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In This Issue

[2014 CDID Officers Election Announcement](#)

[Poster Walk Report](#)

[CDID Mixer Report](#)

[Technical Report](#)

[Technology Report](#)

[Division Speakers Bureau](#)

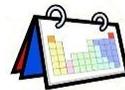
IMMUNOTES

Table of Contents



Message From the Chair

CDID update from Division Chair, Steve Binder



2014 CDID Officers Election Announcement



Poster Walk Report

Dr. Yan Zhang reports on the "Poster Walk" that took place in the Annual AACC meeting in Houston, TX



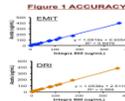
CDID Mixer Report

Dr Evan Ntrivalas presents the highlights of the 2013 CDID Mixer during the Annual AACC meeting in Houston, TX



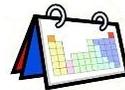
Technical Report

Dr Lori Millner, one of the award winners for outstanding research in Clinical and Diagnostic Immunology in the 2013 AACC meeting in Houston, TX, presents a technical report, based on the results of her abstract. The title of the technical report is "Subtyping circulating tumor cells in breast cancer for personalized therapeutics"



Technology Report

Dr. Yan Zhang presents a technology report that describes the evaluation of hemolysis, icterus and lipemia on acetaminophen assays



Division Speakers Bureau

Information on the initiative by the Division Management Group of the AACC

Message from the Chair



As a chair of the Clinical and Diagnostic Immunology Divisions for 2012-13, it is my pleasure to greet all the members of the division. We have a number of interesting updates for you in this issue of ImmunoNotes and I appreciate the fact that you have taken the time to read them. If you wish to contribute to future issues, or if you have ideas for articles, please contact me directly. You can send e-mails to any divisions officer via the CDID website by clicking on the *Division Officers link*.

Personalized Medicine. The AACC has recognized the importance of Personalized Medicine (PM) by creation of a new division. This division held its first mixer at the Houston 2013 AACC meeting, and there was a lively crowd. While much of the focus with personalized medicine is with DNA assays, there are many examples of protein assays that directly guide patient care, including the PM's "poster child" assay---Her2/Heu.

Recently the Commissioner of the FDA spoke about the importance of personalized medicine and announced the release of new report.

http://blogs.fda.gov/fdavoices/index.php/2013/11/personalized-medicine-the-future-is-now/?source=govdelivery&utm_medium=email&utm_source=govdelivery

2014 Elections. We recently distributed a ballot to all active CDID members, with our nominations for officers in the divisions, as well as some changes in the bylaws. I hope you were able to vote in the election. You should note that we slightly modified the mission statement, which now reads like this:

The mission of the Clinical and Diagnostic Immunology Divisions is to encourage the study, advance the science, and improve the practice of diagnostic immunology and the use of immunological techniques in clinical chemistry.

In support of this mission, we have organized an outstanding symposium for the 2014 AACC meeting, we are creating another certificate program, and we are working to increase our collaboration with other divisions and other groups who share our interests. If you have ideas about how we can "advance the science" and "improve the practice", please get in touch.

CDID Mixer Report

by Evan Ntrivalas, CDID Communications Officer



The CDID mixer was held on Tuesday, July 30 at 5:30 pm, followed by the business meeting at 6:30 pm. This was a networking event in which CDID members had the opportunity to meet old friends and make new ones.



Left to right: Dr Vernon Mustoe, Beckman Coulter representative;
Dr Gyorgy Abel; Dr Eleftherios Diamandis

The CDID mixer is also the place where individuals are recognized for their contribution in their field. One of these awards is the **Carl R. Jolliff Award for Lifetime Achievement in Clinical and Diagnostic Immunology**. This award was instituted in 2006 by the CDID in memory of Carl R. Jolliff. Carl had an exemplary career in both clinical service and education and this award recognizes a member of the AACC with an outstanding achievement in either of those areas.

The 2013 Carl Jolliff Award was given to **Dr. Eleftherios Diamandis**. **Dr. Diamandis** is division head of Clinical

Biochemistry in the department of Pathology and Laboratory Medicine at Mount Sinai Hospital; biochemist-in-chief at the University Health Network and Toronto Medical Laboratories, and division head of Clinical Biochemistry in the department of Laboratory Medicine and Pathobiology at the University of Toronto in Ontario, Canada. He extensively contributed to immunodiagnosics with cancer biomarker discovery and immunoassay development; taught and mentored scores of students, fellows, and other young colleagues nationally and internationally; has served for decades as laboratory director, and provided leadership and service on multiple committees and panels over the past three decades.

Lastly, CDID offers annually two poster presenters an award for outstanding research in clinical and diagnostic immunology. This year the winners of this competition were:



Left to right: Dr Julia Drees; Dr Steve Binder, CDID chair; Dr Lori Millner

Julia Drees from Kaiser Permanente Regional Laboratories, Berkeley, CA for her work *"Four Commonly Utilized Immunoassays Fail to Detect TSH in a cohort of Euthyroid Patients: Are TSH Assays Hyper-Selective?"*

Lori Millner from University of Louisville, Louisville, KY for her work *"A Highly Sensitive and Specific Method for Characterization of Circulating Tumor Cell Subtypes in Breast Cancer Patients"*

The CDID mixer ended with the business meeting where CDID Officers presented the current happenings and future initiatives of the Division.

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2014 CDID Officers Election Announcement

Dear CDI Division Members,

Voting is now open for the division's 2014 election. You can access the online ballot on the [division's web page](#).

You will need to log-in using your AACC member ID and password to vote.
Voting for this election closes on December 16, 2013.

Please Note: Per AACC bylaws, you must be a full or emeritus member to vote.

Regards, Kelsey Blake AACC Membership Coordinator

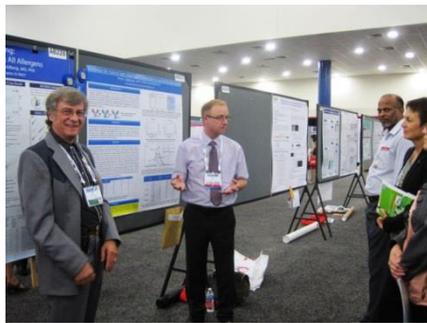
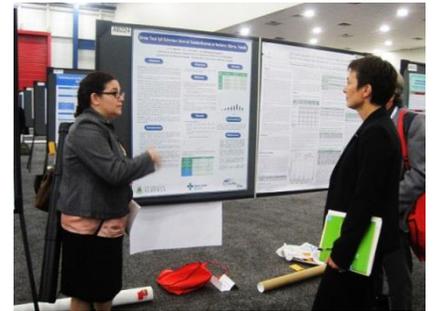
kblake@aacc.org

Poster Walk Report



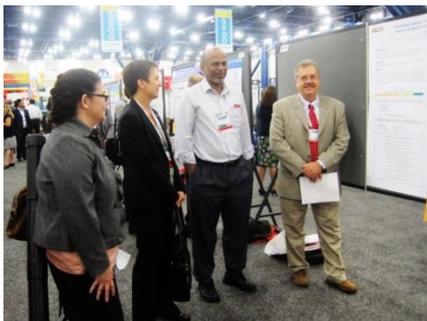
The AACC divisions are piloting an initiative called "Poster Walk" in which small groups of division members will visit some of the posters at the annual meeting together with a volunteer who will select several of the most interesting abstracts and moderate a discussion of the findings. Our CDID Division was privileged to be part of this initiative and our "poster walk" was held on Tuesday July 30, 2013 from 4-5pm with Dr. Yan Zhang, our current division treasurer.

The idea was that the person who leads the "Poster Walk" chose several abstracts that he/she thinks are the most interesting and lead any members (limit to 10 for this initiative) who show up at the appointed time to these selected posters. The person had to summarize the abstract and point out what were the major take-home points and encouraged questions and discussion among the group. Certainly if the presenter was there, introduced him/her and explained what they were doing.



During our one-hour "Poster Walk", we had four posters discussed with two award-winning posters (right, lower picture), and two other highly recognized posters that were revealed during our poster award review process (left).

We had good attendance and a great discussion was generated during this event. During the session, we received some very constructive comments and suggestions for this initiative which had been passed on to the Division Management Group leadership for consideration.



The "Poster Walk" is still in its infancy and most likely it will continue. The division management group plans to expand this idea next year and may need to have an RSVP mechanism. Please keep this in mind when you prepare for your 2014 AACC meeting.

Technical Report

Subtyping Circulating Tumor Cells in Breast Cancer for Personalized Therapeutics



Lori M. Millner, Kevin Goudy, Mark W. Linder, Roland Valdes, Jr. Department of Pathology and Laboratory Medicine, School of Medicine, University of Louisville, Louisville, Kentucky Enumeration of circulating tumor cells (CTCs) in blood is used in breast cancer patients as an independent predictor of outcome. Present methods do not distinguish subtypes and only detect epithelial-type CTCs. This is significant because CTCs experience epithelial to mesenchymal transition (EMT), a process that increases motility, disease progression, and decreases epithelial marker expression. These mesenchymal CTCs likely pose a greater threat to the patient than epithelial CTCs due to their highly aggressive and motile nature.

The goal of this project was to establish the feasibility of enriching, detecting, isolating and performing molecular analysis on single cells after being spiked into whole blood. We did this using a model composed of 4 heterogeneous breast cancer cell lines and a method for capturing and characterizing distinct CTC subsets regardless of EMT status. The 4 breast cancer molecular subtypes were used: luminal (MCF-7), HER2 (SK-Br-3), basal-like (HCC1954), and claudin-low (MDA-MB-231) and were chosen to represent patient CTC heterogeneity. 25,000 or 2,500 cells of each cell line were combined and then identified using a combination of antibodies including HER2, EpCAM, and CD44.

The specificity (separation efficiency) for each subtype was $67.3\% \pm 7.1$ (\pm SE) (HCC1954), $91.7\% \pm 9.7$ (MCF-7), $57.3\% \pm 8.7$ (MDA-MB-231), and $100\% \pm 19.0$ (MCF-7). The overall separation efficiency was $79.4 \pm 5.9\%$ (\pm SE). Spiking experiments of a single mesenchymal-like cell line that does not express EpCAM were conducted in whole human blood, and a sensitivity (percent recovery) of $84.9 \pm 14.6\%$ (\pm SE) was achieved.

Single cells were captured using the DEPArray, a novel separation technology that relies upon capturing cells in individual dielectrophoretic cages. These cages are then individually manipulated to sort single cells with 100% purity. A viewer allows the cells selected to be positively identified and cell identify and integrity is visually monitored throughout the sorting process. These single cells were then interrogated to analyze RNA expression of multiple genes. We achieved 100% sorting efficiency and 100% amplification of all sorted cells. Expression of a house-keeping gene and at least one target was observed for 100% of the cells sorted.

High percent recovery of a spiked mesenchymal-like breast cancer cell line into whole blood was achieved. The combination of antibodies has high separation efficiency with 2 of the 4 cell lines. Enrichment processes and antibody selection are being optimized to improve specificity of all 4 subtypes. The single cell sorting performance of the DEPArray was 100% and molecular analysis on the single cell level was conducted. Single cells from all 4 subtypes were analyzed and expression of a house-keeping gene and at least one target gene was observed for each cell. This data indicates that phenotypically diverse CTCs are capable of being subtyped and characterized on a single cell level. This information will improve prognostic capabilities and will allow therapies to be individually tailored to address each patient's CTCs.

Technology Report

HIL interferences on Acetaminophen Assays: Evaluation of Syva EMIT® and Microgenics DRI® and Roche Assays

Yan Zhang, University of Rochester Medical Center. Acetaminophen is one of the most popular non-prescription pain relievers with a therapeutic range between 10 and 20ug/dL. However, its toxic side effects for patients on chronic acetaminophen therapy and large dose injection on liver damage have been well documented. Accurate measurement of serum acetaminophen levels plays a vital role in the proper management of acetaminophen overdoses and, more importantly, prognosis and treatment with antidotes to avoid hepatic necrosis. We performed a study to evaluate the Syva EMIT and Microgenics DRI acetaminophen assays on Roches cobas c501 analyzer in comparison to the Roche acetaminophen assay as COBAS INTEGRA 800 system with a focus on the hemolytic, icteric, and lipemia interference effects on the assay performance.

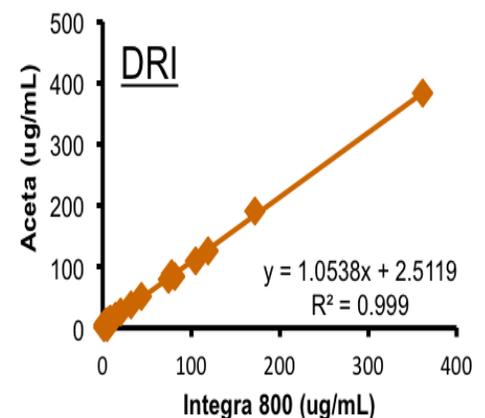
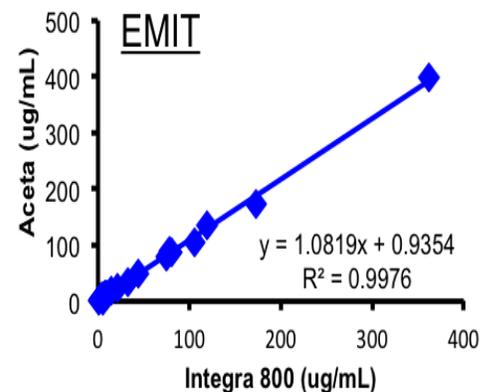
Both Syva EMIT and Microgenics DRI acetaminophen assays are homogenous enzyme immunoassay techniques. The assays are based on the competition between the acetaminophen in the patients' sera and the enzyme glucose-6-phosphate dehydrogenase (G6PDH) labeled acetaminophen with the acetaminophen-specific antibodies (at a fixed amount) in the ready-to-use reagents. The G6PDH enzyme activity is determined by spectrophotometric measurement at wavelength of 340nm by measuring the increased level of NADH converted from NAD in the reagents. The complex of acetaminophen-G6PDH conjugated with the drug specific antibodies inhibits the enzyme activity, which results in decreased absorbance at 340nm when no acetaminophen is present in the serum.

The user-defined parameters were added to cobas c501 analyzer for both assays. Imprecision was evaluated using three levels of controls purchased from BioRad ten times a day (within-day precision) or once a day for 25 days (between-day precision). Accuracy was examined by comparing acetaminophen levels from EMIT and DRI assays from 30 patient sera (acetaminophen level up to 360 ug/dL) to the results from COBAS INTEGRA 800 system. Linearity was calculated based on average results from 5 serum samples at acetaminophen levels of 0, 50, 100, 150, and 200ug/dL measured

in duplicate and the assay performance at 240ug/dL was also tested. Limit of detection (LOD) and limit of quantification (LOQ) was performed by measuring a blank serum sample five times and serum sample at 1, 2, and 2.5ug/dL. LOD was determined by the mean plus three times standard

deviation for the blank serum and LOQ was determined by the lowest acetaminophen concentration which gave no more than 10% coefficient of variation (CV). The investigation of hemolytic (H), icteric (I), and lipemia (L) interferences was carried out by spiking (10% total volume) concentrated hemolyzed sample, bilirubin, and intralipids into serum of

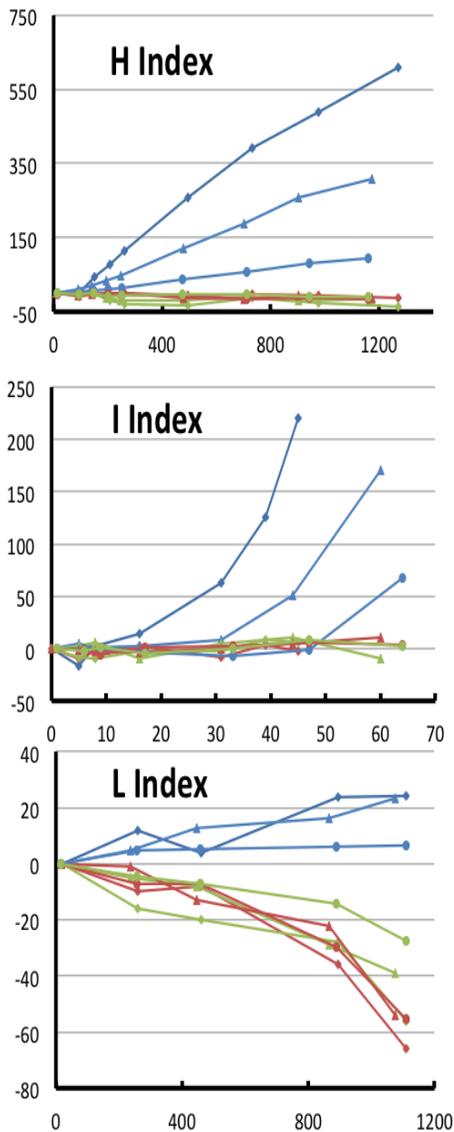
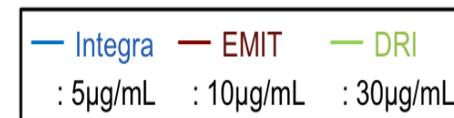
Figure 1 ACCURACY



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acetaminophen levels at 5, 10, and 30ug/dL to achieve H/I/L indices up to 10000, 60, and 10000, respectively.

Figure 2. INTERFERENCE



Our study indicated that the within-day precision for EMIT and DRI at low, medium, and high levels of controls were 5.3%, 2.5%, 4.5%, and 7.3%, 4.9%, 5.4% CV, respectively. The between-day precision for both assays at the three levels of controls were 3.3%, 4.2%, 3.9%, and 7.5%, 5.9%, 6.8% CV, respectively. The correlation between EMIT (y) and INTEGRA (x) was $y = 1.08x + 0.94$ ($R^2 = 0.9976$) and the correlation between DRI (y) and INTEGRA (x) was $y = 1.05x + 2.51$ ($R^2 = 0.9990$) with the highest concentration at 360ug/dL measured on INTEGRA (Figure 1).

LODs for EMIT and DRI were 0.42 and 3.04ug/dL, while LOQs for both assays was 1.14 and 3.09ug/dL. The linear range for EMIT was tested up to 240ug/dL with the same regress parameters as up to 200ug/dL with slope of 0.99 and intercept of 2.71 ($R^2 = 0.9993$). DRI assay was linear up to 200ug/dL with slope of 1.05 and intercept of 1.15 at ($R^2 = 0.9998$).

No hemolytic or icteric interference was observed for EMIT and DRI assays at all levels of acetaminophen for H index up to 10000 and I index up to 60, although acetaminophen assay on INTERGRA was affected significantly at nearly all acetaminophen concentrations for H index as low as 100 (except acetaminophen = 5ug/dL at H = 100 wasn't affected) (Figure 2). It didn't appear to us that the Roche assay was affected by icteric condition until it reached to I = 20 at acetaminophen = 5ug/dL or I reached to about 30 and 50 at acetaminophen = 10 and 30ug/dL. The lipemia level slightly interfered with Roche acetaminophen assay with the higher L index producing higher assay results. To our surprise, however, both EMIT and DRI assays were affected by high L index in a negative fashion with more effect at higher acetaminophen levels.

In conclusion, the user-defined EMIT and DRI acetaminophen assays were evaluated on cobas c501 system and imprecision, accuracy, LOD, LOQ were established. The hemolytic, icteric, and lipemia interference analysis at acetaminophen 5, 10 and 30ug/dL indicated that these two assays were not influenced by H/I indices up to 10000 and 60 respectively, although they were more prone to lipemia interference especially at acetaminophen 10ug/dL and more so at acetaminophen 30ug/dL. In comparison, Roche INTEGRA acetaminophen assay was dramatically affected by hemolytic interference, and highly affected by bilirubin levels at I index beyond 20, 30, and 50 for acetaminophen levels at 5, 10, and 30 ug/dL, respectively. It, however, didn't

appear to be affected by L index up to 10000 on tested acetaminophen levels.

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Division Speakers Bureau

The Division Management Group of the AACC has created a Division Speakers Bureau for division members that would be interested in providing a scientific lecture for a Local Section Event. This will facilitate increased collaboration between Divisions and Local Sections.

If you are interested in participating in this initiative please provide the following information:

Name of Speaker:

E-mail:

Areas of Expertise:

Potential Lecture Titles:

You can return this information to [Dr Evan Ntrivalas](#), CDID Communications Officer.