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- Turning the Tables on the Rollover-Accident Bug
- Blood-Injection-Injury Phobia: Preventative Intervention for Syncope
- Antibody Titration
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Case Description: A healthy 16 year-old male was involved in an all-terrain vehicle (ATV) rollover accident. He was transported to a local hospital with an open fracture of the left distal humerus and an injury of the left brachial artery and upper arm median nerve. An open reduction internal fixation of the humerus was performed with irrigation and debridement, followed by a thrombectomy of the left brachial artery.

Three months later the patient was referred to Cincinnati Children's Hospital Medical Center with suspected brachial plexus palsy on the left side. There was limited improvement of his left upper extremity peripheral nerve injuries sustained in the accident, as well as a recent history of infections of the wound sites. An additional left humerus open reduction internal fixation and a right iliac crest bone graft were performed. Bone pieces and anaerobic and aerobic swab specimens were obtained from the surgical site and submitted to the microbiology laboratory for cultures.

Laboratory Specimen Processing and Analysis
In the laboratory, the bone fragments were placed in approximately 2 mL of sterile saline and were ground using a Precision™ tissue grinder. The ground bone and aerobic swab specimens were each inoculated onto trypticase soy agar with 5% sheep blood, chocolate, and MacConkey agars and enriched thiglycollate medium with vitamin K and hemin. Aerobic solid media was incubated in a 5% CO₂ atmosphere at 35°C, and the thioglycollate medium was incubated at 35°C in ambient air. Bone tissue and anaerobic swabs were also inoculated onto a Brucella Blood Agar, Brucella Laked Blood Agar with Kanamycin and Vancomycin (LKV), and Brucella Blood Agar with Phenylethyl Alcohol (PEA). Anaerobic media was incubated in Whitley A35 Anaerobic Workstation by Don Whitley Scientific Limited. The atmosphere inside the chamber was kept at 5% hydrogen, 5% carbon dioxide, and 90% nitrogen. Gram-stained smears from both specimens showed no organisms and small quantities of white blood cells.
No anaerobic bacteria were isolated. Growth of a single colony morphology was noted on the aerobic media after 24 hours of incubation. Medium sized, smooth, and circular colonies with entire borders were observed. Lactose fermenting, Gram-negative bacilli were observed on the MacConkey agar (Figure 1a). Gram-stained smears from the growth on the blood agar plate showed small to medium length Gram-negative bacilli.

Given the clinical situation and the laboratory findings, what are the three organisms on your list of “most likely” causes?

*See page 8 for case followup and discussion.*
Some important facts about chronic kidney disease (CKD):

- 26 million American adults have CKD and millions of others are at increased risk.
- Early detection can help prevent the progression of kidney disease to kidney failure.
- Heart disease is the major cause of death for all people with CKD.
- Glomerular filtration rate (GFR) is the best estimate of kidney function.
- Hypertension causes CKD and CKD causes hypertension.
- Persistent proteinuria (protein in the urine) means CKD is present.
- High risk groups include those with diabetes, hypertension and family history of kidney failure.
- African Americans, Hispanics, Pacific Islanders, American Indians and senior citizens are at increased risk for CKD.
- Three simple tests can detect CKD: blood pressure, urine albumin and serum cystatin C or serum creatinine.

Chronic kidney disease (CKD) includes several conditions that damage the kidneys and decrease their ability to carry out their functions. CKD may be caused by diabetes, high blood pressure and other disorders. Accompanying CKD are a number of complications including high blood pressure, anemia, weak bones, poor nutritional health and nerve damage. Also, kidney disease increases the risk of having heart and blood vessel disease. These conditions may slowly worsen over a long period. Early detection and treatment may reduce the effects of CKD. When kidney disease progresses, it may eventually lead to kidney failure. The kidney failure requires dialysis or a kidney transplant to maintain life.

The two major causes of chronic kidney disease are diabetes and high blood pressure, which are responsible for up to nearly 70% of the cases. And vice versa: chronic kidney disease can cause high blood pressure.

Other conditions that affect the kidneys are: Glomerulonephritis, a group of diseases that cause inflammation and damage to the kidney’s filtering units. These disorders are the third most common type of kidney disease.

The laboratory can play a pivotal role in both the detection and monitoring of CKD. There are several serum biomarkers that are commonly used as well as several that are under study to determine their role as additions or replacement for the ‘traditional’ markers.

Following is a discussion of creatinine and creatinine clearance, micro-albumin, cystatin C, kidney injury molecule-1 (KIM-1), NAG (N-acetyl-β-D-glucosaminidase), and NGAL (neutrophil gelatinase associated lipocalin). Also included are estimates of glomerular filtration rate (eGFR) using various formulas with either creatinine or cystatin C.

Jeon evaluated the clinical usefulness of cystatin C levels of serum and urine in predicting renal impairment in normoalbuminuric patients with Type 2 diabetes and evaluated the association between albuminuria and serum/urine cystatin C. Creatinine, urinary albumin levels, serum/urine cystatin C and estimated glomerular filtration rate (eGFR by MDRD [Modification of Diet in Renal Disease] and CKD-EPI [Chronic Kidney Disease Epidemiology Collaboration] equations) were determined. The cystatin C levels of serum and urine increased with increasing degree of albuminuria, reaching higher levels in macro-albuminuric patients (P < 0.001).
cally, serum cystatin C was affected by C-reactive protein (CRP), sex, albumin-creatinine ratio (ACR) and eGFR. Urine cystatin C was affected by triglyceride, age, eGFR and ACR. Cystatin C levels of serum and urine were identified as independent factors associated with eGFR < 60 mL/min/1.73m2 estimated by MDRD equation in patients with normoalbuminuria. On the other hand, eGFR < 60 mL/min/1.73m2 estimated by CKD-EPI equation was independently associated with low level of high-density lipoprotein in normoalbuminuric patients. The cystatin C levels of serum and urine could be useful markers for renal dysfunction in type 2 diabetic patients with normoalbuminuria.

Inker10 pointed out that “There does not appear to be a consistent message among published studies as to whether cystatin C-based equations are better than creatinine-based equations in the general population or those with chronic kidney disease (CKD).” And further that “cystatin C could be used in combination with creatinine as a confirmatory test for estimated GFR from creatinine.”

In his analysis, Inker showed that the independent predictors of cardiovascular events in patients with ‘normal’ kidney function were age, hypertension and serum level of cystatin C (higher than 0.88mg/L). Thus, a higher level of serum cystatin C might be another independent risk factor for cardiovascular events, even in those with relatively normal renal function.

Argraw presented data illustrating the combination of CKD, as defined by cystatin C estimated glomerular filtration rate ≤60 mL/min/1.73m2, and metabolic syndrome is a strong predictor of clinical cardiovascular events due to the interaction between CKD and metabolic syndrome.

Urinary interleukin-6, CXCL10/IP-10, NAG, and KIM-1 levels were very low in healthy individuals, increased in type 1 patients with normoalbuminuria, and were highest in diabetic patients that had micro-albuminuria. Low baseline concentrations of urinary KIM-1 and NAG both individually and collectively were significantly associated with the regression of micro-albuminuria over the subsequent 2 years, an effect independent of clinical characteristics. Progression and regression of micro-albuminuria were unrelated to urinary levels of interleukins 6 and 8, CXCL10/IP-10, and monocyte chemoattractant protein-1. The results of this study indicated that lower urinary KIM-1 and NAG levels were associated with the regression of micro-albuminuria in type 1 diabetes mellitus — tubular dysfunction is a critical component of the early course of diabetic nephropathy.

One study10 has shown that the combination of creatinine, cystatin C, and albuminuria improves detection and risk stratification for death, heart failure, cardiovascular events, and ESRD compared with creatinine alone.

The addition of eGFR and albuminuria to the Framingham equation has not been shown to substantially improve overall CVD risk prediction in populations not enriched with CKD.

Conclusions

There exist several useful markers for chronic kidney damage. There does not seem to be a consensus on whether serum creatinine or serum cystatin C is a better marker. None of the many equations for estimating GFR is accurate across the range of GFRs, especially at the lower end.

There are several other markers that are being investigated that may find a place in the lab’s continuing effort to supply more sensitive and specific biomarkers.

References

Questions for STEP Participants

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1. High blood pressure can result in CKD.
   A. True
   B. False

2. At least 20 million Americans have some form of kidney disease.
   A. True
   B. False

3. Which of these has been used as a biomarker for kidney disease?
   A. Protein in the urine
   B. Albuminuria
   C. Cystatin c
   D. All of these

4. CKD can lead to heart disease.
   A. True
   B. False

5. Of all the equations to estimate GFR, the MDRD is the best.
   A. True
   B. False

6. Early detection of CKD can improve the outcomes.
   A. True
   B. False

7. Addition of eGFR to the Framingham equation improves risk assessment.
   A. True
   B. False

8. Genetics plays a role in the development of CKD.
   A. True
   B. False

9. Age has not been shown to affect the incidence of CKD.
   A. True
   B. False

10. The laboratory can play a significant role in the detection and monitoring of CKD.
    A. True
    B. False
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Case Twenty Seven: Turning the Tables on the Rollover-Accident Bug

Scott J. Bielewicz and Joel Mortensen

**CASES IN CLINICAL MICROBIOLOGY**

**Editor’s Note:** Before reading the Case Follow-up and Discussion below, study the Case Description on page 2 of this issue, and formulate your own answers to the questions posed.

**Case Follow-up**

The Biomerieux MALDI-TOF MS plus (Research Use Only database) (bioMérieux, Laurent, Kansas) was used to establish a preliminary identification of the isolate from the bone sample. The top 4 spectra showed a likely match for the identification of *Enterobacter cancerogenes*. Identification using the Vitek 2 system confirmed the presumptive identification from the MALDI system. The bacterial isolate obtained from the swab of the wound site showed an identical identification. The susceptibility profile generated using Vitek 2 is shown in Table 1.

**Introduction**

According to the Consumer Product Safety Commission, there have been more than 11,000 ATV-related deaths from 1982 to 2010. One-quarter of the fatalities were under the age of 16. Of those deaths, 43% were under the age of 12. Additionally, emergency departments have received more than 28,000 visits due to ATV-related injuries in children. A study by Rebecca L Brown, M.D., associate director of Trauma Services at Cincinnati Children’s Hospital Medical Center, showed that almost half of all ATV injuries at the Trauma Center required surgical intervention, and 23% required care in the intensive care unit.

Osteomyelitis is usually suspected in a chronic wound that does not heal with standard treatments. *Staphylococcus aureus* is the principle pathogen in most osteomyelitis cases all age groups. *Enterobacter* species have a smaller role in most osteomyelitis cases, but it is not as age-dependent as other organisms (such as *streptococci*). Commonality of organisms indicated in osteomyelitis cases by age is shown in Table 2. In the age group ranging from 4 to 18 years-old, 80% of the osteomyelitis cases have been attributed to *Staphylococcus aureus*. Of the *Enterobacter* species, the most common implicated in human infection are *E. aerogenes* and *E. cloacae*.

**Table 1. Sensitivities on patient isolate obtained by Vitek 2.**

<table>
<thead>
<tr>
<th>Antimicrobial</th>
<th>MIC</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin</td>
<td>&gt;=32</td>
<td>R</td>
</tr>
<tr>
<td>Ampicillin/Sulbactam</td>
<td>&gt;=32</td>
<td>R</td>
</tr>
<tr>
<td>Cefazolin</td>
<td>&gt;=64</td>
<td>R</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>&lt;=1</td>
<td>S</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>&lt;=1</td>
<td>S</td>
</tr>
<tr>
<td>Cefoxitin</td>
<td>&gt;=64</td>
<td>R</td>
</tr>
<tr>
<td>Cefepime</td>
<td>&lt;=1</td>
<td>S</td>
</tr>
<tr>
<td>Gentamycin</td>
<td>&lt;=1</td>
<td>S</td>
</tr>
<tr>
<td>Tobramycin</td>
<td>&lt;=1</td>
<td>S</td>
</tr>
<tr>
<td>Amikacin</td>
<td>&lt;=2</td>
<td>S</td>
</tr>
<tr>
<td>Trimethoprim/sulfamethoxazole</td>
<td>&lt;=1</td>
<td>S</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>&lt;=0.25</td>
<td>S</td>
</tr>
<tr>
<td>Levofloxacin</td>
<td>&lt;=0.12</td>
<td>S</td>
</tr>
<tr>
<td>Imipenem</td>
<td>&lt;=1</td>
<td>S</td>
</tr>
<tr>
<td>Ertapenem</td>
<td>&lt;=0.5</td>
<td>S</td>
</tr>
<tr>
<td>Meropenem</td>
<td>&lt;=0.25</td>
<td>S</td>
</tr>
<tr>
<td>Nitrofurantoin</td>
<td>&lt;=16</td>
<td>S</td>
</tr>
</tbody>
</table>

Scott Bielewicz is a Medical Lab Scientist at Cincinnati Children’s Hospital Medical Center in the Diagnostic Infectious Disease Testing Laboratory.

Joel Mortensen Ph.D., is the Director of the Diagnostic Infectious Diseases Testing Laboratory.
Enterobacter cancerogenus is a rare opportunistic infection. However, this organism may be underreported as there are limitations of some identification schemes and not all Enterobacter spp. are fully speciated. There is an estimated 17 cases of E. cancerogenus infection reported. They generally involve a crush injury or laceration followed by environmental contamination of the wounds. Five of those cases have been isolated in the patient’s blood cultures.

The crush injuries often seen in ATV accidents can lead to opportunistic infection of the wound sites. Additionally, these opportunistic infections tend to be more complex in children due to an incomplete development of the immune system. Both human and animal studies show that traumatic injury is correlated with a decrease in immune functions that are dependent on T-cell response. For example, there is evidence that there is a significant decrease in T-1 helper function and cytokine production following traumatic injury. Injury leads to changes that influence how innate and adaptive immune systems interact, which is one of a multitude of factors that raise the risk of opportunistic infections.

**Taxonomy**

In 1966, an organism was isolated and presumptively indicated in a canker disease in poplar trees in Czechoslovakia. It was first described by Uroševi? and designated as Erwinia cancerogena referring to the “cancer inducing” effect on the poplars. Positive reactions for arginine and ornithine decarboxylase suggested that Erwinia cancerogena may have been a species of Enterobacter. Robert S. Dickey and Cathy H. Zumoff analyzed additional phenotypic characteristics in a study from 1988. The isolates showed phenotypic characteristics that supported the suggestion that the organism belonged in the family Enterobacteriaceae. The genera Citrobacter, Hafnia, Serratia, and Klebsiella were ruled out due to several characteristics of the organism, including motility, lack of capsulation, gelatin hydrolysis, and production of lipase, urease, and deoxyribonuclease. Based on three strains, the closest relationship for genus assignment was Enterobacter. Enterobacter cancerogenus was proposed by Dickey and Zumoff, with the description of Gram-negative, straight bacilli that are a facultative anaerobe and are motile with peritrichous flagella.

At around the same time, a new opportunistic human pathogen was described from blood in five cases of septicemia and one bacterial meningitis case. A summary of the proposed Enterobacter taylorae was made from the previously described CDC Enteric Group 19. From there the case history of Enterobacter taylorae became more involved, including four cases of severe nosocomial infections acquired within 6 months. In 1994, E. taylorae was found biochemically identical to E. cancerogenus. Thus, the taxonomic shift was made to use only the name E. cancerogenus when referring to the species. There is further speculation that E. cancerogenus strain MSA2 is a plant growth promoting bacterium that may not fit into what is now defined as E. cancerogenus.

Thus, the taxonomic shift was made to use only the name E. cancerogenus when referring to the species. There is further speculation that E. cancerogenus strain MSA2 is a plant growth promoting bacterium that may not fit into what is now defined as E. cancerogenus.

**Clinical Presentation**

In a study by the National Healthcare Safety Network at the Centers for Disease Control and Prevention, it was found that Enterobacter were the fourth most common cause of Gram-stained negative bacteria in hospital acquired infections in the United States. This accounted for 5% of all hospital acquired infections. In pediatric hospitals, Enterobacter species accounts for 9.8% of pneumonia, 9.5% of urinary tract infections, and 6.8% of bloodstream infections in data collected in between 1992 and 2004. The spe-

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<table>
<thead>
<tr>
<th>Age of Patient</th>
<th>Most Common Bacteria Responsible for Osteomyelitis</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;4 Months</td>
<td>Staphylococcus aureus, Enterobacter spp., Streptococcus agalactiae, Streptococcus pyogenes</td>
</tr>
<tr>
<td>4 Months to 4 Years</td>
<td>Staphylococcus aureus, Streptococcus pyogenes, Haemophilus influenzae and Enterobacter species</td>
</tr>
<tr>
<td>4 to 18 Years</td>
<td>Staphylococcus aureus, less commonly Streptococcus pyogenes, Haemophilus influenzae, and Enterobacter spp.</td>
</tr>
<tr>
<td>18 Years +</td>
<td>Staphylococcus aureus, sometimes Enterobacter spp., Streptococcus spp.</td>
</tr>
</tbody>
</table>

**Table 2. Commonality of organisms indicated in osteomyelitis cases by age. Adapted from Burnett**.
cies *E. cloacae* and *E. aerogenes* are the principal human pathogens in this genus. Most of the other species in the genus are mainly isolated from environmental sources and are generally phytopathogenic. The other *Enterobacter* species have routinely been implicated as opportunistic pathogens.

There are case reports that note *E. cancerogenus* as the primary pathogen implicated in an infection, both nosocomial and from environmental sources. Garazzino et al reported on one environmental source in a case of osteomyelitis caused by *E. cancerogenus*. An otherwise healthy 56 year-old male was involved in a severe motorcycle crash, resulting in an open fracture to his right leg. While being treated with amoxicillin-clavulanic acid and metronidazole, the patient showed signs of infection four weeks after the injury. A culture of the purulent wound showed *E. cancerogenus* with a resistance to aminopenicillins and cefazolin. Due to the susceptibility report, the patient’s antimicrobial therapy was switched to include both levofloxacin and ceftriaxone.

Injuries that result in environmental contamination of the wound site account for the majority of the cases reported, but there are cases that describe opportunistic infections of normal microbiota. An example is a case of *Enterobacter cancerogenus* bacteremia that was documented in a 56-year-old with sclerosing cholangitis. While awaiting liver transplantation, he was admitted to the hospital with nausea and hematemesis. Blood cultures were collected on the patient. After a 16 hour incubation they grew *Enterobacter cancerogenus* and viridans group *Streptococcus*. The patient was initially treated with piperacillin-tazobactam, but was switched to ceftiraxone after the organism tested resistant to first-generation cephalosporins, amoxicillin-clavulanic acid, and ampicillin. The patient was observed to have many dental carries and overall poor dentition. Since oral flora had been isolated on three previous occasions within two months, the source of the infection was thought to be oral. Previous studies demonstrate that *E. cancerogenus* is found in dental roots, but this was the first case documented where endogenous mouth flora was implicated in the cause of infection.

**Laboratory identification**

*Enterobacteriaceae* grow well on routine agar, including typticase soy agar with 5% sheep

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| Table 3. Differentiating biochemical properties for *Enterobacter* species. |
|------------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
|                  | Voges-Proskauer | Urease | Acid from arabinose | Acid from allantoin | Arginine dihydrolase | Acid from melibiose | Acid from adonitol | Acid from lactose | Citrate | Methyl-Red |
| *E. aerogenes*    | +   | V    | +    | +    | -    | +    | +    | +    | +    | -    |
| *E. asburiae*     | +   | V    | +    | +    | -    | -    | V    | +    | +    | +    |
| *E. cancerogenus* | +   | +    | -    | -    | +    | -    | +    | -    | -    | +    |
| *E. cloacae*      | +   | V    | +    | +    | +    | +    | V    | +    | +    | -    |
| *E. cowanii*      | +   | +    | +    | +    | -    | +    | +    | +    | -    | -    |
| *E. gergoviae*    | +   | +    | +    | -    | +    | -    | V    | +    | +    | V    |
| *E. hormaechei*   | +   | V    | +    | -    | -    | -    | +    | V    | V    | V    |
| *E. kobei*        | +   | -    | V    | +    | +    | +    | +    | +    | +    | -    |

*Information adapted from Grimont*
blood, chocolate agar, and MacConkey agar.\textsuperscript{13} Most genera in the \textit{Enterobacteriaceae} family demonstrate similar growth. Colonies will appear large and grey, usually within 24 hours.\textsuperscript{13} \textit{Enterobacter} species will be slightly mucoid due to a polysaccharide capsule and will be pink on MacConkey agar (MAC), yellow on Hektoen enteric agar (HE), and yellow on Xylose-lignosine-deoxycholate agar (XLD).\textsuperscript{13} The biochemical profile listed on the bioMérieux API 20E is 3305113 (shown in Figure 2). A search for the biochemical profile resulted in a 99.9\% confidence in an identification of \textit{Enterobacter cancerogenus}.

**Antimicrobial Agents and Therapy**

Initial antibiotic therapy for most enteric Gram negative bacilli would often include either a fluoroquinolone or a third-generation cephalosporin.\textsuperscript{19} Administration of treatment for osteomyelitis is often difficult to ascertain. The observed improvement in a patients condition could be due to the process of debridement, not from the antimicrobials themselves.\textsuperscript{20} Sustained remission must also be demonstrated.\textsuperscript{30} The factors surrounding the cases also tend to be variable and complex, especially in pediatric institutions. While debridement is one of the main avenues of treatment, antibiotics serve as a necessary compliment. Quick identification and susceptibility profiles play an important role in the outcome of the patient, since start time to appropriate antibiotics is imperative.\textsuperscript{21} It has been recommended that treatment should be administered for at least four weeks, and ideally closer to six weeks.\textsuperscript{20} In a recent review, Travakoli recommends that surgery is necessary in children with osteomyelitis who show no improvement within two days of beginning treatment, or when there is pus when bone aspiration takes place.\textsuperscript{22}

\textit{E. cancerogenus} has a natural resistance to amoxicillin, amoxicillin/clavulanic acid, cefazolin, cefaclor, cefazoline, loracarbef, and ce-

### Table 4. Reported antimicrobial sensitivities of \textit{E. cancerogenus} compared with patient isolate information obtained with the Vitek 2 system.

<table>
<thead>
<tr>
<th>Class</th>
<th>Drug</th>
<th>Literature</th>
<th>MIC Interpretation</th>
<th>Patient Isolate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aminoglycoside</td>
<td>Amikacin</td>
<td>S</td>
<td>&lt;=2</td>
<td>S</td>
</tr>
<tr>
<td></td>
<td>Gentamicin</td>
<td>S</td>
<td>&lt;=1</td>
<td>S</td>
</tr>
<tr>
<td></td>
<td>Tobramycin</td>
<td>S</td>
<td>&lt;=1</td>
<td>S</td>
</tr>
<tr>
<td>Antifolate</td>
<td>Sulfonamide</td>
<td>S</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Trimethoprim</td>
<td>S</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Penicillins</td>
<td>Ampicillin/Sulbactam</td>
<td>S,I</td>
<td>&gt;=32</td>
<td>R</td>
</tr>
<tr>
<td></td>
<td>Penicillin</td>
<td>R</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Penacillin</td>
<td>R</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cephalosporins</td>
<td>1st Generation</td>
<td>Cefazolin</td>
<td>R</td>
<td>&gt;=64</td>
</tr>
<tr>
<td></td>
<td>Cephalothin</td>
<td>R</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2nd Generation</td>
<td>Cefotaxin</td>
<td>R</td>
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<td></td>
<td>Cefoxitin</td>
<td>R</td>
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<td></td>
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<td>Ceftazidime</td>
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<td>&lt;=1</td>
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<td>Ceftriaxone</td>
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</tr>
<tr>
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Data adapted from Stock, I. et al. (2002), Grimont et al. (2006)\textsuperscript{18, 23}
It should be noted that in agar diffusion MIC antibiotic studies on five Enterobacter species, (E. cancerogenus, E. amogenus, E. gergoviae, and E. sakazakii), was the only one that demonstrated this resistance pattern. This pattern of resistance indicates the presence of chromosomal AmpC β-lactamases. This resistance is present in the Enterobacter genus, and includes E. cloacae and E. aerogenes.

The drugs of choice for Enterobacteriaceae have long been carbapenems, but increasing infections from Extended-Spectrum Beta-lactamase producing organisms have suggested the use of β-lactam/β-lactam inhibitor combinations. However, in a review by Vardakas et al., these β-lactam/β-lactam inhibitor combinations have resulted in a higher mortality than carbapenems. The current recommendations by the American Academy of Pediatrics for a gram-negative aerobic organism with chromosomally mediated ampC beta-lactamase suggest the use of meropenem in most infections. Meropenem is not suggested for carbapenemase-producing strains.

**Environmental Reservoir**

Enterobacter species are present in environmental samples from all types of ecosystems. Enterobacter cancerogenus has been indicated as a cause of disease in plants other than in poplar trees, including mulberry wilt disease in China. Isolates of Enterobacter species have been reported in environmental samples from studies all over the globe. Given this, the organism’s presence in environmentally contaminated wound sites can be expected. Enterobacter cancerogenus is not solely a cause of disease in plants. As previously mentioned, the E. cancerogenus strain MSA2 has been shown to be a plant growth promoting bacterium. It is not yet been determined if this bacterium will remain being defined as Enterobacter cancerogenus.

**Conclusion**

In the case of the 16-year-old male in the ATV-rollover accident, Enterobacter cancerogenus was likely obtained from the environment. Although rare, the presence and role of Enterobacter cancerogenus in opportunistic infections should be considered when an Enterobacter species is isolated. This is especially true with the potential of resistance to aminopenicillins and cephalosporins.

There is evidence of an increasing number of opportunistic infections involving Enterobacter species, including a novel Enterobacter of clinical relevance described by Harald Hoffmann, et al. Enterobacter ludwigii has been the subject of at least five case reports since 2005. Further analysis of the E. cloacae complex could reveal more taxonomically, phenotypically, and genetically distinct species within the genus Enterobacter.

The E. cancerogenus strain MSA2 described in this article is an example how this species could still soon be split into two, or possibly more, species. The evolving resistance to antibiotics in the family has also been of concern, especially in the case of Carbapenem-Resistant Enterobacteriaceae (CRE). There has been a dramatic increase in CRE in the United States, and it correlates with an overall trend toward pan-resistant organisms with genetic components which may be highly mobile between microbial species.

The 16-year-old patient discussed in this study showed no more signs of infection following the course of treatment. Extensive nerve damage remains, presumably unrelated to the acquired infection. No further infections of the wound site have occurred in the six months following the positive cultures.

**References**


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April 1 is the filing deadline for applications and supporting documents in the AMTIE 2014 undergraduate/graduate scholarship program and for grants to high school graduates pursuing medical technology, medical assisting, dental assisting, or phlebotomy studies.

Up to three $1,500 AMT Member Scholarships may be awarded annually. Applicants must be members in good standing with AMT and enrolled in a college or university accredited by a regional accrediting commission. The course of study should be concerned with the disciplines certified by AMT. Scholarship recipients will be selected by the AMT Institute of Excellence and scholarship committee based on financial need, career goals, and previous academic record.

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All scholarships will be awarded during the 2014 AMT National Convention in Chicago, IL, July 6–11.

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1. In the age group ranging from 4 to 18 years-old, 80% of the osteomyelitis cases have been attributed to what bacteria?
   A. Escherichia coli  
   B. Staphylococcus aureus  
   C. Enterobacter cancerogenus  
   D. Group B Streptococci

2. Traumatic injury is correlated with a decrease in immune functions that are dependent on which of the following?
   A. Complement cascade  
   B. T-cell Response  
   C. Antibodies  
   D. Protein

3. Enterobacter cancerogenus was described under what name?
   A. Haemophilus influenzae  
   B. Bielewilia mortensenii  
   C. Enterobacter taylorae  
   D. Erwinia cancerogena

4. Which order is Enterobacter cancerogenus in?
   A. Enterobacteriales  
   B. Enterobacter  
   C. Enterobacteriaceae  
   D. CDC Enteric Group 19

5. Between 1992 and 2004, around what percent of blood stream infections were caused by Enterobacter species in pediatric hospitals?
   A. 0.1%  
   B. 6.8%  
   C. 9.8%  
   D. 26.5%

6. What are the principal pathogens in the genus Enterobacter?
   A. Staphylococcus aureus  
   B. Enterobacter cloacae  
   C. Enterobacter aerogenes  
   D. Both B and C

7. Which biochemical properties would be representative of Enterobacter cancerogenus?
   A. ONPG +, URE +, SOR +  
   B. ONPG -, URE +, SOR -  
   C. ONPG +, URE -, SOR -  
   D. ONPG -, URE -, SOR -

8. In Enterobacter cancerogenus, resistance to amoxicillin, amoxicillin/clavulanic acid, cefazolin, cefaclor, cefazoline, loracarbef, and ceftoxitin may indicate the presence of
   A. AmpC β-lactamases  
   B. vanA  
   C. vanB  
   D. CRE

9. The current recommendations by the American Academy of Pediatrics for a Gram-negative aerobic organism with chromosomally mediated ampC beta-lactamase suggest the use of __________ in most infections.
   A. meropenem  
   B. amoxicillin  
   C. ampicillin  
   D. cefazolin

10. Enterobacter cancerogenus strain has been indicated in which of the following conditions?
    A. Mulberry wilt disease  
    B. Tumor growth in poplar trees  
    C. Promoting plant growth  
    D. All of the above
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Crossword Puzzle

by Gerard P. Boe, Ph.D.

ACROSS
3  organism that prefers to grow in total absence of atmospheric oxygen
5  slow onset of disease symptoms
6  organism that prefers to grow in atmospheric oxygen
8  an infection of the blood stream
9  sterile or free from microorganisms

DOWN
1  a reaction that takes place in the body
2  destruction of red cells
4  a secretion that accumulates in body tissues
5  an individual who harbors an organism but does not manifest clinical symptoms
7  a substance which has the ability to kill bacteria

Answers on page 33
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- Disease States
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- Colorectal Cancer Screening: Early Detection Can Lead to Prevention
- Medical Assisting: Are You Doing What You are Supposed to be Doing?
- Medical Errors/HIPAA (HITECH Act)
- Clinical Microbiology-Case Studies
- D-Dimer and its Application in Suspected VTE
- Critical Thinking and Problem Solving for Medical Assistants
- Followership: You Choose Your Path
- PSA, Free PSA, Complex PSA and Prostate Cancer
- Vitamin D -- Why all the Fuss?
- Medical Errors in Healthcare Settings
- Dealing with Difficult People
- Update on Aspirin and Plavix Sensitivity and Resistance Testing
- Leadership & Mentorship
- Lean Six Sigma Implementing 5 S in the Laboratory
- ECG and its Use for Diagnosing Cardiac Conditions
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- Improving Listening Skills
- Ethics in Health Care
- The Health Care Law and You
Introduction

Few, if any, medical laboratory personnel working for any length of measurable time can claim they have not encountered needle phobic patients. Whether the fear is exclaimed overtly or quietly concealed in anxious silence, these patients present a particularly difficult challenge for the lab technician. The DSM IV TR (DSM = Diagnostic and Statistical Manual of Mental Disorders) diagnosis of Blood-Injection-Injury Type Specific Phobia (BII) encompasses various fears of seeing blood, receiving injections, or injury in a medical setting (APA, 2000). The specific related phobias include trypanophobia, fear of medical procedures involving the use of needles; belonephobia, fear of sharp objects or instruments; and hemophobia, fear of the sight of blood. A prominent complication with these phobias is the vasovagal response leading to syncope, during phlebotomy procedures. Detrimental consequences of BII phobia are related to the patient resisting routine medical checkups and blood work, putting their general health at risk (APA, 2000). This article will explore the physiological and psychological mechanisms which trigger the vasovagal response, techniques for minimizing patient fear and syncope risk during blood draws, and behavioral treatments mitigating the anxiety and fear associated with BII phobia.

The Vasovagal Response

With an estimated prevalence of 3% to 4%, BII phobia is a common psychiatric disorder (Agras, Sylvester, & Oliveaux, 1969). In 75% of those presenting with BII phobia, a history of syncope when confronted with the feared situation is reported (APA, 2000). In contrast to other specific phobias such as fear of animals or heights, which elevate blood pressure and heart rate, BII phobia is associated with the vasovagal response which causes vasodilation, hypotension, and slowing of the heart (bradycardia) (Mednick & Claar, 2012). Basically, the vasovagal response is a homeostatic dysfunction of the autonomic nervous system. It is sometimes referred to as emotional fainting, and is typically characterized by a two-phase or di-phasic response (Graham, Kabler, & Lunsford, 1961). The initial phase involves an increase in blood pressure and heart rate, similar to other common phobic responses. However, this initial stage of flight and fright, typical of anxiety responses (Guyton & Hall, 2005) is short lived and quickly replaced by the second phase of hypotension and bradycardia (Graham, Kabler, & Lunsford, 1961).

During the vasovagal response, emotional reactions are translated via the hypothalamic mechanisms into autonomic impulses (Wallin, 1993). Whether the emotional response is triggered by fear of needles, disgust prompted by the sight of their own blood, entry of the needle, or the pain associated with the venipuncture, one fact is salient – the emotional unpleasantness of the blood draw is overwhelming to the BII phobic. In a study conducted by Deacon and Abramowitz (2006) of 3,315 patients undergoing phlebotomy procedures, they found 14.8% (n = 490) of the participants experienced anxiety prior to blood draws. The study also found that 4.3% (n = 141) of the participants had lost consciousness in the past while having blood drawn, and 2.2% (n = 72) of the participants qualified for a DSM diagnosis of BII phobia. When comparing those qualifying for a diagnosis of BII phobia with those experiencing no needle fear, the ef-
fect size differences related to fear of fainting ($d = 3.51$) and disgust ($d = 3.26$) were the most significant, while pain ($d = 2.13$) and health concerns ($d = 1.09$) contributed less to the psychological variance between the two groups. Deacon and Abramowitz also noted those with higher ratings on needle disgust tended to psychologically exacerbate pain and vasovagal reactions during needle injection.

Most people having a vasovagal response leading to syncope experience a combination of hypotension and reduced cardiac output. According to Rea and Thames (1993), the hypotension is often the result of a vasodepressor reaction, precipitated by the Bezold-Jarisch reflex. This reflex, triggered by numerous psychological stressors (Sledge, 1978), originates in the heart, where sensory receptors respond to mechanical and chemical stimuli by increasing the C-fiber vagal afferent signaling to the brain. This increases parasympathetic outflow, while inhibiting vasoconstriction efferent sympathetic outflow, resulting in vasodilation and hypotension (Rea & Thames, 1993).

Stimulation of the vagal nerve also causes parasympathetic efferent pathways to release acetylcholine, causing negative inotropic and chronotropic effects (Levy, 1971). The inhibitory action of the acetylcholine essentially causes a slowing of the contraction and conduction rates of the heart, resulting in bradycardia.

Data obtained in a study by Accurso et al. (2001) suggests that blood phobic persons may be predisposed to vasovagal syncope in other non-phobic situations. The study was conducted on 22 participants, with 11 meeting the diagnostic criteria of BII phobia, and 11 control participants with no prior diagnosis of BII phobia. All participants were subjected to a 45 minute tilt table test. During the procedure, 9 of the 11 BII phobic participants experienced syncope or pre-syncope, while only 1 participant in the control group experienced pre-syncope. The study suggests as a hypothesis that BII phobic individuals could be predisposed to syncope, and might develop their phobia not exclusively due to a fear of needles and blood, but rather as a consequence of fearing syncope (Marks, 1988).

**Preventing Syncope During Phlebotomy Procedures**

During the course of a phlebotomist or lab technician’s career, they may become desensitized to the expression of patient fear elicited by the procedures of blood sample collection. In fact, as the skill and confidence of the technician increases, the signs of patient fear may fade from awareness. However, it is imperative the technician not become too cavalier in their assessment of patient anxiety. This becomes a particularly salient point if the patient happens to be a new patient, child, adolescent, or young adult.

While making positive patient identification prior to the blood draw, the patient should be asked if they are fearful of the procedure, or if they have ever become lightheaded or faint during a past blood draw. If the patient indicates he or she has a history of fainting, the technician should insist the patient lie down for the procedure or sit in a reclining chair (McCall & Tankersley, 2008). Placing the patient in a supine position has three advantages. First, with the patient lying horizontally, it reduces the effect of gravity, and provides less resistance for blood flowing from the lower extremities to the brain. Secondly, if the patient was to become light-headed or lose consciousness during the procedure, the danger of them becoming injured due to a fall is abated. Finally, depending on the build and position of the patient, some technicians may find it easier keeping needles, blood, and the procedure out of patient view.

In a perfect world, patients would always identify themselves as someone with BII phobia, and every lab would have a bed, reclining chair, or table for such patients to lie on for blood draws. However, the world is not perfect, and the medical lab is no exception. Eventually, it is almost certain that every lab technician will discover a BII phobic patient in the chair during the course of a blood draw procedure. If the technician is faced with this scenario, it is crucial the patient is carefully observed during the procedure for the prodromal signs of syncope. These early signs include a general uncomfortable feeling, sensations of warmth, abdominal cramping, and mild nausea (Wieling et al., 2009).

If the technician finds himself/herself with a seated patient in the prodromal stage of syncope, there is a simple counter maneuver with empirical support which could avert patient fainting. Studies have indicated the applied tension technique, a term first derived by Kozak and Montgomery (1981), and later detailed by Ost and Sterner (1987), has been shown to raise blood pressure, reduce anxiety, and reduce syncope risk by having the patient tighten skeletal muscles during the blood draw (Holly, Balegh, & Ditto, 2011). One such study by Krediet et al. (2002) tested this procedure by recruiting 21 participants predisposed to vasovagal syncope, and subjecting them to a tilt...
The researchers first taught the applied tension technique to the participants by having them cross their legs, and then repeatedly tense the leg, abdominal, and buttock muscles for 10 to 15 seconds, followed by a 20 to 30 second release without relaxing. Once the tilting experiment began, and participants were in the prodromal stage of syncope, they were instructed to begin the maneuver. In 20 of the 21 participants, syncope was avoided while they performed the maneuver (some repeating it 4-5 times). After participants ceased the maneuver, 5 of the participants continued to avoid syncope in the tilted position, while 15 of them eventually fainted. However, after the maneuver was halted, the average time syncope was delayed for fainting participants was 2.5 minutes. During the maneuver, mean blood pressure was elevated in the group, with systolic increasing from (M = 65, SD = 13) to (M = 106, SD = 16, p < .001), and diastolic pressure increasing from (M = 43, SD = 9) to (M = 65, SD = 10, p < .001). Mean heart rate for the group also increased significantly during the maneuver, going from (M = 73, SD = 22) to (M = 82, SD = 15, p < .01) (Krediet et al., 2002).

By crossing the legs and contracting muscles in the legs, buttocks, and abdomen, the pooled venous blood in the lower extremities is given assistance moving back to the heart, blood pressure is increased, and circulation to the brain is improved. Although the Krediet et al. (2002) study was conducted using a tilt table, the study simulates the position of a patient leaning or reclining back in the drawing chair with the legs extended outward, and is a technique worth considering as a counter measure to vasovagal syncope. Furthermore, given the efficacy of tightening the skeletal muscles to avoid syncope, the empirical research indicates the technician should avoid telling patients to relax when pre-syncope symptoms are detected.

**Psychological Treatments for Vasovagal Syncope**

Human fear of blood and injection injury is not itself psychopathological unless it causes marked distress, and difficulty in the person’s everyday life. If the individual’s fear becomes so pronounced that it interferes with lifesaving procedures or treatments such as dialysis or insulin injection, treatment becomes mandatory (Marks, 1988). Treatment for BII phobia often involves typical behavioral exposure techniques used to treat anxiety disorders, which gradually expose the client to more intense fear stimuli (Barlow, 2002). The exposure therapy can be implemented as a stand-alone treatment, or it can be coupled with coping techniques such as applied tension (Ayala, Meuret, & Ritz, 2009).

To implement a behavioral exposure treatment, the clinician must first ascertain from the patient what provokes the most anxiety and fear in him or her. The clinician can then formulate a plan of prolonged exposure to these fear-inducing stimuli. The most effective habituation or decreased psychological response to the stimulus is accomplished with prolonged exposures to the feared stimulus for at least an hour. In addition, exposure therapy is most effective when in-vivo exposure is utilized, using either real or filmed representations rather than imagined themes. Therapists will often create homework assignments for patients, which they can do on their own, while keeping diaries of their progress (Marks, 1988).

The applied tension technique discussed earlier can also be performed during exposure at the first sign of blood pressure decrease. Educating patients on how to recognize the signs of blood pressure drop is essential when implementing this technique (Ayala, Meuret, & Ritz, 2009). The exposure, applied tension, and tension only technique (tension without exposure) were compared by Ost, Fellenius, and Sterner (1991) for short and long term effectiveness. They found the applied tension and tension only groups showed significantly more improvement at 90% and 80% respectively, while the exposure only group showed a 40% improvement after treatment. The authors indicated the tension aspect of the treatment may be the most important component. Other available studies reveal clinically significant improvements in 70% to 80% of patients in tension and exposure only therapies. Treatments emphasizing exposure tended to reduce injection and other related BII phobic fears, while the tension techniques emphasized coping skills, which gave the patient a sense of control over fainting, but did little to address the patient’s BII phobia specific fears (Ayala, Meuret, & Ritz, 2009).

**Summary**

BII is a common phobia, and one that can potentially threaten an individual’s health by causing avoidance of medical tests and procedures. Unlike other phobias, BII phobia is unique in that it causes blood pressure and heart rate to decrease by means of the vasovagal response, which can potentially
lead to syncope. If BII phobic patients are identified prior to blood draws, preventative measures can be taken such as having the patient lie down, which can lessen the chance of syncope. If prodromal symptoms of syncope occur while the patient is seated, the applied tension technique can help elevate blood pressure and heart rate sufficiently to avoid syncope in some cases. When BII phobia is interfering with the patient receiving vital medical care, treatment is mandatory. This treatment is generally linked to exposure therapy, where the patient is exposed to the feared stimulus for extended periods of time. Research has shown that exposure therapy coupled with the applied tension technique may be the most effective treatment plan for reducing patient fear, and mitigating syncope risk.

References
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1. The vasovagal response is sometimes referred to as
   A. emotional fainting
   B. reactive fainting
   C. passive fainting
   D. active fainting

2. BII is a common phobia with an estimated prevalence of 3% - 4%; according to the APA (2000); how many people reporting this disorder have a history of syncope when confronted with the fear stimulus?
   A. 50%
   B. 75%
   C. 85%
   D. 25%

3. When behavioral exposure techniques are used to overcome the fear of blood-injection-injury, the most effective techniques use
   A. prolonged exposure lasting at least 1 hour
   B. deception by the therapist
   C. in-vivo exposure to the real or filmed feared stimulus
   D. Both A and C

4. Placing the patient susceptible to syncope in a supine position has the advantage/s of
   A. preventing the patient from falling and becoming injured if they faint during the procedure.
   B. providing less resistance for blood flowing from the lower extremities to the brain.
   C. keeping needles, blood, and the procedure itself out of the patient's view.
   D. All of the above

5. If the lab technician encounters a situation where the seated patient begins to experience the prodromal signs of syncope during a blood draw, a counter maneuver which can avert fainting is called the
   A. applied tension technique.
   B. applied relaxation technique.
   C. prolonged exposure technique.
   D. distraction and deception technique.

6. While making positive patient identification prior to a blood draw, the patient should be asked if
   A. they like or dislike having their blood drawn.
   B. they have a high degree of fear concerning the procedure.
   C. they have ever become lightheaded during a blood draw.
   D. Both B and C

7. Deacon and Abramowitz (2006) found those patients with psychologically exacerbating pain and vasovagal reactions during needle injection tended to rate the highest on
   A. fear of the sight of blood.
   B. needle disgust.
   C. health concerns.
   D. fear of medical personnel.

8. According to the research studying the efficacy of the applied tension technique, if the lab technician observes the prodromal signs of syncope in their patient, the first thing they should tell the patient to do is relax.
   A. True
   B. False

9. The vasovagal response is typically characterized by two phases, consisting of
   A. hypotension followed by hypertension.
   B. hypotension followed by increased body temperature.
   C. hypertension followed by hypotension and bradycardia.
   D. hypertension followed by a return to normal blood pressure.

10. According to Marks (1988), psychological treatment for BII phobia becomes mandatory when the patient
    A. reveals his or her fear of needles and blood draw procedures.
    B. makes derogatory remarks about medical personnel during blood draws.
    C. appears apprehensive before blood draw procedures.
    D. avoids lifesaving treatment or procedures such as dialysis or insulin injections.
Dental Assistants
Promote Healthy Smiles

National Dental Assistants
Recognition Week
March 2-8, 2014
Introduction

The blood bank screens for the presence of antibodies on a daily basis. When an antibody is detected the next step is to identify the specificity of the antibody. In some cases it is important to semi-quantitate the concentration of the antibody (titer). Semi-quantitative assays are similar to qualitative assays in detecting the presence or absence of an antibody but then go on to provide a numeric representation of the amount of that antibody in the specimen that is relative to some other standard or value. Reference materials generally do not exist for an antibody reported as a semi-quantitative result, and response ranges may vary significantly between laboratories and or practitioners. Therefore, semi-quantitative numeric results from different performing laboratories and/or practitioners may not be comparable, though the qualitative interpretation of the results will be similar.

When reporting antibody titer results, it may also be important to determine the score or avidity of that antibody.

Titers

Titers are a variation of a semi-quantitative assay and report the relative amount of an antibody within the sample. The assay requires preparation of the specimen into serial dilutions to detect the point at which the antibody is no longer detected. Dilution standards may be set by the performing laboratory and can vary significantly between laboratories or other organizations.

Titers are usually reported as a ratio of the highest dilution of serum that still allows detection of the antibody, for example 1:320, or as the reciprocal of the dilution, which would be 320 in this example. The method involves visual observation by a trained technologist, and therefore will be subjective with some variation from laboratory to laboratory.

Titers results are usually obtained by testing a serial dilution (usually two-fold or doubling) of a serum against selected cells. In the blood bank, the result is expressed as the inverse of the highest serum dilution which causes macroscopically visible agglutination. In the example below (Table 1), a doubling dilution is used and tube 8 is the last tube to show visible agglutination. The dilution of serum in tube 8 is 1 part of serum in 256 total parts; thus, the titer is 256.

Avidity

According to Harmening, avidity is the term used to express bindings of a multivalent antigen with antiserum produced in an immunized individual. Avidity is a measure of the strength or potency of an antibody in acting against a specific antigen (1-4+).

Titers alone can be misleading; thus, determination of the avidity of an antibody is often useful in the clinical setting. Avidity is determined by scoring each individual tube reaction. Once the scores for each tube have been determined, the scores are added together to determine the avidity of the antibody. Score points are assigned according to Table 2 and an example of deter-

<table>
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<th>TUBE</th>
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<td>REACTION</td>
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mining the score is shown in Table 3. A difference of 10 or more is considered a significant difference in reactivity.

The HTLA (High Titer Low Avidity) alias SHIT (Serum Hemolysins of Inscrutable Type) antibodies are distinguished by high titers and low scores (Table 3).

**Prozone Phenomenon**

When performing antibody titrations, the typical expected reaction may not always be noted. When reading the tubes for agglutination, the first tubes containing the most serum (antibody) may appear to be weaker or even negative compared to later tubes that contain smaller amounts of serum (antibody). This is known as the prozone phenomenon. Table 4 below illustrates the prozone phenomenon. The prozone reaction occurs when the clinical sample contains antibody molecules in excess of the number of antigen binding sites available. As the serum is diluted, the number of antibody molecules decrease and ultimately bring the number into equivalence with the number of antigen binding sites available.

When the amount of antibody present in the serum is very small, false negative results may also occur due to postzone phenomenon. The optimum proportion of antigen and antibody is extremely important; however, antibody production varies significantly among individuals. Other factors can also affect these results and must be controlled as tightly as possible to avoid possible false negative test results. When antigen and antibody are in optimal proportions lattice formation results and agglutinates of RBC form to give a positive reaction.

**Titration Indications**

Antibody titration is clinically indicated in 1) prenatal studies, 2) identifying antibody specificity, 3) separating multiple antibodies, and when 4) performing ABO isohemagglutinin titers on apheresis donor platelet units or organs that are to be transplanted across ABO blood types.

**Prenatal Studies**

In prenatal studies where the mother’s serum exhibits the presence of a clinically significant antibody capable of causing hemolytic disease of the newborn (HDN), titration studies are necessary, especially if the father is known to possess the corresponding RBC antigen. Since this is generally an unknown or undocumented factor, antibody titrations should be performed and repeated at intervals throughout the pregnancy to indicate the likelihood of possible damage to the fetus. A rise in concentration or titer by two tubes (four-fold) is considered clinically significant and suggests that the fetal red cells contain the antigen to the corresponding maternal antibody. Once the titer reaches a determined critical level, additional studies such as ultrasound, Doppler sonography, amniocentesis, or cordocentesis may be performed to further indicate the likelihood of progressive anemia indicating the need for intrauter-

<p>| Table 2 |
| SCORING POINTS |</p>
<table>
<thead>
<tr>
<th>AVIDITY</th>
<th>SCORE</th>
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<tbody>
<tr>
<td>12</td>
<td>4+</td>
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<tr>
<td>10</td>
<td>3+</td>
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<td>8</td>
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</table>

| Table 3 |
| AVIDITY |
| TUBE | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| REACTION | 2+ | 2+ | 2+ | 2+ | 2+ | 2+ | 2+ | 2+ | 2+ | 0 |
| TITER | 2 | 4 | 8 | 16 | 32 | 64 | 128 | 256 | 512 | 1024 |
| AVIDITY | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 8 | 72 |

| Table 4 |
| PROZONE |
| TUBE | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| REACTION | — | — | — | — | 1+ | 2+ | 2+ | 3+ | 4+ | 4+ |
| TITER | 2 | 4 | 8 | 16 | 32 | 64 | 128 | 256 | 512 | 1024 |
ine transfusion, early delivery, or other medical interventions.

A titer that remains at a constant level or even decreases over the course of the pregnancy may indicate that there has been no further fetal-maternal hemorrhage or that the baby lacks the antigen that the mother has produced an antibody to.

**Antibody Specificity**

Some antibodies cause universal agglutination when undiluted serum is used, but comparison of titration results may indicate specificity. For example, a potent auto-Anti-I reacts strongly with both adult and cord cells, but the serum reacts at much higher dilutions against adult cells (II) than against cord cell (ii), thus indicating the specificity of the antibody.

Other antibodies often react weakly at the anti-human globulin (AHG) phase of testing, but when titrations are performed, these antibodies produce relatively high titers though they exhibit low avidity. These antibodies are known as high-titer, low-avidity antibodies and are directed against high frequency red blood cell antigen systems that include Chido, Rogers, York, Knopps, McCoy, John Milton Hagen, and others. While these antibodies are not clinically significant as a cause for transfusion reactions, their presence may mask underlying clinically significant alloantibodies that are capable of causing hemolytic transfusion reactions (HTRs). Since these antibodies are directed against high frequency red cell antigens, all donor products will be crossmatch incompatible and physicians will have to sign for incompatible blood products for transfusion.

**Separating Multiple Antibodies**

Sometimes the presence of multiple antibodies can be detected and identified by performing antibody titers. The specimen could then be diluted before testing with reagent RBCs. The dilution could thus remove one antibody and allow identification of the second underlying antibody with the higher titer.

**ABO Isohemagglutinin Testing**

Some, though not all, institutions perform titers of anti-A and anti-B on group O apheresis donor platelets before dispensing for transfusion to non-group O patients since these units may contain large amounts of plasma and high titers of anti-A and anti-B antibodies. Multiple reports exist in the literature of patients who have suffered hemolytic transfusion reactions due to passively transfused ABO antibodies. However, not all institutions have adopted this policy and continue to transfuse group O platelets to non-group O recipients without the benefit of antibody titration due to 1) a lack of a standardized method for performing isohemagglutinin titers, 2) reports of a widely varying range of titers implicated in such reactions, and 3) no formally adopted critical titer value above which these products should be transfused to non-group O patients.

In more recent years immunosuppression medications and apheresis techniques have allowed for successful ABO-incompatible liver and kidney transplants. Performance of ABO titers is critical for determining the effectiveness of pretreatment regimens and if titers are low enough to permit the transplant. However, again as stated above there is no reported uniform safe anti-A or Anti-B titer. Serial determinations of ABO isohemagglutinin levels in the ABO incompatible post-transplant patient are critical since an increase in titer can be an indicator of possible impending rejection.

**Variables Affecting Titration Results**

Titration is a semiquantitative technique and requires meticulous pipetting technique like quantitative analysis. To decrease possible practitioner variability, some institutions have the same practitioner perform all titrations on a given patient. Optimum incubation time, temperature, pH, antigen-antibody ratio, ionic strength, suspending media, and centrifugation times must be determined and used consistently.

Nature of cells used for testing may affect results. When titers of several samples are to be compared, all samples should be tested against fresh red cells from the same donor or against commercially prepared red cells of the same genotype.

Completely reproducible results are virtually impossible to achieve. Comparisons are valid only when specimens are tested simultaneously. In prenatal testing, samples should be aliquoted and stored frozen for comparison with subsequent samples. Do not repeatedly freeze and thaw a large sample since this denatures the antibody protein. Each new sample should be tested in parallel with preceding sample serving as the control serum. Where possible, the same individual should perform all titrations on the patient to decrease likelihood of practitioner variables.
Master Dilution

Because measurements are more accurate with large volumes than with small volumes, a master dilution usually gives more accurate and reproducible and decreases likelihood of analytical error than a dilution with small volumes; volumes of 0.5 mL or more are recommended. If dilutions of a given serum are to be compared for reactivity against several red cell samples, each dilution must be of sufficient volume that the same specimen can be tested against every red cell sample. This prevents introducing further technical error into the test system.

1. Label a set of tubes according to the serum dilution, 1:1, 1:2, and so forth (1:1 means one volume of undiluted serum and 1:2 means one volume of serum in a final volume of two or a 50% solution of serum in a diluent).
2. Deliver 0.5 mL of diluent (e.g., 0.5% BSA) to all tubes except tube 1 (1:1) with a micropipette.
3. Add 0.5 mL of serum to tubes 1 and 2 with a clean micropipette tip (dilutions 1:1 and 1:2).
4. Using the same micropipette with a clean tip, mix the contents of tube 2 several times and transfer 0.5 mL to the next tube (1:4 dilution).
5. Continue the same technique through all dilutions. From the last tube remove 0.5 mL of the diluted serum and save for use in further dilutions if needed.

Testing Methodology

1. Using a micropipette transfer 100 uL of each dilution to another set of labeled tubes. Add 100 uL of a 2-5% red cells suspension to each tube. The red cell suspension should be prepared in fluid used to dilute serum (0.5% BSA).
2. Incubate tubes for 15 minutes at 37°C.
3. Centrifuge for 15 seconds at 3400 RPM.
4. Observe for agglutination and/or hemolysis over a light source.
5. Wash cells four times with normal saline.
6. Add one drop of AHG to each tube, mix, and centrifuge 15 seconds at 3400 rpm.
7. Observe for agglutination as a positive result.
8. The titer is expressed as the reciprocal of the highest dilution of serum giving a macroscopic reaction with the test cells.
9. Gel cards may also be used for antibody titration utilizing the same dilution made as indicated above which are transferred to the gel cards.

Comments

1. Begin reading with the tube containing the least amount of serum (highest dilution; Tube 10) and proceed to the most concentrated sample (Tube 1).
2. If there is agglutination in the tube containing the most dilute serum, an endpoint has not been reached, and it is necessary to prepare and test additional dilutions.
3. In comparative studies, a difference in titer of at least 2 tubes (four-fold change) is required to be considered a significant difference. A change in titer from 128 to 256 (one tube) is insignificant due to variables of procedure. A change from 124 to 512 (2 tubes or four-fold) is a significant change.
4. Antibodies to drugs, additives, and preservatives may cause positive results when reagent red cells are used as distributed in the manufacturer's media. Reactions occur only rarely if the cells are washed with saline prior to use and the autocontrol is negative unless the autologous cells are suspended in the manufacturer's media.
5. The type of gel card, temperature, suspending media, and time of incubation are dependent upon the type of antibody titrated.

Summary

Antibody titrations are important in antenatal evaluations, antibody identifications, separating multiple antibodies, and ABO isohemagglutinin testing. However, standardization of the titer technique has continued to be a problem in the blood bank, and proficiency testing by CAP has demonstrated up to a 5-fold disparity in titration results. The CAP Transfusion Medicine Resource Committee has been able to reduce the interlaboratory variation through the use of a detailed procedure and a weak positive endpoint.

Additionally, each and every staff member performing antibody titration testing, competency testing must be performed twice during the first year of employment and annually thereafter.

Reference

AABB’s Technical Manual, 17th edition
Questions for STEP Participants

AMT strongly encourages you to submit your answers online so that the CE credits can be automatically transferred into your AMTrax account. To do so, go to www.americanmedtech.org, click on the AMT Store on the top navigation bar. Click on STEP Online, then select the article number and purchase the test. Don’t forget to log in to receive the discounted member price of $5 (nonmembers pay $15/test).

If you wish to submit answers manually (only available to AMT members), the fee is $10/test. Please submit a copy of this page with your answers marked, along with a completed order form located elsewhere in this publication. Don’t forget to include payment.

1. Titration techniques would be LEAST useful in:
   A. monitoring prenatal patients.
   B. detecting presence of antibodies.
   C. identifying mixtures of antibodies.
   D. pretransplant testing of kidney recipients.

2. The equivalence zone occurs when:
   A. antigen is in excess.
   B. antibody is in excess.
   C. either antigen or antibody is missing.
   D. antigen and antibody are in equivalent concentrations.

3. Based upon observation of the panel below, what is the antibody titer if a doubling dilution was used in the procedure?

<table>
<thead>
<tr>
<th>TUBE</th>
<th>1</th>
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<tbody>
<tr>
<td>REACTION</td>
<td>4+</td>
<td>3</td>
<td>2+</td>
<td>1+</td>
<td>w+</td>
<td>0</td>
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<tr>
<td>TITER</td>
<td>2</td>
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<td>8</td>
<td>16</td>
<td>32</td>
<td>64</td>
<td>128</td>
<td>256</td>
<td>512</td>
<td>1024</td>
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</table>

   A. 16
   B. 32
   C. 64
   D. 128

4. Observation of the panel below indicates that the antigen and antibody concentrations fall within the:

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<tr>
<th>TUBE</th>
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<tr>
<td>REACTION</td>
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<td>1+</td>
<td>2+</td>
<td>3+</td>
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<td>4+</td>
<td>3+</td>
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<tr>
<td>TITER</td>
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<td>32</td>
<td>64</td>
<td>128</td>
<td>256</td>
<td>512</td>
<td>1024</td>
</tr>
</tbody>
</table>

   A. Prozone
   B. Postzone
   C. Perizone
   D. Equivalence zone

5. Based upon observation of the following panel, the avidity of this antibody would be reported as:

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<tr>
<th>TUBE</th>
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<tbody>
<tr>
<td>REACTION</td>
<td>4+</td>
<td>3</td>
<td>2+</td>
<td>1+</td>
<td>w+</td>
<td>0</td>
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<tr>
<td>TITER</td>
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<td>16</td>
<td>32</td>
<td>64</td>
<td>128</td>
<td>256</td>
<td>512</td>
<td>1024</td>
</tr>
</tbody>
</table>
   | AVIDITY
   | A. 12
   | B. 22
   | C. 30
   | D. 37

6. Observe the titration results from the first and second visits of an obstetrical patient to her physician and tell what the results indicate.

<p>| First Prenatal Visit |</p>
<table>
<thead>
<tr>
<th>TUBE</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
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<tr>
<td>REACTION</td>
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<td>1+</td>
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<td>0</td>
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<tr>
<td>TITER</td>
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<td>4</td>
<td>8</td>
<td>16</td>
<td>32</td>
<td>64</td>
<td>128</td>
<td>256</td>
<td>512</td>
<td>1024</td>
</tr>
</tbody>
</table>

   A. Danger to the baby is imminent
   B. Evidence of fetal-maternal hemorrhage
   C. No evidence of further fetal-maternal hemorrhage
   D. Baby possesses the antigen Mom has produced antibody to

7. A master dilution is utilized in the titration technique for the purpose of:
   A. decreasing analytical error in the procedure.
   B. comparing multiple samples from the same patient.
   C. increasing likelihood of detecting antibody presence
   D. comparing the reactivity of a single antibody against several red cell samples.
8. Which of the following variables would be MOST likely to influence titration results?  
A. Use of a standard suspending medium  
B. Standard incubation time and temperature  
C. Preparation and use of a master dilution  
D. A variation in the incubation temperature

9. Which of the following statements BEST describes the results in the panel below?

<table>
<thead>
<tr>
<th>TUBE</th>
<th>1</th>
<th>2</th>
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<th>4</th>
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</tr>
</thead>
<tbody>
<tr>
<td>REACTION</td>
<td>1+</td>
<td>1+</td>
<td>1+</td>
<td>1+</td>
<td>1+</td>
<td>1+</td>
<td>1+</td>
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<td></td>
</tr>
<tr>
<td>TITER</td>
<td>2</td>
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<td>8</td>
<td>16</td>
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<td>64</td>
<td>128</td>
<td>256</td>
<td>512</td>
<td>1024</td>
</tr>
</tbody>
</table>

A. These reactions are characteristic for HTLA antibodies.  
B. These reactions are characteristic of the postzone phenomenon.  
C. These reactions are characteristic of cold autoantibodies.  
D. These reactions are characteristic of warm autoantibodies.

10. Which of the following statements would NOT describe the results from three different titration techniques on an obstetrical patient with the following results?

First visit titer  8  
Second visit titer  16  
Third visit titer  64  

A. There is evidence of a fetal-maternal bleed  
B. There is no evidence of fetal-maternal hemorrhage  
C. Baby possesses the antigen Mom has produced antibody to  
D. Additional procedures should be performed to monitor progression of HDN
While Hippocrates (460-377 B.C.) was the first to mention bubbles in urine as a diagnostic tool for kidney disease and "long illness," using the appearance and odor of urine to diagnose disease goes back as far as 6000 B.C. There has been a steady progression in the sophistication of uroscopy, or urinalysis by the senses, through the millennia: from ancient Hindus noting that black ants were attracted to sweet urine, to the medieval development of the graduated urine glass known as a matula for determining color; from the first qualitative urine glucose test in 1850 to automated dipstick readers in the 1970s; from Leeuwenhoek's single lens microscope in 1595 to urine particle flow cytometers of today. What has remained constant through this evolution is the need for skilled labor to successfully identify diagnostic issues. And as that pool of skilled talent in the laboratory has continued to shrink, the need for integration, automation, and rapid identification has increased.

What's not to love?

Despite a "tasteful" yet colorful past, urinalysis remains a potent screening tool for the clinician. It is still an "invaluable [tool] in the diagnosis of urologic conditions such as calculi, urinary tract infection and malignancy...[and] the presence of systemic disease affecting the kidneys." No other laboratory test can provide as much information on the status of metabolic, renal, infectious and urologic functions in the body. As a result, physicians use UA for everything from general screening to cancer detection. While test strip technology is what usually comes to mind when one thinks of urinalysis, it has become clear that microscopic examination is a necessary partner. Chemistry alone cannot detect casts, crystals, cells and bacteria which are critical for early diagnosis of numerous disease states. Indeed, in an era when failure to quickly detect a hospital acquired infection, such as catheter-associated urinary tract infection (CAUTI), means loss of significant revenue to a hospital, the need to integrate urine chemistry testing with urine particle analysis is paramount.

Further, there is a need to move away from manual methods of the past 8,000 years to the automation solutions available today. Albeit a "gold standard," manual methods are not precise enough to capture rare, but clinically significant, elements due to inaccuracies inherent to the method. Automation solves many of the issues with traditional manual methods, including discrepancies in sample volume, urine drop size, aged samples, and cellular breakdown. Most importantly, it addresses the labor issues inherent in any manual method: apart from sample handling and technologist time, the vagaries of subjective judgment are avoided. Minimizing human error reading a test strip or scanning an image is key to quality results.

Size matters

In the cost and space-conscious laboratory, urinalysis workstations tend to be compact by necessity. It can be difficult to carve out the space needed for multiple stations, which leads to draconian choices. Rather than eagerly embracing the new integrated, automated platforms available, many labs stick with the tried-and-true, but detested manual methods, missing out on opportunities to significantly reduce labor, turnaround time, and inaccurate results. All three of these directly and indirectly drive total cost; any decision to evaluate urinalysis systems

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"Back to the Future" with Urinalysis: Part 1

Leslie Williams
must include a value calculation for these costs; the benefit of upgrading your urinalysis workstation will offset the space required.

**Raising the Bar (or is that cup?) in Urinalysis**

Fortunately, today’s laboratories looking to save time and money by updating urinalysis have several options. There are standalone analyzers that bring the benefit of partial automation to laboratories with lower volumes and tighter budgets, and integrated systems that fully automate both urine chemistry and particle analysis while streamlining workflow and reducing turnaround times. While a standalone urine test strip analyzer can eliminate some of the mess and all of the subjectivity inherent in the traditional, visual-read test strip method, automating the manual microscopic may be where the true value proposition can be calculated.

The automated urine particle analysis methods available today are digital imaging and fluorescent flow cytometry. Digital imaging analyzers use laminar flow to present the urine particles to a digital camera; the pictures of the unstained particles are processed by onboard software to produce the pre-classifications and quantities of the various cellular elements which are confirmed by technologist review. Fluorescent flow cytometry analyzers, such as the Sysmex UF-1000i™, employ fluorescent flow technology with hydrodynamic focusing and selective cell lysing to allow for better separation of cells, improving the classification and enumeration of formed elements. The UF-1000 is able to analyze a significantly higher volume of each sample than can be done by visual and digital imaging methods, helping to ensure highly accurate and precise results. The UF-1000 measures approximately 9µl of sample and up to 80,000 particles can be analyzed and classified for every sample, reducing the risk of missing a rare but clinically significant formed element. Classification is further enhanced through the use of specific dyes and stains that highlight the RNA, the nucleus and other internal components of the cells.

The precision inherent in the fluorescent flow cytometry method allows for much higher sensitivity and specificity in detecting, classifying and quantitating the cellular elements in urine, providing clinicians the information they need to quickly and accurately diagnose, and perhaps treat the patient.

**Show me the money**

There are several emerging categories of disease that may warrant the combined power of urine chemistry and cellular analysis reports in every instance. While reflex testing is available on most automated platforms, there are a number of situations where a urine chemistry panel will be normal, so the reflexive particle exam will not occur. However, the cells and particles may hold the key to early detection by physicians of situations that constitute sentinel events for a hospital or individual patient. One major area of concern for clinicians in today’s ever-changing healthcare environment is UTI screening and detection.

**UTIs:**

Urinary tract infections are a significant exposure for hospitals on multiple levels. There is a need for rapid screening for outpatients admitted through the ER, particularly patients from skilled nursing facilities. If a patient is admitted with documentation of an existing UTI, then there is less risk of suspicion that the infection may be hospital acquired. Such screening mandates a rapid and sensitive method to detect bacteria, something urine cultures and urine chemistries alone cannot provide. Urine cultures take 24-48 hours and urine chemistry cannot detect all bacteria. Cellular analysis by fully automated urine particle analyzers could detect all bacteria quickly. Manual procedures cannot cope with the quantity of tests such screening would entail.

Further aggravating the situation, hospital acquired CAUTIs (Catheter-Associated UTIs), considered sentinel events, have grown to approximately 450,000 per year in the U.S, adding an average of $1,000 to the care of each patient. The total financial impact on U.S hospitals is $450 million. The financial risk to hospitals is that payers may not cover costs for hospital acquired infections. Automated platforms can report the actual number of bacteria in urine with a level of specificity and sensitivity unmatched by manual and image-based systems, and integrated platforms ensure that the cells are counted within minutes of the chemistry strip testing, providing
a comprehensive report within STAT turnaround time goals.

**Sensitivity, Specificity, Analytically, Mathematically**

The high levels of specificity and sensitivity that automated urine particle counters provide can greatly improve the rates of early detection and intervention of UTIs, including CAUTIs, allowing hospitals to manage their risk and financial exposure while improving patient care. But what do the terms actually mean, and what benefit do higher levels bring to the laboratory?

Sensitivity is simply a calculation of how well the new, or test method, will detect abnormal results compared to the reference (existing, or predicate) method. Also known as the true positive rate (TPR), the sensitivity is a calculated fraction of the number of true positives out of the total number of positives, either in whole numbers (0.93) or as a percent (93%). The ideal sensitivity is 100%, indicating a test method that has 100% probability of detecting the disease.

\[
\text{Sensitivity} = \frac{\text{TP}}{\text{TP} + \text{FN}} \times 100
\]

*TP = True Positive = Abnormal (positive) by both test and reference method

*FN = False Negative = Normal (negative) by test method and abnormal (positive) by reference method

Specificity, in contrast, indicates how well the new method will detect normal results compared to the current method. Specificity is often referred to as the true negative rate, and is the calculated fraction of the number of false positives out of the total number of actual negatives. The ideal specificity is also 100%, indicating a test method that will NOT detect the disease in populations where it is not present.

\[
\text{Specificity} = \frac{\text{TN}}{\text{TN} + \text{FP}} \times 100
\]

*TN = True Negative = Normal (negative) by both the test and reference methods

*FP = False Positive = Abnormal (positive) by the test method and normal by the reference method

Because of the inaccuracies inherent in the manual slide method, and the time and labor involved in culture, it makes sense to consider the type of automation that newer technologies can bring to the oft-neglected urinalysis department. Increased sensitivity and specificity are just two of the benefits that modern urinalysis analyzers can bring to today's laboratory.

**Times they are a changin’**

After 8,000 years, urinalysis is facing a bright, exciting future. New technology for instrumentation and new appreciation for the importance of urinalysis in modern medicine contrive to bring focus back to where laboratory testing all started. While we may not go back to the days of Roman soldiers cleansing wounds with urine, we can all raise our [urinalysis] cups in salute to the brave new world of UA!

**References**

Crossword solution from page 16
Consulting — My Story

by Kathleen Voldish, CLC(AMT)

I started consulting to physician offices back in 1989 after working over 20 years in the field of hematology oncology. Two thousand plus offices later, I still get asked, “How did you get into this?” by my clients. As I head toward retirement and scout for a motivated health care professional to take over my business (ed. note: at the time of this article submission), it may be of interest to know how it started, what worked, what did not work and what to expect.

How It Started

I wanted to work with cancer patients from the time I was in the 8th grade and specialized in hematology oncology right out of hospital training. I worked in private hematology/oncology medical practice as well as being department head of the special hematology department at New Jersey College of Medicine. I attended ASCP programs and read each issue of Laboratory Medicine but most programs and articles were geared to the hospital setting. So in the early 80s, I wrote an article on employment opportunities in physician offices in the field of hematology. Much to my surprise — they published it. It was the mid 80s and expanding CLIA regulations to physician offices were in the early talk stages. I heard about COLA through an internal medicine physician and my lab was chosen to be part of the pilot program for physician office laboratory certification. This pilot program also included participation in the MLE proficiency testing program. This lead to being contacted by the ASCP who was forming a physician office laboratory (POL) committee. Serving on this committee (six years) was a real learning experience. We were on the cutting edge of what going on with CLIA regulations, developed programs to serve the needs of laboratory professionals working in POLs and writing a regular column in Laboratory Medicine. This was really exciting and opened the door to the possibility of consulting to POLs.

The old saying “don’t quit your day job” was the way I went. I started putting my business plan together while still working part time at a hematology/oncology practice. After one year, I was able to only work as a consultant. The following are things that had to be put in place in order to launch my company:

• Name for the company. I named the company POL Consultants. I had the name searched, registered and then incorporated. In 2005 I changed the name to POC — Point of Care Consultants — because it better described my client base.
• What was I going to consult for? My initial target clientele was hematology/oncology practices; after all, this is what I really knew best. This was before the age of the Internet so getting a mailing list was not that easy.
• What geographic area would I cover? New Jersey is not all that big, so covering the entire state was not out of the question.
• How would I market? I developed an attractive tri-fold brochure with services I would offer, a cover letter and a business card. This was before CLIA went into effect, so this was going to be a hard sell! Much to my surprise, two offices responded immediately and I was on my way.
• What would I offer? A customized laboratory procedure manual that had all the required information found in the proposed CLIA regulations and COLA’s certificate list. This was going to take a lot of time, so I started work-
ing on this way before the marketing letters went out. A basic manual was put together and procedures were then added to personalize the manual.

- **A compliance check list** that would document everything that needed to be put in place. The client would get this before I left the office. This worked for many reasons — it cut down on phone calls with “I forgot what you said,” gave them a reference to go back and work on and last, made them know what they paid for!

- **A newsletter** that would keep the office up to date with pending regulations.

- **Continued services**, each visit using a checklist that would be given before leaving the office.

- **Phone support.**

- **Approach to consulting.** Early on I decided my approach to presenting services would be educational and not a scare tactic approach used by some consultants.

  Since I started the company so early on — I did not have any completion in this field (in NJ).

  When CLIA went into law, consultants seemed to pop up all over the place, however, most in NJ did not get their company off the ground and I still have little completion in NJ.

**Getting Clients**

Well, two clients were not going to cut it. You always have to consider, who gets the mail, and who will just throw it out. I tried making appointments like drug reps but this was a waste of time. I wrote to each county medical society and offered a free seminar. The response was good and the seminars got my name and information out there.

I also offered programs to medical supply companies. These programs were for the sales force to make them aware of pending CLIA regulations and their clients who were desperate to know how CLIA would change their laboratory services. In addition, I wrote a monthly article on regulations for the NJ medical society magazine. My articles appeared over 20 times in this journal. This educational-based approach brought in clients and/or sold manuals.

When the government enacted CLIA, clients contacted me in droves since my company was already established. Now the services I had been promoting were law. It was not just the hematology/oncology offices I had initially marketed to, it was every specialty of medical practice that offers tests. Once the OSHA Bloodborne Pathogen Standard went into law, it opened up services to offices that did not perform tests as well as dental and podiatrist offices. Later when HIPAA became law, compliance with medical privacy and security was added to our list of services.

In 1994, I opened a Florida branch of my company using the same business strategy, and it quickly developed a reputation with county Florida medical societies and medical equipment vendors. Since Florida has strong continuing education requirement for medical personnel, I have gotten all my programs accredited for continuing education through the Florida Department of Health Division of Quality Assurance.

**Pricing Services**

This is a tough one. I asked other consultants who were on my POL committee what they charged and the range was wide. I knew how much I needed to make before I could quit my regular job. I also knew from working in physician offices that I had to make it affordable. I initially put my price at twice what I made hourly as a tech and had no problem getting clients. As my company evolved and developed a reputation, fees for services increased. I still consider our services affordable; however, some new clients still get “sticker shock!” I offer a “package” start up (discounted) as well as a-la-carte services. We are not a hard sell, we will work with each office to meet their needs.

**Equipment Needed to Start a Consulting Business**

Financially, what you spend will vary depending on the level of consulting, but all I started with was a typewriter — yes, typewriter (it was 1989) — and a phone. As technology advanced, so did our equipment. The business is still run from a home office.

**Additional Costs**

I started this company with little business experience. You need to know when to seek help, which could be a book keeper, accountant and/or lawyer. Insurance such as health that may have covered you when you worked a regular job may now need to be purchased. I also took out an insurance policy to cover my services to clients. Luckily, I have never had to use this policy. If you are traveling, you need to consider gas and the wear and tear on your car, and the pay for staff needed such as a secretary and additional consultants. The cost of attending education programs to keep you on top of regulations and technology will now come out of your pocket.
Personal Qualities Needed

- You need discipline to run your own business, especially with a home office. Whether on the road seeing clients or in my office, I work a full day and then some. Running your own business gives you the opportunity to take time off and adjust hours, but if you are self-employed, you are not making money unless you are seeing clients.
- Motivation to keep learning. This is an ever-changing and evolving field. Most of it is self taught. You need to read federal registers and continually update the products and services you offer as new regulations or changes are made.
- The ability to work alone is important, and knowing when to admit what you do not know and where to get the answer. Problem solving is a great educational process. Don’t overextend yourself. Do what you know best, expand to other areas when ready.
- The ability to teach and communicate with clients that do not have a strong laboratory or medical background is also important. You will not be dealing with degreed medical technologists in most offices! You must have the ability to really listen to their concerns.

Hiring Help

I have had a secretary to run the office since the first year of the company. My focus is on seeing the clients, not making the appointments, answering the routine calls, sending out information and filling manual orders. I have only had two secretaries in my 20+ years; both have been worth their weight in gold.

I have always had consultants since the early 90s. They work part time as private contractors and can take the “overflow” of clients. I train them for three+ months before they venture out on their own and I provide support whenever they need it. Here I will stress — have a contract that protects your company from a consultant taking the products you have taken years to develop and running off with them. Believe me, this can happen. I learned the hard way. A lawyer can help you write this contract. This is not a bench tech type of job; the consultant must exude a quality of self confidence and be able to represent your company the way you would yourself. I also like consultants who have strong background in areas in which I might be weak. As to what to pay them, it is going to be substantially more than they make at the hospital but not the hourly rate the company is charging.

Cost of Running the Company

For each dollar my business brings in, about 50% of it goes to running the business. A good accountant can tell you what you can and cannot write off to the company. How generous you are to yourself and your staff will come into this equation. The cost of outside help such as a bookkeeper and accountant gets added in. Cost to update computers, phone service, office supplies and copiers all add up.

Closing

As with all careers, circumstances change and opportunities arise. I think how I would have felt if I never attempted starting my company and I’m sure the answer is that I would have always wondered if I could do it. AMT began offering certification in the field of consulting and I was fortunate to meet the qualifications. Have I had any failures? Yes, but not enough to dwell on. Health care has gone through many changes since I started the company and the company and services have had to adjust to these changes.

Consulting is a challenging and rewarding career option for the right individual. For me it has been a great ride!
## EARLY BIRD REGISTRATION FORM

**American Medical Technologists’ 76th Educational Program & National Meeting • July 7 – 10, 2014 • Chicago, Illinois**

### Registrant Information

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**City, State, Zip** _______________________________________________________________________________________

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**E-mail** ____________________________________________

**Membership Information:**  
- [ ] AMT  
- [ ] MT  
- [ ] MLT  
- [ ] RPT  
- [ ] RMA  
- [ ] CMLA/COLT  
- [ ] RDA  
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**Check if not a member of AMT** [ ]

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**Check if 2013 Pittsburgh was your first AMT Convention** [ ]

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**School_______________________________**

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**60-year member** [ ]

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AMT will make every effort to meet your special requirements. *This does not include your hotel restaurant meals or accessibility needs. Please inform the hotel directly of any special requirements.*

Registration Fee must accompany this form. Registration will not be accepted without payment of Registration Fee.

**Cancellation** must be received by June 6, 2014. Refunds minus a $25 processing fee will be sent for all cancellations before June 9. No refunds issued after June 6.

### Registration Fees

**Registration Fees**

(Please check appropriate boxes)

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<th>After May 1</th>
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<td>Monday Lunch of Champions</td>
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</table>

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Register before May 1 and your name will be entered in a drawing for a free meeting registration!

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**Account # ___________________________ Exp. Date ____________**

**Account in name of ___________________________**

**Signature ___________________________**

**Total Registration Fee(s) $__________**

**Donation to Chester Dziekonski Memorial Keynote Speaker Fund (optional) $__________**

**TOTAL PAYMENT $__________**

(Payment must accompany Registration Form)
The Clinical Microbiology Laboratory’s mission is to find the pathogen(s) causing the clinical condition of the patient and provide antibiotic susceptibility testing results, when appropriate. These tasks must be performed in a timely manner in order to provide clinically relevant information. Historically, the process of identification of an organism could take from days for a routine organism to weeks, in the case of a fastidious organism. Mass Spectrometry, an old chemistry technique of identifying a compound by separating it into its components, has recently been adapted as a new approach to identifying microbes. When Cincinnati Children’s Hospital Medical Center began exploring the possibility of identifying organisms with a mass spectrometer, very little data had been published. However, the reports that were available were favorable that the instruments could identify organisms very quickly (less than 10 minutes) and accurately. This article is a summary of our journey to utilizing mass spectrometry in our laboratory.

Background

Clinical Microbiology is a department long rooted in the past. Until the late 1960s all bacterial identifications were done utilizing tubed and plated media, and employed phenotypic characteristics — identification based on morphology, odor, Gram’s stained smears, rate of growth, and biochemical reactions. Organizing the biochemical reactions into metabolic profiles was a “giant” leap for clinical microbiologists. In 1970 the API 20 E® (Analytical Profile Index) was the first biochemical identification system to be developed (fig. 1). The reactions that once took place in numerous large tubes were now taking place in small strips containing dehydrated enzymes and reagents. Biocodes (number sequences) were generated and manuals used to find the corresponding identifications.

In 1980, it was a short hop to automate the interpretation of those biochemical reactions and organize the profiles into a computer database. Thus, microbial identification instruments, such as Vitek®, MicroScan®, and Phoenix™, were born (fig. 2). Automation reduced the need for
hands on time. Once the organism was placed into the machine, the instrument read and interpreted the reactions.

Still, the time to identify an organism required hours — albeit, not 18-24 hours as with tubed biochemical reactions, but 2.5-18 hours, depending on the organism. Moreover, the strength of the automated systems was their databases. Maintaining accurate and current taxonomy was a continuous process with software upgrades.

In the 1980s, scientists started working with the molecular structures of organisms for identifications. Nucleic acid, the substance which carries the genetic material and is found in all living organisms, has highly conserved regions that are specific for each organism. Segments of DNA or RNA labeled with radioisotopes, enzymes, or chemi-luminescent molecules can bind to the complementary nucleic acid of an unknown organism and be used to identify that organism. Particular gene targets specific for each organism can also be amplified, detecting very low numbers of organism. Initially, tests were developed for organisms difficult to culture or sensitive to transport, such as viruses. However, testing was very expensive. This area of molecular microbiology continues to develop, but another branch, proteomics, may also be useful for the identification of bacteria and fungi.

Proteomes are the set of proteins encoded by the genome. Proteomics is the identification, characterization, and quantification of the complete set of proteins made by an organism. Mass spectrometry is one of the tools that can be used to identify an organism using its proteomic signature. The ribosomal proteins are most often utilized since they are plentiful, very concentrated, closely related to the rRNA gene, and stable. A reference strain or group of strains is used to define the unique pattern or fingerprint of an organism’s proteomes. Unknowns are then compared to that fingerprint.

The general principle of mass spectrometry is: an unknown compound is ionized, separated into its elemental components, and, then, by measuring those components, decoding the identity of the compound. How is this concept applied in microbiology? Matrix Assisted Laser Desorption Ionization — Time of Flight or MALDI-ToF is an instrument that uses the mass spectrometric principles to identify organisms. The organism is first mixed with a matrix. The matrix acts to both protect the organism from the laser and to absorb the laser’s energy and transfer that energy to the organism, allowing it to sublime — change from the solid phase to its gaseous phase. The resulting ionized molecules, mostly ribosomal protein fragments, are accelerated using an electric field in the ionization chamber. The speed of the molecules or the time it takes for a molecule to travel through the tube and arrive at the particle detector depends upon the mass to charge ratio. Larger molecules move slower; conversely, smaller molecules move faster. As the accelerated, ionized protein fragments leave the ionization chamber, they are directed through a long tube by deflector plates. The ions strike a particle detector, causing an electrical signal. The computer displays the “hits” as peaks corresponding to the masses of the different fragments. The bigger the fragment, the larger the peak is. The collection of peaks is a spectrum (See Fig 3). After refining the spectrum, computer software compares it to thousands of known spectra of defined organisms in the database.

Although there are two MALDI-ToF instruments on the market, the Bruker Biotyper and bioMerieux Vitek® MS, AnagnosTec was the pioneering company in the field of MALDI-ToF based microbiology. In 1998 AnagnosTec was founded and began building a comprehensive database for bacterial identification. It was called SARAMIS™ — Spectral Archive And Microbial Identification System. Shimadzu, a longstanding global Japanese company specializing in medical equipment, developed a linear MALDI-ToF mass spectrometer called AXIMA. In 2005 Bruker Daltonics began development on the MALDI Biotyper. In 2010 bioMerieux acquired SARAMIS™ from AnagnosTec and joined with Shimadzu to adapt AXIMA to the microbiology laboratory for identification of microbes. For the Bruker Biotyper and Vitek® MS, peaks are obtained then compared to the database using a pattern matching approach which is based on true statistical multi-variant analysis, and includes peak positions, intensities and frequencies ensuring the highest possible levels of accuracy and reproducibility.
across the complete range of microbes. In the very near future, the Vitek MST™ Plus system will be released. This system includes the Advanced Spectra Classifier (ASC) and a process called “binning”. The system looks not only at the spectra produced, but groups peaks together and applies numerical values to the presence or absence of certain peaks forming a database matrix. A score is given for each comparison to a species, and a confidence level is determined. In the new system, there are no spectra in the database.

The early published data comparing MALDI-ToF to current identification systems reported very high percentages of correct identifications for the methodology. In April 2010, a Journal of Clinical Microbiology article reported 99.4% (635/639) of the isolates tested as identified to species level from hospitals in Geneva, Switzerland (Cherkaoui, JCM, April 2010). A study from the Cantonal Institute Microbiology in Switzerland reported 98% of the organisms tested generated the correct identification (Benagli, Plos ONE, January 2011). MALDI-ToF was the hot topic at all of the national meetings. Late in 2011, Cincinnati Children's Hospital Medical Center Diagnostic Infectious Disease Testing Laboratory decided to purchase the Vitek® MS. We were ready to enter the 21st century.

The 21st Century

Installation in early 2012 was a thought-provoking process. The instrument is about the size of a soda machine (See Fig. 5). There are two computers — complete with two monitors, two central processing units, two keyboards, two mice, and one uninterrupted power supply. Because of space issues, the instrument was placed in the main room of the microbiology laboratory and the computers, in an office right off the main room (See Fig. 6). However, to accomplish this, a hole was cut into the wall to allow the wire connections needed between the computers and the instrument. Although there was a phone line in the room, there was no modem line. An information technician was called to install the correct lines. Once construction was finished, the instrument was finally installed.

April 2012, the Diagnostic Infectious Disease Testing Laboratory began training on the Vitek® MS. Two technologists practiced filling in the circles on the 48-welled slides. The quality assurance was mapped out. We would begin the verification of the Vitek® MS to identify organisms with a challenge set of known organisms purchased from ATCC and a twenty day reproducibility study; then, patient isolates of staphylococci, streptococci, and enterococci; and, finally, the Enterobacteriaceae. In June, testing on the organisms began. We started with 25 known organisms, ranging from *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Enterococcus faecalis*, to *Kocuria kristinae*. The isolates were tested with the Vitek® MS and Vitek® 2. If there

![Fig. 5. Vitek® MS (J. Mortensen, 2012)](image)

![Fig. 6. Small office where computers located (B. Deburger, 2013)](image)
were any discrepancies, those isolates were sent for 16S rRNA sequencing and frozen for future use.

Although the staphylococci and enterococci correlated with no statistical differences (Table I), Streptococcus species posed a big problem. Streptococcus species identified 52% (11 of 21) to the species level and another 38% (8 of 21) to the genus level. However, Streptococcus species is a complex and controversial taxonomy. Additionally, Streptococcus pneumoniae did not identify as expected.

Three ATCC isolates were placed on the MALDI-ToF. All three isolates gave results of “Streptococcus mitis/oralis/pneumoniae”. When the patient isolates were tested on the VITEK® MS, eleven of twenty-three patient isolates gave the nebulous “Streptococcus mitis/oralis/pneumoniae” result. Bile solubility, a test somewhat hard to interpret, would still be needed to identify these organisms.

At the same time we began looking at known organisms, a graduate student joined our staff for the summer of 2012. Since the database for the group of organisms known as HACEK (Haemophilus sp., Aggregatibacter sp., Cardiobacterium sp., Eikenella sp., and Kingella sp.) was limited, her goal was to test a number of those organisms and upload the spectra into a database labeled as CCHMC Organisms. Biochemical testing (Remel RapID™ NH), MALDI-ToF identifications, and 16S sequencing was performed on every organism. 140 organisms were tested. Organisms were obtained from area hospitals, purchased ATCC strains, and frozen stocks. MALDI-ToF correctly identified 86% (121 of 140) to the species level (Table 2). More im-

Table I. Comparison of the Vitek® 2 System and the MALDI-ToF System to 16s ribosomal RNA sequencing Gram-Positive cocci

<table>
<thead>
<tr>
<th>Organism (n)</th>
<th>Vitek® 2¹</th>
<th>MALDI-ToF¹</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Species</td>
<td>Genus</td>
</tr>
<tr>
<td><em>S. aureus</em> (43)</td>
<td>42 (98)</td>
<td>1(2)</td>
</tr>
<tr>
<td><em>S. saprophyticus</em> (20)</td>
<td>20 (100)</td>
<td>-</td>
</tr>
<tr>
<td>Coag Neg Staphylococcus (28)²</td>
<td>24 (86)</td>
<td>2 (7)</td>
</tr>
<tr>
<td><em>S. pneumoniae</em> (23)</td>
<td>23 (100)</td>
<td>-</td>
</tr>
<tr>
<td>Beta hemolytic Streptococcus (22)</td>
<td>22 (100)</td>
<td>-</td>
</tr>
<tr>
<td>Streptococcus spp. (21)²</td>
<td>9 (43)</td>
<td>6 (29)</td>
</tr>
<tr>
<td>Enterococcus spp. (55)</td>
<td>52 (95)</td>
<td>2 (4)</td>
</tr>
<tr>
<td>Misc. (13)</td>
<td>10 (77)</td>
<td>-</td>
</tr>
<tr>
<td>Total (225)</td>
<td>202 (90)</td>
<td>11 (5)</td>
</tr>
</tbody>
</table>

¹Number (%) agreement. 16s ribosomal RNA sequencing as reference method
²Staphylococcus lugdenensis 2 of 2 correct by MALDI
³Complex and controversial taxonomy

Table II. Comparison of the Remel NH and the MALDI-ToF System to 16s ribosomal RNA sequencing HACEK.

<table>
<thead>
<tr>
<th>Organism (n)</th>
<th>Remel NH¹</th>
<th>MALDI-ToF¹</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Species</td>
<td>Incorrect ID</td>
</tr>
<tr>
<td><em>H. influenza</em> (26)</td>
<td>25 (96)</td>
<td>-</td>
</tr>
<tr>
<td><em>H. parainfluenzae</em> (20)</td>
<td>10 (50)</td>
<td>1* (5)</td>
</tr>
<tr>
<td><em>H. haemolyticus</em> (11)</td>
<td>7 (64)/ 1**(9)</td>
<td>3 (27)</td>
</tr>
<tr>
<td><em>H. parahaemolyticus</em> (6)</td>
<td>-</td>
<td>2 (33)</td>
</tr>
<tr>
<td>A. actinomycetemcomitans (10)</td>
<td>10 (100)</td>
<td>-</td>
</tr>
<tr>
<td>A. aphrophilus (13)</td>
<td>11(85)</td>
<td>2 (15)</td>
</tr>
<tr>
<td>A. segnis (1)</td>
<td>-</td>
<td>1 (100)</td>
</tr>
<tr>
<td>E. corrodens (29)</td>
<td>29 (100)</td>
<td>-</td>
</tr>
<tr>
<td>K. kingae (20)</td>
<td>-</td>
<td>20 (100)</td>
</tr>
<tr>
<td>K. denitrificans (4)</td>
<td>-</td>
<td>4 (100)</td>
</tr>
<tr>
<td>Total (140)</td>
<td>85 (61)</td>
<td>38 (27)</td>
</tr>
</tbody>
</table>

¹Number (%), 16s ribosomal RNA sequencing as reference method
²ID correct to Genus level; no species identified
** No ID
Importantly, compared to Remel RapID™ NH which misidentified 26% (36/140) of the organisms, MALDI-ToF misidentified only 9% (13/140). The misidentifications were all one species: *Haemophilus hemolyticus*. This species was misidentified as *Haemophilus influenzae* every time.

Ideally, all of the spectra could be added to the research database. However, at this time, we do not have the expertise to accomplish that goal.

Next, we tackled the family Enterobacteriaceae. *Escherichia coli* identified nicely (Table 3). 90% of the *Salmonella* isolates were identified to the subspecies level. Since Vitek® 2 typically identifies *Salmonella* to the genus level, it was unusual to see *Salmonella* identified to the subspecies level. However, after 16S sequencing, the MALDI-ToF identification was confirmed. Unfortunately, no *Shigella* isolate was correctly identified. All were incorrectly identified as *E. coli*. This threw a monkey wrench into the lab of the future. How could we replace Vitek® 2 if a stool pathogen could not be distinguished from the most common organism isolated from stools?

<table>
<thead>
<tr>
<th>Organism (n)</th>
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<th>MALDI-ToF</th>
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</thead>
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</tr>
<tr>
<td><em>E. coli</em> (64)</td>
<td>64 (100)</td>
<td>-</td>
</tr>
<tr>
<td><em>Klebsiella</em> spp. (32)</td>
<td>32 (100)</td>
<td>-</td>
</tr>
<tr>
<td><em>Proteus</em> spp. (25)</td>
<td>21 (84)</td>
<td>4 (16)</td>
</tr>
<tr>
<td><em>Serratia</em> spp. (11)</td>
<td>11 (100)</td>
<td>-</td>
</tr>
<tr>
<td><em>Enterobacter</em> spp. (11)</td>
<td>10 (91)</td>
<td>-</td>
</tr>
<tr>
<td><em>Citrobacter</em> spp. (10)</td>
<td>8 (80)</td>
<td>-</td>
</tr>
<tr>
<td><em>Salmonella</em> spp. (10)</td>
<td>-</td>
<td>10 (100)</td>
</tr>
<tr>
<td><em>Shigella</em> spp. (10)</td>
<td>10 (100)</td>
<td>-</td>
</tr>
<tr>
<td><em>Pseudomonas</em> spp. (19)</td>
<td>19 (100)</td>
<td>-</td>
</tr>
<tr>
<td><em>Acinetobacter</em> spp. (10)</td>
<td>8 (80)</td>
<td>-</td>
</tr>
<tr>
<td><em>Misc</em> (29)</td>
<td>23 (79)</td>
<td>4 (14)</td>
</tr>
<tr>
<td><strong>Total (231)</strong></td>
<td><strong>206 (89)</strong></td>
<td><strong>18 (8)</strong></td>
</tr>
</tbody>
</table>

1 Number (%) agreement. 16s ribosomal RNA sequencing as reference method
2 Vitek®2 and MALDI to genus only
3 16 s sequencing does not accurately differentiate Shigella from *E. coli*
4 Pantoea, Providencia, Aeromonas, Raoltella, Morganella, Edwardsiella, Leclercia, Stenotrophomonas, Pleisiomonas, Yersinia, Vibrio

The identification to the species level was 50% for *Citrobacter* and 64% for *Enterobacter*. When it became apparent that FDA approval was further away than initially thought, testing new organisms slowed.

There were continued reports that yeast identifications correlated very well with traditional identifications (Iriart, JCM, April 2012). Colonial morphologies for most yeast are indistinguishable. Often times, days are spent trying to isolate what appear to be different colony types of yeast. However, after all of the identification testing is performed, only one species of yeast is identified. The ability to quickly test several morphologies at once in a very short time period held an appeal. Therefore, a quality assurance comparing Vitek® 2 and Vitek® MS identifications was drafted. Again, any discrepancies would be sent for 16s sequencing and the isolate frozen.

The data corresponded nicely. See Table IV. For MALDI-ToF, 90% (61 of 68) of the yeasts were correctly identified to the species level. Although 9% (6 of 68) of the yeast did not identify using MALDI-ToF, none were misidentified. However, there are two challenging issues: the organism *Candida albicans* often results in an inadequate identification, i.e., *Candida albicans/Candida africana*/
Candida dubliniensis and the results of some yeast are reported as teleomorphs. Since we report out single species, we do not report the slash line identification from MALDI-ToF, but instead wait for the biochemical identification. Also, technologists need to be aware that the odd result may be a teleomorph of yeast we commonly see, i.e. Clavispora lusitaniae for Candida lusitaniae.

Again, one of the greatest advantages of the MALDI-ToF is the quick turn-around-time. For the less experienced technologists, why not utilize this as a teaching tool? Why not let them put “oropharyngeal flora” on the Vitek® MS to get a better handle on what microbes they are seeing in a sputum, etc. The idea was to use the MALDI-ToF to give them direction for working up respiratory cultures. With that goal in mind, our next study compared identifications of various Gram-negative diplococci using Remel RapID™ H and MALDI-ToF.

The results were favorable for MALDI-ToF (Table V). 94% (49 of 52) of the organisms identified correctly with the Vitek® MS, compared to 83% with the Remel RapID™ NH. For the clinically significant isolates, Neisseria gonorrhoeae, N. meningitidis, and Moraxella catarrhalis, MALDI-ToF correctly identified 100%. Both systems also correctly identified N. lactamica (2 of 2) and N. sicca/subflava (8 of 8), although RapID™ NH panels cannot distinguish between N. sicca and N. subflava. The RapID™ NH system was able to identify N. cinerea (2 of 2) with extra biochemical testing. Without the NO2 result, the RapID™ NH system identified N. cinerea as N. gonorrhoeae. MALDI-ToF was able to identify one N. cinerea isolate to genus level and gave no identification for the other isolate. The RapID™ NH system was not able to identify N. macacae (a newly named organism), while MALDI-ToF identified it to the genus level. The RapID™ NH system misidentified three different Moraxella species [M. nonliquefaciens (3 of 3),

Table IV. Comparison of the Vitek® 2 System and the Maldi-ToF System for Yeast

<table>
<thead>
<tr>
<th>Organism (n)</th>
<th>Vitek® 21</th>
<th>MALDI-TOF1</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Species</td>
<td>Genus</td>
</tr>
<tr>
<td>C. parapsilosis (11)</td>
<td>9 (82)</td>
<td>2 (18)</td>
</tr>
<tr>
<td>C. lusitaniae (4)</td>
<td>3 (75)</td>
<td>-</td>
</tr>
<tr>
<td>C. albicans (20)</td>
<td>19 (95)</td>
<td>-</td>
</tr>
<tr>
<td>C. neoformans (3)</td>
<td>3 (100)</td>
<td>-</td>
</tr>
<tr>
<td>S. cerevisiae (2)</td>
<td>2 (100)</td>
<td>-</td>
</tr>
<tr>
<td>C. tropicalis (7)</td>
<td>6 (86)</td>
<td>1 (14)</td>
</tr>
<tr>
<td>C. krusei (3)</td>
<td>3 (100)</td>
<td>-</td>
</tr>
<tr>
<td>C. glabrata (9)</td>
<td>9 (100)</td>
<td>-</td>
</tr>
<tr>
<td>C. dubleniensis (3)</td>
<td>3 (100)</td>
<td>-</td>
</tr>
<tr>
<td>G. capitatum (1)</td>
<td>1 (100)</td>
<td>-</td>
</tr>
<tr>
<td>C. guillermondii (1)</td>
<td>-</td>
<td>1 (100)</td>
</tr>
<tr>
<td>C. kefyr (1)</td>
<td>1 (100)</td>
<td>-</td>
</tr>
<tr>
<td>C. pulcherrima (1)</td>
<td>1 (100)</td>
<td>-</td>
</tr>
<tr>
<td>R. muciliginosa (1)</td>
<td>-</td>
<td>1 (100)</td>
</tr>
<tr>
<td>Hanseniaspora spp.</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>(opuntiae/uvarum/meyeri)</td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>Total: 68</td>
<td>60 (90)</td>
<td>5 (7)</td>
</tr>
</tbody>
</table>

1Number (%) agreement. 16s ribosomal RNA sequencing as reference method
M. osloensis (2 of 2), and M. lacunata (3 of 3)]. MALDI-ToF was able to identify all eight of the isolates to the species level. Overall, the Vitek MS™ definitely outperformed the Remel RapID™ NH for the identification of the Gram-negative diplococci.

Quality Control and Preventative Maintenance are integral components of our testing at CCHMC. When we first began testing, the guidelines for preventative maintenance and quality control were not well established. *Escherichia coli* ATCC 8739 was used as a calibrator and a QC strain with each run. Yet, there were no guidelines as to what was acceptable and when a run needed to be repeated. Initially, we called bioMerieux whenever we felt the data counts or the percentages of the *E. coli* 8739 were too low. Quality Control logs were utilized to track the percentages and data counts. If we noticed a downward trending of either one, we called bioMerieux for fine-tuning. After 1½ years of testing, we continue to monitor for trending for indications that the instrument needs fine tuning.

Our preventative maintenance was also developed with trial and error. For example, originally, we checked the silica beads weekly for color change. We replaced them only when we saw a change in color. Then, we began changing the beads quarterly. Now, we change the silica beads monthly, as this seems to decrease the time necessary to establish the vacuum. As always, with new instrumentation, QC and PM may not be defined, but discerned as familiarity with the instrument develops.

After listening to my 5th or 6th talk on MALDI-ToF, and how it was revolutionizing the way laboratories identify organisms (completely discarding biochemical testing), I wondered what other laboratories did or, more importantly, we did not do to evaluate MALDI-ToF. Given our experience with MALDI-ToF, we cannot rely solely on the Vitek® MS for identification of organisms without biochemical testing to resolve issues? First, it was not FDA approved. Secondly, not only are there issues with *Shigella* species, *Streptococcus* species, and *Haemophilus haemolyticus*, but *S. pneumoniae* and *Candida albicans* usually present as three or more possibilities. Thirdly, the database may have only one organism as the fingerprint for the spectra. Lastly, there are no clear guidelines for quality control or preventative maintenance.

### FDA Approval

Currently, our laboratory has the SARAMIS™ database. This database has over 1200 organism identifications. It utilizes a SuperSpectra and ReferenceSpectra. On average the SuperSpectra uses 8 isolates/species of an organism to create the spectra used by the instrument to compare the unknown organism. The ReferenceSpectra may use only one strain of an organism as a fingerprint for identification. SARAMIS™ is an open system — meaning laboratories can add their own organism strains specific for their patient population.

FDA approval was announced for the Vitek® MS in September 2013. The new IVD (In Vitro Diagnostics) database has 750 organisms that can be identified. However, only 193 organisms have been approved by the FDA to be reported. Even though the user has access to the other 562 organisms in the

### Table V. Comparison of the RapID™ NH System and the Maldi-ToF System

<table>
<thead>
<tr>
<th>Organism (n)</th>
<th>Species</th>
<th>Genus</th>
<th>No ID</th>
<th>Incorrect ID</th>
<th>Species</th>
<th>Genus</th>
<th>No ID</th>
<th>Incorrect ID</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>N. meningitidis</em> (11)</td>
<td>11 (100)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>11 (100)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>N. gonorrhoeae</em> (10)</td>
<td>10 (100)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>10 (100)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>N. sicca/subflava</em> (8)</td>
<td>8* (100)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>8 (100)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>N. lactamica</em> (2)</td>
<td>2 (100)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2 (100)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>N. cinerea</em> (2)</td>
<td>2** (100)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1 (50)</td>
<td>1 (50)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>N. macaca</em> (1)</td>
<td>-</td>
<td>-</td>
<td>1 (100)</td>
<td>-</td>
<td>1 (100)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>M. catarrhalis</em> (10)</td>
<td>10 (100)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>10 (100)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>M. nonliquefaciens</em> (3)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>3 (100)</td>
<td>3 (100)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>M. osloensis</em> (2)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2 (100)</td>
<td>2 (100)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>M. lacunata</em> (3)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>3 (100)</td>
<td>3 (100)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Total (52)</td>
<td>43 (83)</td>
<td>-</td>
<td>1 (2)</td>
<td>8 (15)</td>
<td>49 (94)</td>
<td>2 (4)</td>
<td>1 (2)</td>
<td>-</td>
</tr>
</tbody>
</table>

1Number (%) agreement. 16s ribosomal RNA sequencing as reference method

*RapID™ NH does not distinguish between N. sicca/subflava. Maldi identified all as N. subflava.

**Further biochemical testing was necessary
IVD database, they are not FDA approved for reporting. If the result is a non-approved FDA organism, the identification will be reported with a symbol to indicate that it is not FDA approved. The user has the option as to whether or not the organism needs further testing. To report the organism without further testing, the result will need a disclaimer.

The IVD database is a closed system. Only those organisms in that database can be identified. It will identify aerobic and anaerobic bacteria and yeast. A newer update will have moulds and mycobacteria. A laboratory will not be able to add organisms to the IVD database.

Even better, the Vitek MS™ Plus is a combined system of the SARAMIS™ and IVD databases. The Vitek MS™ Plus user will have access to both databases. The IVD gives the user the FDA cleared organisms, as well as the other 562 common organisms. The SARAMIS™ gives the user access to spectra for over 1200 organisms. SARAMIS™ identifies Mycobacteria, Nocardia and fungi now. Clinical laboratories may only need the IVD database, but research laboratories will appreciate the full functionality of a closed system with the ability to add spectra to the SARAMIS™ database, as needed.

Also, QC is better developed in the new software. If the program does not find the desired peaks of the E. coli ATCC 8739, the calibration will fail. If the calibration fails, no spots will be analyzed. If calibration is successful, but the identification does not equal 99.99%, the quality control will fail. Quality control serves as verification that there were no technical drifts or environmental changes during the testing.

Preventative maintenance is very clearly defined with the Vitek MS™ Plus. The instrument is air cooled. The temperature should not be >26 °C. A sensor is located in the instrument to monitor the temperature daily. If the temperature is >26 °C, the acquisition station is notified. Weekly, the silica beads are checked. Silica beads dry the air that enters the vacuum. Moisture in the instrument increases the time it takes for the instrument to create the vacuum. Blue silica beads indicate they are still dry. Pink silica beads indicate they need changed. Change the silica beads and recharge as needed, as well as clean the acquisition and prep station monitors, calibrate the touchscreen, and clean the adapter, O-ring, and seal around the door.

BioMerieux will do semiannual preventative maintenance, as well as yearly maintenance. Fine-tuning is done at the yearly maintenance. If the old database is used, both databases must be fine-tuned.

Currently, our laboratory is anxiously awaiting the new upgrade for the Vitek MS™ Plus. A new quality assurance, albeit shorter and less extensive, will be planned and executed for the IVD database. If the quality assurance proves to be successful, work flow throughout the laboratory will be changed, and more technologists will be trained. How will we accomplish that?

- Utilize the same systematic approach to testing each class of organisms.
- Concentrate on testing organisms that did not identify well on the SARAMIS™ database originally, i.e. Streptococcus pneumoniae and Candida albicans.
- Add anaerobic organisms to the selection.
- Test moulds and Mycobacteria with SARAMIS™.
- Train technologists that perform routine organism identification.

References


Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry in Clinical Microbiology Clinical Infectious Diseases August 2013 57:4 564-572

Matrix-assisted laser desorption ionisation time-of-flight mass spectrometry for identification of clinically significant bacteria that are difficult to identify in clinical laboratories J. Clin. Pathol. October 2013 0:2013 jclinpath-2013-201818v1-jclinpath-2013-201818


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