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Stable Isotope Standards for Clinical Mass Spectrometry

Cambridge Isotope Laboratories, Inc.

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Welcome

Cambridge Isotope Laboratories, Inc. (CIL) is the world leader in the production and distribution of stable isotope-labeled compounds, providing labeled compounds for fields spanning from basic analytical chemistry to modern diagnostics. Over the years, CIL has evolved in its breadth and capacity to both produce and characterize a diverse array of organic compounds to support the development of mass spectrometry (MS) methods. Our ever-expanding product offering has been driven by close customer collaborations and partnerships that we've had the privilege of being involved in.

It is with great pride that we present CIL's new "Stable Isotope Standards for Clinical Mass Spectrometry" catalog. This compilation consists of a list of clinically relevant products and a collection of varied content pieces, which comprise researcher perspectives and technical notes that highlight the utility of certain products in specific clinical MS applications. Educational articles are also included, which detail the importance and proper selection of stable isotope standards in clinical MS assays.

The clinical MS field continues to evolve in research scope and application, as well as in instrument advances and their accompanying figures of merit. CIL strives to support your growing efforts by providing well-characterized standards that facilitate your required evaluations. Our offerings in this area have been vastly enhanced by our customer interactions and close collaborations, to which we are truly appreciative of and always open to expanding. Our decades of isotope-chemistry experience in combination with our isotopeseparation facility position us to respond well to the challenging problems in a collaborative manner. Our customer relationships are very important to us, and we look forward to continuing to work with you in an effort to satisfy your needs in the clinical MS field.

Respectfully,

Krista Backiel **Andrew Percy, PhD** *Product Manager, Clinical Mass Spectrometry*

Crystal Belanger *Assistant Product Manager, Clinical Mass Spectrometry*

Marketing Manager Senior Applications Chemist, Mass Spectrometry

You can find all of the information presented in this catalog on isotope.com. Visitors to our website can immediately access updated product information, availability, pricing, and documentation, such as a certificates of analysis (CoA) and safety data sheets (SDS). Visit isotope.com to learn more.

Cambridge Isotope Laboratories, Inc. (CIL) is the world leader in the separation and manufacture of stable isotopes and stable isotope-labeled compounds. Isotope separation is performed at Cambridge Isotope Separations (CIS) in Xenia, Ohio – home of the world's largest 13C isotope separation facility, one of the world's largest ¹⁸O isotope-separation facilities, and the world's only commercial large-capacity D_2O enrichment columns. For over 35 years, CIL has remained the premier supplier of stable isotope standards for MS, NMR, and MRS/MRI research applications. The clinically driven products include bile acids, drugs and their metabolites, free and protected amino acids, MS/MS screening standards and mixes, steroids and hormones, and vitamins and their metabolites. Our products have been specifically designed and tested with the most discerning mass spectrometrists in mind. CIL actively supports the MS community through meeting sponsorships and customer collaborations.

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❛❛**Much like electrons, those extra neutrons make my world go 'round.**❜❜

> *Andy Hoofnagle, MD, PhD Professor of Lab Medicine, University of Washington*

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Importance of Stable Isotope Standards and Their Implementation in Clinical Mass Spectrometry

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The implementation of mass spectrometry (MS) in the preclinical/ clinical laboratory has been garnering more attention over the past couple of decades.¹ Among the reasons for this are the performance benefits that MS-based methods can afford. This pertains to the high specificity, reproducibility, and sensitivity achieved through tandem MS operations (e.g., selected or multiple reaction monitoring). As with any technology, there are a few limitations worth noting. These include the upfront instrument investment and its complexity, as well as the result turnaround time. Nonetheless, as the breadth of instruments and data analysis tools continue to advance, the limitations appear to be diminishing, while the overall merits, relative to historical clinical techniques, are amplifying. Example applications that have capitalized on the analytical power of mass spectrometry include endocrinology,² therapeutic drug monitoring,³ and newborn screening (for inborn errors of metabolism).⁴ The aim of these, and other clinical MS screens, is to help improve the path to diagnosis. From this, specific treatments can be effectively implemented at the earliest time leading to enhanced patient care and longevity.

To facilitate accurate MS-based measurements, stable isotopelabeled standards must be incorporated. The preferred approach

here is to add the labeled standard in a precise and constant amount to both the experimental samples, as an internal standard (IS), as well as the standard curve and QC samples. For utmost accuracy, the curve samples should be generated in an equivalent sample type such that the matrix effects and extraction efficiency are identical. Only by adding the labeled standard as an IS can recovery differences be effectively resolved. With IS use, the type and its point of insertion are two critical factors that a researcher faces in designing a clinically relevant, MS-based method. This is critical to qualitatively evaluate the assay's effectiveness and to help guide corrective measures, as necessary.

The nature of IS can take many forms but is conventionally a compound, or mixture of compounds, that has been labeled with one or more stable isotopes (e.g., ¹³C, ¹⁵N, and/or D). The position and number of stable isotopes in a given compound is predicated on the sample preparation and method of analysis. If, for instance, D-labeling is preferred for a certain metabolite, the labels must be inserted at nonexchangeable positions to mitigate the effects of hydrogen-deuterium exchange. Regardless of the type of isotope incorporated, the labeled standards should ideally bear a total mass shift of 3 Da minimum from its unlabeled counterpart (to enable swift metabolite MS analysis) and be well characterized (e.g., for chemical and/or chiral purity, isotopic enrichment). In terms of the number of labeled standards required for a given experiment, it is recommended that this number equate to the number of target analytes. While this is generally practical for small panel analyses (as would be typical in a clinical experiment), it is common with large panels (as utilized in preclinical experiments) to select certain labeled standards as surrogates for compounds that lack a labeled analogue. This practice is considered acceptable in quantification exercises provided that the surrogates exhibit similar elution times, and thus bear similar physicochemical properties as their native targets.

Given the complexity of human biological samples, in terms of depth and breadth of analytes, it is recommended that the labeled IS be added as early as possible in the analytical workflow. In so doing, losses or modifications that occur during the sample preparation and processing steps can be adequately accounted for. Since the standard is designed to match its native analogue and behave similarly (in terms of its separation, ionization, and

The Importance of Stable Isotope Standards and Their Implementation in Clinical Mass Spectrometry *(continued)*

fragmentation), any changes that occur on one will, in theory, be reflective on the other. Therefore, in analysis, the analyte can be quantified using relative ratios (i.e., unlabeled/labeled) of peak areas as opposed to their absolute values. In addition to the experimental samples, this approach is applied to other sample types, such as standard curve and curve QCs (at low, medium, and high concentrations). While the response of the labeled and unlabeled analyte will differ in curve and QC samples, the point of elution will not (valid particularly with ^{13}C and/or ^{15}N standards), enabling their relative ratios for quantitation or performance assessment to be effectively determined.

The importance of stable isotope-labeled standards in the rapidly evolving clinical MS field is becoming increasingly more recognized.

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- 3. Maurer, H.H. **2018**. Mass spectrometry for research and application in therapeutic drug monitoring or clinical and forensic toxicology. *Ther Drug Monit, 40(4),* 389-393.

This pertains to both small and large molecule analysis,⁵ with applications covering diagnostic testing and drug therapy monitoring, among others. Regardless of the application type, a well-executed clinical MS method should be automated and well controlled. In the assays deemed fit for purpose, the highly characterized standards should be inserted for not only accurate quantitation, but also for system suitability reliance to enable complete accounting of all possible losses or errors.6 This relates to human errors (e.g., improper pipetting), chemical errors (e.g., analyte extraction, hydrolysis), and instrument errors (e.g., ion suppression, matrix effects). The labeled standards that CIL provides can be, and have been, utilized in this realm. The product listing, perspectives, and applications herein provide such examples.

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Benefits of 13C vs. D Standards in Clinical Mass Spectrometry Measurements

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The capabilities of mass spectrometry (MS) have made this analytical technique an invaluable tool in clinical-based developments and applications. As with any clinical test, accurate and precise results are paramount toward correct diagnosis and treatments. In MS testing, reliable results are best achieved by the inclusion of stable isotope-labeled standards. The utility of such standards has been demonstrated in clinical and translational research (see [page 7](#page-6-0) of this catalog for a background article), with their benefits including the ability to help compensate for matrix effects and ion suppression.¹ For optimum results, the standards should be added as early in the analytical workflow as possible, such that they can effectively normalize the variations that may arise throughout the experimental stages. The nature of labeled standards is a critical element of a method and is predicated on its availability/cost, as well as the study design and research aims. Important to recognize in the standard selection process is the isotope differences (e.g., between 13C and D) and the potential impact this may have in the pre-analytical (e.g., storage and handling) and analytical (e.g., sample preparation and processing) phases. As standard selection is not always a straightforward procedure, this article compares the commonly used ^{13}C and D isotopes from production to analysis in an effort to edify the challenges and guide future selections.

Standards labeled with ¹³C (and/or ¹⁵N) have demonstrated broad research utility over the past couple of decades. This stems partly from the chemical stability of its isotope. Its stability ensures that the isotope remains intact irrespective of the experimental methodology employed (e.g., multidimensional LC or derivatizationbased GC prior to MS/MS). In other words, the ¹³C (and ¹⁵N) isotope remains positioned at its point of synthesis throughout all stages of an analytical workflow (includes extraction, derivatization, separation, and analysis in metabolomics). This provides flexibility to the end user as there is no limitation on the choice of sample/ solution preparation nor the mode of MS/MS analysis. Since ¹³C (and/or ¹⁵N) standards have exceptional isotope stability, as compared to their deuterated counterparts, these can be inserted at an early stage of sample preparation. Of additional benefit is that this type of labeled compound co-elutes with its corresponding unlabeled (i.e., native or endogenous) analyte during chromatographic separation. This co-eluting result is optimal in correcting for both ion suppression and matrix effects. Further to the benefits, $13C$ (and/or $15N$) standards are absent from isotope scrambling

or loss during ionization and collisional activation in the mass spectrometer. Owing to these collective merits, ¹³C (and/or ¹⁵N) standards have incurred great value in preclinical and clinical MS applications (from qualification to absolute quantification).

Despite the benefits of 13C (and 15N) labeling, the production of such standards could entail complex and laborious synthesis. While carefully selected structural analogues (with 13C and/or 15N) may instead be used in cases where it is cost or time prohibitive to obtain or synthesize the required standard, deuterated standards are an alternate option to consider. These are comparatively straightforward to prepare, but invoke a number of potential issues at the pre-analytical and analytical phases. The first pertains to the isotope stability. If the D-label is placed at an exchangeable position (i.e., at acidic and polar groups), it could be susceptible to an isotope effect during storage and later in analysis. In this effect, the location of deuterium may scramble or undergo an exchange reaction with protium in solution or in the gas phase. Another situation to consider is deuterium loss on specific compounds from enzymatic reactions (e.g., deuterium abstraction from fatty acids due to fatty acid desaturation).² The impact of these collective effects could be significant and is best illustrated by a hypothetical example. In a complete exchange scenario, for instance, the labeled signal at the mass spectrometer would be unmeasurable, while the unlabeled signal (i.e., M+0) would be elevated. This would provide an invalid view of a patient's biochemistry and a false impression of the assay's fitness, a result that would clearly contribute to "imprecision medicine" in laboratory diagnostics. While this deleterious impact could be overcome by selecting alternate MRM transitions (i.e., at sites verified to have label due to consistent scrambling), a preferred approach would be to incorporate deuterium at chemically inert, nonexchangeable positions. Doing so would aid its stability, but the integrity of the deuterated standards would still need to be validated at all phases of the analytical workflow (from reconstitution through extraction to MS analysis). Complicating these assessments is the difference in physicochemical properties between deuterium and hydrogen. The difference causes deuterated standards to typically exhibit an altered chromatographic retention from its native analogues.³ This elution impact is most pronounced in LC separations, but may also occur in GC separations. The shift could complicate the accuracy/reproducibility of identification and quantification in

Benefits of 13C vs. D Standards in Clinical Mass Spectrometry Measurements *(continued)*

complex biosample analysis, such as human plasma or urine. Only if the stability and effectiveness of deuterated standards are first demonstrated can its subsequent use in large-scale analysis be considered acceptable for critical decision-making studies (e.g., newborn screening, therapeutic drug monitoring, vitamin D deficiency).

To summarize, there are an array of factors to consider in designing experiments and implementing methods. Important amongst them is the type of labeled standard. As described above, $13C$ (and $15N$) standards provide excellent isotope stability and analytical reliability. This means that the position of label is not impacted by the pre-analytical and analytical processes. Since this type of standard has equivalent physicochemical properties as its unlabeled counterpart, we consider these to be ideal toward the accurate and reproducible quantitation of small or large molecules.

References

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Deuterated standards, in contrast, may exhibit isotope instability and an exchange or scrambling effect during storage and the experimental phases. These effects are magnified if the D-label is incorporated at exchangeable positions. Even if deuterium is placed at nonexchangeable positions, development time must be allotted for stability testing (e.g., at storage, in autosampler) and method evaluation (e.g., for mobile phase impact, preferable MRM transitions).4 That said, if validations have been performed and other options (e.g., 13C standards or surrogates) are absent, then this route could be suitable long-term. Overall, although Cambridge Isotope Laboratories (CIL) offer a multitude of variably labeled standards (encompasses vitamins, steroids, and fatty acids/lipids, amongst others), our recommendation is toward a 13C (and/or 15N) variant, when possible, for accurate/reproducible quantification in clinical MS-based analyses.

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Free Amino Acids and Their Derivatives

Amino acids play critical roles in biological functions as building blocks of peptides and proteins, as well as intermediates of various metabolic pathways (e.g., citric acid cycle, urea cycle). These compounds are also reported to influence the pathogenesis and propagation of metabolic disorders/disease, with clinically designed biomarker research aimed to detect disease at the earliest stage.

To aid qualitative and quantitative research, CIL offers an array of unlabeled and stable isotope-labeled free amino acids. These can be used as internal standards or NMR probes in MS- and NMR-based research studies. The amino acids are canonical (e.g., arginine, lysine, phenylalanine) and noncanonical (e.g., beta-alanine, citrulline, ornithine). These are available in their uniform or specifically labeled (with 13C, 15N, D, and/or 18O) forms, in research or MPT grade.

Free Amino Acids and Their Derivatives *(continued)*

❛❛**We concluded that CIL is the best-positioned company in the world to meet our future expected demands in terms of both material quantity and material quality. As we expand the use of our SILK™-based biomarkers beyond research services and into clinical diagnostic** applications, CIL will be an instrumental partner to help us qualify our test kits to produce L-leucine under GMP scaled-up conditions.⁹⁹

> *Joel B. Braunstein, MD, MBA Cofounder, President, and CEO, C2N Diagnostics*

Using Stable Isotopes to Decipher Systems-Level *in vivo* **Metabolism with MS and MRI**

Joseph Ippolito, MD, PhD, Assistant Professor of Radiology Mallinckrodt Institute of Radiology, Washington University School of Medicine, St. Louis, MO USA

In my laboratory, we focus on glutamine and branched chain amino acid metabolism in mitochondria as therapeutic targets for lethal cancers of the prostate and brain. We use 13 C, 15 N, and ²H stable isotopes in concert with MS- and NMR-based workflows to trace central carbon metabolism in cell culture and rodent cancer models. This allows us to combine that information with metabolic imaging strategies to understand cancer metabolism from a systems biology perspective.

Systems biology is a broad field that can be defined as the study of how complex biological systems function through the identification and integration of complex networks at the cellular and molecular levels. The recent revolution in genomics has introduced a wealth of information that has increased our understanding of health and disease. However, biological systems are regulated on multiple levels, indicating that genomics-level analyses will not be sufficient to define the function of a biological system by itself. This also has clinical implications, as the identification of tumor DNA mutations in a patient may not be associated with a readily available, clinically actionable cancer therapy. Therefore, understanding the functional readout of the genome, specifically, how downstream pathways

enhance cell and organ function, may lead to new approaches for diagnosis and treatment of human disease.

One well-known functional readout of the genome is metabolism, or the summation of nutrient-utilization pathways and their contributions to biosynthetic and energetic pathways in the cell. Stable isotopes (notably 2 H, 13 C, 15 N, and 18 O) that exist in thousands of available compounds have been used for decades to probe metabolism in both health and disease on the clinical level. In this scenario, a stable isotope tracer is administered either orally or intravenously to the patient. Once *in vivo*, the labeled tracer undergoes metabolism to the same degree as its equivalent unlabeled form. Tissues or biofluids are then obtained from the patient and analyzed with a MS-based workflow to assess the isotopic enrichment of metabolites within metabolic pathways of interest. Examples of clinically applicable methods using stable isotopes and MS include a breath test for *H. pylori* infection (e.g., with 13C urea), the assessment of body composition and energy expenditure (e.g., with ²H or ¹⁸O-labeled H₂O), and the assessment of macronutrient metabolism (e.g., with 1-13C leucine, ²H₅ phenylalanine, ${}^{13}C_3$ glycerol, 1-¹³C glucose).^{1,2} Despite the exquisite sensitivity and the ability to resolve the tracer and its metabolites, one limitation of MS is the loss of information regarding the distribution of the tracer and its metabolites both within a specific tissue compartment and throughout the body. Imaging mass spectrometry (IMS) takes a next step toward providing this information that can spatially register isotopically labeled metabolites in tissue sections.^{3,4} However, noninvasive systems-level information still cannot be obtained with this method. Thus, there is a pressing need for imaging methods to quantify tracer metabolism. Such methods should provide clinicians with information about metabolic pathways and regulatory networks to help guide the selection of appropriate therapies.

The use of stable isotopes as metabolic contrast agents in magnetic resonance spectroscopic imaging (MRSI) has been gaining traction in the research laboratory and the clinic. Hyperpolarized (HP) 13C MRSI with ¹³C pyruvate is one example. In this imaging modality, the stable isotope, $1^{-13}C$ pyruvate in this case, is dissolved in an aqueous solution and placed in a hyperpolarizer where the stable isotope undergoes dynamic nuclear polarization (DNP). This process "supercharges" the 13C in the pyruvate resulting in signal

enhancement by a factor of $\sim 10^5$. This improves the ability to measure the *in vivo* conversion of pyruvate to its downstream metabolites, including lactate. HP MRSI with 13C pyruvate is currently being used in many active clinical trials for cancer with an emphasis on prostate cancer, cervical cancer, and gliomas, as well as metabolic diseases involving the heart and liver. In addition to 1-13C pyruvate, additional tracers are being developed. This includes, but is not limited to, 2-13C pyruvate, 1-13C acetate, 1-13C alanine, ¹³C urea, ¹³C/¹⁵N₂ urea, ¹³C bicarbonate, and $1,4$ -¹³C₂ fumarate.5-7 HP MRSI still faces many limitations, including expensive instrumentation and lack of compatible isotopes for *in vivo* imaging. Because of these limitations, there is a push to develop alternate metabolic-imaging methods.

An alternative to ¹³C MRSI is ²H-based MRSI or deuterium metabolic imaging (DMI). DMI is rapidly gaining traction within the metabolicimaging community. 8 Recently, DMI with 6,6'-²H₂ glucose and ²H₃ acetate successfully demonstrated noninvasive metabolic maps with high spatial and high temporal resolution in not only rodents, but also in patients with brain tumors.⁹ Although DMI has been successfully performed with only a limited number of compounds, DMI represents a new way to use deuterated stable isotopes to image glycolytic flux rates in living systems. These initial studies of stable isotope imaging focused on glucose metabolism; however, the DMI approach can be expanded to all types of stable

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isotopes to interrogate multiple metabolic pathways. In fact, the development of MRSI with multinuclear capabilities is already in use, suggesting that multiple metabolic pathways may be imaged simultaneously in a living system with 13 C, 15 N, and ²H.

Despite the merits of metabolic imaging, there exists a prevailing need for validating the metabolite chemical shift signals that are acquired during imaging with a "gold standard" for those metabolites in the tissues. Stable isotope mass spectrometry has a clear role in this process, as tissues or biofluids obtained following imaging can then be analyzed and used to validate the imaging data obtained under currently available clinical workflows. This combination of analytical techniques is critical in my lab toward accomplishing the research aims of better understanding metabolism at the systems biology level.

In summary, the combination of conventional MS and imaging using stable isotopes has the potential to advance the research and clinical fields. The synergy of their strengths in providing spatially resolved metabolic flux in real time with coordinated validation and metabolic pathway mapping outweighs any limitations that these techniques face individually. I believe that this integration, and the use of stable isotopes therein, will only grow as the applications evolve.

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Stable Isotope Labeling Kinetics (SILK™) to Measure the Metabolism of Brain-Derived Proteins Implicated in Neurodegeneration

Joel B. Braunstein, MD, MBA, Co-founder, President, and CEO Tim West, PhD, VP of Research and Development C₂N Diagnostics, Center for Emerging Technologies, St. Louis, MO USA

Alzheimer's disease (AD) is a progressive neurodegenerative disease on track to becoming one of the greatest challenges to the healthcare system in the 21st century. AD affects millions of people in one way or another. It causes long-term memory loss, confusion, mood swings, and, eventually, loss of bodily functions. Sufferers from Alzheimer's tend to withdraw from family, friends, and other members of society as symptoms worsen. To date, there are no known cures, and patients and families of patients struggle with symptoms until death. Recent research has shown hope for early diagnosis and treatment. Much of this research has focused on amyloid plaques that are present in the brains of Alzheimer's patients. One approach to studying this unnatural accumulation of amyloid plaques is to monitor synthesis and clearance of the beta-amyloid peptide (Aβ) using L-leucine (${}^{13}C_6$, 99%) (CIL catalog no. CLM-2262-H).

Quantifying alterations in protein synthesis and clearance rates is vital to understanding disease pathogenesis. It also enables a determination of the effects of novel drug treatments on target protein metabolism. The powerful combination of *in vivo* stable isotope labeling and mass spectrometry has made this possible.

Alzheimer's neurons with amyloid plaque.

Specifically, researchers at Washington University have developed a proprietary method to measure the metabolism of Aβ and other proteins in the human central nervous system (CNS). C_2N Diagnostics has commercialized this platform for use in CNS drug development, disease detection, and progression monitoring.

In this method, individuals receive an administration of L-leucine $(^{13}C_6$, 99%) followed by serial cerebrospinal fluid (CSF) and plasma sampling. The clinical site that obtains these biological samples then sends them off to a central laboratory (i.e., C_2N Diagnostics) for processing and analysis. Mass spectrometry quantifies the ${}^{13}C_6$ leucine enrichment of Aβ to obtain rates of amyloid production and degradation. The SILK platform can also assess the kinetics of apolipoproteinE (ApoE) in cell culture as well as the human brain, among other proteins implicated in neurodegeneration. ApoE is the greatest known genetic risk factor for late-onset Alzheimer's disease. Elucidating the metabolism of the various ApoE isoforms is beginning to provide important insights about the role that ApoE plays in the disease progression of AD.

The SILK platform enables the testing of Alzheimer's drugs *in vivo* to determine the effects of the drug on the CNS and other systems in the body. This information is beneficial as a therapeutic biomarker for use in early clinical development. It has the potential to halt undeserving drug candidates early during the development process; thereby, reducing high downstream costs and wasted time to pharmaceutical companies.

Since most leucine-containing proteins are labeled after ${}^{13}C_6$ leucine infusion, this robust and versatile technique can be used as a method to determine the turnover rates for many different proteins. It can identify and quantify potential biomarkers for diseases and metabolic disorders beyond Alzheimer's. Please see the list at right for peer-reviewed references that describe the utility of this method.

SILK™ is a trademark of C_2N Diagnostics.

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Protected Amino Acids

Stable isotope-labeled peptides have demonstrated to be an effective means to quantify endogenous proteins in basic and translational bottom-up proteomics. In these experiments, the labeled peptides are employed as internal standards, where they serve as molecular surrogates of the target proteins enabling relative or absolute protein quantitation.

From a development standpoint, the peptides are produced in a step-wise manner by solid phase peptide synthesis using amino acid building blocks with *N*-terminal, 9-fluorenylmethoxycarbonyl (Fmoc) or *tert*-butoxycarbonyl (*t*-Boc) protecting groups. To help facilitate the synthesis of isotopically labeled peptides, CIL offers an assortment of uniformly or partially labeled Fmoc and *t*-Boc amino acids.

Protected Amino Acids *(continued)*

Protected Amino Acids *(continued)*

❛❛**The commercial availability of stable isotope-labeled amino acids with very high isotopic purity has revolutionized quantitative proteomics. From their use in metabolic labeling of cells and rodents for differential discovery proteomics, to their use in synthetic peptides as internal standards for targeted analysis of proteins, isotopically labeled amino acids make it possible to measure, with very high precision, changes in the levels of peptides and the proteins they are derived from in highly complex samples such as cell lysates, tissue, and plasma. Cambridge Isotope Labs has been and continues to be a leader in the commercial production of labeled amino acids and other labeled compounds.**❜❜

> *Stephen A. Carr, PhD Senior Director of Proteomics Broad Institute of MIT and Harvard*

❛❛**I have been obtaining protected amino acids from CIL for well over a decade. These materials are used to synthesize isotope-enriched peptides, which customers implement in their clinically driven proteomic research. Our clinical customers demand the highest isotopic enrichment, and CIL continues to deliver. In my opinion, CIL is the best isotope company out there – best in quality, best in service.**❜❜

> *Samuel Massoni Founder, President, and CEO New England Peptide*

Preloaded Resins

Through collaboration with New England Peptide, Inc. (NEP), CIL is pleased to offer synthesis-ready, preloaded resins to aid the solid-phase synthesis of stable isotope-labeled tryptic peptides. The resins are prepared from isotopically labeled, protected amino acids with the highest chemical, isotopic, and chiral purity available. Please inquire for pricing and unit sizes.

Antiviral Drugs

Through partnership with Alsachim, CIL is proud to now offer an assortment of antiviral drug standards and metabolites, in their stable isotope-labeled and unlabeled form. These compounds are available in 1 mg units and are adept for use as internal standards in therapeutic monitoring and quantitative analysis exercises. Please inquire for pricing or see [isotope.com](https://shop.isotope.com/category.aspx?id=10033139). *Available in North and South America only.*

For a listing of other "Drugs and Their Metabolites," please see [page 35](#page-34-0) or visit [isotope.com](https://shop.isotope.com/category.aspx?id=10033139).

Bile Acids

The analysis of bile acids (BAs) in biofluids is a developing and growing MS 'omics field. These steroid-like compounds act as a detergent that assists in the breakdown of fats. The primary BAs are synthesized from cholesterol in the liver, while secondary BAs are converted from primary BAs in the colon. The bile acids can also be conjugated with glycine or taurine in the liver, which increase their solubility in water. Bile acids have gained clinical attention by their linkage to colon cancer, liver disease, chronic diarrhea, cholestasis, hyperlipidemia, and gallstones. CIL is pleased to offer an extensive panel of primary and secondary BAs, in their free acid and conjugated salt forms. These research-grade products are available as isotopically labeled and/or unlabeled standards in solution (at 100 µg/mL in methanol) and/or neat form.

Bile Acids *(continued)*

Secondary Bile Acids and Their Conjugated Salts

Caffeine and Its Metabolites

Caffeine is a psychoactive stimulant of the central nervous system that is extensively consumed worldwide. MS-based research into the kinetics/metabolism of this compound and its metabolites (e.g., paraxanthine, theobromine, theophylline) has revealed insight into its health impact and abuse in humans. Studies further suggest an influence on pharmacological activity and neurodegeneration (e.g., Parkinson's disease); thus, strengthening a need for its robust clinical analyses.

CIL offers stable isotope-labeled caffeine and a collection of isotopically labeled metabolites for basic and translational quantitative research. These standards are available in various labeling patterns, with alternate compounds or labels evaluated upon request.

Carbohydrates

Carbohydrates are integral biomolecules to the function and process of living systems (e.g., in cell-to-cell signaling, immune responses, protein folding). Although this family of compounds is structurally diverse and complex, analysis by LC- and GC-MS techniques has been well adopted in the metabolomics field. Clinically, the quantitative analysis of sugars in human biosamples is of increasing importance for such disease screenings as cardiovascular and nonalcoholic fatty liver disease (NAFLD).

In addition to the classic monosaccharides (e.g., glucose, fructose, ribose) and sugar alcohols (e.g., erythritol, sorbitol, xylitol), CIL offers a number of other stable isotope-labeled carbohydrates. The list includes monosaccharides, under the pentose (e.g., arabinose, erythrose) and hexose (e.g., galactose, mannose) classes, disaccharides (e.g., lactose, maltose, sucrose), and polysaccharides (e.g., starch). These compounds are supplied with various labeling patterns as neat standards in research or MPT grade.

Carbohydrates *(continued)*

Carnitine and Acylcarnitines

Carnitine and acylcarnitines play an essential role in fatty acid metabolism. Metabolism disorders of fatty acid oxidation and several organic acidurias impose major clinical manifestations (e.g., hypoketotic hypoglycemia, skeletal myopathy, liver disease, and /or failure). These are largely attributed to enzymatic deficiencies and can be monitored through carnitine/acylcarnitine measurement.

To help facilitate metabolic screening exercises, CIL is pleased to offer a variety of stable isotope-labeled and unlabeled carnitine/acylcarnitine standards. Please refer to [page 58](#page-57-0) for a list of mix offerings; individual standards are noted below.

Carnitine and Acylcarnitines *(continued)*

Drugs and Their Metabolites

The field and scope of drug screening/analysis continues to expand worldwide. Example areas of focus include therapeutic drug monitoring, drugs of abuse, prescription monitoring, and clinical toxicology. The nature of those monitored or identified in the MS-based analysis include psychoactive drugs (e.g., benzodiazepines, cannabinoids, hallucinogens), pain-management drugs (e.g., analgesics, opiates, skeletal muscle relaxants), disorder-related treatment drugs (e.g., anticonvulsants/antiepileptics, antipsychotics, erectile dysfunction), and infectious disease or disease-related treatment drugs (e.g., antibiotics, antiarrhythmics).

CIL is pleased to offer a broad collection of unlabeled and stable isotope-labeled standards to aid the qualitative/quantitative analysis of drugs and their metabolites. These encompass a multitude of classes (e.g., analgesics, benzodiazepines, cannabinoids and its agonists, opiate and opioid analgesics, stimulants). The offerings are individual standards and/or class-specific mixtures in predominantly their concentrated solution form.

For a listing of "Antiviral Drugs," please see [page 27](#page-26-0) or visit [isotope.com](https://shop.isotope.com/category.aspx?id=10033139).

Available from CIL for customers in the US, Australia, Canada, and Switzerland. Contact us for sourcing details for other destinations. Products listed with an asterisk are available globally.

Alchohol Compounds

Amphetamines

**Products listed with an asterisk are available globally.*

Drugs and Their Metabolites *(continued)*

Analgesics

**Products listed with an asterisk are available globally.*
Anesthetics

Antibiotics

Anticonvulsants/Antiepileptics

**Products listed with an asterisk are available globally.*

Antidepressants

**Products listed with an asterisk are available globally.*

Antipsychotics

**Products listed with an asterisk are available globally.*

Barbituates

Benzodiazepines

**Products listed with an asterisk are available globally.*

**Products listed with an asterisk are available globally.*

Cannabinoids and its Agonists

**Products listed with an asterisk are available globally.*

Cardiac Drugs

**Products listed with an asterisk are available globally.*

Cocaine and its Metabolites

Hallucinogens

**Products listed with an asterisk are available globally.*

Immunosuppressants

Opiate and Opiod Analgesics

**Products listed with an asterisk are available globally.*

**Products listed with an asterisk are available globally.*

**Products listed with an asterisk are available globally.*

Other Compounds

**Products listed with an asterisk are available globally.*

**Products listed with an asterisk are available globally.*

Stimulants

**Products listed with an asterisk are available globally.*

**Products listed with an asterisk are available globally.*

Fatty Acids and Lipids

Fatty acids and lipids are important biological compounds that are essential to the regulation and control of cellular functions and metabolic pathways. These biomolecules are also tied to the energetic balance of an organism. Their qualitative/quantitative analysis has emerged to better understand the underlying pathophysiology, as well as to identify new biomarkers or diagnose existing ones.

To aid such research initiatives, CIL is pleased to offer a multitude of stable isotope-labeled and unlabeled fatty acids and lipids. The fatty acids cover saturated and unsaturated classes, while the lipids include ceramides (e.g., *N*-palmitoyl-D-sphingosine, *N*-oleoyl-D-sphingosine), and phospholipids (e.g., dodecylphosphocholine, dipalmitoyl phosphatidylcholine), as well as triacylglycerides (e.g., tripalmitin, tristearin, triolein). These are available in various labeling patterns (i.e., uniform, partial), forms (i.e., free acid, salt, ester), and material grades (i.e., research, MPT).

**Products listed with an asterisk are available globally.*

Fatty Acids and Lipids *(continued)*

Fatty Acids and Lipids *(continued)*

Metabolomics Mixtures and Kits

Metabolomics is an increasingly important and growing area of research. The use of stable isotopes (as internal standards), in combination with analytical techniques such as mass spectrometry, allow researchers to identify and quantify metabolites in a given biological sample. This information can be used to better understand disease mechanisms, evaluate drug responses, and assess putative biomarkers, amongst other targeted applications.

To help facilitate such initiatives, CIL is pleased to offer a variety of mixes and kits. These are designed to aid ease of use in untargeted and targeted metabolomics exercises (e.g., in quantification, qualification, quality control, system suitability). The mixtures are offered neat or as solutions, while the kits are additionally supplied with a user manual. The manuals outline general procedures and processing tables (i.e., platform parameters and conditions), as well as alternate method suggestions and data analysis guides for user reference. Supplemental figures and references in the user manuals provide additional user support.

For complete details, please see the [Metabolomics Mixtures and Kits application page](https://www.isotope.com/applications/subapplication.cfm?sid=Metabolomics%20Mixes%20and%20Kits_8) at isotope.com.

Companion unlabeled standard mixes and kits are also available; please inquire.

❛❛**I have used products from Cambridge Isotope Laboratories (CIL) for well over 15 years because of the high quality provided. Recently, I have collaborated with CIL to develop a new product that would enable improved quality control in MS metabolomics. This process was exciting and engaging. Through collaboration, I found CIL to be a cutting-edge vendor that seeks opportunities to enable scientific discovery and data quality. They seek advice from clients to learn how to better serve them. I always look forward to talking with CIL at conferences and often seek out their booth to visit, not just to talk science, but to also see how their team is doing.**❜❜

> *Timothy J. Garrett, PhD Associate Professor in Department of Pathology, Immunology, and Laboratory Medicine, University of Florida*

MS/MS Screening Mixtures and Standards

The utility of stable isotope-labeled standards for MS/MS screening is gaining traction worldwide. To support such research endeavors and enhance method adoption, CIL is pleased to offer a breadth of high-quality, stable isotope-labeled mixtures. These mixes contain a collection of stable isotope-labeled standards (e.g., 12 amino acids in NSK-A) and are class-specific (e.g., amino acids, carnitine/ acylcarnitines, steroids). These are available in 10-vial sets or single vials and are suitable for metabolite quantification in isotope dilution MS (IDMS) experiments. Also listed here are example individual standards used in MS/MS screening research. Please refer to [page 106](#page-105-0) for CE-mark amino acid and acylcarnitine mixes.

For complete details, please see the [MS/MS Standards application page](https://www.isotope.com/applications/applications.cfm?aid=MS%2FMS%20Standards) at isotope.com.

Mixtures

Individual Standards (Examples)

MS/MS Screening Mixtures and Standards *(continued)*

Stable Isotopes and Mass Spectrometry: An Inseparable Duo

Donald H. Chace, PhD, MSFS, FACB, CSO Medolac Laboratories, Boulder City, NV USA

The importance of stable isotope-enriched compounds in accurate mass spectrometry (MS)-based quantification cannot be underestimated. For context, using ion counts without a reference to quantify a substance is like estimating the temperature based on the time of year. It is simply an inaccurate and imprecise strategy. Stable isotopes have therefore been an important tool throughout my entire career. This stems from the development of a novel MS approach for use in the discovery of drug metabolites to isotope dilution for the quantification of metabolites in neonatal blood spot screens. In both cases, the ability to choose compounds with variable labels (e.g., ^{13}C , ^{15}N , D) and positions that have been produced at high isotopic enrichment (>99%) and chemical purity (>98%) are vital to effective discovery and clinical-based applications. The following highlights this importance with a newborn screening example.

As one of the original developers of the tandem MS application of amino acid and acylcarnitines in dried blood spots of neonates,¹ it became clear early on that three-four standards would be insufficient to accurately measure metabolites in a profile of dozens of compounds from multiple chemical families (i.e., basic, acidic, neutral). As the compounds exhibit vast structural and chemical diversity (in terms of polarity, charge, and functional groups), it became imperative to develop an approach to measure multiple

metabolite standards in a single analysis with each method, processing as many compounds or compound types as possible. Because flow injection MS/MS (without chromatography) is a feature of the screening method, having the ability to pick the right standard (with the correct number of labels and mass shifts from unlabeled) was extremely important. Also important in the standard selection process was the nature of quality control (QC). Only wellcharacterized standards would provide me with the confidence to utilize these on a routine basis in newborn-screening analysis.

CIL was the first to develop a multiplexed standard approach back in the late 1990s. Since then, we have together been making improvements and expanding the metabolite list. Often, as the "real estate" for available mass values in a flow injection analysis becomes more narrow, we require custom synthesis to make the ideal standard and ultimately add to the set of standards in our multiplexed screening panel. Today, CIL still sets the standard by providing a resource to those in metabolic screening, as well as to the rapidly expanding, and clinically relevant, field of metabolomics.2 Although there may not be a kit available immediately for all targets of interest, CIL remains a resource to obtain the candidate or validated biomarker standards for a proper quantitative reference.

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Simultaneous Analysis of Amino Acids, Acylcarnitines, and Succinylacetone in Dried Blood Spots for Research Using Nonderivatized and Derivatized Methods

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Introduction

Flow injection tandem mass spectrometry (FIA-MS/MS) has been frequently used for analysis of amino acids (AA) and acylcarnitines (AC) in dried blood spots for inborn errors of metabolism research.1-3 Established methods for detecting succinylacetone (SUAC) can be laborious because they require additional extraction due to its insolubility in the first extraction solution for AA and AC. In this technical note, a single extraction step was used to simultaneously analyze AA, AC, and SUAC in dried blood spots on the Thermo Scientific™ TSQ Endura™ triple quadrupole mass spectrometer.4

The original sample preparation techniques use butyl esterification (i.e., derivatized) of amino acids and acylcarnitines in dried blood spots (DBS) due to the increased sensitivity that derivatization provides. However, with the improved sensitivity of new mass spectrometry technologies, it is possible to detect both amino acids and acylcarnitines as their native free acids (i.e., nonderivatized). This technological improvement simplifies analytical operation and minimizes the use of corrosive chemicals. In this technical note, both nonderivatization and derivatization sample preparation methods were compared.

Many previous FIA-MS/MS studies on dried blood spots deployed neutral-loss scan mode (acylcarnitines) and precursor ion scan mode (some amino acids) for fast method development. In this technical note, selected reaction monitoring (SRM) was used for all AA, AC, and SUAC data acquisition. The advantage of SRM is that it accurately quantifies analytes and ensures both high selectivity and sensitivity, which especially benefited analysis of analytes that ionize poorly.

Method

Sample Preparation

Sets of isotope-labeled internal standards of amino acids (NSK-A), acylcarnitines (NSK-B and NSK-B-G), and succinylacetone (NSK-T) were purchased from Cambridge Isotope Laboratories, Inc. The daily working internal standard concentration is listed in Table 1. Hydrazine, 1-butanol, and acetyl chloride were purchased from Sigma-Aldrich®. The other reagents were from Thermo Fisher Scientific.

The DBS quality control (QC) samples were kindly provided by the United States Centers for Disease Control and Prevention (CDC) for research purposes. The QC samples contained enriched analytes at three concentrations: low, intermediate, and high.

Table 1. Daily working internal standard concentrations.

The following protocols were used to prepare the DBS samples:

Derivatized

- 1. Punch one 1/8" diameter disc from DBS sample into a 96-well plate.
- 2. Add 100 μL of working internal standard solution (containing internal standards of 12 amino acids, 12 acylcarnitines, and SUAC) to each well.
- 3. Shake the well plate for 45 min at 45°C.
- 4. Transfer the eluates to another well plate and evaporate at 50°C under nitrogen flow.
- 5. Pipet 50 μL of methanol into each sample well and evaporate under nitrogen flow.
- 6. Pipet 50 μL of 3 n-butanol HCl into each sample well and incubate at 65°C for 20 min. Then, evaporate under nitrogen flow.
- 7. Reconstitute each sample well with 100 μL of 50:50:0.02 acetonitrile/water/formic acid.

Nonderivatized

- 1. Punch one 1/8" diameter disc from DBS sample into a 96-well plate.
- 2. Add 100 μL of working internal standard solution (containing internal standards of 12 amino acids, 12 acylcarnitines, and SUAC) to each well.
- 3. Shake the well plate for 45 min at 45°C.
- 4. Transfer the eluates to another well plate and evaporate at 50°C under nitrogen flow.
- 5. Pipet 50 μL of methanol into each sample well and evaporate under nitrogen flow.
- 6. Reconstitute each sample well with 100 μL of 50:50:0.02 acetonitrile/water/formic acid.

Liquid Chromatography

Table 2. Flow gradient.

Mass Spectrometry

Flow injection MS/MS analysis was performed on a TSQ Endura triple quadrupole mass spectrometer. The mass spectrometer conditions were as follows:

Data Analysis

Tandem MS data were analyzed using a meta-calculation software, iRC PRO (2Next srl, Prato, Italy). This offline automated data analysis tool can process peaks and formulas, as well as quantify target analytes from the ion ratios (unlabeled:labeled) of SRM data (see Figure 1).⁵

The metacalculation software improves time effectiveness by eliminating the manual calculation process and removing transcription errors in the post-analytical phase. The processing time is reduced from hours to minutes.

Assay Validation

The intra-assay precision was determined at three concentrations by means of ten successive, independent measurements of DBS samples (n=10). The interassay precision was determined at three concentrations by means of ten independent measurements of DBS samples in seven different test series (n=70).

Figure 1. irC PRO intuitive workflow – icon-based interface.

Continued ➤

Simultaneous Analysis of Amino Acids, Acylcarnitines, and Succinylacetone in Dried Blood Spots for Research Using Nonderivatized and Derivatized Methods *(continued)*

Table 3. SRM parameters (derivatized).

Table 4. SRM parameters (nonderivatized).

Simultaneous Analysis of Amino Acids, Acylcarnitines, and Succinylacetone in Dried Blood Spots for Research Using Nonderivatized and Derivatized Methods *(continued)*

Results

The derivatization process using butanol converted free amino acids and acylcarnitines into the butyric esters and added a mass of 56 (except for aspartic acid, glutamic acid, and C5DC, in which a mass of 112 was added). Figures 2 and 3 show full-scan spectra of derivatized and nonderivatized internal standards, respectively.

Figure 2. Full-scan spectra of derivatized internal standards.

Figure 3. Full-scan spectra of nonderivatized internal standards.

SRM was used to acquire MS/MS data for all the analytes. Collision energy and RF lens parameters were optimized for each target and internal standard to ensure maximum selectivity and sensitivity.

Figure 4. Flow injection analysis (FIA) profiles of SUAC-¹³C₅, SUAC and C5DC- d_3 , C5DC using derivatized method.

SRM allowed acquisition of peaks with good signal-to-noise ratios even for analytes with poor ionization such as SUAC and C5DC regardless of whether derivatization was used (see Figures 4 and 5).

Figure 5. Flow injection analysis (FIA) profiles of SUAC-¹³C₅, SUAC and C5DC-d₃, C5DC using nonderivatized method.

Simultaneous Analysis of Amino Acids, Acylcarnitines, and Succinylacetone in Dried Blood Spots for Research Using Nonderivatized and Derivatized Methods *(continued)*

Intra-assay Precision

For the derivatized method, the average intra-assay precisions (n=10) for 12 AA and SUAC at three concentrations were 7.9% (low), 8.0% (intermediate), and 8.0% (high). The average intraassay precisions for 18 AC at three concentrations were 8.9% (low), 8.3% (intermediate), and 9.0% (high) (see Table 5).

N/A, the analytes were not enriched in QC samples.

For the nonderivatized method, the average intra-assay precisions (n=10) for AA and SUAC at three concentrations were 6.1% (low), 7.2% (intermediate), and 9.8% (high). The average intra-assay

precisions for AC at three concentrations were 7.6% (low), 6.2% (intermediate), and 8.2% (high) (see Table 6).

N/A, the analytes were not enriched in QC samples.

Simultaneous Analysis of Amino Acids, Acylcarnitines, and Succinylacetone in Dried Blood Spots for Research Using Nonderivatized and Derivatized Methods *(continued)*

Inter-assay Precision

For the derivatized method, the average inter-assay precisions (n=70) for 12 AA and SUAC at three concentrations were 13.5% (low), 12.9% (intermediate), and 12.5% (high). The average

inter-assay precisions for 18 AC at three concentrations were 15.0% (low), 15.6% (intermediate), and 16.1% (high) (see Table 7).

N/A, the analytes were not enriched in QC samples.

For the nonderivatized method, the average inter-assay precisions (n=70) for AA and SUAC at three concentrations were 12.8% (low), 12.8% (intermediate), and 12.6% (high). The average inter-assay

precisions for AC at three concentrations were 12.7% (low), 10.5% (intermediate), and 11.8% (high) (see Table 8).

N/A, the analytes were not enriched in QC samples.

Continued ➤

Simultaneous Analysis of Amino Acids, Acylcarnitines, and Succinylacetone in Dried Blood Spots for Research Using Nonderivatized and Derivatized Methods *(continued)*

Method Comparison

The concentration of analytes obtained from nonderivatized and derivatized methods were compared. The average method differences of 12 AA and SUAC between quantitative values resulting from derivatization and nonderivatization methods at three concentrations were 3.8% (low), 4.8% (intermediate), and 3.2% (high). The average method differences of 18 AC at three concentrations were 14.2% (low), 11.4% (intermediate), and 10.5% (high) (see Figure 6). Therefore the two methods were highly correlated. Our data are consistent with the reported results from a comprehensive empirical analysis.6

Figure 6. Comparisons between quantitative values of 12 AA, SUAC, and 18 AC resulting from nonderivatized and derivatized methods.

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Conclusions

- Flow injection-tandem mass spectrometry methods were developed to simultaneously detect and quantify amino acids, acylcarnitines, and succinylacetone in a single extraction process in dried blood spots for research. Rapid data processing was performed using iRC Pro metacalculation software.
- Both derivatization and nonderivatization sample preparation methods were capable of accurately quantifying AA/AC/SUAC on TSQ Endura triple quadrupole MS with a run time of 1.5 min.
- SRM data acquisition mode optimized for each analyte and internal standard guarantees both high sensitivity and high selectivity.
- The TSQ Endura MS system can provide average intra-assay precision (n=10) at three enriched concentrations of less than 10% and average inter-assay precision (n=70) of less than 15% for both nonderivatized and derivatized methods.
- The method difference between quantitative values resulting from nonderivatized and derivatized methods was minor and both methods are highly correlated.

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Neurotransmitters and Their Metabolites

Neurotransmitters are small chemicals in the central nervous system that modulate and regulate brain function. Signals are relayed from neuron to neuron by release, upon stimulation, from a synaptic vesicle into a space where it can bind to a receptor. These molecules can be grouped into several classes, such as amino acids and their derivatives (e.g., γ-aminobutyric acid, glutamate) and biogenic amines (e.g., dopamine, epinephrine, serotonin). MS analysis of neurotransmitters in human biosamples, such as urine, is a clinically relevant area as they mediate homeostatic function, modulate neural activity, and have been correlated to the pathogenesis of neurodegenerative diseases (e.g., Alzheimer's).

CIL offers an array of stable isotope-labeled neurotransmitters. These research-grade materials are available in their solution and/or neat form.

Neurotransmitters and Their Metabolites *(continued)*

Nucleic Acids

Nucleic acids are necessary biomolecules of living systems, being fundamentally important to a multitude of cellular processes. Its basic building blocks are nucleobases (e.g., adenine, cytosine, xanthine), nucleosides (e.g., adenosine, guanosine, inosine), and nucleotides (e.g., ATP, CDP, dGTP). The qualification/quantification of these compounds, and their synthetic analogues (e.g., 5-fluorouracil), in biosamples is performed preclinically and clinically to address a number of purposes. This includes the screening of metabolic errors and the efficacy evaluation of drug treatments (be it anticancer, antiviral, or immunosuppressive), among other target areas.

CIL offers an array of stable isotope-labeled nucleic acid building blocks for MS- or NMR-based research. These standards are available in a variety of labeling patterns and quantities.

Nucleic Acids *(continued)*

❛❛**I have been extremely happy with all products and services that we've obtained from CIL. All stable isotope reagents have exceeded our expectations. We've also obtained custom services from CIL to complete some very challenging studies, and have found CIL flexible and willing to work with us to achieve our goals. In short, I have nothing but positive things** to say about our experience with CIL and their products.^{••}

> *Matthew Steinhauser, MD Principle Investigator, Department of Medicine Brigham and Women's Hospital*

❛❛**When my lab makes labeled RNA, we count on high yields and purity. We have been using 13C/15N ribonucleotide triphosphates (rNTPs) and selectively deuterated rNTPs from CIL for the past 10 years. We have always been very happy with the performance and quality of the CIL rNTPs. They are the gold standard.**❜❜

> *Samuel Butcher, PhD Professor, Department of Chemistry University of Wisconsin-Madison*

Organic Acids and Their Conjugate Salts

Organic acids (OAs) play essential roles in energy metabolism pathways (e.g., glycolysis, tricarboxylic acid cycle), with the short-chained OAs emerging as important regulators of host immune response and transcriptional regulation.

To aid quantitative research in preclinical and clinical studies, CIL is pleased to offer a collection of stable isotope-labeled and unlabeled OAs and their conjugate salts. These encompass monocarboxylic (e.g., acetic, lactic), dicarboxylic (e.g., malic, succinic), and tricarboxylic (e.g., *cis*-aconitic, citric) acids.

Organic Acids and Their Conjugate Salts *(continued)*

Other Compounds

CIL offers a breadth of other compounds that could find utility in qualitative and quantitative, analytical analyses. These are available in neat or solution form in variable unit sizes. Please see [isotope.com](http://www.isotope.com) for a comprehensive listing of additional individual compounds.

Other Compounds *(continued)*

❛❛**CIL has greatly facilitated our clinical research by supplying specially designed labeled compounds. We have made great progress in our** neonatal antioxidant research program by the provision of labeled glutathione by CIL.⁹⁹

> *Frans W.J. te Braake, PhD Erasmus MC, Sophia Children's Hospital*

PeptiQuant™ Plus Assay Kits

Researchers in academia and life science industries continue to adopt a targeted, bottom-up MS-based workflow for protein biomarker evaluation. Biomarker verification/validation requires absolute quantification of surrogate peptides in the sample matrix, a requirement that is best achieved using well-characterized standards. To ensure robust quantitative measurement, quality control (QC) checks should be routinely performed. CIL offers a collection of PeptiQuant™ Assay Kits (from MRM Proteomics Inc.) for QC and biomarker assessment using bottom-up LC-MS/MS methodologies. The QC kits are designed to evaluate the performance of an LC-MS platform, either alone or in combination with a human or mouse plasma proteomic workflow. The biomarker assessment kits (BAKs) are intended to help researchers screen target panels of candidate protein disease biomarkers in human or mouse plasma samples. Platform-specific kits are listed below.

Quality Control (QC) Kits

Biomarker Assessment Kits (BAKs)

*Alternate sets of 125 target proteins are available (see [product flyer](https://www.isotope.com/userfiles/files/assetLibrary/MRM_PQ_PLUS%20flyer.pdf) for details). PeptiQuant is a trademark of MRM Proteomics Inc.

❛❛**The PeptiQuant Plus Platform Performance Kit has proven to be a vital component of our everyday quality assurance that enables us to deliver high-quality targeted proteomics data in an accurate and timely manner. This kit has a 'dilute and shoot' operation and comes with vendor-specific LC-MRM/MS parameters and a Skyline analysis file for quick input and results output. Altogether, the performance** kit is an excellent means to rapidly assess LC-MS performance that should become a routine staple in a proteomic user's toolbox. ⁹⁹

Tasso Miliotis, PhD, Associate Principal Scientist at AstraZeneca Gothenburg

❛❛**PeptiQuant Plus Assay Kits contain all the essential materials, including the standards and methods, for performing absolute protein quantification by LC-MRM/MS in a standardized way. The standard protocol helped us reduce the assay development time, while improve the reproducibility and precision of multiplex protein quantification. In addition to the biomarker assessment kits, the quality control kits enable the instrument performance and assay reproducibility to be monitored and assessed, which ultimately provided us confidence in the reliability of the quantification results.**❜❜

Elaine Wong, PhD, Scientific Officer at Queen Mary Hospital, Fu Lam, Hong Kong

Pharmaceutical and Personal Care Products

Concerns about environmental and human exposure to pharmaceutical and personal care products (PPCPs) has grown significantly over the years. The classification of PPCPs encompasses a broad range of chemicals, ranging from antibiotics to hormones to food and drinking water impurities. Isotope-labeled standards are necessary in the qualitative/quantitative analysis of PPCPs, especially in complex matrices such as sewage sludge and biosamples, as well as in applications where ion suppression or enhancement are of high concern. CIL, with guidance from leading laboratories around the world, works diligently to produce high-quality, native and stable isotope-labeled standards for analysis of PPCPs. Please see [isotope.com](https://shop.isotope.com/category.aspx?id=10032742) for the listing and details of our PPCP standards.

- *• n*[-Alkane Standards](https://shop.isotope.com/category.aspx?id=10032725)
- *•* [Bisphenol Standards](https://shop.isotope.com/category.aspx?id=10032950)
- *•* Chlorinated Paraffin Standards
- *•* [Endocrine-Disrupting Compounds and Xenoestrogen Standards](https://shop.isotope.com/category.aspx?id=10032721)
- *•* [Explosives Standards](https://shop.isotope.com/category.aspx?id=10032724)
- *•* [Food and Drinking Water Impurity Standards](https://shop.isotope.com/category.aspx?id=10032714)
- *•* [Halogenated and Substituted Benzene, Phenol, and Anisole Standards](https://shop.isotope.com/category.aspx?id=10032720)
- *•* Industrial Chemical Standards
- *•* [Nitrosamine Standards](https://shop.isotope.com/category.aspx?id=10032717)
- *•* [Nonylphenol, Nonylphenol Ethoxylate, and Nonylphenol Carboxylate Standards](https://shop.isotope.com/category.aspx?id=10032715)
- *•* Paraben Standards
- *•* Perfluorinated Standards
- *•* [Personal Care Products](https://shop.isotope.com/category.aspx?id=10032759)
- *•* Phthalate and Phthalate Metabolite Standards
- *•* [Perscription and Nonperscription Drugs](https://shop.isotope.com/category.aspx?id=10032728)
- *•* [Sex and Steroidal Hormones](https://shop.isotope.com/category.aspx?id=10032745)
- *•* Tobacco-Specific Nitrosamines and Other Tobacco-Related Standards
- *•* [Veterinary and Human Antibiotics](https://shop.isotope.com/category.aspx?id=10032744)

Steroids and Hormones

Steroids and hormones play vital roles in the regulation of a diverse array of cellular functions and physiological processes. These pertain to development, reproduction, homeostasis, and metabolism, among others. Accurate quantification of this compound class is essential for basic and clinical translation research. This can be achieved by spiking an isotopically labeled steroid standard(s) into a sample of interest, such as plasma or urine, with measurement performed by an MS- or NMR-based approach.

CIL offers a variety of stable isotope-labeled and unlabeled steroids and hormones. These are available in different labeling patterns in their neat and/or solution forms.

**Isotopic enrichment to be advised at time of shipment.*

**Isotopic enrichment to be advised at time of shipment.*

Steroids and Hormones *(continued)*

Steroids and Hormones *(continued)*

Vitamins and Their Metabolites

Vitamins are organic compounds that directly or indirectly participate in organisms' biochemical reactions. These are divided into two classes, based on their solubility in fat (includes A, D, E, and K) and water (includes B and C).

CIL offers unlabeled and stable isotope-labeled vitamins as neat compounds and/or in solution at specified concentrations. These can be used in a wide range of applications, such as metabolism and pathophysiology explorations, as well as disease biomarker evaluation in preclinical and clinical MS studies (e.g., vitamin D deficiency). These standards help facilitate accurate and precise quantification of endogenous metabolites in biological matrices.

Water Soluble

**Products listed with an asterisk are available only in the US, Switzerland, and Australia.*

**Products listed with an asterisk are available only in the US, Switzerland, and Australia.*

Vitamins and Their Metabolites *(continued)*

❛❛**Quantitative analysis in clinical diagnostics using mass spectrometry remains a difficult endeavor particularly for small molecules due to chemical similarity and isobaric forms of many substances. Both chromatography and the use of isotopically labeled internal standards to perform small-molecule quantification are required to obtain good quantitative results in many applications. The use of isotopically labeled internal standards remains the best solution as these standards ideally match the chemical behavior of their analytes, thus** leading to better quantification than obtained when using structure homologues with physicochemical characteristics.[•]

> *David C. Kasper, PhD CEO, ARCHIMED Life Science*

Urea

To complement the growing area of urea-based research in the preclinical and clinical fields (e.g., as biomarker of respiratory and renal diseases), CIL offers a variety of stable isotope-labeled urea compounds. These are available in various labeling patterns and in different material grades (i.e., research, MPT, cGMP). In one example application, a ¹³C urea breath test can be used to accurately and noninvasively diagnose *H. pylori* infections, such as peptic ulcer disease and gastric cancer. This test involves the oral ingestion of cGMP-grade ¹³C urea, with measurement of the ¹³CO₂ to ¹²CO₂ area ratios in the expired breath facilitating diagnosis.

Water

CIL offers a variety of singly and doubly labeled water compounds for use in MS- and NMR-based studies (see list below). These could be applied, for example, in energy-expenditure research or in virtual biopsy methods, as described in the Hellerstein article that follows.

Chemical purity (CP) is 98% or greater, unless otherwise specified.

cGMP (current good manufacturing practice) and MPT (microbiological and pyrogen tested) may be available; please inquire. For research use only. Not for use in diagnostic procedures.

Stable Isotopes in Drug Development and Personalized Medicine: Biomarkers that Reveal Causal Pathway Fluxes and the Dynamics of Biochemical Networks

Marc Hellerstein, MD, PhD

Professor, The Dr. Robert C. and Veronica Atkins Chair in Metabolic Nutrition Berkeley Nutritional Sciences and Technology, University of California, Berkeley, CA USA

The combination of stable isotope labeling with powerful mass spectrometric analytic techniques is providing increasingly important diagnostic tools for drug development and clinical diagnostics in the emerging era of personalized medicine.

The Problem: High Attrition Rates in Contemporary Drug Development

Although it is widely believed that we live in a golden era of breakthroughs in new medicines, the opposite is true. Recent years have witnessed the lowest rate of new drug approvals in a generation, despite greatly increased pharmaceutical industry investment.1 These disappointing facts hold true for all classes of disease but are particularly worrisome for growing epidemics of chronic disease, such as Alzheimer's disease, diabetes, osteoarthritis, and obesity-related disorders.

The problem is not a lack of molecular targets or candidate drugs. The molecular target-based approach to drug discovery, which has dominated pharmaceutical research for the past 20 years, has generated huge lists of genes, proteins, and potential drug therapies. The problem is that the attrition rate of drug leads has gotten worse, not better, with >98% of leads now failing for efficacy or safety reasons, including 90% failure rates in human trials.2,3 This attrition is largely responsible for the high cost of each successful drug eventually approved.

Losing the War with Complexity

Attrition, in turn, is largely due to the unpredictability of the complex networks that comprise living systems in response to targeted interventions at specific nodes.² Unanticipated functional consequences of targeted interventions, both undesirable and beneficial, are the rule rather than the exception in such systems (Figure 1). Pathogenic heterogeneity among individuals within each disease magnifies this problem, requiring different intervention strategies for different subsets of patients. The latter issue is embodied by the notion of personalized medicine.

The Missing Link: Metrics for Navigating through the Complex Biology of Disease

The key missing factors for navigating through the complex biology of disease are objective measures that guide drug developers toward the goals of safe and efficacious outcomes.⁴ These metrics, called biomarkers, must be predictive of clinical outcomes and translatable from preclinical models into humans. The most reliable way to achieve these goals is to capture the underlying biologic processes driving each disease (i.e., the disease modifying pathways or underlying pathogenesis). Metrics of this type can serve to guide rational drug discovery and development and allow monitoring of clinical response.

Figure 1. Losing the war with complexity: unpredictability of complex dynamic networks. Figure 2. Pathway fluxes as the link between molecular targets and clinical outcomes.

Nowhere will this need for functionally informative biomarkers be greater than in the field of "personalized medicine"– the right

Continued ➤

Stable Isotopes in Drug Development and Personalized Medicine: Biomarkers that Reveal Causal Pathway Fluxes and the Dynamics of Biochemical Networks *(continued)*

patient, the right drug, at the right time, and in the right dose. Companion diagnostic tests are extremely high-value examples of this trend.

Stable Isotopes Are Essential for a New Class of Biomarkers: Tests that Predict Clinical Outcomes by Revealing Functionally Interpretable Information about Underlying Disease Processes

A new class of biomarkers is needed that are predictive of clinical outcomes.4,5 The biologic pathways that underlie chronic diseases – the causal processes responsible for initiation, progression, severity, and therapeutic reversal of disease – generally involve the flow of molecules through a pathway that is itself complex and influenced by numerous factors⁵⁻⁸ (Figure 2). Stable isotopic techniques have made all of these causal pathways measurable in higher organisms.

What Stable Isotopes Bring to Diagnostic Biomarkers

Stable isotopes allow fluxes through metabolic pathways and the dynamics of global biochemical networks to be measured, without toxicity and often noninvasively, for two reasons: first, experimental administration of stable isotopes introduces an "asymmetry" in the dimension of time (label not present, then present), which allows the timing of dynamic processes to be measured; and, second, biochemical research over the past century has established the pathways that link molecules in cells and organisms, allowing the fates of labeled substrates to be traced *in vivo*.

Importantly, stable isotopes have been used for over 70 years in humans and experimental animals and have almost no known toxicities. The FDA policy toward stable isotope-labeled products is clear and has been consistent for decades: no regulatory approval is required to administer stable isotope-labeled compounds, beyond what is needed to administer their natural abundance congeners (sterility, pyrogenicity, etc.). It should be noted that stable isotopicmass spectrometric biomarkers are not radiographic imaging techniques, but require a sample from the body (blood, urine, CSF, tissue, saliva).

Two Broad Categories of Stable Isotope-Based Kinetic Biomarkers Are Available

There are two broad categories of stable isotope-based biomarkers that are most useful in drug development and diagnostics: 1) Kinetics of targeted causal pathways and; 2) Interrogation of network dynamics for unbiased discovery of kinetic signatures and unanticipated causal pathways. Both types are available and useful in drug discovery and development.⁵⁻¹⁶

Kinetics of Targeted Causal Pathways as Biomarkers for Drug Discovery and Development

Some common examples of causal pathways in disease are shown

Table 1. **Examples of Causal Pathways: A) Neurobiology**

- Cargo transport through axons
- Amyloid beta synthesis and plaque turnover
- Neurogenesis
- Myelination/remyelination
- Neurotransmitter release and turnover
- Neuronal mitochondrial biogenesis
- Neuroinflammation, microglia activation
- Cytokine release
- Hungtingtin protein turnover
- Prion turnover
- Synaptic plasticity

B) Obesity/T2DM

- Pancreatic beta cell proliferation and mass
- Insulin-mediated glucose uptake
- Hepatic glucose production
- Adipogenesis and TG deposition
- Adipose tissue fatty acid oxidation/brown fat transition
- Adipose tissue remodeling
- Hepatic TG synthesis and release
- Atheroma cholesterol removal and deposition
- Adipose tissue macrophage proliferation and activation
- Muscle mitochondrial beta-oxidation and biogenesis

C) Cancer/Neoplasia

- Tumor cell proliferation and death rate
- Angiogenesis
- Lymphangiogenesis/metastatic spread
- Tumor-specific T-cell proliferation
- DNA methylation/demethylation
- Ribonucleotide reductase activity
- Histone deacetylation
- Precancer evolution to aggressive phenotypic
- Extracellular matrix turnover

(Table 1). These include: synthesis of collagen and extracellular matrix in fibrotic diseases; myelin synthesis and metabolism in multiple sclerosis; turnover of amyloid plaque and synthesis of amyloid beta 1-42 in Alzheimer's disease; synthesis of muscle myosin and biogenesis of mitochondria in sarcopenia; angiogenesis and proliferation and death of tumor cells in cancer; transport of cargo molecules through axons in neurodegenerative conditions; autophagic flux in Huntington's, Parkinson's, and other diseases characterized by protein aggregates; clot formation and lysis in thromboembolic diseases; insulin-mediated glucose uptake and pancreatic beta cell proliferation in insulin-resistant states; adipose tissue lipid dynamics and remodeling in obesity; reverse cholesterol transport in atherosclerosis; activation of the complement cascade in inflammatory states; HIV replication and turnover of CD4+T-cells in AIDS; and many others.

The ability to measure the activity of any of these functionally relevant processes that are believed to play causal roles in disease is potentially transformative for drug discovery and development in these fields (e.g., Parkinson's disease.^{10,11}).

Interrogation of Network Dynamics

Perhaps the most exciting advance in stable isotope biomarkers in recent years is the emergence of "network dynamics": unbiased interrogation of the dynamic behavior of complex biochemical networks that comprise living systems. This has been successfully applied to preclinical models and humans for the dynamics of the global proteome, or dynamic proteomics.^{12,13} This provides a new type of systems biology, with great potential as an unbiased screening tool for biomarker discovery.

Dynamic proteomics represents the most functionally interpretable of the "omics" technologies – i.e., providing not just heat maps or informatics, but functionally interpretable systems biology information. The operational flow chart for measuring the dynamics of a proteome is shown (Figure 3). This approach has been applied with great success to questions such as the effects of calorie restriction of cellular proteostasis, including mitochondrial biogenesis and mitophagy; the proteome dynamic signature of poor prognosis in chronic lymphocytic leukemia tumor cells; differentiating between pancreatic islets successfully compensating for insulin resistance in obese animals vs. islets that are failing and becoming "exhausted"; the effects of exercise on muscle proteome turnover; the effects of neuroinflammation on CSF proteome turnover; the dynamics of the high-density lipoproteins (HDL) proteome in dyslipidemic states; and other questions of interest in physiology and pathophysiology.

'Virtual Biopsy' Approach for Noninvasive Biomarkers of Intracellular Pathways

Unbiased screening of proteome dynamics in a tissue can also lead to discovery of targeted protein biomarkers that are accessible to sampling in a body fluid. Called the "virtual biopsy" technique (Figure 4), this is a powerful method for measuring the rate of protein synthesis or protein breakdown in an inaccessible tissue of origin, such as skeletal muscle, heart, brain, kidney, liver, or a cancer tissue, through a measurement made from an accessible body fluid, such as blood, cerebrospinal fluid, saliva, or urine. The method comprises administering a stable isotope tracer (e.g., deuterium oxide (D, 70%; DLM-4-70); L-leucine (¹³C₆, 99%; CLM-2262); glycine (15N, 98%; NLM-202); spirulina whole cells (lyophilized powder) (U-15N, 98%; NLM-8401)) that is metabolically incorporated into newly synthesized proteins. These proteins then escape into an accessible body fluid, from which they are isolated and analyzed for isotopic content or pattern. The measured replacement rate of the escaped protein reflects the synthesis or breakdown rate of the protein back in the tissue of origin. A "virtual biopsy" of the tissue of origin has thereby been carried out.

The virtual biopsy method has utility for discovering and validating biomarkers for use in drug discovery and development, for identifying disease subsets in personalized medicine and for clinical diagnosis and management of patients. This approach has been developed and applied to blood-based measurements of tissue fibrosis and skeletal muscle protein synthesis and CSF-based measurements of axonal transport of cargo¹⁰ and neuroinflammation. An example is plasma creatine kinase-MM (derived from skeletal muscle), for measuring skeletal muscle protein anabolism from a blood test. Many other applications can be envisioned.

in Situ Kinetic Histochemistry: Combining Histopathology with Stable Isotopes and Mass Spectrometry

It is also now possible to visualize the kinetics of targeted molecules of interest spatially, within a histopathologic specimen.14 Linking spatial histologic information with molecular flux rates provides a remarkable new dimension to pathologic diagnosis and monitoring of disease. This can be carried out by either laser microdissection or physical microdissection of slides (Figure 5). An example of tissue microdissection after introducing stable isotopes has been published for prostate cancer. The proliferative gradient of prostate cells, for example, has been shown to correlate closely with histologic grade in biopsy specimens from men with prostate cancer and is reflected by the proliferative rate of prostate epithelial cells isolated from seminal fluid, as a potential noninvasive biomarker.¹⁴

Kinetic Imaging of Tissue Samples

Kinetic or metabolic flux imaging is now possible by combining stable isotope labeling with mass spectrometric imaging of tissues, through NIMS- or MALDI-based spatial visualization of histologic slides. Spatially defined kinetic lipidomics in cancer models has revealed anatomic differences in tumor behavior that correlate with *in vivo* aggressiveness in mouse mammary cancer models.15

Practical Uses of Stable Isotope-Based Biomarkers in Drug Development

There are many uses for stable isotope-based biomarkers in drug discovery and development (Table 2). These include target

Table 2. Applications of Causal Pathway Metrics

Less guessing about:

- 1. Picking targets
- 2. Choosing chemical class and best compound in class
- 3. Identifying the right patients (excluding nonresponders subsets at risk for toxicities)
- 4. Finding the best dose and regimen for clinical trials
- 5. Selecting intermediate end-points to measure and variability to expect in patients
- 6. Dosing to avoid minimize toxicities
- 7. Testing whether personalization can improve response
- 8. Deciding whether to get out early (quick kill)

Continued ➤

Stable Isotopes in Drug Development and Personalized Medicine: Biomarkers that Reveal Causal Pathway Fluxes and the Dynamics of Biochemical Networks *(continued)*

Figure 3. Dynamic proteomics: measuring proteome kinetics and concentrations via stable isotope labeling *in vivo.*

validation; translating preclinical results rapidly into man; "quickkill" of agents or classes with poor activity against the targeted pathway; identifying the right subsets of patients for treatment; identifying optimal dose, regime, measurement end-points, and intersubject variability of response; medical personalization (companion diagnostics); and anticipating toxicities or avoiding toxicities through dose adjustment. Translational markers that are predictive of disease outcomes also allow the selection of animal models that best reflect human disease, or the de-emphasis or even gradual elimination of animal models from the drug-development process.

Stable Isotope-Based Kinetic Biomarkers Have Advantages over but Are Complementary to Static Biomarkers

Traditional static biomarkers provide information about the concentration, presence, or structure of molecules in a living system. In contrast, kinetic biomarkers reveal the dynamic behavior of the pathways that lead to and from these molecules. The amount of collagen in a tissue, for example, does not reveal the rate at which collagen is being synthesized (fibrogenesis) in a disease setting or after starting a therapeutic intervention.Nor does the content of mitochondrial proteins tell us the degree to which mitochondrial

biogenesis or mitophagy was induced by an intervention. Similarly, the concentration of a protein in the cerebrospinal fluid does inform us the efficiency at which neurons in the brain transported this molecule through axons to nerve terminals. These latter processes all involve, at their core, the flux of molecules through oftencomplex pathways and networks.

The activity of these pathogenic processes or disease pathways are in principle the metrics most closely related to the initiation, severity, progression, and therapeutic reversal of a disease. The only way to measure molecular flux rates is by the introduction of isotopic labels, as noted above. Although static parameters can provide key complementary information, such as pool size and net gain or loss of a molecular component, the functional activity of underlying pathogenic processes can only be revealed through kinetic measurements.

The same considerations apply to "network dynamics," such as dynamic proteomics, when compared to static "-omics" biomarkers, but with an additional point that is worth noting. Protein synthesis and breakdown rates typically represent a proactive decision by a cell or organism that is functionally interpretable in context of health or disease. By way of example for

Figure 4. "Virtual biopsy" technique for kinetic biomarkers. Example of skeletal muscle protein synthesis from plasma creatine kinase M-type (CK-M).

proteins, ubiquitin-proteosome-based removal, transcription factorstimulated synthesis, assembly during biogenesis of an organelle, packaging and secretion in vesicles, modulation through the unfolded protein response, deposition as extracellular matrix, induction as part of a protein signaling cascade, etc. – these can all be thought about in functional terms by physiologists, toxicologists, and clinicians. The same cannot always be said for the simple presence or concentration of a protein. Because of this marriage between intrinsic functional significance and broad, hypothesesfree screening, dynamic proteomics is a particularly powerful technology for biomarker and target discovery.

Summary and Conclusions

In summary, the recent addition of stable isotope-based biomarkers to the diagnostic repertoire has brought a new and rapidly expanding dimension to drug development. These biomarkers provide functionally interpretable, decision-relevant information about the underlying biology of disease, capturing the activity of causal pathways that are the driving forces underlying disease and therapy. Kinetic biomarkers thereby predict clinical response and its relation to target engagement or the effects of a clinical treatment regimen. Stable isotope-based kinetic biomarkers are particularly powerful additions in the emerging era of personalized medicine.

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More information is available at www.kinemed.com.

Stable Isotopes in Drug Development and Personalized Medicine: Biomarkers that Reveal Causal Pathway Fluxes and the Dynamics of Biochemical Networks *(continued)*

Figure 5. Microdissection of normal and tumor tissues for mass spectrometric kinetic analysis.

Related Products

Research Use of Products

CIL manufactures highly pure research biochemicals that are produced for research applications. As a service to our customers, some of these materials have been tested for the presence of *S. aureus, P. aeruginosa, E. coli, Salmonella sp.,* aerobic bacteria, yeast, and mold, as well as the presence of endotoxin in the bulk material by taking a random sample of the bulk product. Subsequent aliquots are not retested. Presence of endotoxin is assessed by determining endotoxin content following established protocols and standardized limulus amebocyte lysate (LAL) reagents. Any materials listed in our catalog or website that are designated as "MPT" in the item product number (e.g., DLM-349-MPT) contain these tests as part of release specifications.

If a product does not have an "MPT" designation, CIL may be able to provide microbiological testing on the product. Depending on the compound and the quantity ordered, an additional fee may apply for the testing. Please note that microbiological-tested products are not guaranteed to be sterile and pyrogen-free when received by the customer, and microbiological testing does not imply suitability for any desired use. If the product must be sterile and pyrogen-free for a desired application, CIL recommends that the product be packaged or formulated into its ultimate dose form by the customer or appropriate local facility. The product should always be tested by a qualified pharmacy/ facility prior to actual use.

CIL research products are labeled "For research use only. Not for use in diagnostic procedures." Persons intending to use CIL products in applications involving humans are responsible for complying with all applicable laws and regulations, including, but not limited to the US FDA, other local regulatory authorities, and institutional review boards concerning their specific application or desired use.

It may be necessary to obtain approval for using these research products in humans from the US FDA or the comparable governmental agency in the country of use. CIL will provide supporting information, such as lot-specific analytical data and test method protocols, to assist medical research groups in obtaining approval for the desired use. An Enhanced Data Package (EDP) is also available (see next page for an overview of the technical package contents).

CIL will allocate a specific lot of a product to customers who are starting long-term projects requiring large amounts of material. Benefits from this type of arrangement include experimental consistency arising from use of only one lot, no delay in shipments, and guaranteed stock. Please note that some CIL products have a specific shelf life and cannot be held indefinitely. If interested, please contact your sales manager for further details.

Because of increasing regulatory requirements, CIL manufactures different grades of materials to help researchers with those requirements. Listed below are the grades of materials that CIL currently manufactures:

For more information on controls in manufacturing and testing of the different grades, see our [Product Quality Designations flyer](https://www.isotope.com/userfiles/files/assetLibrary/Product%20Quality%20Designations%20Rv1[2].pdf).

Images used are for illustrative purposes only and may not be representative of actual product(s).

Enhanced Data Package (EDP)

CIL offers the option of an Enhanced Data Package (EDP). This technical data package is available for most MPT products. It includes all of the data currently included with the MPT products, as well as the additional information listed below. You have the option of purchasing this package at the time of order or at a later date.

Please note that if you choose to purchase at a later date, some of the information listed below may not be available. Also, the EDP may not be available for all lots. In some cases, only a partial EDP may be available. Please confirm availability and content prior to order.

EDP Contents

- Product description: structural formula, stereochemical description, molecular formula.
- Product physical properties: melting point, pH, optical rotation (mix of literature or measured values).
- Outline of the synthesis route (including details of solvents used).
- Data used to confirm structure and chemical purity.
- Additional testing data: products with an EDP have been tested to the specifications /monograph similar to those detailed in the USP or EP, but not using compendia methods.
- Impurities: available data on impurities detected and identified together with the method of detection and the cutoff applied.
- Residual solvents: measured residual solvents from the final synthetic step and purification.
- Certificates of Analysis of raw materials, where appropriate.
- Informal stability data: estimated and measured.
	- This will be either actual shelf-life data, if it can be obtained from CIL history or by analysis of in-stock batches, or
	- If no data is available, CIL will commit to assaying the batch provided after six months and one year. Data will be provided after one year, unless the batch fails assay after six months. This option will not be available if the Enhanced Data Package is ordered at a later date.

cGMP Production Capabilities

With increasing requirements from institutional review boards (IRBs) and governmental agencies, partnering with CIL for your next stable isotope cGMP (current good manufacturing practices) project can help ensure your regulatory compliance. With the world's largest 13C and 18O isotope-separation plants, CIL is able to provide the raw materials necessary for your project. Your compound of interest most likely already appears in CIL's extensive list of research compounds – if not, CIL's team of PhD chemists can determine the best method of synthesis for incorporating 13C, 15N, D, 17O, and/or 18O into your compound.

CIL has manufactured bulk active pharmaceutical ingredients (APIs) since 1994. It recently added a 15,000-square-foot, stateof-the-art cGMP facility to complement its existing cGMP facilities. An additional team of experts – specializing in synthetic chemistry, customer support, quality control, and quality assurance – serves to provide technical guidance from beginning to end of your project. Partner with CIL to help you meet your increasing regulatory compliance requirements.

Products of Interest

Other products may be available as CTM/cGMP. Please inquire for details.

Manufacturing Capabilities

- Dedicated development facility
- Five production and two isolation suites
- Dedicated packaging room
- Production scale from milligrams to multikilograms
- Clinical trials to bulk API
- Customizable projects to meet your needs

Analytical Services

- Fully equipped, cGMP-dedicated analytical facility
- Method development and validation
- Raw material and final product testing
- Wet chemistry and compendial methods
- Stability studies and chambers
- Analytical instrumentation:
	- High-field NMR (1H, D, 13C, 15N, multinuclear)
	- HPLC with UV, RI, ELSD, DA, Pickering, and MS detection
	- GC with FID, ECD, and MS detection
	- KF
	- FT-IR
	- Polarimetry
	- TOC

Quality and Compliance

- Drug master files
- FDA-audited facility
- QA release of API product
- Follows FDA and ICH guidances
- CMC sections for NDA or IND

CTM: manufactured following ICH Q7, Section XIX GMP: good manufacturing practices grade

Newborn Screening Standards

CE Mark *in vitro* Diagnostics (IVD)

Newborn screening (NBS) is an analytical or physical screening process used to test neonates for inherited or congenital disorders related to inborn errors of metabolism (IEM). IEMs are caused by the deficiency, absence, or alteration of specific enzymatic reactions. The goal of NBS is to detect metabolic errors at the earliest stage of development, such that treatment can be initiated and irreversible damage to the central nervous system can ultimately be avoided.

To help facilitate IEM screens (e.g., for phenylketonuria, maple syrup urine disease, medium-chain and very-long-chain acyl-CoA dehydrogenase deficiencies), CIL is pleased to offer two types of CE-marked *in vitro* diagnostic (IVD) medical devices: amino acid reference standards (NSK-A-CE) and carnitine/acylcarnitine reference standards (NSK-B-CE). When used as directed, these devices provide solutions of stable isotope-labeled standards at defined concentrations. The ready-to-use mixes can be implemented to measure the concentrations of target analytes (amino acids in NSK-A-CE; free carnitine/acylcarnitines in NSK-B-CE) in a range of biosamples (e.g., dried blood spot, urine) by a variety of analytical techniques (e.g., FIA-MS/MS, LC-MS/MS).

For sale in European Economic Area (EEA) – EU and EFTA – only.

For professional use only.

Custom synthesis and formulations are also available. Please inquire.

CIL provides additional testing on many products as a service to our customers. CIL also has cGMP capabilities and can manufacture products to meet your increasing regulatory compliance requirements. Please contact us to learn more.

CIL products are labeled "For research use only. Not for use in diagnostic procedures."

Please visit isotope.com for a complete list of isotope-labeled compounds.

Research products are distributed and sold worldwide via our extensive network. CIL's distributor listing is available at isotope.com.

> **To request a quotation or place an order:** North America: 1.800.322.1174 | 1.978.749.8000 | cilsales@isotope.com International: +1.978.749.8000 | intlsales@isotope.com Fax: 1.978.749.2768 | isotope.com

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