



CPAL

Central Pennsylvania Alliance Laboratory

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Tacrolimus Assay

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Effective Date:

April 18, 2011

Mnemonic: TACRO

Performed: Monday through Saturday (Days)

Specimens:

Only whole blood specimens collected in EDTA tubes may be used for the test.

Handling and Storage:

Specimens may be stored for up to 7 days refrigerated at 2-8°C prior to being tested. If testing will be delayed more than 7 days, store frozen (-10°C or colder).

Summary:

Effective April 18, 2011, CPAL will be offering the Tacrolimus assay (Abbott Architect platform). The Architect Tacrolimus assay is a chemiluminescent microparticle immunoassay (CMIA) for the quantitative determination of tacrolimus in human whole blood. The Architect Tacrolimus assay is to be used as an aid in the management of liver and kidney allograft patients receiving tacrolimus therapy.

Reference Range:

No firm therapeutic range exists for tacrolimus in whole blood. *The measurement range for the Architect Tacrolimus assay is 2 ng/mL to 30 ng/mL.* The complexity of the clinical state, individual differences in sensitivity to immunosuppressive and nephrotoxic

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effects of tacrolimus, co-administration of other immuno-suppressants, type of transplant, time post-transplant and a number of other factors contribute to difference requirements for optimal blood levels of tacrolimus. Therefore, individual tacrolimus values cannot be used as the sole indicator for making changes in treatment regimen and each patient should be thoroughly evaluated clinically before changes in treatment regimens are made. Clinicians should be made aware of the date and time of the specimen collection and the time of the last dose prior to that collection.

The Consensus Document has reported that the therapeutic range of tacrolimus is not clearly defined, but the target 12-hour trough whole blood concentrations are 5-20 ng/mL early post-transplant. Higher concentrations are associated with an increased incidence of adverse effects. Twenty-four hour trough concentrations are 33-50% less than the corresponding 12-hour trough levels (1).

Additional Information:

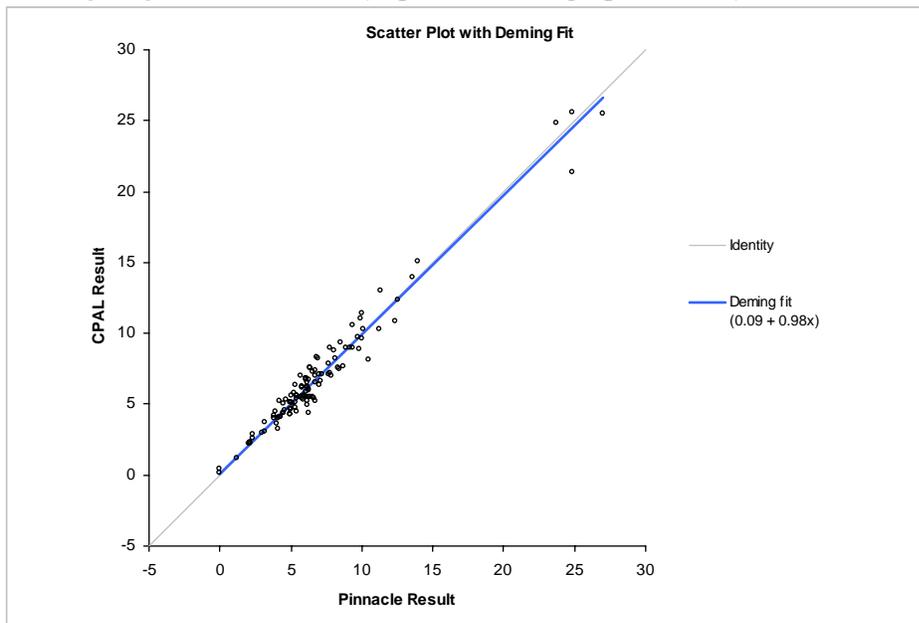
Comparison studies (n=112) were performed comparing tacrolimus results on split specimens obtained from Pinnacle Health System. Testing performed at Pinnacle Health System is the same platform/assay as the method employed at CPAL (Abbott Architect Tacrolimus CMIA Assay). In addition, comparative studies (n=46) were performed comparing split specimen results for testing performed at CPAL (Abbott Architect Tacrolimus CMIA Assay) and Quest Diagnostics (LC/MS/MS assay). In both sample sets, the assays compared as expected (2).

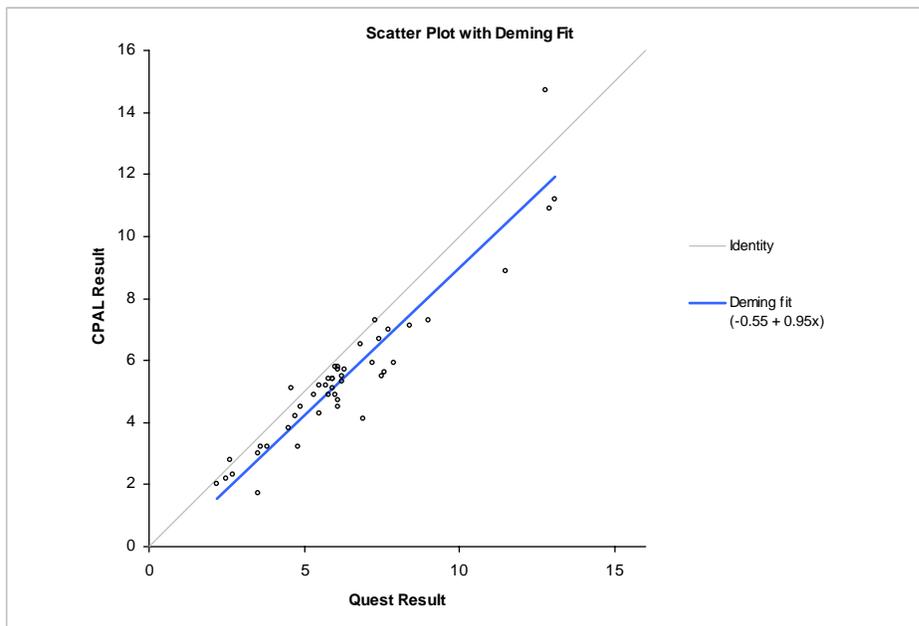
Linear Regression

CPAL vs Quest: slope = 0.9855; $R^2=0.8876$

CPAL vs Pinnacle: slope = 0.9982; $R^2=0.9637$

Deming Regression Results (depicted in the graphs below)





Summary and Explanation of the Test:

Tacrolimus is an immunosuppressive drug discovered in 1984 by the Fujisawa Pharmaceutical Co., Ltd. It has been shown to be effective for the treatment of organ rejection following transplantation. The results of clinical trials with liver and kidney have been published. Clinical studies are continuing for a variety of indications.

Tacrolimus binds to a family of proteins termed FK506 (tacrolimus) binding proteins (FKBPs). The formation of a larger pentameric complex comprised of FKBP, tacrolimus, calmodulin and calcineurins A and B results in the inhibition of the phosphatase activity of calcineurin. The action of transcription factors requiring dephosphorylation for transport to the cell nucleus are thus inhibited leading to blockage of T-cell proliferation and function.

Tacrolimus may be administered IV or orally. Absorption from the gastrointestinal tract is variable and irregular. Pharmacokinetic studies with tacrolimus have shown that there are large inter- and intra-individual differences in its kinetics in organ transplant patients.

Pharmacokinetic studies have also indicated that whole blood rather than plasma may serve as the more appropriate medium to describe the pharmacokinetic characteristics of tacrolimus. Tacrolimus is bound to proteins, mainly albumin and α -1-acid glycoprotein, and is highly bound to erythrocytes. The distribution of tacrolimus between whole blood and plasma depends on several factors such as hematocrit, temperature of separation of plasma, drug concentration, and plasma protein concentration. In a U.S. study, the ratio of whole blood concentration to plasma concentration ranged from 12 to 67 (mean 35).

Tacrolimus is extensively metabolized in the liver and small intestine microsomes utilizing the cytochrome P-450 enzymes. Nine different metabolites of tacrolimus have been identified; several of the metabolites have been found and tested in whole blood.

The use of tacrolimus is associated with serious toxic side effects, primarily nephrotoxicity. At the present time it is not clear whether the nephrotoxicity of tacrolimus is

the result of parent drug, metabolites, or a combination of both. Other adverse side effects include neurotoxicity, hypertension, insomnia, and nausea. (2)

Limitations of the Procedure:

1. Results should be used in conjunction with other data: e.g. symptoms, results of other tests, clinical impressions, etc.
2. If the tacrolimus results are inconsistent with clinical evidence, additional testing is suggested to confirm the result.
3. The concentration of tacrolimus in a given specimen determined with assays from different manufacturers can vary due to differences in assay methods and reagent specificity.
4. Immunoassays are nonspecific and can cross react with metabolites. When elimination of tacrolimus is impaired (e.g. during cholestasis), tacrolimus metabolites may accumulate, the immunoassay may overestimate the concentration of tacrolimus. In such cases, the use of a specific assay (e.g. Liquid Chromatography Mass Spectrometry/Mass Spectrometry [LC/MS/MS]) could be considered.
5. Heterophilic antibodies in human serum can react with reagent immunoglobins, interfering with in vitro assays. Patients routinely exposed to animals or to animal serum products can be prone to this interference and anomalous values may be observed. Additional information may be required for diagnosis.
6. Specimens from patients would have received preparations of mouse monoclonal antibodies for diagnosis of therapy may contain human anti-mouse antibodies (HAMA). Results from specimens containing HAMA may produce anomalous results.

(1) Jusko WJ, Thomson AW Fung J, et al Consensus document: therapeutic monitoring of tacrolimus (FK-506). Ther Drug Monit 1995;17(6):606-14.

(2) Package Insert. Architect System, Tacrolimus. Abbott Laboratories. April 2009.