Protocol

This trial protocol has been provided by the authors to give readers additional information about their work.

PROTOCOL SUPPLEMENTAL APPENDIX

Choice of Skin Antisepsis at Cesarean: a Randomized Controlled Trial

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This supplement contains the following items:

1. Original protocol, final protocol, summary of changes

2. Original statistical analysis plan, final statistical analysis plan, summary of changes
Antiseptic skin preparation for preventing surgical site infection at cesarean delivery: a randomized comparative effectiveness trial

“The Skin Prep Trial”

PROTOCOL

Version 1

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# Table of Contents

A. Specific Aims .................................................................................................................. 3

B. Background and Significance.......................................................................................... 5

C. Research Design and Methods....................................................................................... 10

D. Preliminary Studies........................................................................................................ 19

E. Key Personnel................................................................................................................ 24

F. Protection of Subjects.................................................................................................... 27

G. References..................................................................................................................... 30
A. SPECIFIC AIMS

We propose a randomized controlled clinical trial to determine the comparative effectiveness of chlorhexidine-alcohol and iodine-alcohol preoperative skin preparation for preventing surgical site infections at cesarean section. Cesarean section is the most common major surgical procedure performed on women in the United States and infectious morbidity is one of the most common associated complications. While estimates vary, surgical site infections complicate up to 5 – 10% of all cesarean sections and result in significant human suffering and excess health care costs. The national burden of surgical site infection has led the Institute of Medicine to list research on reducing their incidence in the highest priority group of the initial 100 priorities for comparative effectiveness research. Interventions such as preoperative antibiotic prophylaxis reduce surgical site infections by 60%, but the rate of infection remains high. There is therefore a great need to identify and test other potential interventions to further reduce these infections.

The skin is a major source of pathogens that cause surgical site infection. Therefore, optimizing preoperative skin antisepsis has the potential to decrease postoperative surgical site infections. There is paucity of evidence to guide the choice of antiseptic for skin preparation at cesarean section. To date, only two underpowered trials have been published comparing two methods of preoperative skin preparation at cesarean section. A recent randomized trial in adults undergoing clean-contaminated mostly *general surgical* procedures demonstrated a 41% reduction in surgical site infection with the use of chlorhexidine-alcohol when compared to the more commonly used povidone-iodine. While it is plausible that findings from trials in other clean-contaminated *surgical* procedures may apply to cesarean sections, physiological changes in pregnancy, the peculiar dual microbial source for cesarean-related infections and the hormone-mediated immune-modulation in pregnancy make the validity of such extrapolation uncertain. Thus, superiority of chlorhexidine must be demonstrated in a well-designed, adequately powered comparative effectiveness trial before changing the current use of iodine to the more expensive chlorhexidine for antisepsis at cesarean section.
This study will be potentially practice changing and have a great impact on cesarean-related infections. If chlorhexidine is proven to be more effective than iodine for preoperative antisepsis at cesarean section, wide spread use of a superior method of surgical site antisepsis will reduce infections, improve patient outcomes and reduce health care costs. The study has the following specific aims:

**Primary Aim:** To test the hypothesis that preoperative chlorhexidine-alcohol skin preparation at cesarean section significantly reduces *surgical site infections* compared to iodine-alcohol.

**Secondary Aim 1:** To test the hypothesis that preoperative chlorhexidine-alcohol skin preparation at cesarean section significantly reduces *bacterial contamination* at the surgical site compared to iodine-alcohol.

**Secondary Aim 2:** To determine clinical outcomes and medical costs associated with cesarean-related infections and quantify *potential cost savings* attributable to use of chlorhexidine-alcohol for preoperative skin preparation at cesarean section.
B. BACKGROUND AND SIGNIFICANCE

Burden of cesarean-related infections, prevention as a priority

Surgical site infection is the second most common type of health care associated infections [1]. They are classified as superficial incisional (involving only skin or subcutaneous tissue), deep incisional (involving fascia and/or muscular layers) and organ or space [2]. Approximately two-thirds of these infections involve superficial incisions, and the remainder involve the deeper tissues and organ spaces. Such infections are among the leading causes of preventable death in the United States, accounting for an estimated 1.7 million infections and 99,000 associated deaths [1]. In addition to the substantial human suffering, it is estimated that they result in $28 - $33 billion of excess health care costs each year [3]. Prevention of these infections is, therefore, a top priority of the U.S. Department of Health and Human Services. The national burden of surgical site infection has led the Institute of Medicine to list research on reducing their incidence in the highest priority group of the initial 100 priorities for comparative effectiveness research [4, 5].

Cesarean section is the most common major surgical procedure performed on women in the United States and rates continue to rise. In 2008, 1.4 million (32%) of the 4.3 million deliveries in the United States were by cesarean section [6]. **Infectious morbidity is one of the most common complications of cesarean delivery.** Specifically, surgical site infections complicate 5 - 10 % of cesarean deliveries. One study from our institution reported a 5% rate of surgical site infections based on hospital data [7]. More recent data from a randomized controlled trial revealed a 10% rate of surgical site infection based on both inpatient and outpatient infection surveillance [8]. While costs may vary depending on the location, depth and severity, average attributable cost for each cesarean wound infection is estimated to be $2,800 - $3,400 [9].

Limitations of current prevention measures

Preoperative antibiotic prophylaxis has been established as an effective preventive measure for reducing cesarean-related infections. The most recent Cochrane review suggests a 60% decrease in
surgical site infections when antibiotic prophylaxis is used at cesarean delivery (pooled RR 0.39; 95% CI 0.32 to 0.48) [10]. A meta-analysis including three randomized trials suggests antibiotic administration prior to skin incision, rather than after cord clamping, is associated with a reduced risk of endometritis and a trend towards lower rates of wound infections [11]. Emerging evidence also suggests that extending the spectrum of antibiotics may reduce the frequency of postcesarean wound infections [12]. However, even with optimal use of broad spectrum preoperative antibiotic prophylaxis the rate of postcesarean wound infection remains high [8]. In addition, results of trials on other potential interventions such as supplemental oxygen have been largely disappointing [8, 13]. There is therefore a great need to identify and test other potential interventions to further reduce cesarean-related infections.

Optimizing preoperative skin preparation

The skin is a major source of pathogens that cause surgical site infection. It is estimated that 34% of surgical site infections are attributable to skin flora [14]. Postcesarean wound infections are commonly caused by Staphylococcus aureus, aerobic streptococci, and aerobic and anaerobic gram-negative bacilli [15]. Preoperative antiseptic skin preparation attempts to achieve a sterile surgical field by decreasing the concentration of bacteria colonizing the skin at the incision site. Therefore, optimizing preoperative skin antisepsis has the potential to decrease surgical site infections. The ideal preoperative skin antiseptic agent should significantly reduce microorganisms on intact skin, be non-irritating to the skin, broad spectrum, fast acting, have a persistent effect, remain effective in the presence of organic material, and be cost effective [16]. The Food and Drug Administration has approved several antiseptics including iodine, chlorhexidine and alcohol for skin antisepsis. Chlorhexidine and iodine-based preparations have both been shown to decrease bacterial counts and are widely used. Iodine acts by oxidizing sulphydryl groups and distorting microbial protein structure. Potential disadvantages of iodine are skin irritation and need to dry for optimal action.

Chlorhexidine on the other hand does not require a wait time between application and surgical
incision. However, it is more expensive than iodine and may be associated with increased risk of allergic reactions [17]. Chlorhexidine acts by detroying bacterial cell membrane and precipitating cell contents. Alcohol is believed to act by damaging microbial cell membranes and denaturing protein. It has the advantage of being broad spectrum and fast acting, but lacks persistent activity. It is commonly combined with other antiseptics for a synergistic effect.

Evidence of superiority of chlorhexidine compared to iodine for preoperative skin preparation

Previous studies suggest a greater reduction in bacterial contamination in foot and ankle surgery [18], vaginal hysterectomy [19], and clean and clean-contaminated general surgery [20, 21] following skin preparation with chlorhexidine compared to iodine (pooled RR 0.44 [95% CI, 0.35 - 0.56]) [17]. A recent randomized trial in adults undergoing clean-contaminated mostly general surgical procedures demonstrated a 41% reduction in surgical site infection with preoperative chlorhexidine-alcohol when compared to the more commonly used povidone-iodine (9.5% versus 16.1%, RR 0.59, 95%CI 0.41 – 0.85) [22]. The associated number needed to treat was 17. Of note, chlorhexidine-alcohol was protective against both superficial and deep incisional infections, but not organ or space infections [22]. A subsequent meta-analysis including this and five other trials involving clean contaminated surgical procedures showed a reduction in postoperative surgical site infection when chlorhexidine-alcohol was used (pooled OR 0.68, 95%CI 0.50 – 0.94) [23].

Taken together, these data suggest superiority of chlorhexidine-alcohol for preoperative surgical site antisepsis for clean-contaminated general surgical procedures. Chlorhexidine has a number of properties that may account for its apparent superiority as an antiseptic. It has strong affinity for binding to the skin, high antibacterial activity against gram-positive and gram-negative bacteria including methicillin-resistant staphylococcus aureus, and residual effects for up to 6 hours [24]. In contrast to iodine, chlorhexidine does not become inactivated in the presence of organic materials such as blood and other body fluids [25].

Lack of evidence on preoperative skin preparation at cesarean section
There is paucity of evidence on methods of preoperative skin preparation at cesarean section. A comprehensive review of evidence for different aspects of cesarean delivery concluded that “skin cleansing before cesarean delivery is grossly understudied” [26]. In fact, there have been only two published randomized trials to date on skin preparation at cesarean delivery [27, 28]. The first study compared a one-minute alcohol wash followed by application of an iodophor-impregnated adhesive film to a five-minute iodophor scrub followed by an iodophor wash in a prospective, randomized, controlled study of 79 patients [27]. While no differences in infectious morbidity was noted, this study demonstrated a greater reduction in skin bacterial count of the more rapid method of pre-operative skin preparation compared to a longer procedure. In the other study Magann et al. randomly assigned 50 women to parachlorometaxylenol scrub for five minutes followed by povidone-iodine scrub and paint, or povidone-iodine scrub and paint alone [28]. No significant differences were seen in the incidence of endometritis and wound infections in the two groups. Both studies are limited by the small sample sizes and possible type II errors.

In contrast to the accumulating data on skin preparation in other surgical specialties there is an evidence gap regarding the relative effectiveness of chlorhexidine and iodine skin preparation for reducing skin contamination and surgical site infection at cesarean section. To our knowledge, there is no published trial comparing the two antiseptics for skin preparation at cesarean section. Rauk recently reported a significant reduction in the incidence of overall surgical site infections after cesarean section when a multidisciplinary protocol including chlorhexidine skin preparation was implemented [29]. However, the use of historical controls and the multiplicity of interventions in the protocol (comprehensive staff education and training, skin preparation using chlorhexidine no rinse cloths prior to going to the operating room, chlorhexidine-alcohol for preoperative skin preparation in the operating room, and modified instrument sterilization techniques), make it impossible to attribute the reduction in infections to the chlorhexidine skin preparation alone.

Uncertainty extrapolating findings from other surgical procedures to cesarean section
As a clean-contaminated procedure, it is plausible that findings from trials in other clean-contaminated surgical procedures may apply to cesarean sections. However, physiological changes in pregnancy, the peculiar dual microbial source for cesarean-related infections (genital tract, skin) and the hormone-mediated immune-modulation in pregnancy make the validity of such extrapolation uncertain [30]. Experience with chlorhexidine use for the prevention of infections in other clinical settings gives reason for caution. For example, while a randomized trial showed a significant reduction in catheter-related bloodstream infections in critically ill adults in the intensive care unit with the use of chlorhexidine-impregnated dressings for intravascular catheters [31], a subsequent adequately powered trial in patients with tunneled central venous catheters undergoing hemodialysis failed to show a decrease in bloodstream infections [32]. Therefore, **superiority of chlorhexidine must be demonstrated in a well-designed, adequately powered comparative effectiveness trial** before changing the current use of iodine to the more expensive chlorhexidine for surgical site antisepsis at cesarean section.

**Significance and potential impact of study**

**This study will have a potentially great impact on patient outcomes and health care costs.** A finding that chlorhexidine is more effective than iodine for preoperative antisepsis at cesarean section will be practice changing. With over 1.4 million cesarean sections performed in the United States annually, widespread use of a superior method of surgical site antisepsis will reduce surgical site infections, improve patient outcomes and reduce health care costs. In a cost-benefit model based on data from other types of surgical procedures, Lee et al. demonstrated a net cost savings of $16 – 26 per case and $349,904 - 568,594 per year if the Hospital of the University of Pennsylvania switched from iodine to chlorhexidine [17]. Sensitivity analyses indicated persistence of net cost savings under most circumstances. If such cost savings hold for cesarean sections, a potential $16 – 26 billion could be saved in the United States annually by switching from iodine to chlorhexidine.
C. RESEARCH DESIGN AND METHODS

OVERALL DESIGN

This will be a randomized controlled clinical trial aimed at determining the comparative effectiveness of chlorhexidine-alcohol and iodine-alcohol preoperative skin preparation for preventing surgical site infection at cesarean section.

Rationale for Design

The randomized control trial is the gold standard of research design. Other designs such as case-control, retrospective cohort and prospective cohort are limited by potential bias and confounding. Randomly assigning subjects to different interventions minimizes selection bias. The random assignment also results in groups that are likely to be similar with regards to important confounding variables. This minimizes confounding by both measured and unmeasured factors. While random allocation does not guarantee the groups will be identical, it does ensure that any differences between them are due to chance alone. Finally, randomization produces groups that are random samples of the population. This permits use of standard statistical tests that are based on probability theory.

We will use broad inclusion criteria and analyze all the main outcomes by the intent-to-treat principle. This approach will allow a more conservative estimate of differences between groups and allow a better estimate of effectiveness and public health implications of practice change rather than a pure estimate of efficacy alone [33].

Subject Selection

Inclusion criteria – Women undergoing cesarean delivery at Barnes-Jewish Hospital.

Exclusion criteria - Inability to obtain consent; allergy to chlorhexidine, alcohol, iodine, shellfish; and evidence of infection adjacent to operative site.
Figure 1: Schema of study hypothesis and specific aims. Bold and dashed arrows indicate increasing and decreasing effects of the proximal factors on the distal factor, respectively.

Randomization and Treatment

Enrolled patients will be randomly assigned in a 1:1 ratio using computer-generated randomization sequence to two skin preparation methods:

1. **Chlorhexidine-alcohol**-2% chlorhexidine gluconate with 70% alcohol (ChloraPrep, Cardinal Health) preoperative skin preparation.
2. **Iodine-alcohol**-8.3% povidone-iodine with 72.5% alcohol (Prevail-FX, Cardinal Health)
   
   preoperative skin preparation.

**Blinding**

Blinding both patients and physicians to the antiseptic used for skin preparation (double-blinding) would be ideal. However, it is not feasible in this trial. First, most patients can determine whether they were assigned to chlorhexidine or iodine, as the two antiseptics are of different colors and leave a stain on the skin (pink or brown, respectively). Second, physicians are often in the operating room when the skin is being prepared for cesarean section and will know which antiseptic is used. We will minimize systematic bias by using the same standard procedures of skin preparation, skin culture and assessment of outcomes. All diagnoses of surgical site infection will be verified by chart review using the Centers for Disease Control and Prevention (CDC) National Nosocomial Infections Surveillance System criteria [34] (Figure 2). The principal investigator will verify the diagnoses without knowledge of the group to which the patients were assigned.

**PRIMARY AIM:**

To test the hypothesis that preoperative chlorhexidine-alcohol skin preparation at cesarean section significantly reduces surgical site infections compared to iodine-alcohol

**Outcome measures**

Primary outcome-Proportion of subjects with surgical site infection (superficial incisional [skin, subcutaneous layer] or deep incisional [fascia, muscle]) within 30 days of cesarean delivery. Surgical site infection will be based on diagnosis by the treating physician and verified by chart review in accordance with the CDC Nosocomial Infections Surveillance System definitions [34] (Figure 2).

Secondary outcomes- Length of hospital stay, number of office visits and re-admissions for infection-related complications, endometritis, positive culture from wound culture, skin irritation and allergic reactions.
**Figure 2:** *CDC criteria for defining surgical site infection [34]*

**Methods**

Subjects will undergo cesarean delivery based on the technique selected by the surgeon. The circulating nurse will record information on key variables known to be related to surgical site infection: antibiotic administration (type and timing), type of cesarean section (scheduled or emergent), status of membranes (ruptured or unruptured), duration of surgery, depth of subcutaneous layer (closed or not closed) and skin closure method (subcuticular suture or staples) on data collection forms.

Demographic (age, race, socioeconomic status), obstetric (parity, gestational age, indication for cesarean section, cervical dilation at time of cesarean section, presence of chorioamnionitis, surgical complications) and neonatal (birth weight, Apgar score, cord pH) data will be abstracted from the patients chart.
Subjects will be contacted every 2 weeks (up to 30 days from delivery) to assess symptoms of cesarean-related infections. Patients who report symptoms will be directed to follow up in the emergency department or with their physician to be evaluated for surgical site infection. Wound swabs will be taken for aerobic and anaerobic cultures in all subjects who present at Barnes-Jewish Hospital with wound infection. Medical records will be obtained from treating physicians to determine the diagnosis at each postoperative office visit or readmission within 30 days of cesarean section. All data will be managed in Datstat (Datstat Inc., Seattle, Washington).

Data Analysis

Analyses will be based on the intent-to-treat principle. The primary outcome (proportion of subjects with surgical site infection) and the other categorical variables will be compared across groups using the chi-squared test. Fisher’s exact test will be used for variables in which expected numbers in any of the cells in 2 x 2 tables is <5. We will calculate 95% confidence intervals around the differences in proportions and the relative risk of surgical site infection.

Distribution of continuous variables will be evaluated by visual inspection of histograms and the Kolmogorov-smirnov test. Normally distributed variables will be compared using the unpaired t-tests. If variables are not normally distributed, log transformation will be used in an attempt to achieve normal distribution. If the data is still skewed after log transformation the Mann-Whitney U test will be used to compare groups.

It is anticipated that baseline characteristics will be similar in the two groups. In the event that the groups are unbalanced with regards to variables significantly associated with the primary outcome, supplemental analyses will be performed using stratification on the individual variables and multivariable logistic regression adjusting for multiple covariates.

Planned subgroup analysis will be performed for: i. scheduled and elective cesarean sections, iii. obese and normal weight women, iii. Subcuticular and staple closure, and iv. women with and without chronic medical conditions (diabetes, chronic hypertension, renal disease). Interaction tests will be
used to determine if the effectiveness of the skin preparation methods differ across these subgroups. Tests with \( p < 0.05 \) will be considered statistically significant. Analyses will be performed using Stata version 10.0 (Stata Corp., College Station, TX).

**Sample Size Consideration**

The sample size estimation for the primary aim is based on an assumed baseline surgical site infection rate of 8% and an anticipated clinically significant 50% reduction in surgical site infection. To have 80% power to detect 50% difference in a two-tailed chi-squared test with \( \alpha \) of 0.05, a total of 1084 subjects will need to be randomized. To accommodate a 10% drop out rate, 1,192 subjects will be enrolled (596 chlorhexidine, 596 iodine).

**SECONDARY AIM #1**

To test the hypothesis that preoperative chlorhexidine-alcohol skin preparation at cesarean section significantly reduces bacterial contamination at the surgical site compared to iodine-alcohol

**Outcome measures**

Primary outcome- Proportion of subjects with surgical site skin contamination after antiseptic preparation. Contamination will be defined as \( \geq 5000 \) total colony-forming units per milliliter on aerobic or anaerobic culture.

Secondary outcomes- Types of bacteria cultured before and after skin preparation, concordance of bacteria at surgical site following preoperative skin preparation with bacteria in postoperative surgical site infections.

**Methods**

Two skin swabs will be taken transversely across the suprapubic area, 2 finger breadths above the symphysis pubis immediately before, and 5 minutes after skin preparation. These swabs will be cultured under aerobic and anaerobic conditions. To ensure that the groups at high risk for surgical
site infections are well represented, we will ensure that obese women, diabetics and women undergoing emergent cesarean deliveries are adequately sampled and randomized.

Data Analysis

Analyses will be based on the intent-to-treat principle. The primary outcome (proportion of subjects with surgical site skin contamination after skin preparation) and the other categorical variables will be compared across groups using the chi-squared test. Fisher’s exact test will be used for variables in which expected numbers in any of the cells in 2 x 2 tables is <5. Tests with \( p < 0.05 \) will be considered significant. We will also conduct stratified analysis based on the different risk groups. Finally, we will calculate 95% confidence intervals around the difference in proportions and relative risk of skin contamination after antiseptic skin preparation. Analyses will be performed using Stata version 10.0 (Stata Corp., College Station, TX).

Sample Size Consideration

The sample size estimation for secondary aim #1 is based on the primary outcome of skin contamination following skin preparation. A meta-analysis of data from non-obstetric surgical procedures suggest a contamination rate of 39% after preoperative skin preparation with iodine and a rate of 18% after the use of chlorhexidine [17]. On the basis of an assumed contamination rate of 39% in the iodine group and 50% difference in skin contamination as clinically significant, a total of 168 subjects will be needed (84 chlorhexidine, 84 iodine) to have 80% power in a two-tailed chi-squared test and \( \alpha \) of 0.05.

<table>
<thead>
<tr>
<th>Study or Subgroup</th>
<th>Chlorhexidine Events</th>
<th>Total</th>
<th>Iodine/Iodophor Events</th>
<th>Total</th>
<th>Weight</th>
<th>Risk Ratio M-H, Fixed, 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bibbo 2005</td>
<td>23</td>
<td>60</td>
<td>53</td>
<td>67</td>
<td>31.1%</td>
<td>0.48 [0.34, 0.68]</td>
</tr>
<tr>
<td>Culligan 2005</td>
<td>5</td>
<td>23</td>
<td>17</td>
<td>27</td>
<td>9.7%</td>
<td>0.35 [0.15, 0.79]</td>
</tr>
<tr>
<td>Pacharoen 2009</td>
<td>36</td>
<td>250</td>
<td>78</td>
<td>250</td>
<td>48.4%</td>
<td>0.46 [0.32, 0.66]</td>
</tr>
<tr>
<td>Saltzman 2009</td>
<td>4</td>
<td>50</td>
<td>26</td>
<td>100</td>
<td>10.8%</td>
<td>0.31 [0.11, 0.83]</td>
</tr>
<tr>
<td>Total (95% CI)</td>
<td>383</td>
<td></td>
<td>444</td>
<td></td>
<td>100.0%</td>
<td>0.44 [0.35, 0.56]</td>
</tr>
</tbody>
</table>

Total events: 68, 174

Heterogeneity: \( \chi^2 = 1.19, \text{df} = 3 \) (\( p = 0.76 \)); \( I^2 = 0\% \)

Test for overall effect: \( Z = 6.76 \) (\( p < 0.00001 \))

0.01 0.1 1 10 100
Favours experimental Favours control
Figure 3: Meta-analysis of 4 studies on positive skin culture after skin preparation with iodine or chlorhexidine [17].

SECONDARY AIM #2

To determine clinical outcomes and medical costs associated with cesarean-related infections and quantify potential cost savings attributable to use of chlorhexidine-alcohol preoperative skin preparation at cesarean section

Outcome measure

The outcome for secondary aim #2 is attributable cost saving (if any), defined as the difference in total costs between women with preoperative iodine and chlorhexidine skin preparation.

Methods/Data Analysis

A cost-benefit decision analysis model will be developed depicting the decision of whether to use chlorhexidine or iodine for a patient undergoing cesarean section. The cost of implementing each strategy will include the purchase costs of the antiseptic agents. For each antisepsis strategy, the patient would then have a probability of subsequently developing surgical site infection based on results of the randomized trial under the primary aim. We will calculate cost incurred by patients who did and did not develop an infection.

Costs will be obtained from the Barnes-Jewish Hospital cost accounting database for the surgical admission and any readmission to the hospital and office visits within 30 days after cesarean section. Cost savings, if any, will be the difference between the costs in the two groups. The cost-benefit analysis will be performed using TreeAge Pro 2009 (TreeAge Software).

Sample Size Consideration

No formal sample size estimation is made for secondary aim #2. The cost-benefit analysis will be based on outcomes among the subjects enrolled under secondary aim #2.
### Table 1: TIME LINE OF STUDY ACTIVITIES

<table>
<thead>
<tr>
<th>Dates</th>
<th>Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>August 2011 - February 2013</td>
<td>Recruitment</td>
</tr>
<tr>
<td>April 2012</td>
<td>Interim Analysis</td>
</tr>
<tr>
<td>March 2013</td>
<td>Final Data Analysis</td>
</tr>
<tr>
<td>April - July 2013</td>
<td>Preparation of final manuscripts for publication</td>
</tr>
<tr>
<td>August 2013</td>
<td>Estimated End Date</td>
</tr>
</tbody>
</table>
D. PRELIMINARY STUDIES

i. Preliminary study#1: Cesarean deliveries at Barnes-Jewish Hospital

To determine the feasibility of recruiting the required sample size, we reviewed delivery data at Barnes Jewish Hospital, site for the proposed study, from January 1, 2007 to December 31, 2007. During this one year period, a total of 3981 deliveries were performed. Table 2 shows a breakdown of the deliveries. A total of 1122 deliveries, constituting 28% were by cesarean section. Of the cesarean sections 61% were primary while 39% were repeat cesarean sections. The most common indications for cesarean section were repeat sections, arrest of labor, non-reassuring fetal heart tracing and mal-presentation.

Table 2: Delivery Data at Barnes Jewish Hospital, January 1, 2007 to December 31, 2007

<table>
<thead>
<tr>
<th>Delivery Type</th>
<th>Number</th>
<th>Percent of Deliveries (%)</th>
<th>Percent of Cesarean Sections (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vaginal Delivery</td>
<td>2809</td>
<td>72</td>
<td>-</td>
</tr>
<tr>
<td>Cesarean Sections</td>
<td>1122</td>
<td>28</td>
<td>-</td>
</tr>
<tr>
<td>Primary</td>
<td>684</td>
<td>-</td>
<td>61</td>
</tr>
<tr>
<td>Repeat</td>
<td>438</td>
<td>-</td>
<td>39</td>
</tr>
<tr>
<td>Total</td>
<td>3981</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

Given the broad inclusion criteria we estimate that less than 10% of the patients undergoing cesarean section will need to be excluded (allergy to iodine or chlorhexidine, inability to obtain consent, existing infection at incision site). Based on our previous experience enrolling patients for cesarean section related studies and the universality of skin preparation at cesarean section, we estimate a consent rate of at least 60%. Thus, we estimate that the sample size of 1192 can be achieved in a 2-year period (2 x 1122 x 90% x 60% = 1212).
ii. **Preliminary study #3: Burden and risk factors for cesarean-related surgical site infection** [7].

A retrospective study was conducted at Barnes-Jewish Hospital to identify independent risk factors for surgical site infection after cesarean. A total of 1605 women who underwent low transverse cesarean section during the period from July 1999 to June 2001 were evaluated. Using the International Classification of Diseases, Ninth Revision diagnosis codes for surgical site infection or wound complication and/or data on antibiotic use during the surgical hospitalization or at readmission to the hospital or emergency department, potential cases of surgical site infections were identified in this cohort. Cases of surgical site infection were verified by chart review using the definitions from the CDC National Nosocomial Infections Surveillance System. Control patients without surgical site infection or endometritis were randomly selected from the population of patients who underwent cesarean section. Independent risk factors for surgical site infection were determined by logistic regression.

Surgical site infections were identified in 81 (5.0%) of the 1605 women who underwent low transverse cesarean section. **We consider the surgical site infection rate of 5% as an underestimation, since it was based only on hospital data.** Independent risk factors for surgical site infection included development of subcutaneous hematoma after the procedure (adjusted odds ratio [aOR], 11.6 [95% confidence interval [CI], 4.1-33.2]), operation performed by the university teaching service (aOR, 2.7 [95% CI, 1.4-5.2]), and a higher body mass index at admission (aOR, 1.1 [95% CI, 1.0-1.1]). Antibiotic prophylaxis before or after the operation was associated with a significantly lower risk of surgical site infection (aOR, 0.2 [95% CI, 0.1-0.5]).

iii. **Preliminary study #4: Effect of skin closure method on cesarean wound infection or separation** [35]

We recently published a systematic review and meta-analysis to estimate whether staples or subcuticular suture closure is associated with a higher risk of wound infection or separation when used for transverse skin incisions at cesarean section. We searched electronic databases from 1966
to September 2010 for randomized controlled trials (RCTs) and prospective cohort studies comparing staples to subcuticular sutures at cesarean section. The primary outcome was occurrence of wound infection or separation.

Six studies including 5 RCTs and one prospective cohort study met inclusion criteria. Staple closure (n=803) was associated with a two-fold higher risk of wound infection or separation compared to subcuticular suture closure (n=684) (13.4% versus 6.6%, pooled OR 2.06 [1.43 – 2.98]) (Figure 4).

![Figure 4: Forrest Plot of meta-analysis of studies comparing wound complications with staples versus subcuticular suture [35].](image)

The number needed to harm was 16. The increased risk persisted when analysis was limited to the RCTs (OR 2.43 [1.47 – 4.02]). There was no evidence of significant statistical heterogeneity among studies (χ² =0.74, p=0.327, I²=13.7%) or publication bias (t= -0.86, p=0.439). Staple closure was associated with a small, but shorter duration of surgery in most studies that evaluated operating time.

We concluded that staple closure is faster to perform, but associated with a higher risk of wound infection or separation.
iv. **Preliminary study #5**: Randomized control trial of supplemental oxygen to reduce post cesarean infections[8]

We also recently completed a randomized control trial to investigate whether supplemental oxygen during cesarean delivery and for 2 hours afterwards reduces the incidence of post-cesarean infectious morbidity. Women undergoing cesarean were randomized to receive either 2L of oxygen via nasal cannula during cesarean delivery only (standard care) or 10L oxygen via non-rebreather mask (intervention group) during cesarean and for 2 hours afterward. The primary outcome was infectious morbidity, defined as surgical site infection or endometritis. A total of 585 women were included in the final analysis. Demographic data was similar in the two groups. There was no significant difference in the rate of infectious morbidity between the standard care and intervention groups (RR 1.4, 95% CI 0.9-2.3).

**Table 3**: Maternal Outcomes in women randomized to supplemental oxygen or standard care for prevention of surgical site infections at cesarean section.

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Overall (N=585)</th>
<th>Standard Care (n=297)</th>
<th>Supplemental Oxygen (n=288)</th>
<th>Relative Risk (RR)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Composite</td>
<td>61 (10.4%)</td>
<td>26 (8.8%)</td>
<td>35 (12.2%)</td>
<td>1.4 (95% CI 0.9-2.3)</td>
<td>0.18</td>
</tr>
<tr>
<td>Endometritis</td>
<td>9 (1.5%)</td>
<td>2 (0.6%)</td>
<td>7 (2.4%)</td>
<td>3.6 (95% CI 0.8-17.2)</td>
<td>0.08</td>
</tr>
<tr>
<td>Surgical site infection</td>
<td>59 (10.1%)</td>
<td>26 (8.8%)</td>
<td>33 (11.5%)</td>
<td>1.3 (95% CI 0.8-2.1)</td>
<td>0.28</td>
</tr>
<tr>
<td>Wound hematoma or seroma</td>
<td>33 (5.6%)</td>
<td>17 (5.9%)</td>
<td>16 (5.4%)</td>
<td>1.1 (95% CI 0.6-2.1)</td>
<td>0.79</td>
</tr>
<tr>
<td>Hospital readmission</td>
<td>25 (4.3%)</td>
<td>10 (3.4%)</td>
<td>15 (5.2%)</td>
<td>1.5 (95% CI 0.7-3.4)</td>
<td>0.27</td>
</tr>
</tbody>
</table>

This study demonstrates that supplemental oxygen does not reduce the rate of post-cesarean infectious morbidity. This underscores the need to identify and test other interventions such as skin preparation methods. **The study also establishes a more realistic surgical site infection rate of 10% in our population (Table 3)**, since infections were identified from both hospital data and active
outpatient surveillance. It also confirms our ability to recruit, randomize and follow-up patients in a cesarean-related randomized trial.

v. **Preliminary study #5: Ongoing randomized trial on the effect of omitting the Bladder Flap at cesarean section**

We are currently conducting a randomized control trial to evaluate short and medium term effects of omitting a step (creation of the bladder flap) considered to be unnecessary at cesarean section. So far, we have recruited 228 of the target 258 sample size. This further underscores the ability of the investigators to recruit, randomize and follow-up patients to evaluate outcomes in cesarean-related randomized trial.

vi. **Preliminary study #6: Attributable costs of surgical site infection and endometritis after low transverse cesarean delivery [9].**

Finally, a cost-analysis was conducted at Barnes-Jewish Hospital to determine the attributable costs of surgical site infections and endometritis after cesarean section by two different methods. Using data from 1605 women who underwent low transverse cesarean sections from July 1999 to June 2001, attributable costs of surgical site infections and endometritis were determined by generalized least squares (GLS) and propensity score matched-pairs. For the matched-pairs analyses, uninfected control patients were matched to patients with surgical site infection or with endometritis on the basis of their propensity to develop infection, and the median difference in costs was calculated. The attributable total hospital cost of surgical site infections was $3,529 by GLS and $2,852 by propensity score matched-pairs.
E. KEY PERSONNEL

Methodius G. Tuuli, M.D., M.P.H., a Principal Investigator for this study, will be Assistant Professor (clinician investigator track) in the Department of Obstetrics and Gynecology at Washington University in St. Louis, starting in July 2011. Dr. Tuuli received his undergraduate and M.D. degrees from the University of Ghana Medical School. He then completed a master's degree in public health (MPH) at the University of California, Berkeley, with a concentration in maternal and child health. After residency training in Obstetrics and Gynecology at Emory University in Atlanta Georgia, Dr. Tuuli is completing fellowship training in Maternal-Fetal Medicine at Washington University in St. Louis. He is board-certified in Obstetrics and Gynecology and will be an active candidate for certification in Maternal-Fetal Medicine. During his fellowship, Dr. Tuuli completed two graduate level courses in advanced statistics for clinical researchers at the Washington University Institute for Clinical and Translational Sciences (ICTS). Of direct significance to this proposal, Dr Tuuli is currently completing a randomized control trial on the role of the bladder flap at cesarean delivery. He has 27 publications in high-quality journals including Obstetrics & Gynecology, American Journal of Obstetrics and Gynecology, and the British Journal of Obstetrics and Gynecology. These include 17 peer-reviewed articles, 5 invited reviews, 2 book chapters and 3 other publications. He is also a reviewer for several journals. In addition to his appointment as Assistant Professor, Dr Tuuli will be a Women’s Reproductive Health Research (WRHR) Scholar under an NIH funded career development grant (K12) awarded to the Department of Obstetrics and Gynecology of Washington University.

George A. Macones, M.D., M.S.C.E., a Co-investigator for this study, is the Mitchell and Elaine Yanow Professor and Chair of the Department of Obstetrics and Gynecology at Washington University School of Medicine, Senior Scholar in the Institute of Public Health at Washington University in St. Louis, and founder of the Division of Clinical Research. He is trained in Maternal-Fetal Medicine and Epidemiology, and has been nationally and internationally recognized for his
perinatal outcomes research. Dr. Macones’ expertise in clinical research design and epidemiology has been utilized in his role as reviewer for a variety of NIH/NICHD study sections. His research has been published in high-quality, peer-review journals and has been extensively funded by the NIH and other foundations. He is currently the PI of an RO1 for an observational study investigating the association between LEEP and preterm birth. Dr. Macones is also recipient of an ongoing K24 grant (Midcareer Award in Patient Oriented Research) from NICHD. He is currently an Associate Editor for the American Journal of Obstetrics and Gynecology. He currently serves on the sub-specialty Board of the American Board of Obstetrics and Gynecology for Maternal-Fetal Medicine, and is on the Board of Directors for the Society of Maternal Fetal Medicine. He was recently elected to the American Society of Clinical Investigation. Finally, and specific to this proposal, Dr. Macones has worked closely with Tuuli in his role as fellowship director, research mentor, and collaborator. He will continue to be Dr Tuuli’s mentor under the WRHR scholar program. This outstanding working relationship will aid in the development and performance of this study.

David M. Stamilio, M.D., M.S.C.E., a Co-investigator, is Associate Professor of Obstetrics and Gynecology, and the Division Chief of Maternal-Fetal Medicine at Washington University Medical Center. Dr. Stamilio is also a member of the Division of Clinical Research in the Department of Obstetrics and Gynecology, and a founding member of the interdisciplinary Washington University Center for Preterm Birth Research. He is a formally trained epidemiologist with experience and expertise in the area of perinatal outcomes research, and has chaired the forum for Perinatal Epidemiology at the past two annual national meetings for the Society of Maternal-Fetal Medicine. Dr. Stamilio has published numerous articles in the area of clinical obstetrics, maternal-fetal medicine, economic analysis and perinatal epidemiology. He has been the PI on several research projects, including funding from the Barnes-Jewish Foundation as PI for a randomized-controlled trial examining post-cesarean maternal oxygen exposure to decrease the risk of operative infectious
morbidity. Dr. Stamilio will add his expertise to many areas of this study, including assistance in quality assurance, study logistics, analysis and publication.

Jingxia (Esther) Liu, PhD, the Study Statistician, is Assistant Professor in the Division of Biostatistics. She received her BSc in scientific computing and applied software at the Department of Mathematics of NanKai University in 1997, her MSc in financial mathematics from National University of Singapore in 2002 and a PhD in biostatistics from the Medical College of Wisconsin in 2007. After graduating from the Medical College of Wisconsin, she worked as a lead statistician at the Pharmaceutical Product Development (PPD) Inc. from 2007 to 2009. Since joining Washington University, Dr. Liu has focused her efforts in statistical support for both the ICTS and the Siteman Cancer Center. Dr. Liu’s research interests include propensity score methodology, survival data analysis, study design in clinical trials and missing data methods. She is also interested in cost-effective analysis and methodological issues in observational studies. She was involved in many projects from small to large size and provided statistical consulting on Phase I-IV clinical trials across different functional departments at the Pharmaceutical Product Development (PPD) Inc., which has ensured meeting the regulatory demands of FDA and submission deadlines of pharmaceutical companies. She is a member of the Institute of Clinical and Translational Sciences (ICTS) Research Design and Biostatistics Group (RDBG) and is currently providing statistical support for the projects of the Siteman Cancer Center and the ICTS.

Swarup Sri Varaday, M.D., a Co-investigator is Assistant Professor of Anesthesiology. Dr Varaday works closely with the department of Obstetrics and Gynecology as one of the key providers of anesthesia for labor and cesarean delivery. Since anesthetic management is an integral part of pre-operative procedures, involvement of Dr Varaday will ensure cooperation between the anesthesia and obstetrics teams, and facilitate the smooth conduct of the study.

Cindy Bertolino, RN, a Co-investigator, is a charge nurse of labor and delivery. She will bring a unique nursing perspective to the study and facilitate the recruitment of patients.
F. PROTECTION OF HUMAN SUBJECTS

Assessment of Risks

Subject Characteristics

All women undergoing cesarean section at Barnes-Jewish Hospital during the study period will be eligible. We anticipate enrolling a total of 1192 patients.

Exclusion criteria include inability to obtain consent; allergy to chlorhexidine, alcohol, iodine; and evidence of infection adjacent to operative site. The involvement of pregnant women (a vulnerable population) is inevitable since cesarean section is a procedure performed only in this patient population.

Potential Risks

Patients undergoing cesarean sections are at risk for complications including anesthetic complications, bleeding, need for blood transfusion, injury to surrounding organs such as bowel, bladder, ureters, possible need for further procedures and postoperative infection. The use of chlorhexidine alcohol for skin preparation is not expected to increase these risks. Preliminary studies in other types of surgical procedures suggest a likely reduction in surgical site infection. Potential risks include skin irritation, allergy to the antiseptics and breach of patient confidentiality. Measures outlined below will be employed to minimize these risks. It is also noteworthy that while iodine-alcohol is the standard of care at Barnes-Jewish Hospital, chlorhexidine-alcohol is available and is used in patients who report iodine allergy.

Adequacy of protection against risks

Data and Safety Monitoring Plan

Although risks to study participants are expected to be small, a number of measures are planned to ensure patient safety.
1. The principal investigator (PI) – The PI will be the first layer of monitoring for risk to study subjects. The PI will monitor, document and report to the Institutional Review Board, any adverse events among study participants.

2. Data and Safety Monitoring Board (DSMB) – The DSMB will consist of three individuals with extensive experience in clinic research and who are not directly involved in the study: Anthony Odibo, MD, MSCE; Alison Cahill, MD, MSCI; Anthony Shanks, MD. The DSMB will be tasked with ensuring the overall safety of subjects enrolled in the study. They will also be responsible for interpreting results of the interim analysis and making decisions including stopping the study.

3. Interim analysis – Interim efficacy analyses will be conducted (with surgical site infection as the primary outcome of interest) after 50% and 75% of the patients have been evaluated. Analysis will be performed by the study statistician and presented to the DSMB. The board will make a recommendation regarding further conduct of the study. The principal investigator will not be informed of the results of the interim analysis unless the DSMB determines that some level of unblinding is necessary to make final decisions about the conduct of the study. Possible decisions include stopping the study because efficacy has been achieved or because of futility, and modifying the target sample size.

While early stopping decisions cannot be based purely on a mathematical stopping rule, the Haybittle-Peto stopping rule will be used as a guide. Under this rule the interim analysis of the primary outcome would have to demonstrate a difference between groups in excess of three standard errors (or \( p < 0.001 \)) to justified premature disclosure [36, 37]. This rule has the advantage that the exact number and timing of interim analysis need not be specified. It also preserves the power and overall type I error (0.05). Final decisions will be based partly on more subjective considerations such as the consistency of results across subgroups and secondary endpoints.

4. Protection of patient confidentiality – We will protect patient confidentiality by making sure that only individuals directly involved in the study have access to identifiable patient information. Study files will
be kept under lock and key. Only the study coordinator and PI will have access to the key. Patient information will be coded and stripped of identifiers prior to analysis and publication of study results.

**Potential Benefits/Importance of knowledge gained**

The study is not designed to provide direct benefits to research participants. However, if our hypothesis that chlorhexidine-alcohol is a superior antiseptic for cesarean section is true, those participants randomized to the chlorhexidine-alcohol group will enjoy the benefits of reduced infections. More importantly, results from this study have the potential to reduce cesarean-related infections, improve patient outcomes and reduce health care costs. Since the anticipated risk to participants is minimal, the risks-benefit ratio is very favorable.
G. REFERENCES


INFORMED CONSENT DOCUMENT

Project Title: Antiseptic skin preparation for preventing surgical site infection at cesarean delivery: a randomized comparative effectiveness trial

Principal Investigator: Methodius Tuuli, MD, MPH

Research Team Contact: Shannon Martin, RN 314-362-8523

This consent form describes the research study and helps you decide if you want to participate. It provides important information about what you will be asked to do during the study, about the risks and benefits of the study, and about your rights as a research participant. By signing this form you are agreeing to participate in this study.

• If you have any questions about anything in this form, you should ask the research team for more information.
• You may also wish to talk to your family or friends about your participation in this study.
• Do not agree to participate in this study unless the research team has answered your questions and you decide that you want to be part of this study.

What is the purpose of this study?
This is a research study. We invite you to participate in this research study because you are pregnant and may be undergoing a cesarean section as the mode of delivery of your baby.

The purpose of this research study is to determine the effectiveness of two antiseptics (chlorhexidine-alcohol and iodine-alcohol preoperative) for preventing surgical site infections at cesarean section and to study the DNA of bacteria which cause infections at the surgical site. The information obtained from this study will hopefully in the future reduce infections related to cesareans sections.

What will happen during this study?
If you agree to participate in the study you will be assigned to one of two groups by a method similar to a flip of a coin, called randomization. You will be randomized to one of two skin preparation methods:

1. Chlorhexidine-alcohol-2% chlorhexidine gluconate with 70% alcohol (ChloraPrep, Cardinal Health) preoperative skin preparation.
2. Iodine-alcohol-8.3% povidone-iodine with 72.5% alcohol (Prevail-FX, Cardinal Health) preoperative skin preparation.

Immediately before and 5 minutes after the skin preparation, two skin swabs will be taken across the lower abdomen, 2 inches above the pubic hair line. These swabs will be cultured to see if there are any bacteria on the skin. We will also extract bacterial DNA (the chemicals that provide instructions as to how the bacteria act in our bodies) from the swabs and use it to identify all of the bacteria in the skin or surgical wound. This DNA study is of germs only. We will not determine or study your DNA.

You will be contacted 30 days from delivery to assess symptoms of cesarean-related infections. If you
report symptoms of cesarean-related infections, you will be directed to follow up in the emergency department or with your physician for evaluation of surgical site infection. Wound swabs will be taken and cultured if you present at Barnes-Jewish Hospital with a wound infection.

Your medical records will be reviewed to obtain demographic, your pregnancy, delivery and post delivery information. If you are treated for a wound infection by your physician your medical records will be obtained to determine the diagnosis, treatment and possible readmission within 30 days of cesarean section.

**How many people will participate?**
Approximately 1,200 women will take part in this study conducted by investigators at Washington University.

**How long will I be in this study?**
If you agree to take part in this study, your involvement will last until your 30-day follow-up phone call is complete.

**What are the risks of this study?**
You may experience one or more of the risks indicated below. In addition to these, there may be other unknown risks, or risks that we did not anticipate, associated with being in this study.

The use of chlorhexidine-alcohol or povidone-alcohol for skin preparation has the potential risks of skin irritation or allergy to the antiseptics. These irritations or allergies include symptoms of:

- Likely: temporary orange or brown color on the skin
- Less likely: skin irritation
- Rare: allergic skin reaction

**Risks related to breach of patient confidentiality:**
We strive to maintain confidentiality although rarely a breach of confidentiality may occur. To minimize these risks you will be given a unique study number. The master list associating study number and patient will be kept separate to all study materials. All study related information will be maintained in a locked cabinet in a locked office.

**What are the benefits of this study?**
You are not expected to directly benefit from being in this study. However, we hope that, in the future, other people might benefit from this study because the information obtained may provide additional ways to reduce infections related to cesarean delivery.

**What other treatment options are there?**
Participation in this study is optional and you may choose not to participate. If you choose not to participate in the study the standard antiseptic will be used.
Will it cost me anything to be in this study?
You will not have any additional costs for being in this research study. You and/or your medical/hospital insurance provider will remain responsible for your regular medical care expenses.

Will I be paid for participating?
You will not be paid for being in this research study.

What if I am injured as a result of this study?
Washington University investigators and staff will try to reduce, control, and treat any complications from this research. If you feel you are injured because of the study, please contact Shannon Martin at (314) 362-8523 and/or the Human Research Protection Office at 1-(800)-438-0445.

Decisions about payment for medical treatment for injuries relating to your participation in research will be made by Washington University. If you need to seek medical care for a research-related injury, please notify the investigator as soon as possible.

How will you keep my information confidential?
We will keep your participation in this research study confidential to the extent permitted by law. However, it is possible that other people such as those indicated below may become aware of your participation in this study and may inspect and copy records pertaining to this research. Some of these records could contain information that personally identifies you.

- Government representatives, (including the Office for Human Research Protections) to complete federal or state responsibilities
- Hospital or University representatives, to complete Hospital or University responsibilities
- Washington University’s Institutional Review Board (a committee that oversees the conduct of research involving human participants.) The Institutional Review Board has reviewed and approved this study.

To help protect your confidentiality, we will give you a unique study number, which will be kept in a master list. The master list will be kept separate from all study materials. The master list and all study related materials collected will be maintained in a locked cabinet in a locked office. If we write a report or article about this study or share the study data set with others, we will do so in such a way that you cannot be directly identified.

Protected Health Information (PHI) is health information that identifies you. PHI is protected by federal law under HIPAA (the Health Insurance Portability and Accountability Act). To take part in this research, you must give the research team permission to use and disclose (share) your PHI for the study as explained in this consent form. The research team will follow state and federal laws and may share your health information with the agencies and people listed under the previous section titled: “How will you keep my information confidential?”.

Once your health information is shared with someone outside of the research team, it may no longer be protected by HIPAA.
The research team will only use and share your information as talked about in this form. When possible, the research team will make sure information cannot be linked to you (de-identified). Once information is de-identified, it may be used and shared for other purposes not discussed in this consent form. If you have questions or concerns about your privacy and the use of your PHI, please contact the University’s Privacy Officer at 866-747-4975.

Although you will not be allowed to see the study information, you may be given access to your health care records by contacting your health care provider.

**If you decide not to sign this form, it will not affect**
- your treatment or the care given by your health provider.
- your insurance payment or enrollment in any health plans.
- any benefits to which you are entitled.

However, it will not be possible for you to take part in the study.

**If you sign this form:**
- You authorize the use of your PHI for this research
- Your signature and this form will not expire as long as you wish to participate.
- You may later change your mind and not let the research team use or share your information (you may revoke your authorization).
- To revoke your authorization, complete the withdrawal letter, found in the Participant section of the Human Research Protection Office website at [http://hrpo.wustl.edu/participants//withdrawing-from-a-study/](http://hrpo.wustl.edu/participants//withdrawing-from-a-study/) or you may request that the investigator send you a copy of the letter.
  - **If you revoke your authorization:**
    - The research team may only use and share information already collected for the study.
    - Your information may still be used and shared if necessary for safety reasons.
    - You will not be allowed to continue to participate in the study.

**Is being in this study voluntary?**
Taking part in this research study is completely voluntary. You may choose not to take part at all. If you decide to be in this study, you may stop participating at any time. If you decide not to be in this study, or if you stop participating at any time, you won’t be penalized or lose any benefits for which you otherwise qualify.

**What if I decide to withdraw from the study?**
You may withdraw by telling the study team you are no longer interested in participating in the study or you may send in a withdrawal letter. A sample withdrawal letter can be found at [http://hrpo.wustl.edu/participants/](http://hrpo.wustl.edu/participants/) under Withdrawing from a Research Study.

**What if I have questions?**
We encourage you to ask questions. If you have any questions about the research study itself or if you experience a research-related injury, please contact the nurse coordinator at (314) 362-8523.
If you have questions, concerns, or complaints about your rights as a research participant, please contact the Human Research Protection Office, 660 South Euclid Avenue, Campus Box 8089, St. Louis, MO 63110, 1-(800)-438-0445 or email hrpo@wusm.wustl.edu. General information about being a research participant can be found by clicking “Participants” on the Human Research Protection Office web site, http://hrpo.wustl.edu. To offer input about your experiences as a research subject or to speak to someone other than the research staff, call the Human Research Protection Office at the number above.

The Principle Investigator (PI) may withdraw you from the study without your consent if considered appropriate. For safety, it may be in your best interest to allow follow-up outside of the study. The investigator may terminate your participation in this study if in their best judgment it is in your best interest to do so, or under certain circumstances. For example, if you do not meet the study criteria the PI may withdraw you from the study.

This consent form is not a contract. It is a written explanation of what will happen during the study if you decide to participate. You are not waiving any legal rights by agreeing to participate in this study.

Your signature indicates that this research study has been explained to you, that your questions have been answered, and that you agree to take part in this study. You will receive a signed and dated copy of this form.

**Do not sign this form if today’s date is after** **EXPIRATION DATE: 01/19/16.**

(Signature of Participant)  (Date)

(Participant's name – printed)

**Statement of Person Who Obtained Consent**

The information in this document has been discussed with the participant or, where appropriate, with the participant’s legally authorized representative. The participant has indicated that he or she understands the risks, benefits, and procedures involved with participation in this research study.

(Signature of Person who Obtained Consent)  (Date)

(Name of Person who Obtained Consent - printed)
Antiseptic skin preparation for preventing surgical site infection at cesarean delivery: a randomized comparative effectiveness trial

“The Skin Prep Trial”

PROTOCOL

Final

Date: June 6, 2, 2015

Principle Investigators: Methodius G. Tuuli, MD., M.P.H.

Division of Maternal-Fetal Medicine
Department of Obstetrics and Gynecology
Washington University in St. Louis School of Medicine
660 S. Euclid Ave., Campus Box 8064
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Funding sponsor: National Institute of Child Health and Human Development (NICHD)
Table of Contents

A. Specific Aims........................................................................................................... 3

B. Background and Significance.............................................................................. 5

C. Research Design and Methods........................................................................... 11

D. Preliminary Studies.............................................................................................. 21

E. Key Personnel....................................................................................................... 27

F. Protection of Subjects........................................................................................... 31

References.................................................................................................................. 34
A. SPECIFIC AIMS

We propose a randomized controlled clinical trial to characterize the skin microbiome at Cesarean section surgical sites and to determine the comparative effectiveness of chlorhexidine-alcohol and iodine-alcohol preoperative skin preparation for preventing surgical site infections at cesarean section. Cesarean section is the most common major surgical procedure performed on women in the United States and infectious morbidity is one of the most common associated complications. While estimates vary, surgical site infections complicate up to 5 – 10% of all cesarean sections and result in significant human suffering and excess health care costs. The national burden of surgical site infection has led the Institute of Medicine to list research on reducing their incidence in the highest priority group of the initial 100 priorities for comparative effectiveness research. Interventions such as preoperative antibiotic prophylaxis reduce surgical site infections by 60%, but the rate of infection remains high. There is therefore a great need to identify and test other potential interventions to further reduce these infections.

The skin is a major source of pathogens that cause surgical site infection. Therefore, optimizing preoperative skin antisepsis has the potential to decrease postoperative surgical site infections. There is paucity of evidence to guide the choice of antiseptic for skin preparation at cesarean section. To date, only two underpowered trials have been published comparing two methods of preoperative skin preparation at cesarean section. A recent randomized trial in adults undergoing clean-contaminated mostly general surgical procedures demonstrated a 41% reduction in surgical site infection with the use of chlorhexidine-alcohol when compared to the more commonly used povidone-iodine. While it is plausible that findings from trials in other clean-contaminated surgical procedures may apply to cesarean sections, physiological changes in pregnancy, the peculiar dual microbial source for cesarean-related infections and the hormone-mediated immune-modulation in pregnancy make the validity of such extrapolation uncertain. Thus, superiority of chlorhexidine must be demonstrated in a
well-designed, adequately powered comparative effectiveness trial before changing the current use of iodine to the more expensive chlorhexidine for antisepsis at cesarean section. This study will be potentially practice changing and have a great impact on cesarean-related infections. If chlorhexidine is proven to be more effective than iodine for preoperative antisepsis at cesarean section, widespread use of a superior method of surgical site antisepsis will reduce infections, improve patient outcomes and reduce health care costs. The study has the following specific aims:

**Primary Aim:** To test the hypothesis that preoperative chlorhexidine-alcohol skin preparation at cesarean section significantly reduces *surgical site infections* compared to iodine-alcohol.

**Secondary Aim 1:** To characterize the skin microbiome at the surgical site and test the hypothesis that preoperative chlorhexidine-alcohol skin preparation at cesarean section significantly reduces *bacterial contamination* at the surgical site compared to iodine-alcohol.

**Secondary Aim 2:** To determine clinical outcomes and medical costs associated with cesarean-related infections and quantify *potential cost savings* attributable to use of chlorhexidine-alcohol for preoperative skin preparation at cesarean section.
B. BACKGROUND AND SIGNIFICANCE

Burden of cesarean-related infections, prevention as a priority

Surgical site infection is the second most common type of health care associated infections [1]. They are classified as superficial incisional (involving only skin or subcutaneous tissue), deep incisional (involving fascia and/or muscular layers) and organ or space [2]. Approximately two-thirds of these infections involve superficial incisions, and the remainder involve the deeper tissues and organ spaces. Such infections are among the leading causes of preventable death in the United States, accounting for an estimated 1.7 million infections and 99,000 associated deaths [1]. In addition to the substantial human suffering, it is estimated that they result in $28 - $33 billion of excess health care costs each year [3]. Prevention of these infections is, therefore, a top priority of the U.S. Department of Health and Human Services. The national burden of surgical site infection has led the Institute of Medicine to list research on reducing their incidence in the highest priority group of the initial 100 priorities for comparative effectiveness research [4, 5].

Cesarean section is the most common major surgical procedure performed on women in the United States and rates continue to rise. In 2008, 1.4 million (32%) of the 4.3 million deliveries in the United States were by cesarean section [6]. Infectious morbidity is one of the most common complications of cesarean delivery. Specifically, surgical site infections complicate 5 - 10 % of cesarean deliveries. One study from our institution reported a 5% rate of surgical site infections based on hospital data [7]. More recent data from a randomized controlled trial revealed a 10% rate of surgical site infection based on both inpatient and outpatient infection surveillance [8]. While costs may vary depending on the location, depth and severity, average attributable cost for each cesarean wound infection is estimated to be $2,800 - $3,400 [9].
Limitations of current prevention measures

Preoperative antibiotic prophylaxis has been established as an effective preventive measure for reducing cesarean-related infections. The most recent Cochrane review suggests a 60% decrease in surgical site infections when antibiotic prophylaxis is used at cesarean delivery (pooled RR 0.39; 95% CI 0.32 to 0.48) [10]. A meta-analysis including three randomized trials suggests antibiotic administration prior to skin incision, rather than after cord clamping, is associated with a reduced risk of endometritis and a trend towards lower rates of wound infections [11]. Emerging evidence also suggests that extending the spectrum of antibiotics may reduce the frequency of postcesarean wound infections [12]. However, even with optimal use of broad spectrum preoperative antibiotic prophylaxis the rate postcesarean wound infection remains high [8]. In addition, results of trials on other potential interventions such as supplemental oxygen have been largely disappointing [8, 13]. There is therefore a great need to identify and test other potential interventions to further reduce cesarean-related infections.

Optimizing preoperative skin preparation

The skin is a major source of pathogens that cause surgical site infection. It is estimated that 34% of surgical site infections are attributable to skin flora [14]. Postcesarean wound infections are commonly caused by Staphylococcus aureus, aerobic streptococci, and aerobic and anaerobic gram-negative bacilli [15]. Preoperative antiseptic skin preparation attempts to achieve a sterile surgical field by decreasing the concentration of bacteria colonizing the skin at the incision site. Therefore, optimizing preoperative skin antisepsis has the potential to decrease surgical site infections. The ideal preoperative skin antiseptic agent should significantly reduce microorganisms on intact skin, be non-irritating to the skin, broad spectrum, fast acting, have a persistent effect, remain effective in the presence of organic material, and be cost effective [16]. The Food and Drug Administration has approved several antiseptics including iodine, chlorhexidine and alcohol for skin
antisepsis. Chlorhexidine and iodine-based preparations have both been shown to decrease bacterial counts and are widely used. **Iodine** acts by oxidizing sulfydryl groups and distorting microbial protein structure. Potential disadvantages of iodine are skin irritation and need to dry for optimal action. **Chlorhexidine** on the other hand does not require a wait time between application and surgical incision. However, it is more expensive than iodine and may be associated with increased risk of allergic reactions [17]. Chlorhexidine acts by destroying bacterial cell membrane and precipitating cell contents. **Alcohol** is believed to act by damaging microbial cell membranes and denaturing protein. It has the advantage of being broad spectrum and fast acting, but lacks persistent activity. It is commonly combined with other antiseptics for a synergistic effect.

**Evidence of superiority of chlorhexidine compared to iodine for preoperative skin preparation**

Previous studies suggest a greater reduction in bacterial contamination in foot and ankle surgery [18], vaginal hysterectomy [19], and clean and clean-contaminated general surgery [20, 21] following skin preparation with chlorhexidine compared to iodine (pooled RR 0.44 [95% CI, 0.35 - 0.56]) [17]. A recent randomized trial in adults undergoing clean-contaminated mostly general surgical procedures demonstrated a 41% reduction in surgical site infection with preoperative chlorhexidine-alcohol when compared to the more commonly used povidone-iodine (9.5% versus 16.1%, RR 0.59, 95%CI 0.41 – 0.85) [22]. The associated number needed to treat was 17. Of note, chlorhexidine-alcohol was protective against both superficial and deep incisional infections, but not organ or space infections [22]. A subsequent meta-analysis including this and five other trials involving clean contaminated surgical procedures showed a reduction in postoperative surgical site infection when chlorhexidine-alcohol was used (pooled OR 0.68, 95%CI 0.50 – 0.94) [23].

Taken together, this data suggest superiority of chlorhexidine-alcohol for preoperative surgical site antisepsis for clean-contaminated general surgical procedures. Chlorhexidine has a number of properties that may account for its apparent superiority as an antiseptic. It has strong affinity for
binding to the skin, high antibacterial activity against gram-positive and gram-negative bacteria including methicillin-resistant staphylococcus aureus, and residual effects for up to 6 hours [24]. In contrast to iodine, chlorhexidine does not become inactivated in the presence of organic materials such as blood and other body fluids [25].

Lack of evidence on preoperative skin preparation at cesarean section

There is paucity of evidence on methods of preoperative skin preparation at cesarean section. A comprehensive review of evidence for different aspects of cesarean delivery concluded that “skin cleansing before cesarean delivery is grossly understudied” [26]. In fact, there have been only two published randomized trials to date on skin preparation at cesarean delivery [27, 28]. The first study compared a one-minute alcohol wash followed by application of an iodophor-impregnated adhesive film to a five-minute iodophor scrub followed by an iodophor wash in a prospective, randomized, controlled study of 79 patients [27]. While no differences in infectious morbidity was noted, this study demonstrated a greater reduction in skin bacterial count of the more rapid method of pre-operative skin preparation compared to a longer procedure. In other study Magann et al. randomly assigned 100 women to parachlorometaxylenol scrub for five minutes followed by povidone-iodine scrub and paint, or povidone-iodine scrub and paint alone [28]. No significant differences were seen in the incidence of endometritis and wound infections in the two groups. Both studies are limited by the small sample sizes and possible type II errors.

In contrast to the accumulating data on skin preparation in other surgical specialties there is an evidence gap regarding the relative effectiveness of chlorhexidine and iodine skin preparation for reducing skin contamination and surgical site infection at cesarean section. To our knowledge, there is no published trial comparing the two antiseptics for skin preparation at cesarean section. Rauk recently reported a significant reduction in the incidence of overall surgical site infections after cesarean section when a multidisciplinary protocol including chlorhexidine skin
preparation was implemented [29]. However, the use of historical controls and the multiplicity of interventions in the protocol (comprehensive staff education and training, skin preparation using chlorhexidine no rinse cloths prior to going to the operating room, chlorhexidine-alcohol for preoperative skin preparation in the operating room, and modified instrument sterilization techniques), make it impossible to attribute the reduction in infections to the chlorhexidine skin preparation alone.

Uncertainty extrapolating findings from other surgical procedures to cesarean section

As a clean-contaminated procedure, it is plausible that findings from trials in other clean-contaminated surgical procedures may apply to cesarean sections. However, physiological changes in pregnancy, the peculiar dual microbial source for cesarean-related infections (genital tract, skin) and the hormone-mediated immune-modulation in pregnancy make the validity of such extrapolation uncertain [30]. Experience with chlorhexidine use for the prevention of infections in other clinical settings gives reason for caution. For example, while a randomized trial showed a significant reduction in catheter-related bloodstream infections in critically ill adults in the intensive care unit with the use of chlorhexidine-impregnated dressings for intravascular catheters [31], a subsequent adequately powered trial in patients with tunneled central venous catheters undergoing hemodialysis failed to show a decrease in bloodstream infections [32]. Therefore, superiority of chlorhexidine must be demonstrated in a well-designed, adequately powered comparative effectiveness trial before changing the current use of iodine to the more expensive chlorhexidine for surgical site antisepsis at cesarean section.

Significance and potential impact of study

This study will have a potentially great impact on patient outcomes and health care costs. A finding that chlorhexidine is more effective than iodine for preoperative antisepsis at cesarean section will be practice changing. With over 1.4 million cesarean sections performed in the United States annually, wide spread use of a superior method of surgical site antisepsis will reduce surgical site
infections, improve patient outcomes and reduce health care costs. In a cost-benefit model based on data from other types of surgical procedures, Lee et al. demonstrated a net cost savings of $16 – 26 per case and $349,904 - 568,594 per year if the Hospital of the University of Pennsylvania switched from iodine to chlorhexidine [17]. Sensitivity analyses indicated persistence of net cost savings under most circumstances. If such cost savings hold for cesarean sections, a potential $16 – 26 billion could be saved in the United States annually by switching from iodine to chlorhexidine.
C. RESEARCH DESIGN AND METHODS

OVERALL DESIGN

This will be a randomized controlled clinical trial aimed at determining the comparative effectiveness of chlorhexidine-alcohol and iodine-alcohol preoperative skin preparation for preventing surgical site infection at cesarean section.

Rationale for Design

The randomized control trial is the gold standard of research design. Other designs such as case-control, retrospective cohort and prospective cohort are limited by potential bias and confounding. Randomly assigning subjects to different interventions minimizes selection bias. The random assignment also results in groups that are likely to be similar with regards to important confounding variables. This minimizes confounding by both measured and unmeasured factors. While random allocation does not guarantee the groups will be identical, it does ensure that any differences between them are due to chance alone. Finally, randomization produces groups that are random samples of the population. This permits use of standard statistical tests that are based on probability theory.

We will use broad inclusion criteria and analyze all the main outcomes by the intent-to-treat principle. This approach will allow a more conservative estimate of differences between groups and allow a better estimate of effectiveness and public health implications of practice change rather than a pure estimate of efficacy alone [33].

Subject Selection

Inclusion criteria – Women undergoing cesarean delivery at Barnes-Jewish Hospital.

Exclusion criteria - Inability to obtain consent; allergy to chlorhexidine, alcohol, iodine, shellfish; and evidence of infection adjacent to operative site.
Recruitment

All eligible patients who are scheduled for cesarean section will be approached for consent to participate in the study. Only IRB approved study team members will be involved in the recruitment of study subjects. Patients will be given study information and consent form for review, allowing time for consideration and discussion with family members/friends. For unscheduled patients, consent will be sought concurrently while the patient is being consented for a possible Cesarean section or immediately after.

**Secondary Aim 1:**
To test the hypothesis that chlorhexidine-alcohol skin preparation significantly reduces skin flora compared to iodine-alcohol.

**Primary Aim 1:**
To test the hypothesis that chlorhexidine-alcohol skin preparation significantly reduces surgical site infection compared to iodine-alcohol.

**Secondary Aim 2:**
To determine clinical outcomes, costs and potential cost savings attributable to chlorhexidine-alcohol skin preparation.
**Figure 1**: Schema of study hypothesis and specific aims. **Bold and dashed arrows indicate increasing and decreasing effects of the proximal factors on the distal factor, respectively.**

This will be limited to non-emergent indications, where consent can be obtained without interfering with patient care as in the case of failure for labor to progress. Study consent will be sought prior to the administration of any medications that may impair the patient’s decision-making ability. The vast majority of patients in labor at BJH receive epidural anesthesia for pain control, which does not impair the mental status. Patients who have received intravenous narcotics for pain control, which may affect their ability to give consent, will not be approached for study participation. Once the patient is transferred to the operating room, the circulating nurse will perform the randomization by selecting and opening a randomization prep envelope containing either chlorhexidine-alcohol prep or iodine-alcohol prep. The skin prep will be performed per BJH standards as in all cesarean section patients.

**Randomization and Treatment**

Enrolled patients will be randomly assigned in a 1:1 ratio using computer-generated randomization sequence to two skin preparation methods:

1. **Chlorhexidine-alcohol**-2% chlorhexidine gluconate with 70% alcohol (ChloraPrep, Cardinal Health) preoperative skin preparation.

2. **Iodine-alcohol**-8.3% povidone-iodine with 72.5% alcohol (Prevail-FX, Cardinal Health) preoperative skin preparation.

**Blinding**

Blinding both patients and physicians to the antiseptic used for skin preparation (double-blinding) would be ideal. However, it is not feasible in this trial. First, most patients can determine whether they were assigned to chlorhexidine or iodine, as the two antiseptics are of different colors and leave a stain on the skin (pink or brown, respectively). Second, physicians are often in the operating room
when the skin is being prepared for cesarean section and will know which antiseptic is used. We will minimize systematic bias by using the same standard procedures of skin preparation, skin culture and assessment of outcomes. All diagnoses of surgical site infection will be verified by chart review using the Centers for Disease Control and Prevention (CDC) National Nosocomial Infections Surveillance System criteria [34] (Figure 2). The principal investigator will verify the diagnoses without knowledge of the group to which the patients were assigned.

**PRIMARY AIM:**

To test the hypothesis that preoperative chlorhexidine-alcohol skin preparation at cesarean section significantly reduces surgical site infections compared to iodine-alcohol

**Outcome measures**

Primary outcome—Proportion of subjects with surgical site infection (superficial incisional [skin, subcutaneous layer] or deep incisional [fascia, muscle]) within 30 days of cesarean delivery. Surgical site infection will be based on diagnosis by the treating physician and verified by chart review in accordance with the CDC Nosocomial Infections Surveillance System definitions [34] (Figure 2).

Secondary outcomes—Length of hospital stay, number of office visits and re-admissions for infection-related complications, endometritis, positive culture from wound culture, skin irritation and allergic reactions.

**Methods**

Subjects will undergo cesarean delivery based on the technique selected by the surgeon. The circulating nurse will record information on key variables known to be related to surgical site infection: antibiotic administration (type and timing), type of cesarean section (scheduled or emergent), status of membranes (ruptured or unruptured), duration of surgery, depth of subcutaneous layer (closed or not closed) and skin closure method (subcuticular suture or staples) on data collection forms.
Demographic (age, race, socioeconomic status), obstetric (parity, gestational age, indication for cesarean section, cervical dilation at time of cesarean section, presence of chorioamnionitis, surgical complications) and neonatal (birth weight, Apgar score, cord pH) data will be abstracted from the patients chart.

Criteria for defining surgical site infection (SSI). From US Centers for Disease Control and Prevention

**Superficial incisional SSI**
Infection occurs within 30 days after the operation and infection involves only skin or subcutaneous tissue of the incision and at least one of the following:
1. Purulent drainage, with or without laboratory confirmation, from the superficial incision.
2. Organisms isolated from an aseptically obtained culture of fluid or tissue from the superficial incision.
3. At least one of the following signs or symptoms of infection: pain or tenderness, localized swelling, redness, or heat and superficial incision is deliberately opened by surgeon, unless incision is culture-negative.
4. Diagnosis of superficial incisional surgical site infection (SSI) by surgeon or attending physician.

**Deep incisional SSI**
Infection occurs within 30 days after the operation and infection involves deep soft tissue (e.g. fascial and muscle layers) of the incision and at least one of the following:
1. Purulent drainage from the deep incision but not from the organ/space component of the surgical site.
2. A deep incision spontaneously dehisces or is deliberately opened by a surgeon when the patient has at least one of the following signs or symptoms: fever (>38°C), localized pain, or tenderness, unless site is culture-negative.
3. An abscess or other evidence of infection involving the deep incision is found on direct examination, during reoperation, or by histopathologic or radiologic examination.
4. Diagnosis of a deep incisional SSI by a surgeon or attending physician.

**Organ/space SSI**
Infection occurs within 30 days after the operation and infection involves any part of the anatomy (e.g. organs or spaces), other than the incision, which was opened or manipulated during an operation and at least one of the following:
1. Purulent drainage from a drain that is placed through a stab wound into the organ/space.
2. Organisms isolated from an aseptically obtained culture of fluid or tissue in the organ/space.
3. An abscess or other evidence of infection involving the organ/space that is found on direct examination, during reoperation, or by histopathologic or radiologic examination.
4. Diagnosis of an organ/space SSI by a surgeon or attending physician.

**Figure 2:** CDC criteria for defining surgical site infection [34]

Subjects will be contacted within 30 days from delivery to assess symptoms of cesarean-related infections. Patients who report symptoms will be directed to follow up in the emergency department or with their physician to be evaluated for surgical site infection. Wound swabs will be taken for aerobic and anaerobic cultures in all subjects who present at Barnes-Jewish Hospital with wound infection.
Medical records will be obtained from treating physicians to determine the diagnosis at each postoperative office visit or readmission within 30 days of cesarean section.

All data will be managed in REDCAP.

**Data Analysis**

Analyses will be based on the intent-to-treat principle. The primary outcome (proportion of subjects with surgical site infection) and the other categorical variables will be compared across groups using the chi-squared test. Fisher’s exact test will be used for variables in which expected numbers in any of the cells in 2 x 2 tables is <5. We will calculate 95% confidence intervals around the differences in proportions and the relative risk of surgical site infection.

Distribution of continuous variables will be evaluated by visual inspection of histograms and the Kolmogorov-smirnov test. Normally distributed variables will be compared using the unpaired t-tests. If variables are not normally distributed, log transformation will be used in an attempt to achieve normal distribution. If the data is still skewed after log transformation the Mann-Whitney U test will be used to compare groups.

It is anticipated that baseline characteristics will be similar in the two groups. In the event that the groups are unbalanced with regards to variables significantly associated with the primary outcome, supplemental analyses will be performed using stratification on the individual variables and multivariable logistic regression adjusting for multiple covariates.

**Planned subgroup analysis** will be performed for: i. scheduled and elective cesarean sections, iii. obese and normal weight women, iii. Subcuticular and staple closure, and iv. women with and without chronic medical conditions (diabetes, chronic hypertension, renal disease). Interaction tests will be used to determine if the effectiveness of the skin preparation methods differ across these subgroups. Tests with p <0.05 will be considered statistically significant. Analyses will be performed using Stata version 12.1 (Stata Corp., College Station, TX).
Sample Size Consideration

The sample size estimation for the primary aim is based on an assumed baseline surgical site infection rate of 8% and an anticipated clinically significant 50% reduction in surgical site infection. To have 80% power to detect 50% difference in a two-tailed chi-squared test with α of 0.05, a total of 1084 subjects will need to be randomized. To accommodate a 10% drop out rate, 1,192 subjects will be enrolled (596 chlorhexidine, 596 iodine).

SECONDARY AIM #1

To characterize the skin microbiome at the surgical sites and test the hypothesis that preoperative chlorhexidine-alcohol skin preparation at cesarean section significantly reduces bacterial contamination at the surgical site compared to povidone-iodine

Outcome measures

Primary outcomes-

1. Descriptive characteristics of the type and relative abundance of the skin microbiome at the surgical site at cesarean
2. Proportion of subjects with surgical site skin contamination after antiseptic preparation.
   Contamination will be defined as ≥5000 total colony-forming units per milliliter on aerobic or anaerobic culture.

Secondary outcomes- Types of bacteria cultured before and after skin preparation, concordance of bacteria at surgical site following preoperative skin preparation with bacteria in postoperative surgical site infections.

Methods

Two skin swabs will be taken transversely across the suprapubic area, 2 finger breadths above the symphysis pubis immediately before, and 5 minutes after skin preparation. We will extract microbial DNA from one swab each and perform microbial sequencing using well established 16s rRNA
sequencing procedures at the Genome Institute at Washington University. We will perform post-
sequencing processing to identify the bacteria present in each sample and their relative abundance.
The remaining swabs will be cultured under aerobic and anaerobic conditions. To ensure that the
groups at high risk for surgical site infections are well represented, we will ensure that obese women,
diabetics and women undergoing emergent cesarean deliveries are adequately sample and randomized. In addition to aerobic and anaerobic cultures on wound swabs of women who develop surgical site infections, 16s rRNA sequencing will also be performed and the identity and relative abundance of the bacteria present will be determined.

Data Analysis
Analyses will be based on the intent-to-treat principle. The primary outcome (proportion of subjects with surgical site skin contamination after skin preparation) and the other categorical variables will be compared across groups using the chi-squared test. Fisher’s exact test will be used for variables in which expected numbers in any of the cells in 2 x 2 tables is <5. Tests with p <0.05 will be considered significant. We will also conduct stratified analysis based on the different risk groups. Finally, we will calculate 95% confidence intervals around the difference in proportions and relative risk of skin contamination after antiseptic skin preparation. Analyses will be performed using Stata version 10.0 (Stata Corp., College Station, TX).

Sample Size Consideration
The sample size estimation for secondary aim #1 is based on the primary outcome of skin contamination following skin preparation. A meta-analysis of data from non-obstetric surgical procedures suggest a contamination rate of 39% after preoperative skin preparation with iodine and a rate of 18% after the use of chlorhexidine [17]. On the basis of an assumed contamination rate of 39% in the iodine group and 50% difference in skin contamination as clinically significant, a total of
200 subjects will be needed (100 chlorhexidine, 100 iodine) to have 80% power in a two-tailed chi-squared test and α of 0.05.

![Table showing results of meta-analysis](attachment:image.png)

**Figure 3:** Meta-analysis of 4 studies on positive skin culture after skin preparation with iodine or chlorhexidine [17].

**SECONDARY AIM #2**

To determine clinical outcomes and medical costs associated with cesarean-related infections and quantify potential cost savings attributable to use of chlorhexidine-alcohol preoperative skin preparation at cesarean section

**Outcome measure**

The outcome for secondary aim#2 is attributable cost saving (if any), defined as the difference in total costs between women with preoperative iodine and chlorhexidine skin preparation.

**Methods/Data Analysis**

A cost-benefit decision analysis model will be developed depicting the decision of whether to use chlorhexidine or iodine for a patient undergoing cesarean section. The cost of implementing each strategy will include the purchase costs of the antiseptic agents. For each antisepsis strategy, the patient would then have a probability of subsequently developing surgical site infection based on results of the randomized trial under the primary aim. We will calculate cost incurred by patients who did and did not develop an infection.
Costs will be obtained from the Barnes-Jewish Hospital cost accounting database for the surgical admission and any readmission to the hospital and office visits within 30 days after cesarean section. Cost savings, if any, will be the difference between the costs in the two groups. The cost-benefit analysis will be performed using TreeAge Pro 2009 (TreeAge Software).

**Sample Size Consideration**

No formal sample size estimation is made for secondary aim #2. The cost-benefit analysis will be based on outcomes among the subjects enrolled under secondary aim #2.

**Table 1: TIME LINE OF STUDY ACTIVITIES**

<table>
<thead>
<tr>
<th>Dates</th>
<th>Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>September 2011 - June 2015</td>
<td>Recruitment</td>
</tr>
<tr>
<td>April 2013</td>
<td>Interim Analysis</td>
</tr>
<tr>
<td>March 2014</td>
<td>Interim analysis</td>
</tr>
<tr>
<td>July – August 2015</td>
<td>Final Data Analysis</td>
</tr>
<tr>
<td>July – August 2015</td>
<td>Preparation of final manuscripts for publication</td>
</tr>
<tr>
<td>August 2015</td>
<td>Estimated End Date</td>
</tr>
</tbody>
</table>
D. PRELIMINARY STUDIES

i. Preliminary study#1: Cesarean deliveries at Barnes-Jewish Hospital

To determine the feasibility of recruiting the required sample size, we reviewed delivery data at Barnes Jewish Hospital, site for the proposed study, from January 1, 2007 to December 31 2007. During this one year period, a total of 3981 deliveries were performed. Table 2 shows a breakdown of the deliveries. A total of 1122 deliveries, constituting 28% were by cesarean section. Of the cesarean sections 61% were primary while 39% were repeat cesarean sections. The most common indications for cesarean section were repeat sections, arrest of labor, non-reassuring fetal heart tracing and mal-presentation.

Given the broad inclusion criteria we estimate that less than 10% of the patients undergoing cesarean section will need to be excluded (allergy to iodine or chlorhexidine, inability to obtain consent, existing infection at incision site). Based on our previous experience enrolling patients for cesarean section related studies and the universality of skin preparation at cesarean section, we estimate a consent rate of at least 60%. Thus, we estimate that the sample size of 1192 can be achieved in a 2-year period (2 x 1122 x 90% x 60%=1212).

Table 2: Delivery Data at Barnes Jewish Hospital, January 1, 2007 to December 31, 2007

<table>
<thead>
<tr>
<th>Delivery Type</th>
<th>Number</th>
<th>Percent of Deliveries (%)</th>
<th>Percent of Cesarean Sections (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vaginal Delivery</td>
<td>2809</td>
<td>72</td>
<td>-</td>
</tr>
<tr>
<td>Cesarean Sections</td>
<td>1122</td>
<td>28</td>
<td>-</td>
</tr>
<tr>
<td>Primary</td>
<td>684</td>
<td>-</td>
<td>61</td>
</tr>
<tr>
<td>Repeat</td>
<td>438</td>
<td>-</td>
<td>39</td>
</tr>
<tr>
<td>Total</td>
<td>3981</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>
ii. Preliminary study #3: Burden and risk factors for cesarean-related surgical site infection [7].

A retrospective study was conducted at Barnes-Jewish Hospital to identify independent risk factors for surgical site infection after cesarean. A total of 1605 women who underwent low transverse cesarean section during the period from July 1999 to June 2001 were evaluated. Using the International Classification of Diseases, Ninth Revision diagnosis codes for surgical site infection or wound complication and/or data on antibiotic use during the surgical hospitalization or at readmission to the hospital or emergency department, potential cases of surgical site infections were identified in this cohort. Cases of surgical site infection were verified by chart review using the definitions from the CDC National Nosocomial Infections Surveillance System. Control patients without surgical site infection or endometritis were randomly selected from the population of patients who underwent cesarean section. Independent risk factors for surgical site infection were determined by logistic regression.

Surgical site infections were identified in 81 (5.0%) of the 1605 women who underwent low transverse cesarean section. **We consider the surgical site infection rate of 5% as an underestimation, since it was based only on hospital data.** Independent risk factors for surgical site infection included development of subcutaneous hematoma after the procedure (adjusted odds ratio [aOR], 11.6 [95% confidence interval [CI], 4.1-33.2]), operation performed by the university teaching service (aOR, 2.7 [95% CI, 1.4-5.2]), and a higher body mass index at admission (aOR, 1.1 [95% CI, 1.0-1.1]). Antibiotic prophylaxis before or after the operation was associated with a significantly lower risk of surgical site infection (aOR, 0.2 [95% CI, 0.1-0.5]).

iii. Preliminary study #4: Effect of skin closure method on cesarean wound infection or separation [35]

We recently published a systematic review and meta-analysis to estimate whether staples or subcuticular suture closure is associated with a higher risk of wound infection or separation when
used for transverse skin incisions at cesarean section. We searched electronic databases from 1966 to September 2010 for randomized controlled trials (RCTs) and prospective cohort studies comparing staples to subcuticular sutures at cesarean section. The primary outcome was occurrence of wound infection or separation.

Six studies including 5 RCTs and one prospective cohort study met inclusion criteria. Staple closure (n=803) was associated with a two-fold higher risk of wound infection or separation compared to subcuticular suture closure (n=684) (13.4% versus 6.6%, pooled OR 2.06 [1.43 – 2.98]) (Figure 4). The number needed to harm was 16. The increased risk persisted when analysis was limited to the RCTs (OR 2.43 [1.47 – 4.02]). There was no evidence of significant statistical heterogeneity among studies (χ² =0.74, p=0.327, I²=13.7%) or publication bias (t= -0.86, p=0.439). Staple closure was associated with a small, but shorter duration of surgery in most studies that evaluated operating time.

We concluded that staple closure is faster to perform, but associated with a higher risk of wound infection or separation.
iv. **Preliminary study #5**: Randomized control trial of supplemental oxygen to reduce post cesarean infections[8]

We also recently completed a randomized control trial to investigate whether supplemental oxygen during cesarean delivery and for 2 hours afterwards reduces the incidence of post-cesarean infectious morbidity. Women undergoing cesarean were randomized to receive either 2L of oxygen via nasal cannula during cesarean delivery only (standard care) or 10L oxygen via non-rebreather mask (intervention group) during cesarean and for 2 hours afterward. The primary outcome was infectious morbidity, defined as surgical site infection or endometritis. A total of 585 women were included in the final analysis. Demographic data was similar in the two groups. There was no significant difference in the rate of infectious morbidity between the standard care and intervention groups (RR 1.4, 95% CI 0.9-2.3).

This study demonstrates that supplemental oxygen does not reduce the rate of post-cesarean infectious morbidity. This underscores the need to identify and test other interventions such as skin preparation methods. **The study also establishes a more realistic surgical site infection rate of 10% in our population (Table 3)**, since infections were identified from both hospital data and active outpatient surveillance. **It also confirms our ability to recruit, randomize and follow-up patients in a cesarean-related randomized trial.**
Table 3: Maternal Outcomes in women randomized to supplemental oxygen or standard care for prevention of surgical site infections at cesarean section.

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Overall (N=585)</th>
<th>Standard Care (n=297)</th>
<th>Supplemental Oxygen (n=288)</th>
<th>Relative Risk (RR)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Composite</td>
<td>61 (10.4%)</td>
<td>26 (8.8%)</td>
<td>35 (12.2%)</td>
<td>1.4 (95% CI 0.9-2.3)</td>
<td>0.18</td>
</tr>
<tr>
<td>Endometritis</td>
<td>9 (1.5%)</td>
<td>2 (0.6%)</td>
<td>7 (2.4%)</td>
<td>3.6 (95% CI 0.8-17.2)</td>
<td>0.08</td>
</tr>
<tr>
<td>Surgical site infection</td>
<td>59 (10.1%)</td>
<td>26 (8.8%)</td>
<td>33 (11.5%)</td>
<td>1.3 (95% CI 0.8-2.1)</td>
<td>0.28</td>
</tr>
<tr>
<td>Wound hematoma or seroma</td>
<td>33 (5.6%)</td>
<td>17 (5.9%)</td>
<td>16 (5.4%)</td>
<td>1.1 (95% CI 0.6-2.1)</td>
<td>0.79</td>
</tr>
<tr>
<td>Hospital readmission</td>
<td>25 (4.3%)</td>
<td>10 (3.4%)</td>
<td>15 (5.2%)</td>
<td>1.5 (95% CI 0.7-3.4)</td>
<td>0.27</td>
</tr>
</tbody>
</table>

v. Preliminary study #5: Ongoing randomized trial on the effect of omitting the Bladder Flap at cesarean section

We are currently conducting a randomized control trial to evaluate short and medium term effects of omitting a step (creation of the bladder flap) considered to be unnecessary at cesarean section. So far, we have recruited 228 of the target 258 sample size. This further underscores the ability of the investigators to recruit, randomize and follow-up patients to evaluate outcomes in cesarean-related randomized trial.
vi. **Preliminary study #6: Attributable costs of surgical site infection and endometritis after low transverse cesarean delivery [9].**

Finally, a cost-analysis was conducted at Barnes-Jewish Hospital to determine the attributable costs of surgical site infections and endometritis after cesarean section by two different methods. Using data from 1605 women who underwent low transverse cesarean sections from July 1999 to June 2001, attributable costs of surgical site infections and endometritis were determined by generalized least squares (GLS) and propensity score matched-pairs. For the matched-pairs analyses, uninfected control patients were matched to patients with surgical site infection or with endometritis on the basis of their propensity to develop infection, and the median difference in costs was calculated. The attributable total hospital cost of surgical site infections was $3,529 by GLS and $2,852 by propensity score matched-pairs.
E. KEY PERSONNEL

Methodios G. Tuuli, M.D., M.P.H., a Principal Investigator for this study, will be Assistant Professor (clinician investigator track) in the Department of Obstetrics and Gynecology at Washington University in St. Louis, starting in July 2011. Dr. Tuuli received his undergraduate and M.D. degrees from the University of Ghana Medical School. He then completed a master’s degree in public health (MPH) at the University of California, Berkeley, with a concentration in maternal and child health. After residency training in Obstetrics and Gynecology at Emory University in Atlanta Georgia, Dr. Tuuli is completing fellowship training in Maternal-Fetal Medicine at Washington University in St. Louis. He is board-certified in Obstetrics and Gynecology and will be an active candidate for certification in Maternal-Fetal Medicine. During his fellowship, Dr. Tuuli completed two graduate level courses in advanced statistics for clinical researchers at the Washington University Institute for Clinical and Translational Sciences (ICTS). Dr. Tuuli has been very productive during fellowship, using his skills to carry out several retrospective and prospective studies. Of direct significance to this proposal, Dr. Tuuli is currently completing a randomized control trial on the role of the bladder flap at cesarean delivery. He has 27 publications in high-quality journals including Obstetrics & Gynecology, American Journal of Obstetrics and Gynecology, and the British Journal of Obstetrics and Gynecology. These include 17 peer-reviewed articles, 5 invited reviews, 2 book chapters and 3 other publications. He is also a reviewer for several journals. In addition to his appointment as Assistant Professor, Dr Tuuli will be a Women’s Reproductive Health Research (WRHR) Scholar under an NIH funded career development grant (K12) awarded to the Department of Obstetrics and Gynecology of Washington University.

George A. Macones, M.D., M.S.C.E., a Co-investigator for this study, is the Mitchell and Elaine Yanow Professor and Chair of the Department of Obstetrics and Gynecology at Washington University School of Medicine, Senior Scholar in the Institute of Public Health at Washington University.
University in St. Louis, and founder of the Division of Clinical Research. He is trained in Maternal-Fetal Medicine and Epidemiology, and has been nationally and internationally recognized for his perinatal outcomes research. Dr. Macones’ expertise in clinical research design and epidemiology has been utilized in his role as reviewer for a variety of NIH/NICHD study sections. His research has been published in high-quality, peer-review journals and has been extensively funded by the NIH and other foundations. He is currently the PI of an RO1 for an observational study investigating the association between LEEP and preterm birth. Dr. Macones is also recipient of an ongoing K24 grant (Midcareer Award in Patient Oriented Research) from NICHD. He is currently an Associate Editor for the American Journal of Obstetrics and Gynecology. He currently serves on the sub-specialty Board of the American Board of Obstetrics and Gynecology for Maternal-Fetal Medicine, and is on the Board of Directors for the Society of Maternal Fetal Medicine. He was recently elected to the American Society of Clinical Investigation. Finally, and specific to this proposal, Dr. Macones has worked closely with Tuuli in his role as fellowship director, research mentor, and collaborator. He will continue to be Dr Tuuli’s mentor under the WRHR scholar program. This outstanding working relationship will aid in the development and performance of this study.

David M. Stamilio, M.D., M.S.C.E., a Co-investigator, is Associate Professor of Obstetrics and Gynecology, and the Division Chief of Maternal-Fetal Medicine at Washington University Medical Center. Dr. Stamilio is also a member of the Division of Clinical Research in the Department of Obstetrics and Gynecology, and a founding member of the interdisciplinary Washington University Center for Preterm Birth Research. He is a formally trained epidemiologist with experience and expertise in the area of perinatal outcomes research, and has chaired the forum for Perinatal Epidemiology at the past two annual national meetings for the Society of Maternal-Fetal Medicine. Dr. Stamilio has published numerous articles in the area of clinical obstetrics, maternal-fetal medicine, economic analysis and perinatal epidemiology. He has been the PI on several research projects,
including funding from the Barnes-Jewish Foundation as **PI for a randomized-controlled trial examining post-cesarean maternal oxygen exposure to decrease the risk of operative infectious morbidity.** Dr. Stamilio will add his expertise to many areas of this study, including assistance in quality assurance, study logistics, analysis and publication.

**Jingxia (Esther) Liu, PhD,** the Study Statistician, is Assistant Professor in the Division of Biostatistics. She received her BSc in scientific computing and applied software at the Department of Mathematics of NanKai University in 1997, her MSc in financial mathematics from National University of Singapore in 2002 and a PhD in biostatistics from the Medical College of Wisconsin in 2007. After graduating from the Medical College of Wisconsin, she worked as a lead statistician at the Pharmaceutical Product Development (PPD) Inc. from 2007 to 2009. Since joining Washington University, Dr. Liu has focused her efforts in statistical support for both the ICTS and the Siteman Cancer Center. Dr. Liu’s research interests include propensity score methodology, survival data analysis, study design in clinical trials and missing data methods. She is also interested in cost-effective analysis and methodological issues in observational studies. She was involved in many projects from small to large size and provided statistical consulting on Phase I-IV clinical trials across different functional departments at the Pharmaceutical Product Development (PPD) Inc., which has ensured meeting the regulatory demands of FDA and submission deadlines of pharmaceutical companies. She is a member of the Institute of Clinical and Translational Sciences (ICTS) Research Design and Biostatistics Group (RDBG) and is currently providing statistical support for the projects of the Siteman Cancer Center and the ICTS.

**Swarup Sri Varaday, M.D.,** a Co-investigator is Assistant Professor of Anesthesiology. Dr Varaday works closely with the department of Obstetrics and Gynecology as one of the key providers of anesthesia for labor and cesarean delivery. Since anesthetic management is an integral part of pre-
operative procedures, involvement of Dr Varaday will ensure cooperation between the anesthesia
and obstetrics teams, and facilitate the smooth conduct of the study.

Cindy Bertolino, RN, a Co-investigator, is a charge nurse of labor and delivery. She will bring a
unique nursing perspective to the study and facilitate the recruitment of patients.
F. PROTECTION OF HUMAN SUBJECTS

Assessment of Risks

Subject Characteristics

All women undergoing cesarean section at Barnes-Jewish Hospital during the study period will be eligible. We anticipate enrolling a total of 1192 patients. Exclusion criteria include inability to obtain consent; allergy to chlorhexidine, alcohol, iodine; and evidence of infection adjacent to operative site. The involvement of pregnant women (a vulnerable population) is inevitable since cesarean section is a procedure performed only in this patient population.

Potential Risks

Patients undergoing cesarean sections are at risk for complications including anesthetic complications, bleeding, need for blood transfusion, injury to surrounding organs such as bowel, bladder, ureters, possible need for further procedures and postoperative infection. The use of chlorhexidine alcohol for skin preparation is not expected to increase these risks. Preliminary studies in other types of surgical procedures suggest a likely reduction in surgical site infection. Potential risks include skin irritation, allergy to the antiseptics and breach of patient confidentiality. Measures outlined below will be employed to minimize these risks. It is also noteworthy that while iodine-alcohol is the standard of care at Barnes-Jewish Hospital, chlorhexidine-alcohol is available and is used in patients who report iodine allergy.

Adequacy of protection against risks

Data and Safety Monitoring Plan

Although risks to study participants are expected to be small, a number of measures are planned to ensure patient safety.
1. The principal investigator (PI) – The PI will be the first layer of monitoring for risk to study subjects. The PI will monitor, document and report to the Institutional Review Board, any adverse events among study participants.

2. Data and Safety Monitoring Board (DSMB) – The DSMB will consist of three individuals with extensive experience in clinic research and who are not directly involved in the study: Anthony Odibo, MD, MSCE; Alison Cahill, MD, MSCI; Anthony Shanks, MD. The DSMB will be tasked with ensuring the overall safety of subjects enrolled in the study. They will also be responsible for interpreting results of the interim analysis and making decisions including stopping the study.

3. Interim analysis – An interim efficacy analysis will be conducted (with surgical site infection as the primary outcome of interest) after half the patients have been evaluated. Analysis will be performed by the study statistician and presented to the DSMB. The board will make a recommendation regarding further conduct of the study. The principal investigator will not be informed of the results of the interim analysis unless the DSMB determines that some level of unblinding is necessary to make final decisions about the conduct of the study. Possible decisions include stopping the study because efficacy has been achieved or because of futility, and modifying the target sample size.

While early stopping decisions cannot be based purely on a mathematical stopping rule, the Haybittle-Peto stopping rule will be used as a guide. Under this rule the interim analysis of the primary outcome would have to demonstrate a difference between groups in excess of three standard errors (or p <0.001) to justified premature disclosure [36, 37]. This rule has the advantage that the exact number and timing of interim analysis need not be specified. It also preserves the power and overall type I error (0.05). Final decisions will be based partly on more subjective considerations such as the consistency of results across subgroups and secondary endpoints.

4. Protection of patient confidentiality – We will protect patient confidentiality by making sure that only individuals directly involved in the study have access to identifiable patient information. Study files will
be kept under lock and key. Only the study coordinator and PI will have access to the key. Patient information will be coded and stripped of identifiers prior to analysis and publication of study results.

**Potential Benefits/Importance of knowledge gained**

The study is not designed to provide direct benefits to research participants. However, if our hypothesis that chlorhexidine-alcohol is a superior antiseptic for cesarean section is true, those participants randomized to the chlorhexidine-alcohol group will enjoy the benefits of reduced infections. More importantly, results from this study have the potential to reduce cesarean-related infections, improve patient outcomes and reduce health care costs. Since the anticipated risk to participants is minimal, the risks-benefit ratio is very favorable.
REFERENCES


20. Paocharoen, V., C. Mingmalairak, and A. Apisarnthanarak, Comparison of surgical wound infection after preoperative skin preparation with 4% chlorhexidine [correction of chlohexidine]


# SUMMARY OF CHANGES TO PROTOCOL

<table>
<thead>
<tr>
<th>Change</th>
<th>Rationale</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Addition of microbiome analysis of skin swabs to secondary aim 1</td>
<td>Advances in microbiome analysis permit a more complete assessment of organisms in several body niches. We included microbiome analysis to enable us evaluate in a more comprehensive manner the bacteria at the site of cesarean incisions</td>
<td>Page 4 &amp; 17 - 18</td>
</tr>
<tr>
<td>2. Change of data management system from DATSTAT to REDCAP</td>
<td>A new data management system was used by the division of clinical research</td>
<td>Page 16</td>
</tr>
<tr>
<td>3. Change of data Analysis software from STATA version 10.0 to STATA version 12.1</td>
<td>A new version of the data management software became available by the end of the study and was used for analysis</td>
<td>Page 16</td>
</tr>
<tr>
<td>4. Modification of the time line of study activities</td>
<td>Slower recruitment than anticipated necessitated adjustment to the time line</td>
<td>Page 20</td>
</tr>
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</table>
Original Statistical Analysis Plan

Data Analysis

Analyses will be based on the intent-to-treat principle. The primary outcome (proportion of subjects with surgical site infection) and the other categorical variables will be compared across groups using the chi-squared test. Fisher’s exact test will be used for variables in which expected numbers in any of the cells in 2 x 2 tables is <5. We will calculate 95% confidence intervals around the differences in proportions and the relative risk of surgical site infection.

Distribution of continuous variables will be evaluated by visual inspection of histograms and the Kolmogorov-smirnov test. Normally distributed variables will be compared using the unpaired t-tests. If variables are not normally distributed, log transformation will be used in an attempt to achieve normal distribution. If the data is still skewed after log transformation the Mann-Whitney U test will be used to compare groups.

It is anticipated that baseline characteristics will be similar in the two groups. In the event that the groups are unbalanced with regards to variables significantly associated with the primary outcome, supplemental analyses will be performed using stratification on the individual variables and multivariable logistic regression adjusting for multiple covariates.

Planned subgroup analysis will be performed for: i. scheduled and elective cesarean sections, iii. obese and normal weight women, iii. Subcuticular and staple closure, and iv. women with and without chronic medical conditions (diabetes, chronic hypertension,
renal disease). Interaction tests will be used to determine if the effectiveness of the skin preparation methods differ across these subgroups. Tests with p <0.05 will be considered statistically significant. Analyses will be performed using Stata version 10.0 (Stata Corp., College Station, TX).

**Sample Size**

**Consideration**

The sample size estimation for the primary aim is based on an assumed baseline surgical site infection rate of 8% and an anticipated clinically significant 50% reduction in surgical site infection. To have 80% power to detect 50% difference in a two-tailed chi-squared test with α of 0.05, a total of 1084 subjects will need to be randomized. To accommodate a 10% drop out rate, 1,192 subjects will be enrolled (596 chlorhexidine, 596 iodine).
**Final Statistical Analysis Plan**

**Data analysis**

The primary data analyses followed the intention-to-treat principle in which subjects were analyzed in the group to which they were randomized, regardless of whether or not they received the assigned intervention. Descriptive and univariable statistics were used to characterize the study subjects and investigate comparability of the two groups at baseline.

We compared the primary outcome and other categorical outcomes between groups, and estimated relative risks with 95% confidence intervals. We also used time-to-event analysis to compare the interval from surgery to diagnosis of surgical site infection in the two groups. Kaplan-Meier estimates of freedom from infection were compared in the two groups using the log-rank test. We conducted four prespecified (scheduled versus unscheduled cesarean, obese versus non-obese, subcuticular suture versus staple skin closure) and one post hoc (diabetic versus non-diabetics) subgroup analyses for the primary outcome. We used the Mantel-Haenszel test of homogeneity to assess if the relative effectiveness of antiseptics differed across subgroups. We also conducted analysis limited to patients who completed the 30 days of follow up.

In addition to the prespecified secondary outcomes (length of hospital stay, physician office visits and re-admissions for infection-related complications, endometritis, positive wound culture, skin irritation and allergic reactions) we assessed other secondary outcomes, post hoc, other wound complications (including skin separation, seroma, hematoma, cellulitis), emergency room visits for wound
complications, additional wound surgery, use of home health or wound clinic, and duration of wound care.

All tests were two-sided and $P<0.05$ was considered statistically significant. Data analysis was conducted using Stata version 12.1 (Stata Corp., College Station, TX).
<table>
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<tr>
<th>Change</th>
<th>Rationale</th>
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<tr>
<td>1. Addition of time-to-event analysis to compare the interval from surgery to diagnosis of surgical site infection.</td>
<td>This was not specified in the initial plan, but deemed important in assessing whether the two antiseptics have a differential effect on time to infection.</td>
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<tr>
<td>2. Addition of analysis limited to patients who completed the 30 days of follow up.</td>
<td>This was added as a sensitivity analysis to assess the impact of patients lost to follow up (5% in each group) on the primary intention-to-treat analysis.</td>
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<tr>
<td>3. Addition of one post hoc subgroup analysis</td>
<td>We specifically assessed if the presence of diabetes, a major risk factor for surgical site infection, affects the relative efficacy of the two antiseptics.</td>
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<tr>
<td>4. Assessment of other secondary outcomes post hoc</td>
<td>This was performed to assess the impact of type of antiseptic on other important outcomes that were not prespecified.</td>
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