Duke/UNC Prevention Epicenter Program

The Antiseptic Scrub Contamination and Transmission (ASCOT) Trial: a 3-Arm Randomized Crossover Trial

Protocol Date:	September 1, 2015
Protocol Version:	1.0
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STATEMENT OF COMPLIANCE

This study will be conducted in compliance with the applicable principles and regulatory requirements from the United States Code of Federal Regulations (CFR), including 21 CFR 56 (institutional review board [IRB]) and 21 CFR 50 (informed consent) and to the principles outlined in applicable ICH guidelines.

STUDY PRINCIPAL INVESTIGATOR SIGNATURE

The signature below documents the review and approval of this protocol and provides the necessary assurances that this study will be conducted according to all stipulations of the protocol, including all statements regarding confidentiality, and according to local legal and regulatory requirements and to the principles outlined in applicable U.S. federal regulations and ICH guidelines.

Deverick Anderson, MD, MPH
_____

Study Principal Investigator

_____   9/1/15
Signature   Date

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LIST OF ABBREVIATIONS

AB	Acinetobacter
DICON	Duke Infection Control Outreach Network
DUH	Duke University Hospital
HCP	Healthcare provider
MICU	Medical intensive care unit
MDR	Multidrug resistant
MDRO	Multidrug resistant organism
MSSA	Methicillin susceptible *Staphylococcus aureus*
MRSA	Methicillin resistant *Staphylococcus aureus*
SICU	Surgical intensive care unit
VSE	Vancomycin susceptible *enterococci*
VRE	Vancomycin resistant *enterococci*

PROTOCOL SYNOPSIS

Protocol Title:	**The Antiseptic Scrub Contamination and Transmission (ASCOT) Trial: a 3-Arm Cluster Randomized Crossover Trial**
Phase:	Not Applicable
Products:	Not Applicable
Objectives:	To determine if antiseptic-impregnated surgical scrubs decrease the burden of HCP clothing contamination compared to standard, control surgical scrubs following a 12-hour ICU shift
Study Design:	Prospective, blinded, 3-arm randomized controlled trial with crossover design
Study Population:	Nurses who work in the Duke University Hospital SICU and MICU
Number of Participants:	40
Number of Sites:	1
Duration of Participant Participation:	3 12-hour shifts
Dose Schedule:	Not Applicable
Estimated Start:	August 1st 2015
Estimated Time to Complete Enrollment:	6 months

Schematic/Description of Study Design

Figure. Crossover design for each enrolled nurse subject.
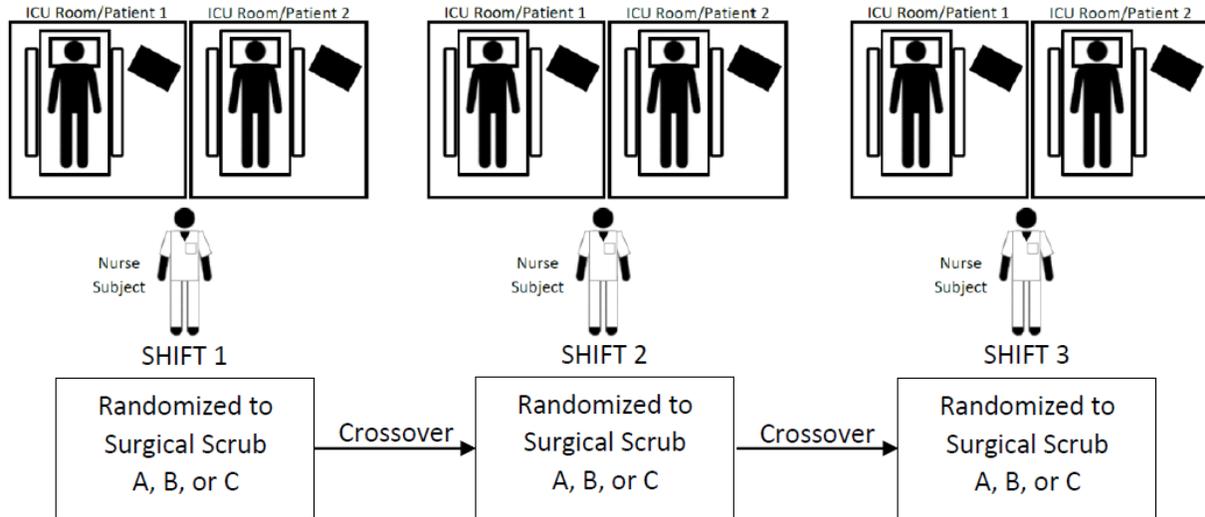
1 KEY ROLES

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2 BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE

2.1 Background Information

Approximately 1 in 25 patients contract a healthcare-associated infection (HAI) each year in the US, and 75,000 die in the hospital as a result of their HAI.[1] In order to develop better prevention measures, we must learn more about complex interactions involved in pathogen transmission between patients, healthcare providers (HCP), and the environment. This proposal focuses on three aspects of this complex transmission process: HCP clothing, personal protective equipment (PPE), and environmental risk factors.

2.2 Scientific Rationale

HCP clothing quickly becomes contaminated during routine clinical duties.[2,3] For example, 54% of the surgical scrubs worn by 57 nurses became contaminated with VRE, MRSA, and/or *C. difficile* at the end of a standard shift in one study.[4] The highest burden of contamination of clothing worn by HCPs occurs at points of frequent contact, such as pockets and ends of sleeves.[5] Contaminated clothing can also lead to contaminated HCP hands and patient environment.[6] The pathogens on contaminated HCP clothing, hands contaminated by clothing, or in the contaminated environment may in turn be transmitted to patients during routine care.

Pre-clinical data. Pre-clinical data suggest that antiseptic-impregnated textiles are efficacious.[7-9] Our study will investigate two types of antiseptic textiles with significant in vitro activity against *S. aureus*, *C. albicans*, Acinetobacter, vegetative *C. difficile*, and *K. pneumonia*e. **Vestex** (Vestagen Technical Textiles) is a cotton-polyester blend impregnated with an organosilane-based quaternary ammonium antiseptic and a hydrophobic fluoroacrylate copolymer emulsion that repels liquids (http://vestagen.com/).[10,11] **PurThread** fabrics (PurThread Technologies) contain a complex element compound with a silver-alloy embedded in its fibers (http://www.purthread.com/).[12]

Clinical data. Data to support the use of antiseptic-impregnated textiles in clinical practice are limited. Two clinical studies have been performed on the textiles included in our proposal. Schweizer et al. performed a double-blinded RCT to evaluate the median time to first contamination of hospital curtains in ICUs; 15 curtains were formulated with PurThread fabrics, and 15 curtains were made with standard polyester fabrics.[13] Both types of curtains became contaminated with MRSA, VRE, Acinetobacter, Pseudomonas, and/or *E. coli*. The median time to first contamination was longer for the antiseptic-impregnated curtains than controls (14 days vs. 2 days; p<0.01). The overall rate of contamination was 29% lower for antiseptic curtains than controls.

Bearman et al. performed a blinded, crossover RCT trial comparing antiseptic-impregnated surgical scrubs made of Vestex fabric to standard surgical scrubs.[14] Surgical scrubs were cultured for MRSA, VRE, and GNRs once weekly at random times. While the proportion of scrubs that were contaminated with one or more target pathogens was not different between the antiseptic and control scrubs, the bacterial burden was lower on the scrubs made with Vestex fabric. Furthermore, use of surgical scrubs made with Vestex fabric led to a >4 log reduction in the burden of MRSA on the HCP (p<0.01). No significant differences in the amount of VRE or GNR contamination were observed.

Three studies have evaluated other types of antiseptic-impregnated clothing – all failed to show significant decreases in bacterial contamination. Burden et al. performed a prospective RCT comparing two different types of antiseptic-impregnated scrubs to control scrubs and found no major differences in the median amount of bacterial contamination: 99 CFU (control) v. 137 CFU (scrub A) v. 138 (scrub B) (p=0.36).[15] Gross et al. compared the use of silver-impregnated

clothing to standard clothing worn by ambulance personnel. No differences in total CFUs were observed at days 0, 3, or 7 in this small pilot study.[16] Finally, Boutin et al. performed a prospective, crossover trial comparing the use of scrubs impregnated with a chitin-based antiseptic against the use of standard scrubs in 90 HCP. No differences were observed. The overall prevalence of bacterial contamination in both groups was approximately 30% for all pathogens, 16% for *S. aureus*, and 15% for GNRs.[17]

In contrast to the above studies, our study will include simultaneous measurements of the bacterial burden in the environment and on the patient. Both of these sources are key confounders to the assessment of contamination of clothing worn by HCPs. Second, our proposal to undertake a prospective, RCT with a crossover design is unique. Our crossover design will allow us to better adjust for HCP behavior. Our approach of enrolling nurses prior to each of three 12-hour shifts performed on 3 consecutive shifts will decrease the likelihood that unique patient care factors will impact the measurements obtained for any individual subject, as the median duration of ICU stay in our hospital is > 3 days. Third, the primary outcome of earlier studies was the total burden (CFU) of contaminating organisms *or* the identification of specific, targeted pathogens. In contrast, we will analyze total CFU as a primary outcome *and* include targeted organisms in secondary analyses. Importantly, our outcomes include the burden of organisms such as MSSA and vancomycin-susceptible Enterococcus that are as epidemiologically important as MDR pathogens. Finally, our study will include two types of antiseptic-impregnated scrubs with pre-clinical and early clinical signs of efficacy.

2.3   Potential Risks and Benefits

2.3.1 Potential Risks

For both nurses and patients, we consider this to be a minimal risk study. There is no risk to the subject from culturing his/her clothing. There are two small risks for nurse subjects. The first is the potential for skin irritation, itching or rashes from the 2 antiseptic scrubs. These side-effects have been reported during earlier studies involving these scrubs. The second is a small potential risk for loss of confidentiality, though no PHI will be obtained from the nurses.

There is also minimal risk to the subjects' patients. Swabs will be obtained from these patients from the anterior nares, peri-rectal area, and from the skin using techniques previously used and approved by the DUHS IRB (IRB# Pro00036470). Specimens obtained during the course of routine clinical care will also be analyzed. Our approach for obtaining nasal, skin, and peri-rectal swabs is similar to policies for surveillance for important multidrug-resistant organisms that is already performed at the above hospitals (e.g., methicillin resistant *staphylococcus aureus* (MRSA) and/or vancomycin-resistant enterococcus (VRE)). Nasal cultures will be obtained using standardized CDC surveillance methodology involving the culturing of both nostrils. The nasal surveillance culturing method is physically non-invasive but there may be some extremely mild discomfort during the procedure which lasts approximately 5-10 seconds. The nose culture will feel to the patient as if he/she is being tickled in the nose. There are no risks to the patient in obtaining these cultures. The nasal swab will not be inserted deeply into the nose or into the throat. The peri-rectal cultures will be obtained using standardized CDC clinical surveillance methodology and standardized technique which involves swabbing the peri-rectal area in a circular motion. The peri-rectal culture will feel similar to wiping the area with toilet tissue. There are no risks to the patient in obtaining these cultures. The peri-rectal swab will not be inserted into the anus. Integument cultures will be obtained from the following areas (in order of availability): tracheostomy site, PEG tube site, wound drain site, axillae, and groin.

2.3.2 Benefits

Nurses may benefit from wearing clothing that becomes less contaminated while they perform routine duties. Patients may benefit if these clothes decrease the risk of transmission of epidemiologically important pathogens.

3   OBJECTIVES

The purpose of the study is to perform a prospective, blinded, randomized, controlled trial (RCT) with a crossover design to determine if antiseptic-impregnated surgical scrubs decrease the burden of HCP clothing contamination compared to standard, control surgical scrubs following a 12-hour ICU shift.

3.1   Study Hypotheses and Objectives

3.1.1   Hypotheses

Primary Hypothesis

Antiseptic-impregnated scrubs become less contaminated than standard scrubs during a single ICU shift

Secondary Hypotheses

1. HCP clothing becomes contaminated with important pathogens during a single shift.
2. HCP clothing becomes contaminated with pathogens present in the environment of the patient during a single shift.

3.1.2   Primary Objective

1. To determine if antiseptic-impregnated surgical scrubs become less contaminated than standard surgical scrubs after being worn by nurses during an ICU shift.
2. To determine the number and type of "transmission events" between the patient, the environment, and the nurse during a shift and compare across type of surgical scrubs.

3.1.3   Secondary Objectives

1. To estimate the type and amount of bacterial contamination that occurs on nurse clothing during a standard ICU shift and compare across type of surgical scrubs.
2. To determine the type and amount of MDR pathogens that contaminate nurse clothing at the end of an ICU shift and compare across type of surgical scrubs.
3. To determine HCP perceptions of each type of surgical scrub

4   STUDY DESIGN

We will perform a 3-arm prospective, blinded, randomized, controlled trial with a cross-over design to determine if HCP who wear antiseptic-impregnated clothing (i.e., surgical scrubs) will acquire and transmit fewer pathogens than HCP wearing standard clothing.

A total of 40 intensive care unit (ICU) nurses (from the MICU and SICU in the Duke Medical Pavillion) will be enrolled and sign a consent form to participate in the study. Each subject will wear control (non-antiseptic) scrubs (Arm 1) and two different types of antiseptic-impregnated scrubs (Arms 2 and 3). Subjects will be randomized to one of 6 strategies (Table 1). Each nurse typically cares for two patients each day and will be enrolled for three consecutive shifts. Once the nurses have consented to participate in this project, the study coordinator will provide the 3 sets of scrubs and will label them with symbols, thus blinding the nurses. The nurse will wear the scrubs on the 3 pre-arranged shifts.

Microbiological specimens will be obtained from the nurse's clothing and the environment at the beginning and end of each shift. Patients will be cultured once during each shift.

Table 1. Randomization schema

	Approach					
	1	2	3	4	5	6
Shift/Day 1	C	C	1	1	2	2
Shift/Day 2	1	2	C	2	C	1
Shift/Day 3	2	1	2	C	1	C

C=Control; 1=antiseptic scrub #1; 2=#2

4.1   Study Population

4.1.1 Selection of the Study Population

Our main study subjects will include only nurses who volunteer and consent to participate in the study. Pregnant nurses may consent to participate in the study since there is no risk to the subject from culturing her clothing.

The corresponding ICU patients of the subject nurses will also be included in this study. We will obtain a waiver of consent & HIPAA authorization in order to culture the patients' rooms and to obtain swabs from 3 patient body sites. All ICU patients being cared for by the participating nurse will be eligible for room cultures and swabs, including pregnant women and prisoners. No pediatric subjects or patients will be included.

4.1.2 Inclusion/Exclusion Criteria

Inclusion criteria: All nurses working in the MICU and SICU at DUH will be eligible for enrollment.

Exclusion criteria: Not a nurse working in the MICU or SICU at DUH.

4.1.3 Treatment Assignment Procedures

Subjects will be randomized to one of 6 strategies (Table 1). Nurse subjects will wear a different scrub type during each of the three shifts being monitored. Scrubs will be provided to nurses as described below. Nurses will be blinded to the type of scrub during the study. The randomization scheme is included in Appendix 1.

4.1.4 Strategy Descriptions

Nurses will wear one of three types of scrubs:

1. Control scrubs – These cotton-polyester scrubs will be purchased from the Duke University Bookstore.
2. Intervention scrubs #1 – These scrubs will be donated by PurThread and will contain silver fabric, as described above.

3. <u>Intervention scrubs #2</u> – These scrubs will be donated by Vestagen and will contain a quaternary ammonium disinfectant and hydrophobic component, as described above.

All scrubs will be "Duke blue". All brands will be removed to ensure blinding of nurses. Scrubs will be marked with the symbols ▲, ■, or ● in order to avoid implication of ranking.

4.1.5 Termination of Study

This study may be terminated at any time by the principal investigator (PI) in consultation with the CDC. Otherwise, the study will be terminated at the end of enrollment, analysis, and publication of findings.

5   STUDY PROCEDURES

5.1   Data Collection

Data will be collected on the following:

> Nurse background
>
> Patient characteristics
>
> Microbiological assessment of
>
>> Nurse scrubs
>>
>> Environmental surfaces in ICU rooms
>
> Nurse perceptions of each type of surgical scrub

Endpoints

Primary endpoints:

- The difference in total acquired contamination (Total CFU) on HCP clothing at the end of a 12-hour ICU shift.

  o Results from each intervention arm will be compared against results from the control arm

  o Total CFU will be determined by adding the CFU detected by individual cultures to yield a single value

Secondary endpoints (compared between each intervention arm and the control arm):

- The presence or absence of individual specific pathogens: *S. aureus* (MRSA or MSSA), *Enterococci* (VRE or VSE), *Acinetobacter* spp., *Pseudomonas* spp., and *Enterobacteriaceae* of interest such as *E. coli* and *Klebsiella* spp.

- The proportion of positive cultures – overall and at each culture location

- The number and proportion of suspected and confirmed "transmission events"

  o A "potential transmission event" will be defined as the sequential identification of the same pathogen species from HCP clothing, the patient, and/or the environment. A "confirmed transmission event" will be defined as the sequential identification of the identical pathogen, as confirmed by molecular techniques, from HCP clothing, the patient, and/or the environment.

- HCP perceptions of clothing

Exploratory endpoints

- None

Data Collection Strategy and Sources

1. Nurse – The following information will be gathered by directly interviewing the nurse subject
    a. Date
    b. Shift
    c. Assigned rooms for the day
    d. Wearing correct scrubs per randomization scheme
    e. Has a pet at home. If so, what type?
2. Patient characteristics – Nurses typically care for 1-4 patients per shift (average=2). The following data for each patient will be obtained from the medical record:
    a. Room number
    b. Hospital admission date
    c. ICU admission date
    d. Hospital length of stay
    e. ICU length of stay
    f. On contact precautions
        i. If so, for which organism?
    g. Percutaneous feeding tube present?
    h. Drain present?
    i. Diarrhea
    j. Rectal tube present?
    k. On mechanical ventilation?
    l. Wound present?
    m. Surgical wound present?
3. Microbiological specimens – see Section 5.2.1
4. Nurse perceptions – Nurse subjects will be asked to respond to the following questions using a 5-point Likert scale:
    a. These scrubs felt like wearing my normal scrubs.
    b. Did you experience any itchiness with the scrubs you wore today?
    c. Did you experience any redness or rash with the scrubs you wore today?
    d. Did you experience any feeling of heaviness or poor breathability with the scrubs you wore today?
    e. Any other comments? (Free text response)

Data Monitoring

No formal interim analyses involving hypothesis testing is planned.

5.2 Other Study Procedures

Study activities with enrolled subjects will take place over a concentrated period of time (3 shifts). No data will be obtained after these shifts, so no follow-up of final study visits are required. In the event a subject does not complete all three arms, this subject's data will be removed from the study and an additional subject will be included.

We will not enroll patients as part of this protocol. Therefore, the following sections are not applicable:

1. Screening
2. Enrollment/baseline
3. Follow-up
4. Final study visit
5. Follow-up safety phone call
6. Early termination visit
7. Unscheduled visit
8. Laboratory evaluations

5.2.1 Microbiological Procedures

General Procedures - The study coordinator will arrive to the ICU prior to the beginning of each shift to obtain Rodac plates cultures from the nurse's scrubs (sleeve, abdomen and pocket, each in triplicate) as well as specified, "high touch" surfaces in the rooms (bedrail, bed and supply cart) of that nurse's current patients (up to 2 patients). These samples will be repeated at the end of each shift. Finally, swabs will be taken from the patients that the enrolled nurse is caring for during each day of the study. Swabs will include nares, peri-rectal and integument (See Figure 1). No swabs will be taken directly from the skin of nurses.

Environmental cultures of the patient rooms and the HCP clothing will be obtained using protocols previously used and validated by our group. RODAC plates (Becton Dickinson, Sparks, MD) containing DE Neutralizing Agar (Becton Dickinson, Sparks, MD) will be used for environmental and textile cultures, as previously validated.[18-20] These plates will be aerobically incubated at 37°C for 48 hours. CFU of organisms on each plate will be quantified to estimate the "overall bioburden." Each RODAC plate culture will be evaluated specifically and quantitatively for microorganisms of interest. Each location will be cultured in triplicate, providing a culture surface area of 75 cm^2 for each location.

1-6: Environmental sites (bedrail, bed, supply cart)
7-9: HCP scrubs (sleeve, abdomen, pocket)
10-15: Patient (nares, peri-rectal, integument)

Cultures will be obtained from locations in the patient rooms and HCP clothing at the beginning and end of each shift (**Figure**); 54 cultures will be obtained each shift for each enrolled nurse; 18 cultures will be obtained from three "high touch" locations in each patient room before
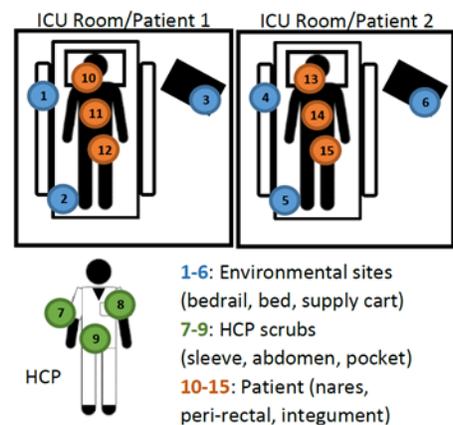
the nurse begins his/her shift (locations **1-6** in triplicate); cultures from these same locations will be obtained at the end of the shift. Similarly, 9 cultures will be obtained from three locations on HCP clothing (sleeve, abdomen, and pocket - locations **7-9,** in triplicate) at the beginning and end of each shift. In total, 162 environmental cultures will be obtained for each nurse; 6,480 environmental cultures will be obtained during the study.

In addition, swab cultures will be obtained daily from each of the patients for whom each enrolled nurse is providing care (locations **10-15**). Cultures will be obtained from the anterior nares, peri-rectal area, and from at the integument (wounds, drains, or axilla) to provide the highest sensitivity of detecting bacterial colonization.[21-23] Finally, results of specimens obtained during the course of routine clinical care will also be analyzed.

Cultures for a nurse/patient/room grouping will be analyzed for the presence of similar species. When a potential transmission event is identified, microorganisms will be sub-cultured and analyzed using standard molecular techniques, including pulsed field gel electrophoresis (PFGE), ribotyping, and/or multilocus sequence typing (MLST) to confirm or exclude a transmission event.

Our study will thus include a minimum of 240 unique nurse clothing/patient/room "triangles." We will obtain 6,480 cultures from the nurse clothing, patients, and patient rooms during this randomized controlled trial to evaluate bacterial contamination and transmission using microbiological and molecular methodologies.
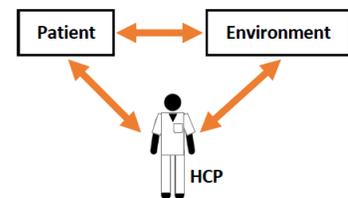
The swabs and culture specimens will be processed in the research laboratories in the Fowler Microbiology and Molecular Laboratory. Bacterial species will be identified using standard microbiological techniques and tested for similarity using standard molecular typing techniques (see Appendix 2). No patient or human DNA sequencing will be performed.

6 STUDY PRODUCT DESCRIPTION

Not applicable

6.1 Concomitant Medications/Treatments

Not applicable

7   ASSESSMENT OF SAFETY

We are not enrolling patients; therefore, the following sections are not applicable:

1. Specifications of safety parameters
2. Methods and timing for assessing, recording, and analyzing safety parameters
3. Guidelines for assessing intensity of an adverse event
4. Guidelines for determining causality
5. Discontinuation due to adverse events
6. Reporting procedures (for AE)
7. Type and duration of follow-up of participants after adverse events
8. Halting rules
9. Safety oversight

8   CLINICAL MONITORING

ICH E6 states that the purpose of monitoring is to ensure the rights of subjects, obtain accurate data, and conduct trial in accordance with protocol and applicable regulations. Routine procedures in our study group and through the research infrastructure at DUHS ensure be the qualification of hospital personnel to conduct the trial, regulatory requirements (e.g. IRB review), protocol training, data quality monitoring procedures, hospital data completion expectations (e.g. completeness, frequency, etc.). Rights of subjects will be maintained at all times as outlined in the Privacy section.

9 STATISTICAL CONSIDERATIONS

9.1 Design and Sample Size Considerations

This prospective, blinded, cluster randomized trial with crossover design will allow us to test hypotheses regarding the amount and type of contamination that occurs on HCP clothing during a single shift in the ICU. The primary and secondary analyses will include hypothesis testing and estimation of the difference of the two interventions to the control.

Power calculation. Assuming a mean 2-log increase from the beginning to the end of the shift for the control arm (SD=2)[14,15] and a correlation of 0.5 given the crossover design of the study, we will have 90% power to determine a mean 1-log decrease (SD=1) in HCP clothing contamination among 40 subjects and 120 (40 subjects x 3 days) repeated measures (alpha=0.025 for multiple comparisons).

9.2 Planned Interim Analyses

There will be no planned interim analyses for safety in this protocol.

9.3 Participant Enrollment and Follow-Up

40 nurse subjects will be enrolled. No follow-up will be performed following the completion of the three shifts.

9.4 Analysis Plan

9.4.1 Analysis

Data will be summarized using standard statistical methods.

Primary analyses

We will utilize linear mixed models to compare relative differences in the amount of contamination between arms at the end of the shift to adjust for our crossover study design. Mixed effects logistic regression models will be used to compare proportions. All calculations will be adjusted for the amount of environmental contamination observed during the shift and contamination on HCP clothing in the beginning of the shift. Statistical significance for two primary comparisons (each antiseptic scrub versus control) will be corrected for multiple comparisons.

Secondary analyses

The analyses of the secondary objectives will consist of multiple analyses to address the hypotheses. The endpoints required for these analyses, include, but are not limited to:

- The presence or absence of individual specific pathogens: *S. aureus* (MRSA or MSSA), *Enterococci* (VRE or VSE), *Acinetobacter* spp., *Pseudomonas* spp., and *Enterobacteriaceae* of interest such as *E. coli* and *Klebsiella* spp.

- The proportion of positive cultures – overall and at each culture location

- The number and proportion of suspected and confirmed "transmission events"

- o A "potential transmission event" will be defined as the sequential identification of the same pathogen species from HCP clothing, the patient, and/or the environment. A "confirmed transmission event" will be defined as the sequential identification of the identical pathogen, as confirmed by molecular techniques, from HCP clothing, the patient, and/or the environment.
- HCP perceptions of clothing

Data Acquisition.   See above

10   LIMITATIONS AND POTENTIAL SOLUTIONS

The study has potential limitations. First, our study will rely on enrollment of ICU nurse volunteers. We do not anticipate difficulty enrolling subjects, however, as we have extensive experience with subject enrollment and specific experience[24] and long-standing good relationships with the staff and leadership of ICUs at DUH. All of the ICU nurses we approached about this study in a preliminary poll were interested in participating. A second potential limitation relates to the efficacy and yield of the technique we will utilize for cultures. However, our study personnel have extensive experience with both environmental and patient cultures and the methods used in these prior studies will be utilized in the current study. Third, we will not include the detection of *C. difficile* in our study design because of the additional workload required (essentially doubling the cultures required) and the limited time available for completion of the study. Finally, we will not include all sources of pathogen transmission in our analysis. Our study will focus on nurses and not other HCP or family member that enter the rooms.

11   IMPLICATIONS

Completion of this study will lead to the most detailed analysis of the utility of antiseptic-impregnated clothing in preventing transmission of important pathogens. We will also provide important data on the transmission dynamics of pathogens among all three components of the "transmission triangle." As such, this work will a) potentially change the way that care is provided in ICUs and b) provide data that may lead to subsequent interventions that improve care and outcomes of all hospitalized patients.

12   PARTICIPANT CONFIDENTIALITY

Informed consent will be obtained from all subjects. Appropriate waivers of consent and HIPAA authorization will be obtained to access patient data.

13   INFORMED CONSENT PROCESS

All ICU nurses are eligible to participate in this study. Thus, we will not approach or enroll subjects who do not have capacity to give legal consent. Informed consent will be obtained from nurse subjects using IRB-approved consent forms.

14   SOURCE DOCUMENTS AND ACCESS TO SOURCE DATA/DOCUMENTS

No source documents will be used by this protocol.

15 QUALITY CONTROL AND QUALITY ASSURANCE

The principal investigator will ensure that all study personnel are appropriately trained and applicable documentations are maintained.

16   ETHICS/PROTECTION OF HUMAN PARTICIPANTS

16.1 Institutional Review Board

The investigator will ensure that the protocol is reviewed and approved by the DUHS IRB prior to the start of any study activities. The IRB will be appropriately constituted and will perform its functions in accordance with US regulations, ICH Good Clinical Practice guidelines, and local requirements as applicable.

16.2 Informed Consent

All ICU nurses are eligible to participate in this study. Thus, we will not approach or enroll subjects who do not have capacity to give legal consent. All nurse subjects will provide informed consent using IRB-approved forms.

16.3 Data Confidentiality

This is a minimal risk study and we have no safety concerns from the patients or nurses as relates to obtaining swabs and room cultures, nor with wearing the scrubs and having them cultured. Data will be stored on encrypted Duke Medicine servers (participant log & study IDs) and/or in our REDCap database (all other data collected for the study).

16.4 Study Discontinuation

This study may be terminated at any time by the principal investigator (PI) in consultation with the ARLG.

17   DATA HANDLING AND RECORD KEEPING

17.1 Data Management Responsibilities

The study coordinator will enter data into the ASCOT database via REDCap (see Data Capture Methods below). Other data will come from the Fowler microbiology laboratory database.

Data Capture Methods

REDCap is a toolset and workflow methodology for electronic collection and management of research and clinical trial data. Both REDCap and REDCap Survey systems provide secure, web-based applications that are flexible enough to be used for a variety of types of research, provide an intuitive interface for users to enter data and have real time validation rules (with automated data type and range checks) at the time of entry. These systems offer easy data manipulation with audit trails and reporting for reporting, monitoring and querying patient records, and an automated export mechanism to common statistical packages (SPSS, SAS, Stata, R/S-Plus).

The REDCap program will serve as the portal for data entry by the study coordinator. Data entered into this database will be password protected and only accessible by study personnel. All access to this secure separate database will be monitored and logged.

Specific Data Management. As above, four types of data will be collected. All data will ultimately be entered into the ASCOT database via REDCap.

17.2 Study Data Retention

Research records and data will be kept for a minimum of 6 years after final reporting or publication.

17.3 Protocol Deviations

Deviations from the study protocol (e.g., randomization scheme) will be documented.

18 PUBLICATION POLICY

Following completion of the study, the investigator will publish the results of this research in a scientific journal.

19 REFERENCES

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2.   Burden M, Cervantes L, Weed D, Keniston A, Price CS, Albert RK. Newly cleaned physician uniforms and infrequently washed white coats have similar rates of bacterial contamination after an 8-hour workday: a randomized controlled trial. Journal of hospital medicine : an official publication of the Society of Hospital Medicine 2011;6:177-82.
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20 APPENDIX 1. RANDOMIZATION SEQUENCE FOR ASCOT TRIAL

Subject	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3	3 types of scrubs
1	B	A	C	○	Δ	□	A= Δcontrol/standard cotton-poly
2	A	B	C	Δ	○	□	B=○PurThread scrubs
3	A	C	B	Δ	□	○	C=□Vestex scrubs
4	C	A	B	□	Δ	○	
5	C	B	A	□	○	Δ	
6	B	C	A	○	□	Δ	
7	B	A	C	○	Δ	□	
8	C	B	A	□	○	Δ	
9	A	B	C	Δ	○	□	
10	B	C	A	○	□	Δ	
11	A	C	B	Δ	□	○	
12	C	A	B	□	Δ	○	
13	C	B	A	□	○	Δ	
14	B	C	A	○	□	Δ	
15	A	B	C	Δ	○	□	
16	A	C	B	Δ	□	○	
17	B	A	C	○	Δ	□	
18	C	A	B	□	Δ	○	
19	B	A	C	○	Δ	□	
20	C	A	B	□	Δ	○	
21	C	B	A	□	○	Δ	
22	A	B	C	Δ	○	□	
23	A	C	B	Δ	□	○	
24	B	C	A	○	□	Δ	
25	A	B	C	Δ	○	□	
26	A	C	B	Δ	□	○	
27	C	A	B	□	Δ	○	
28	B	A	C	○	Δ	□	
29	B	C	A	○	□	Δ	
30	C	B	A	□	○	Δ	
31	A	B	C	Δ	○	□	
32	C	B	A	□	○	Δ	
33	A	C	B	Δ	□	○	
34	C	A	B	□	Δ	○	
35	B	C	A	○	□	Δ	
36	B	A	C	○	Δ	□	

37	C	B	A	□	○	Δ
38	C	A	B	□	Δ	○
39	B	C	A	○	□	Δ
40	A	B	C	Δ	○	□
41	B	A	C	○	Δ	□
42	A	C	B	Δ	□	○
43	C	A	B	□	Δ	○
44	A	C	B	Δ	□	○
45	C	B	A	□	○	Δ
46	B	C	A	○	□	Δ
47	A	B	C	Δ	○	□
48	B	A	C	○	Δ	□
49	A	B	C	Δ	○	□

21 APPENDIX 2. MICROBIOLOGICAL APPROACH FOR ASCOT TRIAL.

Environmental Samples

1) Incubate D/E Neutralizing RODAC plates at 37C for 48 hours.
>-After 48 hours incubation the plates can be left at room temperature for 24 hours
>-OR left at 4C for 48 hours

2) Count the total amount of colonies forming units (CFUs) on the plate using the Colony Counter Pen.

3) Identify different types of colonies based on morphology (size, color, etc.), and count the total number of colonies of each morphology, designating different morphologies with a unique letter (a, b, c, etc.)

4) Do not count non-target organisms.

Target organisms:
 Staphylococcus aureus (methicillin resistant-MRSA and methicillin sensitive-MSSA), *Enteroccus sp. (*vancomycin resistant -VRE, and vancomycin sensitive- VSE*), Acinetobacter sp, Pseudomonas sp., Stenotrophomonas sp. and Enterobacteriaceae (Esherichia coli and Klebsiella sp.)*

Commonly found Non-Target organisms:
Micrococcus sp., Bacillus sp., coagulase negative Staphylococcus sp. Diphtheroids, Molds, and Fungi.

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5) Divide a sheep blood (SB) agar plate into sixths and label each sixth with a unique letter (a, b, c, etc). If there are more than six colony types use additional SB plates.

6) Subculture one colony of each morphology type identified to the appropriate place on the SB plate and incubate for 24 hours at 37°C.

7) After 24 hrs, review the growth on the SB plates and use the specific tests listed below to confirm the identity of any possible target organisms.

MSSA and MRSA

1) Perform the Staphaurex test to confirm colonies are *S. aureus*.
- Positive tests show clumping within the solution and negative tests do not.

2) Suspend several colonies of *S. aureus* from an 18-24hr culture in trypticase soy broth and adjust turbidity to a 0.5 McFarland standard. Inoculate the oxacillin plates with 10 ul of the prepared suspension and incubate for a full 24 hours at 35°C.

3) Growth on the oxacillin plates after 24 hours indicates methicillin resistance (MRSA); no growth indicates methicillin sensitivity (MSSA)

Negative
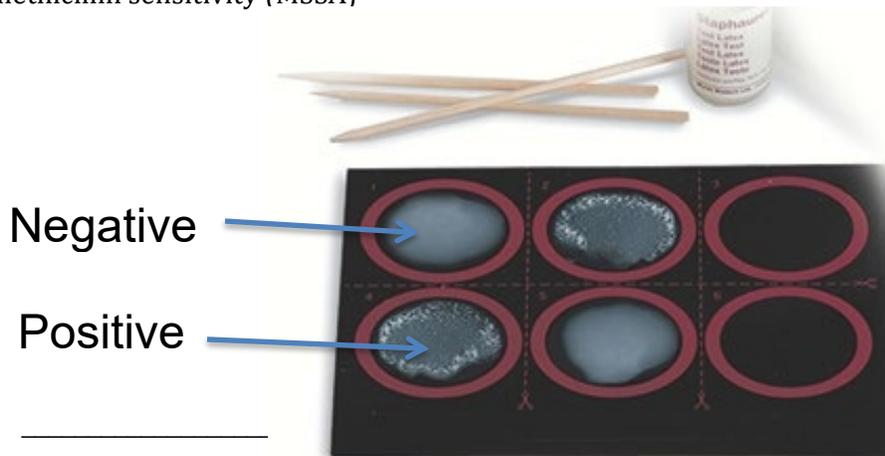
Positive

VRE and VSE

1) Inoculate a bile esculin (BE) slant with 3-4 colonies of suspected Enterococcus sp. from the SB plate and incubate for 48 hours at 37°C.

- After 48 hours, if half of the slant is black the test is positive. If less than half of the slant is black, incubate for an additional 24 hours at 37°C before calling negative.

2) Inoculate a 6.5 % NaCl (salt) tube with 3-4 colonies of suspected Enterococcus sp. from the SB plate and incubate for 24 hours at 37°C.

- If the tube is turbid the test is positive.

3) Enterococcus sp. is confirmed if both the BE slant and salt tube are positive.

4) Confirm vancomycin resistance by suspending several colonies of *Enterococcus sp.* from an 18-24hr culture in trypticase soy broth and adjust turbidity to a 0.5 McFarland standard. Inoculate the vancomycin plates with 10 ul of the prepared suspension and incubate for a full 24 hours at 35°C.

- Growth on the vancomycin plate indicates resistance (VRE), no growth indicates sensitivity (VSE).
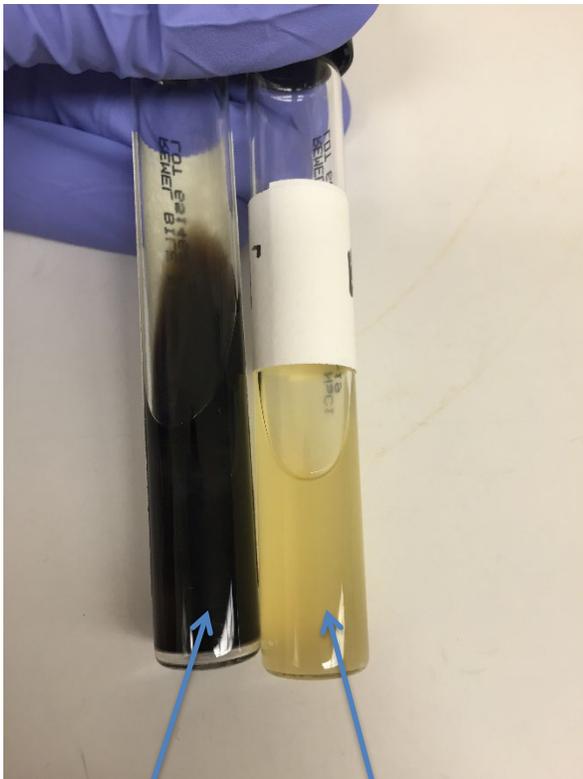
Positive Slant: Black color on > half the slant

Positive Salt Solution: Turbid (Cloudy)
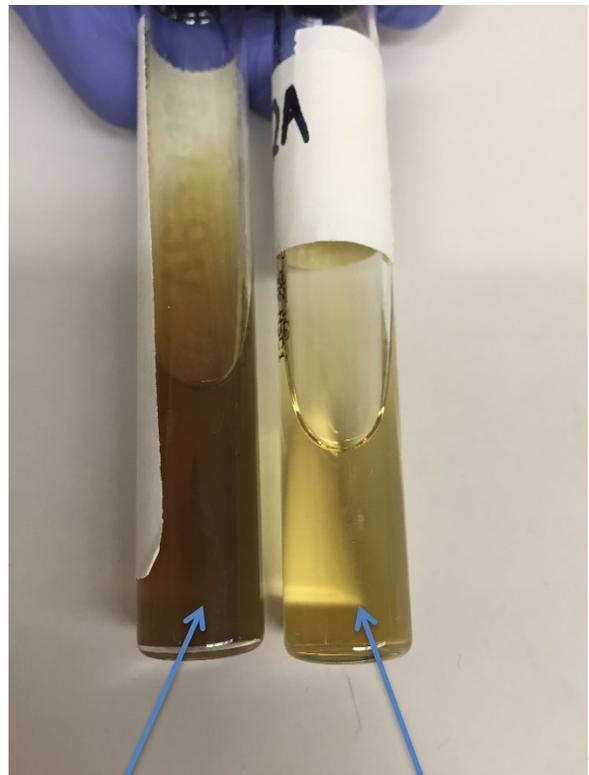
Negative Slant: brown/no

Negative Salt solution: Non-turbid (Not cloudy)

Gram-negative Rods

1) Subculture suspected Gram-negative rods to a MacConkey (MAC) plate and incubate for 24 hours at 37°C.

2) Subculture pink (lactose fermenters-LAC+) and colorless (lactose non-fermenters-LAC-) colonies to separate SB plates and incubate for 24 hours at 37°C.

3) Identify GNRs to species level using Matrix Assisted Laser Desorption Ionization-Time of Flight (MALDI-TOF)

Summary
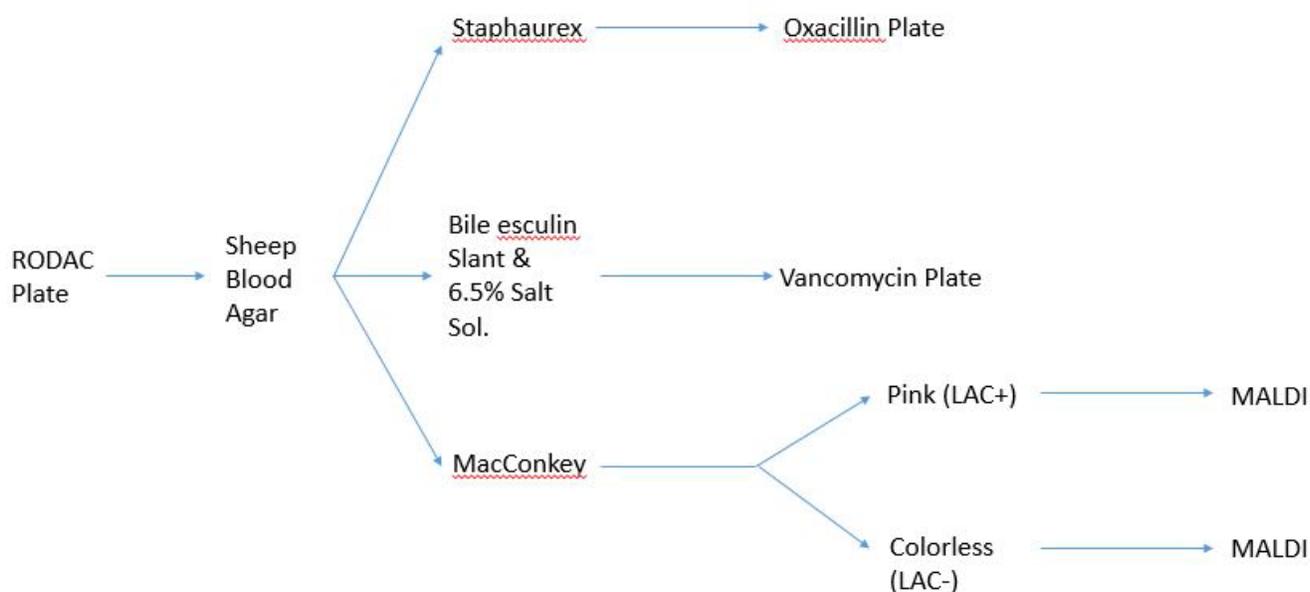
Clinical Samples

Nares (MRSA/MSSA)

1) Inoculate a mannitol salt agar (MSA) plate with the nasal swab, streak for isolation and incubate at 37°C for 24 hours.

2) After 24 hours, subculture yellow colonies to SB plate and incubate for 24 hours at 37°C.

3) Confirm the colonies are *S. aureus* using the catalase and Staphaurex tests.

4) Suspend several colonies of *S. aureus* from an 18-24hr culture in trypticase soy broth and adjust turbidity to a 0.5 McFarland standard. Inoculate the oxacillin plates with 10 ul of the prepared suspension and incubate for a full 24 hours at 35°C.

5) Growth on the oxacillin plate after 24 hours indicates methicillin resistance (MRSA); no growth indicates methicillin sensitivity (MSSA)
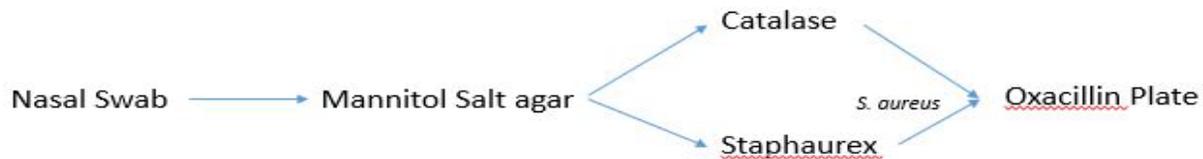
Peri-rectal (VRE, ESBL, Pseudomonas, Acinetobacter, MRSA)

NOTE: Two peri-rectal samples will be collected. Each sample contains two swabs for a total of four swabs.

1) Use one swab to inoculate a MacConkey plate, streak for isolation and incubate at 37°C for 24 hours.

- Subculture colorless (lactose non-fermenters) colonies to a SB plate and incubate for 24 hours at 37°C . Use a separate SB plate for each colony type if there are multiple types.
- Identify GNRs to species level using Matrix Assisted Laser Desorption Ionization-Time of Flight (MALDI-TOF)

3) Use one swab to inoculate a Columbia CNA plate, streak for isolation and incubate at 37°C for 24 hours.

Staphylococcus aureus

- Subculture suspected *S. aureus* colonies onto a mannitol salt plate and incubate for 24 hours at 37°C.
- After 24 hours, subculture yellow colonies to SB plate and incubate for 24 hours at 37°C.
- Confirm the colonies are *S. aureus* using the catalase and Staphaurex tests.
- Suspend several colonies of *S. aureus* from an 18-24hr culture in trypticase soy broth and adjust turbidity to a 0.5 McFarland standard. Inoculate the oxacillin plates with 10 ul of the prepared suspension and incubate for a full 24 hours at 35°C.
- Growth on the oxacillin plate after 24 hours indicates methicillin resistance (MRSA); no growth indicates methicillin sensitivity (MSSA)

Enterococcus sp.

- Subculture suspected *Enterococcus sp.* colonies to a SB plate and incubate for 24 hours at 37°C.
- Confirm *Enterococcus sp.* using a bile esculin slant and a 6.5% salt tube as described in the VRE and VSE section.
- Confirm vancomycin resistance by suspending several colonies of *Enterococcus sp.* from an 18-24hr culture in trypticase soy broth and adjust turbidity to a 0.5 McFarland standard. Inoculate the vancomycin plates with 10 ul of the prepared suspension and incubate for a full 24 hours at 35°C.
  - Growth on the vancomycin plate indicates resistance (VRE), no growth indicates sensitivity (VSE).

4) Use one swab to inoculate a HardyCHROM ESBL agar, streak for isolation and incubate at 37°C for 24 hours.

- Pink colonies are *E. coli,* Large dark blue colonies are *Klebsiella* and *Enterobacter spp. and d*ark blue colonies with a rose halo are *Citrobacter spp.*
- Identify GNRs to species level using Matrix Assisted Laser Desorption Ionization-Time of Flight (MALDI-TOF)
- Freeze these suspected target species for later identification using MALDI-TOF
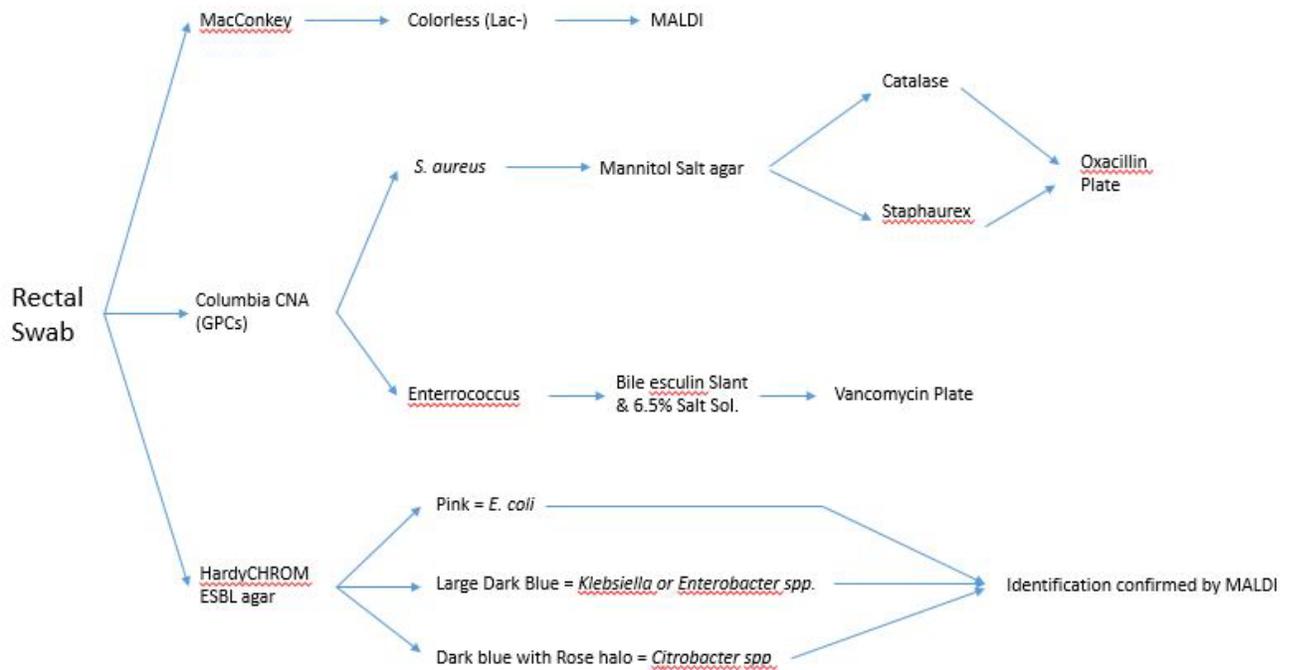
Wound culture

1) Inoculate a SB, MacConkey and Columbia CNA plate with the wound swab, streak for isolation and incubate for 24 hours at 37°C.

2) From the MAC plate:

- Subculture pink (LAC⁺) and colorless (LAC⁻) colonies to separate SB plates and incubate for 24 hours at 37°C.
- Identify GNRs to species level using Matrix Assisted Laser Desorption Ionization-Time of Flight (MALDI-TOF)

3) From the Columbia CNA plate:

Staphylococcus aureus

- Subculture suspected *S. aureus* colonies onto a mannitol salt plate and incubate for 24 hours at 37°C.
- After 24 hours, subculture yellow colonies to SB plate and incubate for 24 hours at 37°C.
- Confirm the colonies are *S. aureus* using the catalase and Staphaurex tests.
- Suspend several colonies of *S. aureus* from an 18-24hr culture in trypticase soy broth and adjust turbidity to a 0.5 McFarland standard. Inoculate the oxacillin plates with 10 ul of the prepared suspension and incubate for a full 24 hours at 35°C.
- Growth on the oxacillin plate after 24 hours indicates methicillin resistance (MRSA); no growth indicates methicillinsensitivity (MSSA)

Enterococcus sp.

- Subculture suspected *Enterococcus sp.* colonies to a SB plate and incubate for 24 hours at 37°C.
- Confirm the colonies are *Enterococcus sp.* using a bile esculin slant and a 6.5% salt tube as described in the VRE and VSE section.
- Confirm vancomycin resistance by suspending several colonies of *Enterococcus sp.* from an 18-24hr culture in trypticase soy broth and adjust turbidity to a 0.5 McFarland standard. Inoculate the vancomycin plates with 10 ul of the prepared suspension and incubate for a full 24 hours at 35°C.
  - o Growth on the vancomycin plate indicates resistance (VRE), no growth indicates sensitivity (VSE).