



Drug Screening in Serum Samples with the Dade Behring Viva-E™ System



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Introduction:

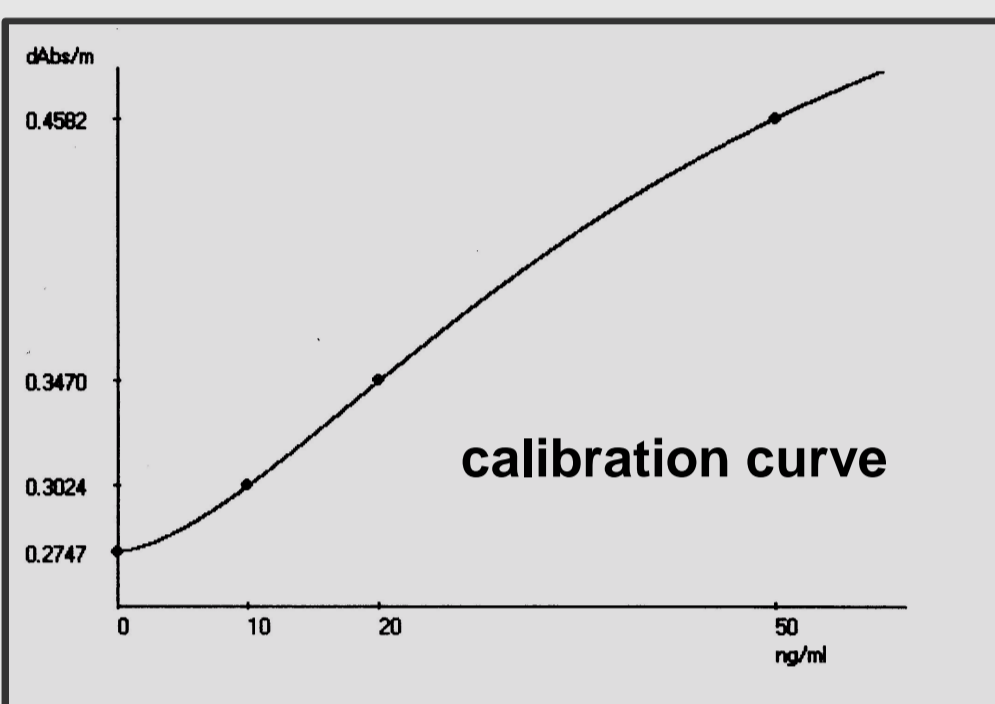
The object of the study was the evaluation of the Emit[®] II Plus urine tests for the detection of drugs in serum samples with special consideration of the substances listed in § 24a of street traffic law in Germany (THC, morphine, benzoylecgonine, amphetamine, MDMA, MDEA).

Methods:

To enhance discrimination of low concentration, additional calibration points were added in the lower ranges of the calibration curves. Within-run precision was determined using calibrators of a low and a high concentration and a positive serum pool (n = 20). Between-day precision was calculated measuring the same calibrators and serum pools in duplicate on 20 days. Serum samples were measured within a few days after taking or after storage at -20°C for up to 3 years. Drug free serum samples were used to detect the sensitivity limits which served as cut-off values. The results were compared to those of a gas chromatography/mass spectrometry analysis. To enhance the sensitivity for amphetamines and ecstasy, the ratio of sample to buffer volume and the antibody amount was modified, and finally, serum samples were concentrated prior to measurement.



Results:

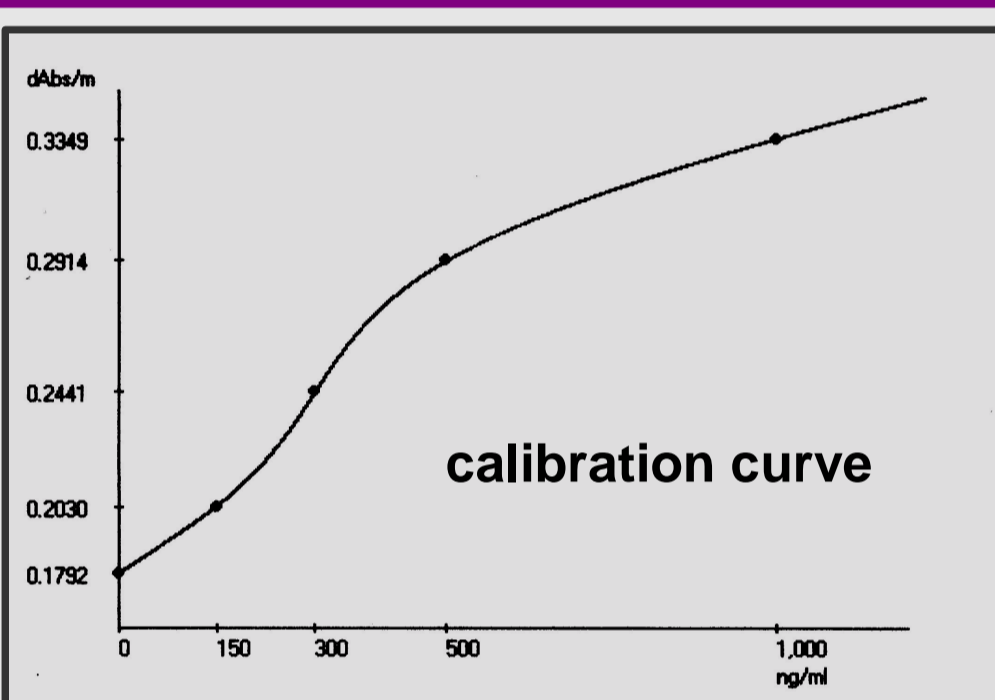


Cannabinoids

	[ng/mL]	CV [%]	
		within-run	between-day
high calibrator	40	3,35	3,67
low calibrator	10	5,07	5,64
serum pool	47	1,44	2,71

GC/MS* (n = 128)	[ng/mL]	n	Emit results [ng/mL]	
			0	0 < sl < 1,82 > 1,82
0	20	18	0	2
0 < c < 10**	17	1	0	16
>= 10	91	0	0	91

* sum of THC, THC-OH, and THC-COOH
** normally the sum is > 10 ng/mL if THC is present in amounts > 1 ng/mL
sl = sensitivity limit
(mean of 28 negative samples + 3s)

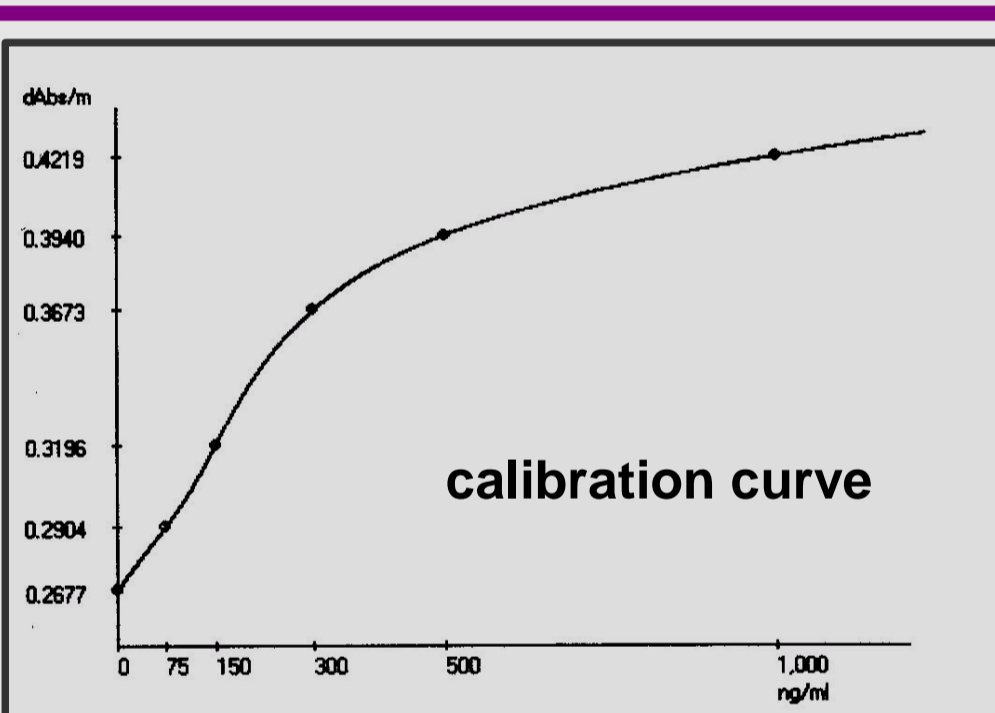


Opiate

	[ng/mL]	CV [%]	
		within-run	between-day
high calibrator	500	2,43	2,18
low calibrator	150	2,28	3,30
serum pool	527	2,44	3,38

GC/MS* (n = 122)	[ng/mL]	n	Emit results [ng/mL]	
			0	0 < sl < 47,5 > 47,5
0	22	8	14	0
0 < c < 10	11	2	2	7
>= 10	89	1	2	86

* sum of free morphine and codeine
sl = sensitivity limit
(mean of 29 negative samples + 3s)

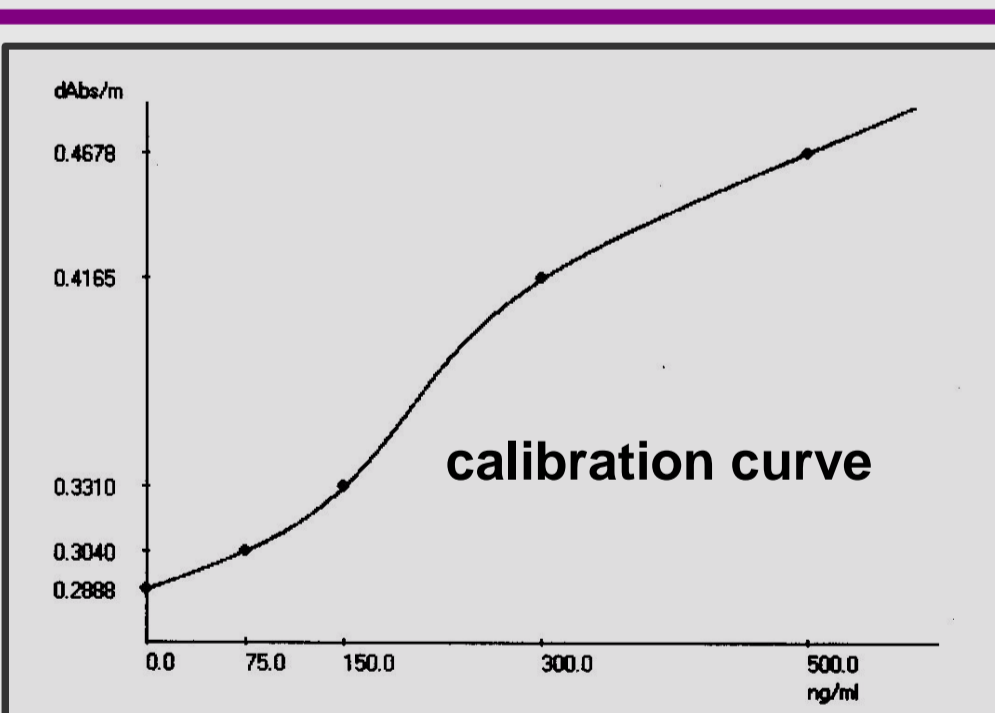


Cocaine Metabolite

	[ng/mL]	CV [%]	
		within-run	between-day
high calibrator	500	6,24	9,96
low calibrator	75	5,31	9,61
serum pool	112	4,90	7,36

GC/MS* (n = 101)	[ng/mL]	n	Emit results [ng/mL]	
			0	0 < sl < 33,8 > 33,8
0	24	15	9	0
0 < c < 75	20	10	7	3
>= 75	57	0	5	52

* sum of cocaine, BE, and EME
sl = sensitivity limit
(mean of 24 negative samples + 3s)

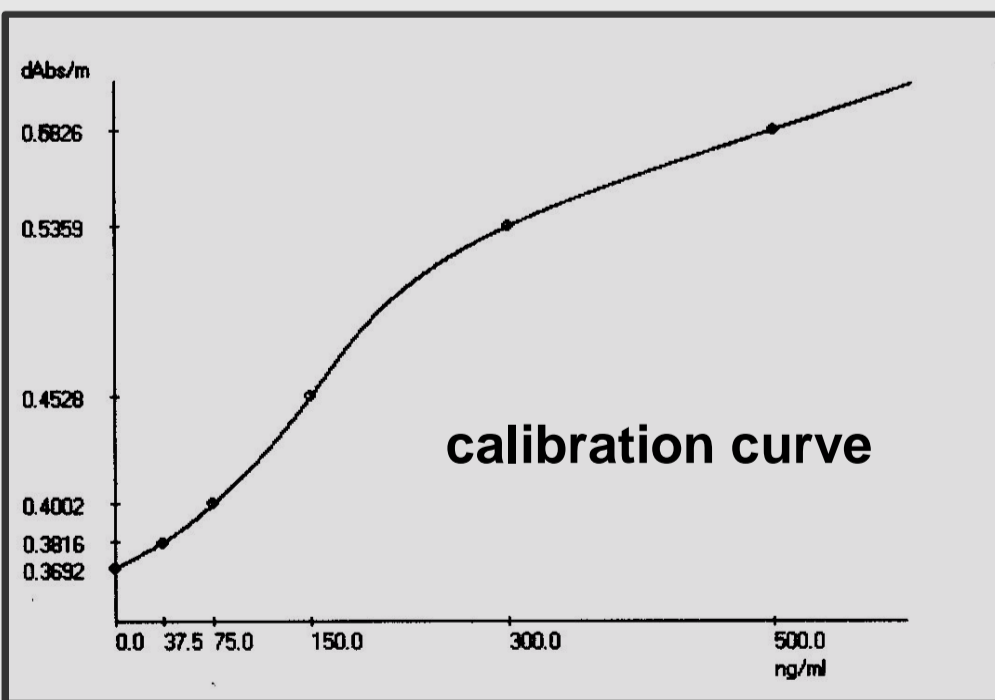


Amphetamines

	[ng/mL]	CV [%]	
		within-run	between-day
high calibrator	300	2,16	1,85
low calibrator	75	7,17	11,98
serum pool	149	2,55	3,77

GC/MS* (n = 204)	[ng/mL]	n	Emit results [ng/mL]	
			0	0 < sl < 1,6 > 1,6
0	62	50	1	11
0 < c < 25	43	40	0	3
>= 25	99	52	1	46

* sum of amphetamine and methamphetamine
sl = sensitivity limit
(mean of 40 negative samples + 3s)
If GC/MS >= 100 ng/mL (37 samples), Emit showed results above the sl in 33 cases.



Ecstasy

	[ng/mL]	CV [%]	
		within-run	between-day
high calibrator	300	4,91	7,17
low calibrator	37,5	8,91	30,61
serum pool	130	3,41	14,62

GC/MS* (n = 204)	[ng/mL]	n	Emit results [ng/mL]	
			0	> 0
0	129	125	4	
0 < c < 25	14	13	1	
>= 25	61	9	52	

* sum of MDMA, MDEA, and MDA
sl = sensitivity limit = 0
(mean of 40 negative samples)
If GC/MS >= 100 ng/mL (42 samples), Emit showed results above the sl in all 42 cases.

Extraction of Amphetamines and Ecstasy:

Alkalis 1 mL of serum with 0.5 mL of NaOH (20%). Add 2 mL of cyclohexane and vortex gently for appr. 1 min. Centrifuge at 4000 rpm for 5 min. Remove the organic phase (= upper layer). Add 1 drop of HCl in Methanol (1:5, v/v) to the extract. Evaporate the solvent (30°C, compressed air). Reconstitute in 150 µL of NaCl-solution (0.9%).

Combined test

GC/MS* (n = 26)	[ng/mL]	n	Emit results [ng/mL]**	
			0	> 0
0	10	10	0	
0 < c < 25	1	1	0	
>= 25	15	2	13	

* sum of amphetamine, methamphetamine, MDMA, MDEA, and MDA
sl = sensitivity limit = 0,
(mean of 10 negative samples)
** sum of both Emit tests
If GC/MS >= 100 ng/mL (9 samples), Emit showed results above the sl in all 9 cases.

Conclusion:

- The Emit[®] II Plus tests designed for urine can be used for the analysis of serum samples as well, if the assays are calibrated at much lower concentrations than those recommended by the manufacturer for urine samples.
- The cut-off values can be markedly reduced because serum samples showed very low background.

- The tests are sensitive enough to screen for cannabinoids, opiates, and cocaine at concentrations which satisfy the demands of § 24a of German street traffic law (recommended cut-off values: 1 ng/mL THC, 10 ng/mL morphine, 75 ng/mL benzoylecgonine) without requiring prior sample preparation.
- The assays for amphetamine and ecstasy proved to be less sensitive. Changing assay parameters led to a slight improvement. To detect concentrations in the range which is recommended for § 24a of German street traffic law (25 ng/mL), serum samples have to be extracted prior to analysis under standard conditions.