INTRODUCTION

This is the first resubmission of 1R01 NR05197, "Staff Hand Hygiene and Nosocomial Infections in Neonates", and includes (bolded and in italics) changes made in response to reviewers' careful critique. There were a number of specific recommendations made, and these fell into five major categories, summarized below.

First, reviewers questioned whether this study would add in any significant way to the already large body of information regarding the effectiveness of hand hygiene regimens. They noted that a number of studies have already demonstrated that alcohols are effective antimicrobial agents and are milder to the skin and that there have been studies comparing hand hygiene regimens. They questioned whether this study was adding sufficiently to what is already known. Because of the high cost of conducting a clinical trial such as this one, its clinical relevance and potential contribution must be clear. Hence, we will attempt to clarify why we think this study is not only important and new, but also essential for evidence-based practice in the field of skin hygiene.

While laboratory data and controlled, short-term studies indicate that alcohol-based products are superior microbiologically and physiologically, there has been only one prospective clinical trial in which the outcome of nosocomial infections was evaluated. It is quite possible for a product to perform well at reducing microbial counts on the hands and improve skin condition, but have little effect on infection rates in patients. Thus, clinical studies evaluating clinically relevant outcomes are essential and required by the Food and Drug Administration. We found seven clinical comparative studies of the effect of hand hygiene regimens on infections, but only one (Doebeling, 1997) compared alcohol and the antiseptic agent most frequently used in hospitals today, chlorhexidine gluconate (CHG) (others compared various other products or regimens which are not commonly used in many hospitals). Further, this one study was fraught with methodologic problems, including potential confounding by handwashing frequency and gloving practices. Results of this single study, in fact, were opposite what would have been expected from all other previous work (i.e. it found that there were significant reductions in infection rates during use of the CHG agent), but the authors and the accompanying editorial speculated that the results were due to confounding. Because there is a new interest in the U.S. in changing to alcohol products for hand hygiene, we believe it is essential at this time to determine whether this previous finding was an artifact of confounding and bias.

The field of infection control is fraught with untested or inadequately tested traditions and rituals (handwashing, surgical scrubbing, isolation precautions, and wearing masks, for example) which, once inculcated into practice, are exceedingly difficult to change. Handwashing is a long accepted standard of practice, but prospective outcome studies are glaringly lacking. Hence, the question of what are the most effective and efficacious hand hygiene regimens in actual clinical practice remains unresolved. A waterless form of hand hygiene without the need for running water and drying represents something of a revolution in the U.S. If we are to have evidence-based practice in infection control, it is essential to study the important question of the efficacy of hand hygiene using alcohol in reducing infection rates under long-term clinical use conditions before it becomes a national standard. Unfortunately, most studies of "effectiveness" are short-term, laboratory-based and funded by industry for the sole purpose of specific product testing. For several decades, the FDA has requested long-term outcome studies of effects of products and/or regimens on infection rates, but none have been forthcoming. It is inappropriate that these be funded by manufacturers because of their strong
special interest in their own brands. A definitive study of this sort must come from the scientific rather than the commercial sector. We have added narrative in Sections B.1, B.4, and B.6 to explicate this more clearly.

Second, the reviewers raised concerns that the potential confounding effects of gloving could weaken the study's ability to assess the direct effect of hand hygiene regimens on nosocomial transmission, particularly if there were differences in gloving practices between the two study periods or units. We certainly agree that, if not controlled and accounted for, this could be a fatal flaw in the study. We have made a number of attempts to prevent and identify any such confounding, as described in sections D.5.3 and D.7. A related question that might be raised is that since gloves are used so frequently today, might this then negate the need for or importance of hand hygiene? During our pilot study we noted that gloves were consistently changed between patients. Nevertheless, based on recent outbreaks in our own and other institutions which have been traced to the hand flora of nursing personnel, it seems clear that hand hygiene, even in this era of frequent gloving, still brings added protection (see Appendix 4 for examples). And because gloving is becoming increasingly common, allergic reactions and other untoward effects such as skin damage through shearing are also becoming more common. Hence, the need to study not only the microbiologic effects of hand hygiene agents, but their long-term effects on skin health when used in conjunction with gloves.

A third concern raised by the reviewers was whether there would be sufficient variation in infection rates to meet Aim 1 (comparing differences in infection rates between two regimens). Our power calculations (D.2.2) provide us with a high degree of security that we will have sufficient variation and sufficient sample size, given our current incidence rates on the study units, to identify clinically meaningful changes in infection rates. The infection rates we used for the power analysis are realistic, based on data over several years in the study units. Even though infection rates are low (e.g. 3-6%), our power is still excellent, given our sample size. In fact, our power analysis was quite conservative. Doebelling (1992) reported nearly double the infection rate when one regimen was compared with the other, and retrospective evidence from outbreak investigations provide evidence that effects of hand hygiene on infection rates can be detected. Therefore, if there is a clinically meaningful effect of one of the regimens being studied, we are very confident that we will find it.

Fourth, concerns were raised about the nurses' diary recordings and the cost analysis. As clarified in Section D.5.3, each nurse's diary recording will be completed on a specific day, based on a master schedule maintained each month by the research assistant. Compliance with diary recording will be monitored with direct observation during the scheduled day by the research assistant. This was the process we tested during our pilot study, and it worked well. Using this procedure, we will have >3,600 person-days of data regarding frequency of hand hygiene, gloving, and lotion use. Regarding the cost analysis, one reviewer stated that there was insufficient evidence that costs were likely to vary between the regimens or that cost would play a factor in deciding between hand hygiene regimens. In fact, there is strong evidence that there may be a significant difference in costs (Voss & Widmer, 1997) between the two hand hygiene regimens we propose to study. We have clarified in Sections D.5.4 and D.8.3 how direct costs will be determined, including salary and products. We have also added copies of the observation tools we will use for calculating time and quality of the two hand hygiene regimens (Appendix 2).

Fifth, one reviewer noted that we would not be able to determine specific sources of skin flora of endemic infections because we are only studying nurses, and not visitors or other members of the staff. This is correct, and we have
clarified the narrative in the Significance section and discussed why we selected nurses for study. Our primary rationale for studying nurses is (a) in our pilot study we found that 70% of the direct touching of neonates was done by the nursing staff (the other 30% by a variety of others); (b) the causative agents of several recent outbreaks in the study NICUs and others in the U.S. have been traced to nurses' hands (even when there is a policy of frequent gloving); and (c) nurses are the only staff members who are consistently present on the study units over long periods of time, and are therefore the only staff members whose hand flora can tracked accurately over time. Hence, while we will not be including everybody, we should get an excellent idea of the sources of endemic flora by studying nurses, since they are the primary "touchers" and permanent staff members.

The transcription error of computer costs in the budget has also been corrected and the time line updated.

Research Plan

A. Specific Aims

Nosocomial infections are one of the most serious complications of health care, costing over $1 billion/year, and the fifth leading cause of death in acute care hospitals. Yet at least one-third of such infections are preventable (Jarvis, 1996). The hands of healthcare personnel frequently serve as vectors for the transmission of organisms between patients and are also a major reservoir for pathogens with antimicrobial resistance (Larson, 1988; Larson, McGinley, Foglia, et.al., 1992; Larson, Hughes, Pyrek, Spartks, Cagatay, & Bartkus, 1998). Although hand hygiene is one of the most important interventions to reduce risk of transmission of infectious agents, health care professionals routinely wash significantly less often and for shorter durations than recommended, and approaches to change their behavior have not been effective (Handwashing Liaison Group, 1999; Larson & Kretzer, 1995). Thus there is an urgent need to explore other potential means to improve hand hygiene and reduce nosocomial infections. There is not a national standard for hand hygiene products, but antiseptic detergents are most commonly used in adult and pediatric critical care settings. However, the need for frequent handwashing and glove changing among staff in intensive care units causes skin damage (Larson, Friedman, Cohran, Treston-Aurand, & Green, 1997; Larson, Hughes, Pyrek, Sparks, Cagatay, & Bartkus, 1998). Ironically, irritant contact dermatitis and other skin problems occur among staff who practice appropriate hand hygiene vigorously. This skin damage leads to changes in normal flora and shedding of more organisms into the environment, actually increasing the risk of nosocomial transmission of pathogens from the hands of personnel to patients (Meers & Yeo, 1978; Meers, 1980). Thus, the challenge that faces the health care community is to maximize the antimicrobial effectiveness of hand hygiene practices while minimizing damaging changes to the skin's health or microflora in order to reduce nosocomial infection rates.

The primary goal of this study is to compare effects of two hand hygiene protocols used by staff in neonatal intensive care units (NICUs) on nosocomial infection rates among infants. We hypothesize that a reduction in infections can be effected by two intervening variables--improved skin health and a decrease in the number and types of potential pathogens on hands of care providers. In addition, others have reported that alcohol-based regimens are less expensive than traditional handwashing. Hence the aims and hypotheses of the study are:

<table>
<thead>
<tr>
<th>Study Aim</th>
<th>Hypotheses</th>
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<tbody>
<tr>
<td>1. To compare the impact of two hand hygiene regimens used by NICU staff on</td>
<td>• Nosocomial infections among neonates are reduced when NICU staff use a</td>
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nosocomial infection rates, particularly endemic rates, among the infants

hand care regimen which includes an alcohol-based degerming agent as compared with a traditional detergent-based antiseptic.

- The number of nosocomial infections in which pathogens detected on nurses' hands are clonally and epidemiologically linked to infected neonates is reduced when an alcohol-based degerming agent is used as compared with a detergent-based antiseptic.

<table>
<thead>
<tr>
<th>2. To compare the impact of two hand hygiene regimens used by NICU staff on skin condition of nurses' hands</th>
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<tbody>
<tr>
<td>• The skin condition of nurses' hands is improved when a skin regimen which includes an alcohol-based degerming agent is used as compared with a detergent-based antiseptic.</td>
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<tr>
<th>3. To compare the impact of two hand hygiene regimens used by NICU staff on skin microbiology (numbers and types of organisms) of nurses' hands</th>
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<tr>
<td>• The skin microbiology of hands of the nurses using a regimen which includes an alcohol-based degerming agent differs from the skin microbiology of those using a traditional detergent-based antiseptic in the following ways:</td>
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<tr>
<td>• average number of colonizing flora is significantly lower;</td>
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<td>• mean number of species is fewer;</td>
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<tr>
<td>• antibiotic-resistant flora (methicillin-resistant Staphylococcus aureus (MRSA) or vancomycin-resistant enterococci (VRE)), gram-negative bacteria, and yeast are isolated less frequently.</td>
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4. To compare the direct costs of two hand hygiene regimens

• Direct costs of the alcohol-based hand care regimen are lower than those of the traditional handwashing regimen.

B. Background and Significance

B.1. Transmission of nosocomial infections by contaminated hands.

Although others before Semmelweis (1861) recognized the infectious nature of puerperal sepsis and the importance of the hands of care attendants in spreading disease (Holmes, 1843; Campbell, 1822), he was the first to demonstrate the role of hand hygiene in the prevention of person-to-person transmission of infection (Wiese, 1930). There is little other published work specifically related to hand hygiene until the mid-twentieth century. During the 1960s investigators demonstrated that Staphylococcus aureus was spread by the airborne route only 6-10% of the time, but 54% of babies in a newborn nursery handled by a 'carrier' nurse with unwashed hands subsequently became colonized with her strain of S. aureus (Wolinsky, Lipsitz, Mortimer, et.al., 1960). When non-carrier nurses handled a baby colonized with S. aureus and then handled another baby without handwashing, the transmission rate from the nurses' hands was 43%. Antiseptic handwashing subsequently reduced the transmission rate to 14% (Mortimer, Wolinsky, Gonzaga, et.al., 1966). Furthermore, 92% of babies attended by a nurse colonized with S. aureus who did not wash her hands before touching the baby acquired her staphylococcal strain as compared with 53% of babies handled in the
same manner with washed hands. Colonization took four times longer among infants handled by nurses with washed hands (Mortimer, Lipsitz, Wolinsky, et.al., 1962). Thus, these investigators were among the first to demonstrate that while \textit{S. aureus} is normal flora found in the anterior nares, it is rarely airborne, it is almost always transmitted by direct touch, and handwashing reduces its transmission several fold.

In a review of the Medline database (1879-Nov 1998) using the keyword "handwashing", 676 citations were found, but only 39 (5.8\%) were focused primarily on the role of hand hygiene in the prevention of infection. The majority of these studies were retrospective designs--case-control or outbreak investigations--which demonstrated a correlation and temporal relationship between improved handwashing and reduced rates of infection, and the causal evidence was often weak or anecdotal (Larson, 1988; Bryan, Cohran, & Larson, 1995). Between 1977-1998, there were 16 published quasi-experimental studies designed to examine the effects of a handwashing intervention on the risk of infection. Six of these were in schools or day care centers (Bartlett, Jarvis, Ros, et.al., 1988; Black, Dykes, Angerson, et.al., 1981; Butz, Larson, Fosarelli, et.al., 1990; Early, Battle, Cantwell, English, Lavin, & Larson, 1998; Kimel, 1996; Master, Hess, Longe, &Dickson, 1997), three in Bangladeshi communities (Khan, 1982; Shahid, Greenough, Samadi, Huq, Rahman, 1996; Stanton & Clemons, 1987), and seven in hospitals, summarized below.

### Quasi-Experimental Hospital-Based Studies of the Effect of Hand Hygiene Practices on Risk of Infection

<table>
<thead>
<tr>
<th>Year</th>
<th>Author</th>
<th>Setting</th>
<th>Significant Results</th>
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<tbody>
<tr>
<td>1977</td>
<td>Casewell</td>
<td>Adult critical care (U.K)</td>
<td>Reduced rates from endemic \textit{Klebsiella}</td>
</tr>
<tr>
<td>1982</td>
<td>Maki</td>
<td>Adult critical care</td>
<td>Reduced rates</td>
</tr>
<tr>
<td>1984</td>
<td>Massanari</td>
<td>Same</td>
<td>Reduced rates in some units</td>
</tr>
<tr>
<td>1990</td>
<td>Simmons</td>
<td>Same</td>
<td>No effect</td>
</tr>
<tr>
<td>1992</td>
<td>Doebelling</td>
<td>Same</td>
<td>Significant differences in rates between the two regimens</td>
</tr>
<tr>
<td>1994</td>
<td>Webster</td>
<td>NICU</td>
<td>Elimination of MRSA</td>
</tr>
<tr>
<td>1995</td>
<td>Zafar</td>
<td>Newborn nursery</td>
<td>Elimination of MRSA</td>
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</table>

In 6/7 of these studies, there were statistically significant results indicating that improved hand hygiene practices (\textit{for example, improving technique or frequency of handwashing or changing products}) had a beneficial impact on infection rates. Although this body of evidence is impressive, only two studies reported an acceptable power calculation, several lacked adequate controls and none were randomized or blinded. Unfortunately, it is not feasible in the patient care setting to blind subjects or investigators to hand hygiene regimen, since most products have distinctive characteristics. The demands of patient care and limitations of the physical setting also make randomization or separation of patients into study groups infeasible. Hence, it is not possible to rule out bias or confounding as explanations for results in previous studies. While laboratory data and controlled, shortterm studies indicate that alcohol-based products are superior microbiologically and physiologically, there has been only one clinical trial in which the outcome of \textit{NOSOCOMIAL INFECTIONS} was evaluated. It is quite possible for a product to perform well at reducing microbial counts on the hands and improve skin condition, but have
little effect on infection rates in patients. Thus, clinical studies evaluating clinically relevant outcomes are essential and required by the Food and Drug Administration (1994). Only one (Doebelling, 1997) of the seven studies noted above compared alcohol and the antiseptic agent most frequently used in hospitals today, chlorhexidine gluconate (CHG) (others sought to improve handwashing frequency or compared various other products which are not commonly used in many hospitals). Further, this one study was fraught with methodologic problems, including potential confounding by handwashing frequency and gloving practices. Results of this single study, in fact, were opposite what would have been expected from all other previous work (i.e. it found that there were significant reductions in infection rates during use of the CHG agent), but the authors and the accompanying editorial speculated that the results were due to confounding. Hence, although improved hand hygiene in general (i.e. more frequent washing) has been shown to be associated with reduced infection risk, the most efficacious regimens are simply unknown. Further, no studies have examined the relationship between the care providers' skin health and hand flora and infections in patients, and none of these studies used molecular epidemiologic techniques to link the isolates from staff skin flora with patient isolates. An "ideal" experimental design to examine the effects of hand hygiene on infection rates may not be feasible for the reasons described above. However, the cumulative results of decades of descriptive and quasi-experimental studies indicate that a carefully designed clinical trial comparing different hand hygiene protocols and incorporating molecular epidemiology and assessment of skin condition is an essential next step to further explicate the role of hand hygiene in the prevention of nosocomial infections.

B.2. Nosocomial infections in neonates. Nosocomial infections are one of the most devastating and common complications of infants requiring hospitalization. Recent outbreaks of nosocomial infections have resulted in neonatal deaths and closing of NICUs in several states, but these outbreaks represent just the tip of the iceberg (Baltimore, 1998). Up to one-fourth of neonates in NICUs develop nosocomial infections; between 1976 and 1996 individual NICUs reported 8-25 nosocomial infections/100 discharges (Beck-Sague, Azimi, Fonsecz, et.al., 1994; Ford-Jones, Mindorff, Langley, et.al., 1989; Goldmann, Durbin, & Freeman, 1981; Hemming, Overall, & Britt, 1976). In a cohort of 7,861 very low birth weight infants (<1500 grams) from 12 centers followed in the National Institute of Child Health and Human Development Neonatal Research Network, nosocomial bloodstream infections occurred in about 25% of babies. Most infections were caused by gram-positive cocci, particularly coagulase-negative staphylococci (which can be true pathogens, especially in very low birth weight infants). The smallest infants had the highest rates of infection (Stoll, Gordon, Korones, et.al., 1996).

The largest national database is maintained by the Centers for Disease Control and Prevention (CDC) through the National Nosocomial Infections Surveillance System (NNIS), which currently receives data from about 120 NICUs across the country (NNIS Report, 1998). Data from over 13,000 NICU infections were reported through the NNIS system from October 1985-Sept 1994 and summarized recently (Gaynes, Edwards, Jarvis, Culver, Tolson, & Martone, 1996). Infection rates were correlated with length of stay and device use (such as central venous catheters and ventilators). Bloodstream infections were the most frequent type of infection in all birth weight categories, and 88% of these were associated with use of umbilical or central venous catheters. The next most common infections were pneumonia and eye, ear, nose, and throat infections (mostly conjunctivitis). The most common causative agents were coagulase-negative staphylococci, S. aureus, enterococci, Enterobacter spp, and Escherichia coli. The most recent published NNIS summary, Jan 1990-May 1999, reported pooled mean catheter-associated bloodstream infection rates of 4.5-12.2/1000 catheter days.
and ventilator-associated pneumonia rates of 2.8-4.9/1000 ventilator days, the highest rates being in the lowest birth weight categories (NNIS, 1999). Mortality rates among infected neonates are several times higher than among noninfected infants of comparable birthweight and risk. Nosocomial infections add significantly to costs of care, length of stay, and clinical management among hospitalized infants (Saiman, in press, 2000, see Appendix 4; Baltimore, 1998).

**B.3. Impact of hand care practices on skin condition.** The most superficial layer of the epidermis, the stratum corneum, is composed of flattened dead cells (corneocytes or squames) attached to each other to form a tough horny layer of keratin mixed with several skin lipids. This horny layer has been compared to a wall of bricks (corneocytes) and mortar (lipids), and serves as the primary protective barrier. Lipids are an important component in maintaining the hydration, pliability, and the barrier effectiveness of the skin. There are about 15 layers of stratum corneum. A new layer is formed approximately daily, and it is completely replaced about every two weeks (Schaefer & Redelmeier, 1996). About $10^7$ particles are disseminated into the air each day from healthy skin, and 10% of these skin squames contain viable bacteria (Noble & Davies, 1965).

The superficial skin layers absorb or lose water and under normal circumstances retain sufficient moisture to keep the skin soft and pliable. Water is the plasticizer of the stratum corneum, and with increased hydration comes increased diffusibility. One important function of the intercellular lipids is to prevent dehydration of the corneocytes. Depending upon the product used, washing can raise the pH of the skin, and long term changes in skin pH may alter the antibacterial characteristics of the skin associated with normally acidic pH (Marples, 1965, p 179). In one report, the pH increased 0.6-1.8 units and then gradually declined to baseline levels over a period of 45 min-2 hr after a 1-2 min handwash with plain soap (Klauder & Gross, 1951). With more prolonged soap contact, skin pH can reach 7.0-8.5 and remain high for 3-4 hrs (Kirk, 1966). Some soaps are associated with longstanding changes in skin pH, reduction in fatty acids, and, subsequently, changes in resident flora such as Propionobacter spp. (Korting, Kober, Mueller & Braun-Falco, 1987; Hoffler, Gloor, Peters, Ko, Brautigam, Thurn & Pulverer, 1980).

In a study examining the effect of repeated use of two different washing agents, all skin function tests (stratum corneum capacitative resistance, lipids, transepidermal water loss, pH, laser Doppler flow, and skin reddening) were markedly changed after a single wash and after one week of washing, further damage was noted (Grunewald, Gloor, Gehring & Kleesz, 1995). Wilhelm, et.al. (1994) tested irritant skin reactions induced by three different surfactants used in soaps and detergents and found that damage was present for days; complete skin repair was not achieved until 17 days post exposure.

Soaps and detergents, particularly if they are anionic or cationic, are the most damaging of all substances routinely applied to skin (Jarrett, 1978, p 1747; Dugard & Scheuplein, 1973), and increased concentrations of surfactant result in more rapid and severe damage (Scheuplein & Ross, 1970). Kirk (1966) concluded that

“It is generally agreed that removal of a certain amount of contaminated surface fats and of bacteria attached to superficial epidermal cells is an essential hygienic feature. However, the lipid and cell removal through washing should be somewhat limited to avoid damage to lower layers of the epidermis” (p 88).

Each time the skin is washed, it undergoes profound changes. Most of these changes are transient, but among individuals in occupations such as health care in which frequent handwashing is required, long term changes in the skin can result in chronic damage, irritant contact dermatitis and eczema, and concomitant changes in microbial flora.

**B.4. Impact of hand care practices on skin microbiology.** Several decades ago, Finnish investigators demonstrated that nurses working in a NICU who practiced
frequent handwashing with an antiseptic soap reached a point at which their hands become damaged and posed greater risk to themselves and patients than if they had not washed as frequently (Kolari, Ojajarvi, Lauharanta, & Makela, 1989; Lauharanta, Ojajarvi, Sarna, & Makela, 1991). Physiological factors which control the bacterial skin flora include humidity, water content, skin lipids, temperature, and rates of desquamation (Aly & Maibach, 1981; McBride, Duncan & Knox, 1975): washing results in changes in all of these factors. It was demonstrated years ago that bathing, showering and washing increases microbial dispersal by many fold (Meers & Yeo, 1978; Meers, 1980). Staff showering immediately before surgery is no longer recommended, but national guidelines continue to recommend frequent handwashing. The dispersal of organisms is greater in males than in females and varies between individuals using the same hygienic regimen by as much as 5-fold (Noble, 1965). Although the loss of the first few layers of stratum corneum reduces numbers of bacterial colonies shed from hands, counts remain stable with removal of subsequent layers, indicating that resident skin flora is located throughout skin layers in the deeper regions of hair follicles and sebaceous glands (Brown, Wenzel, & Hendley, 1989). The loss of the outermost layers of the skin from washing is accompanied by an increase is transepidermal water loss, indicating reduced barrier function (Schaefer & Redelmeier, 1996, p 44). Washing defats the skin and the rate of lipid replenishment on the dorsum of the hands is only about 20% after 1 hr and 50% after 3 hrs (Scheuplein & Ross, 1970). Fatty acids in the horny layer also have fungicidal and bactericidal activity important in modulating the skin flora (Marples, p 121).

Irritant contact dermatitis associated with frequent handwashing is one of the most prevalent occupational risks for health care professionals. The prevalence ranges from about 10-45% (Holness, Tarlo, Sussman, & Nethercott, 1995; Munksgaard, Hansen, Engen, & Holm, 1996; Sproat & Uveges, 1995; Stingeni, Lapomarda, & Lisi, 1995). Damaged skin more often harbors increased numbers of potential pathogens. Further, washing damaged skin with either plain or antiseptic soap is less effective at reducing numbers of bacteria on hands than washing normal skin, and numbers of organisms shed from damaged skin are often higher than shed from healthy skin (Ojajarvi, Makela, & Rantsalo, 1977; Parry, Hutchinson, Brown, Wu, & Estreller, 1980; Richards, Williams, Warner, et.al., 1993).

While numerous studies have shown alcohol-based formulations (isopropyl, ethyl, or n-propanol in concentrations of 60-90% v/v) to be equivalent or superior to antiseptic detergents for microbial killing (Larson, Eke, & Laughon, 1986; Lilly, Lowbury, & Wilkins, 1979; Ojajarvi, 1980; Tanaka, Kuman, Hirata, Yamamoto, Fujita, 1988 Morrison, Gratza, Cabzudo, Wenzel, 1986; Stratton, 1986; Rotter, 1984; Rotter & Koller, 1992; Hobson, Woller, Anderson, & Guthery, 1998), clinical evidence of their efficacy in reducing infections is lacking. In addition, alcohols with appropriate emollients are at least as tolerable on skin as antiseptic detergents (Ojajarvi, 1980; Newman & Seitz, 1990; Rotter, Koller, & Neumann, 1991; Larson, Butz, Gullette, & Laughon, 1990). Alcohols are rapid acting, broad spectrum, and require no washing or drying, reducing damage due to mechanical friction. Alcohol formulations are commonly used in Europe, but have not yet been readily adopted in the U.S.

Meers (1980) reported that microbial counts on hands were reduced satisfactorily by either a surgical scrub or an alcoholic lotion, but that there was no increase in skin shedding after alcohol application as compared with an 18-fold increase after scrub. The reduction in skin damage associated with a change from antiseptic detergent wash to alcohol lotion plus the reduced shedding led Meers to conclude that "a spirit based hand lotion should be used as a substitute for handwashing when this is done for degerming rather than cleaning" (p. 17), but the longterm effects of these products on skin health AND on infection rates have not been tested in a clinical trial.

B.5. Importance of emollients. In the 1960s and 70s, in response to reports of nosocomial infections traced to contaminated lotion (France, 1968; Morse, Williams, Grann, et.al., 1967), lotions were generally banned from hospitals and their use by staff
strongly discouraged. In the 1980s and 90s, the increased prevalence of skin problems associated with more gloving and washing as well as better product packaging to reduce the risk of contamination of lotions has resulted in the realization that emollients applied to hands are desirable, perhaps even essential, in clinical practice.

As early as the 1950s and 60s, an antiseptic hand cream was shown by British investigators to control cross-infection (Gillespie, Simpson, Tozer, 1958; Murray & Calman, 1955) and to be more effective than alcohol alone or antiseptic detergent in reducing microbial skin counts (McBride, Montes, Knox, 1975). More recent data from the U.S. indicate that an antiseptic ingredient in the emollient may not even be necessary for protective effects. Application of inert ointments or lotions (as long as extrinsic contamination is prevented) has been associated with reduced colonization rates in adults (Wilson, Ayers, & Stone, 1986) and notably in neonates. In two prospective trials, positive bloodstream and cerebral spinal fluid cultures were significantly reduced after the skin of very low birthweight babies was treated with a preservative-free topical ointment (Nopper, Horii, Sookdeo-Drost, Want, Mancini, & Lane, 1996; Wallace, Lindado, Bedrick, Moravec, & Nieto, 1998). Investigators speculated that this ointment supplemented the ineffective epidermal barrier of the neonatal skin. On the other hand, this same ointment was a source of bloodstream infection in neonates after it became contaminated (Ramsey, et.al., 1998). Skin emollient, with or without antiseptic, greatly reduces dispersal of bacteria from the skin for up to four hours (Hall, Mackintosh, & Hoffman, 1986), so some of its effectiveness in reducing infections may be the result of simply preventing the bacteria present on skin from shedding.

There is biologic evidence to support the hypothesis that the use of emollients on skin of healthcare professionals may be protective against cross-infection. Moisturizers prevent dehydration, damage to barrier properties, desquamation, and loss of skin lipids, restore the water-holding capacity of the keratin layer, and increase the width of corneocytes (Grunewald, Gloor, Gehring, Kleesz, 1995; Lachapelle, Wahlberg, & Maibach, 1996; Zelickson, Zelickson, & Zelickson, 1982). There is growing interest in the use of barrier creams and lotions that not only shield damaged skin but also restore its structure and/or function. In a Swedish single-blind study, a moisturizing cream was found to accelerate the rate of improvement of surfactant-damaged skin (Loden, 1997).

In a recent randomized, double-blind trial comparing scheduled use (four times/day) of two hand lotions in 54 nurses with severe hand irritation, McCormick, Buchman, & Maki (1998) reported marked, sustained improvements in skin condition in both groups. Significantly more improvement occurred in the group using an oil-based product. However, oil-containing lotions may degrade latex gloves and increase the transfer of allergenic glove proteins to skin (Beezhold, Kostyal, & Wiseman, 1994). For these reasons, the National Institute of Occupational Safety and Health (NIOSH) recommends that oil-containing hand products not be used when latex gloves are worn.

B.6. Significance. Despite the devastating impact of nosocomial infections in neonates, most attention to the problem has been focused on outbreaks (Potter-Bynoe, et.al., 1998). Although the majority of nosocomial infections are not associated with identified outbreaks (i.e. they are endemic, occurring at a lower, persistent rate) and are caused by skin flora, few attempts have been made to reduce rates of endemic infections. In addition, no studies have yet identified the specific sources of the skin flora causing these infections (i.e. whether infecting organisms originate from the hands of nurses or others). This study will fill a gap in our knowledge of the epidemiology of endemic neonatal infections (as well as "outbreaks") and how they might be prevented by identifying the role of nurses' hand flora. Nurses are the focus of this study because they comprise the majority of healthcare professionals providing direct patient care, their hand flora has been associated with previous outbreaks of...
nosocomial infections in NICUs, and in our pilot study, nurses made 70% of the direct contacts to neonates.

This is a timely study since there is great emphasis in the U.S. on changing hand hygiene practices to improve antimicrobial effectiveness as well as staff skin condition. The PI gets several inquiries each week and the email discussion group (listserv) of the Association for Professionals in Infection Control and Epidemiology (APIC) has included widespread discussion in the past few months indicating that many health care facilities are considering a change from antiseptic wash products to waterless alcohol rinses. Unfortunately, plans to change practice are not accompanied by plans to evaluate these changes. Thus, this study will provide new clinical data for evidence-based practice in skin hygiene. The field of infection control is fraught with untested or inadequately tested traditions and rituals (handwashing practices, surgical scrubbing, isolation precautions, and wearing masks, for example) which, once inculcated into practice, are exceedingly difficult to change. If we are to move infection control toward evidence-based practice, it is essential to study the important question of the efficacy of hand hygiene using alcohol in reducing infection rates under longterm clinical use conditions before it becomes a national standard. Much of the research in this area is conducted by corporate sponsors for the purpose of selling products, and clinical decisions are often based on data on intervening variables such as reductions in microbial counts provided by the manufacturers of these products rather than outcome and clinical data generated by the scientific community. Hence, the major objective of this study is to provide a sound scientific basis for clinical decision making regarding hand hygiene practices.

Although we know from previous studies that certain aspects of hand hygiene, particularly improved frequency, can reduce infection rates, we do NOT know which regimens are most efficacious. Theoretically, alcohol based formulations should be most effective in reducing nosocomial infection rates since they most rapidly reduce skin microbial counts. However, results from the only study to compare alcohol and CHG conflicted with other available evidence and showed the opposite effect (Doebelling, 1992). The authors attributed this to potential confounding (e.g. subjects washed their hands more often during the CHG phase). Because alcohols are beginning to be used for hand hygiene in the U.S., it seems vital to confirm (or refute) their clinical effectiveness before their use becomes widespread.

This proposal is unique and will make several important contributions toward the prevention of nosocomial infections: it is the first clinical trial to examine effects of nurses’ hand hygiene on infections in a NICU population, to try to improve both the skin health of patient care providers and infectious complications in patients, to extensively use molecular epidemiologic techniques to link the skin flora of nursing staff to infections in neonates, to focus on endemic infections rather than outbreaks, and to emphasize scientific concepts of skin antisepsis rather than product testing. The conduct of a clinical trial to examine the influence of hand hygiene on infections among neonates is fraught with numerous challenges and potential design difficulties. Among high risk populations such as neonates there are multiple factors associated with the development of an infection, many which cannot be controlled. For this reason, such a trial has not previously been conducted. But because of the many years of background and preliminary research conducted by this research team and because our pilot study demonstrated that such a study is feasible, we are able to submit this proposal with confidence that we can add to the important body of knowledge about the role of hand hygiene in the prevention of infections. If the results of this study were only applicable to a few NICUs or a single clinical setting, the costs would not be justified. However, the clinical outcomes and findings outlined above can be applied to hand hygiene and other prevention strategies in many health care settings and are far reaching in their
C. Preliminary Studies

Over the past two decades, the PI has published over 40 research papers related to hand hygiene, including clinical trials which compared antimicrobial effectiveness of various antiseptic and other hand hygiene products, descriptive studies of the flora of hands of various populations, factors which influence handwashing behavior, intervention trials to change handwashing practices of health care professionals and school children, effects of hand care practices on the skin of nurses' hands, and integrated reviews of the literature. The PI is currently Chair of the CDC Healthcare Infection Control Practices Advisory Committee (HICPAC) which develops the U.S. guidelines for infection prevention and control in healthcare settings. Recent guidelines include isolation precautions, prevention of vancomycin resistance, personnel health guideline, prevention of surgical site infection and of IV therapy-related infections. She is also the author of an extensive research-based professional guideline, APIC Guideline for Handwashing and Hand Antisepsis in Health Care Settings (Larson, 1995).

In several recent investigations, research team members have demonstrated their proficiency with the molecular epidemiologic techniques proposed for this study. Examples include an investigation of scalded skin syndrome with isolates from symptomatic and asymptomatic neonates determined by pulsed field gel electrophoresis to be genetically identical or related to isolates from three staff; a study of an increased incidence of nosocomial infections in a NICU caused by Pseudomonas aeruginosa, with fingerprinting which identified a particular clone colonizing the hands of a nurse; a large fungal surveillance study conducted in collaboration with CDC; and a descriptive study of the molecular epidemiology of S. epidermidis blood isolates from NICU patients (Marco, Lockhart, Pfaller, et al., 1999; Rangel-Frausto, Wiblin, Blumberg, et al., 1999; Saiman, Jakob, Holmes, et al., 1998; Saiman, Ludington, Pfaller, et al., in press. See Appendix 4 for summaries of some of these studies).

C.1. Validity, reliability and feasibility testing of proposed methods and protocols. A pilot study was conducted in Jan-Feb 1999 to determine the feasibility and reliability of proposed data collection techniques, assess subject compliance, and gather some preliminary data for more precise estimation of sample size needs and acceptability of potential hand care regimens. Two hand care regimens were selected for pilot testing using the following criteria: evidence from research literature and preliminary studies of current practice (Regimen A) and modifications in current practice shown independently in well designed studies to have potential to improve skin condition and/or reduce microbial transmission (Regimen B); regimens that would be feasible, acceptable to users, and efficient in terms of time and material costs; regimens that could be readily generalized for use in other settings and health care institutions.

The choice of particular products to use posed a dilemma for several reasons. First, although there are certain generic active ingredients that have been shown in previous research to be effective (e.g., chlorhexidine gluconate (CHG) or alcohol), products are formula-dependent and vary by manufacturer. Second, we wanted to maximize the generalizability of our findings and assure that this clinical trial was not just testing specific products. Since there are a number of products available on the market containing the same active ingredients, we used the following selection criteria for products: (a) available in vitro and clinical data using FDA-recommended protocols on the specific product demonstrating the highest available levels of antimicrobial activity; (b) manufactured by a national or international firm adhering to good manufacturing practices; (c) readily available throughout the U.S. in health care settings and/or over-the-counter; (d) as representative (generic) as possible of the class of antiseptic/active ingredient being tested; and (e) cost competitive with comparable products.
While there are no national databases describing what skin care products are used for hand hygiene, all 41 nurses surveyed in a study in 1996 who worked in four NICUs in three states reported that their units used a liquid or foam detergent-based product containing CHG for hand scrubbing and washing (Larson, et.al., 1997). In preparation for this grant submission, the PI called infection control staff in 17 hospitals located in nine states, all of whom reported using a CHG-containing product in the NICU. Alcohol and CHG-containing products are among the few designated as safe and effective for a range of topical applications ((FDA, 1982; FDA, 1994). Products containing CHG were used in two published crossover studies assessing the correlation between handwashing and infection rates among adults in critical care units (Maki & Hecht, 1982; Massanari & Hierholzer, 1984), and are currently used in both of the proposed study units. Hence, Regimen A was chosen because it reflects the most prevalent current practice in the U.S.; Regimen B reflects changes in practice which have shown promise in other scientific studies, and is consistent with common practice in many European countries.

Final regimens tested were:

**Regimen A:**
- a 2-minute scrub using a sponge containing a 4% CHG-containing detergent (Foam Care Surgical Hand Scrub, Ballard, Draper, UT) when coming on duty;
- handwashing with a 2% CHG product (Foam Care) throughout the working shift;
- liberal use of CHG-compatible lotion (Prima-Kare Lotion, Steris, St. Louis, MO) during working hours (activity of CHG is neutralized by most lotions [Frantz, Haines, Azar, Ward, Homan, & Roberts, 1997], and therefore must be used only with specially formulated non-ionic moisturizing products);
- no restrictions or modifications of skin hygiene practices at home.

**Regimen B:**
- a short (15 sec) wash with mild, non-antimicrobial liquid detergent soap (Kindest Kare Body Wash and Shampoo, Steris, St. Louis, MO) followed by a 15 sec application of a 60% isopropanol preparation containing emollients (Cal-Stat, Steris, St. Louis, MO) when coming on duty (no scrub);
- use of the mild, non-antimicrobial soap at home and at work to remove soil;
- a short application (10-15 secs) of the alcoholic preparation throughout the working shift when hand degerming was indicated;
- liberal and scheduled (four times/day and as needed) use of an oil based skin emollient/moisterizer (Curel, Bausch & Lomb, Rochester, NY);
- no use of other antimicrobial skin products at home or work.

Following approval by the Institutional Review Board at Columbia University Medical Center, 16 NICU nurses were randomly assigned to one of these two regimens for a 4 week trial period. Nurses were eligible for the pilot study if they worked fulltime on either day or night shift, had no diagnosed dermatologic problems such as psoriasis or latex hypersensitivity, were not taking steroids or antibiotics, and had no vacation during the 4-week study period. Hand cultures and measures of skin condition (Visual Scoring of Skin Condition, VSS, Hand Skin Assessment form, HSAF, described in D.4.2) were obtained at baseline, 2 and 4 wks. One working shift each week, nurses kept a diary of each handwash, application of alcohol, lotion, and glove use (Diary form in Appendix 2). The Mann Whitney U test was used to compare differences between Regimens A and B at each of the three test points.

All subjects worked fulltime on 12-hr shifts, 11 on days and 5 on nights. None wore artificial nails, two (12.5%) reported routinely having a professional manicure, and four (25%) routinely wore nail polish. Average time in nursing was 18.3 years, not significantly different between the two groups (p=.64). All subjects readily adhered to the assigned protocol (measured by weekly checks on volume of products used), and there were no dropouts. Daily random spot checking confirmed that diaries were maintained throughout the working shift.
### Pilot Study Results: Hand Hygiene Practices (n=16 nurses)

<table>
<thead>
<tr>
<th>Practice</th>
<th>Regimen A: mean (+/-stand. deviation)</th>
<th>Regimen B: mean (+/-stand. deviation)</th>
<th>Mann-Whitney U P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Handwashes/shift</td>
<td>21.2 (8.4)</td>
<td>15.4 (5.3)*</td>
<td>.08</td>
</tr>
<tr>
<td>Lotion applications/shift</td>
<td>2.6 (1.5)</td>
<td>4.4 (2.6)</td>
<td>.19</td>
</tr>
<tr>
<td>Alcohol uses/shift</td>
<td>Not applicable</td>
<td>8.4</td>
<td></td>
</tr>
<tr>
<td>Glovings/shift</td>
<td>12.4 (8.0)</td>
<td>12.2 (5.8)</td>
<td>.80</td>
</tr>
</tbody>
</table>

*Note that nurses using alcohol did fewer "traditional" washes with soap and water because they used alcohol for degerming.

In 92 hand cultures, 150 isolates were obtained: 1-5 isolates/sample (median: 2).

### Pilot Study Results: Species Isolated from Hands (n=150 isolates)

<table>
<thead>
<tr>
<th>Species</th>
<th>Frequency: # isolates (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coagulase-negative staphylococci (CNS)</td>
<td>74 (49.3%)</td>
</tr>
<tr>
<td>Diphtheroids/ micrococi</td>
<td>50 (33.3%)</td>
</tr>
<tr>
<td>Gram-negative bacteria: 6 Enterobacter spp, 2 each of Pseudomonas aeruginosa, Klebsiella spp, Acinetobacter spp</td>
<td>12 (8%)</td>
</tr>
<tr>
<td>Candida albicans</td>
<td>7 (4.7%)</td>
</tr>
<tr>
<td>Enterococci</td>
<td>4 (2.7%)</td>
</tr>
<tr>
<td>Methicillin-sensitive Staphylococcus aureus</td>
<td>3 (2%)</td>
</tr>
</tbody>
</table>

### Pilot Study Results: Skin Condition and Microbial Colony Forming Units (CFU)

<table>
<thead>
<tr>
<th>Test Mean</th>
<th>Regimen A</th>
<th>Regimen B</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>VSS (range 0-6): Baseline</td>
<td>5.0</td>
<td>4.25</td>
<td>.44</td>
</tr>
<tr>
<td></td>
<td>Mid-point</td>
<td>4.0</td>
<td>4.75</td>
</tr>
<tr>
<td></td>
<td>End (wk 4)</td>
<td>3.25</td>
<td>5.12</td>
</tr>
<tr>
<td>HSAF (range 4-28): Baseline</td>
<td>20</td>
<td>20</td>
<td>.5</td>
</tr>
<tr>
<td></td>
<td>Mid-point</td>
<td>19.25</td>
<td>24.12</td>
</tr>
<tr>
<td></td>
<td>End (wk 4)</td>
<td>16.25</td>
<td>25.5</td>
</tr>
<tr>
<td>Log CFU: Baseline, pre- wash</td>
<td>3.69</td>
<td>3.21</td>
<td>.44</td>
</tr>
<tr>
<td>Baseline, post-wash</td>
<td>3.65</td>
<td>3.19</td>
<td>.99</td>
</tr>
<tr>
<td>Mid-point, pre-wash</td>
<td>3.69</td>
<td>3.85</td>
<td>.57</td>
</tr>
<tr>
<td>Mid-point, post-wash</td>
<td>3.84</td>
<td>3.4</td>
<td>.88</td>
</tr>
<tr>
<td>End, pre-wash</td>
<td>3.71</td>
<td>3.67</td>
<td>.96</td>
</tr>
<tr>
<td>End, post-wash</td>
<td>3.68</td>
<td>3.58</td>
<td>.57</td>
</tr>
</tbody>
</table>

There were no significant differences in CFU counts between the two regimens, but the skin condition of hands in Regimen B significantly improved over the 4-week study. From this pilot work, we concluded that both regimens were feasible and
acceptable to personnel, both had at least equivalent microbiologic activity, and data collection methods were reliable and yielded accurate information. Based on these findings, slight changes in both regimens were made. Changes in Regimen A are designed to assure that the two proposed study sites are comparable (i.e., replace 4% with 2% CHG for initial 2 min scrub and eliminate the sponge). The soap used in Regimen B was extremely well tolerated. Nurses reported that the alcohol hand rinse, although acceptable, was somewhat drying and caused discomfort in the presence of skin abrasions. The lotion used was petrolatum based and not compatible with latex gloves (which were not in use in the pilot study unit). Even though the study units do not use latex gloves, a latex compatible product would be more generalizable to other settings. Hence, we made the following changes in Regimen B in the final protocol: (1) retain use of the same mild soap; (2) replace the hand rinse with a more recent formulation containing added emollients; (3) use a non-oil based lotion. Finally, we concluded that attempts to regulate skin care practices at home were feasible, but would limit generalizability in a larger study so we will use both regimens only during working hours.

C.2. Validating definitions of nosocomial infections in neonates. Definitions for nosocomial infections have been developed by the CDC (Garner, Jarvis, Emori, Horan, & Hughes, 1988). These definitions are the best validated available, the only nationally standardized definitions and used by all hospitals enrolled in the NNIS system. But there are specific problems in neonates, who do not necessarily exhibit the usual signs and symptoms of infection. Further, the NNIS definition for nosocomial pneumonia is undergoing revision, so we based our modifications on the latest draft revision. Lastly, it will not be possible to blind the surveillance officer to the regimen being used. Hence, we examined additional methods to improve reliability and validity of the traditional surveillance protocols and definitions.

An expert consultant recommended by the NNIS staff, a pediatric infection control nurse with >20 years experience in surveillance of neonatal infections (B. Stover, Louisville, KY), made a site visit in March 1999. She has served as a pediatric consultant to CDC for their nosocomial definitions for more than 10 years. She reviewed our surveillance protocols and definitions in meetings with the clinical research team and clinicians, and spent a day conducting chart reviews with two experienced infection control nurses in both study units. Subsequently at both study sites the surveillance staff again conducted chart reviews using these modified definitions. Meetings were held with the clinician members of the study team and study units (neonatologists, nurses, infectious disease physicians) to discuss and validate the definitions. Based on input from the consultant and expert clinicians and the clinical chart reviews, the NNIS definitions were modified slightly to increase sensitivity and specificity for neonates. These modifications were then sent to NNIS staff for final review and validation. The final surveillance definitions are included in Appendix 3. We feel confident, and the NNIS staff agreed, that these changes represent an important improvement and clarification over current definitions for use in high risk, low birth weight or premature neonates. For this study we will be using both the most current NNIS definitions as well as our definitions modified for neonates to allow a comparison of the sensitivity and specificity of the two variations.

D. Research Design and Methods

D.1. Design. This clinical trial uses a sequential, crossover design to examine the effects of two staff hand care regimens on nosocomial infections in critically ill neonates. We had hoped that it would be possible to design a randomized clinical trial. However, based on the pilot study we determined that it will not be feasible to randomly assign staff to one of two different regimens because it would not be possible to geographically segregate neonates (neonates are assigned to staff and rooms based on
severity of illness and special needs). Additionally, the chances of misclassification or failure to adhere to the assigned regimen would be greatly increased if several products were available on the units at the same time. Therefore, a crossover design will be used in which Regimen A or B is used by one of two study units for 12 months, and then the two study units will alternate regimens for a second year. The initial regimen to be followed will be randomly selected for one of the study units and the second unit will use the alternate regimen. This crossover allows sufficient time and statistical power (see D.2.2) to identify significant changes in outcomes during each regimen while accounting for seasonal variations in skin condition and infection trends. We considered having a larger number of shorter intervals with more crossovers, but based on preliminary studies it became clear that more frequent changes would be impractical and more likely to result in misclassification. We have set up a careful system of interim analysis so that if there are significant changes in infection rates or staff compliance during one of the two regimens, we will stop the study early (see D.5.2a, Data and Safety Monitoring Board).

All staff, visitors and others in the NICU will use the assigned regimen while on the units. Only permanent nursing staff will be studied with regard to skin health and microbiology because they are the only group permanently assigned to the study unit, are a very stable group, and have the most intimate and prolonged contact with the neonates.

D.2.1. Setting and sample. The study will be conducted in two NICUs in NYC which are part of the New York Presbyterian Hospital System: one 50-bed unit at the Cornell campus and a second 47-bed unit at Babies & Children’s Hospital, Columbia campus. While this is a sample of convenience, it is similar to the NICUs in the NNIS database in terms of types and rates of infections, distribution of birth weights and other relevant characteristics of the patients, and nurse:patient staffing ratios. All infection control policies and procedures, including those for surveillance and hand hygiene, are the same in both units. All permanent nursing staff and all neonates hospitalized for at least 48 hours will be included in the study.

<table>
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<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Cornell</td>
<td>50</td>
<td>11,300</td>
<td>569</td>
<td>19.7 days</td>
<td>76</td>
<td>3.86</td>
</tr>
<tr>
<td>Columbia</td>
<td>47</td>
<td>12,000</td>
<td>557</td>
<td>25 days</td>
<td>77</td>
<td>4.60</td>
</tr>
</tbody>
</table>

D.2.2. Statistical power. The proposed design essentially fixes the sample size both in terms of the number of neonates and the number of nurses. We expect all nurses (approximately 75 at each site) to participate, and expect approximately 550 neonates per year per site (a total of 2200). These numbers ensure that power to detect clinically important differences is excellent. For example, power exceeds 90% to detect a significant regimen difference in terms of neonatal infections if the true rates are 6% for one regimen and 3% in the other, or 6% for one regimen and 10% for the other. Our power calculations provide us with a high degree of security that we will have sufficient variation and sufficient sample size, given our current incidence rates on the study units, to identify clinically meaningful changes in infection rates. The infection rates we used for the power analysis are realistic, based on data over several years in the study units. Even though infection rates are low (e.g. 3-6%), our power is still excellent. In fact, our power analysis was quite conservative. Doebeling (1992) reported nearly double the infection rate when one regimen was compared with the other, and retrospective evidence from outbreak investigations provide evidence that effects of hand hygiene on
infection rates can be detected. Therefore, if there is a clinically meaningful effect of one of the regimens being studied, we will find it.

The large sample also ensures good power to detect differences for repeatedly recorded outcomes. For example, power exceeds 90% to detect a difference of 5% versus 10% on a serially recorded dichotomous outcome (such as "damaged" hands or the presence of antibiotic resistant isolates) assuming correlations between repeated measurements of 0.50. (See formula on p. 31 of Diggle, Liang, and Zeger, 1994). All calculations are based on 0.05 level two-tailed tests and ignore the likely increase in power to be obtained by adjusting for covariates and thereby reducing the variance of outcomes.

D.3. Intervention: Skin Care Regimens

D.3.1 Definitions. (a) Non-antimicrobial soap. A detergent-based formulation in liquid, bar, or powder form without antiseptic or antibacterial active ingredients (except as preservatives). Also called "plain soap" or "soap" and used primarily for physical cleaning and removal of soil and transient microbes. (b) Antiseptic (antimicrobial or antibacterial) product. A product designed for topical application to the skin which has cidal activity against microbial flora. It may be a detergent-based liquid, foam or bar (usually containing either CHG, triclosan, para-chloro-meta-xylene (PCMX), or an iodophor) or a waterless liquid, gel, or foam (most of these products contain either ethyl or isopropyl alcohol). Some of these products are available over-the-counter for the general public, and may be labelled as "antibacterial". Antiseptic products which meet specified FDA testing criteria (Food and Drug Administration, 1994) may use the designation of healthcare personnel handwash or surgical hand scrub.

D.3.2 Regimen A. Regimen A will consist of:
• a short (10-15 sec) wash with a 2% CHG-containing detergent (Foam Care, Ballard, Draper, UT) when coming on duty;
• handwashing throughout the working shift for 10-15 sec with the same product;
• as needed use of CHG-compatible lotion (Prima-Kare Lotion, Steris, St. Louis, MO) during working hours.

D.3.3 Regimen B. Regimen B will consist of:
• a short (10-15 sec) wash with mild, non-antimicrobial liquid detergent soap (Kindest Kare Body Wash and Shampoo, Steris Corporation, St. Louis, MO) followed by a 10-15 sec application of a 60% isopropanol preparation containing emollients (Avagard Instant Antiseptic Hand Sanitizer, 3M HealthCare, St. Paul, MN) when coming on duty;
• use of the mild, non-antimicrobial soap throughout the working shift when hands are soiled (ratio of soap wash to alcohol application is usually about 1:6);
• a short application (10-15 secs) of the alcoholic preparation for deggering throughout the working shift;
• scheduled (at least two times/day and as needed) use of a non-oil based non-ionic moisturizing protectant lotion (DermaShield, Benchmark Medical, Salt Lake City, UT) throughout the working period. This product in a previous study was associated with a reduction in infection rates and improved skin condition, and has no detrimental effects on glove integrity (Malone & Larson, 1996; Larson, Anderson, Baxendale, & Bobo, 1993);

D.3.4 Gloves and lotion use. Changes in gloving practices or lotion use could be major confounding factors in this study if not controlled. Hence, we have taken several precautions to avoid this. First, both units will be free of latex gloves, and the same glove brands will be used at both sites (Delta Powderfree Vinyl Exam Gloves, Norwood, MA; Neolon neoprene gloves (sterile), Maxxim Medical Inc., Clearwater, FL; Triflex Sterile Vinyl Exam Gloves, Baxter Healthcare Corporation, Deerfield, IL). Latex-free gloves are selected because of the increasing prevalence
of latex hypersensitivity among staff and patients. All staff will be provided with the same lotion for use at work. Second, the protocols for use of lotion and for gloving are identical for both units during both regimens, and we have received a commitment from administrative personnel that no changes in these procedures or products will be made during the study. Third, records of frequency of gloving and lotion use will be kept by each study participant (and confirmed by direct observation, see D.5.3). Finally, glove use/month/unit will be measured by obtaining glove purchasing data from the central supplier to the units. These variables will be included in the multivariate analysis to identify and control for any residual confounding.

A related question that might be raised is that since gloves are used so frequently today, might this then negate the need for or importance of hand hygiene? Based on recent outbreaks in our own and other institutions which have been traced to the hand flora of nursing personnel, it seems clear that hand hygiene, even in this era of frequent gloving, still brings added protection (see Appendix 4 for examples). And because gloving is becoming increasingly common, allergic reactions and other untoward effects are also becoming more common. Hence the need to study not only the microbiologic effects of hand hygiene regimens, but also their longterm impact on skin health when used in conjunction with current gloving practices.

D.4. Measurement of Outcome Variables

D.4.1. Nosocomial infections. Nosocomial infections are the most important outcome variable in this trial and their valid and reliable measurement is crucial. Both in national data (Baltimore, 1998; Gaynes, 1996) and in our own units, three types of infection--bloodstream, pneumonia, and conjunctivitis--represent more than 75% of nosocomial infections in neonates and will be the only infections included in our surveillance. Surveillance will be prospective, with the surveillance officer visiting the study units at least three times/week. Sources of data will include laboratory, radiology, and pharmacy records, patient charts, information from physician and nursing staff, and direct observation of neonates. Definitions adapted for use in neonates from the NNIS system as described in Preliminary Studies will be used (See Appendix 3 for surveillance definitions, decision trees, and a list of variables to be collected on all neonates, including those with and without infection). Microbiologic culturing patterns in the two sites are similar; in 1998 an average of 1.2 and .95 blood and eye cultures/neonate were obtained at Columbia and Cornell sites respectively. With surveillance to include pneumonia and conjunctivitis as well as bloodstream infections, we anticipate about 260 neonatal bloodstream, pneumonia and eye infections during the study period.

The experienced surveillance officer will be specifically trained to do surveillance in both study units. In addition to routine laboratory and clinical surveillance, we will use Clinical Event Monitoring, a free service available at the Columbia campus, to set up computerized triggers/alerts to identify the neonates who need to be assessed for possible nosocomial infection. These will include, for example, changes on chest X-ray. These algorithms will be programmed on the hand-held computer being used by the surveillance officer and all numerator and denominator data collected on-line. Infections will be reported with appropriate denominators: bloodstream infections/1000 catheter days and per 1000 patient days, pneumonia/1000 ventilator days, nasal cannula continuous positive airway pressure (CPAP) days and/or patient days (as appropriate), and conjunctivitis/1000 patient days. Relevant risk factors will be prospectively recorded for all neonates as noted in Appendix 3.

The CDC NNIS staff have served in an active advisory capacity throughout the planning of these protocols and have agreed to continue to provide ongoing monitoring. Also, to monitor interrater reliability and to prevent potential rater bias, a subset of
records (25 randomly selected records of neonates judged by the surveillance officer to have an infection and 50 randomly selected records of neonates determined not to have a nosocomial infection) will be retrospectively reviewed quarterly by a member of the CDC NNIS staff or an experienced pediatric infectious disease specialist who is blinded to both the regimen being used and the surveillance officer's rating (see letter of support from Dr. Robert Gaynes, Director of the NNIS program).

D.4.2. Skin condition of nurses' hands. While there are physiologic techniques to assess skin condition, many are not practical or reliable in clinical settings. These techniques include measurement of transepidermal water loss (TEWL), electrical impedance, image analysis, and corneocyte shedding (Grove, 1982; Leyden, McGinley, Kaminer, Bakel, Nishijima, Grove & Grove, 1991; McGinley, Marples, & Plewig, 1969; Hassing, Nater, & Bleumink, 1982). Some tests require subjects to be in a resting metabolic state in a controlled environment for prolonged periods of time, so subjects would have to leave the unit and go to a testing site. To address these limitations, over the past decade the PI and other investigators at the Skin Study Center, University of Pennsylvania, have developed several non-invasive tools with high reliability and validity to measure skin condition and irritant contact dermatitis. For this study, two instruments which are well validated and practical in clinical settings will be used.

D.4.2.a. Visual Scoring of Skin Condition (VSS) scale. The VSS is a 6-point scale using stereomicroscopic examination of the hands at three times magnification, and correlates well with other physiologic measures of skin condition (Highley, Savoya, O'Neill, & Ward, 1976). The scores range from 6 (normal, no observable scale or irritation) to 0 (extensive cracking of skin surface, widespread reddening or occasional bleeding). In previous studies, including validation with dermatologist ratings (Larson, 1997; Larson, 1998), as well as in our pilot work, an interrater agreement of >95% within a score +/-1 was consistently obtained over a large spectrum of damaged and undamaged hands of various skin types.

D.4.2.b. Hand Skin Assessment Scale (HSAF). Previous studies have confirmed that self-reported symptoms combined with objective clinical measures of skin irritation such as the VSS described above provide valid and reliable measures of skin condition of hands that are reproducible and have a high level of sensitivity and specificity (Simion, Rhein, Morrison, Scala, Salko, Kligman, & Grove, 1995). The HSAF is a self-rating scale developed in the 1980s for subjects to assess the condition of their hands. Subjects give themselves a score from 1-7 in four dimensions: appearance, intactness, moisture content, and sensation. The possible range of scores is 4-28, with 28 indicating totally healthy skin. In previous studies, scores correlated significantly with other physiologic measures of skin damage (Larson, Leyden, McGinley, Grove & Talbot, 1986a; Larson, McGinley, Grove, Leyden, & Talbot, 1986b). These data have been recently revalidated in a series of studies conducted at by Dr. Gary Grove, The Skin Study Center, University of Pennsylvania (manuscript in preparation). Hence, we are using the best validated, state-of-the-science instrumentation to measure the condition of skin of hands. These two instruments used together (VSS and HSAF) provide both subjective and objective assessment of skin condition, minimizing bias and providing an ongoing assessment of reliability. For this study we have added an affective assessment to the HSAF to monitor subjects' responses to individual components of each regimen, since their reactions to components of the either regimen could influence their adherence.

D.4.3. Microbial flora of hands.

D.4.3.a. Culturing technique. To assess the effects of the hand regimens on colonizing rather than transient flora, cultures will be obtained on clean hands. Prior to sampling, subjects will cleanse the hands for 10 secs using the product assigned in the current regimen (either CHG- or alcohol-containing product). A modified glove-juice technique will be used for sampling. The subject inserts the dominant hand into a sterile polyethylene bag containing 50 ml of sampling solution
(.075M phosphate buffer, pH 7.9, containing 0.1% polysorbate 80, 0.1% sodium thiosulfate, and 0.3% lecithin). This solution neutralizes any residual antiseptic on the skin and facilitates removal of microorganisms by dispersing the macrocolonies into single cells, which can then be counted as colony-forming units (CFU). The entire hand is massaged by the data collector through the wall of the bag for 1 min. Cultures will be obtained by the graduate research assistant (GRA) and processed by the microbiology technologist specifically trained for this task (she also participated in the pilot study). In pilot tests we stored a subset of samples in the sampling solution for >15 hours, and found no significant change in bacterial recovery for more than 8 hours. Hence, all samples will be processed within 8 hours (usually much less).

D.4.3.b. Microbiologic isolation techniques. All microbiologic isolation, identification and quantification, antibiotic susceptibility screens and molecular analysis will be performed by the Clinical Microbiology Service of the Columbia campus. Undiluted and diluted (10-fold and 100-fold) aliquots of sampling solution will be inoculated onto 5% sheep blood agar (BBL, Becton Dickinson Microbiology Systems, Cockeysville, MD.) for total counts, MacConkey (BBL) (selective for gram negative rods), Colistin-Nalidixic Acid (CNA) with 5% sheep blood (BBL) (selective for gram positive bacteria), Sabouraud’s with chloramphenicol and gentamicin (Remel, Lexara, KS) (selective for fungi) and Bile Esculin (BBL) (selective for enterococci). The media will be incubated at 35°C in 5% CO₂ and observed daily for growth up to 48 hours for all media except Sabouraud’s, which is incubated at 30°C. The CFU will be enumerated and recorded. After subculture, screening plates will be used for the detection of MRSA (Mueller Hinton Agar with 4% NaCl and 6 mcg/ml oxacillin, Remel, Lexara, KS) and for VRE (CNA with 6 mcg/ml vancomycin, BBL). The identification systems for staphylococci and enterococci will be Staphaurex (Murex Biotech Limited, Dartford England) and MicroScan Pos Combo Panel Type 12 (Dade Behring Inc., West Sacramento, CA), respectively, and yeast by Yeast ID (API, Murex) and germ tube formation.

D.4.3.c. Molecular epidemiology. Molecular epidemiology will be used to provide more rigorous evidence supporting our hypothesis (i.e. the number of nosocomial infections in which pathogens detected on nurses' hands are clonally and epidemiologically linked to infected neonates will be reduced when an alcohol-degerming agent is used as compared with a detergent-based antiseptic), assess the frequency of transmission between staff and neonates, and to identify possible intervention strategies. All clinical bacterial and fungal isolates from infected infants and all isolates from hands of NICU nurses (~3,000/year) will be stored (-70°C) for future epidemiologic investigation. To determine whether there is a link between isolates causing neonatal infections and isolates from nurses' hands, appropriate strains will be fingerprinted by pulsed field gel electrophoresis technology (PFGE) using the GenePath system (BioRad, Hercules, CA). Pulsed field gel electrophoresis is a "gold standard" molecular typing method (Olive & Bean, 1999) in which bacterial or fungal strains are processed to isolate large fragments of chromosomal DNA which allows the "fingerprints" of these strains to be compared. Because the cost to fingerprint all isolates would be prohibitive and the results difficult to interpret, a specific protocol will be used to determine which nurse and neonatal isolates to process. For each clinical infection in a neonate, a review will be conducted by the surveillance officer and pediatric infectious disease physician or neonatologist member of the research team using case-control and other surveillance methods to determine which staff members are epidemiologically linked to the baby. Data used for this review will include nurse assignment sheets, laboratory and clinical records. Neonatal and nurse isolates will be fingerprinted when one or more nurses is epidemiologically linked to the infected neonate and if the nurse’s hand isolate is the same genus and species and has similar antimicrobial susceptibility patterns to the isolate causing the infection in the neonate. Most of the clinical isolates causing
neonatal nosocomial infections in the study units are associated with organisms potentially spread by direct contact (about 60% are staphylococci, 17% gram negative bacteria, 9% yeast). In a recent study of P. aeruginosa infections in one of the study units (abstract in Appendix 4), we found that each neonate was cared for by an average of 17 nurses, but only two in case/control analyses were found to be significantly associated with infection in the neonates. In order to link the clinical isolates to isolates from nurses’ hands, the molecular epidemiology will be conducted for two weeks before and after the bimonthly hand cultures obtained from nursing staff. We will also fingerprint isolates from other neonates who are temporally or geographically linked to examine the potential for baby-to-baby spread. Hence, we anticipate that about 260 patient isolates and 520 isolates from nurses’ hands (an average of two/neonatal infection) will meet the criteria for fingerprinting.

Our research team has extensive experience in these techniques, as noted in Preliminary Studies and in published papers/abstracts in Appendix 4. Although PFGE can identify related strains, a limitation is that no determination can be made without additional temporal or other causal evidence regarding whether transmission was from neonate to nurse, nurse to neonate, or both from a different common source. Nevertheless, this technique will provide prospective and concurrent (rather than retrospective) data on the frequency with which specific clones are shared between nurses and patients on the unit. Our epidemiologic investigations will provide additional causal evidence.

D.5. Procedures

D.5.1. Orientation and monitoring of subjects. At the onset of the study and prior to the changeover in regimen after the first year, meetings will be held for all staff, including physicians, nurses, respiratory therapists, and consulting services to discuss the purposes and general procedures for the study. These meetings will be conducted jointly by the co-investigators and the medical and nursing administrative directors of the units. During the week before the changeover of regimens, staff will be notified by poster, memo, staff meeting, and during patient rounds of the pending change. On the first day, all soap and lotion dispensers will be changed. A week-long “phase in” period will be allowed before infections or skin condition are attributed to the new regimen. Because adherence to the study regimens is so vital to this study, major, proactive efforts will be made to assure that everyone who touches infants is completely familiar with the assigned regimen. In our preliminary work, we determined that the vast majority of physical contact with the neonates is by nursing personnel (>70% of contacts). The permanent nursing staff have very low turnover (the average duration of employment is well over 10 years and only a few new staff are hired each year), and the nursing and medical leadership and staff on both units have committed to helping the research team assure that nobody touches a baby until they are familiar with the hand hygiene regimen in use (see Appendix 5 for letters of support from the nurse coordinators and medical directors of both units).

A member of the research team will visit study units daily to monitor compliance with regimens and respond to questions and concerns. The units will be inspected daily on day and night shifts, and any non-protocol products present will be removed. Any nurse subject who develops dermatologic problems during the study will be evaluated through the Occupational Health Service. If a participant is unable to tolerate the assigned regimen, an alternative product or practice will be provided, and the subject will be excluded from the skin condition and microbiology components of the study. Based on current experience, this event is unlikely. The following methods will be used to monitor compliance with the assigned hand care regimen:

- Measurement of the amount of soap, towels, alcohol rinse, and lotion used during both regimens;
- Daily direct observation by a member of the research team to include review of nurse diary recordings, environmental rounds to assure that only regimen products
are available, and one-on-one demonstration and instruction for new staff (primarily house officers) and visitors.

D.5.2. Training of data collectors and quality monitoring. Four research team members will be specially trained and will work at both sites: (a) The microbiology technician will be responsible for processing the nurses' hand cultures, for obtaining clinical isolates from infected neonates from clinical laboratories, for processing and cataloging all specimens to be saved for further molecular epidemiology, and for doing the PFGE under the direction of Dr. Della-Latta. This individual has worked with the research team on four previous studies of hand flora and is expert in the microbiologic protocols. (b) The project coordinator, with support from the GRA, will oversee the day-to-day operations and supervision of study staff, collect data on nurses' skin condition, monitor compliance with the prescribed regimens including use of the diary, obtain nurses' hand cultures, and serve as the day-to-day contact for staff. (c) The surveillance officer will conduct surveillance for infections as described in D.4.1. (d) The Systems Analyst will oversee the front end development of the relational databases (see Database Hierarchy in Appendix 3) and will supervise the downloading and interface of all data collection. All members of the research team who will be monitoring or collecting data will undergo inter-rater reliability testing and practice with all instruments at the beginning of the study and quarterly to assure consistency in techniques and inter-rater reliability agreement of 90% or greater. The research team will meet weekly throughout the course of the study to review data, address issues of concern, and keep on track. Quality monitoring protocols for microbiology include random sterility testing of equipment and supplies such as hand culture bags and sampling solution, spot checks of identification techniques by dual processing, and standard quality checks for PFGE. For infection surveillance, NNIS personnel and/or pediatric infectious disease experts will monitor protocols and definitions as described in D.4.1.

D.5.2a. Data and Safety Monitoring Board. A 3-member Data and Safety Monitoring Board will provide unbiased, concurrent review and advice regarding the progress of the study. The purpose of the Board is to assure that there are no untoward events or safety threats to neonates or staff and to review interval analyses to determine whether study endpoints indicate an early stop date due to increased nosocomial infections associated with one regimen. Members will include Robert Gaynes, MD, Director, NNIS, CDC; Mary Castle, PhD, Associate Professor, UCSF School of Nursing; and Richard V. Goering, PhD, Professor and Interim Chair, Dept of Medical Microbiology, Creighton University School of Medicine. Within the first 3 months of the onset of the study and during the second year, the Board members will visit both sites, meet with the research team, and review data. They will subsequently receive quarterly progress reports and data to review.

D.5.3. Control and monitoring of potential confounding factors. Potential confounding variables are anticipated in the study. First, there may be unit-specific differences in infection rates or other outcomes, so nosocomial infection rates and other outcomes will initially be stratified by unit in the analysis. Second, two neonate variables--birth weight and use of devices such as umbilical and central intravascular catheters--change the risk of nosocomial infection. Both will be recorded for all neonates and accounted for in the analysis. Four nurse variables--frequency and quality of handwashing, lotion use, and gloving--may affect both infection rates as well as skin condition and microbiology of the hands. A particular concern in this study would be if nurses during one of the two hand care regimens differentially changed the frequency of their handwashing, lotion or gloving practices. In fact, this problem occurred in a 1992 clinical trial of two handwashing products, making it impossible to determine whether the differences found were attributable to study protocol or frequency of washing (Doebelling, 1992). Therefore all nurse subjects in this study will use a diary card to record these practices during one 12-hour shift/month (sample in Appendix 3). In our pilot study, we tested the diary and reporting protocol and found
that nurses were able and willing to do this, and that their reporting was valid and reliable. The GRA will maintain a monthly calendar on which each nurse is scheduled to record diary data on an assigned day. The GRA will provide each nurse with the diary card, monitor compliance with recording throughout the scheduled day by making rounds to visit each nurse at least twice at random points throughout the shift, and collect the cards at the end of each working shift. If the nurse has not started completing the card or has forgotten, she/he will be asked to start recording on the next scheduled working shift rather than attempt to recall the hand hygiene practices retrospectively. This procedure worked successfully in our pilot study, and compliance with completing the cards was >95% every week. Frequency of handwashing, gloving, and lotion use will be measured as the number of times in each 12-hour shift that the nurses self-report these episodes. This will provide us with >3,600 person-days of data regarding frequency of hand hygiene, gloving, and lotion use. Finally, actual glove and lotion use will be measured by obtaining purchasing data from the central supplier. The quality of hand hygiene will be measured by direct observation as described in Section D.5.4 below.

D.5.4. Cost analysis. Voss and Widmer (1997) calculated, based on recommended standards, that traditional soap and water handwashing would consume 16 hours of time/shift for every 12 ICU personnel and would likely interfere with patient care or require additional staff. This compared with a requirement of 3 hours/shift if alcohol hand rinses were used. They make a compelling case for a significant cost differential between these two regimens. Since cost is one important consideration for making selections between products or regimens, it is an important variable in evidence-based practice. We postulate that the direct costs of the alcohol regimen will be less, but to confirm this, direct costs of both hygiene regimens will be compared by tracking the costs of supplies (soaps, alcohol, lotions, gloves, towels) and staff time (by direct observation) during each regimen. The mean hourly salary and fringe benefits for registered nurses working on each study unit will be used to calculate time costs (i.e. time spent in a hand hygiene episode [in secs] X salary and fringe benefits for that time period = staff cost/hand hygiene episode). Product costs and amounts (soap, alcohol, lotion, paper towels) used on study units will be obtained from the central suppliers of both units on a monthly basis.

Time required for hand hygiene will be estimated by direct observation, using an adapted tool which has undergone rigorous psychometric testing and found to be valid and reliable (Larson & Lusk, 1985; See Appendix 2). This tool will also provide a measure of the "quality" of the hand hygiene on a 5-point scale. The decision regarding which episodes to be observed will be made in the following manner. One randomly selected shift (selected by computer generated randomization) each month will be set aside for observations. During that shift, an investigator will observe hand hygiene events of subjects as they occur sequentially on the unit for at least 2 hours and until at least 10 episodes are recorded, even if that requires >2 hours. That will yield a minimum of 120 observed hand hygiene episodes during each crossover period. From these data, an average quality index will be computed for the two study periods.

D.6. Data collection intervals and time schedule

<table>
<thead>
<tr>
<th>Study Population: Variable</th>
<th>Instrument</th>
<th>Data Collector</th>
<th>Time Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nurse: skin condition</td>
<td>Visual Scoring of Skin Condition (VSS)</td>
<td>Project coordinator/ GRA</td>
<td>Monthly</td>
</tr>
<tr>
<td>Hand Skin</td>
<td>Subject self-report</td>
<td></td>
<td>Monthly</td>
</tr>
<tr>
<td>Assessment (HSAF)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>------------------</td>
<td>------------------</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nurse: skin microbiology</td>
<td>Microbiologic culture</td>
<td>Project coordinator/ GRA</td>
<td>Every 2 mths</td>
</tr>
<tr>
<td>Nurse: frequency of handwashing and gloving</td>
<td>Diary</td>
<td>Subject self-report, with spot checking by GRA</td>
<td>One shift/month</td>
</tr>
<tr>
<td>Neonate: nosocomial infection or not</td>
<td>Continuous surveillance of all neonates</td>
<td>Experienced surveillance officer</td>
<td>Member of CDC NNIS staff</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cost data</td>
<td>Measurement of supply costs: soap, towels, gloves, alcohol hand rinse, lotion</td>
<td>Calculation of staff time costs</td>
<td>Project coordinator/GRA</td>
</tr>
</tbody>
</table>

**Time schedule** for the study is:

<table>
<thead>
<tr>
<th>Time Period</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jan-Mar 99</td>
<td>Pilot study (completed)</td>
</tr>
<tr>
<td>Jan-Mar 2001</td>
<td>Recruitment, training and interrater reliability testing with research team</td>
</tr>
<tr>
<td>Apr 2001-May 02</td>
<td>Recruitment, orientation of NICU staff and beginning of randomized Interval #1*</td>
</tr>
<tr>
<td>Jun 2002-Jul 03</td>
<td>Regimen crossover to Interval #2</td>
</tr>
<tr>
<td>Aug-Oct 2003</td>
<td>Data cleaning and analysis</td>
</tr>
<tr>
<td>Nov-Dec 2003</td>
<td>Manuscript preparation, submission and implementation of dissemination plan</td>
</tr>
</tbody>
</table>

*The first regimen (A or B) will be randomly selected for one site and the alternate regimen used in the other site simultaneously.

**D.7. Threats to Validity and Limitations.** Major threats to validity and limitations in this study include potential for misclassification or dilution between the two study regimens; difficulties with attempting to change longstanding ritualized practices such as hand hygiene and concomitant lack of staff compliance; potential for investigator and/or nurse subject bias (for example, nurses may believe that one regimen is superior to the other and therefore change their hand hygiene practices to compensate, or the ratings of skin condition by members of the research team may be influenced by knowledge of the regimen being used); and the possibility that changes in outcomes such as infection rates or skin condition are influenced by changes over time (for example, new medical practice, changes in the physical environment, new physician staff) rather than the hand regimen. A further limitation is that only nursing, but not other staff, will be assessed in the skin condition and microbiology components of the study. We have attempted to minimize these potential problems, as summarized below:
<table>
<thead>
<tr>
<th>Potential Limitation/Bias/Validity Threat</th>
<th>Design Feature to Minimize</th>
</tr>
</thead>
<tbody>
<tr>
<td>Potential for misclassification, dilution or mixing between the two study regimens</td>
<td>Initial education plan; Assure that only assigned products are available for all staff; Prominent signage; Daily monitoring and direct observation; Ongoing orientation for staff.</td>
</tr>
<tr>
<td>Poor or inadequate adherence to assigned regimen.</td>
<td>Completed pilot study to confirm staff willingness to adhere to assigned regimens; Daily monitoring throughout data collection period; Strong administrative support for study.</td>
</tr>
<tr>
<td>Potential investigator or subject bias</td>
<td>Standardized, reliable and valid instruments and protocols; Careful training of all data collectors; Inter-rater reliability testing; Prospective infection surveillance using standardized national definitions and procedures and conducted by expert; Ongoing consultation and inter-rater reliability testing by CDC staff blinded to regimen and surveillance officer rating.</td>
</tr>
<tr>
<td>Confounding due to frequency or quality of handwashing</td>
<td>Recording of handwashing frequency and quality with control for differences in analysis; Measurement of amount of products used.</td>
</tr>
<tr>
<td>Changes in outcomes associated with the passage of time rather than hand care regimen</td>
<td>Both regimens used in each site and season to account for seasonal changes; Trend analysis of national infection rates in NICUs over the same time period; Recording of any other changes over time (staffing ratios, invasive devices and new therapies); Analytic strategy to control/account for potential confounding factors related to time trends.</td>
</tr>
<tr>
<td>Lack of generalizability</td>
<td>Use of more than one study site and standard definitions of infections Study sites comparable to NICUs nationally.</td>
</tr>
<tr>
<td>&quot;Contamination&quot; (bias) of subjects involved in pilot study (16 nurses at Columbia site)</td>
<td>Commitment from nursing leaders and staff from both study sites that both regimens will be followed; Ongoing monitoring of regimen compliance.</td>
</tr>
</tbody>
</table>
### Confounding due to changes in gloving or lotion use practices

| **Same glove and lotion brands and protocols used at both study sites;**
| **No change in brands or protocols during entire study;**
| **Recording of use frequency by all subjects;**
| **Monthly records of glove and lotion use obtained from central suppliers;**
| **Inclusion of gloving and lotion practices in multivariate analysis as possible confounders**

### D.8 Data analysis.

The biostatistician member of the research team has been actively involved in planning the analytic strategy since before the pilot study. In collaboration with the Systems Analyst, data will be downloaded from the several databases (Appendix 3), and analyzed using SAS. The principle aims of this study are to estimate and test differences in neonatal infection rates and nurse skin condition and microbiology associated with two hand hygiene regimens. The study design, selected because it addressed a series of logistical concerns previously discussed, allows each nurse to be assigned to each regimen and therefore act as her/his own control. The correlated structure of these repeated measures for each nurse are analyzed by repeated measures analysis of variance methods, and by logistic regression with parameter estimation via generalized estimating equations (GEE). In each of these models the average differences between regimens, and not the correlation structure, is of primary interest.

### D.8.1 Aim 1: To compare the impact of two hand hygiene regimens used by NICU staff on nosocomial infection rates, particularly endemic rates, among neonates.

Logistic regression analysis will be used to estimate differences in risk of nosocomial infection during the use of the two hand hygiene regimens. The presence or absence of a nosocomial infection in each treated neonate is the dependent variable in the regression. The independent variables include an indicator of hygiene regimen; potential confounding variables characterizing the neonate: birthweight, device utilization (e.g. ventilators, umbilical and central line catheters), and length of stay prior to infection; potential confounding variables characterizing nurse behavior: frequency and quality of hand washing, glove and lotion use (the respective average self-reported frequency in each period); and study unit and time period (first or second year). Potential interactions of regimen with site and time period will be explored.

The logistic model is of the form:

\[
y (\text{presence or absence of nosocomial infection}) = \text{site} + \text{regimen} + \text{time period} + \text{confounders}.\]

In the absence of any regimen interaction (e.g. if one regimen worked differently by site), the exponentiated regression coefficient indicating the regimen will represent the adjusted (for the confounders and for site and period) increase in risk of nosocomial infection among neonates for one hygiene regimen compared to the other. In the absence of any regimen interaction, the null hypothesis of no difference in hygiene regimens will be tested by a normal approximation test based on the ratio of the estimated regression coefficient to its standard error. Likewise, a 95% confidence interval estimate of the difference in the adjusted risk of nosocomial infection between treatments will be computed by exponentiating the bounds computed by adding and subtracting 1.96 standard errors to the estimated regression coefficient of the skin hygiene regimen indicator. If significant interactions are present, we will describe the effect of regimen (for example, using a 95% confidence interval) for each level of the interacting factor.

We will also examine the relationship between the skin condition of nurses' hands and the nosocomial infection rates of neonates. Data on skin condition will be collected via two scales, the VSS and HAS, monthly for a total of 24 times. The average of the each of these scales over all nurses will be used as independent variables in a
simple linear regression model along with site predicting the neonatal infection rate observed in the preceding month (i.e., we will fit a model of the form: Infection rate = skin condition + study unit + error). The number of episodes in which organisms on nurses’ hands are clonally and epidemiologically related to a neonatal nosocomial infection will also be compared between the two regimens.

D.8.2 Aim 2: To compare the impact of two hand hygiene regimens on the skin condition of nurses’ hands. Each nurse will provide 24 longitudinal observations (one a month for two years) on each of two scales (VSS and HSAF) measuring skin condition. We will treat each of these scales as continuous variables and account for the correlation of observations within nurses by analyzing each outcome separately with repeated measures analysis of variance (RMANOVA) methods. Each of the two RMANOVA models (one for each scale) will include an indicator of hygiene regimen and time period as within subject factors, and site as a between subjects factor. The principle interest is in the regimen effect and with the potential interaction of regimen with site or time period. An additional analysis of skin condition will be performed based on categorizations of nurses’ hands as “damaged” or not according to criteria tested by Larson (1997). At each monthly assessment, hands will be classified as “damaged” if the VSS score is four or less (indicating an observer rating of clinical scaling, roughness, dryness) and the HSAF score is sixteen or less (indicating a self-report of skin redness, dryness, roughness, and/or discomfort). Otherwise, hands will be categorized as undamaged. We will use logistic regression analysis, with a generalized estimating equation (GEE) approach to parameter estimation. The GEE approach takes account of the correlated structure of the data (i.e. the fact that each nurse will produce 24 observations) and yields correct estimates of standard error for the parameter estimates.

D.8.3 Aim 3: To compare the impact of two hand hygiene regimens on skin microbiology (numbers and types of organisms). Counts of CFU from each of the six periodic assessments per regimen will be log transformed and analyzed by repeated measures analysis of variance analogously as described for each of the skin condition scales (VSS and HSAF) to test for a difference in CFU by regimen. A similar repeated measures analysis of variance of regimen differences will be conducted for the number of antibiotic-resistant strains present.

D.8.3 Aim 4: To compare direct costs of the two hand hygiene regimens. Direct costs (supplies and salary and fringe benefit costs for time required for hand hygiene during both regimens) will be compared using the Wilcoxon Signed Rank Test. Supply and time costs will also be compared per 100 handwashes between regimens, using modelling developed and described by Voss & Widmer (1997).

D.8.4 Alternative hypotheses. We considered the implications for clinical practice if our primary research hypotheses are not supported, i.e. if there is no effect of hand hygiene regimen on infection rates and/or skin health. Possibilities include: (1) No effect on skin condition but reduced infections and same or less cost: change practice; (2) No effect on infections but improved skin condition and same or less cost: change practice; (3) No effect of either regimen on any dependent variables. Assuming that we will demonstrate rigorous implementation of protocols and control of confounding and bias, we can conclude that the elements of the tested regimens had little effect. Reasons for this could include: (1) host factors are overwhelmingly important in high risk neonates and hand hygiene plays little role; (2) other environmental factors (e.g. antimicrobial prescribing patterns, intensity of device use, crowding, etc.) are more important than hand hygiene as predictors of risk for infection; (3) other elements of hand hygiene not manipulated (frequency or duration of washing or glove use patterns) are more important than the regimens studied; or (4) the relevant healthcare providers were not tested in this project. The data we collect will allow us to
examine each of these alternative hypotheses and to make relevant recommendations for next steps in clinical practice and research.

**Human Subjects**
This study has been approved by the IRBs of both the Columbia and the Cornell sites.

1. Subject population. All staff and visitors in the study NICU will use the assigned hand hygiene regimen when in the study units. The nurses (~153) are all registered nurses, primarily female (96%), about 40% non-Caucasian (African-American, Asian, Latino). The inclusion of high risk neonates is justified because of their high rate of nosocomial infections and because both hand hygiene regimens being tested have been shown to be effective and safe and are standard practice.

2. Sources of materials being collected:

<table>
<thead>
<tr>
<th>From Nurses</th>
<th>Specifically For Research?</th>
<th>From Neonates</th>
<th>Specifically For Research?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Visual examination of skin of hands</td>
<td>Yes</td>
<td>Nosocomial infection rates</td>
<td>No</td>
</tr>
<tr>
<td>Recording of frequency of handwashing and gloving</td>
<td>Yes</td>
<td>Birth weight, length of stay</td>
<td>No</td>
</tr>
<tr>
<td>Self-assessment of skin condition</td>
<td>Yes</td>
<td>Device utilization days</td>
<td>Yes</td>
</tr>
<tr>
<td>Microbiologic culture of hands</td>
<td>Yes</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

3. Plans for recruitment and consent procedures. The medical and nursing administrators of the NICU have all been actively involved in planning this study, and are supportive of the interventions. Hence, this is a unit-wide endeavor. Because all staff who work in or enter the NICU must follow handwashing regimens set by unit policy and use the products provided, individual written informed consent is unnecessary and inappropriate for the hand hygiene component of the study. On the other hand, the permanent nursing staff in the unit will be asked to provide information specifically for this study (assessments of skin condition, recording of hand hygiene practices and cultures of the hands). Therefore, explicit written consent will be obtained from each member of the permanent nursing staff. Nurses who elect not to participate will use the assigned hand care regimen, but will not participate in the skin condition and microbiologic data collection. No consent will be obtained from parents of the babies.

4. Potential risks and seriousness. The risks to nurse subjects are a small additional commitment of time at work, possible concerns regarding confidentiality of personal data, and the need to change potentially longstanding personal handwashing habits. It is also possible that staff may be intolerant of or find unacceptable one or more of the products used in the assigned regimens. There are no added risks to the neonates from this study beyond that associated with routine care in the unit.

5. Procedures to protect against or minimize potential risks. The additional time required by nurse subjects in this study will be kept to a minimum, and all data collection will take place on the unit at the convenience of the nurses. The monthly assessments of skin condition take about 2 min. The bimonthly skin culture takes about 2 min (including the preparatory wash). In our preliminary studies, nurses estimated that recording the frequency of their gloving and washing required an additional 5 min/working shift. Even including orientation time and checks with the research team, we estimate an additional time commitment of less than 10 min/month/nurse. With regard to confidentiality, staff will be given confidential code
numbers. Only the PI will be privy to the master code sheet, which will be stored in a locked cabinet in her office. Only code numbers will appear on microbiology samples. Nurses who find any product unacceptable during the study, or who develop dermatologic symptoms, will be assessed by occupational health staff. If indicated, they will receive alternative products for their own use. Although it will not be possible to separate these individuals from the regimen assignment with regard to nosocomial infections, they will not participate in the skin health and microbiology assessments. In our meetings with the subjects all of these precautions will be discussed.

6. Risk/benefit ratio. We feel that the minor inconvenience and risks, which are primarily to nurse subjects, are fully justified in order to determine which (if any) hand hygiene regimen is more effective in controlling nosocomial infections and more beneficial to skin health. The high prevalence of irritant contact dermatitis in critical care nurses indicates that they are at considerable occupational risk for dermatologic problems.

**Vertebrate Animals.** Not applicable

**Literature Cited.**


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**Consortium/Contractual Agreements.** Although the two hospital entities, Columbia and Cornell, have merged to form The New York Presbyterian Hospital, the academic entities are still separate. For that reason, a subcontract is necessary to establish the collaborative arrangement for the two-site study and to articulate the responsibilities of Dr. Mirjana Nesin who serves as the primary liaison and coordinator of the Cornell NICU site. The subcontract pays for 10% of Dr. Nesin’s salary and fringe benefits. See following “Statement of Intent to Establish a Consortium Agreement”.

**Consultants.** See letters of support and agreement to consult from members of the Data and Study Monitoring Board, Drs. Gaynes, Castle, and Goering.