Antimicrobial Susceptibility Testing:
Why We Do What We Do in Microbiology

The primary role of the clinical microbiology laboratory is to provide information with which physicians can diagnose and treat infectious disease. The most important issues are whether an infectious agent is present and which antimicrobial agents will provide adequate therapy.

Mechanisms of bacterial resistance are complex and not completely understood. Likewise, antimicrobial susceptibility testing has become more challenging with the continued emergence of unique resistance mechanisms. The goal of the microbiology laboratory in antibiotic susceptibility testing is to provide standardized *in vitro* susceptibility tests that can be reproduced from day to day and from laboratory to laboratory. Without reproducibility there is no scientific basis for therapy. Standardized guidelines for susceptibility testing are published and updated annually by the Clinical Laboratory Standards Institute (CLSI). These guidelines provide susceptibility testing methods that have been validated as accurate, reproducible, clinically relevant and predictive of clinical efficacy based on pharmacokinetic and outcome data. Regulatory agencies, e.g. CLIA and CAP, expect microbiology laboratories to comply with CLSI guidelines for susceptibility testing.

Susceptibility testing of a presumed pathogen is indicated when its response to antimicrobial agents is not predictable. However, not all microbial pathogens and antimicrobial agents have been studied and validated by CLSI, and hence, the Microbiology laboratory does not routinely provide susceptibility results for these organisms. The most common reasons that susceptibility tests are not performed include:

1. Antimicrobial-organism combination does not require testing because all strains are known to be either susceptible or resistant (e.g. group A strep vs. penicillin).
2. Pathogen is so rarely recovered that too few clinical studies exist to establish testing standards (e.g. *Vibrio* species).
3. Antimicrobial-organism combination is found to give erroneous and misleading susceptibility results (e.g. Enterococcus vs. cephalosporins).
4. Organism does not grow well enough on standardized susceptibility media for testing to be performed.
5. Drug-organism combination does not have adequate clinical response data to define MIC breakpoints (e.g. *Corynebacteria* and *Bacillus* species).
6. Organisms recovered from culture represent normal human flora from the site of collection, or mixed flora from contamination of the collection site (e.g. skin flora from a wound swab or multiple gram-negative rods from a urine culture). Susceptibility testing is not indicated for normal flora or contaminating bacteria that are not responsible for the infection.

The majority of susceptibility testing performed by Saint Luke’s Microbiology laboratory is done by microbroth dilution. Serial dilutions of each antibiotic are inoculated with a standardized suspension of the bacteria being tested, then monitored for growth. The minimum inhibitory concentration (MIC) for a particular bacteria/antibiotic combination is defined as the lowest concentration of antimicrobial agent in micrograms per milliliter that prevents the *in vitro* growth of bacteria.

Saint Luke’s Microbiology susceptibility reports include MIC data along with an interpretation of S, I, or R. In addition to the actual MIC number, other information that is critical in choosing an appropriate antibiotic includes half-life and achievable concentration at the site of infection. The physician should keep in mind that the antibiotic with the lowest MIC is not always the most appropriate choice of therapy.
The most useful means for assessing the adequacy of antimicrobial treatment in many infections is the clinical response of the patient to treatment and, if needed, demonstration by repeated culture that the infecting organism either has been eliminated or still persists. Antibiotic susceptibility tests are intended to be a guide for the clinician, not a guarantee that an antimicrobial agent will be effective in treatment, as many other in vivo factors may alter a patient’s response to therapy. Consultation with an infectious disease physician is recommended for assistance with complex antimicrobial therapy.

**New Swine-Associated Flu**

As of August 17, 2012 the CDC noted that 224 cases of influenza H3N2v have been reported from seven states including Ohio, Indiana, Pennsylvania, West Virginia, Illinois, Wisconsin, and Michigan with >90% occurring in children. This virus contains genes from avian, swine, and human viruses including a portion of the 2009 H1N1 pandemic gene. Intense surveillance is underway, due to the potential for human-to-human transmission. So far, all infections with this strain have been acquired by direct or indirect contact with pigs, primarily at agricultural fairs. No cases of human-to-human infection have been documented to date.

The symptoms and severity of illness following infection with H3N2v are similar to seasonal influenza and include fever, cough, sore throat, headache and myalgia. CDC has advised that persons at increased risk for complications from influenza (children, elderly, chronic medical conditions, or immunocompromised) should avoid pig barns and swine exhibits for the remainder of the season. Prompt antiviral therapy with oseltamivir or zanamivir should be considered for patients with suspected or confirmed H3N2v infection.

Conventional testing, including most rapid influenza tests and PCR, has been shown to detect H3N2v strains. Saint Luke’s Regional Laboratories utilizes the Xpect test for rapid influenza, which detected all H3N2v isolates in a recent analysis by CDC (MMWR early release (61), 8/10/12). In general, a negative rapid influenza test should not be considered conclusive for ruling out influenza infection. All patients with suspected H3N2v infection should be reported to the local or state public health agency regardless of initial test results.

**HCV Testing is Booming**

Hepatitis C Virus (HCV) persists as a chronic infection in 75 to 85% of individuals. Approximately 20% of infected persons will progress to cirrhosis within 20 years and up to 5% will die from HCV-related liver disease. Today, HCV infection is the leading indication for liver transplantation.

In 1998, CDC recommended HCV testing for individuals at high risk for HCV transmission, including those who had injected drugs, been hemodialysed, transfused or transplanted before July 1992, or received clotting factor concentrates produced before 1987. Screening also was recommended for persons with occupational sharps exposures, children born to HCV-infected mothers and individuals with persistently elevated ALT levels and individuals infected with HIV.

Unfortunately, this risk-based testing strategy has had limited success, as evidenced by the substantial number of HCV-infected persons who remain unaware of their infection. Of the estimated 2.7–3.9 million persons infected with HCV in the United States, 45%–85% are unaware of their status.

A recent analysis of NHANES data determined that the prevalence of HCV antibody among persons in the 1945–1965 birth cohort was 3.25%, compared to 1.0 – 1.5% in the general population. People within this age cohort account for approximately three fourths of all chronic HCV infections.

CDC recently published new birth-year based recommendations that target the baby boomer generation (MMWR August 17, 2012 / 61:1-18). These birth-year-based recommendations are intended to augment, not replace, the 1998 HCV testing guidelines. In addition to testing adults of all ages at risk, CDC now recommends that all adults born during 1945–1965 should be tested one-time with an HCV antibody test (anti-HCV).

An immunocompetent person without risk factors who tests anti-HCV negative is not HCV-infected and does not require additional testing. Repeat testing should be considered for persons with ongoing risk behaviors. A person whose anti-HCV test is reactive should be tested for HCV RNA to distinguish active from cleared infection.