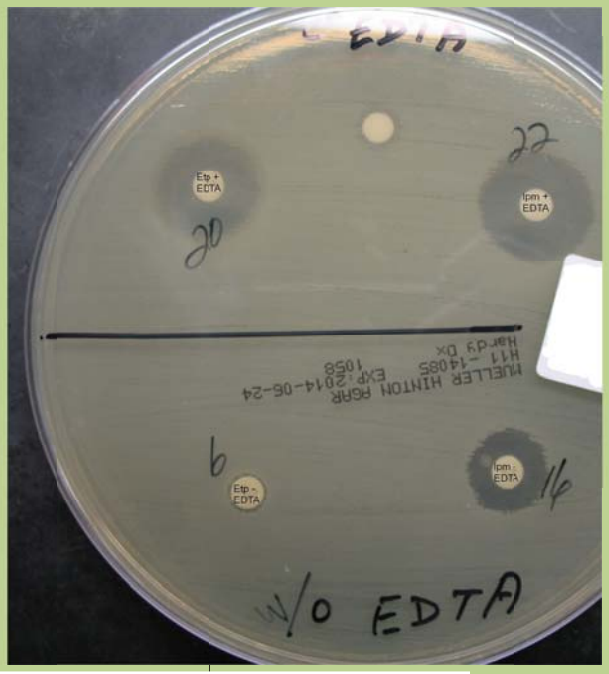

Carbapenem Susceptibility Testing -
Recommendations for Microbiology Laboratories

Utah Department of Health

Division of Disease Control and Prevention

Bureau of Epidemiology

HAI Program

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The Utah Department of Health's *Carbapenem Susceptibility Testing - Recommendations for Microbiology Laboratories* was modeled after the Oregon CRE Toolkit (http://public.health.oregon.gov/diseasesconditions/communicabledisease/reportingcommunicabledisease/reportingguidelines/documents/cre_iguide.pdf) but includes Utah-specific definitions, recommendations and protocols.

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http://health.utah.gov/epi/ARO/CRE_Lab.pdf

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Recommendations for Microbiology Laboratories

Determine carbapenem susceptibility following the CLSI recommended procedures and interpretive criteria. Between 2010 and 2012, CLSI adjusted susceptibility breakpoints for testing Enterobacteriaceae to carbapenems (see Table below). The 2012 breakpoints increased the sensitivity for carbapenamase detection; laboratories using the 2012 breakpoints do not need to perform a "confirmatory" Modified Hodge Test (MHT) for patient management.

The Utah Department of Health (UDOH) administered a statewide survey in December 2013 and found that most Utah microbiology laboratories used CLSI breakpoints predating the 2010 update and did not perform MHT for confirmatory carbapenamase testing. This is a potential gap in our ability to detect and report carbapenemase-producing CRE (CP-CRE). We recommend that laboratories using pre-2010 breakpoints perform carbapenemase screening and confirmation via MHT. If those laboratories are unable to do their own carbapenemase screening and confirmation, we advise that they send their specimens to a reference laboratory that is able to test (**Appendix A**).

	Breakpoints Predating 2010 Update (µg/ml)[1] (through Jan. 2010; M100-S19)			2012 Breakpoints (µg/ml) (revised Jun. 2010 and Jan. 2012[2]; M100-S22)		
	Susceptible	Intermediate	Resistant	Susceptible	Intermediate	Resistant
Doripenem	n/a	n/a	n/a	≤1	2	≥4
Ertapenem	≤2	4	≥8	≤0.5	1	≥2
Imipenem[3]	≤4	8	≥16	≤1	2	≥4
Meropenem	≤4	8	≥16	≤1	2	≥4

In May 2013, the UDOH required the reporting of carbapenem non-susceptible *Acinetobacter, E coli,* and *Klebsiella* species. Use the UDOH case definition as follows:

Confirmed:

- Isolation of Acinetobacter species and:
  - MIC to Imipenem or Meropenem of ≥4 µg/mL, or
  - PCR positive for carbapenemase gene
- Isolation of Klebsiella species or Escherichia coli and:
  - Intermediate or resistant MIC to a carbapenem (refer to table for breakpoints) or
  - Positive Modified Hodge test, or
  - PCR positive for carbapenemase gene

Screening cultures

CRE screening cultures for outbreak investigations should be performed as recommended by local facility's Infection Prevention and Control staff in consultation with UDOH. The number of surveillance cultures requested will be based on pertinent epidemiology.

- The recommended protocol for screening cultures is included (**Appendix B)**. If your laboratory does not have ertapenem (preferred) or meropenem (alternative) disks, contact UDOH HAI epidemiologist. Confirm candidate CRE organisms via routine identification (UPHL should be able to type specimens) and susceptibility (specimens will be sent to a reference lab); keep confirmed CRE isolates until further notification by UDOH.
    - Screening cultures should NOT be billed to the patient; discuss billing with your facility's Infection Prevention and Control department in consultation with UDOH.
    - Facilities may want to consider allocation of 'emergency funds' to pay for active screening cultures in the event of an outbreak or identification of rare organism.
    - Discuss how results of screening cultures will be reported with your facility's Infection Prevention and Control department.

How to send isolates to UPHL for typing only:

Use the General Microbiology Request Form (**Appendix C**).

- In "tests requested", check "other" under isolate identification and write "CRE/CRAB".
- In "comments", please indicate genus and species.
- Send specimen….on a slant; a plate is also acceptable.
- Include collection date, source of specimen, and patient medical record number.

"Unusual" Isolates

To send any *E. coli, Klebsiella spp*., or *Acinetobacer* spp. or other isolates that are 'unusual*' to the Utah Public Health Laboratory (UPHL) for further testing at CDC please contact the UDOH HAI Epidemiologist at 801-538-9182. The HAI Epidemiologist will work with you and UPHL to forward the isolate to the Centers for Disease Control and Prevention for further analysis if warranted (not real-time). UPHL will fax results to your laboratory within 3 business days of receipt of test result.

*Unusual: Rare or unknown isolate.

Carbapenem-resistant *Acinetobacter* species (CRAB) Laboratory Response Diagram

Acinetobacter species isolate identified that meets case definition

Is this your facility's first CRAB?

Yes

No

Report to Local Health Department
•Submit Lab Report
•Submit susceptibilities
Notify Infection Prevention and Control

Results confirmed?

No

Confirm results

Negative

Do not report

Positive

Yes

Report lab results to Local Health Department
•Submit updated lab report
•Submit susceptibilities
Notify Infection Prevention and Control

***Acinetobacter* species**
Case definition

Confirmed:
Isolation of *Acinetobacter* species and:

- MIC to Imipenem or Meropenem of ≥4 µg/mL, or
- PCR positive for carbapenemase gene

Note: If using FDA-cleared instruments, follow instrument (FDA) breakpoints for CRAB.

Escherichia coli and *Klebsiella* species Laboratory Response Diagram

Escherichia coli and *Klebsiella* species isolate identified that meets case definition

Report to Local Health Department
•Submit Lab Report
•Submit susceptibilities
Notify Infection Prevention and Control

Did your laboratory test for carbapenemase production?

Yes

No

Result confirmed carbapenemase producer?

Submit isolate to reference laboratory (Appendix A) for Confirmatory testing

Yes

Report updated lab results to Local Health Department
•Submit updated lab report
•Submit susceptibilities
Notify Infection Prevention and Control

No

Yes

Report updated lab results to Local Health Department
•Submit updated lab report
•Submit susceptibilities
Notify Infection Prevention and Control

Escherichia coli* and *Klebsiella species
Case definition

Confirmed:
Isolation of Klebsiella species or Escherichia coli and:

- MIC to Imipenem, or Meropenem of ≥8 µg/mL, Ertapenem of ≥4 µg/mL (if using pre-2010 CLSI breakpoints), or
- MIC to Imipenem, Meropenem, or Doripenem of ≥2 µg/mL, Ertapenem of ≥1 µg/mL (if using post-2012 CLSI breakpoints as described in the table above) or
- Positive Modified Hodge test, or
- PCR positive for carbapenemase gene

Note: If using FDA-cleared instruments, follow instrument (FDA) breakpoints for CRE.

Appendix A.

List of Utah Laboratories - CRAB/CRE molecular confirmation or Carbapenamase production

LABORATORIES

Salt Lake City	**ARUP** 500 Chipeta Way Salt Lake City, UT 84108 801-583-2787 **Haleina Muir - Lab Supervisor** **Phone:** 583-2787 x 2229	**INTERMOUNTAIN CENTRAL LABORATORY** 5121 S. Cottonwood St. Salt Lake City, UT 84107 801-507-2244 **George Hinde - Lab Supervisor** **Phone:** 801-507-2244	**PRIMARY CHILDRENS HOSPITAL** 100 N. Mario Cappechi Drive Salt Lake City, UT 84113 801-662-2140 **Abby Phillips - Lab Supervisor** **Phone:** 801-662-2140
	Testing Provided:	**Testing Provided:**	**Testing Provided:**
	NDM **KPC** **PCR test**	**NDM** **KPC** **OXA** **IMP** **VIM** **CTXM type ESBL**	**KPC**
	E-Mail: haleina.m.muir@aruplab.com	**E-Mail**: george.hinde@imail.org	**E-Mail**: Abby.Phillips@imail.org

Laboratory Protocol for Detection of Carbapenem-Resistant or Carbapenemase-Producing, *Klebsiella* spp. and *E. coli* from Rectal Swabs

Purpose

To identify patients colonized with carbapenem-resistant or carbapenamase-producing Enterobacteriaceae in the intestinal tract. Patients who grow these organisms should be placed on Contact Precautions (5) to prevent transmission of the resistant bacteria. The procedure described below is a modification of the procedure described by Landman et al. (4). See the procedural notes for steps in the procedure which can be modified.

Background

Carbapenem-resistant Enterobacteriaceae (CRE) are usually resistant to all β-lactam agents as well as most other classes of antimicrobial agents. The treatment options for patients infected with CRE are very limited. Healthcare-associated outbreaks of CRE have been reported. Patients colonized with CRE are thought to be a source of transmission in the healthcare setting (1). Identifying patients who are colonized with CRE and placing these patients in isolation precautions may be an important step in preventing transmission (6).

Carbapenem resistance in Enterobacteriaceae occurs when an isolate acquires a carbapenemase or when an isolate produces an extended-spectrum cephalosporinase, such as an AmpC-type β-lactamase, in combination with porin loss. In the United States, the most common mechanism of carbapenem resistance is the *Klebsiella pneumoniae* carbapenemase (KPC).

Detection of carbapenemase production is complicated because some carbapenemase-producing isolates demonstrate elevated but susceptible, carbapenem MICs. CLSI has published guidelines for detection of isolates producing carbapenemases (CLSI document M100) (2). For isolates that test susceptible to a carbapenem but demonstrate reduced susceptibility either by disk diffusion or MIC testing, performing a phenotypic test for carbapenemase activity, the Modified Hodge Test (MHT), is recommended.

Carbapenem resistance and carbapenemase-production in any species of Enterobacteriaceae is an infection control concern. However, the methodology described here focuses on the detection of carbapenem-resistant or carbapenemase-producing *Klebsiella spp* and *E. coli* as this facilitates performance of the test in the microbiology laboratory and, more importantly, because these organisms, especially *Klebsiella* spp. represent the vast majority of CRE encountered in the United States (3).

Reagents
5 ml Trypticase Soy Broth
10-µg carbapenem disks
MacConkey agar

Equipment
Vortex
35 ± ºC, ambient air

Supplies
100 µl calibrated pipettes
Forceps
Sterile loops

Specimen
Rectal swab or perianal swab specimen in suitable transport media

Special safety precautions
Biosaftey Level 2

Quality Control (QC)
The carbapenem disks that are used in this procedure should be quality control tested using disk diffusion methods and quality control strains as described in the CLSI guideline documents M2 and M100 (2,(2). For example, if the10-µg/mL meropenem disk is used in this procedure, test *E. coli* ATCC 25922 by the disk diffusion method using meropenem disks from the same lot. An acceptable control test will yield a zone size between 28-34 mm. Follow CLSI guidelines for frequency of disk QC testing and corrective action if results are out of range.

Procedure

Step 1 Day One	Aseptically, place one 10-µg ertapenem or meropenem disc in 5 ml trypticase soy broth (TSB) (see procedure note 1) Immediately inoculate the broth with the rectal culture swab Incubate overnight at 35 ± 2ºC, ambient air
Step 2 Day Two	Vortex and subculture 100 µl of the incubated broth culture onto a MacConkey agar plate (see procedure note 2) Streak for isolation Incubate overnight at 35 ± 2ºC, ambient air
Step3 Day Three	Examine the MacConkey agar for lactose-fermenting (pink-red) colonies. More than one colony morphology may represent different species of Enterobacteriaceae (see procedure note 3). It may be necessary to subculture representative colonies of each morphology type to a non-selective media for isolation and/or for susceptibility testing. Screen representative isolated colonies using a phenotypic test for carbapenemase production, such as the Modified Hodge Test (MHT) or test for carbapenem susceptibility using a standardized method and follow the CLSI guidelines for identification of carbapenemase-producing Enterobacteriaceae (see procedure note 4).
Step 4 Day Four	For CRE and/or MHT-positive isolates, perform species-level identification.

Interpretation/Results

Report all cultures that are positive for CRE or carbapenemase-producing Enterobacteriaceae to the appropriate infection control personnel. Contact Precautions should be implemented for all patients with positive cultures for CRE or carbapenemase-producing Enterobacteriaceae.

Quality assurance

The ability to recover CRE using this procedure could be assessed as follows: Inoculate 5mL of TSB containing the 10-ug carbapenem disk with a swab that was used to sample a known CRE-negative stool specimen. In addition, inoculate the TSB with 0.5 mL of a 1 x 10^5 CFU/mL suspension of a known carbapenemase-producing isolate (e.g., *K. pneumoniae* ATCC BAA-1705), (see procedural note 5 for suspension preparation) Proceed with Step 2 of the procedure. The carbapenemase-producing *K. pneumoniae* should be recovered on the MacConkey agar.

To test for specificity of the procedure, use a carbapenem susceptible *Klebsiella pneumoniae,* (e.g. ATCC 700603) and follow the same steps. The carbapenem susceptible *K. pneumoniae* isolate should not grow on the MacConkey agar.

Method limitations

1. Patients may be colonized with CRE or carbapenemase-producing Enterobacteriaceae at a concentration that is not detectable by this method. Studies described by Landman et al. and studies performed at the CDC suggest that the lower limit of detection is between ranges from 1 x 10^2 CFU/ mL to 1 x 10^6 CFU/ mL (4).

2. Non-fermenting gram-negative bacilli with intrinsic mechanisms of carbapenem-resistance, such as *Acinetobacter* spp. and *P. aeruginosa*, will be detected on the MacConkey agar. These isolates should be identified as non-lactose fermenters on the MacConkey agar and therefore would not be picked for characterization. If carbapenem-resistant non-fermenters are present at high concentration, they could overgrow CRE or carbapenemase-producing Enterobacteriaceae on the media and prevent detection of colonization.

3. Enterobacteriaceae can be resistant to carbapenems by mechanisms other than a carbapenemase, the most common of which is expression of an extended-spectrum cephalosporinase, such as an AmpC-type enzyme or an ESBL, combined with porin loss. These isolates will also grow on the MacConkey agar and be identified as carbapenem-intermediate or resistant by standard susceptibility testing but these isolates are negative by the MHT. For isolates that test intermediate or resistant to carbapenems, it may not be necessary to distinguish between these mechanisms of resistance because all carbapenem-nonsusceptible Enterobacteriaceae produce a broad-spectrum β-lactamase, and are therefore an infection control concern. Implementing Contact Precautions for patients colonized with these bacteria would be appropriate. Laboratories may choose to test carbapenem-intermediate or resistant isolates with the MHT to identify carbapenemase-production for epidemiological purposes.

Procedure notes

1. The procedure described by Landman et al. (4) describes using a 10-µg imipenem disk for step 1. However, there are species of Enterobacteriaceae which have intrinsic mechanisms of resistance to imipenem other than a carbapenemase (See CLSI document M100, Appendix G)(2). Therefore, ertapenem or meropenem may provide more specific selection for acquired carbapenem resistance in Enterobacteriaceae.

2. Some laboratories performing cultures for isolation of CRE from rectal specimens place a 10-µg carbapenem disk in the first quadrant of the MacConkey plate. Only colonies growing "close" to the carbapenem disk are picked for characterization. No clear criteria for "close" have been established. However, it may be helpful to use either a meropenem or ertapenem disk and then apply the CLSI disk diffusion screening criteria to identify potential carbapenemase-producing isolates (i.e., an ertapenem or meropenem disk zone ≤ 21 mm). Note: These zone size criteria

were validated for standardized disk diffusion testing methods as described in CLSI document M2.

3. Carbapenemases are known to exist in several different species of gram-negative bacilli including species of Enterobacteriaceae and *Pseudomonas aeruginosa*. However, carbapenemases are more common in lactose-fermenting species of Enterobacteriaceae (e.g., *K. pneumoniae* and *E. coli*) than in non-lactose fermenting Enterobacteriaceae (e.g. *Serratia marcescens* and some *Enterobacter* spp.) and *P. aeruginosa*. In this procedure, it is suggested that laboratories focus their efforts on detection of resistant lactose-fermenting bacteria to reduce workload. Healthcare facilities that have identified clinical infections with carbapenemase-producing non-lactose fermenting gram-negative species should consider altering this procedure to include characterization of colonies with a morphology that is consistent with those species.

4. The exact procedure for confirmation of CRE or carbapenemase-production should be laboratory-specific and chosen based upon laboratory workflow and the types of isolates causing clinical infections in the patient population served. It may be helpful to refer to the CLSI guidelines for identification of carbapenemase production in isolates that test susceptible to carbapenems in document M100 (2).

5. A 1 x 10^4 CFU/mL suspension of the known carbapenem-resistant or carbapenem-susceptible isolates could be prepared as follows: Dilute 0.1 mL of a 0.5 McFarland standard suspension (equals approximately 1 x 10^8 CFU/ mL), in 9.9 mL sterile water or saline for a 1:100 dilution. From the 1:100, dilute 1.0 mL in 9.0 mL water or saline for a 1:1000 dilution. Add 0.5 mL of the 1:1000 dilution (equals approximately 1 x 10^5 CFU/mL), suspension to the 5 ml TSB for a final concentration of approximately 1 x 10^4 CFU/mL.

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5. **Siegel, J. D., E. Rhinehart, M. Jackson, L. Chiarello, and HICPAC.** 2007. Guideline for Isolation Precautions: Preventing Transmission of Infectious Agents in Healthcare Settings 2007. www.cdc.gov/ncidod/dhqp/pdf/isolation2007.pdf

6. **Siegel, J. D., E. Rhinehart, M. Jackson, L. Chiarello, and HICPAC.** 2006. Management of Multidrug-Resistant Organisms in Healthcare Settings 2006. www.cdc.gov/ncidod/dhqp/pdf/ar/mdroGuideline2006.pdf

Appendix C Date in use: 2/18/2014

INFECTIOUS DISEASE TEST REQUEST FORM

UTAH PUBLIC HEALTH LABORATORY
4431 SOUTH 2700 WEST
TAYLORSVILLE, UTAH 84129
TELEPHONE: (801) 965-2400
FAX: (801) 965-2551
http://health.utah.gov/lab/infectious-diseases

FOR UPHL USE ONLY

LAB# DATE STAMP

PLEASE PRINT CLEARLY FOR ACCURACY.

PATIENT INFORMATION:

PATIENT STATE OF RESIDENCE:	PATIENT COUNTY OF RESIDENCE:	ZIP CODE:	DATE OF BIRTH (mm/dd/yyyy)	AGE	SEX
			_____/_____/_____		M F

PATIENT NAME (Last, First):

Is Patient Insured? [ ] Yes [ ] No

STI TESTING ONLY: Is patient MSM? [ ] Yes [ ] No

PATIENT ID #

ETHNICITY
[ ] Hispanic
[ ] Non-Hispanic

RACE
[ ] White [ ] Black or African American [ ] American Indian or Alaska Native
[ ] Asian [ ] Native Hawaiian or other Pacific Islander

PROVIDER INFORMATION
Provider Code:

Physician: _____
Provider Phone: _____
Provider Email: _____
Secure Fax #: _____

SPECIMEN COLLECTION DATE AND TIME
(mm/dd/yy) _____/_____/_____
Time: _____

SPECIMEN SOURCE/SITE (CHOOSE 1):

[ ] Blood	[ ] Environmental (specify):_____	[ ] Plasma	[ ] Urethra
[ ] Body Fluid (specify):_____	[ ] Food (specify):_____	[ ] Rectum	[ ] Urine
[ ] Bronchoalveolar lavage	[ ] Isolate (source):_____	[ ] Serum	[ ] Vagina
[ ] Bronchial aspirate/wash	[ ] Lesion (site):_____	[ ] Sputum (natural / induced)	[ ] Vomitus
[ ] Cerebrospinal Fluid	[ ] Liquid Pap	[ ] Stool	[ ] Wound/Abcess
[ ] Cervix	[ ] Nasal (aspirate /swab / wash)	[ ] Throat swab	[ ] Other (specify): _____
[ ] (Endo)tracheal aspirate/wash	[ ] Nasopharyngeal swab	[ ] Tissue (specify):_____	

BACTERIOLOGY/TUBERCULOSIS TESTS

Bacteriology Specimen
REQUIRED Shipping Temperature:_____
[ ] Bacterial Culture
[ ] Bacterial ID / Referral
Presumptive ID:_____
[ ] Mycobacterial culture
[ ] Mycobacterial referral
Presumptive ID:_____
[ ] Other (specify): _____

BIOTERRORISM TESTS

(Notify Lab before submitting)

[ ] Bacillus anthracis Detection/Identification
[ ] Brucella species Detection/Identification
[ ] Brucella antibody
[ ] Burkholderia mallei/pseudomallei Detection/ID
[ ] Clostridium botulinum culture & toxin
[ ] Coxiella burnetii Detection
[ ] Francisella tularensis Detection/Identification
[ ] F. tularensis antibody
[ ] Orthopox viruses Detection
Virus Suspected:
[ ] Vaccinia virus
[ ] Varicella zoster virus
[ ] Variola virus
[ ] Yersinia pestis Detection/Identification
[ ] Yersinia pestis antibody
[ ] Other (specify):

VIROLOGY / IMMUNOLOGY TESTS

[ ] C. trachomatis and N. gonorrhea by NAAT
[ ] Patient is a partner of a 15-24 year old female

[ ] Herpes/VZV PCR (HSV-1, HSV-2, VZV)

[ ] Virus Identification (culture)
Virus suspected _____

[ ] Cytomegalovirus

[ ] Varicella zoster virus

[ ] Multi-Pathogen Respiratory Panel
(Includes Adenovirus, Coronovirus, Human Metapneumovirus, Rhino/Enterovirus, Influenza A, Influenza B, Parainfluenza 1-4, RSV, Bordetella pertussis, C. pneumoniae, M. pneumoniae)

[ ] Influenza A & B virus PCR (with subtyping)
[ ] Hospitalized w/ Influenza-like illness
[ ] Other (i.e., cluster investigation)
Cluster location: _____
Other reason for testing: _____

[ ] West Nile virus IgM (Human)

[ ] QuantiFERON-TB Gold
REQUIRED information:
Blood draw date/time:_____
Incubation at 37°C completed? [ ] Yes [ ] No
Signature:_____
Incubation starte date/time:_____
Incubation end date/time:_____

[ ] Syphilis IgG EIA (includes confirmatory testing)
[ ] RPR (suspect acute infection/previous positive)

[ ] HIV Antigen/Antibody (includes confirm. testing)
[ ] Previous positive

[ ] Hepatitis C Antibody
[ ] Add HCV RNA Testing if Positive

[ ] Hepatitis C RNA
(Qualitative; Antibody screen not included)

[ ] Hepatitis B Antibody

[ ] Hepatitis B Antigen

[ ] Hantavirus (Sin Nombre) IgG/IgM
[ ] Acute Serum (mm/dd/yy)_____/_____/_____
[ ] Convalescent serum (mm/dd/yy)_____/_____/_____

ADDITIONAL INFORMATION

[ ] Other Diseae Suspected: _____

[ ] Referral Test to CDC (form **REQUIRED**) specify:_____
Contact UPHL for CDC form

COMMENTS:

Utah Department of Health
Healthcare-Associated Infections
Prevention & Reporting Program
P.O. Box 142104
Salt Lake City, UT 84114
http://health.utah.gov/epi/diseases/HAI/index.html
PH 801-538-6191 • FAX 801-538-9923