PURPOSE.
At the direction of State epidemiology offices or CDC’s Division of Healthcare Quality Promotion, Regional Laboratories will conduct CRE colonization testing from rectal swab specimens as part of response to detection of epidemiologically important CRE or an outbreak response. This Guidance document identifies options for CRE colonization assays and details recommended procedures for isolate storage, handling, and reporting.

GENERAL CONSIDERATIONS.
- This Guidance outlines recommended options for laboratory testing methods. Methods used by laboratories are not limited to these options, but other testing methods may require in-house validation prior to use with clinical samples.
- All testing should be implemented in compliance with Clinical Laboratory Improvement Amendments (CLIA) regulations.
- Regional Laboratories should maintain databases of test results and retain these results for a minimum of 7 years.
- Regional Laboratories should report results back to submitting healthcare facilities, jurisdictional state Department of Health (DOH), and CDC within 1 working day of results.
- Regional Laboratories should store colonizing CRE isolates recovered from culture-based testing for a minimum of 2 years.

CRE COLONIZATION SCREENING RATIONALE.
As described in the 2015 Update to the CDC CRE Toolkit (http://www.cdc.gov/hai/pdfs/cre/CRE-guidance-508.pdf), if a culture from a patient not previously known to be CRE colonized grows epidemiologically-important CRE (e.g., carbapenemase-producing CRE [CP-CRE]), facilities should consider screening patient contacts to identify transmission. Patients considered contacts might vary from setting to setting; however, at minimum should include any roommates of the index patient for the duration of their stay.

For patients with long stays prior to their positive culture, during which they might have had undetected CRE colonization, or if CRE transmission is documented, consideration should be given to screening a broader group of patients to identify ongoing transmission. Facilities could perform one or multiple point prevalence surveys during which all the patients on a particular unit or ward are screened. If transmission is documented on a point prevalence survey, follow-up surveys should be conducted to document that transmission is halted. Generally, transmission is considered halted when at least two point prevalence surveys conducted at least one week apart do not identify new patients that are colonized or infected with the strain of concern.
Specimens should only be submitted to a Regional Laboratory for colonization testing if a state epidemiologist (i.e., from the jurisdictional state of the affected healthcare facility) and the Regional laboratory approve the testing request.

General guidelines on appropriate and inappropriate use of colonization testing are below; however, we encourage flexibility and use of best-judgement to determine which requests to grant (based on testing capacity and differences in endemicity)

<table>
<thead>
<tr>
<th>Appropriate Use (One-Time Testing)</th>
<th>Non-Priority or Inappropriate Use (Ongoing Testing)</th>
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</thead>
<tbody>
<tr>
<td>Screening roommates of new cases</td>
<td>Regional lab has exceeded capacity</td>
</tr>
<tr>
<td>Screening contacts of contaminated device</td>
<td>Routine inpatient screening</td>
</tr>
<tr>
<td>Point prevalence study (PPS) for new resistance or first identification of CRE</td>
<td>Admission screening</td>
</tr>
<tr>
<td>PPS when transmission suspected</td>
<td>Testing not linked to a facility or network prevention program</td>
</tr>
</tbody>
</table>

If the testing is approved, the state or local health department will request the collection of rectal swab samples among patient contacts. These samples will be sent to Regional Laboratories for testing. Testing should be done in a timely manner (initiated upon receipt of samples; results reported within two days of sample receipt). Results will be simultaneously reported to the submitting healthcare facility and the jurisdictional state DOH (including designated contacts from state public health laboratory and hospital-associated infections program). A monthly compilation of testing results will be reporting to CDC.

**SAMPLE COLLECTION AND TRANSPORT.**

**Sample Collection**

- **Use of Cepheid dual swab collection kit (Cepheid catalog #900-0370) is encouraged.** This is the manufacturer-recommended sample collection device for use with the Cepheid GeneXpert Carba-R assay, the recommended primary testing method. Additionally, the use of dual swabs allows simultaneous testing using both PCR and culture methodology directly from rectal swabs when needed (e.g., when CRE mechanism of interest will not be detected by PCR and/or if molecular typing is required). Other body sites may be sampled at the discretion of the state epidemiologist.
  - Cepheid swabs will be purchased in bulk by CDC and distributed to ARLN regional labs.
  - Regional labs will distribute Cepheid dual swab collection kits to healthcare facilities upon initiation of colonization screening.
- **If stool specimens are collected, a swab sample must be prepared from the stool once received in the laboratory, prior to testing with either the Cepheid or culture assays.**
- **If IMP is identified in index case using CDC PCR protocol, index case isolate should be forwarded to the regional lab for preliminary testing on Cepheid to confirm sensitivity for the type of IMP detected before testing swabs sent for colonization screenings (Cepheid detects IMP-1 group only)**
• Coordinate with jurisdictional health department and laboratory to forward the index case isolate
• Swabs collected for colonization screening should be processed after confirming Cepheid detects IMP in index isolate

Transport
• Rectal swabs collected using the Cepheid dual-swab which are intended for testing using the Cepheid assay should be inserted in the tube’s universal transport media and shipped at room temperature (15-28°C) to the Regional Laboratory within 1 day of collection.
• Rectal swabs or stool specimens intended for testing by culture should be transported in a suitable bacterial transport medium (e.g., Amies, Stuart’s, Cary Blair) and shipped on cold packs to the Regional Laboratory within 1 day of collection.

LABORATORY TESTING.
Methods
This guidance recommends and discusses two options for laboratory testing of CRE colonization. The Cepheid Xpert Carba-R molecular assay is recommended as the primary assay because of its quick turnaround time and ease of use. The Direct MacConkey Plate Culture method is recommended as the secondary assay. A brief description, methods overview, and limitations of each assay is given below.

• Cepheid Xpert Carba-R
  • Cepheid Xpert Carba-R is the only FDA-approved, commercially available CRE detection assay. This methodology requires the use of the Cepheid Gene Xpert system, Carba-R analysis software, and proprietary kit reagents. This molecular assay has a short turn-around time of ≤1 day.
  • The protocol involves a simple workflow and an easily interpretable results read-out. Briefly, the rectal swab specimen is added to the reagent, vortexed, and a volume of suspension is added to the Cepheid cartridge for analysis with the Cepheid Gene Xpert system. This molecular assay uses real-time PCR technology to detect genes for the most common carbapenem resistance mechanisms: KPC, NDM, VIM, IMP-1 group and OXA-48 (also covering OXA-181 and OXA-232).
  • Limitations
    o Molecular testing eliminates recovery of a bacterial isolate for further testing (e.g., isolate identification, antimicrobial susceptibility testing or molecular typing).
    o Detection of resistance mechanisms is restricted to the target capacity of the assay’s primer and probes.
  • Note: If isolates are needed for further characterization, or if the index case isolate possesses a novel or unusual carbapenem-resistance mechanism, then the culture-based assay described below would be the more suitable choice. The Cepheid assay can be used to test a derived CRE bacterial isolate from the culture method.

• Direct MacConkey Plate Culture
  • Direct MacConkey Plate Culture is a culture-based method in which the rectal swab sample is streaked directly onto a MacConkey agar plate. The turn-around time for results using this culture assay is ≤4 days.
The protocol involves a streaking a rectal swab onto MacConkey agar plate for isolation and incubating overnight in the presence of a meropenem disk. Organisms growing within the defined zone of inhibition are identified as potential CRE.

Isolates identified as CRE should be further characterized with PCR using the Cepheid assay (inputting bacterial samples direct from the culture plate) or using an in-house validated PCR assay (using DNA extracted from bacterial culture).

**Limitations**
- For determination of mechanism, the turn-around time is approximately 48 hours
- Validation studies are ongoing; the protocol may change

**Note:** Although the Direct MacConkey Plate Culture assay is not recommended as the primary method, it may be required if:
  1. isolates are needed for molecular typing;
  2. a representative, random sampling of culture isolates from a point-prevalence survey is desired
  3. isolate from the index patient is known or anticipated to harbor a carbapenemase-resistance mechanism that cannot be detected using the primary Cepheid assay.

**Laboratory Testing Workflows**

**Primary: Cepheid Xpert Carba-R**
- Clinical Sample (Dual Rectal or Fecal Swab)
- Xpert Carba-R Kit Reagents
- Xpert Carba-R Reaction Cartridge
- Gene Xpert Platform
- Computer Read-Out Of Resistance Mechanism
- Report within 1 day of results

**Secondary: Direct MacConkey Plate Culture**
- Clinical Sample (Rectal or Fecal Swab)
- Streak for Isolation On MacConkey Agar
- Overnight Incubation with Meropenem disk
- Subculture Colonies In Zone of Inhibition for simultaneous bacterial ID and AS
- PCR to identify Resistance Mechanism
- Re-streak for Isolation
- Overnight Incubation with Meropenem

**OR**
- In-house Validated PCR Assay
- Report within 1 day of results

**REQUIRED REPORTING.**
- Regional Laboratories will simultaneously report testing results back to the submitting healthcare facility, state public health laboratory, and coordinating state epidemiologist via secure communications (i.e., established protocols that may include utilization of standardized data exchange platforms and systems, secure fax, or encrypted e-mail).
• **Results from Cepheid testing (primary assay) should be reported within 1 working day of results.** If the Direct Mac Test is being used to further characterize positive results of Cepheid testing, preliminary results of that positive Cepheid test should be reported within 1 working day.

• **Results from the Direct MacConkey Plate culture assay (secondary or confirmatory assay) should be reported within 1 working day of results.**

• **Regional laboratories will provide a monthly report to CDC summarizing testing activities.** This report will be submitted through the APHL AIMS portal.

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**STORAGE AND SUBMISSION OF CERTAIN ISOLATES.**

- **Isolate storage:** CRE-positive isolates obtained from culture-based testing should be stored by the Regional Laboratories at -70°C for a minimum of 2 years.

- **Submission of Certain Isolates:**
  - CDC may request bacterial isolates from a single patient of interest, or group of associated patient contacts, for further testing or banking. An aliquot of these isolates should be shipped on dry ice within 1 week of request.
  - If a Regional Laboratory is seeking additional confirmation testing by CDC, the CDC laboratory should be informed, and an aliquot of the indicated isolate shipped on dry ice to CDC.

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**LABORATORY TRAINING, PROFICIENCY, AND ASSAY VALIDATION**

- **Laboratory Training:** CDC will provide training to regional laboratory staff on all testing, shipping, and reporting protocols.

- **Proficiency testing:** CDC will assist in proficiency testing by providing isolates and/or specimens once per year.

- **Assay validation:** CDC will assist in the Laboratory’s validation of a selected testing method by providing an isolate panel and, if needed, assistance or expertise in results interpretation.
CONTACT INFORMATION.
For questions or further information, please contact Allison Brown (acbrown1@cdc.gov) or ARLN@cdc.gov.
CDC AR LABORATORY NETWORK:
Guidance for Colonization Testing by Cepheid GeneXpert

PURPOSE.
The Cepheid GeneXpert Carba-R test is the only FDA-approved commercial test for the molecular detection of carbapenem resistance in human rectal swab specimens and has a rapid turnaround time for results. Proper sampling and handling is critical for obtaining accurate results. This guidance provides reference for:

1. rejection criteria
2. procedure for sample collection
3. acceptable vs unacceptable sampling
4. labeling requirements
5. storage and packaging
6. line list/manifest
7. shipping and transport
8. FAQs from patients
9. verbal consent

REJECTION CRITERIA.

Unacceptable specimens include, but are not limited to:

- Incorrect swab type
  - Swabs must be made by Copan (Cepheid Sterile Transport Dual Swab, catalog # 900-0370)
- Swab is overloaded with fecal material (see Figure 3)
- Specimen is non-fecal (tracheal, sputum, environmental, etc.)
  - Contact state/local health department if there is an investigation need for non-fecal specimens
- Specimen is unlabeled or insufficiently labeled
- Primary or secondary receptacle is leaking, cracked, or otherwise unsafe for handling
- Failure to comply with shipping instructions
- Specimen is received by testing lab >1 day after date of collection
- Unauthorized specimens that are sent without prior approval from the state epidemiologist and recipient regional laboratory
PROCEDURE FOR COLLECTION.

EQUIPMENT AND MATERIALS NEEDED FOR COLLECTION

- Appropriate personal protective equipment (PPE) as indicated by the patient’s clinical care team
  - e.g. gloves, gown, mask

- Culture collection and transport system
  - Cepheid dual swab collection device (Cepheid catalog # 900-0370)
  - Stocks of these swabs are available through regional ARLN labs

PROCEDURE

1. The individual should be informed about why it is recommended that they be screened and informed about the procedure for collection of a rectal swab. Permission from the patient or guardian is needed prior to collection of the rectal swab. The patient or guardian should have an opportunity to ask questions. A list of frequently asked questions and sample scripts for health departments and healthcare facilities may be found at the end of this document. A copy of these FAQs should be left with the patient and contact information for the state or local health department if any questions arise.

2. Before beginning, perform hand hygiene and wear appropriate PPE as indicated by the patient’s clinical care team
   a. e.g. gloves, gown, mask

3. Open the outer plastic packaging on the end that says “PEEL HERE,” opposite end from the cotton tips.

4. Carefully remove the tube from the plastic packaging and label the tube (see label instructions below)
   a. While labeling, leave the dual swab enclosed in the plastic packaging to prevent contamination.

5. Pull the dual swab from the plastic packaging, being careful not to touch the cotton tips.

6. The dual swab may be moistened with sterile saline or transport medium only
   a. Do NOT use tap water or lubricating gel
7. Insert the swab 1 cm (½ inch) beyond the anal sphincter and gently rotate against the walls of the rectum 3 times (Figure 2).
   i. Diapered infants: For diapered infants, the cotton swab may be used to swab the stool present in the soiled diaper.
   ii. Patients with an ostomy: Use the cotton applicator to obtain specimen from the stoma site
   iii. Neutropenic patients: Use perianal swab

![Figure 2. Proper swab depth](image)

8. Confirm swab is not overloaded or underloaded (Figure 3).
9. Remove cap from tube by twisting.
10. Insert dual swab into tube and firmly close cap (Figure 4a).

![Acceptable Specimens](image)

![Unacceptable Specimens](image)

*Figure 3. Acceptable vs unacceptable specimens.*
LABELING REQUIREMENTS
Specimen must be clearly labeled with:
1. A **minimum of two unique patient identifiers**. Acceptable identifiers include:
   - Patient’s full name
   - Date of birth
   - Medical record number
   - Sample ID number
2. **Date of specimen collection**
3. **Site of collection**

STORAGE AND PACKAGING
- Cepheid swabs can be stored at room temperature (15-28°C)
- Please be sure to ship swabs within 1 day of collection
- Coordinate timing of colonization swabbing and specimen shipping with recipient regional laboratory’s testing schedule.
  - **Testing must be performed within 5 days of collection**

Primary Receptacle
- Primary receptacles must be in a plastic container
- Specimens collected with Cepheid swabs are in an acceptable primary receptacle (Figure 4a)
- The screw cap of the swab collection container must be sealed
  - Parafilm can be used to ensure containment of swab

*Figure 4. (A) Collection device and (B) acceptable packaging of multiple swabs in a bag.*
Secondary Receptacle
- Secondary receptacles must be watertight and leak-proof
- Generally, sealed plastic bags are accepted as a secondary receptacle
- Multiple collection tubes may be placed in the same bag (Figure 4b)
  - To minimize cross-contamination, please individually bag each collection tube before combining into one large bag
- Do not overfill the bag with too many tubes; the bag **MUST** be sealed completely
- Absorbent material (e.g. absorbent pads, paper towels, or cotton balls) should be placed in the bag with the tubes in case of leakage

Sturdy Outer Packaging
- Once swabs are secured in a sealed plastic bag, place bag into a box
- FedEx shipping requires the outer packaging box be made of corrugated fiberboard or wood (Figure 5)
- Foam boxes, paper bags, and envelopes are **not** acceptable as an outer package for FedEx shipping of these specimen types
- The box should be an appropriate size to encase the bag of swabs; not too big and not too small

Acceptable Outer Packaging

Unacceptable Outer Packaging

*Figure 5. Outer packaging.*

Line List/Manifest of Specimens
- Required in shipment
- Must be placed between the secondary receptacle and the outer package
- See example on next page
Submitter___________________________
Submitter point of contact___________________________
Submitter contact information___________________________

Recipient___________________________
Recipient point of contact___________________________
Recipient contact information___________________________

Prior approval name___________________________

Test Order Name___________________________
Methodology___________________________
Suspected organism___________________________
Specimen description (e.g. patient room #) ___________________________
Date of collection (MM/DD/YYYY) ___________________________
Index isolate organism (genus, species) ___________________________
Index isolate gene of interest___________________________

<table>
<thead>
<tr>
<th>Unique ID</th>
<th>Patient ID 1</th>
<th>Patient ID 2</th>
<th>Specimen Source</th>
<th>Swab Lot #</th>
<th>Swab Expiration Date</th>
</tr>
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SHIPPING AND TRANSPORT

Guidelines may vary from transporter to transporter; however, guidelines below are catered to FedEx requirements because CDC is using FedEx as the designated transporter for ARLN specimens.

Cepheid swab specimens fall under the International Air Transport Association’s (IATA) “Category B”. Category B is defined as “an infectious substance not in a form generally capable of causing permanent disability or life-threatening or fatal disease in otherwise healthy humans or animals when exposure to it occurs”. The IATA guidelines require three receptacles to package and ship Category B substances that are highlighted in this guideline.

A special permit/certification is not required to ship a Category B substance; however, training is necessary to ensure proper packaging of shipments and to reduce risks of exposure during transportation. Training may be informal, consisting of a documented review of Category B shipping practices.

General Considerations for Shipping

- Please ensure that employees tasked with packing and/or shipping swabs are properly trained
  - Employers/leadership must be responsible for training employees shipping Cepheid swabs
- Cepheid swabs can be transported at room temperature (15-28°C)
  - Dry ice should not be used for shipment
  - Cold packs may be considered if shipping temperatures are expected to exceed 28°C.
- Shipment must include line list/manifest of specimens being shipped
- Multiple bags of swabs can be placed in one box but the weight should not exceed 4kg
- Packages containing biohazards should NEVER be dropped off at a FedEx Express® Drop Box
- Must be labeled with:
  - UN 3373 label -- labels can be purchased from Fisher Scientific (catalog numbers 22-130-067 or 22-130-069, based on size requirements) or the “Appropriate Label” example below may be printed and adhered to the outer packaging with clear packing tape).
  - 6-mm-high text stating “Biological Substance, Category B” adjacent to the label UN 3373 shown below:
- The shipper’s and recipient’s name, address, and telephone number must be displayed clearly on the box
  - For CDC,

Jane Smith
Centers for Disease Control and Prevention
1600 Clifton Road, NE
Specimen Triage and Tracking (STAT), MS G-xx
ATTN: Unit# 154
Atlanta, GA 30329
Phone #: xxx-xxx-xxxx

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1 Definition derived from Transporting Infectious Substances Safely Department of Transportation.; 2006
Figure 6. Biological substance, Category B marking requirements.
Figure 7. Biological substance, Category B label.
Figure 8. Labels on box.
INSTRUCTIONS TO HEALTH DEPARTMENTS AND HEALTHCARE FACILITIES

The following FAQs and scripts are resources for health departments and healthcare facilities performing patient screening for multidrug-resistant organisms (MDROs) such as carbapenem-resistant Enterobacteriaceae, *Acinetobacter*, or *Pseudomonas*. Content is provided in an editable format so it can be tailored for different settings and scenarios.

Health departments that have questions about MDRO screening should email the Centers for Disease Control and Prevention (CDC) Division of Healthcare Quality Promotion (DHQP) healthcare-associated infection outbreak mailbox: HAIoutbreak@cdc.gov

FAQS ABOUT SCREENING TESTS FOR RARE ANTIBIOTIC-RESISTANT GERMS THAT COLONIZE THE GUT, SUCH AS CARBAPENEM-RESISTANT ENTEROBACTERIAEAE (CRE)

Your [insert healthcare facility e.g., hospital or nursing home] has identified a person with a type of bacteria (a kind of germ) that is resistant to important antibiotics drugs that are used to treat infections. When bacteria are resistant to an antibiotic, it means that the drug will not work to treat infections caused by those bacteria.

Why have I been contacted?
To make sure this type of resistant bacteria does not spread further, the healthcare facility or health department is contacting people who might have had contact with this bacteria. They are requesting that these people get a screening test to make sure they are not also carrying the bacteria.

Why is it important for me to be tested for this bacteria?
It is important for you to be tested for this germ so that the healthcare facility and health department can prevent it from spreading. Preventing the spread of these bacteria is very important so that these resistant bacteria don’t become common in your community.

What happens if these bacteria are found in or on me?
The results of the test will be kept confidential to the extent allowed by law. The results will be shared with you and your healthcare providers and might be shared with the health department.

The risk to you from this germ is low. Most people carry these bacteria and never get sick from them. If you receive medical care, your healthcare providers may take extra steps to protect you and make sure they do not spread the bacteria to other patients.

How can I be tested for this germ?
People carry this kind of germ in their gut or stool, so the best way to test for these bacteria is to swab your rectum. If you agree to be tested, a healthcare provider will gently insert just the tip of a soft swab that looks like a Q-tip into your rectum, gently rotate it, and then remove the swab. The procedure is not painful and there should be no side effects. The swab will be sent to a lab and, within a few days, the lab will report the result to your healthcare provider.
Do I have a choice to be tested?
Yes, providing a swab is voluntary. You can choose to decline testing. However, if you decline testing and you receive medical care, your healthcare providers might take extra precautions, such as wearing a gown and gloves when caring for you, since they will not know if you have this germ.

I want to be tested, but I am not comfortable having a rectal swab collected. Is there an alternative test?
At this time, there are no alternative tests because they may decrease our ability to find these bacteria in your body if they are present.

If my test is positive, what will I need to do?
The risk of spreading this germ to your family and friends is very low, but family and visitors should wash their hands well after caring for you or visiting you to decrease the chance of getting the germ. You should also wash your hands frequently, especially after using the bathroom and before eating or preparing food.

If you receive medical care at a healthcare facility such as a hospital or nursing home, be sure to let your healthcare providers know about the results so that they can take steps to prevent spreading the germ to others.

If my test is positive, will I need treatment?
If the test is positive, it means you are carrying the germs in your gut. Since they are not making you sick (causing infection), you will not need antibiotics. Many people stop carrying these bacteria over time, but this depends on many factors. Taking antibiotics can increase the time these germs are carried in your gut. So, in consultation with your doctor, antibiotics should be used appropriately and should be taken exactly as prescribed.

Your healthcare providers might recommend you get an additional test at a later time to see if the germ is gone. However, a follow-up test will not be recommended for everyone.
EXAMPLE VERBAL CONSENT FOR COLLECTION OF RECTAL SWAB TO ASSESS COLONIZATION WITH ENTERIC BACTERIA

Hi, my name is [insert name] and I work for [insert organization]. I’m here to talk to you about some screening the [insert healthcare facility e.g., hospital or nursing home] is doing to check for a rare germ. Recently, we identified this germ that is rare in the U.S. in a patient who was cared for at this facility. The germ is called carbapenem-resistant Enterobacteriaceae, or “CRE” for short.

We are screening patients for this germ because some people can carry this germ in the gut without knowing it and they can spread the germ to others without knowing it.

The chance that you carry this germ is very low, and fortunately, most people who do carry it never get sick from it. But to make sure this germ has not spread, the health department would like us to screen patients to make sure they don’t have it.

If you agree to be screened, the process is very simple and takes just a few seconds. We would need to swab inside your rectum. To do that, we would gently insert just the tip of a soft swab, which looks like a Q-tip, into your rectum, gently rotate it, and then remove it. The process is not painful and there shouldn’t be any side effects.

The swab will be sent to a lab to test for the bacteria, which will take a few days. If they find the germ, someone will contact you to discuss what to do. The results of the test will be kept confidential to the extent allowed by law. Providing a swab is completely voluntary and you can choose not to.

Do you have any questions? [pause for questions]

Is it OK if we collect the swab?

ADDITIONAL RESOURCES

1. The rationale for CDC screening recommendations
2. How to screen appropriately based on risk
3. Information on shipping Category B substances
   a. FedEx UN3373 Shipping Guidelines

ACKNOWLEDGEMENTS

Images used from Cepheid, Maryland Public Health Lab, & University of New Hampshire

CONTACT INFORMATION

For questions or further information, please contact ARLN@cdc.gov or Dr. Sarah Malik.