Methicillin-resistant *Staphylococcus Aureus*: A Seek and Destroy Approach

Canadian hospital spending has soared to in excess of $100 million a year for the treatment of patients with hospital acquired antibiotic resistant infections. In a special series covering the “Invisible Dangers” of Methicillin-resistant *Staphylococcus Aureus* (MRSA), Clostridium difficile and Vancomycin resistant enterococci (VRE), the CBC reports that patients infected with these nosocomial bacteria spend on average an additional 39 days in hospital recovering. MRSA incidence alone has increased tenfold in less than a decade reaching what some have called the tipping point for disease crisis. As an infection becoming dramatically harder to treat, we can expect both the costs associated with MRSA (estimated at $59 million annually in 2001) and hospital waiting lists to increase as patients utilize beds recovering from this infection.

As a worldwide crisis not limited to Canada there have been reports that the Netherlands and Denmark hospitals are winning their fight against antibiotic-resistant infections, thanks to strict patient isolation policies and a “seek-and-destroy” approach to infection control. All foreign patients who have been treated at hospitals outside the Netherlands are isolated to private rooms at Dutch hospitals until they can be “tested” and shown to be free of infections such as MRSA and VRE. This demonstrates a distinct and valuable relationship between laboratory services and infection control.

Early screening of patients for MRSA carriage can contribute to effective infection control programs. However, current or traditional laboratory techniques used to detect MRSA require a time intensive culture step involving the isolation of pure colonies followed by either oxacillin susceptibility testing, detection of the meca gene or detection of the penicillin binding protein (PBP 2a) encoded by the meca gene. This methodology puts the time to identify MRSA carrier status at a minimum of 16 hours and a
Methicillin-resistant *Staphylococcus Aureus* continued from page 1

The rapidity at which *S. aureus* infections can spread, a faster method that identifies MRSA carriage on the day of admission would provide a much more compelling advantage for infection control programs.

The IDI-MRSA™ is a novel laboratory technology that utilizes real time PCR (polymerase chain reaction) to detect the unique molecular sequence of MRSA directly from the patient swab providing results within one hour. Highly sensitive, half of the identified sequence comes from the *S. aureus* chromosome and the second half is within the genetic element carrying methillin resistance (the SCCmec cassette specimen) conferring double specificity.

This unique IDI-MRSA™ detection system offers the critical levels of rapidity and performance required for optimal management of MRSA colonized patients. Distinct cost advantages are realized as isolation periods are limited during the screening process and risk of infection is detected prior to admission. Unquestioned advantages come from rapid identification should the organism appear on hospital wards.

Detailed information on the IDI-MRSA™ technology and advances in MRSA detection are available through Somagen Diagnostics.

Health Canada approves this technology for diagnostic use and has not limited the procedure as research only. Testing for additional organisms including VRE on the IDI platform is in currently in development for release 2006.

1 Infect Control Hosp Epidemiol 2001;22:99-104

**New Streck Cyto-Chex BCT- Stabilizing Whole Blood for up to 7 days for Immunophenotyping Procedures**

As with any testing done in the laboratory, ensuring reliable results begins with optimizing the sample for analysis. In the area of flow cytometry, a technology that determines the types and quantities of antigens on the surface of white blood cells, this becomes very critical. These antigens present on the cell surface signal activation of the immune response system, and the technique of identifying these antigens through their cluster of differentiation (CD) patterns have become an essential part of diagnosing and monitoring a wide range of conditions, including transplant acceptance, cancers, and infectious diseases. The CD4+ lymphocyte count, in particular, has emerged as the gold standard for monitoring HIV/AIDS antiretroviral treatment.

The key challenge to the clinical lab arises from the fact that these surface epitopes can begin to deteriorate very rapidly, leading to sample integrity issues. Often within 48 hours, whole blood samples collected into conventional K3EDTA tubes display loss of stability, resulting in variable antibody binding and leading to inaccurate measurement of CD markers. The critical time line involved in analyzing patient specimens can become costly, forcing weekend / holiday staffing calls, expensive sample transport choices, and patient redraws.

To address the WBC preservation problem, Streck Laboratories applied its cell stabilization technology, developed over many years, to the production of a new blood collection tube, Cyto-Chex®BCT. These standard size, vacuum assisted tubes are specifically designed for flow cytometry applications, effectively preserving surface epitopes for up to 7 days. The 13x75mm tube is configured for a 5mL draw, and includes 60µL of concentrated reagent with a cell preservative and anticoagulant. While stabilizing whole blood samples at room temperature to accommodate batching or shipping needs, the small volume of reagent allows direct analysis from the collection tubes without the need for dilution corrections.

Streck Laboratories selected the HIV marker panel defined by the CDC for studies that included 20 HIV-positive and 25 non-infected donors. All samples were maintained at room temperature throughout the 7 days of the studies. Samples drawn into K3EDTA tubes were used as controls for both the infected and non-infected groups. Analysis was performed on the BD FACSCalibur flow cytometer. The absolute counts of CD3+, CD4+, and CD8+ lymphocyte subsets in whole blood drawn into Cyto-Chex BCT were compared at 7 days post-draw to the values obtained 6 hours after collection in a K3EDTA tube. The results for typical patients are shown in figure 1. For each marker in both normal and HIV donor blood, the cell counts at 7 days in the Cyto-Chex BCT are nearly identical to those at 6 hours in EDTA. When a statistical comparison of 6 hours EDTA versus 7-day BCT data for all 25 normal and 20 HIV-infected patients is carried out, R2 values shown in table 1 convincingly demonstrate that for the markers tested, the Cyto-Chex BCT can preserve peripheral whole blood immune epitopes for up to 7 days.

Of the CD3, CD4, CD8, CD19, CD45 and CD 16+56 immune epitopes tested, all showed uncompromised results up to 7 days when drawn into the Cyto-Chex BCT. In normal and infected donor types, 7-day Cyto-Chex BCT results are indistinguishable from 6-hour K3EDTA results based on correlation coefficients.

Table 1  

<table>
<thead>
<tr>
<th>Epitope</th>
<th>Normal</th>
<th>HIV</th>
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</thead>
<tbody>
<tr>
<td>CD4</td>
<td>0.9714</td>
<td>0.9892</td>
</tr>
<tr>
<td>CD3</td>
<td>0.9812</td>
<td>0.9799</td>
</tr>
<tr>
<td>CD8</td>
<td>0.9896</td>
<td>0.9619</td>
</tr>
<tr>
<td>CD19</td>
<td>0.9925</td>
<td>N/A</td>
</tr>
<tr>
<td>CD16+56</td>
<td>0.9609</td>
<td>N/A</td>
</tr>
<tr>
<td>CD45</td>
<td>0.9779</td>
<td>N/A</td>
</tr>
</tbody>
</table>

Cyto-Chex BCT demonstrates a simple but profound technological advance in blood collection that enables both improvement in both sample integrity and laboratory workflow.

Reference:  
Is it Allergy? Or not...

Studies have shown that up to two-thirds of people who think they are allergic, are actually not. Adding to this challenge is that patients and physicians often rely on the clinical response to over the counter or prescription antihistamines as an indication of allergic disease.

Appropriate treatment of the allergic patient begins with an accurate assessment of specific IgE antibodies. This can be achieved through a simple blood test.

When atopy (personal or familial tendency to produce IgE antibodies in response to low doses of allergens) is present, avoidance is considered the number-one therapeutic approach by leading allergy associations. Newer anti-IgE medication may also be beneficial, but guidelines recommend specific IgE testing prior to use, as this medication works only on the truly allergic patient.

Allergy testing via ImmunoCAP

Pharmacia Diagnostics has long been the leader in allergy testing. As developers of the first RAST testing system, their technology has evolved and matured into a reliable standard. This new and improved, next generation ImmunoCap™ system, is now optimized for the special requirements involved in the task of making precise measurements of IgE antibodies.

Only by perfecting each component in the test system, is it possible to achieve a high degree of precision in an immunoassay as complex as allergen-specific IgE measurements. The ImmunoCap™ systems, from Pharmacia are the result of a continuous development experience spanning more than 30 years.

Allergen-specific IgE is NOT just another Immunoassay!

Assaying IgE antibodies is considerably more complicated than performing most other immunoassays and require considerable experience in this specific area. There are a number of complicating factors such as:

• The concentration of IgE antibodies in the blood is extremely low in comparison to most other substances assayed (i.e IgG), even in highly sensitized individuals.
• Each main allergen (i.e tree pollen, food or dander from a pet) contains a large number of different allergenic components (proteins). The assay must therefore be sensitive enough to capture antibodies to all relevant components, even if these are present only in a very minute amount in the main allergen.
• The assay must have high enough capacity to bind “all” IgE antibodies to an allergen in competition with other antibodies with the same specificity, from other immunoglobulin classes present in higher concentration.
• Most allergen sources are complex mixtures of biological material. Allergen sources of one and the same species often show variation caused by geographical differences, seasonal variations etc. To achieve a precise and reproducible test system, total control of the allergen source material is necessary, both in content and in allergenic activity, thus reassuring lot to lot reproducibility.

Proven Reliability…
the optimal solid phase

Quantitative and reliable measurement of specific IgE depends on the excess availability of relevant allergen components. To provide quantitative results and high sensitivity, it is crucial that the solid phase has sufficient capacity to bind all clinically relevant proteins of the allergen, including those present in low amounts in the allergen source material.

ImmunoCap™ solid phase remains unrivalled as an optimal solid phase for allergy diagnostics. The hydrophilic, highly branched, three dimensional cellulose polymer provides an ideal microenvironment for allergens, binding them irreversibly while maintaining their native structure. This cellulose structure provides an extremely large inner surface, ensuring the coupling of a maximum amount of allergen material.

ImmunonCap™ has an extremely high total binding capacity, achieved through a high binding capacity per mg cellulose in combination with an optimal amount of cellulose in each solid phase. This ensures binding of all relevant antibodies, regardless of antibody affinity, still giving low non-specific binding. This allows high sensitivity of very low concentrations of IgE antibodies to be detected. The excellent stability of ImmunoCap™ allergens (2 years for most allergens) is also due to the beneficial chemical characteristics of the solid phase.

Reliance on response to allergy medication seems an inappropriate means of diagnosis in 2005. In addition, the contraindications listed for most medications, over the counter or prescribed provide a compelling reason to pursue proper diagnostic certainty prior to treatment. The ImmunoCap™ blood test provides a precise and easy vehicle to help get the correct answer.

2 American Academy of Allergy, Asthma, and Immunology (AAAAI) and the American Association of Otolaryngic Allergy (AAOA).
Frequently emergency departments see patients who present with altered mental status and other non-specific symptoms. This may be the result of the ingestion of drugs or multiple other causes. The range of symptoms and multitude of causes create a compelling argument for rapid response diagnostics. In many of these time sensitive conditions, the ability to quickly differentiate drug induced causes from those non-drug induced allows the Physician to get the patient on the appropriate care pathway.

Biosite™ Diagnostics introduced the Triage® Drugs of Abuse Panel in 1992 which quickly became a standard of practice in assessing patients suspected of drug induced symptoms. As the first to offer a urine based TCA method they continue to pioneer the rapid drugs of abuse screening market by developing the first qualitative urine Acetaminophen assay Triage® TOX+APAP (N-acetyl-p-aminophenol) for use on the Biosite Triage® Meter. This highly sensitive assay will be able to detect as little as two regular strength Tylenol as early as 1/2 an hour after ingestion. Since 80-95% of all Acetaminophen screens are negative, Triage® TOX + APAP will provide a rapid, real time rule out.

Biosite’s commitment to rapid diagnostics continues further with the addition of the Triage® MeterPlus and symptom-defined testing panels expanding the ability to provide ER physicians with the definitive results they need to quickly make sense of non-specific yet critical and time sensitive symptoms. Panels include the Triage® TOX Drug Screen, Triage® Cardiac Panel, Triage® Profiler (includes Cardiac markers and BNP) and soon to be available Triage® Shortness of Breath Panel (to include Cardiac Markers, BNP and D-dimer). Enhanced software features provide maximum user defined testing parameters.

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