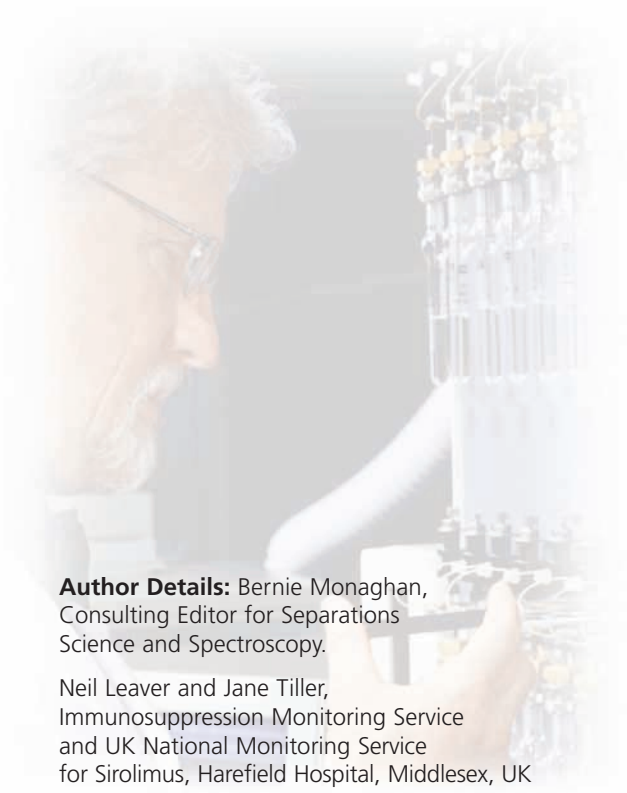
Spectroscopy Focus

CHALLENGES INVOLVED IN RUNNING MS BASED ASSAYS IN CLINICAL ENVIRONMENTS

Bernie Monaghan, Neil Leaver and Jane Tiller

Diagnosis by separation science techniques has never been overly common within the therapeutic drug monitoring community for a variety of reasons. These include the capital cost of equipment, the lack of either separations science applications knowledge within clinical laboratories and the lack of clinical knowledge / relevance of results within the separations and spectroscopy communities. Yet the use of admittedly sophisticated equipment such as LC or GC-MS systems can be utilised to provide highly specific, rugged, fast and accurate assays within clinical laboratories providing the correct strategy for implementation is devised. Indeed with "Black Box" Instrumentation, "Sample in, results out – and don't worry about what's going on in between" the lack of specialist knowledge as outlined above may not be a problem and the benefits of chromatography and spectroscopy can be applied to the service that the Clinicians supply. Bernie Monaghan our Separations Science and Spectroscopy editor paid a visit to Neil Leaver, Head of Department, and Jane Tiller, BMS3, at the Immunosupression Monitoring Service at Harefield Hospital. We discussed how they actively embraced the benefits that LC-MS brings to their service, how they set about implementing their systems and how they see the future.



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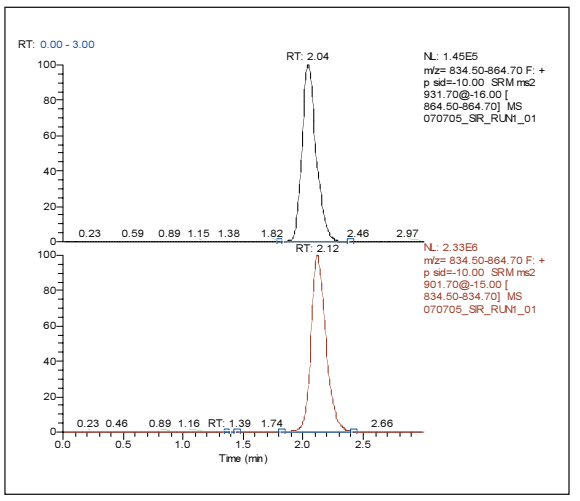
Bernie Monaghan: Can you tell me a little background to the service you provide?

Neil Leaver/Jane Tiller: The services that we provide are vital to the care of patients who have undergone solid organ transplants including heart, kidney, lung and liver. Specifically we measure the levels of immunosuppresant drugs (administered to prevent donor organ rejection), which transplant recipients must take for the remainder of their lives following a transplant. The regular monitoring of the trough blood levels of these immunosuppressants is essential to allow optimised treatment to be given. In early post transplantation stages monitoring may be required anywhere from twice daily to twice weekly to monthly monitoring for life. Since 1980 when Professor Sir Magdi Yacoub performed the first heart transplant at Harefield Hospital over 2,600 transplants have taken place. These include in Heart, lungs, heart/lungs and multi-organ transplants such as heart/kidney and heart/lung/liver. Typical lifetimes of recipients of donor hearts are 9.5 years and some recipients have survived over 20 years. Currently this Department analyses 26,000 samples per year. We offer monitoring of Cyclosporin, Tacrolimus & Sirolimus by MS, Mycophenolic Acid by EMIT, lymphocyte phenotyping by flow cytometry and CMV antigenaemia.

BM: So what kind of assays needed to be developed to cover this requirement and how did you arrive at a MS based assay?

NL/JT: Basically the first drug that was administered with any success as an immuosuppressant was the calcineurin inhibitor Cyclosporin in the early 1980's. This was followed by Tacrolimus (1993) and Sirolimus (2001). Typical chromatograms of a Sirolimus patient sample (upper) and internal standard (lower) are shown in the graph above. Exact experimental conditions can be found at www.sirolimus.org.uk. Early monitoring systems for Cyclosporin utilised polyclonal radioimmunoassay (RIA) in plasma followed by mono specific RIA methods using whole blood and giving higher specificity. The 1995 Lake



Louise International Consensus Conference on Immunosuppressive Drugs[1] recommended that Cyclosporin analysis should be completed on the same day and parent compound should be measured in whole blood. For many years after the introduction of Tacrolimus there was only a single commercial microparticle enzyme immunoassay kit available.

Sirolimus was licensed in 2001 but a commercial kit has only recently become available in 2004. In 1997 we approached a commercial MS company to explore the feasibility of developing a clinical service based on an ion-trap MS system that would allow the measurement of immunosuppressive drugs. Initial work centred around an assay to measure both Cyclosporin and Tacrolimus simultaneously on protein precipitated blood samples using rapid acetonitrile/water gradients.

Following validation a full clinical service was introduced in 1999.This programme allowed Harefield to become the first UK Hospital to introduce routine 7-day week MS based services for immunosuppressives. Following on from this the Department was requested to establish a National Monitoring Service for Sirolimus covering the UK and Eire.

The service (www.sirolimus.org.uk) is provided by the Royal Brompton and Harefield NHS Trust and handles 8,000 samples per year for this assay.

The IMS team for Sirolimus, Harefield Hospital

The department's newest instrument currently used for the National Monitoring Service for Sirolimus.

BM: What resources within the Department do you have to cover the current and expanding workload?

NL/JT: We are a small department staffed by 6 fulltime scientists and one part time administrator.

The Department also offers an advisory service for other Hospitals wanting to set up similar screening services using MS based assays.

We explain the need for clinical risk management strategies, training of technical staff, maintaining stock of spare parts and the need for training of system specialists who are fundamental to the successful operation of an MS based service.

The department currently operates the following instruments:

- Thermo Finnigan LCQ Classic, (1997) used for method development and back up.
- Thermo Finnigan Deca XP Plus, (2001) used for cyclosporin/ tacrolimus analysis
- Thermo Finnigan Quantum Discovery (2004) used for Sirolimus assays.

Each assay system is developed on the LCQ Classic and cross validated on each instrument so that we have a comprehensive backup plan.

BM: It appears that all too few Hospitals that monitor immunosuppressants are using MS based assays. What advantages do they bring over the commercial kits?

NL/JT: The problem with traditional commercially available immunoassay kits is that they are based on monoclonal antibody technology and there are known cross reactivities with non-biologically active metabolites.

Many hospitals with adequate workload do not realise the financial advantages of moving to MS based systems. For example, prior to implementing the single MS instrument we were spending £140K on kits. By leasing the MS instrument we saved £50K in year one whilst improving the quality of service.

MS based assays offer higher specificity and improvements in areas such as LOQ, accuracy, precision and speed of analysis. Our specification was to develop a method with a maximum analytical time of 3 minutes per sample in order to meet the required turnaround times and current workload. For cyclosporin and tacrolimus we operate a 3-hour turnaround of results and for sirolimus all samples submitted by 11am are reported that day.

BM: Clearly with such potentially critical issues resting on the quality of your results how do you safeguard your levels of service?

NL/JT: As with any planning and risk management strategies, a clear understanding of the problems associated with the operation of a clinical MS service are of paramount importance. In our risk assessments we evaluate every possible scenario to develop corrective and preventive action plans as required by our ISO9001: 2000 quality management system. For example we might develop an action plan for the mechanical failure of all 3 MS systems even though it is extremely unlikely.

In terms of staff resources required for the Clinical risk management, it is essential that at least two individuals be trained as system specialists. They would be required to provide unsocial hours cover for telephone support and attend to make repairs if necessary. If a 365/24/7 service is offered then it must be resourced adequately. Hospitals running services on a single instrument must also develop adequate backup plans. These may take the form of networking with other local laboratories to provide analytical support or maintaining commercial assay systems.

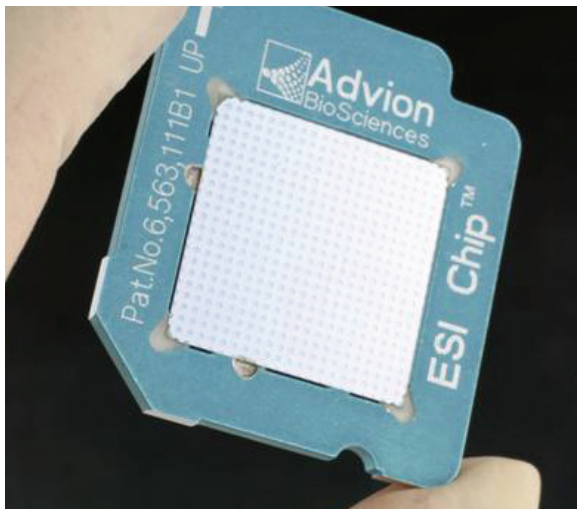
When we implemented with a single instrument we kept stocks of commercial kits, the instrumentation was maintained on a daily basis, the assay system was calibrated regularly and we submitted MS and kit data to the proficiency testing schemes. At Harefield we now have no commercial kits in use (or as back up) as the chances of all 3 systems failing at the same time is considered remote and manageable.

BM: So why do so few clinical laboratories take up this option when, from what you are saying it has more benefits than commercial kits and there is growing interest in moving in this direction? More importantly how do they overcome the apparent hurdles?

NL/JT: There appear to be two main issues, one is financial and the other is the immediate lack of expertises necessary to run the service. Newcomers to the technology may be led to believe that they will have an operational system within weeks of instrument installation. In our experience getting an MS based assay up and running and fully validated would take between 3 – 6 months.

For a laboratory with no skills in LC-MS this would obviously be significantly longer as there is an extremely steep learning curve required to become familiar with the technology and its application to the clinical environment. Our experience would suggest 12-18 months is not unrealistic from starting the project to full implementation of the MS system.

Justification of the financial advantages of MS based systems must include the possibility of an extended implementation in the business plan. We have shown that savings can be made if systems are leased rather than purchased and as the workload increases the savings become higher annually. However, budgets may be exceeded during the first year of implementation due to running parallel systems i.e. both instrument and commercial kit costs. Historically MS systems have been designed for more research based applications. Manufacturers have yet to design instrumentation specifically for clinical markets and unfortunately we are some way off a dedicated TDM "Black Box" MS system which would require minimal method development.



The ESI Chip: Array of 400 independent nanoelectrospray nozzles

BM: What do you see as the future for this technology and what are you working on?

NL/JT: In our experience over 80% of the problems we encounter are with the HPLC system and this has led us to consider the possibilities of 'chromatography free' analysis. In particular robotic nanospray is an emerging technology that has potential for a future "Black Box" type clinical analyser. We have experimented with a Chip- based infusion technique using a disposable ESI Chip (see above) integrated into a Nanomate TM 100 Robotic System. Our initial study resulted in a single drug assay for Cyclosporin but we have recently developed an assay that allows simultaneous quantification of both Cyclosporin and Tacrolimus with an analytical time of 1 minute per sample. Although we are still in the early stages of development good correlation was obtained between chip-based infusion and LC-MS/MS methods.

BM: Summarising then what do you see as the major challenges Hospitals need to overcome to become familiar with MS based assays?

NL/JT: Clearly the first issue is the building up of LC-MS expertise within the NHS and the application of that expertise to clinical needs. Secondly we need to establish communication pathways for integrating the clinical expertise within the NHS with that of the hardware/ software specialists working for the instrument manufacturers so that instruments can be designed specifically for use in clinical laboratories. This is not to suggest that every district hospital will ultimately have an MS based system on site, as this will be dependant on workload for TDM. However, if an instrument could be developed that required little or no separation science expertise then who knows? It was only 20-25 years ago that the concept of a desktop or hand held computer would have been inconceivable based on technology at that time. Lab-on-a-Chip technologies may yet open doors and miniaturised MS instruments, that have long been discussed[2], are now in routine use in the space program.

References:

1. Lake Louise Consensus Conference on Cyclosporin Monitoring in Organ Transplantation: Report of the Consensus Panel. TDM. 1995, 17, 6, 642-655.

2. http://pubs.acs.org/hotartcl/ac/99/apr/shrink.html