in later stages of the disease, or when the tumor becomes metastatic. The similarity to the LD pattern of brain is worth noting.

References


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Improved Procedures for Enzyme Immunoassay of Tacrolimus (FK506) in Whole Blood

To the Editor:

Tacrolimus (FK506) is a new, highly potent immunosuppressant now undergoing clinical trials for liver, kidney, and bone marrow transplantation. The protocols call for therapeutic monitoring of plasma and (or) whole-blood concentrations of the drug (1–3).

Tamura et al. initially reported a sensitive double-antibody enzyme immunoassay (EIA) procedure for quantifying tacrolimus in 1987 (1). Cadoff et al. adapted this method and utilized C18 Sep-Pak™ cartridges (Analytichem) to extract FK506 from plasma (2). Jusko and D’Ambrosio presented a methylene chloride extraction procedure, which allowed analysis of both whole-blood and plasma samples by EIA (3). Warty et al. (4) and Wallemacq et al. (5), using the Sep-Pak method, reported that the EIA could be performed with 2-h incubations to shorten the procedure. We describe a third extraction procedure, with adaptations that considerably enhance sensitivity and turnaround of the assay.

The following differences from previous methodology (3) were involved: Whole-blood calibrators, controls, and samples (20 or 200 μL, rather than only 20 μL) are placed in 1.5-mL polypropylene centrifuge tubes (Laboratory Product Sales; no. 509). Then 700 μL of method (Baxter Scientific; 230-43D) is added to each tube (rather than extraction for 30 min with 9 mL of methylene chloride). The tubes are capped, placed in a rack, and then forcefully inverted to mix their contents. All are vortex-mixed for 30 s on a multitube vortex-type mixer (Baxter Scientific), and then centrifuged at 10,000 g, in a microcentrifuge (Herneus Biofuge) for 5 min. From each tube 550 μL of supernate is transferred to 13 × 100 mm glass tubes (VWR no. 60825-571) and evaporated.

The assay then proceeds as described previously (3) except that all wells (rather than only nonperipheral wells) of the microtiter plates are used and plates are shaken (speed 5, Titer Plate Shaker; Lab-Line Instruments) for 2 h (rather than overnight) at room temperature (rather than at 4°C).

To determine analytical recovery, we mixed 10 mL of FK506 containing 400 000 or 40 000 counts/min [3H]di-hydro-FK506 with 2 mL of whole blood. Either 20 (n = 5) or 200 μL (n = 2) of these samples was extracted with methanol. Average recoveries were calculated from data obtained by liquid scintillation counting.

Assay comparisons were made with 110 patients’ whole-blood samples containing FK506. For the lower concentration range (0.05–20 μg/L), 58 patients’ samples were also assayed undiluted and 10-fold diluted. For all comparisons of results, we used a per-adapted least-squares technique, wherein both variables are considered to be subject to error (θ).

The EIA standard curves were sigmoidal, and the four-parameter fitting typically yields predicted concentrations within 30% of the nominal value(s) and r² ≥0.99. Absorbances ranged from 0.1 to 2.9 at 490 nm, and nonspecific binding absorbances averaged 0.060 ± 0.01 A. These ranges are characteristic of the Nunc Maxisorp plate utilized; other plates exhibit lower absorbances due to less binding.

The performance characteristics for 20-μL whole-blood samples showed limits of quantitation (LOQ) and detection (1.0 μg/L each) comparable with those of the methylene chloride method. The limit of detection is the FK506 concentration producing absorbances statistically different from blank samples; the LOQ is the lowest concentration with a CV <30%. Extraction of 200 μL of whole blood improved the LOQ to 0.1 μg/L.

Repeatability yields intrassay CVs ranging from 3.7% to 11.1% (n = 6). Repeated measurements of control samples (n ≥ 20) over several days (n ≥ 5) yielded CVs of 6.5% to 25%, with the lowest concentrations typically exhibiting the highest variation.

The percentage recovery of FK506 from 20 and 200 μL of whole blood was 81.4% ± 1.5% and 73.4% ± 5.8% (mean ± SD). This is slightly lower than the 80–90% recovery found with 30 min of methylene chloride extraction (7). Extraction time with methanol is briefer (<1 min vs 30 min).

The methylene chloride and methanol assay results for 110 patients’ whole-blood (20–μL) samples are depicted in Fig. 1. This comparison shows acceptable functioning of the two methods: the slope was close to 1, the intercept was insignificant and below the LOQ (<1 μg/L), and the correlation was good.

FK506 concentrations were measured in patients’ samples by utilizing

![Fig. 1. Comparison of patients’ results for FK506 concentrations (μg/L) in 20 μL of whole blood after methylene chloride or methanol extractions.](image-url)

The line of identity is shown (slope = 1.0). Regression analysis (θ) yielded a slope of 0.95, intercept of 0.15 μg/L, and r² = 0.88.
methanol extraction and the 0.05 to 20 
µg/L range of standards, with and 
without 10-fold sample dilution. Re-
gression of the assayed concentrations 
for diluted vs undiluted samples 
yielded a slope of 0.10, indicating ex-
cellent accuracy at the lower end of 
the standard curve range. The inter-
cept, 0.03, is below the LOQ (0.1 µg/L); 
the r² was 0.78.

Validation results were generated 
for a 2-h room temperature precoating 
of plates with polyclonal antibody. The 
means of outer vs inner wells were not 
significantly different for four experi-
ments. This indicates that the tech-
niques produce an identical product, 
but the 2-h procedure allows more as-
says to be run per plate (increased 
from 12 to 30).

The two extraction methods were 
applied to blood samples from rats and 
rabbits that had been given FK506. 
For both species, we found slopes near 
1.0 and intercepts near 0.0.

A simple methanol extraction 
method with 2-h room-temperature 
incubation for EIA of tacrolimus (FK506) 
in whole blood thus produces analytical 
data comparable with that obtained af-
after methylene chloride extraction. The 
sensitivity and LOQ in whole blood are 
decreased 10-fold, from 1.0 to 0.1 µg/L. 
The use of peripheral wells of EIA 
plates increases the number of un-
known samples per plate from 12 to 30.

Overall, the assay time is reduced 
from 36 h to ~5 h. These modifications facili-
tate use of the EIA procedure for phar-
macokinetic studies and therapeutic 
monitoring of FK506.

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Serum Creatine Kinase MB After 
Acute Myocardial Infarction in a 
Patient with IgA–CK–BB Complex

To the Editor:

A few years ago, we described a pa-
tient (1) who had an IgA–creatinine 
kinase isoenzyme BB (CK–BB) complex 
with an electrophoretic mobility the 
same as for CK-MB. This patient was 
recently admitted to our hospital with 
an acute myocardial infarction.

The electrocardiogram (EKG) at the 
time of admission showed definitive 
changes, and at 5 days after ad-
mision, another EKG revealed new 
changes consistent with reinfarction.

After admission, the electrophoretic 
pattern of creatine kinase (CK; EC 
2.7.3.2) isoenzymes (Corming Medical, 
Halstead, UK) revealed two bands: 
The band with skeletal muscle type 
(CK-MM) mobility was 46% of the 
total CK and the band with heart type 
(CK-MB) mobility was 54%. In con-
trast, CK-MB mass concentration by 
the IMx immunometric method (Ab-
bot Laboratories, North Chicago, IL) 
was borderline (5 µg/L), and no 
CK-MB activity was detectable by an 
immunoprecipitation method (Roche 
Diagnostics, Nutley, NJ). Six hours 
later, total CK, lactate dehydroge-
nase, and aspartate aminotransferase 
activities (determined on a Hitachi 
717, with reagents from Boehringer 
Mannheim, Mannheim, Germany) 
were at 7.8, 1.4, and 3.8 times the up-
per limits of the reference intervals, 
respectively. Peak concentrations of 
CK isoenzymes were reached 12 h af-
after admission, and a second increase 
occurred later, matching the fifth-day 
changes in the EKG.

We assessed the presence of IgA– 
CK–BB complex as described else-
where (1), with minor variations.

Briefly, two samples were collected at 
admission and 6 h later, and were 
fractionated on a Sephadex G-200 column 
(Pharmacia, Uppsala, Sweden). The 
CK activity eluted in two peaks in 
both samples, suggesting complex-re-
lated activity in the peak closer to the 
void volume. Fractions of each eluted 
peak were mixed, concentrated, and 
electrophoresed directly, after treat-
ment with antibodies to subunit M 
and after treatment with antibodies to 
IgA. The procedure showed CK-MM 
activity (farther from the void volume) 
and false CK-MB (IgA–CK–BB) activity 
(closer to the void volume) in both 
samples, but true CK-MB activity 
(farther from the void volume) was 
present only in the sample taken 6 h 
after admission. Fig. 1 shows the 
profiles for total CK activity and CK-MB 
analysed by the three methods in most 
of the patient’s serum samples. Note 
that electrophoretic results are pre-
sented as a percentage of the total CK 
activity, and display a different pat-

![Fig. 1. Time plots for total CK activity and CK-MB activity and mass in serum determined by IMx mass measurement, immunoprecipitation, and electrophoresis for the patient.](image)