BioStrands

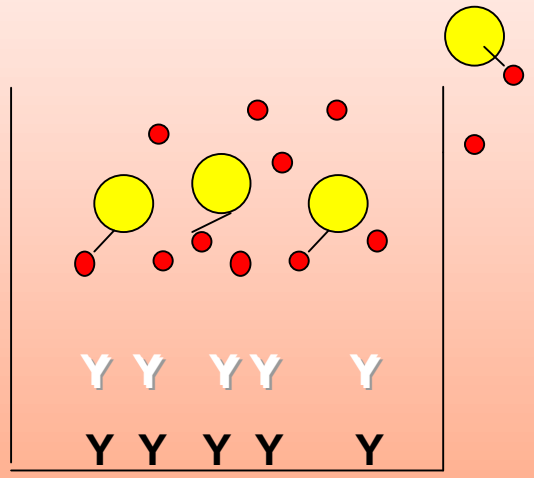
TDM ELISA

General Information

- TDM-ELISA consists of a colorimetric competitive enzyme immunoassay for the determination of HIV-1 protease inhibiting (PI) drugs in human plasma
- Correct Plasma concentrations of PI are needed for an efficient therapy and to avoid side effects
- PI's monitoring adds an important information in the management of HIV-positive patients

ELISA outline

I. COMPETITION



Enzyme-drug Conjugate

Free drug

Anti-drug antibody

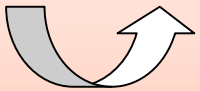
Anti-rabbit antibody

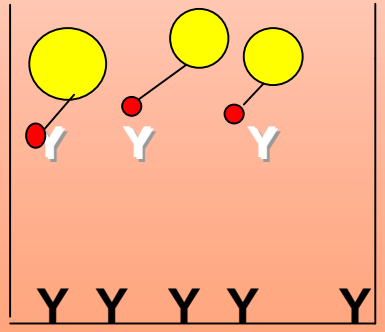
Solid phase common to all TDM ELISA

Automation possible

II. Detection Reaction

Chromogenic Substrate





Anti-drug antibody

Anti-rabbit antibody

Hereafter is shown an outline of the preparation steps for a clear fresh plasma sample.

	LPV	APV	NFV	RTV booster
Dilution in absolute methanol	✓	✓	✓	
Centrifugation	✓		✓	
Dilution in water	✓		✓	
Dilution in 30% methanol	✓		✓	✓

Samples can be pre-centrifuged, aliquoted and stored at –20°C avoiding repeated freezing and thawing

Summary

Description and code	Sample Preparation	Assay procedure
TDM-ELISA LPV Cod. 2678	100µl of plasma + 300µl of MetOH – Vortex for 10-15sec Centrifugation : 10min at 10000 g in a microcentrifuge Dilute 100µl of clear surnatant with 150µl of water. Vortex Add to 100µl of the diluted surnatant 150µl of MetOH 30% Vortex Use 20 µl for testing	20µl of pre-treated sample + 80µl of specific enzyme + 100 µl specific Antiserum <u>Incubate 1h at RT</u> Wash wells 5 times with Washing Buffer Add 200µl of chromogenic solution (TMB) diluted in the development solution <u>Incubate 30min (RT in the dark)</u> Add 50µl Of stop solution Read at 450nm on a microplate reader
TDM-ELISA RTV booster Cod. 7678	10µl of + 990 µl of MetOH 30% Vortex Use 20 µl for testing	
TDM-ELISA APV / Fos APV Cod. 1678	10µl of plasma + 990 µl of sample diluent Vortex Use 20 µl for testing	
TDM-ELISA NFV Cod. 3678	100µl of plasma + 300µl of MetOH – Vortex for10-15sec Centrifuge for 10min at 10000 g in a microcentrifuge Dilute 100 µl of clear surnatant with 50 µl of distilled water. Vortex Add to 10 µl of the diluted surnatant 990µl of 30%.Methanol Vortex Use 20 µl for testing	

Applications

This technology allows the determination of PI concentration in plasma samples of patients in HAART treatment

TDM-ELISA is especially useful in the following conditions:

- **Pregnant women and children**
- **Patients with suspected significant pharmacologic interactions which might induce dose-related adverse effects or which could cause a reduction of treatment efficacy**
- **Physiopathological conditions significantly modifying liver or kidney's function and inducing alterations in absorption, distribution, metabolism or clearance of the drug**
- **Patients with viral strains resistant to treatment and needing different dosage schedules whose efficacy has not yet been proved in clinical trials**
- **Dose-related toxicity**
- **Lack of virologic response in "naive" patients**

IMPORTANCE OF TDM - ELISA

A normal TDM result is always an important information for the clinician because it allows to exclude abnormal drug levels as cause of toxic symptoms or uneffective therapy

Data from Medizinische Laboratorien Düsseldorf (Germania) - dr Kuschak

TDM-ELISA LPV

Table 1 - Summary

7 control samples were used from Ring Test at known concentration for 7-10 times on the same plate

# 1	# 2	# 3	# 4	# 5	# 6	# 7	# 8	# 9	# 10	Target Value	Mean	St Dev	CV	Inaccuracy
8240	7649	7516	8842	8681	8681	7709	8681	6962		8195	8106,8	668,5	8,2	-1,1
# 1	# 2	# 3	# 4	# 5	# 6	# 7	# 8	# 9	# 10	Target Value	Mean	St Dev	CV	Inaccuracy
7085	7827	7440	7287	7709	8053	6387	7515	7929		7990	7470,2	511,5	6,8	-6,5
# 1	# 2	# 3	# 4	# 5	# 6	# 7	# 8	# 9	# 10	Target Value	Mean	St Dev	CV	Inaccuracy
3155	3171	2863	3194	3185	3521	3528	3478	3444	3419	2930	3295,8	216,1	6,6	12,5
# 1	# 2	# 3	# 4	# 5	# 6	# 7	# 8	# 9	# 10	Target Value	Mean	St Dev	CV	Inaccuracy
1569	1808	1831	1822	1776	1873	1822	1799	1849	1901	1639	1805,0	90,4	5,0	10,1
# 1	# 2	# 3	# 4	# 5	# 6	# 7	# 8	# 9	# 10	Target Value	Mean	St Dev	CV	Inaccuracy
785	792	864	1011	841	854	892	869	900	864	737	867,2	63,0	7,3	17,7
# 1	# 2	# 3	# 4	# 5	# 6	# 7	# 8	# 9	# 10	Target Value	Mean	St Dev	CV	Inaccuracy
3057	3329	3049	3027	3393	3777	3768	3486	3328	3695	3296	3390,9	290,9	8,6	2,9
# 1	# 2	# 3	# 4	# 5	# 6	# 7	# 8	# 9	# 10	Target Value	Mean	St Dev	CV	Inaccuracy
15950	16080	15860	16210	16760	17760	18210				16214	16690	939,8	5,6	2,9

TDM-ELISA APV

Summary table 2

Evaluation on 4 Ring Test samples repeated 9 - 10 times (intra-assay)

# 1	# 2	# 3	# 4	# 5	# 6	# 7	# 8	# 9	# 10	Target Value	Mean	St Dev	CV	Inaccuracy
5131	7394		6484	6862	7510	7451	5756	6183	3376	5720	6238,6	1347,6	21,6	9,07
# 1	# 2	# 3	# 4	# 5	# 6	# 7	# 8	# 9	# 10	Target Value	Mean	St Dev	CV	Inaccuracy
3887	2489	2409	3077	4357	3560		2660	3609	2896	3600	3216,0	674,1	21,0	-10,67
# 1	# 2	# 3	# 4	# 5	# 6	# 7	# 8	# 9	# 10	Target Value	Mean	St Dev	CV	Inaccuracy
3039	1984	3361	2930	2711	3248	2700	2516	3659	2023	2860	2817,1	547,0	19,4	-1,5
# 1	# 2	# 3	# 4	# 5	# 6	# 7	# 8	# 9	# 10	Target Value	Mean	St Dev	CV	Inaccuracy
1596	2010	1505	2010	1721	1991	2120	2581	2085	2023	1800	1964,2	304,3	15,5	9,1

TDM-ELISA RTV Booster

Table 3 - Summary

7 Ring Test samples at known concentration were use for 10 times on the same plate (intra-assay)

# 1	# 2	# 3	# 4	# 5	# 6	# 7	# 8	# 9	# 10	Target Value	Mean	St Dev	CV	Inaccuracy
1039	1127	1250	927	986	1067	1144	1177	1144	1160	1100	1102	100,4	9,1	0,2
# 1	# 2	# 3	# 4	# 5	# 6	# 7	# 8	# 9	# 10	Target Value	Mean	St Dev	CV	Inaccuracy
492	447	511	477	500	614	492	573	532	532	550	517,0	51,0	9,9	-6,0
# 1	# 2	# 3	# 4	# 5	# 6	# 7	# 8	# 9	# 10	Target Value	Mean	St Dev	CV	Inaccuracy
197	187	186	218	169	187	209	210	190	210	220	196,3	15,2	7,7	-10,8
# 1	# 2	# 3	# 4	# 5	# 6	# 7	# 8	# 9	# 10	Target Value	Mean	St Dev	CV	Inaccuracy
1025	1122	1210	1115	1115	1161	971	1087	947	965	1172	1071,8	90,0	8,4	-8,5
# 1	# 2	# 3	# 4	# 5	# 6	# 7	# 8	# 9	# 10	Target Value	Mean	St Dev	CV	Inaccuracy
77,4	88	95,7	85,9	85,3	84,8	49,7	91,9	77,7	112	110	84,8	15,8	18,7	-22,9
# 1	# 2	# 3	# 4	# 5	# 6	# 7	# 8	# 9	# 10	Target Value	Mean	St Dev	CV	Inaccuracy
55,2	51,8	48,5	46,1	83	48,1	50,2	50,6	51,3	50,6	73,3	53,5	10,6	19,9	-27,0
# 1	# 2	# 3	# 4	# 5	# 6	# 7	# 8	# 9	# 10	Target Value	Mean	St Dev	CV	Inaccuracy
30,5	37,7	45,9	39,7	38,5	39,4	44,3	43,3	43,6	40,8	52,4	40,3	4,7	11,6	-23,0

Results of the evaluation

Accuracy and Precision

The data obtained with TDM-ELISA are equivalent to the data which characterize the performance of HPLC

(Drose J.A. et. Alt., 32, 287-291 – JAIDS 2003)

TDM-ELISA
Specificity

- No cross-reactivity has been observed of each TDM with respect to the other peptidic PI

  (APV, LPV, RTV, NFV e M8, ATV, IDV, SQV)

  and with **TIPRANAVIR** (new drug of a new class of non-peptidic PI*)*

- No cross-reactivity has been observed with other drugs used in HAART therapy

  (NNRTIs: Nevirapine, Efavirenz; NRTIs: Zidovudine, Abacavir and Lamivudine)

Detection limit in matrix

TDM - ELISA	ng/ml
LPV	123
APV	400
RTV	40

Measuring Range

- In agreement with the American Guidelines (US Therapy – DHHS) of October 2006

  the kits have measuring ranges based on the significant therapeutic limit

Dynamic measuring ranges of the different kits

Drug	Quantification Range Accuracy within 20 % (concentrations: µg / ml)
LPV	1 – 8
NFV	0,8 - 3
APV	0,4 - 5
RTV booster	0,09 – 0,9
RTV	0,5 - 5

Conclusions

ELISA TDM for their low cost and simplicity can be a reliable and useful method for routine monitoring of TDM in any laboratory

Availability

- Available Products :

TDM-ELISA APV	**cod. 1678**
TDM-ELISA LPV	**cod. 2678**
TDM-ELISA NFV	**cod. 3678**
TDM-ELISA RTV booster	**cod. 7678**
TDM-ELISA ATV*	**cod. 5678**