THE RISKS OF METHEMOGLOBINEMIA WITH THE USE OF TWO ANTIMICROBIALS IN LOW BIRTH WEIGHT INFANTS

By

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The purpose of this study was to determine the degree of risk of methemoglobinemia and extent of skin breakdown when 0.5% chlorhexidine was used as a topical antimicrobial in a population of low birth weight (LBW) infants. Differences in the measures of methemoglobin (MetHb) levels and observed appearance of the skin for breakdown were sought. As the first investigation of the risks of the use of 0.5% chlorhexidine in LBW infants, the study employed an experimental design of repeated measures. The use of chlorhexidine 0.5% was compared with the conventional practice of using 70% isopropyl alcohol to test the hypothesis that MetHb levels are significantly higher in LBW infants treated with chlorhexidine than those treated with isopropyl alcohol. Daily skin assessments using the Scale for Integrity of Neonatal Skin (SINS) were collected to test the hypothesis that the skin integrity scores of LBW infants treated with chorhexidine are significantly higher than those treated with isopropyl alcohol. Surface electrical capacitance (SEC) measurements (which
positively correlate with transepidermal water loss) were taken to test the hypothesis that SEC is correlated with MetHb levels.

Data were collected on LBW infants who were less than 29 days old, with birth weight between 750 and 1500 grams. Data collection began at no later than 7 days of age and were collected daily throughout the neonatal period until the subjects were 28 days old. There was a statistically significant difference in means of MetHb levels between those infants treated with alcohol and those treated with chlorhexidine (F= 4.41, p =0.0378). Subjects treated with chlorhexidine had statistically more skin breakdown than those treated with isopropyl alcohol (F = 4.38, p = 0.0375). There was no correlation between the methemoglobin levels and surface electrical capacitance (r = -.0206, p = 0.4088). Although the methemoglobin levels were statistically higher in the treatment group, they were within normal limits. The researcher advises cautious use of 0.5% chlorhexidine as a topical antimicrobial in a population of low birth weight infants greater than 750 grams, and only with diligent attention to its removal after 30 seconds.
CHAPTER ONE
INTRODUCTION

Statement of the Problem

Pathogens systemically invade low birth weight (LBW) infants through many portals of entry clinicians create to sustain the delicate balance of life. Direct portals are made by endotracheal tubes and chest tubes to sustain ventilation, and umbilical and peripheral central lines to sustain fluid balance and nutrition. Application and removal of adhesives used to secure these tools of technology often contribute to barrier disruption with multiple breaks in the skin. The skin of the full term neonate is more robust and less prone to infection because the stratum corneum and dermis are much thicker than in the preterm infant. The skin is virtually sterile at birth, but colonization occurs rapidly. Within six weeks, the skin of a full term neonate has microbial flora levels comparable to that of an adult (Young, 1995).

The immaturity of the LBW infant skin is a significant risk factor for nosocomial infection because the dermal-epidermal junction, although structurally complete, is not strong (Smith, Sakai, Burgeson, & Holbrook, 1988). Further, an incompetent immune system exists because gestational age is
inversely proportional to immunological maturity. Preterm infants are born with inadequate immunoglobulins, which usually are transferred from the mother during the last trimester. Routine nursing care may also increase risk of nosocomial infection in the LBW infant, such as frequent blood sampling and removal of adhesives used to secure endotracheal tubes, intravenous catheters, and monitoring equipment such as pulse oximetry.

Immature LBW infant skin is also readily penetrated by applied topically drugs (Barrett & Rutter, 1994). Agents pass through the skin by passive diffusion along a concentration gradient (Rutter, 1987). Clinicians commonly use topical antimicrobials to reduce the risk of nosocomial infection in the immune-deficient infant, but this practice predisposes the LBW infant to risks associated with absorption of topically applied antimicrobials. All topically applied agents expose the LBW infant to some degree of risk, but those associated with chlorhexidine are not known to date.

**Background of the Problem**

Infants born with a birth weight less than 2500 grams are 40 times more likely to die in the first month of life (CityMatCH, 1997), and are more likely to be developmentally or physically handicapped than infants born at term (Paneth, 1995). Low birth weight is a major public health problem not only because it imposes societal burden, but also because low birth weight is an important
predictor of preterm infant mortality. Infant mortality rates decrease when either
the percentage of LBW decreases, or the birth weight-specific mortality rates
decrease (Guyer et al. 1997). The most common risk factor of LBW is preterm
delivery, which is defined as birth prior to 37 weeks of completed gestation.
Other risk factors associated with LBW are listed in Appendix A.

Low birth weight is a global problem, but the United States (US) ranks
higher than many developed nations. The US counts all live births regardless of
the gestational age, which may explain the difference. Many non-viable LBW
infants are counted as live births in the US. Further, there has been a surge of
births occurring in multiples of three, four, five and even eight, pushing the limits
of viability every day. Even compared against itself, however, the percentage of
LBW in the US continues to rise. The percentage of LBW in the United States
was 7.4% in 1996, its highest level since 1975 (Guyer et al. 1997). In 1998, the
rate of LBW in the United States rose again to 7.6%, and is influenced in part by
the increase in multiple births (National Center for Health Statistics, 2000).
There are no simple solutions to the problem of LBW. A significant difference in
mortality rates between African-American and white populations persists
(National Center for Health Statistics, 1995). A significant cause of infant
mortality in developed nations is preterm delivery (Paneth, 1995). Preterm
delivery may be a result of placental insufficiency, maternal hypertension, and
poor maternal nutrition, but also inadequate prenatal care, maternal illness such as diabetes or infection, and trauma (National Center for Health Statistics, 1995). Technical medical advancements have had little impact in infant mortality rates in the last ten years in the United States (Paneth, 1995).

Infants in newborn nurseries have one of the lowest rates of infection, while infants in NICUs have one of the highest rates (Parvez & Jarvis, 1999). Low birth weight infants are exposed to many infectious pathogens during their hospitalization. Prematurity is a significant risk factor for nosocomial infection in the newborn intensive care unit (NICU) because gestational age is directly related to immunological maturity. Premature infants born at gestational ages of 34 weeks or less have immature immunological systems, and as the limits of viability are approached protection is practically non-existent. The immunological system of full term infants is supplemented by transference of immunoglobulins during the last trimester from the mother. Infants less than 34 weeks gestation are born without full maternal protection, in addition to the immature immunological response to pathogens.

Low birth weight infants are highly susceptible to infection. Their immature immune systems are unable to localize infection. Sepsis is a generalized bacterial infection in the bloodstream that LBW infants are highly susceptible to because they have diminished inflammatory and humoral
immunity. Impaired phagocytosis, delayed chemotactic response, minimum or absent IgA and IgM, and decreased complement levels are characteristic of the LBW infants' immunologic immaturity. The local response to infection that occurs in a portal of entry in an older infant results in symptoms such as redness, swelling, and inflammation. Low birth weight infants are unable to mount a specific response to infection, and may only have vague, nonspecific symptoms such as temperature instability or irritability. Factors that further increase the risk of infection in the LBW infant include bottle feeding (pathogens introduced from environmental contamination) and use of steroids in the treatment of lung disease (Wong, Hockenberry-Eaton, Winkelstein, Wilson, D., Ahmann, & DiVito-Thomas, 1999). Sepsis that occurs in the LBW infant 1 to 3 weeks after birth often represents a nosocomial infection, and the organisms involved include staphylococci, streptococcus, klebsiella, enterococci, and pseudomonas. Bacterial invasion occurs through the umbilical stump (in close proximity to the perineum), skin, mucous membranes of the eyes, nose, pharynx, and ear, and internal systems such as the respiratory, nervous, urinary, and gastrointestinal systems. Infection may be acquired by cross-contamination from other infants, nursery personnel, or objects in the environment.

Topical application of antimicrobial solution to reduce bacteria on the skin is a common aseptic practice in all areas of health care. It is desirable to reduce
bacteria around the insertion site of an invasive procedure to minimize the possibility of infecting the blood stream, soft tissue, or bone with normal skin flora. The antimicrobials available in the NICU are 70% isopropyl alcohol, povidone-iodine, and chlorhexidine. Of the three, the most effective is chlorhexidine (Garland et al. 1995) which has been widely used throughout the world since 1954. It has been extensively studied for its antimicrobial and toxicological properties (Smythe & Ross, 1961; Rountree, Loewenthal, Tedder, & Gye, 1962; Hugo & Longworth, 1964). Unfortunately, alcohols are known to enhance percutaneous absorption when used as a vehicle. McCormack and colleagues (1982) first demonstrated that the preterm infant’s skin had a permeability 3 to 50 times that of the term infant.

Chlorhexidine is a topical antiseptic which is an effective agent against Gram-positive and Gram negative bacteria, and some yeasts (Maki, Ringer, & Alvarado, 1991), properties which render it useful in the NICU (Siegfried, 1999). Chlorhexidine absorption in the preterm infant, however, may outweigh the benefits. Premature infants have a transient deficiency of soluble cytochrome-β₅ and cytochrome-β₅ reductase, which are responsible for the enzymatic activity that reduces hemoglobin oxidized from a variety of endogenous and exogenous agents (Oski & Naiman, 1982; Roberts, 1997). An early study documented percutaneous absorption in preterm infants exposed to chlorhexidine in daily...
baths (Cowen, Ellis, & McAinsh, 1979). A small quantity of 4% chlorhexidine solution was directly applied to the infants’ head and body and then rinsed with water. High levels of chlorhexidine were found and attributed to sample contamination by chlorhexidine left on the skin. Hjelt and colleagues (1995) investigated an outbreak of methemoglobinemia that occurred in their newborn intensive care unit. They discovered that chlorhexidine was used inadvertently as a disinfectant for incubators, and surmised that the preterm infants most likely experienced prolonged topical exposure from aerosolized droplets on their skin. The possibility also exists that the aerosolized droplets of chlorhexidine were inhaled. All infants fully recovered after treatment and removal from the exposure. This is the only study to date that directly links methemoglobinemia with chlorhexidine.

Chlorhexidine has also been tested as an antiseptic for cord care (Belfrage, Enocksson, Kalin & Marland, 1985; Smales, 1988; & Verber & Pagan, 1993). Detectable serum levels were documented in preterm infants whose umbilical cords were treated with 1% chlorhexidine in an unspecified concentration of alcohol every four hours for 5 to 9 days (Siegfried, 1999). A similar study tested absorption of chlorhexidine in a 3% zinc oxide powder, and reported no significant absorption in a group of preterm infants (Aggett, Cooper, Ellis, & McAinsh, 1981).
Methemoglobinemia is the condition when more than 1% of the total hemoglobin is methemoglobin (Tse et al. 1995), although other definitions include 5% (Law et al. 1996) and 7% methemoglobin (Davidson et al. 1998). Transformation of the heme portion of the red blood cell reduces the oxygen carrying capacity of the red blood cell. High concentrations of methemoglobin (MetHb) cause low oxygenation states due to erythrocytes delivering less oxygen and a leftward shift of the hemoglobin dissociation curve. Organs with high oxygen demands, such as the central nervous and cardiovascular systems, are usually the first systems to manifest toxicity. Methemoglobin is unable to bind and transport oxygen, and causes cyanosis. Hemoglobin that is oxidized is unable to release oxygen molecules required by tissue for energy. Although the blood may be fully saturated (100%) with oxygen, the tissue will be hypoxic and the infant cyanotic. Methemoglobinemia may occur congenitally, but more commonly occurs from absorption or inhalation of an oxidizing substance (Appendix B). Disinfectants and aniline marker dyes (methylene blue) used in the nursery cause methemoglobinemia (Weinberg, 1931; Neuland, 1921), as well as prilocaine (Taddio et al. 1997), benzocaine (Mandel, 1989) and nitric oxide (Heal & Spencer, 1995). Methemoglobinemia has not been specifically associated with Vitamin E, an anti-oxidant, which is a common additive to total parenteral nutrition (TPN). Chaney and colleagues (1981) investigated potential
changes in Vitamin E levels in human subjects exposed to nitrogen dioxide, but found no significant changes. Similarly, Chow (1992) studied the effects of oxidative damage to the red cells of vitamin E-deficient rats. He reported levels of glutathione and catalase activity decreased faster in the vitamin E-deficient cells, and that the vitamin E-supplemented cells were resistant to oxidative damage.

When methemoglobinemia is detected early, the prognosis is favorable for full recovery. The prognosis of infants who develop severe cases of methemoglobinemia is predicted on the degree of anoxic end organ damage. Serum levels greater than 50% are usually lethal in infants (Roberts, 1997). Newborns, especially those in intensive care, are unusually susceptible to methemoglobinemia. Additionally, premature infants have a transient deficiency of soluble cytochrome-\textit{b}_5 and cytochrome-\textit{b}_5 reductase, which are responsible for the enzymatic activity that reduces hemoglobin oxidized from a variety of endogenous and exogenous gents (Oski & Naiman, 1982; Roberts, 1997). Preterm infants in intensive care units tend to have greater exposure to oxidants that outpace their ability to keep up with the rate of methemoglobin production. Infant levels are reported to be between 50 and 60% that of adult levels (Tse et al. 1995). Normal concentrations of methemoglobin in preterm and term infants were first established by Kravitz and colleagues (1956). They compared
venous and capillary samples, and reported no statistical differences between them. The highest values were in preterm infants up to 7 days old.

**Conceptual Framework**

The two proposed conceptual frameworks for this study were the pertinent aspects of Levine’s (1967) conservation principles, and Bradin & Sidanis’ (1998) theory in effectiveness research. Levine’s grand theory had great potential for empirical testing in the population of low birth weight infants, because it described a framework for nursing activities that guides care of the preterm infant. Bradin and Sidanis (1998) provide a theory-driven approach in the evaluation of nursing interventions to optimize opportunity for causal explanation, which nursing needs to build the knowledge base for clinical practice.

**Conservation Principles**

Levine (1967) defines conservation as to keep together. The major goal of conservation is health, with the focus on the integrity of “oneness” of the individual. Nursing interventions must keep together the individual resources that each person brings to their situation. Nursing interventions based on the four conservation principles were guided by scientific knowledge and served to support adaptation of the patient to the situation of illness. When nursing intervention influences adaptation favorably, the nurse acted therapeutically;
when the response was unfavorable, the nurse added supportive care. All nursing interventions were provided within the internal and external patient environment. The internal environment pertained to the physiological and pathophysiological aspects of the patient, while the external environment included perceptual, operational, and conceptual levels. The perceptual environment included aspects of the world that we engaged in with our senses. The operational level contained things that affected us physically, such as microorganisms. The conceptual level emerged from a spiritual existence, and was mediated by the symbols of language, thought, and history.

The first principle, conservation of energy, guided nurses towards atraumatic care. All nursing interventions should be scaled to make the least additional demands possible on patients, since patient energy is challenged by processes such as illness and healing. The second principle, conservation of structural integrity, refers to nursing interventions that limited the amount of tissue involved in disease by early recognition of structural and functional changes. These nursing interventions were guided by scientific knowledge and skills to identify potential problems and begin early intervention. The third principle, conservation of personal integrity, refers to nursing interventions that were guided by respect and value of the patient that enabled the patient to resume private, independent life. Finally, the fourth principle of conservation of
social integrity refers to nursing interventions that supported patient social and personal relationships with other persons.

Levine's grand theory with the principles of energy and structural conservation provided the theoretical framework for this study, because of the four principles, these had the greatest potential for empirical testing in the population of low birth weight infants. Low birth weight infants were fragile, easily stressed, and quickly entered a catabolic state as their energy requirements exceeded that which can be provided. Every organ system was challenged by the nature of their prematurity, and physiologic adaptation was dependent upon astute clinical observations and interventions. Their fragility was demonstrated by structural and functional changes such as intraventricular hemorrhage due to swings in blood pressure, pneumothorax due to small changes in mechanical ventilation, and skin loss due to application and removal of adhesive tapes used to secure the tools of technology. Stress that was demonstrated by behavior or vital signs consumes energy reserves that were critical for growth and development. Virtually every intervention in the newborn intensive care unit was stressful to the LBW infant, and had the potential to rob the infant of the energy necessary for survival. Indeed, the LBW infant was at great risk for structural and functional changes, therefore conservation of energy and structural integrity were essential to survival.
Bradin and Sidanis's Theory in Effectiveness Research

The second organizing framework was one formulated by Bradin and Sidanis (1998) and focused on evaluation of the two interventions (chlorhexidine and isopropyl alcohol) compared in this study. The purpose of theory in effectiveness research was to organize, describe, predict, explain, and aid in the understanding and controlling of an intervention (Bradin & Sidanis, 1998). The theory is a group or series of statements that provide a systematic view about phenomena that occur in life situations. Since effectiveness research is concerned with understanding why and how an intervention works, the theory explained the processes that mediated the causal relationship between the intervention and the desired outcomes. The six elements of this theory helped to guide the design of the study interventions.

The first element was that the problem definition must specify which condition is treatable, in which populations, and under what circumstances. The problem may be real or potential, with varying levels of severity among populations or conditions. Most importantly, the intervention must be aimed, or have a direct connection with the problem. The problem being investigated in this study was the risk of topical absorption of a dilute (0.5%) concentration of chlorhexidine in LBW infants, when left on the skin for a timed period before being wiped off. Using a dilute concentration and wiping it off the skin after a specific time period was designed to minimize opportunities for absorption.
The second element that must be considered was the critical inputs, which delineated the practical and prescriptive aspects of the intervention. All the specific components of the intervention were defined, such as the strength or dose, mode of delivery, frequency, and duration. The critical input to the chlorhexidine intervention was the vigorous back and forth scrub (mode of delivery), for five times (dose), using 0.5% dilution (strength), and removal after 30 seconds (duration).

Mediating processes, the third critical element of intervention theory, refers to the intervening variables to which the process is linked. In this study, the number of daily chlorhexidine applications on each infant depended upon the infants' need for procedures. Some preterm infants required as many as 20 applications of chlorhexidine daily. Maintaining intravenous access often required 5 to 10 intravenous attempts daily, and every 2 to 3 hour blood glucose monitoring by heelstick was not unusual. The youngest preterm infants required the most number of invasive procedures, usually within the first 28 days of life. As they matured, they relied less on intravenous feedings, and required fewer invasive procedures.

The fourth element was the expected outcomes which included the expected timing or pattern of changes and interrelationships among the outcomes. In this study the expected outcome of using 0.5% chlorhexidine as
the antimicrobial with preterm infants was that the risks of methemoglobinemia
and skin breakdown would increase as gestational and postnatal ages decrease
and transepidermal water losses (TEWL) increase. A second possible expected
outcome was that the level of methemoglobin would be higher in infants with the
most frequent chlorhexidine applications.

The fifth element is the extraneous factors which include contextual or
environmental factors and client characteristics that significantly affected
treatment processes and intervention outcomes. They may have influenced the
outcomes directly or moderate the intervention effects. Extraneous factors
considered in this study included staff adherence to the antimicrobial scrubbing
protocol and potential addition of humidity to the microenvironment. Staff in the
NICU attended an inservice program explaining the study protocol, and were
regularly observed during the study period for adherence to the protocol.

Preterm infants whose mothers were treated with antenatal corticosteroids were
expected to present with skin that has matured beyond the level expected of
their gestational age (Kalia, Nonato, Lund, & Guy, 1998). If this occurred, there
might have been protection against topical absorption. Information about
prenatal corticosteroid therapy was collected from a maternal history. Data from
these infants was analyzed separately to detect differences in outcomes
between those exposed to antenatal steroids and those who were not.
The final element is the implementation issues which refer to resources or setting of the study required for implementing the intervention. The resources included the setting, equipment, and personal and professional characteristics of the person providing the intervention. The clinical setting of this study was noteworthy and assured the investigator of an adequate number of potential subjects in the LBW infant population. More than 150 LBW infants were admitted during the previous year, and it was estimated that 10 would be available during the months of data collection. The required equipment was a point of care (POC) device called a Dermal Phase Meter 9003®, commonly labeled “NOVAmeter”, (NOVA Technology Corporation, Portsmouth, NH) to measure surface electrical capacitance (SEC), a DELL™ laptop computer to intake data from the DPM 9003®, and a POC device called an AVOXimeter 4000® (Diametrix Corporation) to measure methemoglobin levels at the bedside.

Purpose of the Study

The purpose of this study was to determine the risks of methemoglobinemia and extent of skin breakdown when 0.5% chlorhexidine was used as a topical antimicrobial in a selected population of LBW infants. Isopropyl alcohol 70%, the conventional antimicrobial agent, served as the antimicrobial for the control group. Differences in the measures of methemoglobin levels and observed appearance of skin breakdown was sought.
between groups. Low risk elevates 0.5% chlorhexidine as the preferred choice of antimicrobial in the NICU.

The risk of methemoglobinemia and skin breakdown may be higher in those LBW infants with high levels of desorption (as measured by surface electrical capacitance) and extremely immature skin barriers. The aim was to make a determination based on the scientific evidence of the circumstances when 0.5% chlorhexidine may be safely used to disinfect the skin of LBW infants. The variability of the levels of methemoglobinemia risk was correlated to gestational age, number of applications, and surface electrical capacitance.

**Definition of Terms**

Preterm Infants: infants born before 37 weeks gestational age.

Low Birth Weight Infants: birth weight between 750 and 1500 grams.

Independent variables:

0.5% chlorhexidine gluconate as the treatment intervention.

Isopropyl alcohol as the control intervention.

Dependent variables:

Methemoglobinemia Level: A POC test of blood to measure the level of MetHb present in the LBW infant.

Gestational Age: Gestational age recorded by the physician in the maternal history or reported by Dubowitz examination (23 to 34
weeks) performed by the admitting physician or nurse practitioner.

Postnatal Age: age in days from day of birth.

Surface Electrical Capacitance: Surface electrical capacitance was measured by the NOVAmeter on the forehead or temporal area of the neonate. The head is the only area not routinely covered by Aquaphor®, a product commonly used to reduce insensible water loss and protect the skin of LBW infants.

Skin Breakdown: The daily determination of skin breakdown was measured using the Scale of Integrity of Neonatal Skin (SINS).

**Significance of the Study**

Chlorhexidine is known to be efficacious as an antimicrobial in the NICU population of high risk infants (Aggett, Cooper, Ellis, & McAinsh, 1981; Champagne et al. 1984; Garland et al. 1995), but has been associated with serious toxic events. Risks associated with topical chlorhexidine use in premature infants include skin breakdown, burns, and methemoglobinemia (Hjelt et al. 1995). A case report of adrenergic nerve damage was reported concerning an infant who ingested of large amounts of chlorhexidine (Quinn & Bini, 1989). The mother used an unspecified concentration of chlorhexidine spray to cleanse her breasts prior to breast feeding. Reversible adrenergic nerve damage had previously been reported in an animal model (rat) injected with chlorhexidine (Henschen & Olsen, 1984).
The investigator conducted a survey of NICU nurses who are members of the Internet list-serv NICU-NET, an international list-serv. The purpose of the survey was to determine if nurses who used topical chlorhexidine in the neonatal intensive care unit (NICU) expressed any concern about absorption. The six respondents represented six facilities in the United States. The survey focused on two questions: 1) What is the antimicrobial used in your NICU to prepare IV insertion sites and how is it used? 2) Is there any concern about using chlorhexidine topically on low birth weight infants? The findings of the survey revealed no common practice among NICU nurses about skin disinfection procedures. Respondents (n=6) each reported using a different procedure when using isopropyl alcohol, povidone-iodine, or chlorhexidine. Chlorhexidine was used in concentrations of 0.5%, 0.75%, 2%, and 4%. Most respondents (n=5) did not report that they wiped it off the skin.

The survey results also enabled two nurses to share their concerns about the risk of absorption of chlorhexidine in extremely LBW infants. One NICU nurse expressed frustration by the lack of reported research on this antimicrobial in this population. Another NICU nurse reported that her facility was considering a future study to measure the absorption rate of chlorhexidine in low birth weight infants. Four respondents from different facilities who used chlorhexidine did not acknowledge any concerns with absorption. Yet two respondents reported
infants who suffered burns, one with use of 4% and the other with 0.5% chlorhexidine. The nurses in these two NICUs acknowledged that removal of chlorhexidine after application was not part of their conventional procedure.

In one facility, NICU nurses used 4% chlorhexidine as the antimicrobial for all neonates. Testing for methemoglobinemia was done on only those infants who also received nitric oxide treatment. These test results were within normal limits. This same facility officially reported a blood stream infection rate of 3/1000 patient days. In the acute level III NICU, this level is considered a desirable and low rate. Based on the literature that associates skin breakdown with topical chlorhexidine in LBW infants and the comments from the nurses who participated in this survey, the researcher designed a study to test the most dilute preparation of chlorhexidine available commercially to minimize skin breakdown and opportunities for burn.

Assumptions

This research was based on three assumptions. First, the most dilute preparation of chlorhexidine is probably the least toxic to the LBW infant. Since 0.5% chlorhexidine is an effective antimicrobial (Garland et al. 1995), ethical concerns of exposing LBW infants to higher concentrations are eliminated. Second, LBW infants are at greatest risk because of their extremely immature epidermal barriers, which are extremely permeable. Third, LBW infants who
require more applications of chlorhexidine are likely to have a higher risk of methemoglobinemia. Finally, LBW infants who are exposed to other hemoglobin oxidants (such as prilocaine) will be at higher risk to develop methemoglobinemia, and were therefore excluded from this study.

**Research Hypotheses**

The following hypotheses were tested in this study:

1. Methemoglobin levels are significantly higher in LBW infants treated with 0.5% chlorhexidine (treatment) than those who are treated with 70% isopropyl alcohol (control).

2. The skin integrity scores of LBW infants of those treated with 0.5% chlorhexidine are significantly higher than those treated with 70% isopropyl alcohol.

3. Surface electrical capacitance (SEC), which is positively correlated with transepidermal water loss, is positively correlated with Methemoglobin levels.
CHAPTER TWO
REVIEW OF THE LITERATURE

This chapter reviews the published research in areas related to the research study. Past research on the permeability of LBW infant skin, absorption of topical chlorhexidine in neonates, LBW infant skin integrity, permeability of LBW infant skin, transepidermal water loss, and topical antimicrobials are described. The research on desorption measurements of LBW infant skin is also described to support the importance of this measurement in this study, as well as the past and current technology of methemoglobin (MetHb) testing.

Low Birth Weight Infant Skin Integrity

Permeability

It is well known that drugs readily penetrate the skin of preterm infants (Rutter, 1987; Barrett & Rutter, 1994; Kalia, Marino, & Guy, 1998). Agents pass through the skin by passive diffusion along a concentration gradient (Rutter, 1987). A high degree of water and lipid solubility is necessary for intracellular transport through the lipoprotein cell membranes and aqueous cell contents (Rutter, 1987). The rate of drug absorption depends on the surface area of the
skin in contact with the drug. Since LBW infants have a high surface area-to-weight ratio in comparison to a child or adult, they are particularly susceptible to absorption of many topically applied and potentially harmful agents.

Recent developments in neonatal care have uncovered new challenges of extremely immature skin. As the limits of viability have been pushed further back, the functions of organs previously taken for granted in preterm infants have become a limiting factor of viability. The skin of the LBW infant appears red, shiny, and glistening. The glistening reflects a moist skin surface caused by a high transepidermal water loss (Rutter, 1996). Maturational changes greatly accelerate after delivery presumably triggered by the change from an aqueous to a gaseous environment. Within 14 days, the fragile skin of those LBW infants who manage to survive develop a mature appearance with mature characteristics.

**Physiologic Differences**

Because the skin is the largest organ of the body, disruptions in its integrity have significant impact on organism homeostasis. Thermoregulation is compromised when the infant is exposed to transcutaneous heat loss through radiation, convection, conduction, and evaporation. Preterm infants born before 30 weeks gestation do not have brown fat, the substrate that provides a source of energy to maintain thermoregulation. Susceptibility to nosocomial infection is
another life-threatening effect of LBW infant skin integrity. Opportunistic infectious organisms such as candida, serratia, pseudomonas, and staphylococcus invade broken skin readily, and quickly escalate to systemic infections due to the incompetent immune system of the premature infant. Further, antibiotic resistant strains pose a major threat to this population. The prevalence of antibiotic resistant organisms such as methicillin resistant staphylococcus aureus (MRSA), gentamicin and vancomycin resistant staphylococcus aureus are increasing (Haddad et al. 1993; Reboli et al. 1989; McNeeley et al. 1996; Matrai-Kovalskis et al. 1998; Krediet et al. 1999).

A recent study reported that the epidermal barrier of low and extremely low birth weight infants born at 23 to 25 weeks gestational age does not attain functional maturity until 5 to 7 weeks after delivery (Kalia, Nonato, Lund, & Guy, 1998). These researchers combined TEWL measurements and impedance spectroscopy to document the maturation process in a cohort of LBW and ELBW infants born between 23 to 32 weeks gestational age. Impedance spectroscopy provided an added dimension of ion flow across the stratum corneum when an alternating electric field is applied to the skin. Ion flow was measured at low and high frequencies to provide information about the structural changes taking place in the stratum corneum. Findings indicated an inverse correlation between low frequency impedance and TEWL as barrier function developed. At low
gestational and postnatal ages, they reported the highest TEWL measurements (60-70 g per m² per hour) and the lowest skin impedance. Interestingly, the one infant in the study who received antenatal steroids (betamethasone) prior to delivery at 30 weeks gestation had TEWL comparable to those of adults and full-term infants, and impedance measurements at the mature range. The two infants treated postnatally with dexamethasone had TEWL and impedance measurements which rapidly accelerated after the medication was discontinued.

Use of Topical Antimicrobials in NICU

Topical antimicrobials are used in the NICU to reduce colonized bacteria and the normal flora of the skin before an invasive procedure. The following section provides the historical perspective of antimicrobial use to illustrate the special problems faced by LBW infants.

Hexachlorophene: The first experience with topical antimicrobials use in newborn infants produced tragic effects (Anderson et al. 1981; Powell et al. 1973). After its introduction in the 1950s, hexachlorophene (pHisohex™) was widely used for neonatal bathing for twenty years. It is extremely effective in preventing colonization of Staphylococcus aureus. Reports appeared in the literature in 1970s describing severe neurotoxic effects, especially in preterm infants. An animal (rat) model study demonstrated acute encephalopathy in newborns, with autopsy reports describing vacuolation of myelin (Kimbrough &
Gaines, 1972). These published findings led to a moratorium on the use of hexachlorophene in all newborn infants.

**Isopropyl alcohol:** Isopropyl alcohol is commonly used as a topical antimicrobial in the NICU. Clear, colorless, volatile, with a characteristic strong odor, isopropyl alcohol has been found to be drying and to de-esterify the skin (Harpin & Rutter, 1982). Even though the alcohol evaporates before being absorbed into skin in most situations, there is a recent report of isopropyl alcohol poisoning in a 21-day-old full term infant whose mother applied isopropyl alcohol pads to the umbilicus every diaper change (Vivier et al. 1994). This is a routine nursing intervention taught to mothers during discharge teaching. Upon admission to the emergency department, the infant presented with hypotonia, lethargy and intermittent lack of response to pain. Urine and serum drug screens revealed high levels of isopropyl alcohol and acetone. The infant fully recovered after 3 days of supportive care in the hospital.

When isopropyl alcohol does not evaporate and there is prolonged exposure on the skin, serious local and systemic damage results. Hemorrhagic necrosis of the skin (Hodgkinson et al. 1978), chemical burns of the skin (Schick & Milstein, 1981), and skin necrosis (Watkins & Keogh, 1992) were reported after preterm infants lay supine in an alcohol soaked bed during a prolonged procedure to place umbilical lines.
**Povidone-iodine:** Povidone-iodine is widely used as a topical antimicrobial in the NICU (Siegfried & Shah, 1999; Munson, Bare, Hoath, & Visscher, 1999). It is not effective in killing bacteria unless allowed to dry. Povidone-iodine usually takes at least 30 seconds to dry, but it can take as long as 2 to 3 minutes depending upon the amount of solution applied. The rate of absorption is linked to the time the agent is on the skin. The longer the skin contact, the more absorption occurs. Adverse effects of topically applied antimicrobial containing iodine have been known for more than twenty years (Pyati, Ramamurthy, Krauss, & Pildes, 1977). Roberts (1984) described a case report of necrosis due to prolonged skin contact in a preterm infant. Systemic toxic effects include transient hypothyroxinemia, hypothyroidism and goiter (Chabrolle & Rossier, 1978; Gordon, Rowitch, Mitchell, and Kohane, 1995; Parravicini, Fontana, Paterlini, Tagliabue, Rovelli, Leung, & Stark, 1996). Clinicians can minimize absorption by wiping the skin with sterile water or saline to remove the povidone-iodine before the skin puncture. Linder and colleagues (1997) reported significantly elevated (p < .01) thyrotropin levels in 2 groups of preterm infants when skin was routinely treated with a povidone-iodine antimicrobial before an invasive procedure. Elevated urine iodine levels were found reflecting an abnormally high iodine absorption rate. Thyroid hormones have a critical role in normal neurological development, and even transient disturbances may be detrimental to LBW infants.
Chlorhexidine: Chlorhexidine has been also been used as an antimicrobial in infants in the NICU. Necrosis of the skin was anecdotally reported in 8 infants with birth weight less than 1200 grams by neonatologists (Wilkinson et al. 1981). They suspected prolonged application of chlorhexidine during umbilical line placement. After recognizing this new problem, the physicians changed their practice by removing the chlorhexidine before draping the patient with sterile towels, after which they reported no further problems. They warned that excessive use of disinfecting fluids can be toxic to the thin skin of very preterm infants.

Garland and colleagues (1995) also reported skin reactions to topical chlorhexidine. Their study was a comparison of the BIOPATCH® antimicrobial dressing with povidone-iodine scrub for central venous catheter sites. BIOPATCH® is a chlorhexidine (4%) impregnated foam patch which releases chlorhexidine over a period of several days. Nine of the first 300 infants entered into the study developed local skin reactions. Seven of these 9 infants were between 22.5 and 26.5 weeks gestational age, which is associated with a very thin to non-existent stratum corneum. The BIOPATCH® dressings were placed on infants at 1 and 8 days of age, with a mean of 4.7 days. Reactions were severe contact dermatitis and pressure necrosis. They revised the study protocol to limit enrollment to LBW infants who were greater than 26 weeks
gestation and at least 7 days old. Despite these precautions, 3 more skin reactions occurred in 75 infants who enrolled in the BIOPATCH® arm of the study. Prolonged exposure to immature skin increased the risk of skin disruptions. Similar skin reactions were reported by Karwowska and colleagues (1995) in a group of infants weighing less than 1000 grams.

In a study of 1% chlorhexidine powder for use in umbilical cord care, Aggett and colleagues (1981) reported detectable, increasing plasma chlorhexidine levels in preterm infants. The chlorhexidine powder was reconstituted in an unspecified concentration of alcohol and applied every 4 hours for 5 to 9 days. The researchers suspected the alcohol enhanced absorption of chlorhexidine. They reported no adverse effects, except for detectable levels of chlorhexidine.

**Absorption of Topical Chlorhexidine in Neonates**

Topical chlorhexidine has been determined to be safe in adults because its protein-binding characteristics do not allow it to be absorbed through intact skin (Case et al. 1976). Since the stratum corneum of full term infants is fully developed, topical chlorhexidine should pose no risk. This hypothesis was tested with a 4% chlorhexidine solution in 51 full term neonates (O'Neill et al. 1982). Newborns were assigned one of four groups: sponge bath with 4% chlorhexidine followed by a sterile water rinse on Day 1, or on Days 1 through 3;
and sponge bath with 4% chlorhexidine without rinse on Day 1, or on Days 1 through 3. Serum chlorhexidine levels were measured within 60 minutes after the bath, but none was detected in any sample. The investigators did not consider methemoglobinemia, but reported no treatment-related side effects during active exposure to chlorhexidine, or during the one-year follow up period.

An earlier investigation focused on absorption in preterm infants (Cowen et al. 1979). Subjects were preterm infants with gestational ages between 28 and 37 weeks, birth weight ranging from 700 to 3020 grams, and postnatal ages between 4 and 84 days. Once entered into the study, all subjects were bathed daily with 4% chlorhexidine followed by a water rinse. Serum levels of chlorhexidine were measured at one hour by heelstick in Group 1, at four hours by venipuncture in Group 2, and at 12 hours by venipuncture in Group 3. The investigators reported that 3 infants in Group 2 had significantly higher levels of chlorhexidine, and these infants were 7 days old, and between 33 and 35 weeks gestational age. They also found positive results in Group 1, but determined that the serum levels reflected contamination inherent in the heelstick sampling technique. These authors also did not test for methemoglobinemia, nor did they report any unusual effects which could be attributed to chlorhexidine during the study period.
In summary, LBW infants at high risk of nosocomial infection in the NICU are those who require the most invasive interventions and procedures. Clinicians must use the most efficacious antimicrobial to remove as many pathogens as possible from potential portals of entry. The thin, permeable skin of LBW infants, however, does not provide an effective barrier against any topically applied agent. Serious adverse events are associated with alcohol, povidone-iodine, and chlorhexidine, raising concern about the specific risks of chlorhexidine associated with LBW infants.

**Transepidermal Water Loss**

The stratum corneum is the major barrier to transepidermal water loss, and its maturity is dependent upon the gestational age of the LBW infant. The stratum corneum is barely developed in the extremely low birth weight (ELBW) infant, commonly defined as infants with birth weight less than 750 grams. These infants are born at the limits of viability at less than 25 weeks gestation, and the limit is currently progressing towards 20 weeks gestation. According to Elias and colleagues (1981), barrier function is related to the thickness or number of cell layers in the stratum corneum, and there is an inverse relationship between the lipid weight percentage and the permeation of a particular skin site to water. Mechanisms that contribute to transepidermal water loss in the ELBW infant are physiologic and environmental. Physiologic mechanisms are the rapid rate of
breathing, and thin, permeable skin (Horns, 1994; Kalia, Nonato, Lund, & Guy, 1998). The amount of transepidermal water loss in preterm infants is enormous, and the most significant physiologic factor is the thin permeable skin of the extremely LBW infant. Transepidermal water loss can be directly measured with an Evaporimeter® (Nilsson, 1977). The body composition of infants born between 22 and 25 weeks gestation are approximately 85% water. Insensible water loss is extremely high in these infants, often reaching more than 10 cc per kilogram per hour. Transepidermal water loss occurs because the stratum corneum of the epidermis may only be a single cell layer thick, and has not yet undergone any significant keratinization (Fairley & Rasmussen, 1983). Water is transported freely through the skin until the stratum corneum develops (O'Rahilly & Müller, 1996). When transepidermal water loss is measured below 10gm per m² per hour, the stratum corneum is providing an effective barrier. Values above 20 gm per m² per hour indicate an ineffective barrier. Immature epidermal barriers facilitate topical absorption of any drug or product (Rutter, 1987). The major criticism and limitation of the Evaporimeter® as a measurement device is that it is expensive (> $10,000), slow, and most importantly, susceptible to error due to convective air currents (Okah, Randall, Pickens, & Hoath, 1995).
Surface Electrical Capacitance Measurement of the Stratum Corneum

Surface electrical capacitance (SEC) is a technology that has been used to measure wound healing in adults (Goretsky, Supp, Greenhalgh, Warden, & Boyce, 1995), to predict skin disorders in nursing home residents (Schnelle, Adamson, Cruise, Al-Samarrai, Sarbaugh, Uman, & Ouslander, 1997), and tested on mice as a noninvasive index of epidermal barrier (Boyce, Supp, Harriger, Pickens, Wickett, & Hoath, 1996). The surface electrical capacitance technology has recently been applied to the low birth weight population (Okah, Randall, Pickens, & Hoath, 1995). The impedance of the skin reflects its ability to restrict ion flow (Kalia, Merino, & Guy, 1998). Impaired barrier function enables ions to be transported more freely, and results in lowered impedance. Impedance is not a direct measurement of transepidermal water loss, but has been shown to correlate with TEWL measurements in a population of LBW and ELBW infants (Wickett, Nath, Tanaka, & Hoath, 1995). Surface electrical capacitance measures skin surface hydration and its changing hydration by occlusion. Elevated surface electrical capacitance measurements indicate a very hydrated skin surface, which is associated with pronounced permeability (Ryatt, Mobayen, Stevenson, Maibach, & Guy, 1988). The high permeability of LBW infant skin increases the risk of absorbing substances that are internally toxic.
The usefulness of surface electrical capacitance as an index of barrier maturation in LBW infants was reported by Okah and colleagues (1995). They used the NOVA Dermaphase Meter, Model 9003 (NOVA Technology Corporation, Hanover, NH) to obtain baseline measurements of skin surface hydration and continuous readings to provide an indirect measure of transepidermal water movement. They tested 40 infants at 12 to 24 hours of life for 5 days. The infants were between 25 and 40 weeks gestation, and had birth weights between 840 and 3200 grams. The baseline skin hydration was significantly related to the gestational age of the subjects (p = .0009). The highest surface electrical capacitance measurements were recorded in those infants less than 30 weeks gestation. They also reported significant changes in surface electrical capacitance measurements in the least mature infants (p < .05). These results support previous studies of TEWL, which demonstrated an inverse relationship between TEWL and gestational age (Sedin, Hammarlund, Nilsson, Stromberg & Oberg, 1985; Wilson & Maibach, 1980; Hammarlund & Sedin, 1982; Hammarlund & Sedin, 1979).

Okah and colleagues (1995) reported preliminary data that supported a study period of 12 seconds was sufficient for continuous surface electrical capacitance measurements to reach a plateau. They also chose the forehead as the measurement site because it is flat, accessible, and not routinely covered with products used to protect LBW infant skin.
Although the cost of the NOVAmeter® is high ($7500), it is easy to use and calibrate, and is not affected by convective air currents. Data collected by the EDWINA™ software easily converts to the EXCEL® spreadsheet where it is quickly analyzed. Most infants were not disturbed by the surface electrical capacitance measurement, evidenced by their sleeping through the procedure.

Chemical Determination of Methemoglobin

Hemoglobin converts to methemoglobin when its iron is oxidized, and is characterized by a brown pigment. Normal concentrations of methemoglobin are less than 1.5% of the total hemoglobin. Infants with congenital methemoglobin due to methemoglobin reductase deficiency present with blood levels as high as 40-55% of the total hemoglobin (Porter, 1999). Concentrations greater than 50-60% are usually lethal. Because the absorbance spectrum of methemoglobin is a small characteristic peak at 630-635 nm, it may be analyzed using laboratory techniques (Porter, 1999).

The blood specimen should be fresh and anticoagulated. Methemoglobin is not stable at room temperature. If not able to be analyzed immediately, it should be put on ice or refrigerated. Food or fluid restriction is unnecessary prior to testing. The reagents needed for the procedure are potassium ferricyanide, potassium cyanide solution, and potassium phosphate buffer. The laboratory procedure is listed in Appendix C.
Measurement of Methemoglobin by Light Spectrography

Light spectrography is a technology used to isolate discrete portions of the spectrum for purposes of measurement (Evenson, 1999). Most simply, a light beam passes through a cell containing a solution of a compound that absorbs light of certain wavelength. A reference cell identical to the sample cell but without the compound of interest is used to focus attention on the compound of interest. Slits are used to isolate a narrow beam of light and improve chromatic purity. As the light passes through the cuvet (absorption cell), unabsorbed light is transmitted to the detector, which converts light energy into electrical energy (Figure 2-1). This energy is registered on a meter, recorded, or digitally displayed. The AVOXimeter 4000® displays results digitally, and may be printed. Cuvets must be handled carefully to avoid damage. They should be kept in an airtight container at room temperature, and care should be taken to avoid extremes in temperature.

Figure 2-1: Major components of a single-beam spectrophotometer (Evenson, 1999) (permission pending)
Beer's Law (Evenson, 1999) states that if several light-absorbing compounds are present in a solution, the concentration of each compound can be deduced if the compounds differ in their optical absorbances and if optical density is measured at as many wavelengths as there are compounds present. For example, the optical density (OD) of three compounds X, Y, and Z measured at three different wavelengths (λ) can be expressed in the following set of equations, given unknown concentrations (c), optical path length (R) and the extinction coefficients (ε):

\[
\begin{align*}
\text{OD}_{\lambda_1} &= \epsilon_{x,\lambda_1} c_x R + \epsilon_{y,\lambda_1} c_y R + \epsilon_{z,\lambda_1} c_z R \\
\text{OD}_{\lambda_2} &= \epsilon_{x,\lambda_2} c_x R + \epsilon_{y,\lambda_2} c_y R + \epsilon_{z,\lambda_2} c_z R \\
\text{OD}_{\lambda_3} &= \epsilon_{x,\lambda_3} c_x R + \epsilon_{y,\lambda_3} c_y R + \epsilon_{z,\lambda_3} c_z R
\end{align*}
\]

Conventional whole-blood oximeters use two wavelengths to measure the relative concentrations of oxyhemoglobin and deoxyhemoglobin, assuming the total hemoglobin concentration consists entirely of oxy- and deoxyhemoglobin. If other hemoglobin species such as methemoglobin are present, oximeter measurements of oxyhemoglobin saturations would be in error (Evenson, 1999).

The AVOXimeter 4000® uses many wavelengths to obtain accurate measurements of total hemoglobin by measuring the presence of methemoglobin, oxyhemoglobin, and carboxyhemoglobin (AVOXimeter 4000®)
Product Manual). Because of the additional wavelengths and corrections, interference due to bilirubin, hemolysis (Gong, 1995) and fetal hemoglobin (Speakman, Boyd, & Bruns, 1995) are also eliminated. Fifty microliters (less than 2 drops) of whole blood can be collected with a syringe from a central line, or a collection device from a peripheral heel stick. The sample is gently introduced into the cuvette, taking care not to overfill the chamber. The cuvette should be inserted into the AVOXimeter 4000® within ten seconds, because methemoglobin is not stable at room temperature. Specimens may be kept on ice or refrigerated, but not frozen. Evidence exists that significant decreases in methemoglobin concentrations after 4 to 8 hours (Porter, 1999). The AVOXimeter 4000® analyzes the sample within ten seconds. A companion printer provides a hard copy of the results.

**Summary**

At this point, research connects methemoglobinemia with use of topical chlorhexidine in preterm infants, but only in excessive applications (bathing) or unconventional applications such as ingestion and inhalation. The risk of methemoglobinemia has not been investigated when chlorhexidine is used as a topical antimicrobial on preterm infants. Since preterm infants have immature skin, they are at greater risk for absorbing chlorhexidine than full term infants. Review of the literature on percutaneous absorption supports limiting the
exposure of chlorhexidine to preterm infants, since they readily absorb many other topically applied chemicals and medications. None of the researchers who tested chlorhexidine on preterm infants as an antimicrobial reported noticing a chocolate brown color of the blood, which is characteristic of methemoglobinemia. Blood color is an important clinical indicator that is extremely obvious to experienced NICU clinicians. This research will build on previous research by providing data about this gap identified in the literature, which may increase knowledge and understanding of the safety of chlorhexidine as an antimicrobial in the preterm infant population.
CHAPTER THREE
MATERIALS AND METHODS

This chapter describes the methodology for this research. Specifically, design, sample, setting, measures, data collection procedures, instrumentation, and protection of human subjects are discussed. Pilot testing to test the research design is also presented.

**Design**

An experimental design with repeated measures was used. In this study, subjects were selected from a convenience sample and randomized to the treatment or control group. The treatments were not blinded, which is a threat to validity. A double-blind design was not feasible since both chlorhexidine and alcohol wipes have distinctive odors.

The independent variable was 0.5% chlorhexidine gluconate as the antimicrobial intervention. The dependent variables included the methemoglobinemia level as measured by the AVOXimeter 4000®, gestational age determined by the physician from the maternal history or reported by Dubowitz examination performed by the admitting physician or nurse practitioner, desorption measured by the DPM 9003® on the forehead skin of the
neonate, and skin breakdown measured using the Scale of Integrity of Neonatal Skin (SINS).

Sample

A convenience sample of LBW infants were recruited from a NICU in the southeastern United States and followed until postnatal day 28. Sample size was determined to be 10 subjects who had at least 13 measurements of methemoglobin (MetHb) each. This sample size was based on a formulation of 83% power, an effect size of 0.20, 3 response variables, and a significance of 0.05 for a one-tailed test.

Inclusion criteria

Subjects were invited to participate in the study if they were infants with birth weight between 750 and 1500 grams, between day of birth and 7 days old, and whose parents who speak and understand English.

Exclusion criteria

Potential subjects were excluded from the study if they were infants born with surgical emergencies such as gastrochisis, ommphalocele, mylomeningocele or other congenital anomalies which may alter the basic barrier property of the skin. Infants exposed to other known agents that increase the risk of methemoglobinemia, such as aniline marker dyes, prilocaine, benzocaine, and nitric oxide were also excluded. Infants who were critically ill at the time of their admission were also excluded.
Setting

The setting was a 216-bed tertiary care, pediatric teaching hospital located in the southeastern United States. The hospital was licensed by the state for 24 neonatal intensive care and 36 neonatal intermediate care beds. The average daily census was 50 neonates, and approximately 600 were admitted yearly. During fiscal year 1998, approximately 150 preterm infants were admitted to NICU with birth weight less than or equal to 1500 grams. Because all infants were transported into the facility, parents were not usually immediately available. The first visit by the mother often did not occur until 2 or 3 days after NICU admission.

Measures

The following instruments were used to determine physiologic measures of methemoglobin levels, surface electrical capacitance, and environmental humidity.

Physiologic Measures

AVOXimeter 4000® (Diametrix Corporation) was used to determine MetHb concentrations. This device is a whole-blood oximeter that measures MetHb concentration designed for POC testing. A sample of blood was collected and inserted into a disposable cuvette, which was inserted into the AVOXimeter 4000®. No sample preparation was required, and results were available in less
than 10 seconds. The investigator performed and documented visual quality control measures daily according to manufacturers directions. In November 1999, a correlational study of 11 neonatal samples from the study facility was performed using the AVOXimeter 4000® with known analytes over a wide range of concentrations against a reference analyzer. Methemoglobin linearity analysis revealed $R^2 = 0.0002$. Diametrix Corporation provided the device for the duration of the study.

Dermaphase Meter (DPM) 9003® (Nova Technology, Hanover, New Hampshire), commonly known as the NOVAmeter®, was the device used to measure surface electrical capacitance (SEC) by impedance. The impedance of the skin reflects its ability to restrict ion flow. Ion transport occurs more easily with immature barrier function (Kalia, Merino, & Guy, 1998). These data provided a measure of "desorption" of water from the skin. The term desorb was defined as the process of water being expelled from the skin. The flat end (similar to the end of a stethoscope but dime-sized) of a small wand was placed on the infant skin and held in place for twelve seconds. The instrument collected data about water desorption ten times per second while a software package (EDWINA®) enabled measurements to be recorded using a laptop computer. The DPM 9003® and software were easy to use, more affordable than the Evaporimeter®, and less susceptible to changes in environmental conditions than the
Evaporimeter® (Okah, Wickett, Pickens, & Hoath, 1995). This device enabled the operator to document changes in desorption associated with developing skin maturity. An automated internal calibration mechanism provided quality control before every measurement session.

Environmental Measure

Hygrometer (Traceable®, Control Company, Friendswood, Texas) was used to measure the ambient temperature and humidity of the microenvironment. Once measured and calibrated, the electronic digital hygrometer/thermometer is reported to maintain its accuracy.

The following instrument was used to assess the extent of skin breakdown among the subjects.

Scale of Integrity of Neonatal Skin (SINS) was used to measure the relative amount of skin breakdown present at the time of assessment. A score of 0 indicates intact skin, and higher scores represent increasing severity of skin integrity. Face validity of this investigator-developed scale was established by a panel of 10 expert neonatal nurses and clinical nurse specialists. It is currently being tested for validity and reliability in a privately funded, multi-center study. A reproduction of the tool appears as Appendix D.

The following products were used during the procedure of antimicrobial application and removal.
Isopropyl alcohol 70% (Kendall) is supplied in 1.5 inch by 1.5 inch sterile swabs. Hibistat® Chlorhexidine gluconate 0.5% is supplied as a towelette, packaged in 2 x2 inch sterile packets by Zeneca. Klear-Wipe™ (CAS Medical Systems, Inc, Branford, CT) is a sterile water swab supplied as a 1.25 inch by 1.5 inch sterile pad saturated with 100% Deionized Water.

Protection of Human Subjects

This study was approved prior to its implementation by the University of Florida’s Institutional Review Board (IRB) and the All Children’s Hospital Nursing Research Committee and Institutional Review Board. A copy of the informed consent is found in Appendix E.

Informed consent was obtained from the parents of LBW infants who met inclusion criteria. Parents were asked to consent to their infant being randomized to receive either isopropyl alcohol 70% or 0.5% chlorhexidine. Parents were told that daily serum samples of 1 to 2 drops will be collected for methemoglobin testing, surface electrical capacitance measured, and skin assessment performed. All data were coded to ensure anonymity. There was no direct benefit to the subject for participating in this study, and there was minimal risk to subjects. Parents were informed they may remove their infant at any time during the study without loss of any benefit or medical care. A decision
was made by the investigator that should methemoglobin levels exceed 7%, the subject would be removed from the study, although the data would be included in the analysis. Methemoglobin levels did not exceed 7%, and it was not necessary to remove any subject from the study. Finally, data were reported in aggregate.

Piloting the Procedures for Collecting Data

A pilot study was conducted to test and refine the data collection procedure using the study instruments. A single subject was recruited with informed consent from the parents on postnatal age 1. The infant was randomized to the treatment arm, and data were collected for 27 consecutive days.

Data Collection Procedures

The baseline sample of methemoglobin was 2.3%, and subsequent samples on this patient ranged from 1.2% to 2.7%. One result was determined to be false (5.1%) because of poor technique (air in cuvet). Practice was needed to develop skill in obtaining a good sample using the blood collection device, and in filling the cuvet carefully to avoid air pockets. A total of 15 methemoglobin samples were measured during the pilot study period.

The pilot subject was a relatively healthy preterm infant who no longer required multiple daily laboratory studies by day 10. The physician ordered
laboratory samples weekly, which were scheduled between 0400 - 0800 hours so they were available during medical rounds for review. A nurse assigned to the night shift was trained to use the AVOXimeter 4000® to take advantage of the early laboratory sampling.

Study designs of previous researchers limited surface electrical capacitance data collection to a single investigator (Okah, Wickett, Pickens, & Hoath, 1995). The pressure of the wand against the skin may influence the surface electrical capacitance measurements. The design of this study requires daily surface electrical capacitance measurements on 10 patients for approximately 28 days, which may be an unrealistic goal for a single investigator. Instead, a co-investigator who was trained in surface electrical capacitance measurement labeled the data she collected with I-2 (investigator #2), while the principal investigator labeled the data she collected with I-1 (investigator #1). Data were analyzed to determine if there were significant differences in the data collected by the two investigators.

**Instruments**

The NOVAmeter® was used daily to measure surface electrical capacitance. Two wands were included in the purchase price of the DPM 9000®. The smaller-sized head was difficult to obtain occlusion with, and required multiple sessions to obtain an uninterrupted measurement. The larger-sized
head sat flatly on the forehead of this subject, despite his small (775 grams) size.

Although the NOVAmeter® was marketed to enable ten samples per second, the investigator discovered the recordings were not measuring at that rate because the software provided did not include that feature. On day 5 of data collection, updated software was tested on the pilot study patient and no further problems were encountered. Representatives from Nova Technology made a site visit 6 weeks after data collection began and validated correct use of the instrument and software.

Site Selection for the Probe: The forehead of the pilot subject was not available between days 7 and 12 of data collection, because an intravenous line was inserted in his scalp, and tape completely covered his forehead. The investigator determined that surface electrical capacitance measurements should be restricted to the head when the forehead was not available. Alternatively, surface electrical capacitance measurements were taken on the temporal bone near the eye when the forehead was not available. Measurements were repeated if readings were “zero” at any time during the twelve second measurement period. Readings of “zero” indicated that the probe was not occluding the skin. The subject was not disturbed by the surface electrical capacitance measurements.
A hygrometer was used to measure humidity and ambient temperature because high humidity levels decrease insensible water losses in LBW infants. A switch turned it on, a digital reading appeared immediately, and measurements stabilized within 10 seconds. When not in use, it was turned off and stored in its plastic case, which was recommended by the manufacturer to optimize battery life and calibration.

The Scale for Integrity of Neonatal Skin (SINS) was used once daily to document assessment of skin integrity. The investigator and co-investigator achieved an inter-rater reliability of .90 on the pilot subject. The SINS was developed to provide an overall assessment of skin integrity, rather than capture differences in scores associated with alcohol and chlorhexidine. The investigator determined the SINS was valuable because the antimicrobials were over the entire skin surface of LBW infants, and not limited to any specific sites.

In summary, the changes in the data collection procedures as a result of pilot testing were:

a) attention to development of technical skill in using the blood collection device and surface electrical capacitance measurements;
b) identifying the investigator collecting the surface electrical capacitance measurements;
c) adding another surface electrical capacitance data collection site, namely, the temporal bone near the eyes;
d) collecting data on humidity and ambient temperature, and;
e) training a nurse to perform the methemoglobin on subjects who were scheduled for laboratory studies during the early morning hours.

The data collection procedures used in the study are described below.

Data Collection Procedures of the Study

Parents of LBW infants meeting inclusion criteria were approached by the investigator to explain the study and obtain informed consent. A baseline methemoglobin level was obtained first to assess presence of congenital methemoglobinemia. If not present, the infant was randomized by a coin flip to the control or treatment group. Surface electrical capacitance and SINS data were collected on the subjects every day until they reached postnatal day 28. Samples for methemoglobin were taken only when the subject was scheduled for laboratory studies ordered by the physician, but no more than once daily. Methemoglobin levels greater than 4% were to be re-sampled and analyzed in the hospital chemistry laboratory to compare or confirm results. Data collected daily were recorded on the Daily Log (Appendix F). Surface electrical capacitance data were downloaded to an Excel97 database as each subject completed the study and data were prepared for analysis. Descriptive data about the maternal and fetal history was documented once for each subject (Appendix G).
**Control Group**

The skin of LBW infants in the control group was prepared with the conventional antimicrobial agent, 70% isopropyl alcohol, for peripheral intravenous catheters and peripheral skin punctures for laboratory sampling. The site was scrubbed five times with one swab in preparation for skin puncture. The skin was then entered without contaminating the field. Procedures such as placement of percutaneous intravenous central catheters (PICC), chest tube placement, needle aspiration of chest, blood culture, and lumbar puncture conventionally require a scrub with povidone-betadine according to the NICU protocol. For these procedures in the control group, the skin was prepared first with povidone-iodine, and then wiped off with sterile water in the conventional manner. The subjects' bedside was marked indicating participation in the study so clinicians, therapists, and laboratory support staff were alerted to the study procedures. The alcohol wipes were kept in a green covered, plastic disposable container clearly labeled with green "Alcohol" stickers and directions for application. All staff who performed these procedures recorded the number of applications on the daily record to log each application of alcohol and povidone-iodine (Appendix H).
Treatment Group

The skin of preterm infants in the treatment group was prepared with the treatment antimicrobial agent, chlorhexidine gluconate 0.5% aqueous solution, for peripheral intravenous catheters and punctures for laboratory sampling. The site was scrubbed five times with one chlorhexidine swab. After thirty seconds, the skin was wiped off with a sterile water swab. The skin was then entered without contaminating the field. Procedures such as placement of percutaneous intravenous central catheters (PICC), chest tube placement, needle aspiration, blood culture, and lumbar puncture that conventionally require an additional scrub with povidone-betadine was prepared with a liquid preparation of chlorhexidine 0.5%, left for 30 seconds, then wiped off with a sterile water. The subjects' bedside was marked indicating participation in the study so clinicians, therapists, and laboratory support staff were alerted to the study procedures. The liquid preparation was prepared by the NICU pharmacist by diluting a 4% solution of chlorhexidine to 0.5%. A small bottle (30 mL) was kept in a red covered, plastic disposable container with a supply of chlorhexidine towelettes and sterile water wipes clearly labeled with red “Chlorhexidine” stickers and directions for application. All staff who performed these procedures recorded the number of applications on the daily record to log each application of chlorhexidine (Appendix I).
**Daily Measurements**

All subjects had point of care testing for methemoglobin levels at the time of regular daily laboratory sampling. The samples were usually taken at the same time each day, about twenty-four hours apart. The frequency of daily applications of each antimicrobial solution was recorded by the bedside nurse and counted by the investigators. Daily surface electrical capacitance measurements were recorded with the NOVAmeter® using a site on the forehead or temporal bone near the eye. These measurements were usually done at the same time each day, so subjects were not disturbed during sleep. Nursing assessment of skin breakdown was recorded daily using the SINS by the investigator.

**Training**

NICU nurses and phlebotomists attended an inservice to learn the study protocol. They were taught to scrub back and forth or in a circular motion 5 times in an area approximately the size of the swab. They were taught to leave the chlorhexidine solution on the skin for 30 seconds, then wipe it off using a circular pattern beginning at the center of the cleaned site. Conventional procedure prohibits the site from being touched again, except by a sterile catheter or Tenderfoot®. The phlebotomist supervisor requested a listing of patients who were subjects to use as notification for phlebotomists assigned to the NICU.
Appendix J was electronically mailed to the supervisor every time a new subject was entered.

Clinicians such as neonatal nurse practitioners, attending physicians, and resident physicians were taught the same protocol using the liquid chlorhexidine solution for blood cultures, lumbar punctures, needle aspirations, chest tubes or PICCs. Specifically, they were taught to scrub the area with 0.5% chlorhexidine in the same manner they use povidone-iodine, wait 30 seconds, and cleanse the site with a sterile water swab or solution. Again, conventional procedure prohibits the site from being touched again, except by a sterile catheter or sterile towels. All clinicians were observed routinely for adherence to study protocol by the investigator.

The principal and co-investigator assessed and documented the skin integrity of subjects using the SINS (Appendix D). Once daily one of the investigators assessed the preterm infants skin, and documented the score on the study collection tool (Appendix F). Interrater reliability of .9 was obtained between the investigator and the co-investigator.

**Data Analyses**

Data were collected by the principal investigator and entered into the SAS program in preparation for analysis. Demographic data on maternal, fetal, and neonatal variables were analyzed to generate descriptive statistics using mean scores, normal distributions, and variance.
The hypotheses were tested using multiple regression to determine the most valid variables for describing the relationship between the dependent and independent variables. Univariate analysis of repeated-measures was used to examine the covariates of desorption, ambient temperature and humidity, and surface electrical capacitance in relation to levels of methemoglobin. Adjusted means and F scores were examined. The general linear model procedure was also used to detect significant effects of repeated antimicrobial applications with methemoglobin levels and desorption, and methemoglobin levels and skin breakdown.

**Summary**

This chapter presented the research design, sample criteria, and data collection procedures for this study. Results of pilot testing were discussed. Finally, the data analysis methods for the research hypotheses were presented.
CHAPTER FOUR
FINDINGS

A description of the subjects and the results of the study are presented in this chapter. The results are discussed in relation to the hypotheses. In this experimental study, the investigator was not blinded to the randomized treatments of the subjects. Data were collected daily on newly born, preterm infants until postnatal age day twenty-eight. All data analyses were computed using SAS statistical program. Descriptive statistics of the sample were computed using Excel97. Statistical significance was set at \( p < .05 \).

Characteristics of the Patient Sample

The parents of twenty-one patients were approached and invited to participate in the study. Ten parents declined. One father of twins strongly preferred isopropyl alcohol as the antimicrobial to be used on his infants. The others who declined cited concern over the blood sampling required. One infant admitted during the study period was excluded by the investigator because her twin died in utero.

Nine parents consented, one the parents of twins. All subjects had baseline methemoglobin (MethHb) levels within the normal range (1.1% - 3.2%). Data collected included 128 methemoglobin, 220 SINS, and 219 surface
electrical capacitance (SEC) observations. Subjects were entered into the study between day one and day four of life and followed until they were twenty-eight days old. All completed the study.

**Sample Characteristics**

Six males and four females participated in the study. Three subjects were Caucasian, five were African-American, and the twins were bi-racial (African-American and Caucasian). Two Caucasian subjects were randomized to the control group and the third to the treatment group. Three African-American subjects were randomized to the control group and four to the treatment group. Half of the subjects were exposed to antenatal steroids prior to delivery (Figure 4-1). Baseline SINS scores ranged between 0 and 3, except for one infant whose baseline score was sixteen. She was transferred into the NICU on day three of life, and her high score reflected an excoriated abdomen from aggressive use of tape at the referring facility. All subjects had a relatively benign respiratory course in the NICU. A paired t-test was performed to determine significant differences between the groups for birth weight (p = 0.41) and gestational age (p = 0.47). The paired t-test was also used to determine significant differences between the groups for daily applications (p=0.3569), ambient temperature (p = 0.139), and ambient humidity (p = 0.2356). No significant differences were found between the groups.
The mothers' age ranged from 15 to 35 years (mean = 24.7). Five women had premature rupture of membranes, seven had normal vaginal deliveries, and two delivered by cesarean section. One mother reported smoking a total of less than one pack of cigarettes daily during her pregnancy, and the others denied smoking.

Figure 4-1: Subjects born to mothers who were treated with antenatal corticosteroids.
Table 1

Summary Measures of Sample Characteristics

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean</th>
<th>SD</th>
<th>Min.</th>
<th>Max.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Background Variables</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Birth weight (grams)</td>
<td>1063</td>
<td>235.39</td>
<td>798</td>
<td>1441</td>
</tr>
<tr>
<td>Gestational Age (weeks)</td>
<td>28.6</td>
<td>2.05</td>
<td>26</td>
<td>32</td>
</tr>
<tr>
<td>Baseline Methemoglobin</td>
<td>2.02</td>
<td>0.601</td>
<td>1.1</td>
<td>3.2</td>
</tr>
<tr>
<td>Baseline SEC</td>
<td>128</td>
<td>32.2</td>
<td>92</td>
<td>210</td>
</tr>
<tr>
<td>Baseline SINS</td>
<td>2.2</td>
<td>4.96</td>
<td>0</td>
<td>16</td>
</tr>
</tbody>
</table>

Table 2

Summary Measures of Sample Characteristics by Group

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control Group</th>
<th>Mean</th>
<th>SD</th>
<th>Min.</th>
<th>Max.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Daily Methemoglobin</td>
<td></td>
<td>1.78</td>
<td>0.7112</td>
<td>0</td>
<td>3.7</td>
</tr>
<tr>
<td>Daily SEC</td>
<td></td>
<td>117.6</td>
<td>30.56</td>
<td>90</td>
<td>302</td>
</tr>
<tr>
<td>Daily SINS</td>
<td></td>
<td>0.167</td>
<td>0.409</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Daily Applications (isopropyl alcohol)</td>
<td></td>
<td>3.56</td>
<td>3.19</td>
<td>0</td>
<td>14</td>
</tr>
<tr>
<td>Ambient Temperature</td>
<td></td>
<td>77.19</td>
<td>3.03</td>
<td>72</td>
<td>89.5</td>
</tr>
<tr>
<td>Ambient Humidity</td>
<td></td>
<td>45.8</td>
<td>7.99</td>
<td>27</td>
<td>80</td>
</tr>
</tbody>
</table>

**Note:** Min. = minimum, Max. = maximum

(Table continues)
### Table 2 (Continued)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean</th>
<th>SD</th>
<th>Min.</th>
<th>Max.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment Group</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Daily Methemoglobin</td>
<td>2.05</td>
<td>0.491</td>
<td>1.1</td>
<td>3.6</td>
</tr>
<tr>
<td>Daily SEC</td>
<td>121.9</td>
<td>32.22</td>
<td>90</td>
<td>282</td>
</tr>
<tr>
<td>Daily SINS</td>
<td>0.369</td>
<td>1.508</td>
<td>0</td>
<td>16</td>
</tr>
<tr>
<td>Daily Applications (chlorhexidine)</td>
<td>3.49</td>
<td>2.43</td>
<td>0</td>
<td>13</td>
</tr>
<tr>
<td>Ambient Temperature</td>
<td>76.7</td>
<td>3.431</td>
<td>72</td>
<td>95</td>
</tr>
<tr>
<td>Ambient Humidity</td>
<td>44.98</td>
<td>6.92</td>
<td>28</td>
<td>61</td>
</tr>
</tbody>
</table>

**Note:** Min. = minimum, Max. = maximum

A two-sample t-test was performed to determine significant differences between the groups for birth weight (p = 0.41) and gestational age (p = 0.47). The Pearson r product moment correlation was used to detect correlation between the number of chlorhexidine applications and methemoglobin (p = 0.4275), and to detect correlation between skin breakdown and gestational age (p = 0.1300). No significant differences were found between the groups.
Analysis of Data in Relation to the Hypotheses

Hypothesis 1

Hypothesis 1 stated that methemoglobin levels were significantly higher in LBW infants treated with 0.5% chlorhexidine (treatment) than those who are treated with 70% isopropyl alcohol (control). The mean methemoglobin in the control group was 1.85 (SD = 0.726). The mean methemoglobin in the treatment group was 2.06 (SD = 0.434). There was a significant difference in the means using a univariate repeated-measures analysis of variance (F = 4.41; \( p = 0.0378 \)). These results support the hypothesis, indicating that methemoglobin levels in the two groups were significantly different.

When methemoglobin levels were adjusted for surface electrical capacitance, there was not a significant difference in the mean methemoglobin using a univariate repeated-measures analysis of variance (F = -1.77884; \( p = 0.0779 \)). The Pearson product moment correlation was performed to evaluate correlation between methemoglobin and the number of chlorhexidine applications (\( r = 0.00; 1 \ p = 0.4996 \)). Further analysis of variance was performed to evaluate significant differences of mean methemoglobin between subjects (F = 2.04; \( p = 0.0938 \)), and to evaluate significant interaction of mean methemoglobin between subject and group (F = 0.73; \( p = 0.5722 \)). Analysis of variance was performed to evaluate significant differences of mean
methemoglobin for ambient temperatures ($F = 2.56; p = 0.1121$), and to evaluate significant differences in mean methemoglobin for ambient humidity ($F = 0.53; p = 0.4691$). These tests were statistically nonsignificant.

**Hypothesis 2**

Hypothesis 2 stated that the skin integrity scores of LBW infants treated with 0.5% chlorhexidine were significantly higher than those treated with 70% isopropyl alcohol. The mean SINS of subjects in the control group was 0.148 (SD = 0.411), and the mean SINS of subjects in the treatment group was 0.406 (SD = 1.671). There was a significant difference in the means using a univariate repeated-measures analysis of variance ($F = 4.38; p = 0.0375$). These results support the hypothesis, indicating that SINS in the two groups were significantly different. Further analysis of variance adjusted for temperature and humidity found a significant difference in the SINS between groups ($F = 2.0931; p = .0375$). In addition, analysis of variance was performed to evaluate significant differences of mean SINS between subjects ($F = 4.75; p = 0.0011$), and to evaluate significant interaction of mean SINS between subject and group ($F = 1.15; p = 0.3350$). Analysis of variance was performed to evaluate significant differences of mean SINS among ambient temperatures ($F = 0.01; p = 0.9335$), and to evaluate significant differences of mean SINS among ambient humidity ($F = 1.06; p = 0.3037$). The Pearson product moment
correlation was performed to assess risk of skin breakdown for gestational age 
\( r = 0.0673; \ p = 0.1497 \), and to assess risk of skin breakdown for surface 
electrical capacitance \( r = 0.137931; \ p = 0.0188 \).

**Hypothesis 3**

Hypothesis 3 stated that surface electrical capacitance (SEC) was 
significantly higher in LBW infants with higher methemoglobin levels. This 
hypothesis was tested using the Pearson product moment correlation 
\( r = -0.0206; \ p = 0.4088 \), indicating there was not a statistically significant 
correlation between surface electrical capacitance and methemoglobin. A 
univariate repeated-measures analysis of variance was also used to determine 
any significant difference between the groups for surface electrical capacitance 
with adjustment for temperature and humidity \( F = 0.7256; \ p = 0.4689 \). The 
adjusted mean surface electrical capacitance in the control group was 126.13 
\( \text{SD} = 30.6 \), and the adjusted mean surface electrical capacitance in the 
treatment group was 122.57 \( \text{SD} = 32.88 \). These results indicated that there 
were no significant differences in surface electrical capacitance between the 
control and treatment groups, indicating similar skin maturity among all subjects. 
Further analysis of variance was performed to evaluate significant differences of 
mean surface electrical capacitance between subjects \( F = 1.40; \ p = 0.2362 \), 
and to evaluate significant differences of mean surface electrical capacitance
between groups (F = 0.53; p = 0.4689). In addition, analysis of variance was performed to evaluate significant differences of mean surface electrical capacitance among ambient temperatures (F = 0.21; p = 0.6441), and to evaluate significant differences of mean surface electrical capacitance among ambient humidity levels (F = 0.81; p = 0.3687). Analysis of variance was also performed to evaluate significant interaction in the mean methemoglobin concentration between subject and group (F = 5.61; p = .0003). The Pearson product moment correlation was performed to test for correlation between antenatal steroids and baseline surface electrical capacitance (r = 0.9996; p =0.0001).

Additional Research Questions

Measurements taken by the NOVAmeter probe require a light touch while maintaining full contact with the skin surface. Hoath and colleagues (1995) adapted their study design of surface electrical capacitance measurements so a single investigator collected those data, thus avoiding problems associated with inter-rater reliability. Analysis of variance was performed to evaluate significant differences of mean surface electrical capacitance among the principal investigator and two co-investigators who collected these data (F = 0.22; p = 0.8034). There were no significant differences among the surface electrical capacitance measurements taken by the investigator and co-investigators.
Analysis of variance was performed to evaluate significant differences of mean SINS assessed by the three investigators who collected these data ($F = 0.24; p = 0.7836$). There were no significant differences among the SINS taken by the investigators.

Summary

The results of statistical analysis were presented in this chapter. Two hypotheses were supported by the data. These data indicated that methemoglobin levels were significantly higher for those LBW infants treated with topical 0.5% chlorhexidine. There was also significantly more skin breakdown in those infants treated with topical 0.5% chlorhexidine. The third hypothesis was not supported, indicating no correlation between methemoglobin levels and surface electrical capacitance.
CHAPTER FIVE
CONCLUSIONS AND RECOMMENDATIONS

The purpose of this experimental, repeated measures design study was to determine the degree of risk of methemoglobinemia and extent of skin breakdown when 0.5% chlorhexidine was used as a topical antimicrobial in a population of low birth weight infants. Methemoglobin (MetHb) levels and skin integrity scores were compared in two groups of LBW infants.

A non-random sample of 10 subjects admitted to the Neonatal Intensive Care Unit at a large tertiary pediatric hospital in the southeastern United States completed the study. Subjects were recruited between the first day of life and 7 days of age, and informed consent was obtained from the parents. The subjects were screened for congenital methemoglobinemia, and then randomized to isopropyl alcohol 70% or chlorhexidine gluconate 0.5% for all antimicrobial applications prior to invasive procedures. Surface electrical capacitance (SEC) and skin integrity data using the Scale for Integrity of Neonatal Skin (SINS) were collected on the subjects every day until they reached postnatal day 28. Samples for methemoglobin were taken when the subject was scheduled for laboratory studies ordered by the physician, but no more than once daily.
The skin of LBW infants in the control group was prepared with the conventional antimicrobial agent, 70% isopropyl alcohol, for peripheral intravenous catheters and punctures for laboratory sampling. The site was scrubbed five times with one swab in preparation for skin puncture. Procedures such as placement of PICCs and chest tubes, needle aspiration of chest, blood culture, and lumbar puncture conventionally require a scrub with povidone-betadine according to the NICU protocol. In the control group, these procedures were prepared first with povidone-iodine, and then wiped off with sterile water. The alcohol wipes were kept in a covered, plastic disposable container clearly labeled with green “Alcohol” stickers and directions for application. All clinicians used the daily record to log each application of alcohol and povidone-iodine (Appendices H & I). The Investigator reviewed the medical record daily to verify application counts on the logs.

The skin of preterm infants in the treatment group was prepared with the treatment antimicrobial agent, chlorhexidine gluconate 0.5% aqueous solution, for peripheral intravenous catheters and punctures for laboratory sampling. The site was scrubbed five times with one chlorhexidine swab. After thirty seconds, the skin was wiped off with a sterile water swab. Procedures such as placement of peripheral intravenous central catheters (PICCs) and chest tubes, needle aspiration, blood culture, and lumbar puncture that conventionally require an
additional scrub with povidone-betadine was prepared with a liquid preparation of chlorhexidine 0.5%, left for 30 seconds, then wiped off with a sterile water swab. The liquid preparation was prepared by the NICU pharmacist by diluting a 4% solution of chlorhexidine to 0.5%. A small bottle (30 mL) was kept in a covered, plastic disposable container with a supply of chlorhexidine towelettes and sterile water wipes clearly labeled with red “Chlorhexidine” stickers and directions for application. All clinicians used the daily record to log each application of chlorhexidine (Appendix I). The investigator reviewed the medical record daily to verify application counts on the logs.

The independent variables were 0.5% chlorhexidine gluconate as the antimicrobial intervention and isopropyl alcohol. The dependent variables were the methemoglobinemia level as measured by the AVOXimeter 4000®, surface electrical capacitance measured by the NOVAmeter® on the forehead skin of the neonate, and skin breakdown measured using the Scale of Integrity of Neonatal Skin.

It was hypothesized that methemoglobin levels would be significantly higher in LBW infants treated with 0.5% chlorhexidine than those who are treated with 70% isopropyl alcohol. Additional hypotheses proposed that the skin integrity scores of LBW infants treated with 0.5% chlorhexidine would be significantly higher than those treated with 70% isopropyl alcohol. It was further
hypothesized that surface electrical capacitance would be positively correlated with methemoglobin levels. Univariate repeated-measures analysis of variance was used to test differences in methemoglobin levels and SINS between the groups. The Pearson r product moment correlation was used to test the correlation between methemoglobin and surface electrical capacitance.

The first section of this chapter discusses the research findings of this study. The theoretical links between the research findings and Levine’s Conservation Principles is shown. The theoretical links between the research findings and the effectiveness of theory-driven evaluation are also demonstrated. Conclusions and recommendations for practice, education and research are presented in the latter part of this chapter.

**Discussion of Findings**

**Risk of Methemoglobin with 0.5% Chlorhexidine**

Methemoglobin levels were significantly higher in subjects treated with chlorhexidine than those treated with alcohol. The most dilute chlorhexidine product commercially available that provides appropriate topical antimicrobial action was used in the procedure to minimize chemical exposure. Levine’s first principle, conservation of energy, guides nurses towards atraumatic care (1967). The nursing intervention to prepare LBW infant skin for an invasive procedure was designed to cause the least additional demands possible on subjects.
Examining these results using the organizing framework formulated by Bradin and Sidanis (1998) enables a systematic view about the phenomena that occurred. The elements of this theory that helped to guide the design of the study interventions are discussed in relation to the first hypothesis. The first element was that the problem definition must specify which condition is treatable, in which populations, and under what circumstances. In this study, a dilute (0.5%) concentration of chlorhexidine was used and wiped off the skin after a thirty second period to minimize opportunities for absorption. Since chlorhexidine is a known agent that causes methemoglobinemia, it was expected that topical absorption would occur and cause significantly higher levels in those LBW infants whose skin was disinfected with it. Removal after thirty seconds most likely minimized topical absorption, yet higher levels of methemoglobin still occurred with use of chlorhexidine. The chlorhexidine was applied in the immediate newborn period between the first day of life and day twenty-eight, when the LBW infant skin is most permeable. A related finding was no significant difference in methemoglobin means when the methemoglobin was adjusted for surface electrical capacitance. These results indicate that the subjects with similar skin maturity and permeability had similar methemoglobin levels.
Similar results were reported with use of Eutectic Mixture of Local Anesthetics (EMLA) cream, used for analgesia in newborn circumcision (Law et al. 1996). These researchers also reported a statistically significant increase in methemoglobin ($p = 0.049$) in newborns who were exposed to 1 gram of EMLA cream on the foreskin. The highest serum concentration they measured was 3%, and no infant demonstrated clinical signs of methemoglobinemia. They concluded that EMLA was safe to use as a local anesthetic in term neonates. Lander and colleagues (1997) also reported higher methemoglobin levels in newborns treated with EMLA cream for circumcision, but none required treatment.

The second element defined by Bradin and Sidanis (1998) was the critical input which delineates the practical and prescriptive aspects of the intervention. The critical input of the chlorhexidine intervention was the vigorous back and forth scrub (mode of delivery), for five times (dose), using 0.5% dilution (strength), and removal after 30 seconds (duration). This procedural aspect of the study was not directly measured. Nurses and laboratory support staff were routinely observed for adherence to the protocol, and in only a few instances required reminding. Staff often waited to obtain blood samples until they verified the correct procedure.
Mediating processes, the third critical element of intervention theory, referred to the intervening variables to which the process was linked. Intervening variables in this study included ambient temperature and humidity. There were no significant differences of mean methemoglobin for the variables ambient temperature or ambient humidity. These were expected findings because it is unlikely that methemoglobin would be influenced by these types of environmental conditions.

Another mediating process was the removal of the chlorhexidine after 30 seconds. Since the chlorhexidine is clear, it was impossible to know when all of it had been removed. There is a possibility that traces of chlorhexidine were still on the subjects' skin when methemoglobin levels were sampled, as described by Cowen & colleagues (1979). The residual chemical may have oxidized the hemoglobin while the sample was being collected and transferred into the cuvet. Methemoglobin levels would have been higher in the chlorhexidine group, since alcohol does not affect the hemoglobin in the same manner.

The fourth element was the expected outcomes which included the expected timing or pattern of changes and interrelationships among the outcomes. In this study the expected outcome of using 0.5% chlorhexidine as the antimicrobial with preterm infants was that the level of methemoglobin may be higher in infants with the most frequent chlorhexidine applications. Each
support staff were routinely observed for adherence to the protocol, and reminded of it when necessary. Methemoglobin levels were not correlated to the number of chlorhexidine applications \((r = 0.0222, p = .4275)\). Chlorhexidine did not cause a significant increase in mean methemoglobin in the treatment subjects, and neither of the dependent variables related to absorption (surface electrical capacitance, number of applications) tested at statistical significance. The number of daily chlorhexidine applications on each subject was variable depending upon the subjects' need for procedures. The subjects in the treatment group required as many as 13 daily applications \((\text{mean} = 3.5, \text{SD} = 2.43)\) to prevent infection during procedures. The control group subjects had similar requirements with a range between 0 and fourteen applications \((\text{mean} = 3.56, \text{SD} = 3.19)\). There were no significant differences in these means using a paired t-test \((p = 0.4213)\). These results also help confirm that the two populations of subjects were similar.

The fifth element was the extraneous factors which included contextual or environmental factors and client characteristics that significantly affected treatment processes and intervention outcomes. Extraneous factors anticipated in this study included addition of humidity to the microenvironment. This extraneous factor did not occur, except in one instance. One control subjects' microenvironment had added humidity (80%) for a single day. Hygrometer
microenvironment had added humidity (80%) for a single day. Hygrometer measurements were low (30-40%) when the study began in the Fall of 2000, and increased (40-50%) in the Spring of 2000.

Topical application of 0.5% chlorhexidine left on for 30 seconds and then wiped off with a sterile water swab increases the risk of methemoglobinemia in LBW infants with birth weight between 750 and 1500 grams. The results do not appear to be related to the number of chlorhexidine applications. They may be related to the postnatal or gestational age of the infant. The differences in means, however, are clinically small and still within normal levels for term infants. There were no significant differences of mean methemoglobin between subjects or between subject and group, indicating that the statistical differences of means between groups may not be clinically relevant. Despite the significant difference in the mean methemoglobin levels, the methemoglobin levels measured in the treatment group were safe and clinically acceptable. These results should raise concern however, for nursing professionals who use chlorhexidine in greater concentrations, in repeated applications, for durations greater than 30 seconds, and without removing it completely.

Chlorhexidine is commercially available in 2% and 4% preparations. Respondents of the NICU-NET list-serve survey indicated that these preparations are being used with little regard of absorption. Although the
difference in the means are probably not clinically significant for LBW infants treated with 0.5% chlorhexidine, the same cannot be assumed for greater concentrations, or use in smaller, more immature infants. These results should also raise concern for nursing professionals who do not routinely remove the chlorhexidine after the antimicrobial effect has been achieved. The results of this study support strict removal of 0.5% chlorhexidine after a thirty second interval to avoid increases in methemoglobin to potentially unsafe levels. Unlike povidone-betadine, chlorhexidine is colorless. Once applied to the skin, it is impossible to see where it was placed. There are no identifying markers to alert clinicians to its presence so it may be removed completely. The clinician who applies the chlorhexidine must know to wipe down the same area with sterile normal saline or water wipes within 30 seconds to remove it.

Risk of Skin Breakdown with 0.5% Chlorhexidine

There was a significant increase in skin integrity scores of LBW infants treated with 0.5% chlorhexidine in comparison to those treated with 70% isopropyl alcohol. Levine’s second principle, conservation of structural integrity, was operationalized by limiting the amount of time the LBW infant skin was exposed to the potential damaging effects of the chlorhexidine, yet a significant difference in skin breakdown was detected. The SINS was used to assess the overall skin integrity of the subjects, rather than assess the specific sites where
as described in the literature (Garland et al. 1995) should be easily assessed with this tool, but skin breakdown was not reported with the use of chlorhexidine in this study. Areas where chlorhexidine was applied included the feet, ankles, and antecubital sites. The most significant skin breakdown documented occurred on one subject’s abdomen, and that occurred before entry into the study.

Significant differences in SINS scores were not found between subjects. The one high SINS score (16) reflected severe excoriation on the subject’s abdomen due to aggressive use of adhesive at the referring facility, and was assessed at the time of entry to the study. The SINS assessment was not related to the use of chlorhexidine in this subject, although she was in the treatment group. Her abdominal skin healed quickly and was given a score of 1 on day 3 of the study. All but one of the other infants were given SINS scores of zero or one during the study period. The last subject was also admitted with peeling, fragile skin. The high SINS score in this treatment subject was three. There was no interaction between subject and group in SINS. There were no significant differences in the SINS between groups when adjusted for temperature and humidity. These findings reflect the importance of humidity for good skin health in this population.
Another expected outcome (Braden and Sidanis, 1998) of using 0.5% chlorhexidine as the antimicrobial with preterm infants is that the risks of skin breakdown would increase as gestational age decreased and measurements of surface electrical capacitance increased. There was no correlation found, however, between skin breakdown and gestational age. The small sample size may not have enabled detection of differences among the gestational ages. There was a significant correlation between skin breakdown and surface electrical capacitance ($p = 0.01$). These findings indicate that LBW infants with the least mature skin are at most risk of skin breakdown, results consistent with Lund’s extensive review of the literature (1999). The small sample size also did not enable analysis of any potential gender differences in skin breakdown or skin maturity (surface electrical capacitance).

**Relationship Between Surface Electrical Capacitance and Methemoglobin**

Hypothesis 3 stated that surface electrical capacitance is significantly higher in LBW infants with higher methemoglobin levels. Surface electrical capacitance has been inversely correlated with transepidermal water loss (TEWL) as barrier function develops (Okah et al. 1995), and high TEWL is strongly correlated to immature stratum corneum barriers (Kalia, Nonato, Lund, & Guy, 1998). Data analysis did not support the hypothesis, indicating there was not a correlation between surface electrical capacitance and
methemoglobin. The immature stratum corneum of the LBW infant provides a feasible transdermal drug delivery route (Kalia, Merino, & Guy, 1998; Benis, 1999). Kalia, Nonato, Lund, & Guy (1998) contend that premature, LBW infants have an increased susceptibility to percutaneous uptake that provides opportunity for therapeutic, beneficial, and atraumatic administration of medication. Since chlorhexidine is a potentially noxious chemical that compels minimal skin exposure, 30 seconds was not enough time to absorb enough chemical to enable correlation with surface electrical capacitance. There was also no significant difference in surface electrical capacitance between and among the control and treatment groups, indicating similar skin maturity among all subjects.

The finding that there were no significant differences in mean surface electrical capacitance among ambient temperatures was expected. Preterm infants typically require high ambient temperatures to establish and maintain a neutral thermal environment. When humidity is added, the ambient temperature drops when the infant's temperature is managed by servo-control. Ambient humidity levels are correlated with TEWL (Hammarlund & Sedin, 1979; Wilson & Maibach, 1980; Hammarlund & Sedin, 1982; Sedin et al. 1985), but in much higher ranges (> 80%) than were measured in this study. Only one patient (control) had added humidity, and only for a single day. Interestingly, when
neutral thermal environment. When humidity is added, the ambient temperature drops when the infant’s temperature is managed by servo-control. Ambient humidity levels are correlated with TEWL (Hammarlund & Sedin, 1979; Wilson & Maibach, 1980; Hammarlund & Sedin, 1982; Sedin et al. 1985), but in much higher ranges (> 80%) than were measured in this study. Humidity was not routinely added to microenvironments in the NICU where the study took place. Only one patient (control) had added humidity, and only for a single day. Interestingly, when ambient humidity in this subjects’ incubator increased from 43% to 80%, the subject’s surface electrical capacitance dropped from 302 to 202.

The subjects were born equally distributed to mothers who were treated with antenatal corticosteroids. There was a positive correlation between antenatal steroids and baseline surface electrical capacitance, which was expected. Aszterbaum and colleagues (1993) reported significant acceleration of the epidermal barrier function in fetal rats whose mothers were treated with antenatal glucocorticoids. Antenatal glucocorticoids were also associated with significant acceleration of the epidermal barrier function in a study of preterm infants (Kalia, Nonato, Lund, & Guy, 1998). Subjects whose mothers were treated with antenatal steroids to accelerate surfactant development had lower baseline TEWL, reflecting acceleration of the stratum corneum barrier.
Conclusions

These results were interpreted using the elements described by Bradin and Sidanis (1998), providing a systematic approach to interpretation of the findings. The expected outcomes included the timing or pattern of changes and interrelationships among the outcomes. In this study the expected outcome of using 0.5% chlorhexidine as the antimicrobial with preterm infants was shown to increase the risks of methemoglobinemia and skin breakdown. Although the risks of methemoglobinemia were significantly increased in the chlorhexidine group, serum levels were within normal limits. The many precautions taken to protect the subjects from unnecessary harm were likely necessary to keep the methemoglobin levels within normal limits. The differences in the methemoglobin levels may have occurred when the samples were contaminated by traces of chlorhexidine on the subjects' skin. Clinicians who use chlorhexidine in low birth weight infants should be vigilant about removing it after thirty seconds, and using the least dilute concentration available. It should never be used on excoriated skin where absorption is increased.

The variables related to absorption (surface electrical capacitance and number of applications) were not predictive of methemoglobin levels. Neither of these variables demonstrated a significant difference in their means between the groups. Infants in both groups had the same skin maturity and the same number...
of daily antimicrobial applications. Further investigation is needed to determine if surface electrical capacitance actually reflects the ability to absorb chemical agents administered topically. Serum measurement of chlorhexidine would provide an accurate reflection of topical absorption.

Recommendations for Further Research

Sample

This study was limited to a small population of high risk infants in the NICU. These subjects have immature skin in comparison to fullterm newborns, but their skin is not as immature as extremely low birth weight (ELBW) infants. Extremely LBW infants are typically described as those with birth weight less than 750 grams. They are usually born at less than 26 weeks gestational age. This population is at most risk for topical absorption of antimicrobials due to their severely immature skin barrier properties. Although methemoglobin levels were significantly higher in the LBW infant population treated with 0.5% chlorhexidine, the measured levels were safe. It is recommended that the study be replicated in a group of ELBW infants. Since ELBW infants are at highest risk for nosocomial infection, testing the safety of the most effective antimicrobial available appears to be in their best interest. It is expected that this population will have more skin breakdown, since their skin is extremely immature.
expected that this population will have more skin breakdown, since their skin is extremely immature. The design should enable determination of skin breakdown that occurs as a direct result of chlorhexidine, in addition to skin breakdown that occurs from other interventions, such as use of adhesives.

Although the design did not exclude Caucasian infants, only three entered and completed the study. One set of twins was biracial. Replicating this study with a greater number of Caucasian infants is necessary to examine differences in risk of methemoglobinemia between the races. African-American infants may also have less skin breakdown than Caucasian infants. Hanley and colleagues (1996) have demonstrated gender differences in epidermal barrier formation in fetal rats based on hormonal differences. Two of the three infants with baseline SINS scores greater than one were Caucasian.

A study is needed to examine differences in baseline methemoglobin levels in preterm infants between infants of smoking and non-smoking mothers. In this study, only one mother reported smoking, at a rate of less than one pack of cigarettes daily. Smoking increases methemoglobin levels in adults (Imbriani, Melotti, & Ghittori (1987), and it is logical to assume that maternal methemoglobin levels are shared with the fetus. There may be a significant difference in baseline mean methemoglobin levels in LBW infants whose mothers smoked cigarettes during their pregnancy and those who did not.
methemoglobin levels for preterm infants. The appearance of measurable baseline levels raised three research questions during the course of the study: Are there significant differences among baseline methemoglobin levels in this population of preterm infants? Are there significant differences in baseline methemoglobin levels in preterm infants between 25-28 weeks and 29-32 weeks gestational age? Do maternal smoking habits significantly increase methemoglobin levels in LBW infants? These questions require a larger sample size, and the results have the potential to contribute to a scientific knowledge base that is sparse in this topic area.

**Research Design and Measurement**

The majority of methemoglobin data were collected in the first 2 weeks of life in this study. There were limited opportunities to measure methemoglobin after 2 weeks of life, since the subjects no longer required frequent laboratory sampling. Nurses were helpful notifying the investigator when blood glucose checks or blood gas analysis were planned, but many forgot, and sampling opportunities were lost. An alternative design would focus data collection during the first two weeks of life in the LBW infant population. There are many opportunities for laboratory sampling in this time period, and the most significant changes in epidermal barrier occur. The changes in methemoglobin and surface electrical capacitance means during the first two weeks of life may be more robust.
opportunities for laboratory sampling in this time period, and the most significant changes in epidermal barrier occur. The changes in methemoglobin and surface electrical capacitance means during the first two weeks of life may be more robust.

The SINS provided a broad assessment of the skin integrity, rather than a focused assessment of the areas where the antimicrobial swabs were used. The SINS was chosen because the sites of antimicrobial application were extraneous variables that could not be controlled. Many sites were swabbed daily. The researcher believed the SINS would be useful in identifying differences of skin integrity between the two group, which it was able to do. Two subjects had high (3 and 16) SINS scores, and both were in the treatment group. The SINS assessment was not related to the use of chlorhexidine in these subjects because the skin breakdown occurred before admission to the NICU. The design would be improved by changing the procedure to document and assess all sites of chlorhexidine application. Skin breakdown that occurs before admission or entry into the study should be excluded from assessment of skin breakdown related to chlorhexidine application.

The surface electrical capacitance measurement did not reflect the absorption properties of the skin as predicted. It is a direct measure of impedance that reflects the water desorption from the skin. Skin that desorbs
Finally, the samples of methemoglobin from peripheral sites may have been contaminated with chlorhexidine, causing the slightly higher levels in the treatment group. It is possible that blood was contaminated with chlorhexidine as it was collected into the blood collection device, where it immediately began to oxidize. Replicating this study on a sample of LBW infants with central access such as an umbilical arterial catheter would help to eliminate this confounding variable. The same study controlling for site collection would enable comparison of methemoglobin levels sampled from central and peripheral sites. Central sites are not prepared with antimicrobial agents because they are accessed through a 3-way stopcock.

**Recommendations for Practice and Education**

Methemoglobin levels were significantly increased in LBW infants treated with 0.5% chlorhexidine, but the levels were within normal limits. Many NICU facilities currently use topical chlorhexidine in different concentrations as the standard antimicrobial for all their patients. Based on the results of this study, the investigator cautions the use of 0.5% chlorhexidine in LBW infants. It appears to be a safe practice, but only if completely removed after 30 seconds. Chlorhexidine is most likely very safe in full term newborns who do not have alteration in skin integrity (Lund, Kuller, Lane, Lott, & Raines, 1999).

Chlorhexidine should be avoided in those infants who have exposure to other
agents known to cause methemoglobinemia because the risks of interaction between two agents have not studied. The most common agents in the NICU today that cause methemoglobin are nitric oxide and EMLA cream.

Chlorhexidine is the most effective antimicrobial available in the United States, and as such, has the potential to significantly reduce the incidence of nosocomial infection in the NICU. Infants admitted to the NICU have one of the highest rates of nosocomial infection among hospital patients (Parvez & Jarvis, 1999) because they are exposed to many infectious pathogens during their hospitalization. The cost of chlorhexidine towelettes is about $0.13 each. Isopropyl alcohol is so inexpensive (< $0.01 each) that chlorhexidine will certainly increase the cost of NICU hospitalization. The costs associated with prolonged NICU hospitalization due to nosocomial infection, however, is staggering. The risks and benefits of all nursing interventions that prevent nosocomial infection should be considered. This intervention has the potential to reduce the incidence of nosocomial infection, which would decrease length of stay and decrease costs of hospitalization.

Results of this study serve to emphasize the value of education for all NICU personnel. Education is recommended to all NICU staff who currently use chlorhexidine, or who are considering implementing its use. The use of chlorhexidine significantly increased the methemoglobin levels in LBW infants.
Isopropyl alcohol is inexpensive (< $0.01 each) that chlorhexidine will certainly increase the cost of NICU hospitalization. The costs associated with prolonged NICU hospitalization due to nosocomial infection, however, are staggering. The risks and benefits of all nursing interventions that prevent nosocomial infection should be considered. This intervention has the potential to reduce the incidence of nosocomial infection, which would decrease length of stay and decrease costs of hospitalization.

Results of this study serve to emphasize the value of education for all NICU personnel. Education is recommended to all NICU staff who currently use chlorhexidine, or who are considering implementing its use. The use of chlorhexidine increased the methemoglobin levels in LBW infants during the first month of life, although not significantly. Because the levels were within normal limits, no subject required treatment for methemoglobinemia. Elevated levels occurred using the most dilute preparation of chlorhexidine available, timing exposure for only 30 seconds, and removing it completely with sterile water. Timing the exposure and removing the chlorhexidine may represent two additional steps for many hospital clinicians as they prepare a site for a procedure. These two steps, however, are vital to protect the LBW infant from increased methemoglobin levels previously associated with topical chlorhexidine (Cowen et al. 1979; Hjelt et al. 1995). Other important considerations for
education include restricting its use to LBW infants greater than 750 grams, and
those with intact skin. The liquid preparation of 0.5% chlorhexidine was not
needed at any time during this study, and was therefore not evaluated.

The Scale for Integrity of Neonatal Skin (SINS) is recommended for use in
general neonatal skin assessment. It provided a standardized assessment of
overall skin integrity in a population of LBW infants. High scores reflected
alterations in skin integrity, which decreased as skin integrity improved. The
SINS provided a common language among clinicians with which to describe the
extent of skin breakdown. Content validity has been established by a panel of
national experts, and a study is currently underway to establish validity and
reliability. The SINS is easy to use, only requiring a brief orientation to the
descriptors and scoring procedure. It was used once daily in this study, but could
be incorporated into the routine nursing assessments performed two or three
times daily to evaluate the outcomes of nursing care. Skin care is a fundamental
role of nursing professionals, yet a paucity of standardized skin care protocols
exist (Lott & Hoath, 1998). The SINS may enable evaluation of neonatal skin
care protocols by providing a consistent measure of overall skin integrity. It was
very helpful in assessing the impact of 0.5% chlorhexidine on LBW infant skin.
Limitations of the Study

The design did not test chlorhexidine solutions stronger than 0.5%, because it was assumed that a stronger solution is potentially more toxic to this population. Indeed, methemoglobin levels were measurably higher with 0.5% chlorhexidine, although the difference did not achieve statistical significance. This aspect of the design limits the generalizability of these results, since many NICU personnel use concentrations of .75%, 2%, and 4% chlorhexidine on LBW and ELBW infants. The risk of methemoglobinemia using these concentrations remains unknown.

The design also limited the sample to LBW infants with birth weight between 750 and 1500 grams. Infants with birth weight less than 750 grams are at greatest risk for nosocomial infection, skin breakdown associated with use of chlorhexidine (Garland et al. 1995), and topical absorption of chemicals (Kalia, Marino, & Guy, 1998). Chlorhexidine of even the most dilute concentration should not be used in the extremely LBW infant population (less than 750 grams) unless methemoglobin levels are measured serially to assure safety. Great care must also be taken to remove chlorhexidine with a non-toxic solution such as normal saline or sterile water.

It was not possible to blind nurses or investigators to the comparison of two antimicrobials when they assessed skin breakdown. Investigators anticipated this potential bias to be modified by discussing nursings' assessment
of the skin and using that assessment to rate the infant’s skin integrity together. The skin assessment was performed by the bedside nurse and communicated to the investigator before a SINS was assigned. The SINS was not significantly higher in the treatment group. The skin breakdown that was documented occurred before entry into the study.

Finally, the data collection did not discern the cause of skin breakdown in subjects. Medical interventions known to cause skin breakdown in LBW infants such as use of adhesives were not documented. Similarly, many sites were used by care providers for IV insertion or laboratory sampling, such as fingers, heels, scalp, antecubital and saphenous veins. The sites chosen for preparation with chlorhexidine were not documented. The SINS score provided an overall assessment of the skin integrity, which was not always related to use of alcohol or chlorhexidine.

Summary

This chapter presented the conclusions and recommendations of the investigator. Suggestions for further research, including sample and design considerations, were discussed. Recommendations for implementation into practice and the education required were also discussed. Finally, the limitations of the study were discussed and should be considered in any replication.
APPENDIX A
RISK FACTORS OF LOW BIRTH WEIGHT IN THE UNITED STATES

low socioeconomic status
women under age 16 or over 35
African-American women
maternal smoking
acute or chronic maternal illness
multiple-gestation births
obstetric factors
fetal conditions
iatrogenic early delivery
maternal activity requiring long periods of standing or substantial amounts of physical stress

Source: Pursley & Cloherty, 1998
APPENDIX B
AGENTS THAT OXIDIZE HEMOGLOBIN

1. Direct Oxidation: Ferricyanide, Copper, Hydrogen peroxide, Hydroxylamine
   Others: Chromate, chlorate, nitrogen trifluoride, tetranitromethane, quinones, dyes.

2. Interaction with Oxygen: Nitrites, nitroglycerin, Hydrazines, Thiols
   Others: Arsine, aminophenols, arylhydroxylamines, N-hydroxyurethane, phenylenediamines

3. Requiring Biochemical Transformation
   Aniline, dyes (diaper & laundry inks, red wax crayons)
   Sulfonamides, Procaine derivatives, 4,4'-Diaminodiphenylsulfone (dapsone)
   8-Aminoquinolines: primaquine and pamaquine
   N-Acylarylamines: acetanilid, penacetin

APPENDIX C
THE PROCEDURE FOR DETERMINATION OF METHEMOGLOBIN

1. Prepare a blank cuvet containing 1.5 mL of phosphate buffer and 1.5 mL of H₂O₂. Designate the cuvet C₁.

2. Pipet 0.1 mL of whole blood into a test tube containing 3.9 mL of distilled H₂O₂; swirl to mix.

3. Add 4.0 mL of potassium phosphate buffer and mix thoroughly.

4. Transfer 3 mL of hemolysate to each of two cuvets; designate these C₂ and C₃.

5. To cuvette C₃, add 0.1 mL of potassium ferricyanide solution. Cover with Parafilm, mix by inverting three times, and measure its absorbance at 2 minutes.

6. Measure the absorbance at 630 nm for cuvets C₂ and C₃, using C₁ as blank. Record as A₂ and A₃.

7. Add 0.1 mL of KCN to all cuvets. (Use a safety pipet or add 2 drops from a transfer pipet fitted with a rubber bulb). Mix by inverting 3 times, and allow to stand for 5 minutes.

(Appendix continues)
APPENDIX C (continued)

8. Measure absorbance at 630 nm for cuvets C<sub>2</sub> and C<sub>3</sub> with C<sub>1</sub> as blank.

Record as A<sub>2b</sub> and A<sub>3b</sub>.

9. Calculate:

\[
\text{MethHb (percent of total pigment)} = 100 \frac{A_{2b} - A_{2b}}{A_{3b} - A_{3b}}
\]

APPENDIX D
SCALE FOR INTEGRITY OF NEONATAL SKIN
(SINS)

Directions:

Add the scores associated with the descriptors for a total score of all visible skin breakdown, including the front and back of the patient. Determine measurable areas of skin breakdown by measuring square centimeters of breakdown with a paper tape.

Date: _____________ Time: _______________________

<table>
<thead>
<tr>
<th>Descriptor</th>
<th>Scale</th>
<th>Score</th>
<th>Sites</th>
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<tr>
<td>Intact</td>
<td>0</td>
<td></td>
<td>□ R / L hand □ R / L foot □ R / L leg</td>
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<td>□ R / L arm □ groin / perianal □ buttocks</td>
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<td>□ chest</td>
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<td>□ abdomen □ neck □ lower back □ upper back</td>
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<td></td>
<td></td>
<td>□ face □ head □ other</td>
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<tr>
<td>Discolored: Red, Blue, White,</td>
<td>1 for each area not connected</td>
<td>Score</td>
<td>Sites</td>
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<td>Black, or other:</td>
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<td>Granulation</td>
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<tr>
<td>Glistening: shiny or blistering</td>
<td>2 for each area not connected</td>
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<td>skin</td>
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<tr>
<td>Skin Loss: loss of epidermis.</td>
<td>square centimeters of skin loss</td>
<td>Score</td>
<td>Sites</td>
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<td>□ R / L hand □ R / L foot □ R / L leg</td>
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APPENDIX E  
INFORMED CONSENT

You are being asked to participate in a research study. This form provides you with information about the study. The Principal Investigator (the person in charge of this research) or his/her representative will also describe this study to you and answer all of your questions. Read the information below and ask questions about anything you don’t understand before deciding whether or not to take part. Your participation is entirely voluntary and you can refuse to participate without penalty or loss of benefits to which you are otherwise entitled.

What is the purpose of this study?

The purpose of this study is to compare the risks of topically applied 0.5% chlorhexidine with isopropyl alcohol 70% in preterm infants. Because preterm infants are very susceptible to infection, we want to use the skin cleanser that removes the most bacteria. We know that chlorhexidine is the best skin cleanser for infants, and need to test if it is as safe as alcohol. The time your infant will need to spend in this research study will be about 4 weeks.

What will be done if your baby takes part in this research study?

If you consent, your infant will be assigned to either the alcohol or chlorhexidine skin cleaner. Your infant’s skin will be wiped with that cleaner before IV insertion and lab samples taken from the skin. The skin will be scrubbed five times with one swab, left for thirty seconds, then wiped off with a sterile normal saline swab. If your baby is assigned to the alcohol group, the skin will be first cleaned with betadine for procedures such as placement of percutaneous intravenous central catheters (PICC), chest tube placement, needle aspiration, blood culture, and lumbar puncture. Once every day, we will take 2 drops of blood when other lab work is being done. We will also measure the skin maturity and assess the skin condition.

Your infant should not participate in this study if any of the following apply: congenital methemoglobinemia; surgical emergencies such as gastroschisis, and exposure to other known agents that increase the risk of methemoglobinemia, such as aniline marker dyes, prilocaine, and nitric oxide.
What are the possible discomforts and risks?

There is a small risk of methemoglobinemia if your baby’s skin is cleaned with chlorhexidine for procedures. Methemoglobin is a problem since it binds to red blood cells and decreases the amount of oxygen that red blood cells release to tissues. This condition happens slowly and is reversible. We will keep the risk low by wiping off the chlorhexidine after 30 seconds and checking 2 drops of your baby’s blood every day. The risk also decreases every day as your baby gets older. In some other life-saving treatments that cause methemoglobinemia, the levels are allowed to get as high as 20% without a problem for the babies. In this study, the highest we will let the methemoglobin get is 7%. A baseline level of methemoglobin will be tested to see if your baby has congenital methemoglobinemia. There is no known risk of methemoglobinemia if your baby’s skin is cleaned with alcohol for procedures. There is a small risk of skin irritation with chlorhexidine, and this will be decreased by wiping it off after 30 seconds.

There may be some discomfort associated with taking 2 drops of blood, but we will only do the test when blood is being taken during regular daily labs. There is no known risk associated with the skin assessment, and no known risk associated with testing the skin for maturity.

The investigators will immediately tell you if during the study they discover that chlorhexidine causes other new and unknown side effects. If the new findings make it unwise for your infant to continue, the investigators will stop your infant’s treatment. They will then offer you other suitable treatment for your infant’s procedures.

If you wish to discuss the information above or any other discomforts your infant may experience, you may ask questions now or call the Principal Investigator listed on the front page of this form.

What are the possible benefits to you or your infant?

We don’t think that you or your infant will benefit directly from being in this study, if at all. On the other hand, by taking part in this research study, your infant may help to increase our overall knowledge of preterm infant skin and how to treat future patients.

If you choose to take part in this study, will it cost you anything?

There are no costs to you if you choose to have your infant participate in this study.
Will you receive compensation for your participation in this study?

You will not receive any payment or compensation for your infant participating in this study.

What if you are injured because of the study?

If your infant experiences an injury that is directly caused by this study, only professional medical care that your infant receives at the University of Florida Health Science Center will be provided without charge. However, hospital expenses will have to be paid by you or your insurance provider. No other compensation is offered.

If you do not want to take part in this study, what other options or treatments are available to you?

If you choose not to participate, your infant’s skin integrity will be evaluated by the staff according to the standard of care. Participation in this study is entirely voluntary. You are free to refuse to be in the study, and your refusal will not influence current or future health care you or your infant receive at this institution.

How can you withdraw your infant from this research study?

If you wish to stop your infant’s participation in this research study for any reason, you should contact Denise Poirier Maguire, RN at [phone number]. You are free to withdraw your consent and stop participation in this research study at any time without penalty or loss of benefits to which you are otherwise entitled. Throughout the study, the researchers will notify you of new information that may become available and that might affect your decision to remain in the study.

In addition, if you have any questions regarding your rights as a research subject, you may phone the Institutional Review Board (IRB) office at [phone number].

How will your privacy and the confidentiality of your research records be protected?

Authorized persons from the University of Florida, the hospital or clinic (if any) involved in this research, and the Institutional Review Board have the legal right to review your research records and will protect the confidentiality of those records to the extent permitted by law. If the research project is sponsored or if it is being conducted under the
authority of the United States Food and Drug Administration (FDA), then the sponsor, the
sponsor’s agent, and the FDA also have the legal right to review your research records.
Otherwise, your research records will not be released without your consent unless
required by law or a court order.

If the results of this research are published or presented at scientific meetings, your
identity will not be disclosed.

**Will the researchers benefit from your participation in this study (beyond
publishing or presenting the results)?**

The researcher will not benefit from your infant’s participation in this study.

**Signatures**

As a representative of this study, I have explained the purpose, the procedures, the
benefits, and the risks that are involved in this research study:

______________________________  ______________________________
Signature of person obtaining consent  Date

You have been informed about this study’s purpose, procedures, possible benefits and
risks, and you have received a copy of this Form. You have been given the opportunity to
ask questions before you sign, and you have been told that you can ask other questions at
any time. You voluntarily agree to participate in this study. By signing this form, you are
not waiving any of your legal rights.

______________________________  ______________________________
Signature of Subject  Date

______________________________  ______________________________
Signature of Witness (if available)  Date

If you are not the subject, please print your name and indicate one of the following:

______________________________
_____ The subject’s parent  _____ A durable power of attorney

______________________________
_____ The subject’s guardian  _____ A proxy

______________________________
_____ A surrogate  _____ Other, please explain:
# APPENDIX F
DATA COLLECTION: DAILY LOG

<table>
<thead>
<tr>
<th>Date</th>
<th>Patient:</th>
<th>Group: □ CHG □ Alcohol</th>
<th>Date</th>
<th>Patient:</th>
<th>Group: □ CHG □ Alcohol</th>
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<tbody>
<tr>
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APPENDIX G
DATA COLLECTION: DEMOGRAPHIC RECORD

INCLUSION CRITERIA: BW 750-1500 gm; 0-7 days old; English speaking parents.

EXCLUSION CRITERIA: exposure to aniline marker dyes, "caines", nitric oxide; surgical emergencies; congenital anomalies that alter basic barrier property of the skin.

Date entered into study: Subject #:

<table>
<thead>
<tr>
<th>EGA (dates)</th>
<th>EGA (exam)</th>
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</thead>
<tbody>
<tr>
<td>birth weight</td>
<td>Postnatal age</td>
</tr>
<tr>
<td>grams</td>
<td>Days</td>
</tr>
<tr>
<td>Base MethHb</td>
<td>Base SEC</td>
</tr>
<tr>
<td>Base SINS</td>
<td>Apgar Score 1: 5:</td>
</tr>
<tr>
<td>Group</td>
<td>□ Alcohol □ CHG Aquaphor? □Yes □ No</td>
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<tr>
<td>Humidity?</td>
<td>□Yes □ No</td>
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</table>

Procedures completed before entry:
□ UA/UV □ CT □ LP □ Bld Cx □ PICC □ PIV

Maternal History:

<table>
<thead>
<tr>
<th>Age</th>
<th>PROM □Y □N</th>
<th>Race of Mo</th>
<th>C B A H Bi Other:</th>
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</thead>
<tbody>
<tr>
<td>Grav</td>
<td>Smoker □Y □N</td>
<td>Race of Fa</td>
<td>C B A H Bi Other:</td>
</tr>
<tr>
<td>Para</td>
<td>Packs 1 2 3 4</td>
<td>Delivery</td>
<td>□NVD □ C/S</td>
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</tbody>
</table>
Antenatal steroids: X 1 2 3 4 doses type
APPENDIX H
DAILY COUNT OF ALCOHOL APPLICATIONS

Directions: Keeping track of the number of alcohol swab applications will help establish the "dose" or exposure to the antimicrobial. Please keep track of how many alcohol swabs are used on this patient's skin every day. Mark one tick for every swab used, using the fifth to cross off a group. Use the betadine column for those highly invasive procedures such as LP, CT insertion, etc, where betadine solution is used instead of alcohol. For example, four applications looks like this: ₁ ₁ ₁ ₁, and five applications looks like: ₁ ₁ ₁ ₁ ₁.

NAME:

<table>
<thead>
<tr>
<th>Date</th>
<th>Betadine</th>
<th>Alcohol wipes</th>
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APPENDIX I
DAILY COUNT OF HIBISTAT APPLICATIONS

Directions: Keeping track of the number of Chlorhexidine (Hibistat) applications will help establish the "dose" or exposure to the antimicrobial. Please keep track of how many Hibistat swabs are used on this patient's skin every day. Mark one tick for every swab used, using the fifth to cross off a group. Use the .5% soln' column for those highly invasive procedures such as LP, CT insertion, etc, where a diluted solution of Chlorhexidine is used instead of the Hibistat wipes. For example, four applications looks like this: ||||, and five applications looks like: |||||.

NAME:

<table>
<thead>
<tr>
<th>Date</th>
<th>.5% Soln'</th>
<th>Hibistat wipes (5 scrubs, wait 30 seconds, wipe off with sterile H₂O)</th>
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APPENDIX J
NICU PATIENTS CURRENTLY ENROLLED

<table>
<thead>
<tr>
<th>NAME</th>
<th>GROUP</th>
<th>DATE ENROLLED</th>
<th>EXPECTED TO COMPLETE ON:</th>
<th>BED SPACE</th>
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REFERENCES


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BIOGRAPHICAL SKETCH

Denise Poirier Maguire was born in Bronxville, New York. She graduated from Mary Immaculate High School, Ossining, New York in 1972. Denise attended Niagara University, Niagara University, New York, and received a Bachelor of Science in Nursing in 1976. A Master's of Science in parent-child nursing was received from Boston University, Boston, Massachusetts, in 1984. Denise's nursing specialty area is newborn intensive care. She is a member of Sigma Theta Tau, the International Honor Society for Nursing, and the National Association of Neonatal Nurses.

Denise's dissertation research was supported by the Mead Johnson Nutritionals Perinatal Grant of Sigma Theta Tau, the International Honor Society for Nursing. She is currently employed as a Clinical Nurse Specialist and Nurse Researcher in the Department of Nursing Education, Research, and Program Development, All Children's Hospital, St. Petersburg, Florida.
I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Kathleen A. Smyth, Chair
Professor of Nursing

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Rose Nealis
Clinical Associate Professor of Nursing

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Hossein Yarandi
Associate Professor of Nursing

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Charles E. Wood
Professor of Physiology

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Maureen Keller-Wood
Associate Professor of Pharmacodynamics
This dissertation was submitted to the Graduate Faculty of the College of Nursing and to the Graduate School and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

May 2000

[Signature]

Dean, College of Nursing

[Signature]

Dean, Graduate School