



LAL TIMES



Vol. 4, No. 3, October, 1997

EDITOR'S COMMENTS

Endosafe became the leader in kinetic turbidimetric assay (KTA) technology with the introduction of Endosafe® KTA in 1991. This formula brought to kinetic ranks the same level of interference resistance that was found in gel-clot reagents. With its unique clarity and reactivity, both gel-clot and KTA analyses were possible with the same reagent.

This newsletter reports an improvement of the original formula that extends the linear range of KTA to 3 logs (4 points) by increasing its speed and reactivity. Although Endosafe® KTA² is approved for polynomial regression with qualifying software, the extended linearity will minimize the need for this alternative analysis.

Other newsworthy items are a review of the international collaborative endotoxin study, a report on the Charleston LAL Workshop, and an acknowledgment of the distinguished public service career of Don Hochstein, who played a pivotal role in development of endotoxin standards and regulation of LAL producers.

Dr. James F. Cooper, Global Scientific Director

NEW PRODUCT

KTA² FORMULA APPROVED FOR ENDOSAFE

J.F. Cooper and F.T. Jordan

Charles River Endosafe announces the availability of Endosafe® KTA², a powerful new endotoxin analysis tool for kinetic turbidimetric assay (KTA). The new KTA product from Endosafe's development team has unparalleled performance characteristics for a turbidimetric reagent. KTA² matches attributes hitherto assigned only to kinetic chromogenic assay (KCA) reagents, but without the costly color substrate.

The original Endosafe® KTA product has gained an enviable reputation for its robust, interference-resistant formula. Endosafe® KTA² performance advances over the original product include greater speed, sensitivity and linearity. This discussion will highlight the attributes of KTA².

GREATER SPEED

Endosafe® KTA is a true gel-clot reagent as well as a kinetic turbidimetric lysate, a property unmatched elsewhere in the LAL industry. This unique attribute benefits LAL users who wish to use gel clot and kinetic methods interchangeably or make the transition from gel-clot to kinetic technology in a deliberate, orderly fashion. This option is still available as Endosafe continues to offer the dual application KTA reagent in 0.015, 0.03 and 0.06 EU/mL sensitivities.

Endosafe® KTA² labeling will not bear a gel-clot sensitivity because its reactivity surpasses the conventional gel-clot range. The term speed relates to the time required for all standard points to react, that is, exceed the pre-set optical density. Therefore, the greater sensitivity and faster reaction of this reagent means that a test is completed in the shortest possible time. In commonly used multi-tube readers and incubating microplate readers with endotoxin-specific software, KTA² reaches 0.005 EU/ml within 60 minutes with proper instrument settings. Of course, reaction times vary depending on choice of reader, threshold optical density setting and type of incubation system.

Since there is no formulation change, **no revalidation is required to move KTA applications to Endosafe® KTA².**

SUPERIOR LINEARITY

The most significant feature of the KTA² formula is the greater linearity of assays over two-log (3 point) and three-log (4 point) standard curve ranges with linear regression. This refinement improves accuracy and minimizes the enhancement of the positive product control (PPC). Tables 1 through 3 present the results of routine water assays using representative kinetic chromogenic and turbidimetric assays with a three-log (4 point) standard curve. Assay conditions included default settings for the specified systems and use of vendor instructions and lot-specific control standard endotoxins. In this 3-log range, KTA² is clearly equal in performance to kinetic chromogenic assays in speed and recovery. Note that the test was completed in less than an hour and that recovery was within $\pm 25\%$, even when the range was extended to 0.005 EU/mL. When recovery of the water spike was within $\pm 25\%$, the use of polynomial regression was unnecessary and was not applied.

THE KTA INTERFERENCE MYTH

Endosafe has led KTA technology for interference resistance since introducing Endosafe® KTA in 1991. There is a misunderstanding on the part of a few that KCA (kinetic chromogenic analysis) reagents have less inhibition problems than KTA. This scenario was true ten years ago when chromogenic assays were compared with KTA reagents used on the LAL-5000. However, the high inhibition associated with LAL-5000 assays was not a limitation of the tube reader or reagent; rather, it was due to the manner in which parenteral samples were prepared for assay. To provide sufficient volume for the reader, the analyst needed to mix 1 part LAL and 4 parts test sample. This 4:1 ratio exaggerated the inhibition properties of the sample, requiring greater sample dilution and low-range standard curves to compensate for the increased inhibition.

In contrast to this scenario, it is well known that Endosafe® gel and KTA need the least product dilution to reach compatibility. Therefore, microplate readers and tube readers (such as the Ati 320 and Toxinometer) work on a 1:1 ratio and capitalize on the low interference attributes of Endosafe® kinetic reagents.

POLYNOMIAL REGRESSION OPTION

In a previous LAL Times we revealed potential hazards to using polynomial regression (PR) routinely. We showed how PR reduces enhanced recovery by minimizing non-linearity associated with kinetic LAL methods. However, in its presently approved form, PR obscures standard problems or kinetic reagents with poor linearity performance. Endosafe has produced reagents with high linearity to minimize or eliminate the need for PR (Tables 1-3). A means for applying prudent safeguards to PR will be presented in a forthcoming Newsletter.

For exceptional applications where a 4-log (5-point) standard curve is required (e.g., in-process testing), KTA² provides an option for PR. This option extends the KTA curve to a range previously attainable only by kinetic chromogenic assays (KCA). Therefore, KTA² is ideally suited for those who wish to substitute more economical KTA reagents for KCA, and continue to use a wide range standard curve on Bio-Tek and K-QCL systems.

LABELING REQUIREMENTS FOR POLYNOMIAL REGRESSION IN LAL

The supplement approval granted to Endosafe by CBER allows Endosafe's KTA² and Endochrome-K reagents to be used for polynomial regression with FDA-qualified software (e.g., Biolise 2.0 CRE™ and WinKQCL®). Polynomial regression is not allowed in the FDA's LAL-Test Guideline or Bacterial Endotoxin section of the European Pharmacopeia. Therefore, instructions for applying this PR option are found only in the package insert for approved kinetic reagents. CBER required that the following conditions be met before qualifying endotoxin-specific software for the PR option:

1. Linearity (r) exceeds or equals the value of -0.980;
2. Standard curve is monotonic over the entire concentration range, that is, there is protection against a curve that provides multiple answers to a reaction time; and
3. A report cannot be generated with LR or PR if the correlation coefficient is less than -0.980.

SUMMARY

Endosafe® KTA² is a refined kinetic turbidimetric reagent with great economy and features equivalent to KCA. The improved linearity, sensitivity, interference resistance, and wide-range application option provide a powerful new tool to LAL users.

Table 1. Performance on Biolise 2.0 CRE in Standard Range of 5 to 0.005 EU/mL

<i>Product</i>	<i>Type</i>	<i>Linearity</i>	<i>LR Recovery</i>	<i>PR Recovery</i>	<i>Time low pt</i>
KTA ²	T	-0.999	108	NA	56 min
Endochrom.K	C	-0.999	100	NA	54 min

Legend: T = turbidimetric; C = chromogenic; LR = linear regression; PR = polynomial regression

Conditions: Assay by vendor instructions using default settings on Bio-Tek Elx808.

Table 2. Performance on WinKQCL in Standard Range of 5 to 0.005 EU/mL

<i>Product</i>	<i>Type</i>	<i>Linearity</i>	<i>LR Recovery</i>	<i>PR Recovery</i>	<i>Time low pt.</i>
KTA ²	T	-0.999	95	NA	49.5 min
KQCL	C	-0.998	126	110	77 min
PG 5000	T	-0.998	140	105	63.5 min

Legend: T = turbidimetric; C = chromogenic; LR = linear regression; PR = polynomial regression

Conditions: Assay by vendor instructions using default settings on the Kinetic-QCL reader.

Table 3. Performance on Biolise 2.0 CRE in Standard Range of 10 to 0.01 EU/mL

<i>Product</i>	<i>Type</i>	<i>Linearity</i>	<i>LR Recovery</i>	<i>PR Recovery</i>	<i>Time low pt</i>
KTA ²	T	-0.999	118	NA	49.5 min
Endochrom.K	C	-0.999	119	NA	45 min
KTA 0.03	T	-0.996	134	93	56 min

Legend: T = turbidimetric; C = chromogenic; LR = linear regression; PR = polynomial regression

Conditions: Assay by vendor instructions using default settings on Bio-Tek Elx808.

MEETINGS -- EXHIBITS

PDA Annual Meeting: NOV. 10-12, 1997 -, Philadelphia, PA, USA

Visit us at BOOTH #317.

PUBLICATION REVIEW

Dr. Poole and associates¹ reported the results of an international collaborative study to define the potency of a candidate endotoxin standard. Study results support the assignment of 10,000 IU/vial and an equivalence of 1 IU = 1 EU. Gel-clot and photometric assays were contributed by 26 laboratories globally.

1. S Poole, P Dawson, RE Gaines Das, Second international standard for endotoxin: calibration in an international collaborative study, *J Endotoxin Res*, 4:221-231, 1997.

HOCHSTEIN RETIRES FROM PUBLIC SERVICE

An afternoon reception on 25 September 1997 honored Dr. H. Donald (Don) Hochstein who retired from 41 years of distinguished public service. As Deputy Director of the FDA's Division of Product Quality Control, Bureau of Biologics, Don was responsible for batch-release of licensed LAL reagents and for maintenance of reference standards. Don led early collaborative research with Cooper and Seligmann that defined the role of LAL testing for human serum albumin (HSA), influenza vaccines and other injectable biologics. During his tenure, specifications were finalized for LAL-reagent licensure; in-process testing was required for HSA products; and national endotoxin standards EC-2 and EC-5 were produced. It is noteworthy that endotoxin levels in HSA products were reduced more than 100-fold during the period that Don was responsible for LAL-testing for biologics.

Don became a global spokesperson for the FDA on LAL regulations and endotoxin standards. Of his 45 publications, 25 pertain to LAL applications and endotoxin standards. Don reported that he will continue writing and will be spending more time with family and hobbies. It was a pleasure to have this opportunity to show special tribute to Don and to reminisce with Don and Ed Seligman

ANNUAL CHARLESTON LAL WORKSHOP: The energetic and enthusiastic response of participants from four continents resulted in a highly successful 1997 Annual LAL Workshop in Charleston, August 6-14. Many innovative improvements were incorporated in this workshop format such as problem solving lab exercises. Improvements will continue based on changes in the industry and response to customer needs.

Borrowing a phrase from Maurice O'Sullivan, I feel that our Annual LAL Workshop has been "Twenty Years A-Growing" – I presented the first Charleston LAL Workshop in 1978 and look forward to special preparations to mark the 20th anniversary workshop in 1998. Watch for details in future newsletters and mailings.

INFORMATION: For information regarding training schedules, contact Frances Cooper at: 803-795-7316 or Fax: 803-795-7221.