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### Director of Infectious Diseases Division– James T. Rudrik, Ph.D.

Dr. James T. Rudrik, former Manager of the Microbiology Section, has accepted the permanent position as Director of the Division of Infectious Diseases effective March 24, 2014.

Dr. Rudrik brings a wealth of knowledge and a vast amount of valuable experience in the practice of laboratory medicine to this position. He has more than 30 years of service in the laboratory testing field, both in private as well as public institutions and he has been with the Michigan Department of Community Health Bureau of Laboratories for the past 15 years.

Dr. Rudrik will continue to work collaboratively with clinical partners and other state and federal agencies to ensure that we are providing the highest quality of laboratory testing for our public health partners and Michigan residents.

Please feel free to contact Dr. Rudrik regarding any issues related to infectious disease laboratory testing. He can be reached at (517)335-8067.

Director,  
Bureau of Laboratories  
Sandip Shah, Ph.D.,  
HCLD(ABB)





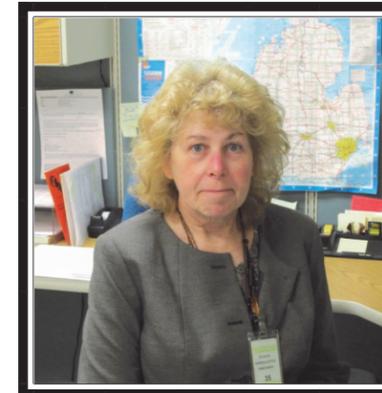
## MDCH Bureau of Laboratories Web Portal for Electronic Test Ordering and Results Delivery

The Michigan Department of Community Health Bureau of Laboratories (MDCH BOL) instituted a web portal for Electronic Test Ordering and Results (ETOR) Delivery at the start of 2014. ETOR is an alternate, voluntary method for clients to order laboratory tests and receive test results. Participating clients are able to log into the web portal and electronically fill out their test request forms; print a packing list to be submitted with the sample to the MDCH BOL for testing; track their samples through the MDCH laboratory; and look up, download or print pdf copies of final reports. Clients using the web portal no longer have to hand write or submit test request forms. This information is collected in the web portal and imported into the BOL Laboratory Information Management System (LIMS) upon arrival of the specimen. Clients have the option to continue receiving final reports via fax or hard copy, as they do now. Web portal result delivery is also available to the client. Upon request, any client may discontinue their fax or hard copy reporting system in favor of receiving only web portal results. Access to the ETOR web portal is through a State of Michigan Single Sign On (SSO) Account. Clients who currently have a SSO account will only need to request the ETOR application link. Clients without SSO accounts will need to register for one. Complete instructions can be found in the ETOR Manual (a link to this manual can be found at <http://www.michigan.gov/mdchlab>).

Current tests available to be ordered through ETOR are:

<i>C. trachomatis</i> & <i>N. gonorrhoeae</i> Non-Culture	Measles IgG
<i>C. trachomatis</i> Non-Culture	Measles IgM
Hepatitis A Antibody (IgM)	Mumps IgG
Hepatitis B Antibody (Anti-HBsAg)	Mumps IgM
Hepatitis B Surface Antigen (HBsAg)	Rabies AB Serology
Hepatitis B Surface Antigen (HBsAg) – Exposure	Rubella IgG
Hepatitis C Antibody	Rubella IgM
HIV AB - Oral Mucosal Transudate	Student/Employee Immune Status Panel
HIV Ag/Ab - Serum	Syphilis (USR)
Lead – Filter Paper	<i>Trichomonas vaginalis</i> Non-Culture (Fee-for-service only)
Lead – Whole Blood	Varicella Zoster IgG
AFB Identification - Isolate ID	AFB Nucleic Acid Amplification
Fungal Identification - Isolate ID	

## New Virology Section Manager at the Bureau of Laboratories



The Michigan Department of Community Health Bureau of Laboratories is pleased to announce the appointment of Janice Matthews-Greer, Ph.D., D(ABMM), as the Virology Section Manager in the Bureau of Laboratories.

Dr. Matthews-Greer comes to MDCH BOL from the Louisiana State University Health Science Center at Shreveport where she served as the Director of Diagnostic Virology for 10 years and held appointments as Professor of Research in

Pediatrics and Professor of Clinical Pathology. She is certified as a Diplomat of the American Board of Medical Microbiology with a specialty in Medical Virology and Public Health and is active in the American Society for Microbiology (ASM) serving as Council Representative At Large, on the ASM Professional Affairs Committee, and as an ASM Branch Lectureship Speaker. Dr. Matthews-Greer is also an accomplished researcher with over 36 articles in peer-reviewed journals and 77 abstracts presented at local, national and international meetings.

Dr. Matthews-Greer started in her new position on April 28, 2014. She may be contacted on issues related to virology at 517-335-8099.

## More About ETOR

Additional tests are being added as they complete their pilot phases. As new tests become available, the list of tests will be updated in the ETOR manual. **If you are interested in using ETOR for a test not listed above, please contact us at [LIMS\\_Help@michigan.gov](mailto:LIMS_Help@michigan.gov) (underscore between LIMS and Help) and we can prioritize a pilot for that test.**

Please note, at this time, ETOR is available only for specimens submitted to the Lansing laboratory. However, *C. trachomatis* & *N. gonorrhoeae* Non-Culture tests may be ordered through ETOR for samples submitted to the Lansing OR Saginaw laboratories.

Questions may be sent to [LIMS\\_Help@michigan.gov](mailto:LIMS_Help@michigan.gov) (underscore between LIMS and Help).



## Antimicrobial Resistance Microbiologist at the Bureau of Laboratories



The MDCH Bureau of Laboratories (BOL) is happy to announce the appointment of Carrie Anglewicz as the Antimicrobial Resistance Microbiologist in the Laboratory Systems Section.

Carrie has worked as a Senior Microbiologist in the Enterics/STD/Chromatography Unit in the Microbiology Section at the BOL where she primarily worked with food borne disease organisms. Carrie has also served as adjunct faculty for Michigan

State University's Biomedical Laboratory Diagnostics Program training students in laboratory methods during their clinical rotation at the BOL. In her new position, Carrie will serve as a laboratory advisor on antimicrobial resistance issues. Carrie may be contacted via email at AnglewiczC@michigan.gov or by phone at 517-335-9654.

## MALDI-TOF at MDCH

*The MALDI-TOF MS has recently been validated for use in the Bacteriology Unit at MDCH. MALDI-TOF MS (matrix-assisted laser desorption/ionization – time of flight mass spectrometry) replaces the routine use of 16s sequencing and biochemical identification of microorganisms.*

Currently, biochemical tests form the basis of most techniques used to identify microorganisms. Organisms are added to media containing specific carbohydrates or substrates which lead, for example, to a characteristic color change of the added reagent. This color change can be compared with reference values. Correlating the results of a number of such tests on a sample culture provides an indication of the organism's identity. The Bruker™ MALDI Biotyper uses a different methodology: it identifies microorganisms by analyzing the expression of their intrinsic proteins using mass spectrometry. This mass spectral pattern of protein expression is compared with reference patterns in a database.

The starting material for a classification using the MALDI Biotyper is an individual colony from a culture on an agar plate. The sample is transferred to a position on a MALDI target and air-dried. After drying, a small quantity of matrix solution is added. The organic solvent in the matrix solution extracts proteins from the microorganisms; the extracted proteins are mainly ribosomal proteins, which are present in high concentrations. Once the matrix has crystallized, sample preparation is complete and the samples can be analyzed. If this straightforward sample preparation method is unsuccessful, a supplementary protocol containing short chemical extraction steps can be used to increase the range of organisms that can be identified by MALDI analysis. ►

MALDI-TOF mass spectrometry forms the basis of MALDI Biotyper analyses. A laser in the MALDI-TOF mass spectrometer irradiates the matrix/sample mixture, evaporating the matrix and releasing positively charged proteins in a so-called “soft” ionization process. The ability of the matrix to absorb UV light and transfer protons onto the extracted proteins is crucial to this process. The protein ions are electrostatically accelerated over a short distance and arrive in the flight tube at a speed that is proportional to their mass. Protein ions with different masses arrive at the detector after different time periods. By simply measuring the time between pulsed acceleration and the corresponding detector signal (in the nanosecond range), the speed of the ions can be measured very precisely and converted into an accurate molecular mass. MALDI Biotyper analysis generates a characteristic mass and intensity distribution of the mainly ribosomal proteins. Because this mass spectrum is species-specific for a large number of microorganisms, it represents a 'molecular fingerprint'. Unknown microorganisms can be identified by comparing their 'fingerprint' with the thousands of patterns in the reference database.



Figure 1. The MALDI-TOF target plate has ninety-six spots that can be inoculated using an applicator such as a toothpick. The spot is overlaid with the matrix solution and after drying, it is loaded into the analyzer. The whole process takes just minutes. A longer time may be necessary if an extra extraction process is needed adding approximately an hour.

Often, the turnaround time for identification is within a day of receiving the isolate. Previously, using biochemical testing and 16s sequencing it could take days or even a week or two for final identification.

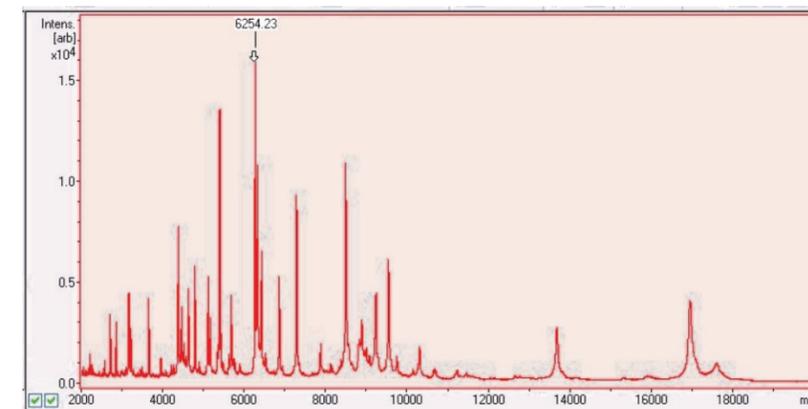


Figure 2. Example of the spectral pattern produced for Escherichia coli.

A spectral pattern is compared to the database in the software's library generating identification. When an identification cannot be obtained using our laboratory algorithm the organism will be sequenced and submitted for biochemical analysis.

The identification using MALDI-TOF has reduced the cost per test to less than a dollar compared to more than forty dollars with previous methods. We will continue to expand the use of MALDI-TOF in other areas of the laboratory in the near future.

## Non-conforming Event Leads to Product Recall and Process Improvements

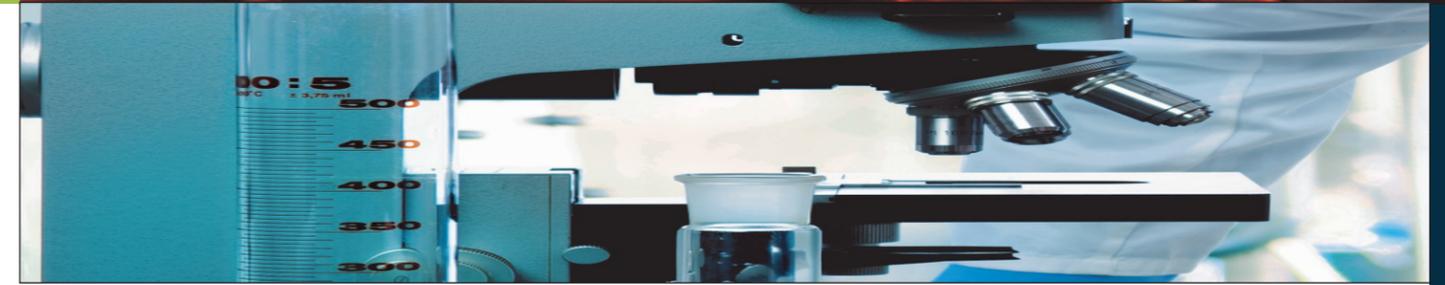


Peggy Casey, BS, MT (ASCP)  
Kristine Smith, BS, MT (ASCP)

*The discovery of a non-conforming event following the completion of complement fixation (CF) fungal antibody testing and subsequent root-cause analysis, resulted in a product recall and the implementation of process improvements at the MDCH Bureau of Laboratories (BOL) Virology Section and the manufacturer.* Inconsistent Histoplasma yeast CF antibody test results detected during paired analysis alerted the Virology staff of a potential reagent defect. All commercially obtained CF controls were within acceptable limits and there was no indication of testing failure to explain these unusual findings. Troubleshooting and root cause analysis were performed to determine the exact cause of these discordant results and to implement any value added process improvements. Results of the investigation convinced the manufacturer to recall the specific CF Histoplasma antigen lot number from the market. The manufacturer implemented additional quality control measures to ensure consistent lot to lot results and created custom packaging to reduce the amount of quality control testing performed at the BOL.

A serum specimen from a 36 year old male patient, Patient A, drawn on 1/4/13, was received at BOL for CF testing on 1/9/13. The BOL routinely checks for the existence of a previous specimen (acute) on each patient. If an acute specimen had been tested at BOL within 8 weeks of the convalescent draw date, the acute specimen is run in parallel with the current (convalescent) specimen to detect a four-fold change in antibody titer. In this case, Patient A had an acute specimen drawn on 12/18/12 with a reported Histoplasma yeast titer of 1:128. Therefore both acute and convalescent specimens were tested in parallel on the same day for comparison. Unexpectedly, both the acute and convalescent specimens tested negative for the presence of Histoplasma yeast antibody. The Histoplasma yeast antisera (positive control) provided by the manufacturer was within acceptable limits indicating all reagents were working properly. Since discrepant results were noted, repeat testing was initiated, patient specimens were not reported, the supervisor was notified, and a full investigation ensued.

To determine causation both specimens were retested the next day using an unopened 5 ml vial of Histoplasma CF antigen of the same lot and shipment date as the suspect lot. Surprisingly, both specimens now tested positive and were within one dilution of the expected titer of 1:128, pointing to a



problem with the particular vial of Histoplasma yeast antigen used the day before. All remaining 8 unopened Histoplasma antigen vials of this suspect lot number were tested against both the vendor supplied kit controls and two known positive (confirmed cases) patient sera (titers 1:64 and 1:256). The kit control, which is an antibody produced in goats, gave the expected titer with all 8 vials. However, only a few of the vials gave expected titers using the two previously tested patient sera. BOL then contacted the manufacturer to alert them of the inconsistent antigen results and to seek resolution and reimbursement. Cooperative discussions with the manufacturer's technical staff followed by multiple specimen exchanges along with new antigen lot number testing revealed improper mixing or dispensing of antigen into the 5 ml vials during the manufacturing process which led to these inconsistent results. Due to this discovery, the defective lot number was pulled from the market to prevent future false negative Histoplasma yeast CF antibody results. The manufacturer also instituted random testing of each new antigen lot number at the final 5 ml dispense step and utilized known positive human sera for quality control testing to ensure accurate, consistent antigen. Root cause analysis also revealed that an alteration in antigen packaging volumes would represent a value added improvement to our testing process at BOL. Upon request, the manufacturer agreed to package all future antigen shipments in custom 40 ml vials, as opposed to the current 5 ml vials. This decreased the number of quality control analyses performed on new antigen lot numbers from 20-30 to less than 3 per year. The acceptable CF positive control result is still unexplained. The vendor theorized that the positive CF control contained non-specific goat antibodies against soluble yeast antigens that are shed from yeast cells and are inadvertently used in the immunization process. In summary, the detection of a non-conforming event, discordant Histoplasma yeast antibody results in an acute serum specimen in comparison to results obtained during paired analysis, led to a valuable root cause analysis. Since the defective antigen was discovered early on, there were no incorrect patient results reported by the BOL. This unusual event led to multiple process improvements at the manufacturer and the BOL. Due to custom antigen packaging, the amount of quality control testing required for each new lot number of CF antigen has been greatly reduced. The manufacturer reimbursed the BOL for all defective product and reagents used during the investigation.



## Middle East Respiratory Syndrome Coronavirus (MERS-CoV)

Bruce A. Robeson MT(ASCP)

As we have all heard by now, there have been two confirmed imported cases of Middle East Respiratory Syndrome Coronavirus (MERS-CoV) in the United States.

The first Middle East Respiratory Syndrome Coronavirus (MERS-CoV) case in the United States was confirmed on Friday, May 2, 2014 in a traveler from Saudi Arabia to the U.S. The traveler has since recovered and has been released from the hospital. Public health officials have contacted healthcare workers, family members and travelers who had close contact with this patient. So far, none of these contacts have exhibited any symptoms of infection with MERS-CoV.

On May 11, 2014, the second imported case of MERS-CoV was confirmed in a traveler who came to the U.S. from Saudi Arabia. This patient is hospitalized and doing well. People who had close contact with this second patient are currently being contacted. These two U.S. cases are not linked.

So far, including this latest U.S. importation, there have been 698 confirmed cases of MERS-CoV infection with 285 deaths, 54 active cases and 359 recovered individuals in 14 countries. To date, all reported cases have originated in six countries in the Arabian Peninsula. Most of these people developed severe acute respiratory illness, with fever, cough and shortness of breath. There is no available vaccine or specific treatment recommended for the virus.

MDCH Bureau of Laboratories is an approved state public health laboratory for MERS-CoV testing using the CDC rRT-PCR assay. Contact your local health department or MDCH at 517-335-8165 to seek approval to coordinate testing.

**Authorization for testing can be obtained by contacting MDCH Communicable Disease Division at: 517-335-8165 during normal business hours or 517-335-9030 after normal business hours.**

To date, little is known about pathogenic potential and transmission dynamics of MERS-CoV. To increase the likelihood of detecting an infection, CDC recommends collecting multiple specimens from different sites at different times after onset of symptoms, if possible. Lower respiratory tract specimens should be a priority for collection and testing by PCR. ►

### Points to consider when determining which specimen types to collect from a patient

#### under investigation for MERS include:

1. The number of days between specimen collection and onset of symptoms
2. Symptoms at the time of specimen collection

#### Additional points to consider:

1. Maintain proper infection control measures when collecting specimens
2. Use approved collection methods and equipment when collecting specimens
3. Handle, store and ship specimens by following appropriate protocols

Lower respiratory specimens are preferred, but collecting nasopharyngeal and oropharyngeal (NP/OP) specimens, as well as stool and serum, are strongly recommended depending upon the length of time between onset of symptoms and specimen collection. For example, if symptom onset for a PUI (Patient Under Investigation) with ongoing lower respiratory tract infection was 14 or more days ago, a single serum specimen for serologic testing in addition to a lower respiratory specimen and an NP/OP specimen are recommended.

Respiratory specimens should be collected as soon as possible after symptoms begin – ideally within 7 days and before antiviral medications are administered. However, if more than a week has passed since onset of symptoms and the patient is still symptomatic, respiratory samples should still be collected, especially lower respiratory specimens since respiratory viruses can still be detected by rRT-PCR. For short periods ( $\leq 72$  hours), most specimens should be held at 2-8oC rather than frozen. For delays potentially exceeding 72 hours, freeze specimens at -70oC as soon as possible after collection (with exceptions as noted below). Label each specimen container with the patient's ID number, specimen type and the date the sample was collected.

#### Respiratory Specimens

##### A. Lower respiratory tract

##### **Bronchoalveolar lavage, tracheal aspirate, pleural fluid**

Collect 2-3 mL into a sterile, leak-proof, screw-cap sputum collection cup or sterile dry container.

Refrigerate specimen at 2-8oC up to 72 hours; if exceeding 72 hours, freeze at -70oC and ship on dry ice.

##### **Sputum**

Have the patient rinse the mouth with water and then expectorate deep cough sputum directly into a sterile, leak-proof, screw-cap sputum collection cup or sterile dry container. Refrigerate specimen at 2-8oC up to 72 hours; if exceeding 72 hours, freeze at -70oC and ship on dry ice. (Continued on next page) ►

► Continued from previous page

## **B. Upper respiratory tract**

### **Nasopharyngeal AND oropharyngeal swabs (NP/OP swabs)**

Use only synthetic fiber swabs with plastic shafts. Do not use calcium alginate swabs or swabs with wooden shafts, as they may contain substances that inactivate some viruses and inhibit PCR testing.

Place swabs immediately into sterile tubes containing 2-3 ml of viral transport media. NP/OP specimens can be combined, placing both swabs in the same vial. Refrigerate specimen at 2-8oC up to 72 hours; if exceeding 72 hours, freeze at -70oC and ship on dry ice.

### **Nasopharyngeal wash/aspirate or nasal aspirates**

Collect 2-3 mL into a sterile, leak-proof, screw-cap sputum collection cup or sterile dry container. Refrigerate specimen at 2-8oC up to 72 hours; if exceeding 72 hours, freeze at -70oC and ship on dry ice.

### **Serum (for rRT-PCR testing)**

For rRT-PCR testing (i.e., detection of the virus and not antibodies), a single serum specimen collected optimally during the first week after symptom onset, preferably within 3-4 days, after symptom onset, may be also be beneficial.

Children and adults Collect 1 tube (5-10 mL) of whole blood in a serum separator tube. Allow the blood to clot, centrifuge briefly, and separate sera into sterile tube container. The minimum amount of serum required for testing is 200 µL. Refrigerate the specimen at 2-8oC and ship on ice- pack; freezing and shipment on dry ice is permissible.

Infants A minimum of 1 mL of whole blood is needed for testing of pediatric patients. If possible, collect 1 mL in an EDTA tube and in a serum separator tube. If only 1 mL can be obtained, use a serum separator tube.

### **EDTA blood (plasma)**

Collect 1 tube (10 mL) of heparinized (green-top) or EDTA (purple-top) blood. Refrigerate specimen at 2-8oC and ship on ice-pack; do not freeze.

### **Stool**

Collect 2-5 grams of stool specimen (formed or liquid) in sterile, leak-proof, screw-cap sputum collection cup or sterile dry container. Refrigerate specimen at 2-8oC up to 72 hours; if exceeding 72 hours, freeze at -70oC and ship on dry ice.

All specimens must be pre-packed to prevent breakage and spillage. Specimen containers should be sealed with Parafilm® and placed in ziplock bags. ►

► Place enough absorbent material to absorb the entire contents of the Secondary Container (containing Primary Container) and separate the Primary Containers (containing specimen) to prevent breakage. Send specimens with cold packs or other refrigerant blocks that are self-contained, not actual wet ice. This prevents leaking and the appearance of a spill. When large numbers of specimens are being shipped, they should be organized in a sequential manner in boxes with separate compartments for each specimen. Specimens should be stored and shipped at the temperatures indicated above. If samples are unable to be shipped within 72 hours of collection, they should be stored at -70°C and shipped on dry ice.



LabLink is published quarterly by the Michigan Department of Community Health, Bureau of Laboratories, to provide laboratory information to Michigan health professionals and public health community.

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