Disinfection of Eyelid Speculums for Retinopathy of Prematurity Examination

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Objective: To evaluate the effectiveness of 70% isopropyl alcohol swabs in disinfecting eyelid speculums after examination for retinopathy of prematurity.

Methods: Two phases. Phase 1: 46 autoclave-sterilized eyelid speculums randomized into either a cleaned or control group following examination for retinopathy of prematurity. Speculums in the cleaned group were disinfected with a 70% isopropyl alcohol swab while control speculums were not cleaned. Bacterial and fungal cultures were then obtained. Phase 2: 20 autoclave-sterilized eyelid speculums inoculated with a clinically relevant dilution of adenovirus serotype 5 or herpes simplex virus type 2. Inoculated speculums were randomized into either a cleaned or control group.

Results: Phase 1: 17 (70.8%) of 24 cultures from the cleaned group yielded bacteria compared with 21 (95.5%) of 22 controls. Fungi were isolated from only 1 control and from no cleaned speculums. Phase 2: all speculums inoculated with adenovirus supported growth of the organism irrespective of cleaning with 70% isopropyl alcohol swabs. None of 5 cleaned speculums inoculated with herpes simplex virus type 2 supported viral growth, compared with 3 (60%) of 5 cultures positive for growth in the control group.

Conclusion: Cleaning eyelid speculums with 70% isopropyl alcohol swabs provided inadequate disinfection against bacteria following examination for retinopathy of prematurity and against adenovirus in a laboratory simulation.

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**METHODS**

**PHASE 1**

Forty-six autoclave-sterilized Alfonso pediatric eyelid speculums (Storz Inc, Ophthalmic Instrumentation Division, Cleveland, Ohio) were randomized into a cleaned or control group following examinations for ROP in the neonatal intensive care unit. Using sterile gloves, the examiner (D.K.C. or E.A.P.) placed a sterile speculum within the eyelids of a neonate and performed indirect ophthalmoscopy with scleral depression in the usual manner of examination for ROP. To minimize possible contamination by the examiner, a separate pair of sterile gloves was used to remove the speculum. Speculums in the cleaned group were cleaned thoroughly with a 70% isopropyl alcohol preparation pad (Webcol, Kendall Co, Mansfield, Mass) for 10 seconds and allowed to air dry. An attempt was made to clean all surfaces of the speculum. Each speculum was then transferred in a sterile manner to a 100-mL sterile container that held 10 mL of trypticase soy broth with 5% Fildes Enrichment (Becton Dickinson Microbiology Systems, Cockeysville, Md), which completely covered the instrument. Speculums in the control group were allowed to air dry and then placed in identical containers without cleaning. Last, 5 sterile, unused speculums were cleaned in the above fashion immediately after removal from the sterile packaging, allowed to air dry, and placed into the culture media to test for possible contamination inherent to the cleansing technique itself. The speculums were then incubated at 35°C in 5% to 10% carbon dioxide and observed for 7 days. If the broth became turbid during the 7-day incubation period, a Gram stain was performed and the broth was inoculated to trypticase soy broth with 5% SRBC, chocolate II agar, and MacConkey II agar (all from Becton Dickinson Microbiology Systems). Organisms were then isolated and identified using standard laboratory methods. After the incubation period, a Gram stain was done on each broth to detect any organisms not previously recovered. The Fisher exact test was used for statistical analysis. This protocol adhered to the Declaration of Helsinki.

**PHASE 2**

Stock strains of adenovirus serotype 5 and herpes simplex virus type 2 (HSV-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 other studies of viral disinfection. Ten eyelid speculums were assigned to each of the 2 viruses. The sterile speculums were then immersed in their respective viral suspensions for 1 minute. Using sterile forceps and gloves, the speculums were removed, separated, and placed on a sterile platform. Five speculums from each group were randomly chosen as controls and allowed to air dry under a biologic safety cabinet and fluorescent lighting. The remaining speculums from each group were thoroughly swabbed with a 70% isopropyl alcohol pad for 10 seconds and allowed to air dry. Separate sterile forceps were used to transfer each speculum to individual containers of viral transport media sufficient for its immersion. Meticulosus sterile technique was maintained to prevent contamination. The solution was then agitated and a 0.2-mL aliquot of media was removed from each container and inoculated onto cell culture monolayers of human foreskin fibroblasts, rhesus monkey kidney, and A549 cells. The inoculum was allowed to adsorb for 4 hours, washed, and refed with media. The cell cultures were incubated on a roller drum in a warm airflow incubator at 37°C, observed daily for cytopathic effect, and the results recorded. Preliminary identification of each virus was by cytopathic effect with confirmation by immunofluorescence assay, using an adenovirus monoclonal antibody reagent (Bartels, Inc, Issaquah, Wash). The Fisher exact test was used for statistical analysis.

**COMMENT**

Examinations for ROP are often performed sequentially on multiple infants. Equipment that comes into contact with the infants during examination must be adequately disinfected between examinations to eliminate the risk of transmission of iatrogenic infectious disease. Several recent studies have demonstrated that Goldmann tonometer tips can be adequately disinfected with an alcohol swab against adenovirus, HSV, and human immunodeficiency virus. Corboy and Borchardt found certain bacteria inoculated onto tonometer tips to be susceptible to simply wiping with a dry tissue. Many nonsurgical examination instruments, such as Goldmann tonometer tips, Schiotz tonometers, diagnostic contact lenses, etc, can be sufficiently cleaned using an alcohol swab technique. These findings and practices may encourage practitioners to use this cleaning technique for eyelid speculums following examination for ROP. However, important differences between the instruments to be cleaned and the patient populations make direct comparisons problematic. For instance, there is a fundamental difference in the shape and configuration of an eyelid speculum compared with a Goldmann tonometer tip. An eyelid speculum is bent in several places, making much of its surface difficult to clean with a swab, whereas a Goldmann tonometer tip has a flat applanation surface making the entire contact area readily amenable to swabbing.

Additionally, a cleaning technique that is adequate for instruments used on healthy adults may not be adequate for instruments used on premature infants. Neonates at risk for ROP are typically immunocompromised and commonly exhibit extremely low birth weight, and such infants are at significantly increased risk for life-threatening infections. Organisms often disregarded as “normal flora” in healthy patients can be devastating to the premature infant. For instance, CONS, a
common environmental organism,\textsuperscript{10} was responsible for as many as 49% of the cases of neonatal sepsis in 2 major centers.\textsuperscript{11,12} Coagulase-negative \textit{Staphylococcus} can also cause other life-threatening infections in this population, including meningitis, pneumonia, and endocarditis, as well as ocular infections such as conjunctivitis, keratitis, and endophthalmitis. Our data demonstrate that cleaning an Alfonso eyelid speculum with a 70% isopropyl alcohol swab is ineffective against this organism, as more than two thirds of the cleaned speculums yielded cultures positive for CONS. Not only does the design of the speculum render cleaning difficult, but CONS-related factors may also contribute to problems associated with this method of disinfection. Certain strains of \textit{Staphylococcus epidermidis}, a commonly isolated member of the CONS family, produce a mucoid substance, or slime, that makes the bacteria highly adhesive.\textsuperscript{13,14} This material may allow organisms to more firmly adhere to regions on the speculum that are difficult to clean with an alcohol pad.

Fungal (yeast) growth was confirmed in only 1 culture of the control group and in none of the cleaned speculums. Fildes Enrichment was used to fortify the culture broth in an effort to promote the growth of any fungal organisms adherent to the speculums, but the low fungal growth rate in both the control and cleaned groups does not allow for conclusions about fungal growth to be made.

Cleaning with 70% isopropyl alcohol swabs proved completely ineffective against adenovirus serotype 5 in our laboratory simulation. The prolonged survival of adenovirus has been previously demonstrated on inorganic surfaces and some investigators have recovered this organism from plastic and metal surfaces as long as 1 to 7 weeks after inoculation.\textsuperscript{15,16} Nevertheless, the poor performance of this cleaning technique on eyelid speculums was surprising, as others have clearly demonstrated alcohol wipes to be quite effective against adenovirus on Goldmann tonometer tips.\textsuperscript{4,5} This difference is most likely because of the structural design differences of the 2 instruments. The clinical relevance of these findings is apparent when one considers that adenovirus not only causes keratoconjunctivitis, but it can produce severe pneumonia and enteritis with potential life-threatening complications in the neonatal population. Our data on HSV-2 corresponds well with Goldmann tonometer data.\textsuperscript{6} The higher kill rate for HSV-2 may be related to the fact that this virus is enveloped and therefore more susceptible to the disinfectant properties of isopropyl alcohol.\textsuperscript{17}

In summary, cleaning Alfonso pediatric eyelid speculums with 70% isopropyl alcohol swabs was ineffective in disinfecting them against potentially dangerous bacteria in a clinical study and ineffective against adenovirus in a laboratory simulation. These organisms not only pose a serious threat to vision but have the potential to
cause serious systemic disease in the population at risk for ROP. Although we have not attempted to demonstrate an increase in actual infection rates, the mere presence of these organisms on cleaned speculums warrants discontinuation of this particular cleaning method for eyelid speculums used during examinations for ROP. We recommend the use of a new or autoclave-sterilized speculum on each patient examined for ROP and discourage the use of other common cleaning methods until their efficacy against bacteria, fungi, and viruses has been demonstrated in a controlled study.

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REFERENCES


A look at the past . . .

This report of BURNETT is that which was sent to Chibret as chairman of the committee appointed by the French Ophthalmological Society to collect statistics on trachoma. Letters of inquiry were sent to various oculists in all regions of the United States, asking their observation as to the frequency of trachoma among different races, the effect of altitude, employment, contagiousness, etc., a distinction being made between true trachoma and follicular conjunctivitis. Thirteen answers were received, which are printed in full. The conclusions which Burnett draws from the information thus furnished is that in the United States altitude seems to exercise but little influence on the frequency of the disease. The Jew, the Irish, and Italians are greatest sufferers, though the American of the central region of southeastern Kentucky, West Virginia, and North Carolina forms an important element in the statistics both as to frequency and virulence. The negro is reported as being practically immune even in communities where the disease is very prevalent among the white. Opinions are divided as to contagiousness.