When method validation gives an unexpected outcome

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In a clinical laboratory, there is always the danger that an analytical error could bring physical harm. Up to 70% of diagnostic mistakes occur during the pre-analytical steps of sample collection, transportation, preparation, and storage (Plebani, 2012). For patients that need constant monitoring within the walls of a treatment facility, every procedure from pipetting to centrifugation influences their care.

Immunosuppressive drug monitoring

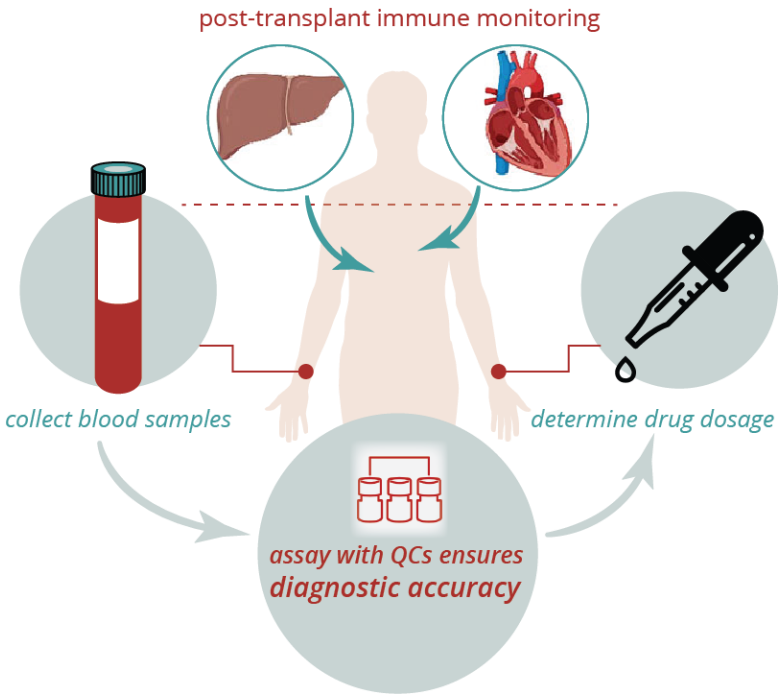
Organ transplantation is a prime example of physicians relying on laboratory clinicians for rapid, accurate results. Immunosuppressant drugs (ISDs) such as everolimus, tacrolimus, mycophenolic acid (MPA), and cyclosporine A (CsA) are used for prophylaxis against tissue rejection after liver, heart, and kidney transplantation (Kahan, Keown, Levy, & Johnston, 2002). CsA has additional uses in bone marrow transplantation, as well (Kaplan, Yüksel, Evliyaoglu, & Basarali, 2015).

These treatments have narrow therapeutic ranges. Besides the immunological dangers, side effects can be neurological, hepatic, and renal. Appropriate dosage relies on regular whole-blood monitoring.

Optimization of analytical strategies in therapeutic drug monitoring

Over the years, multiple immunoassays have been introduced to optimize the quantification of ISDs in whole blood (Morelle, Wallemacq, Caeneghem, & Goffin, 2011). Immunoassays play a central role in laboratory medicine for their ease of use, multi-analyte capabilities, and high throughput.

In addition, automated immunoassay testing offers the benefit of expediting clinical decision-making. A streamlined workflow reduces sample turnaround time. This leads to earlier, more informed medical treatments that help to maximize therapeutic efficacy.



Strategies to ensure high quality and standardization

The results of immunoassays can only be confirmed by internal and external quality controls (QCs). Whatever the drug of interest, there can be no conclusive results without corresponding reference materials. A reference material verifies the traceability of an assigned value to a measured value in a given procedure (Miller, Myers, & Rej, 2006).

Reference materials can take on any number of forms: calibrators, external quality assessment samples, proficiency testing samples, and QC samples are a few examples (CLSI, 2014). These nonpatient samples must be commutable, or behave like patient samples, in the intended measurement procedures.

Serum, plasma, and anticoagulated whole blood are commonly employed as clinical samples for therapeutic drug monitoring. Before being subjected to most immunoassay tests, samples undergo a pre-treatment of cellular and protein precipitation that is expected to remove most endogenous antibodies. Since immunoassays are based upon a binding reaction between a molecule of interest and reagent antibodies, there is always the risk that human antibodies in a patient's bloodstream could interfere with the assay (Morelle, Wallemacq, Caeneghem, & Goffin, 2011).

The relationship between supplier quality and test performance

Control samples are subject to the same preparatory procedures as fresh biological samples. As such, the matrices for corresponding QC samples also undergo some processing. Beyond the difficulties of co-existing antibodies, QC materials must attain longevity and robustness for proper functionality.

This is certainly true when designing the manufacture, qualification, and distribution of immunosuppressive controls at More Diagnostics, Inc. The production workflow for More's Cyclosporine C2 (CSAE) Control relies on stabilizers to preserve the whole blood matrix, reconstituted reference standards to spike in the analyte, and cycles of freeze/thaw to ensure the product will perform after shipping and storage.

Translating protocols into patient testing

When determining a course for post-operative treatment, clinical laboratories need quality indicators at every step of the way to ensure that drug measurements are accurate, precise, and standardized. Suppliers are always releasing improvements on protocols or techniques to aid in faster turnarounds. Before enjoying the benefits, laboratories need to validate the updates.

In 2003, researchers at the University of California-Los Angeles (UCLA) evaluated the performance of a new reagent to quantify CsA in blood by comparing transplant patient samples to More's CSAE controls (Butch & Fukuchi, 2003).

Because the EMIT 2000 CsA specific assay (Siemens Healthineers, Erlangen, DE) requires off-line steps prior to analysis, the new technique would simplify operation and improve stability.

The authors Butch et al. made two discoveries. First, they found that the new pretreatment reagent procedure gave unexpected results with commercial CSAE controls. Measured CsA recovery values using the previous, more laborious methanol extraction were higher than values of the CSAE controls after using the new pretreatment. The pretreatment had resulted in a negative shift from the target values. Second, they found that the new pretreatment could produce the same results as the precursor methanol-based protocol in patient samples. Unlike with the CSAE QC materials, the two methods gave statistically equivalent CsA readings for patient samples.

A question of methods vs. materials

The authors of the investigation at UCLA had to take a second look at the pretreatment and EMIT system. Knowing the importance of verifying assays with preset concentrations of a drug, they pooled in-house blood samples and spiked them with CsA to test as controls. There was excellent correlation in CsA measurements between the two pretreatment methods.

These findings indicated the discrepancy arose from the commercial CSAE controls. When the results from an assay cannot be legitimately compared with the assigned value, there are a number of possibilities. Could there be a bias in the calibration? Have the methods been properly examined? Is there a matrix effect?

In this case, the methods used differed from routine clinical practices. Reference controls are assayed for performance and to determine published recovery ranges. If the assay is tested with a different set of extraction reagents and separatory steps in the pretreatment procedure, then there is no valid comparison between the observed and expected results.

Fit for the purpose?

The demonstration, evaluation, validation, and verification of QCs is a costly and time-consuming process. Manufacturers such as More Diagnostics, Inc. invest significant resources in demonstrating the commutability of control materials through internal reference methods and external value assignments with regulatory-compliant laboratory partners. Guidelines for precise, accurate performance form the basis of quality assurance for the ensuing reference materials.

Outside of the production site, it is up to the laboratorian to ensure that QCs are implemented with the proper reagents, tools, and assays. The trend of fully automated instrumentation in immunoassay development has helped avoid the pitfalls of method-based errors that might introduce variation between users, time periods, and test sites. In the instance that automation does not include an extraction step, care must be taken to prove that any external materials or methods are shown to be robust against interference from endogenous compounds and non-physiologic molecules.

In the grand scheme of ISD testing, clinical workers are grappling with a bewildering level of complexity. The medical sample, analyzer, testing components, and control activities all impact the quality of laboratory medicine. Moreover, the control materials used for each drug analyte indicate performance issues, discrepancies, or shifts that could stop laboratories from achieving high standards for patient care. More Diagnostics, Inc. focuses on producing trustworthy diagnostic controls that are designed and assessed to give accurate results for all methods listed on the value sheet. With years of industry-recognized expertise, the menu of immunosuppressant QCs at More offers multiple solutions to meet today's needs for transplant and autoimmune patient testing.

References

Butch, A. W., & Fukuchi, A. M. (2003). Evaluation of a new pretreatment reagent for use with the EMIT 2000 cyclosporine assay. *Clinical Biochemistry*, 313-316.

CLSI. (2014). *Evaluation of Commutability of Processed Samples, 3rd Edition.* Clinical and Laboratory Standards Institute.

Kahan, B. D., Keown, P., Levy, G. A., & Johnston, A. (2002). Therapeutic drug monitoring of immunosuppressant drugs in clinical practice. *Clinical Therapeutics*, 330-350.

Kaplan, I., Yüksel, H., Evliyaoglu, O., & Basarali, M. K. (2015). Effects of Storage Temperature and Time on Stability of Serum Tacrolimus and Cyclosporine A Levels in Whole Blood by LC-MS/MS. *International Journal of Analytical Chemistry*, 1-5.

Miller, G. W., Myers, G. L., & Rej, R. (2006). Why Commutability Matters. *Clinical Chemistry*, 553-554.

Morelle, J., Wallemacq, P., Caeneghem, O. V., & Goffin, E. (2011). Clinically unexpected cyclosporine levels using the ACMIA method on the RXL dimension analyser. *Nephrology Dialysis Transplantation*, 1428-1431.

Plebani, M. (2012). Quality Indicators to Detect Pre-Analytical Errors in Laboratory Testing. *The Clinical Biochemist Reviews*, 85-88.