

Immunosuppressants by Tandem Mass Spectrometry

IMPROVED MONITORING OF CYCLOSPORINE, TACROLIMUS, SIROLIMUS, AND EVEROLIMUS THROUGH TANDEM MASS SPECTROMETRY (LC-MS/MS)

Test Highlights

- Tests performed by liquid chromatography/tandem mass spectrometry (LC-MS/MS) to determine concentrations in whole blood of the following commonly used immunosuppressant drugs:
 - Cyclosporine (cyclosporin A, Gengraf[®], Neoral[®], Sandimmune[®])
 - Everolimus (SDZ-RAD, Certican[®])
 - Sirolimus (rapamycin, Rapamune[®])
 - Tacrolimus (FK506, Prograf[®])
- LC-MS/MS avoids inaccuracies in results common to immunoassays that cross-react with drug metabolites.
- No need to order a separate test for specimens collected at times other than trough/pre-dose (e.g., C2 cyclosporine).
- Minimum blood sample volume requirement reduced to 250 μ L.

Indications for Ordering

- Immunosuppressant dose optimization
- Failure to respond to immunosuppressants
- Signs or symptoms consistent with inadequate or excessive immunosuppression
- Changes to concomitant medications or other variables that affect pharmacokinetics

Methodology

- The immunosuppressants are first separated from other constituents in the blood by high-performance liquid chromatography (HPLC) and are then detected and quantitated by tandem mass spectrometry (MS/MS).
- LC-MS/MS is considered the most sensitive, specific, and precise technology for monitoring immunosuppressants.

Clinical Background

- Immunosuppressants are used for a wide variety of clinical conditions, but primarily for solid organ and bone marrow transplantation.
- Variable pharmacokinetics and a narrow therapeutic range support a need for routine therapeutic drug monitoring (TDM) for patients who receive immunosuppressive therapy. The traditional methodology used for immunosuppressant TDM is immunoassay.
- The immunosuppressants are extensively metabolized. For example, more than 25 metabolites of cyclosporine are known. The metabolites have limited-to-no pharmacological or toxicological significance, yet many cross-react with antibodies used in TDM immunoassays. It is not possible to predict which patients will generate and accumulate cross-reacting metabolites.

As such, bias in immunosuppressant TDM by immunoassay may affect patients consistently, intermittently, after chronic therapy, or not at all.

- A clinically significant, positive bias in immunosuppressant TDM may lead to inappropriate dose adjustments. LC-MS/MS technology is recognized to minimize bias and improve accuracy of results.

Limitations

- For serial sample collections from the same patient, it is best to utilize the same analytical technology. Results generated by LC-MS/MS may not be comparable to results generated by immunoassays. Results generated by LC-MS/MS are generally lower than those obtained by immunoassay. Immunoassay methods for immunosuppressants tend to cross-react with inactive metabolites, which contributes to a positive bias. Consult the specificity of the detection antibodies involved in the immunoassay of interest when comparing results generated by an immunoassay with results from other technologies.
- Clotted specimens cannot be analyzed.

Interpretation

- Therapeutic ranges for immunosuppressants depend on many factors and should be determined by the transplant clinic. Variables to consider include the organ transplanted, time post-transplant, comorbidities, concomitant medications, drug formulation and route of administration, age and ethnicity of the patient, and method of analysis.
- Generally accepted therapeutic ranges for maintenance therapy, based on specimens collected 12 hours post-dose, are:
 - Cyclosporine: 100–400 ng/mL
 - Everolimus: 3–8 ng/mL (kidney transplant); 5–10 ng/mL for subependymal giant cell astrocytoma (SEGA) with tuberous sclerosis

- Sirolimus: 4–12 ng/mL (proposed range); 12–20 ng/mL for liver transplant
- Tacrolimus: 5–15 ng/mL

References

1. Wallemacq P, et al. Opportunities to optimize tacrolimus therapy in solid organ transplantation: report of the European consensus conference. *Ther Drug Monit.* 2009;31:139–152.
2. Taylor AL, et al. Immunosuppressive agents in solid organ transplantation: mechanisms of action and therapeutic efficacy. *Crit Rev Oncol/Hematol.* 2005;56:23–46.
3. Wong SHY. Therapeutic drug monitoring for immunosuppressants. *Clinica Chemica Acta.* 2001;313:241–253.
4. Holt DW, et al.. International Federation of Clinical Chemistry/ International Association of Therapeutic Drug Monitoring and Clinical Toxicology Working Group on immunosuppressive drug monitoring. *Ther Drug Monitor.* 2002;24:59–67.

Test Information

0070035	Cyclosporine A by Tandem Mass Spectrometry
0090612	Tacrolimus by Tandem Mass Spectrometry
0092118	Everolimus by Tandem Mass Spectrometry
0098467	Sirolimus by Tandem Mass Spectrometry

For specific collection, transport, and testing information, refer to the ARUP website at www.aruplab.com.

For information on test selection, ordering, and interpretation, refer to ARUP Consult® at www.arupconsult.com.

AUTHORS

Gwen A. McMillin, PhD

Kamisha Johnson-Davis, PhD