual manner of examination for ROP. Specula assigned to the cleaned group were washed with a sterile gauze sponge soaked in 4% chlorhexidine gluconate (Zenca Pharmaceutical, Wilmington, Del) for 1 to 2 minutes, rinsed with tap water, and then dried with a sterile gauze sponge. Tap water, rather than sterile water, was used because we believed it would be unrealistic to routinely use sterile water to rinse specula in the clinical setting. Each speculum was then transferred in a sterile manner to a 100-mL sterile container containing 10 mL of brain-heart infusion broth with a nutritional supplement (5% Fildes Enrichment; Remel, Lexena, Kan). Care was taken to completely cover the surface of each speculum that had been in contact with the patient’s eye. Specula in the control group were allowed to air dry, and were placed into identical media without cleaning. Last, 5 sterile and unused specula were cleaned and dried in the previously described fashion immediately after removing them from sterile packaging, and placed into culture media to test for possible contamination inherent to the chlorhexidine gluconate and tap water cleaning technique itself.

All specula were then incubated at 35°C in 5% to 10% carbon dioxide, and observed for 7 days. All observers were masked as to whether the specula were “cleaned” or “control.” If the broth became turbid during the 7-day incubation period, an aliquot of the broth was inoculated to trypticase soy agar II with 5% sheep blood (blood agar), chocolate agar, and Mac Conkey II agar (Becton Dickinson Microbiology Systems, Cockeysville, Md). Isolated colonies were identified using standard laboratory methods. If no turbidity was visible by incubation day 6, an aliquot of the broth was inoculated to blood agar and incubated overnight to detect any organisms not previously recovered.

**PHASE 2**

Stock strains of adenovirus serovar 5 and herpes simplex type 2 were grown in cell culture and diluted in minimal essential media to 10^3 log virus, which was thought to be a clinically relevant titer of virus and comparable to that used in other studies of viral disinfection. Ten eyelid specula were assigned to each of the 2 types of virus. The sterile specula were then immersed in their respective viral suspensions for 1 minute. Using sterile forceps and gloves, the specula were removed, separated, and placed on a sterile platform. Five specula from each group were randomly chosen as controls and allowed to air dry under a biological safety cabinet and fluorescent lighting. The remaining specula from each group were disinfected with chlorhexidine gluconate in a manner similar to that described for phase 1; however, sterile water (rather than tap water) was used to rinse the specula.

Sterile water was used for the virological portion of this experiment to avoid contamination of viral cell cultures, which must be sterile and toxin free. Specula were allowed to air dry and were then transferred to viral transport media. The solution was then agitated, and a 0.2-mL aliquot of media was removed from each container and inoculated into cell culture monolayers, which were observed daily for cytopathic effect. All observers were masked as to which cultures were cleaned or controls. Confirmation was by immunofluorescence assay using an adenovirus monoclonal antibody reagent (Bartels Inc, Issaquah, Wash).

**RESULTS**

**PHASE 1**

None of the 20 cultures of the cleaned group demonstrated microbial growth, whereas all 20 cultures of the control group showed growth. Of the cultures positive for growth in the control group, 10 yielded coagulase-negative Staphylococcus; 5, Staphylococcus aureus; 4, Bacillus species; 3, Enterococcus species; 3, other gram-positive cocci; 1, α-hemolytic Streptococcus; and 1, gram-negative bacillus (Figure). There was no fungal growth. All cultures obtained from specula cleaned immediately after removal from the sterile packaging were negative for organisms.

**PHASE 2**

Five (100%) cleaned and 5 (100%) control specula inoculated with adenovirus serovar 5 were positive for the virus. Of the 10 specula inoculated with herpes simplex type 2, none of the specula that were cleaned with chlorhexidine gluconate showed viral growth, but all 5 controls were positive for the virus.

**COMMENT**

Our data suggest that chlorhexidine gluconate is effective in eliminating common bacteria and lipid-enveloped viruses such as herpes simplex type 2 from neonate eyelid specula, but is ineffective in eliminating non–lipid-enveloped viruses such as adenovirus. This is an important finding because serial examination of neonates undergoing ROP examinations is commonplace. Although nosocomial infection in any setting should be
avoided, it can be particularly dangerous in premature neonates who are relatively immunocompromised. In a recent publication, wiping eyelid specula with 70% isopropyl alcohol swabs proved to be an ineffective method for disinfecting eyelid specula of bacteria and adenoviruses. This result was somewhat unexpected since other studies have demonstrated that Goldman tonometer tips and other instruments can be adequately disinfected of bacteria and viruses with a 70% isopropyl alcohol swab or even simply by wiping with a dry tissue. Nevertheless, given the more complex shape and configuration of the eyelid speculum compared with the tonometer tip, these results are not entirely surprising. This study concluded that the use of a new or autoclave-sterilized speculum for each patient examined for ROP is recommended, and the use of other common cleaning methods should be discouraged.

Although theoretically preferred, the use of a new or autoclave-sterilized speculum and scleral depressor for each patient may be difficult to achieve in the clinical environment. In a busy nursery, 10 or more neonates may require screening examinations on a given day. Such a large volume of autoclave-sterilized instruments may not be available. We set out to determine whether an alternative, practical cleaning technique using chlorhexidine gluconate would provide better disinfection than a 70% isopropyl alcohol swab. Chlorhexidine gluconate is readily available in the clinical setting and has been shown to have a broad spectrum of activity against many common infective organisms, including bacteria, fungi, viruses, and Acanthameoba trophozoites. For these reasons, we propose its use as an alternative to 70% isopropyl alcohol swabs when autoclave sterilization is unavailable.

The results of our study suggest that chlorhexidine gluconate is more effective than 70% isopropyl alcohol against commonly encountered bacteria and lipid-coated viruses. In a similarly designed study, Woodman et al. cultured coagulase-negative Staphylococcus and Bacillus cereus from specula that had been cleaned with 70% isopropyl alcohol wipes. In our study, none of the cultures from specula cleaned with chlorhexidine gluconate grew either of these bacteria despite the fact that coagulase-negative Staphylococcus was recovered from 50% and Bacillus species from 20% of control specula. Since coagulase-negative Staphylococcus is an important source of sepsis in major neonatal centers and has been shown to cause other life-threatening infections in neonates, chlorhexidine gluconate appears to be preferred over 70% isopropyl alcohol swab disinfection for its ability to eliminate common bacteria from eyelid specula. In an effort to make our technique clinically practical, we chose to use tap water rather than sterile water to rinse the specula in phase 1 of our study. In Charleston, SC, tap water is treated with chloramines and tested regularly for total coliform bacteria. During 1998, of 2400 samples tested, less than 1% were positive for bacteria. No bacterial growth was seen in our control cultures, suggesting that chloramine-treated tap water may be safely used to rinse specula with minimal risk of bacterial contamination. Viral studies could not be performed with tap water because other additives would have interfered with viral cell cultures.

Although chlorhexidine gluconate has a broad spectrum of activity against bacteria, its antiviral activity is variable and may be limited to lipid-enveloped viruses, such as herpes simplex, cytomegalovirus, and human immunodeficiency virus. Because chlorhexidine gluconate is a cationic bisbiguanide that derives its antimicrobial action by causing disruption of microbial cell membranes and precipitation of cell contents, nonenveloped viruses (such as adenovirus and poliomyelitis virus) are often resistant. Although adenovirus and other lipid-enveloped viruses have been shown to be resistant to chlorhexidine in vitro, other studies have demonstrated that the mechanical action of wiping even with a water-moistened gauze alone was effective in removing all detectable adenovirus. Unfortunately, an in vivo viral study would be difficult, if not impossible, to carry out since congenital viral infections are relatively infrequent.

Since adenovirus is an important infectious agent of ocular and nonocular morbidity, we cannot categorically recommend chlorhexidine gluconate for routine disinfection of neonate eyelid specula. There may, however, be a place for its use in situations in which autoclave sterilization is impractical, and it should certainly be preferred over wiping used instruments with a 70% isopropyl alcohol swab. Caution must be exercised when using chlorhexidine gluconate around the eye. Several clinicians have reported severe corneal toxicity and permanent corneal scarring after preoperative facial preparation with chlorhexidine gluconate. To avoid toxicity to the eye, instruments must be carefully rinsed after disinfection with chlorhexidine gluconate.

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REFERENCES


Ophthalmological Numismatics

A look at the past . . .

Frans Cornelius Donders, 1818-1889, served as professor of anatomy and physiology at the University of Utrecht, and director of the eye hospital in Utrecht, the Netherlands, but is best known for his work on refraction. His classic work On The Anomalies of Accommodation and Refraction of the Eye, published in 1864, provided a scientific basis for the practice of refractive correction.

This medal was issued in 1888, in honor of Donder’s 70th birthday, and was engraved by Junger, Menger, and Schammer. The obverse (Figure 1) depicts the head of Donders facing right and surrounded by an inscription; the reverse (Figure 2), a laurel wreath with inscriptions both within the wreath and surrounding it.

Courtesy of: Jay M. Galst, MD, 30 E 60th St, New York, NY 10022.