The Association for Clinical Biochemistry & Laboratory Medicine

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Top Tips for the measurement of Immunosuppressive Drugs by LC-MS/MS

Section/Committee: ACB Liquid-Chromatography Tandem Mass Spectrometry – Special Interest Group

- Always use appropriate internal standards:
    - Cyclosporin A – Cyclosporine D (analogue) or deuterated Cyclosporin.
    - Tacrolimus - Ascomycin (analogue) or deuterated Tacrolimus.
    - Sirolimus - desmethoxyrapamycin (analogue) or deuterated Sirolimus.
    - Everolimus – desmethoxyrapamycin or deuterated Everolimus.
    - Mycophenolic Acid (MPA) - indomethacin or deuterated MPA.
    - Always check the purity of new batches of deuterated internal standard to ensure it is not contaminated with unlabelled molecule.
    - Always use commercial whole blood calibrators and controls, preferably from different manufacturers to minimise risk of calibration drift between batches.
    - Always participate in the international proficiency testing schemes for immunosuppressive drugs operated by an official accredited laboratory.
    - Always be aware that there is a matrix difference between fresh whole blood and frozen/ lyophilised whole blood calibrators and controls. Periodically run duplicate analysis of frozen material to directly compare differences between fresh / frozen. For in-house methods, fresh extracting reagent often gives higher extraction efficiency.
    - Be aware that the introduction of new therapies in the patient population can introduce ion suppression effects that were not present during method validation. This is particularly important when using electrospray ionisation sources(ESI) which are more prone to such effects than atmospheric chemical ionisation sources (APCI).

- When introducing commercial MS assay kits do not assume they will work out of the box. They will have to be optimised and validated for each individual ms system.

- Regularly run mass calibration material on each MS system to reduce the risk of mass drift.
- If running multiple analysers or using back up laboratories, comparative data should be generated at least monthly.
- Whether using guard or standard columns for trapping experiments, these should be replaced regularly and before performance deteriorates. Running 0.2u filters before the column and back flushing columns with high organic overnight can often help maintain performance for longer periods.
- Do not develop multi-drug assays where drugs are measured in different matrixes. For example Cyclosporine, Tacrolimus, Sirolimus and Everolimus are measured in whole blood and so can be measured simultaneously. However, mycophenolic acid is measured in plasma requiring different calibrators and controls making it appropriate to run them as a separate assay.
- When using multi-drug assays it is essential to bear in mind that the sample time may not be appropriate for all drugs, for example some patients may be prescribed cyclosporine and sirolimus but these are taken at different times. The Sirolimus dose is usually taken 4 hours after the cyclosporine dose due to an interaction. Hence, a sample specifically taken for a sirolimus pre-dose would be 4 hrs post dose cyclosporine, this could lead to misinterpretation of the results.
- When developing methods for mycophenolic acid it is necessary to chromatographically separate MPA and the glucuronide metabolite, MPAG. This is because the ionisation process can break down MPAG to MPA producing inappropriately elevated MPA results,