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Limitations of Accelerated Stability Model Based on the Arrhenius Equation for Shelf Life Estimation of In Vitro Diagnostic Products

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Guest: Dr. Alireza Ebrahim is the Associate Vice President of R&D at Bio-Rad Laboratories in Irvine, California.

Bob Barrett: This is a podcast from *Clinical Chemistry* sponsored by the Department of Laboratory Medicine at Boston Children's Hospital. I'm Bob Barrett. If you're like most people, you'll check the sell-by date on that milk container before putting it in your cart. Well, it's much the same in the laboratory: a longer shelf-life minimizes changing lots and the overhead required to validate such changes. But how are those expiration dates established? Well, a lot has to do with an equation developed by a 19th century Swedish chemist named Svante Arrhenius.

His equation is a formula for predicting the temperature dependence of reaction rates and it is also widely used to calculate the time dependence of various storage conditions. A paper appearing in the April 2021 issue of *Clinical Chemistry* examined the applicability of this equation and had some surprising results in its ability to predict storage. The lead author for that study is Dr. Alireza Ebrahim. He is the Associate Vice President of R&D at Bio-Rad Laboratories in Irvine, California and he is our guest in this podcast. So, first of all, Dr. Ebrahim, why do we use and perform accelerated aging and accelerated stability studies?

Alireza Ebrahim: Good question. Accelerated stability studies are used in different fields and industries such as pharmaceutical industry, food, and clinical diagnostics. These studies are designed and used to predict expiration date or shelf life for products. Many pharmaceutical and diagnostic products have long shelf lives in the order of one to three years.

The shelf lives for these products are first estimated with accelerated stability of studies during the product development process which usually take a couple of months and later supported with real-time stability of studies that take several years. These studies are designed and performed at elevated temperatures, compared to normal storage conditions for products with storage temperature on product label to observe changes in product performance, mainly a stability, more rapidly than what would be seen under normal conditions.

Bob Barrett: What is the basis of the accelerated aging studies and models?

Alireza Ebrahim: The science and the chemistry behind the accelerated stability of study is very simple and it is based on the Arrhenius equation which describes the mathematical relationship between the rate constant of a chemical reaction, the reaction temperature, and the activation energy for the molecule under a study.

Generally, for every 10-degree rise in temperature (and that is in degrees Celsius), the reaction rate doubles therefore by increasing the temperature in which the product is stored at, you can increase the reaction rate and degradation rate of a molecule or a product. For example, storing a product at 35 degrees Celsius for 30 days is equivalent to storing the same product at 5 degrees Celsius for three years in terms of degradation grade.

Also, 10 days of storage at 35 degrees Celsius is equivalent to one year of storage at 5 degrees Celsius and also 20 days of storage at 35 degrees Celsius is thermodynamically equivalent to two years of storage at 5 degrees Celsius. That is how you can accelerate and shorten the duration of the stability testing. In this example that I just used; you can shorten the duration of stability study from three years to one month by exposing the product to elevated temperatures to determine the stability of the product.

Bob Barrett: How accurate are these models in predicting and estimating the shelf life of products?

Alireza Ebrahim: In general, these models are accurate in predicting a stability and shelf life and there is reasonable and acceptable agreement between the actual shelf life from real-time stability of studies which are performed over several years and the estimated shelf life from accelerated and stability of studies which are performed in a shorter period of time, like over a couple of months.

And that is why accelerated stability of studies are referenced in several standard and guidance documents such as EN 23640, which is a European standard for stability testing of in vitro diagnostic reagents and CLSI EP25 which is a guidance document from the Clinical & Laboratory Standards Institute for establishing shelf life claims for diagnostic reagents such as test kits, calibrators and controls.

Bob Barrett: As a quick aside, were you surprised to find that storage at increased temperatures differed so much from real-time stability studies?

Alireza Ebrahim: No, because over time -- over the past few years, we have seen some data but we wanted to publish the results to share our findings with our colleagues.

Bob Barrett: And what are some of the limitations of the accelerated aging studies?

Alireza Ebrahim: The key factor in Arrhenius equation is temperature. So, if the mode of decomposition is not thermal degradation, accelerated stability study may not work. For example, some molecules are photosensitive and degrade when exposed to sources of light. Bilirubin, one of the analytes that we have studied extensively in our laboratory, is a very good example of photodegradation.

This mode of degradation, this mechanism of decomposition, is not considered when using Arrhenius model. Also, in our laboratory, we have looked at real-time and accelerated stability testing for several hundred analytes including antigens, antibodies, enzymes, hormones, and small and large molecules over the past few years and generally, we have observed good agreement between the two studies. However, we have also seen a number of examples that show poor agreement between the results of real-time and accelerated stability studies.

We shared the results for three molecules in our recent *Clinical Chemistry* paper where the model either overestimated or underestimated the stability of the analyte and we provided explanation for the observed discrepancies. For example, in the case of BNP B-type natriuretic peptide, one of the biomarkers that we studied, shorter stability was estimated by the accelerated stability study, then the real-time stability study. We hypothesized that this peptide is susceptible to proteolytic degradation and proteases involved in the degradation process have optimum activity at increased temperatures.

Therefore, if you have BNP in a human set on base matrix and it is stored at minus 20 degrees Celsius and then you perform two stability studies, one real-time at minus 20 degrees Celsius and one accelerated stability at temperatures like 16 degrees Celsius or 25 degrees Celsius. You will get a shorter stability prediction versus real-time stability because of the increased proteolytic degradation that occurs in the liquid phase at higher temperatures versus when the peptide is in the frozen form and is in the solid phase.

So, the phase change that occurs because of the exposure of the product or biomarker to elevate the temperature may have a significant impact on accuracy of the prediction because of the reasons that we just talked about.

Bob Barrett: Finally, Dr. Ebrahim, what are some of the key conclusions and recommendations from your article?

Alireza Ebrahim: The message that we want to convey with our recent article is the exposure of reagents. Whether you're talking about test kits, calibrators, or controls, to raise temperatures during accelerated stability study may create conditions or produce degradation products that may not be typically observed during real-time stability when the product is stored under normal and unstressed storage conditions. This is especially true when the normal storage temperature of the product is at minus 10 or lower which means the product is frozen and is in a solid phase and accelerated stability studies are performed at higher temperatures, which means the product is now in the liquid phase.

For this reason, the prediction from accelerated stability model may not be accurate and may result in overestimation or underestimation of shelf life. Our recommendation is for the researchers to assess the applicability of the model to their molecule of interest by performing a few simple and quick experiments and consider the shortcomings of the model prior to fully utilizing the model to estimate or establish product shelf life.

Bob Barrett: That was Dr. Alireza Ebrahim, Associate Vice President of R&D at Bio-Rad Laboratories at Irvine, California. He has been our guest in this podcast on stability models based on the Arrhenius equation for shelf life estimation of in-vitro diagnostic products. He is the lead author of a paper examining such studies that appears in the April 2021 issue of *Clinical Chemistry*. I'm Bob Barrett. Thanks for listening.