*AN INTERVIEW WITH...*


**Veronika Wills**
*Manager, Technical Services*
*Associates of Cape Cod, Inc.*


ASSOCIATES OF CAPE COD INCORPORATED

*Specialists in Endotoxin and Glucan Detection*

***Veronika Wills** has been working in the endotoxin industry for the past 15 years and currently manages the Technical Services department at Associates of Cape Cod, Inc.*

*Veronika is a specialist on testing complex samples, method development, regulatory aspects of endotoxin testing, development and delivery of educational and technical contents.*

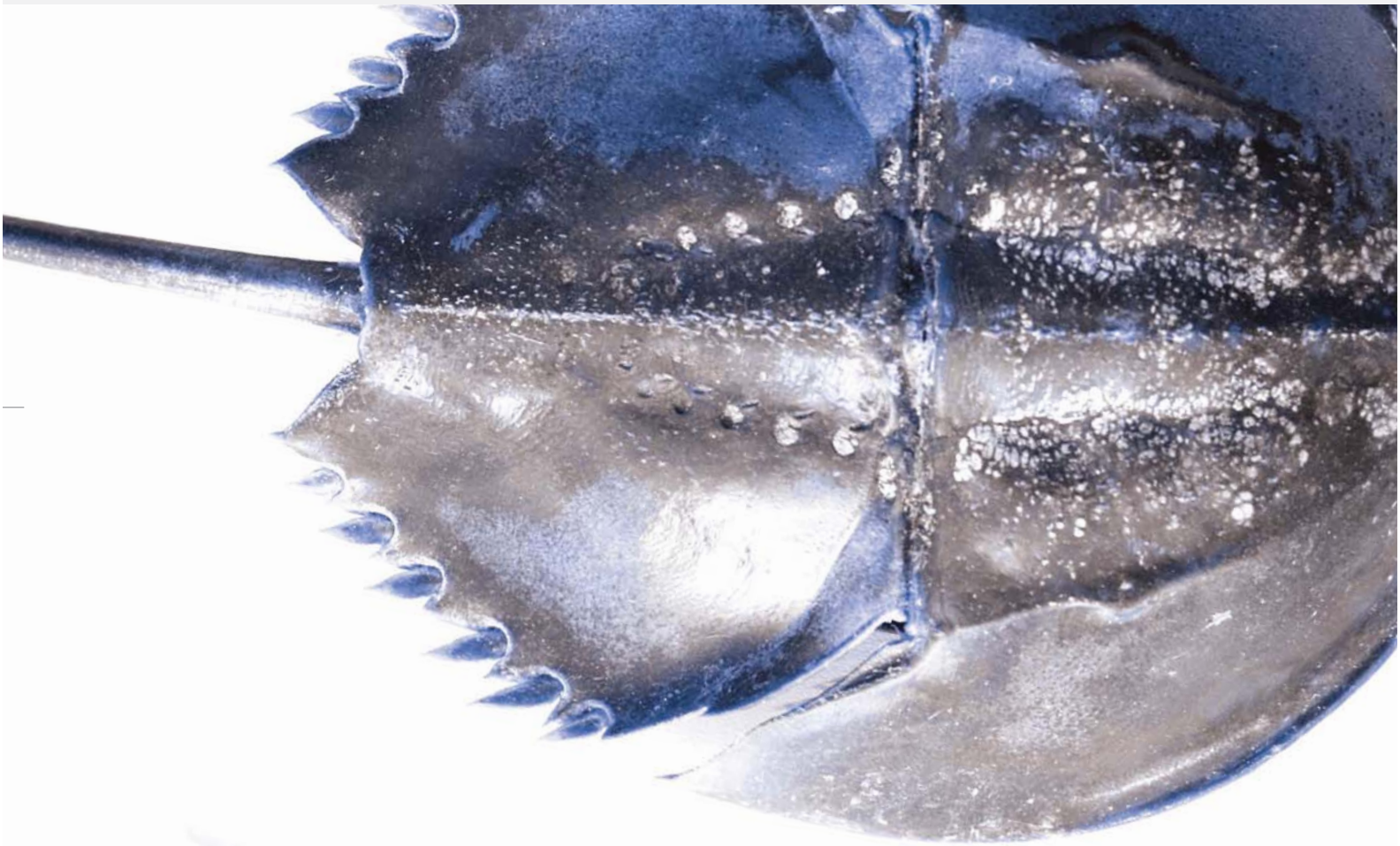
*She attends and speaks frequently at scientific conferences and seminars globally and is an established expert on endotoxin testing. Veronika holds a master's degree in Biochemical Engineering from the Institute of Chemical Technology in Prague, Czech Republic.*

## What are the current accepted test methods for BET?

The methods that are currently defined in the harmonized Bacterial Endotoxins Testing (BET) chapters are all based on the use of animal derived lysates of amebocytes (either Limulus amebocyte lysate (LAL) or Tachypleus amebocyte lysate (TAL)). These biological methods are broken down into two groups: gel clot and photometric methods (where photometric methods can be done using either turbidimetric or chromogenic reagent). In addition, the European Pharmacopeias added another chapter describing BET – this is chapter 2.6.32 which specifically describes testing for endotoxin using recombinant Factor C reagents (rFC). However, it is of note, that all EP monographs continue to refer to chapter 2.6.14 (the BET chapter using LAL reagents).

## Tell us about emerging test methods such as Recombinant Factor C (rFC) and Recombinant Cascade Reagent (rCR). What are the benefits of rCR?

From the practical standpoint, the recombinant methods bring many significant advantages to the pharmaceutical industry, the Quality Control laboratories in particular: improved specificity and increased reproducibility of the signal of the endotoxin response. For many global companies it is also vitally important that neither recombinant reagent requires the harvest of live animals for the collection of the raw material, thus the reagents are and will be sustainably manufactured for years to come. In addition, the recombinant Cascade Reagent inherently provides other advantages to the end user – because the rCR is a chromogenic method, just like one of the LAL-based methods. This means that both LAL chromogenic and rCR chromogenic methods rely on the use of the cascade enzymes from the LAL reagent where the pro-clotting enzyme reacts with the chromogenic substrate to trigger the increase in measured absorbance at 405nm. This makes assessment and feasibility studies of the rCR extremely user friendly as the end user will use the same preparation steps, software protocol and instrument to perform the recombinant test.



## What needs to be done in order for global regulatory bodies to incorporate rCR test methods into their technical standards?

All the pharmacopeias base the implementation of new, improved, rapid methods into the standards based on unbiased data. The data must be available from a statistically significant data set where equivalency of the new (thus alternative) method is under evaluation compared to the benchmark method. For the recombinant reagents for BET, this is referred to as comparability data in the US. This means that the recombinant methods must be first used within the industry to help generate a significant data set on relevant samples. This process is fairly time-consuming, but we believe that it is well under way. ACC has recently shared a large-scale water comparability study performed on over 80 samples to study the equivalency of rCR to LAL but also LAL to LAL results. The equivalency of rCR to LAL was shown higher than equivalency of LAL to LAL results.

## Based on this information, what is the best way to move forward to ensure the rCR test method becomes the preferred test method?

We believe that the best way to move forward is early implementation of rCR on in-process samples (such as in-process water) which are not technically considered to be compendial samples. Testing in-process water samples by both methods (rCR and LAL) during a pre-defined period of time will allow generating onsite data studying the equivalency of the reagents.

## Can you tell us about the PyroSmart NextGen® system including its method validation and how its features and benefits are moving rCR testing forward?

PyroSmart NextGen® reagent, as the first commercial GMP-manufactured recombinant cascade reagent for BET has many advantages that were confirmed and validated by first adopters. PyroSmart NextGen®, as a chromogenic reagent, provides a smooth transition from either photometric LAL reagent on any absorbance reader and software (whether it is a plate reader or tube reader), while utilizing the same standard operating procedures. It also provides the highest sensitivity available for the recombinant reagents 0.001 EU/mL in half the time of the LAL reagent. And it was found to be at least as suitable for testing a wide range of finished drug products of different types, or even more suitable than the LAL. Thus, thanks to the reproducibility and specificity, PyroSmart NextGen® can be used as a tool to troubleshooting BET assays.