ANIMAL CLINICAL CHEMISTRY DACC NEWS

AACC Better health through laboratory medicine.

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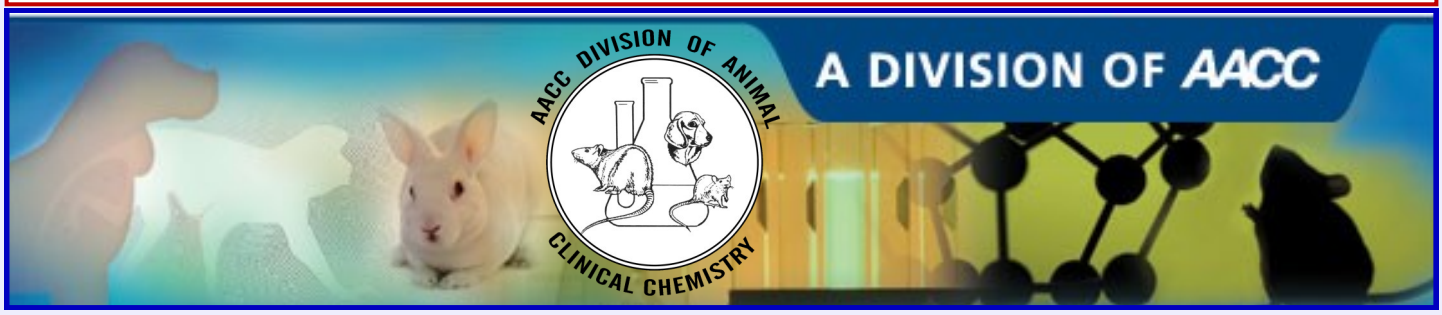
2019 Summer Edition



Message from the Chair

The Division of Animal Clinical Chemistry has been instrumental in bringing together scientists working in interdisciplinary fields of industry, academia, and governmental laboratories for the last several decades. In the past few years, there have been seismic changes in how laboratory medicine is conducted across the industry. Priorities change. Business model changes. Budget allocation changes. It would be ingenuous not to face the reality that these changes in the industry influence us, as professionals in clinical laboratory science. As the cliché goes, 'Change brings Opportunity'. Now, more than ever, it is time for our membership to connect, introspect, brain storm together and come up with ideas that can facilitate our storied organization to be change-agile and transform in structure, ways of engaging the membership so that we can continue to influence improvements in clinical laboratory science. The organization is only as strong as it members. I would like to take this opportunity to extend this invitation to our membership to voice your thoughts, ideas and suggestions to help this goal. I look forward to hearing from all of you.

In This Issue:

Annual Meeting Program

Annual Meeting Poster Titles

Award for Outstanding Contributions

Lunch and Learn Session

We have an exciting agenda for the DACC events at the AACC annual meeting including keynote presentation at Lunch and Learn by Denise Bounous, followed by talks by DACC veteran, Doug Thudium and an international speaker, Fidelis Olumese from Nigeria. Please join us at the awards reception to honor the recipient of the esteemed DACC award for outstanding contributions to animal clinical chemistry.

We also have an upcoming Fall face-to-face meeting, scheduled for Oct 2019. Details to be followed. As we continue to work on this important opportunity to engage the membership, please contact me with your ideas—Do you have a subject in mind that you would like to learn more about? What hot topic do you want to hear about?

I look forward to seeing all of you in a few weeks!

Madhu Sirivelu

DACC *NEWS* Editorial Staff

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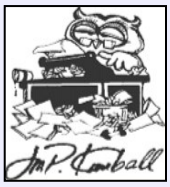

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Membership
Volunteers Needed!

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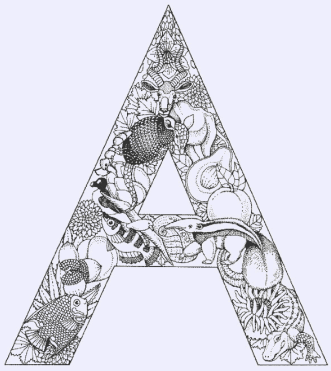
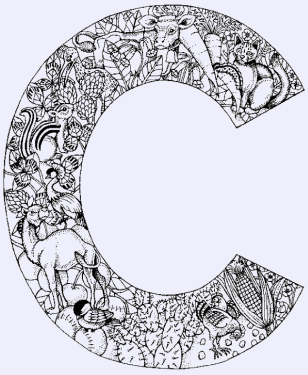
DACC Supporters

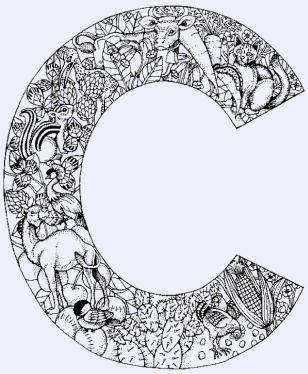
The Division of Animal Clinical Chemistry (DACC) welcomes all persons interested in advancing clinical laboratory science in animals by promoting and encouraging the study, exchanging knowledge, and expanding and improving the practice of clinical laboratory science in animals.



Our division provides a forum for sharing of information relevant to methods of analysis, reference intervals for different animal models, selection of appropriate species for research, and feasibility of transferring relevance of the data to human medicine. The DACC supports the development of methods to detect specific target organ effects and cellular changes associated with xenobiotic agents and/or disease processes.



The Division publishes this newsletter and sponsors regional and national meetings as a means for promoting education, research, and service. We encourage members to pursue professional interactions with AACC as well as within other professional groups. Our membership represents scientists working in interdisciplinary fields, industry, academia, and governmental laboratories.



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SUNDAY– AUGUST 4, 2019

6:45 PM - 8:30 PM

AACC Opening Mixer and Division Networking Event
Grand Plaza, Anaheim Convention Center
Open to All Registrants. Enjoy Networking with DACC Members!

TUESDAY - AUGUST 6, 2019

9:30 AM - 5:00 PM

Animal Clinical Chemistry Posters
Anaheim Convention Center, Hall A

11:00 AM - 3:00 PM
Lunch Provided

DACC General Business Meeting and Lunch & Learn Session
Platinum 10 (Lobby Level), Anaheim Mariott
Generously Supported by Sysmex America, Inc
Open to All Members! Come Join in the Planning of DACC Activities!

Register Soon! Please let us know even if you are thinking about joining us.
Please **RSVP** to : madhu.sirivelu@gmail.com

Keynote address— Evolution of Clinical Pathology in the Pharmaceutical Environment
Denise Bounous - *Bristol-Myers Squibb*

Comparison of Commercially Available Multiplex Rat Cytokine Methods
Doug Thudium - *Merck Research Laboratories*

Effects of *Uvaria chamae* Root Extracts on Cortisol, Insulin, and Testoterone in Normal and Streptozotocin-Induced Diabetic Rats
Fidelis Olumese, College of Medical Sciences, University of Benin, Benin, Nigeria

Verification of the Sysmex XN-V Multispecies Hematology Analyzer for Use in Mouse Sample Analysis
Arlene Love - *Bristol-Myers Squibb*

7:30 PM - 9:30 PM
Dinner Reception

DACC Award Presentation and Reception for the Annual Award for Outstanding Contributions to Animal Clinical Chemistry

Sponsored by DACC and Generously Supported and Hosted by Siemens Healthineers
Location TBD

TUESDAY – AUGUST 6, 2019

9:30 AM - 5:00 PM Anaheim Convention Center, Hall A

Animal Clinical Chemistry Session Posters and other Posters of Potential Interest

A-027. Urine derived renal cells as tools to diagnose salt sensitivity
K. A. Sarpong, R. A. Felder, J. J. Gildea. *University of Virginia School of Medicine, Charlottesville, VA*

A-324. Level of serum lipid in ovarian cancer patients: A hospital-based study
N. K. Yadav[1], R. Pandey[2], D. R. Pokharel[1], P. Pandit[2], M. Lamsal[2], N. Gautam[2], N. Chhimal[2], S. K. Baral[3]. *[1]Manipal College of Medical Sciences (MCOMS), Pokhara, Nepal, [2]Pokhara University, Pokhara, Nepal, [3]B.P. Koirala Memorial Cancer Hospital, Bharatpur, Chitwan, Nepal*

A-325. Dietary Quercetin Modulates Ouabain-Sensitive Na^+/K^+- ATPase in Mammalian Brain Model
T. Ogundajo. *Obafemi Awolowo University Teaching Hospital Complex, Ile-Ife, Osun State, Nigeria*

A-326. Effects of *Uvaria chamae* Root Extracts on Cortisol, Insulin, and Testosterone in Normal and Streptozotocin-Induced Diabetic Rats
F. Olumese. *Department of Medical Biochemistry, College of Medical Sciences, University of Benin, Benin, Nigeria*

A-327. Comparison of Commercially Available Multiplex Rat Cytokine Methods
T. Philip, K. Schorle, W. Glaab, D. Thudium. *Merck & Co., Inc., West Point, PA*

A-329. Role of RNase L in Kidney Recovery
N. Alghamdi, A. Zhou. *Cleveland State University, Cleveland, OH*

A-330. Validation of the qPCR from conjunctival swab for the diagnosis of canine visceral leishmaniasis. M. A. Almeida, T. Vital, A. P. Teixeira, D. M. Silva, B. C. de Carvalho, B. S. Dallago, G. S. C. Paz, L. Hagström-Bex, R. Pratesi, G. A. S. Romero, M. M. Hecht, N. Nitz. *Universidade de Brasília, Brasília, Brazil*

A-331. Verification of the Sysmex XN-V Multispecies Hematology Analyzer for Use in Mouse Sample Analysis. A. Love, R. Riley, J. DiPiero, D. Bounous. *Bristol-Myers Squibb, Princeton, NJ*

A-332. Pharmacological inhibition of the metabolic pathway that converts epoxy- to dihydroxy-metabolites of omega-6 and omega-3 fatty acids attenuated alcohol-induced liver injury in mice via reduction of ER stress. J. Warner, D. Warner, Y. Song, C. McClain, I. Kirpich. *University of Louisville, Louisville, KY*

B-144. Decreased endogenous ω-6 PUFAs induced intestinal mucosa transcriptional reprogramming that contributed to amelioration of intestinal and liver injury in mice in a context of systemic inflammation and chronic ethanol exposure. D. Warner, J. Warner, Y. Song, C. McClain, I. Kirpich. *University of Louisville, Louisville, KY*

A-345. Novel P-glycoprotein Biosensor Technology Detects Six Anticancer Modulators of the Transporter through a Fluorescence-Based Drug Profiling. B. Osa-Andrews, K. Tan, S. Iram. *South Dakota state university, Brookings, SD*

B-014. Impact of Homocysteine and C-reactive protein on Neurological Function Change in Acute Ischemic Stroke Patients after Cerebrovascular Stenting. Q. Chen. *Zhongshan City Peoples Hospital Clinical Laboratory, Zhongshan City, China*

B-267. Stability of Amphetamines in Dried Blood Spots Using GC Method
H. Alkhalaf[1], K. Mohammed[2], M. Elramady[2]. *[1]Prince Sultan Military Medical City, Riyadh, Saudi Arabia, [2]Naif Arab University for Security Science, Riyadh, Saudi Arabia*

Denise I. Bounous, BA/MT(ASCP), DVM, PhD, Diplomate ACVP to Receive the 2019 DACC Award for Outstanding Contributions to Animal Clinical Chemistry

The Division of Animal Clinical Chemistry (DACC) Award for *Outstanding Contributions to Animal Clinical Chemistry* was established in 1992 and constitutes a major milestone for our professional society. This Award is now in its 27th year of recognizing significant contributions including teaching, training, practicing and research. This award acknowledges activities and achievements of a scientist who has significantly benefited and advanced the science, as well as enhanced the public awareness and understanding of the discipline of animal clinical laboratory medicine.

The DACC Executive and Award Committees proudly announce ***Denise I. Bounous, BA/MT(ASCP), DVM, PhD, Diplomate ACVP*** as the 2019 recipient of this prestigious DACC Award. This recommendation reflects Dr. Bounous' extensive contributions in science, education and leadership in several areas of clinical pathology ranging from the *Normal avian hematology: chicken and turkey* (in: Feldman BF, Zinkl JG, and NC Jain, Eds. *Schalm's Veterinary Hematology,* 5th *ed*. Baltimore: Williams & Wilkins, 2000); bone turnover markers of laboratory animal species; preclinical markers of cardiac injury; the clinical pathology of pregnant and growing laboratory animals; clinical pathology of nonroutine laboratory animal species; and unique applications of the Siemens Advia (®) hematology analyzer, to *STP Best Practices for Evaluating Clinical Pathology in Pharmaceutical Recovery Studies* [Tox Path, 2016].

Dr. D. I. Bounous has been an innovator and leader in veterinary clinical pathology with a wide diversity of valued contributions in animal clinical pathology. Highly experienced and qualified, some of her accomplishments in research include publications in a variety of research and diagnostic applications including: *Best Practices for The Evaluation of The Bone Marrow In Non-Clinical Toxicology Studies*, [Tox Path, 2011], *Nontraditional applications in clinical pathology* [Tox Path, 2014]; *Renal biomarker qualification submission: a dialogue between the FDA-EMEA and predictive safety testing consortium*. [Nat Biotechnol, 2010]; *Markers of renal function and injury* [in Kurtz DM and Travlos GS (ed): The Clinical Chemistry of Laboratory Animals, 3rd Edition. New York, CRC Press, 2017] and the Hematopoietic System. [in: Haschek W (ed): Haschek and Rousseaux's Handbook of Toxicologic Pathology, Third Edition, 2013].

Dr. Bounous is presently Senior Veterinary Pathology Fellow, Discovery Toxicology, at Bristol-Myers Squibb Co. R & D in Lawrenceville, NJ having advanced since 2002 from Director, Clinical Pathology Drug Safety Evaluation. Prior to joining the pharmaceutical research and development arena at Bristol-Myers Squibb in 2002, Dr. Bounous obtained a BA/MT(ASCP) at Baptist Memorial Hospital School of Medical Technology, Memphis, TN (1974), her DVM (1986) at Oklahoma State University College of Veterinary Medicine, and PhD in Veterinary Science/Immunology at Louisiana State University College of Veterinary Medicine (LSU;1990). She became a Diplomate, American College of Veterinary Pathologists, Clinical Pathology Specialty in 1992. Following her DVM Dr. Bounous enjoyed academic posts at LSU and University of Georgia College of Veterinary Medicine in Clinical Pathology. Dr. Bounous previously also served as Clinical Pathologist in Safety Assessment at DuPont Pharmaceuticals (Newark, DE) and at AstraZeneca (Wilmington, DE).

Dr. Denise I. Bounous has authored or co-authored over 69 publications peer-reviewed manuscripts (some cited above), 14 books/chapters and 92 abstracts/presentations. As an *invited expert* she has presented on the various aspects of animal clinical chemistry over 90 times at national and international scientific congresses and professional society meetings.

Denise I. Bounous DVM, PhD, DACVP has had many career distinctions, including the Tyler Award for Innovative Teaching at Univ. Georgia, and Phi Zeta Veterinary Honor Society, and remains actively engaged in a number of major veterinary professional societies including the Society of Toxicologic Pathology (STP), American Society for Veterinary Clinical Pathology (ASVCP), the American Association for Clinical Chemistry/Division of Animal Clinical Chemistry (AACC/DACC), American College of Veterinary Pathology (ACVP), American Veterinary Medical Association (AVMA), and the American Society of Clinical Pathology (ASCP).

In summary, Dr. Denise I. Bounous' career has been exemplary in her accomplishments and contributions to the practice of and education, teaching, training and advancing research in animal clinical laboratory medicine. Please join the DACC Awards and Executive Committee in celebrating Denise I. Bounous, BA/MT(ASCP), DVM, PhD, Diplomate ACVP as the 2019 recipient of the DACC Award for Outstanding Contributions to Animal Clinical Chemistry.

~ Jon P. Kimball, PhD, Chair, DACC Awards Committee

2019 DACC Lunch and Learn Session
Presentation Abstracts

Evolution of Clinical Pathology in the Pharmaceutical Environment

Denise Bounous - *BA/MT(ASCP), DVM, PhD, Diplomate ACVP*
Bristol Myers Squibb

Over the last 20 years (or so), Clinical Pathology laboratories, their data production and interpretation, and contributions to drug development have evolved and grown with the changing demands and needs of the industry and the regulatory agencies. This is both challenging and stimulating for those of us who work in this environment. We have moved from a setting that was rather similar to a hospital lab to one that can incorporate more research capabilities, and provide specific support to different programs.

Comparison of Commercially Available Multiplex Rat Cytokine Methods

Doug Thudium
Merck Research Laboratories

Background: The increase of therapeutic biologics and vaccines in drug development has increased the demand for cytokine measurements in non-clinical species. However, available cytokine assays for animal samples are notoriously lacking in standardization, consist of different antibody pairs, and values vary significantly across vendors. To identify the most reliable assay, we examined several rat multiplex cytokine kits with the intent of finding the best method for measuring frequently requested cytokines. In addition, rat strain, sample type, collection site, control and positive samples were investigated.

Methods: To investigate the effect of sample collection site on cytokine values, blood was collected from three sites (jugular vein, vena cava, tail vein) into EDTA plasma or serum tubes. Matched serum and EDTA plasma sets from control Wistar rats were collected from jugular vein and vena cava sites and serum and EDTA plasma were collected from the jugular of Sprague Dawley (SD) rats. Positive plasma samples were obtained from in vivo treatment with agents known to induce cytokines. IL-6, TNF-a and IFN-g were analyzed on the Bio-Plex 200 using magnetic microsphere-based rat cytokine methods from Bio-Rad (Bio-Plex Pro™), Millipore (Milliplex®), Thermo Fisher (ProcartaPlex™), and R&D Systems (Magnetic Luminex® Assay). Additionally, the Meso Scale Discovery (MSD) Proinflammatory Rat V-PLEX kit (multi -spot 96 well format) was evaluated.

Results: The performance of the assays varied considerably, and their performance was further influenced by the sample collection site and the sample matrix examined. Initial evaluation of the Millipore, R&D Systems, and Thermo Fisher rat assays identified issues of sensitivity, or dilutional linearity that did not warrant further investigation; thus, the evaluation was across these platforms, cytokine values were relatively higher and more variable using the Bio-Rad rat kit as compared to the MSD kit. Furthermore, serum samples were more variable than EDTA plasma samples, and vastly lower cytokines values were noted for terminal vena cava EDTA samples compared to interim jugular or tail vein collections. Finally, serum and plasma cytokine values for Wistar and SD rats were similar.

Conclusion: Our comprehensive evaluation of sample type, collection site, and available cytokine assays demonstrated that the least variable and most reliable data was generated from vena cava EDTA plasma samples. Rat serum resulted in considerably higher and more variable values. The Bio-Rad kit provided consistent data for the terminal EDTA plasma samples; however, interim jugular samples were highly variable. The MSD platform demonstrated the lowest and least variable values, and could be considered if interim sampling (e.g., jugular) is needed. Although our findings suggest guidelines for cytokine measurements in rat samples, careful monitoring of the performance of existing assays is recommended due to the potential variability observed with these cytokine assays.

Effects of *Uvaria chamae* Root Extracts on Cortisol, Insulin, and Testosterone in Normal and Streptozotocin-Induced Diabetic Rats

Fidelis Olumese, University of Benin, Nigeria

Background: Uvaria chamae is a medicinal plant that is used in many parts of the world in the treatment of diabetes and many other diseases. *Uvaria chamae* contains some chemicals like C-benzylated monoterpenes, aromatic oils, flavanones, and C-benzylated dihydrochalcones. There is paucity of information on the use of this plant extracts in the treatment of diabetes scientifically. In this study, we evaluated the serum levels of glucose, insulin, testosterone, cortisol and histopathological changes in the pancreas of normal and diabetic rats administered aqueous or ethanolic extract of *Uvaria chamae* root.

Methods: Thirty-six Sprague Dawley rats were assigned by weight into six groups [6 rats per group, average body weight 265.23 ± 7.20 g]: Normal Control (Healthy rats received de-ionized water); Normal plus Aqueous Extract (Normal rats received aqueous extract); Normal plus Ethanolic Extract (Normal rats received ethanolic extract); Diabetic Control (Diabetic rats received de-ionized water); Diabetic plus Aqueous Extract (Diabetic rats received aqueous extract), and Diabetic plus Ethanolic Extract (Diabetic rats received ethanolic extract). Diabetes was induced using a single injection of

Continued on Page 8

Continued from Page 7

streptozotocin (Sigma-Aldrich, 60 mg/kg body weight in 0.05M-citrate buffer, pH 4.5) intraperitoneally. Normal and diabetic rats were administered the aqueous or ethanolic extract (300 mg/kg body weight per day) for 35 days. Animals were then euthanized by decapitation and blood collected for glucose, and hormonal assays. The pancreas was collected and preserved in buffered formalin for histopathological evaluation.

Results: The blood glucose levels decreased by 38% and 53% in the diabetic rats administered aqueous or ethanolic extract respectively compared to the increase in the diabetic control (45%). There was a significant decrease in serum glucose/insulin ratio and upregulation of insulin in the diabetic animals administered aqueous or ethanolic extract when compared to the diabetic control. The cortisol levels were significantly decreased in the diabetic animals administered aqueous or ethanolic extract, while testosterone was significantly increased in the diabetic rats treated with ethanolic extract when compared to diabetic control. The pancreas of normal control groups treated with aqueous or ethanolic extract showed resurgent pancreatic islet cells compared to the untreated normal control. Similarly, the diabetic groups treated with aqueous or ethanolic extract showed resurgent pancreatic islet cells compared to the diabetic control group.

Conclusion: The resurgence of pancreatic islet cells and the enhanced hormones are indicative of extracts potential benefits in the management of diabetes and its associated complications.

Verification of the Sysmex XN-V Multispecies Hematology Analyzer for Use in Mouse Sample Analysis.

Arlene Love. Bristol-Myers Squibb

Introduction: The Sysmex XN-V series is an automated hematology analyzer with software that is capable of analyzing animal EDTA whole blood samples. The instrument operates under principals of electrical impedance, light scatter, and fluorescent dye binding to quantify and identify cell populations found in EDTA whole blood samples.

Objective: A verification and method comparison study was performed specifically for mouse sample analysis using the Sysmex XN-V Multispecies Hematology analyzer and the Advia 2120i Multispecies Hematology analyzer.

Method: The verification of the Sysmex XN-V Analyzer included the following components: Accuracy, Precision, Reportable Range, RBC Extended Linearity, Carryover, and Method Comparison. The Sysmex XN-V provides 3 different methods of platelet for analysis: PLT-I (Impedance), PLT-O (Optical), and PLT-F (Fluorescence). All 3 methods were compared to the Advia 2120i platelet values. Additionally, the RDW-SD parameter versus the RDW-%CV parameter was evaluated.

Results: The Sysmex XN-V demonstrated acceptable Accuracy, Precision, Reportable Range, RBC Extended Linearity and Carryover for the purpose of mouse hematology sample analysis.
Method comparison studies between the Sysmex XN-V and Advia 2120i showed variable results with the following red blood cell parameters: HGB, HCT, MCV, MCHC, and RDW-%CV. The Sysmex XN-V provides a five part white blood cell differential compared to the Advia 2120i, which provides a six part differential due to the reporting of Large Unstained Cell category.
Differences in the instrument methodologies most likely account for the findings listed above. The manufacturer offers flexibility in the Manual Gating feature to redefine cell populations in the white blood cell differential scattergram.
It was determined that PLT-F, fluorescence method, would be the reportable parameter for platelet values. This is due to due to the increased specificity of the fluorescent dye used in the method and the claim that this parameter results in less platelet clump flags, which are frequently noted in rodent samples.
It was also decided that the RDW-SD, would be the reportable parameter rather than the traditional RDW-%CV. The RDW-SD is a direct and more accurate measurement because it is independent of MCV values used in the RDW-%CV calculations.

Conclusion: It is best practice with new instrumentation, and for the reasons stated above, in-house reference ranges will be established for mouse hematology analysis using the Sysmex XN-V. The Manual Gating feature will be utilized to optimize mouse gating strategies and manual differential results will serve as the reference method.

DACC FALL FACE-TO-FACE MEETING & SIEMENS USER GROUP MEETING
OCTOBER 2019

DETAILS TO FOLLOW

DACC memories

Looking Back to the last AACC meeting in Anaheim, CA

2010 DACC Award Presentation—Anaheim, CA. Award recipient **David Eckersall** (left) and DACC Chair – **Mike Bieraugel** (right)

2010 DACC Award Reception and Dinner - hosted by **Siemens** at Tortilla Joe's, Anaheim, CA

Short Scripts

European Society of Toxicologic Pathology
estp

17th European Congress of Toxicologic Pathology
Cologne, Germany
Sept. 17-20, 2019

The Membership of the DACC Extends a HUGE THANK YOU to
Siemens Healthineers
For Hosting
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And to
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At AACC Annual Meeting 2019 in Anaheim, CA

SOT
Anaheim, California
59th Annual Meeting
& ToxExpo
March 15–19, 2020

American College of Toxicology
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American College of Toxicology's
40th Annual Meeting
Phoenix, AZ
Nov 17-20, 2019