Editorial

Biological Matrix Supply Chain Shortages: More Matrices Are Now Rare—the Case for Surrogate Matrices

Evan A. Dubiel,[1] Heather Myler,[1,7] Mark E. Arnold,[2] Patrick Bennett,[3] Jeff Gatz,[4] Elizabeth Groeber,[5] Seema Gupta,[1] Cheikh Kane,[6] Fumin Li,[1] William Mylott,[1] Courtney Noah,[4] Mark O'Dell,[2] Eric Tewalt,[1] Dominic Warrino,[6] and Andrew Vick[5]

Abstract. The COVID-19 pandemic has strained the biological matrix supply chain. An upsurge in demand driven by numerous COVID-19 therapeutic and vaccine development programs to combat the pandemic, along with logistical challenges sourcing and transporting matrix, has led to increased lead times for multiple matrices. Biological matrix shortages can potentially cause significant delays in drug development programs across the pharmaceutical and biotechnology industry. Given the current circumstances, discussion is warranted around what will likely be increased use of surrogate matrices in support of pharmacokinetic (PK), immunogenicity, and biomarker assays for regulatory filings. Regulatory authorities permit the use of surrogate matrix in bioanalytical methods in instances where matrix is rare or difficult to obtain, as long as the surrogate is appropriately selected and scientifically justified. Herein, the scientific justification and possible regulatory implications of employing surrogate matrix in PK, immunogenicity, and biomarker assays are discussed. In addition, the unique challenges that cell and gene therapy (C&GT) and other innovative therapeutic modalities place on matrix supply chains are outlined. Matrix suppliers and contract research organizations (CROs) are actively implementing mitigation strategies to alleviate the current strain on the matrix supply chain and better prepare the industry for any future unexpected strains. To maintain ethical standards, these mitigation strategies include projecting matrix needs with suppliers at least 6 months in advance and writing or updating study protocols to allow for additional matrix draws from study subjects and/or re-purposing of subject matrix from one drug development program to another.

KEY WORDS: regulatory authorities; matrix; pharmacokinetics; immunogenicity; biomarkers.

INTRODUCTION

The COVID-19 pandemic has created an array of supply chain shortages and other challenges across the biotechnology industry (1). Supply shortages have included laboratory staffing, personal protective equipment (PPE), laboratory consumables, reagents, and large animals (purpose bred beagles, cynomolgus monkeys, etc.). Supply chain demand has resulted in a 10–100% increase in the cost of goods, depending on supply/demand ratios. There is currently a growing shortage of various biological matrices. New COVID-19 therapies have simultaneously increased demand for animals for safety testing and created challenges sourcing, storing, and transporting animals, and animal matrices for bioanalytical assays (2). Likewise, for human matrices, an increase in demand, cancellation of blood drives, low donor turnout, and a recent shortage of blood specimen collection tubes is creating growing concerns about human matrix availability (3). Matrix supply chain shortages are highly rate limiting in PK, immunogenicity, and biomarker analysis, which can result in significant delays in drug development.

Current matrix supply chain issues are causing previously more common matrices to now be considered "rare," necessitating further discussion on the use of surrogate matrices. For example, procurement of non-human primate

[1] PPD Clinical Research Services, Thermo Fisher Scientific, 2244 Dabney Road, Richmond, Virginia 23230-3323, USA.

[2] Labcorp Drug Development, 8211 SciCor Drive, Indianapolis, Indiana 46214, USA.

[3] Alliance Pharma Inc., 17 Lee Blvd., Malvern, Pennsylvania 19355, USA.

[4] BioIVT LLC, PO Box 770, Hicksville, New York 11802, USA.

[5] Charles River Laboratories, 1407 George Rd, Ashland, Ohio 44805, USA.

[6] KCAS Bioanalytical and Biomarker Services, 12400 Shawnee Mission Parkway, Shawnee, Kansas 66216, USA.

[7] To whom correspondence should be addressed. (e–mail: Heather.Myler@ppd.com)

(NHP) matrices has seen lead times increased from 3–4 weeks to 3–4 months, in some instances, almost instantaneously. Current lead times for larger quantities (100–500 mL) and individual lots are up to 6 months for NHP serum and plasma, and between 1 and 3 years for NHP cerebral spinal fluid (CSF). The cost of some NHP matrices has also increased up to tenfold within the last 12 months. NHP matrices were not considered rare before the COVID-19 pandemic. Surrogate matrices have been studied previously (4, 5). In cases where matrix may be difficult to obtain, current regulatory guidance states a surrogate matrix may be acceptable for analytical method validation (6, 7). Surrogate matrix in the context of this article is defined as any biological matrix substituted for the healthy sample matching matrix that is used to develop and validate a bioanalytical method. Such surrogate matrix should be selected and justified scientifically for use in the analytical method. The purpose of this editorial is to address the scientific, regulatory, and logistical challenges the pharmaceutical, CRO, and matrix supply industries are facing because of the COVID-19-induced matrix supply chain shortage. Mitigation strategies the industry is actively taken to address the matrix shortage are presented.

PROLOGUE: MATRIX SHORTAGE

Matrix Suppliers

The COVID-19 pandemic created massive disruptions for biological specimen providers, and several challenges emerged to deliver the specimens to meet customer need. At donor centers and clinical sites, restrictions were placed on the number of people that could be in waiting rooms and screening practices were implemented to prevent the collection of samples from anyone exhibiting flu-like symptoms. Those constraints, coupled with donors choosing to stay home, led to fewer collections. Fortunately, centers did remain open throughout the pandemic and there was only minor impact on the delivery of human blood-derived matrices. The pandemic also made it harder to access additional NHPs. Export restrictions, permit delays and trade relations between the USA and China further compounded the issue. As the demand for NHP CSF, plasma, and serum surged, there was no short-term solution to mitigate extended lead times in fulfillment due to the mass influx of requests.

Contract Research Organizations

The bioanalytical services segment of the CRO industry has recently experienced considerable growth. Until recently, this growth trend has been secular, driven by C&GT innovations and more drug makers outsourcing their bioanalytical lab work to CROs. As of December 2021, the onset of the COVID-19 pandemic has resulted in more than 2500 trials testing therapies for COVID-19 and over 1300 trials testing vaccines (8). Together, these trends have increased demand for biological matrices, stretching their supply chain to concerning levels. Unlike the PPE and laboratory consumable supply chains which became stretched shortly after the onset of the COVID-19 pandemic, matrix supply chain shortages started to become apparent to CROs mid-2021.

The sparsity of commercially available matrix seems to impact the robustness of ligand binding assay (LBA) data potentially due to the quality of the matrix that is available. One lab has observed an unusual trend of high failure for selectivity testing in PK and anti-drug antibodies (ADA) assays across multiple drug development programs. Testing demonstrated that NHP matrix purchased in 2021 (New Matrix) was failing selectivity acceptance criteria (80–120% recovery), whereas the NHP matrix purchased in 2019/2020 (Old Matrix) was passing selectivity within the same assay. This is resulting in expanding the percentage recovery criteria to 25% or up to 30% to proceed with validation and requiring evaluation of in-study baseline samples to confirm that the root cause of the selectivity failure is indeed due to the commercially available NHP matrix and not the method. Similarly, another lab observed selectivity failure in human CSF. The root cause of the selectivity failure was determined to be poor-quality remnant CSF samples that happened to be the only matrix available at the time. Further evaluation of Old Matrix *vs* New Matrix would be interesting to get a broader industry perspective on this issue. Putatively, the New Matrix lots may contain residual therapeutics if re-purposed from in-life studies, pre-existing ADA, augmentation of immune function (both innate and adaptive), and supply chain delays effecting the integrity of the NHP matrix.

There have been efforts by the European Bioanalysis Forum (EBF) and others encouraging the use of surrogate matrix to advance the 3R (replacement, reduction, refinement) approach to minimize the use of animals, without compromising scientific integrity (9–12). These 3R strategies can be directly applied to mitigate the current matrix shortage caused by the COVID-19 pandemic. The scientific justifications and regulatory impact of employing a surrogate matrix in bioanalytical methods will be discussed herein with suggestions for the use of surrogate matrices.

SCIENTIFIC AND REGULATORY IMPLICATIONS OF SURROGATE MATRICES

Pharmacokinetic

For clinical PK studies, it is rather difficult to scientifically justify the use of surrogate matrix in bioanalytical method validation (BMV). However, for preclinical pharmacology, PK, and toxicology studies, surrogate matrix use could be more easily justified. Current BMV guidance(s) recommend the sponsor should prepare the calibration standards and QCs in the same biological matrix as the samples in the intended study (6, 7). However, the ICH draft M10 BMV guidance also states that when obtaining blank matrix identical to that of the study samples is difficult, a surrogate matrix may be acceptable for analytical method validation should the selected surrogate matrix be justified scientifically (7). Furthermore, the FDA BMV states, "When surrogate matrices are necessary, the sponsor should justify and validate the calibration curves" (6).

A validation employing surrogate matrix may consist of a full validation in the surrogate matrix and a partial validation in the primary matrix (i.e., the same matrix as in-study samples). Table I lists examples of primary matrices and their possible surrogate matrices. Consideration towards the

Table I Examples of Surrogate Matrix Substitution for Rare Primary Matrix

Primary matrix	Surrogate matrix
Cynomolgus monkey CSF	Human CSF
Cynomolgus monkey serum/plasma	Human serum/plasma
	Rhesus monkey
Transgenic mouse serum/plasma	CD-1® mouse serum/plasma
Sprague Dawley® rat serum/plasma	Wistar rat serum/plasma
	Lewis rat serum/plasma

sensitivity of the surrogate matrix supply chain should be taken when switching animal species. For example, since more than 95% of all NHP matrix requests are for cynomolgus monkey, should a large number of cynomolgus monkey bioanalytical methods use rhesus monkey as a surrogate, it is likely that the rhesus monkey supply chain would be insufficient to accommodate all methods. The partial validation is built upon a full validation using an appropriate surrogate matrix where the intent is to conserve the rare matrix. Scientific justification, as well as meeting regulatory expectations for BMV, is established using quality controls (QC) prepared in the primary matrix, specificity/ selectivity in multiple lots of primary matrix, and linearity/ parallelism evaluated using an above the upper limit of quantitation (ULOQ) QC prepared in primary matrix.

Rare matrix can primarily be conserved in PK assays by preparing the calibrators and diluting study samples above the ULOQ in a surrogate matrix. BMV requires freshly prepared calibration curves for each validation run (6), which creates a significant blank matrix volume burden. Therefore, to minimize such burden for rare matrices, calibration curves can be prepared in surrogate matrix which reduces the amount of primary matrix needed for validation by approximately half. To further conserve usage of QCs prepared in rare matrices for stability experiments, it is advisable to take six aliquots from one tube instead of two aliquots from three individual tubes (13). This approach has been successfully implemented and can be principally applicable to mitigate matrix shortage issues in support of PK studies.

An example of a validation employing a surrogate matrix could be (1) performing accuracy/precision with calibrators prepared in the surrogate matrix and QCs (i.e., ULOQ, high, mid, low, and LLOQ) prepared in the primary matrix; (2) selectivity and fortified specificity samples prepared from multiple lots/individuals (e.g., $n = 6$) of primary and surrogate matrix; (3) parallelism and dilutional linearity evaluated by preparing a QC in primary matrix at a concentration above the ULOQ and diluted using surrogate matrix; and (4) QCs prepared in primary matrix to evaluate extract storage stability as applicable, benchtop stability, freeze/thaw stability, and long-term storage stability. While this approach significantly reduces the volume of primary matrix needed and is scientifically justified, if frozen calibration curves will be used for sample analysis, it requires additional testing such as benchtop, freeze/thaw, and long-term storage stability in the surrogate matrix to support the surrogate matrix calibration curve. In addition, it is recommended that selectivity and

fortified specificity also be performed for the surrogate matrix. Performing selectivity and fortified specificity in the surrogate matrix is necessary to ensure suitability over multiple lots/individuals and not only a particular lot/ individual tested during validation, in the case additional volume is required during sample analysis. These additional validation tests in primary matrix are justified because the quantity of primary matrix conserved during sample analysis would be substantial. In cases where even a surrogate matrix could be difficult to obtain (e.g., ocular matrices), buffer matrices with bulking agents may be considered.

The aforementioned validation example applies to preclinical pharmacology, PK, and toxicology studies. The majority of clinical BMVs are done in healthy serum or plasma matrix with selectivity in the disease state or rare matrix. Thus, rare matrix conservation is already intrinsically incorporated in most BMVs. For clinical studies in rare matrices such as CSF or ocular fluid with a high number of samples, some rare primary matrix can be conserved through diluting samples that quantitate above the ULOQ in rare matrix supplemented with a small amount of the buffer that is used to perform the minimal required dilution (MRD). The regulatory implications should be minimal if the diluent for samples above the ULOQ is composed of at least 95% of the rare primary matrix. This approach is akin to current practices used to prepare fortified specificity samples in PK BMV where an intermediate of the target concentration for example, twentyfold the high QC, is prepared and spiked at 5% v/v into the rare matrix individual sample.

Immunogenicity

ADA detection methods can be developed and validated to better conserve rare matrix, provided that the surrogate matrix appropriately represents the target population for the study. Current immunogenicity guidance recommends a multi-tiered ADA detection approach: screening (tier 1), confirmatory (tier 2), and titer (tier 3) (14). To conserve rare matrix, it may be possible to minimize tier testing given adequate scientific justification. For example, in some cases, it may be advisable to go directly to the titer tier. This decision should be driven by a thorough risk assessment and should be clearly justified during method validation. Preclinical studies frequently only employ tier 1 which should help alleviate the current shortage of NHP matrix and surrogate matrices can be considered for this testing, such as using human matrix in place of NHP matrix provided comparability can be demonstrated. In addition, consideration of the current matrix shortage is recommended during the pre-clinical stage of biologic development for implementation of a clinical immunogenicity strategy (15). This can help ensure additional strain is not placed on the supply chain performing less value added assessments.

According to immunogenicity guidance, assay cut points should be generated from around 50 individual matrix samples (14). For clinical studies in rare disease or matrices, fewer individuals may be used to establish cut point (16). In these cases, due to the decreased number of individuals used for cut point determination, the full variability typically determined by evaluating 50 individuals may not be observed and could result in an unrepresentative cut point requiring

greater in-study diligence. In-study samples should be applied throughout the various phases of clinical development to monitor the adequacy or need to re-evaluate cut points. There is less risk applying this approach in pre-clinical studies for which significantly higher ADA responses are usually expected.

Conservation of rare matrix in ADA assays could also be done by modifying dilution schemes and diluent for titer (tier 3) assessment. For example, to conserve rare matrix titering may be done in matrix supplemented with buffer provided this does not impact reported titer levels. This approach particularly applies when high levels and incidence of pre-existing antibodies are observed, and extensive titering of pre-dose and post-dose samples is necessary to differentiate treatment-boosted ADA from treatment-induced ADA.

Biomarkers

Since biomarker assays quantify an endogenous substance in a biological sample, a surrogate matrix is often used to prepare calibrators and QCs when matrices void of the analyte of interest are not easily obtainable (17, 18). Thus, there has already been much discussion around use of a surrogate matrix in biomarker assays (19, 20). Surrogate matrices can either be a compatible matrix of another species or ideally a stripped matrix that is lacking the analyte of interest. However, given the current supply chain issues with obtaining matrices, these approaches can be difficult. An additional approach is to select a protein-containing buffer that can be used as a surrogate matrix to prepare both the buffer QCs and calibrators and additionally in immunoassays, to dilute the samples for the MRD.

Because the surrogate matrix may not have the same composition as the study samples, it is necessary to evaluate the effects of using it on the sample results. Additionally, the recombinant protein reference standards, diluted in surrogate matrix to prepare the calibrators and buffer QCs, might differ in their reactivity to the critical reagents and in their stability as compared to the endogenous analyte. Hence, experiments justifying the selected surrogate matrix are necessary. These include parallelism/dilutional linearity, spike-and-recovery, and accuracy/precision. Poor assay performance as demonstrated by spike-and-recovery and parallelism/dilutional linearity experiments indicate non-comparability of the surrogate matrix with the biological sample. In these circumstances, further optimization of the assay is necessary by re-examining the choice of the surrogate matrix, consideration of potential interferants, and higher sample dilutions to avoid matrix interference.

The regulatory implications on the selection of surrogate matrices should be minimal if the required experimentation is conducted to assess and validate the assay's ability to differentiate between (1) diseased and normal population for diagnosis/prognosis purposes and (2) drug-treated *vs* placebo samples for drug development purposes. Such sample differentiation supersedes the assay's ability to measure the true (i.e., accurate) amount of target analyte in the sample. If the surrogate matrix approach is used, demonstration of similar matrix effects and extraction recovery in both the surrogate and primary matrix is required. This should be investigated in an experiment using QCs spiked with analyte

in the primary matrix against the surrogate calibration curve and should be within ± 15% for small molecule chromatographic assays and within ± 20% for LBA and LBA-LC-MS/MS assays.

MATRIX DEMAND OF CELL AND GENE THERAPHY MODALITIES

There has recently been an upsurge in C&GT modalities to treat diseases that were previously thought to be undruggable by standard small molecules and biologics (21). Of importance is the generation of PK, biomarker, and immunogenicity data not only in standard liquid matrices such as plasma and serum, but also at the intended site of action of the therapeutic. For example, CSF is frequently used to understand distribution in the brain and spinal cord for diseases of the central nervous system. Extensive tissue analysis to understand biodistribution has also become commonplace. With that in mind, the assessment of these endpoints and anatomical distribution, especially in preclinical models, is of the utmost importance. The data is used to establish correlations to plasma/serum and to further guide dosing regimen in clinical trials, where tissue biopsies are less feasible. Preclinically, where the therapeutic is quickly taken up by target organs, these tissues may become the primary measure of exposure as opposed to plasma or serum. Likewise, preclinical studies permit the addition of biodistribution in investigational new drug (IND) enabling studies which may aid regulatory approval. Full characterization may require sub-sectioning organs to gain a full understanding of drug uptake. For example, cerebellum, stem, and prefrontal cortex may all be relevant and distinct tissues of interest for a brain-penetrating therapeutic. Thus, a host of preclinical liquid and tissue-based matrices that may be in short supply is required to support clinical dosing decisions and the application of conservation mechanisms without compromising data quality is of key importance.

To ensure relevant clinical translation, C&GT rely heavily on NHP models for safety assessment. Recent supply chain issues have put a strain on the availability of most commercial preclinical matrices, particularly NHP. As mentioned earlier, lead times for cynomolgus monkey plasma and serum can be upwards of 6 months, to over a year for tissues and CSF. Establishing matrix surrogacy using alternate species tissues and artificial CSF would ease supply chain constraints and shorten the timelines for establishing drug safety.

MITIGATING THE MATRIX SHORTAGE

Matrix Suppliers

Numerous vendors of biological matrices have been consulted on their plans for expansion; understanding the critical need to meet business demand. To better supply researchers with human plasma and serum, new donor centers have been opened and current donor centers have been expanded allowing for increased donor visits. To meet the high demand for matrix of patients infected with COVID-19, vendors have added mobile collection services that allow sourcing from homebound patients. In order to increase the supply of NHP matrices, vendors are preparing SOPs and

working to gain Institutional Animal Care and Use Committee (IACUC) approval to scale up collections of rare matrices such as CSF. This should double the NHP facility's matrix collection capacity, which will greatly reduce the lead times. Efforts are also in progress to address the increase in demand for rodent and canine matrix.

Proactively anticipating matrix demand is critical, and supply chain managers and vendors can leverage a wealth of publicly available data including drug approvals, research portfolios and investments, and other market indicators. Strong communication between vendors, CROs, drug makers, and other customers can help alleviate supply chain bottlenecks. Customers are encouraged to forecast their matrix needs at least 6 months in advance to ensure everyone is well positioned to support newly emerging R&D programs.

Contract Research Organizations

In addition to employing surrogate matrices to mitigate biological matrix shortages, CROs are also actively strategizing to maintain and expand their in-house inventory using a multiple pronged approach. Generally, this includes possible extension of expiration dates on existing rare matrix inventory, drafting of study protocols to allow for additional draws, and banking and re-purposing matrix from completed past studies to be used in future studies.

To extend the expiration date of matrix, the appropriate bridging experiments must be performed comparing the "expired" matrix to recently sourced matrix. The general goal of such experiments is to ensure the bioanalytical method can equivalently measure the analyte of interest in each matrix. This approach is akin to extending the expiration dates of critical reagent and reference standard lots. Extension of matrix expiration dates is critical for reducing waste and maintaining inventory when demand unexpectedly surges. This helps to alleviate the current pressure on matrix suppliers' supply chain, particularly for NHP and other animal matrices.

The CRO industry is also consulting their clients to draft and modify their study protocols to allow for additional draws of liquid matrices such as blood and CSF. These additional draws can be taken from placebo or pre-dose subjects. In addition, with the appropriate updates to preclinical study protocols, or consent in clinical studies, pre-dose study samples could also be banked so such matrix can be re-purposed and used for other studies. Such a strategy provides clients with their own dedicated matrix supply chain when situations arise where long lead times could result in hindering current studies or postponing future studies. Longitudinal data from multiple pre-dose samples may also be beneficial in the preservation of matrix needed for immunogenicity evaluations.

It is recommended that prior to using samples from any study, the study should be complete to eliminate the risk of samples being needed for reanalysis. Before using any samples, all must be screened to ensure they are appropriate for use in the bioanalytical method in question. Once the samples are screened, they can then be combined to create a pool that may be used for method development, validation, and to create calibrators and QCs for sample analysis or to be used for sample dilution. Once utilized for any activities, continuous monitoring should be put into place to ensure appropriate method performance, potentially including trending and comparison to historical data to ensure no issues arise. As these caveats complicate the bioanalytical process, these practices should be carefully managed and limited to cases where conventional strategies are not possible.

SUMMARY

The COVID-19 pandemic has resulted in a dramatic decline in the supply and availability of many biological matrices due to increased demand and logistical challenges in sourcing. The increase in demand is partially driven by a large number of COVID-19 therapeutic and vaccine development programs to combat the pandemic. In addition, the emergence of C&GT and other innovative therapeutic modalities has also led to increased demand in biological matrix. NHP matrix that was not considered rare or limited prior to the pandemic is now scarce. Given the current circumstances, NHP and many other matrices may now be considered rare or available in limited quantities, warranting the use of surrogate matrices as a strategy to avoid delays in drug development programs.

Current regulatory guidance(s) allow the use of surrogate matrices with scientific justification when the primary matrix is difficult to obtain. A goal of this editorial is to suggest alternatives and the validation experiments necessary to scientifically justify the use of surrogate matrix in order to decrease potential burden on sponsors and reviewers during the submission process. Another goal is to propose mitigation strategies in order for study sponsors to build their own dedicated biobank that could be used should the matrix supply chain be strained for a longer period of time than anticipated. Therefore, the primary strategy should be strong forecasting of material needs and active communication with partners and suppliers. It is important to use the same matrix as study samples when available, even if limited quantities increase their cost. This should remain the gold standard for PK, immunogenicity, and biomarker method validation. Secondary strategies involve updating study protocols and/or consent forms to allow for additional draws and/or the re-purposing of study samples from one drug development program to another.

While the COVID-19 pandemic has placed significant stress on the biological matrix supply chain, the mitigation strategies of suppliers, CROs, and drug makers can improve sourcing and conservation of all types of matrix; providing a framework to not only effectively manage the current matrix supply chain issue, but any future unanticipated matrix shortages that may present themselves. Based on the expansion plans and current initiatives underway of suppliers, it is hopeful that supply chain limitations will eventually resolve and most matrices will be readily available to meet the demands of all drug development programs going forward.

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