

SDS-PAGE applied to EPO detection

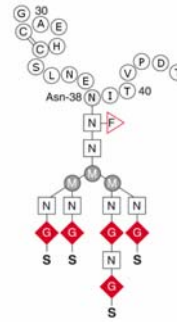
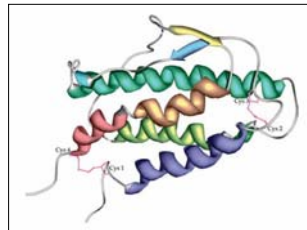
C. Reichel

Overview

- **SDS-PAGE method**
- **Detection of EPO-doping by SDS-PAGE (case studies)**
- **Implementation, advantages**

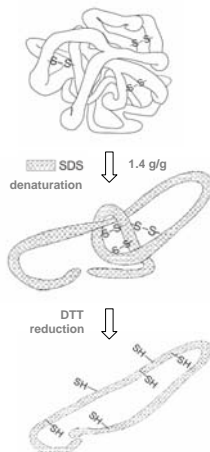
Electrophoresis of Erythropoietin

- Glycoprotein (165 AA, ca. 40% glycans (3N, 1O), ca. 30.4 kDa)
 - many *glycoforms* (cell and tissue specific, process specific)
 - charge differences (IEF-PAGE)
 - mass differences (SDS-PAGE)

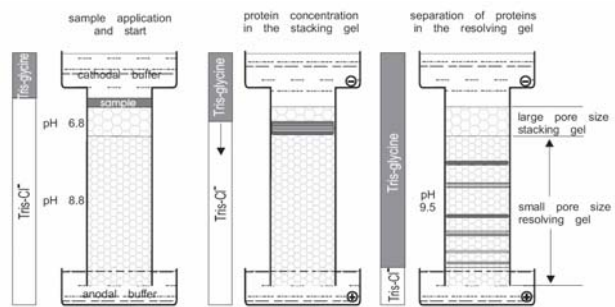


F. Lottspeich, J.W. Engels (Elsevier, 2006)

SDS-PAGE



R. Westemeier (Wiley, 2004)



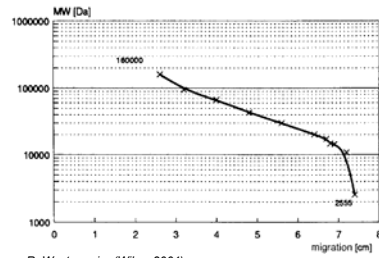
R. Westemeier (Wiley, 2004)

Stacking gel (e.g. 5% T): isotachopheresis → same velocity but different ion mobilities

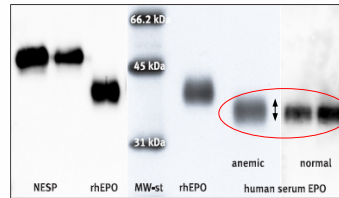
Separating gel (e.g. 10-12% T): zone electrophoresis → different relative migration distances

SDS-PAGE

- Separation according to differences in **molecular mass**



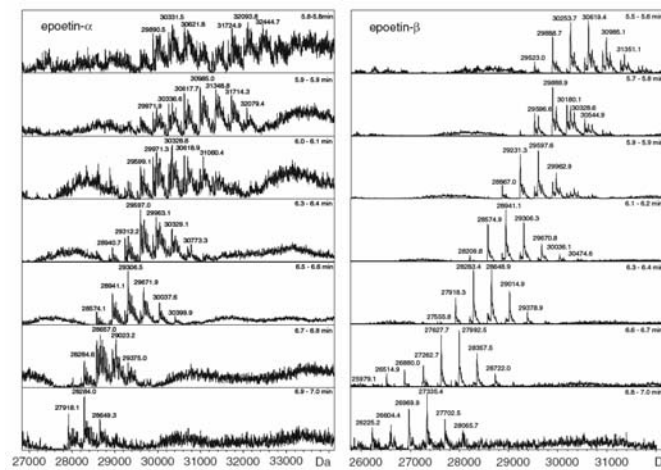
R. Westermeyer (Wiley, 2004)



V. Skibell et al. (DYNALogue 2003, 1, 13-15)

- EPO: mass differences between uhEPO/shEPO and rhEPOs
- Lower sensitivity for MIRCERA than for other epoetins

Band broadness reflects glycoform distribution

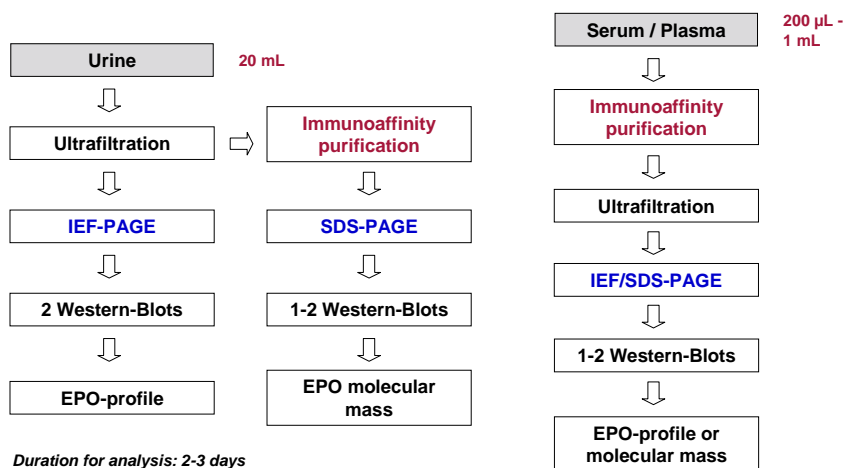


E. Balaguer, C. Neusüß (Chromatographia 2006, 64, 351-357)

Comparison between SDS-PAGE and IEF-PAGE of EPO

Criterion	SDS-PAGE	IEF-PAGE
▪ PAG	e.g. 10-12% T (precast)	e.g. 5% T (homemade)
▪ Separation	mass	pI
▪ Denaturing	SDS (0.1%) or SAR (0.1%)	Urea (7 M)
▪ Reducing	e.g. DTT	no
▪ Western blot	1-2	2
▪ Bands	1 (2)	e.g. 14-15
▪ Matrix	Urine, serum	Urine (serum: MIRCERA)
▪ Evaluation	TD2009EPO	TD2009EPO

Matrices for EPO-analyses

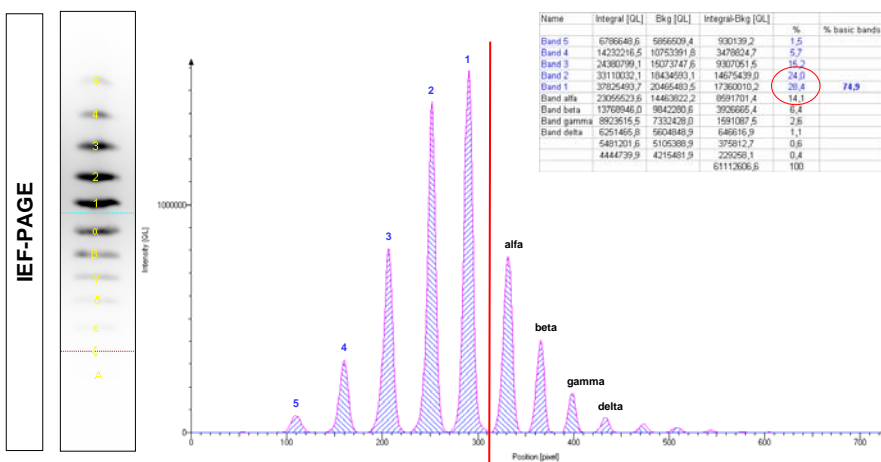


Detection of EPO-doping by SDS-PAGE

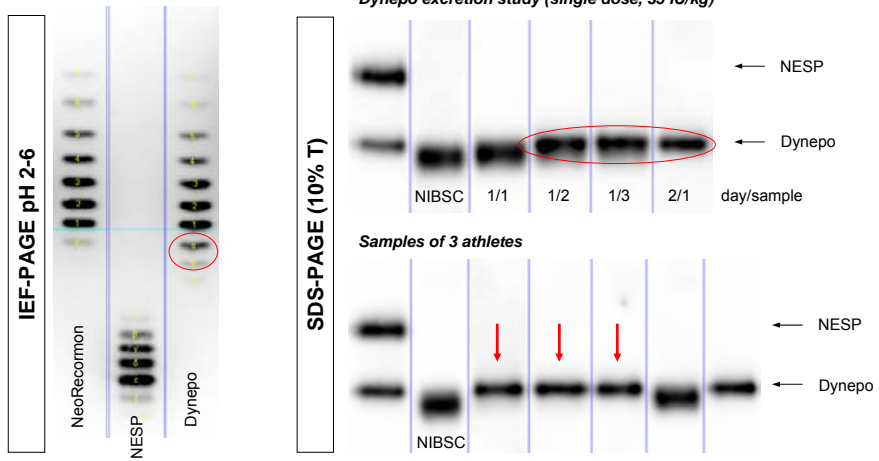
Case Studies

1. Dynepo

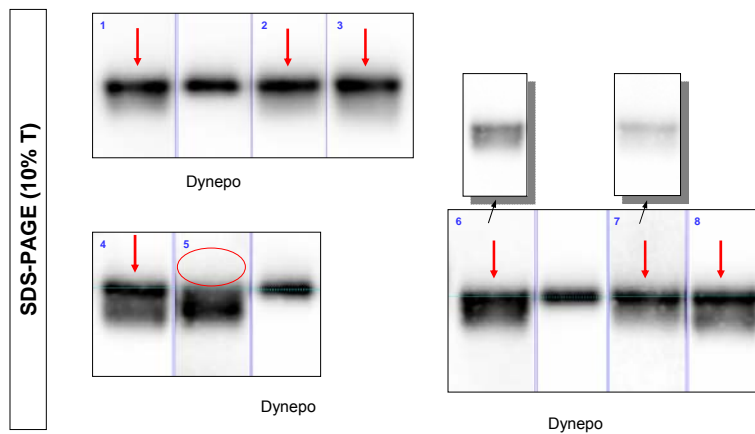
Dynepo-positive case on IEF-PAGE



Dynepo-positive cases on SDS-PAGE



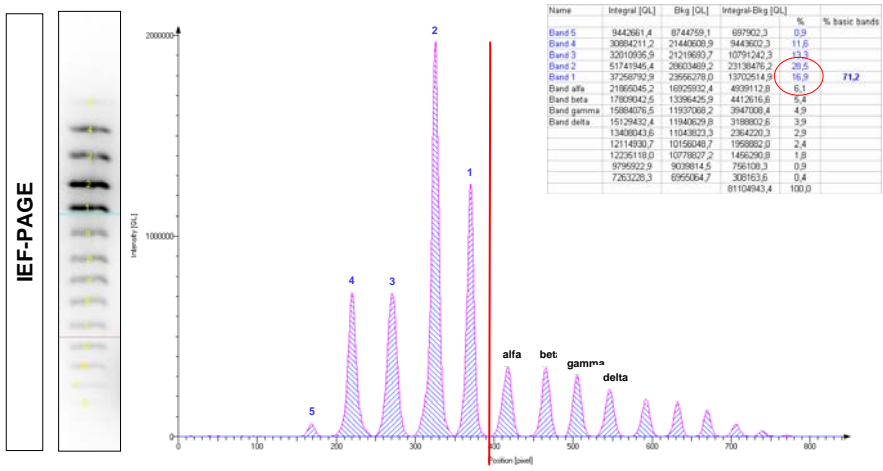
Dynepo-positive cases on SDS-PAGE



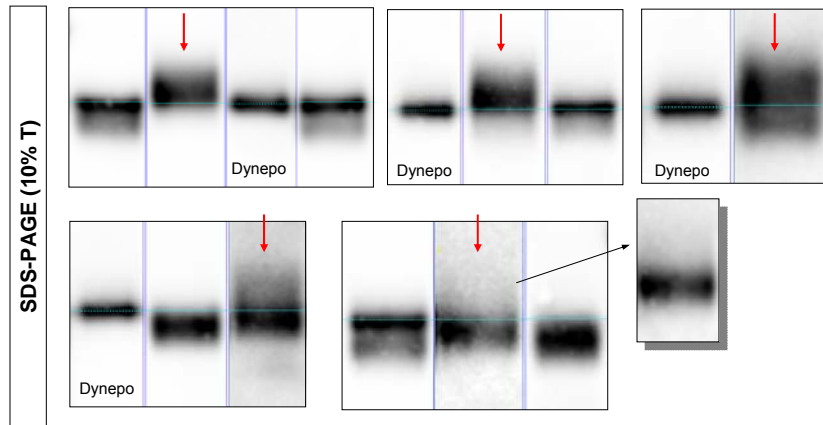
Case Studies

2. Epoetin alfa/beta

Epoetin alfa/beta-positive on IEF-PAGE



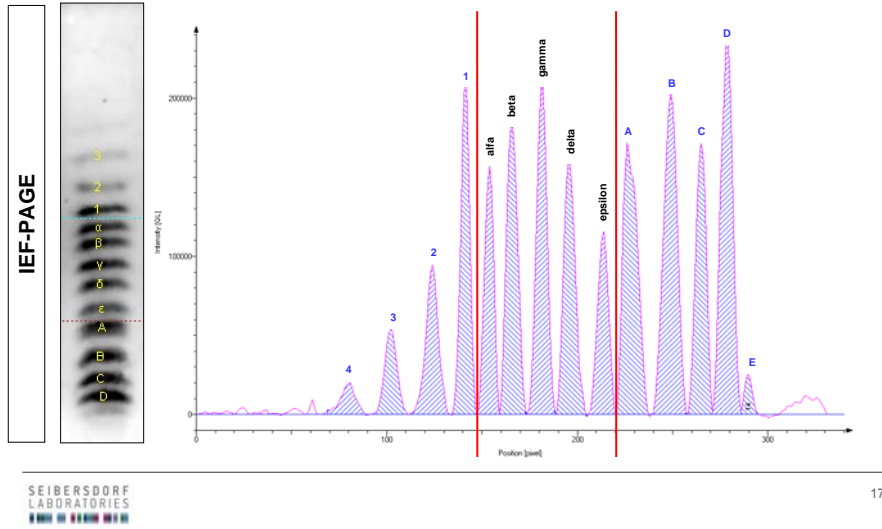
Epoetin alfa/beta-positive cases on SDS-PAGE



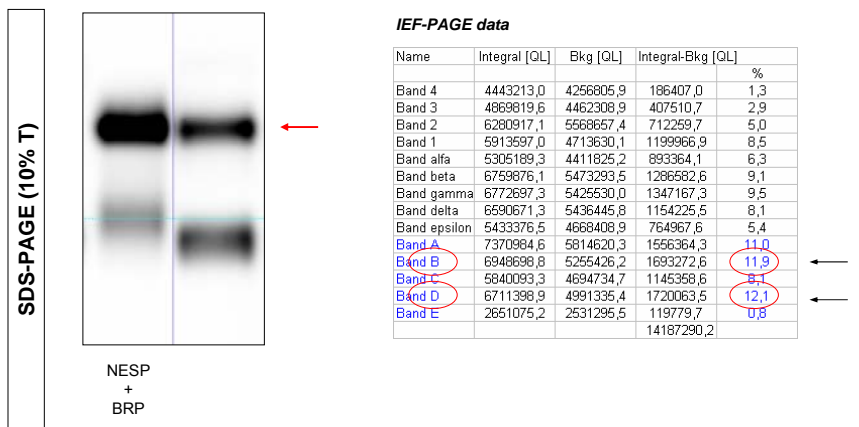
Case Studies

3. Darbepoetin alfa (NESP)

NESP-positive case on IEF-PAGE



NESP-positive case on SDS-PAGE



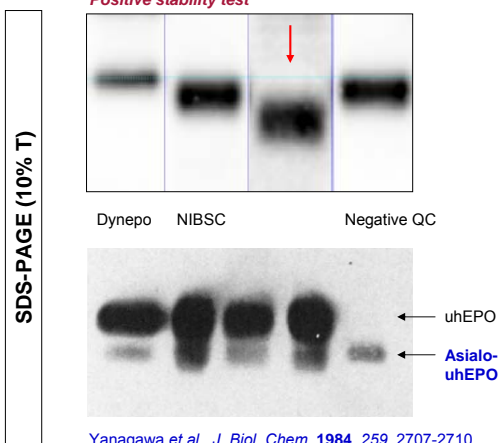
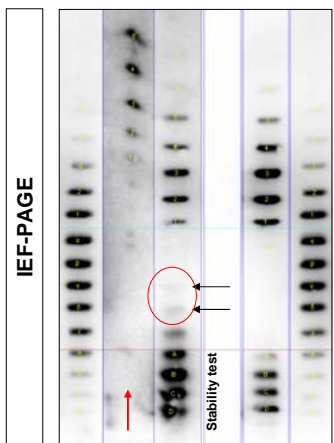
IEF-PAGE data

Name	Integral [QL]	Bkg [QL]	Integral-Bkg [QL]	%
Band 4	4443213,0	4256805,9	186407,0	1,3
Band 3	4869819,6	4462308,9	407510,7	2,9
Band 2	6280917,1	5568867,4	712259,7	5,0
Band 1	5913597,0	4713630,1	1199966,9	8,5
Band alfa	5305189,3	4411825,2	893364,1	6,3
Band beta	6759876,1	5473293,5	1286582,6	9,1
Band gamma	6772697,3	5425530,0	1347167,3	9,5
Band delta	6590671,3	5436445,8	1154225,5	8,1
Band epsilon	5433376,5	4668408,9	764967,6	5,4
Band A	7370984,6	5814620,3	1556364,3	11,0
Band B	6948698,8	5255426,2	1693272,6	11,9
Band C	5840093,3	4694734,7	1145358,6	8,1
Band D	6711398,9	4991335,4	1720063,5	12,1
Band E	2651075,2	2531295,5	119779,7	0,8
			14187290,2	

Case Studies

4. Active Urine
 (“Active AND Negative”)

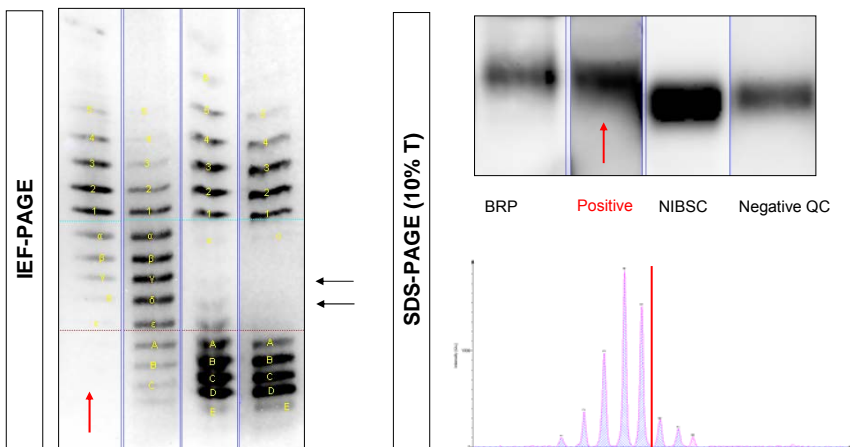
Active Urine: IEF-PAGE and SDS-PAGE



Yanagawa et al., J. Biol. Chem. 1984, 259, 2707-2710.

Case Studies
5. Active Urine
(“Active BUT Positive”)

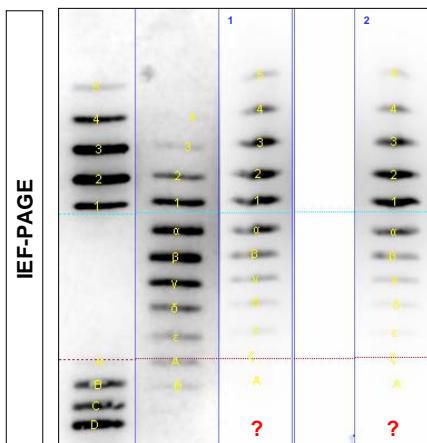
Active Urine: IEF-PAGE and SDS-PAGE



Case Studies

6. Effort Urine

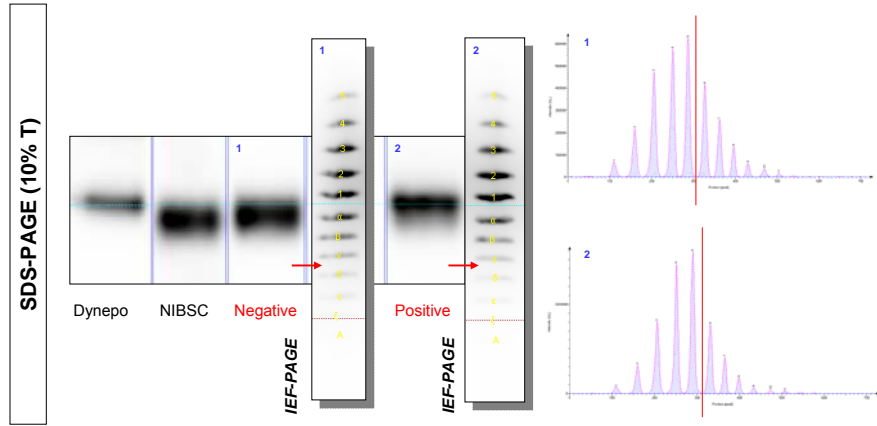
Effort Urine: IEF-PAGE



Name	Integral [OL]	Bkg [OL]	Integral-Bkg [OL]	Integral-Bkg [%]	%	% basic bands
Band 5	8189730,67	7326013,98	863716,6956		2,6	
Band 4	12018849	9420074,44	2598774,541		7,9	
Band 3	18416936	12662446,4	5754489,697		17,4	
Band 2	19028926,7	12354100	6674746,705		20,2	
Band 1	19272633,7	12215662,6	7056971,088		21,4	69,5
Band alpha	15162385,2	10388144,7	4774240,514		14,5	
Band beta	10095452,1	8247433	2730029,078		9,3	
Band gamma	8354586,93	7003619,51	1350967,425		4,1	
Band delta	7064440,17	6420272,41	644167,7534		1,9	
Band epsilon	5747733,51	5332592,82	415140,6868		1,3	
	5196988,01	5031300,38	165687,6331		0,5	
			33036931,71		100	

Name	Integral [OL]	Bkg [OL]	Integral-Bkg [OL]	Integral-Bkg [%]	%	% basic bands
Band 5	7898984,4	6964811,9	934072,5		1,5	
Band 4	14489700,0	11807875,3	3478824,7		5,7	
Band 3	25221872,1	15912549,9	9309322,2		15,2	
Band 2	34376216,3	19700777,3	14675439,0		24,0	
Band 1	37276390,0	19916299,4	17360090,6		28,4	74,9
Band alpha	23354015,6	14761173,7	8592841,9		14,1	
Band beta	13946351,3	10014021,3	3931530,1		6,4	
Band gamma	8630471,0	7039383,5	1591087,5		2,6	
Band delta	6251485,0	6204840,9	466616,9		1,1	
Band epsilon	5481201,6	5105388,9	375812,7		0,6	
	5165337,1	4932444,4	232892,7		0,4	
			61128530,7		100,0	

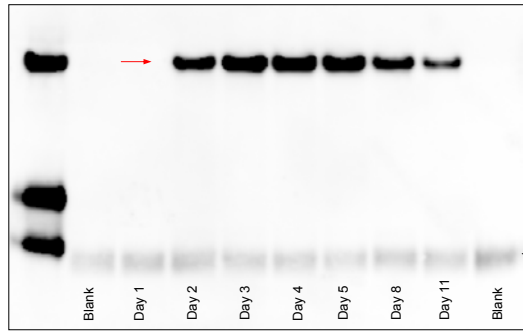
Effort Urine: SDS-PAGE



Case Studies

7. Detection of EPO in Blood

Detection of MIRCERA in Serum



200 µL serum

← MIRCERA

Study design:

- Single dose injection (sc)
(50 µg, i.e. 0.67 µg/kg)
- Collection of blood for 11 days

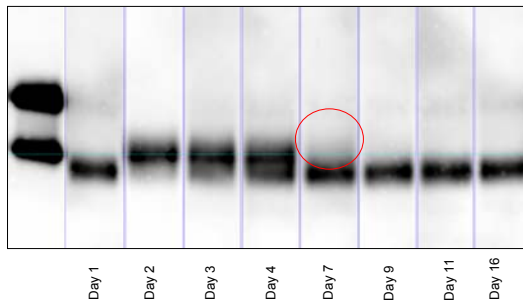
← NESP

← Dynepo

← shEpo

shEpo lanes individually contrast optimized

Detection of EPO alfa/beta in Serum



200 µL serum

Study design:

- Single dose injection (sc)
(NeoRecormon, 5000 IU,
i.e. 66 IU/kg)
- Collection of blood for 16 days

← NESP

← Dynepo

← shEpo

Conclusions SDS-PAGE method

- Suitable for blood and urine
- Not prone to „active“ and „effort urines“
- Femtogram sensitivity (e.g. 10 amol)
- Higher sensitivity for Dynepo
- Criteria of positivity simpler: evaluation of 1 band (qualitative criteria or relative mobility values)
- „1 Matrix – 1 Method“ approach (serum → MIRCERA)
- **200 µL sample volume** (serum)

