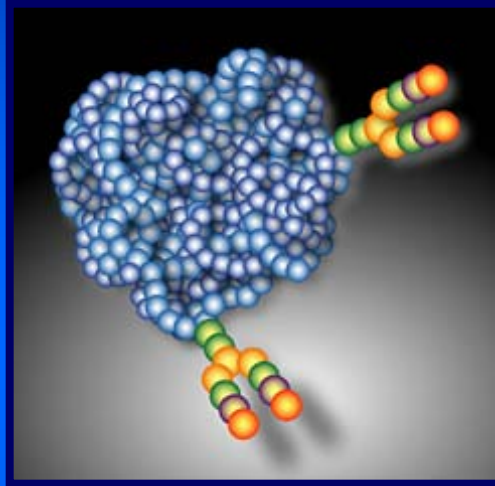


# MAIA method for EPO detection



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**Stockholm Doping Control Laboratory**

**MAIA Diagnostics**

**Sweden**

# MAIA technology platform

## Membrane Assisted Isoform ImmunoAssay

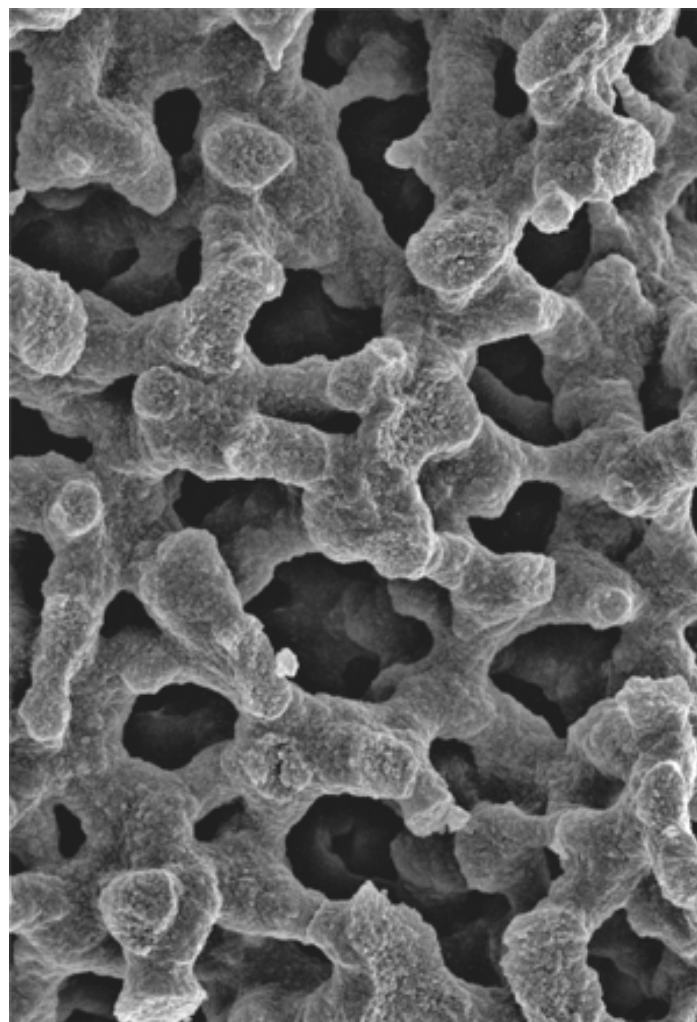
### ■ MAIA

- A new, unique test technology for determination of protein isoforms
- Applications in biological, clinical research and IVD
- A lateral flow based miniaturized chromatography with on line immunoassay detection
- Very sensitive, highly specific, quantitative, rapid, easy to use and versatile
- Suitable for research, large scale laboratory use and POCT

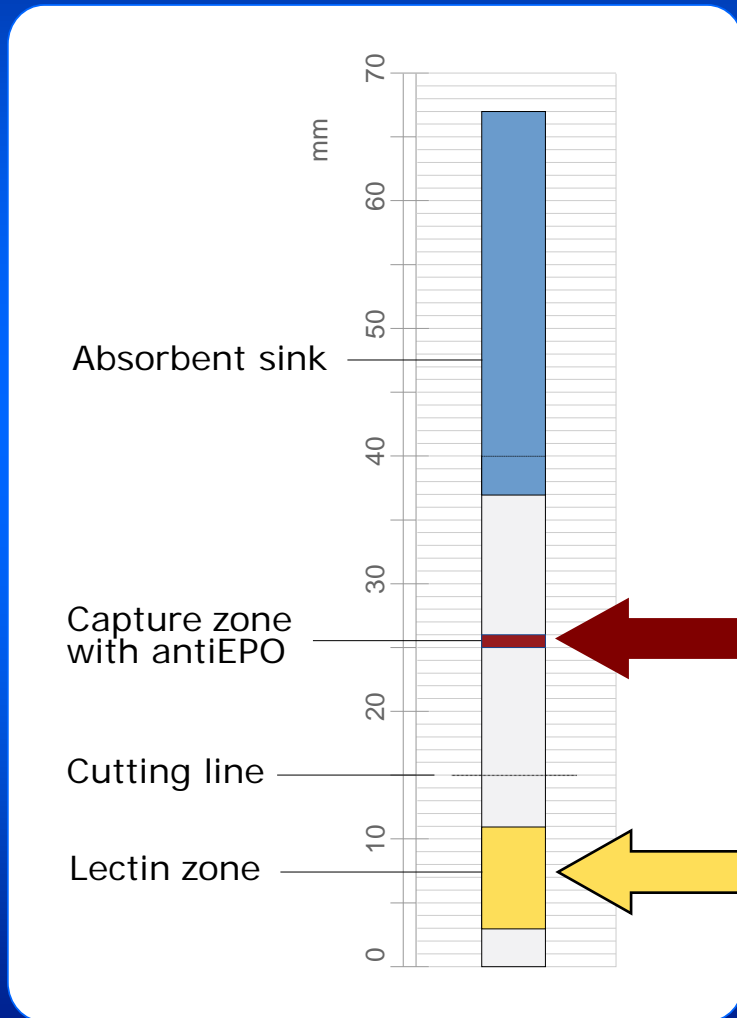
### ■ EPO

- A challenging model analyte
- Low concentration
- Complex isoform pattern
- Widely used pharmaceutical drug
- Illicitly used in sports

# Sample pretreatment with disposable anti-EPO monoliths, ~65% EPO recovery from urine and plasma



# The EPO MAIIA microcolumn - the key reagent in the test-kit



EPO **WGA** MAIIA  
for epoetins

EPO **AbQ** MAIIA  
for EPO analogues

*Capturing and detection zone*

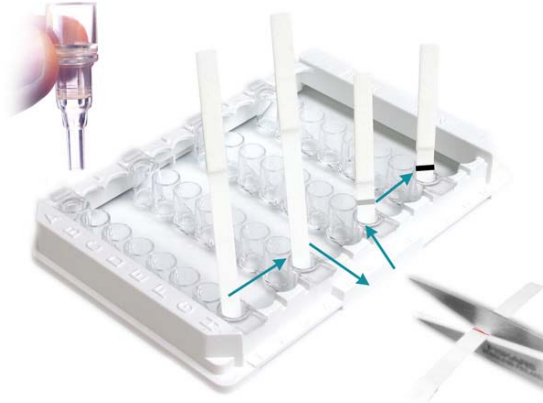
- Anti-EPO AFFINITY
- AbQ MAIIA

*Separation zone*

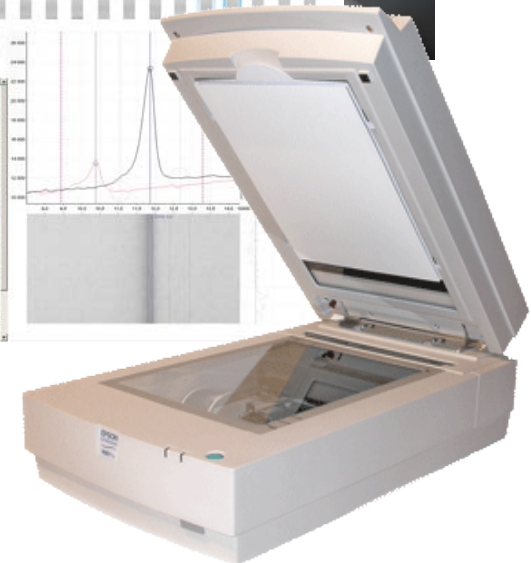
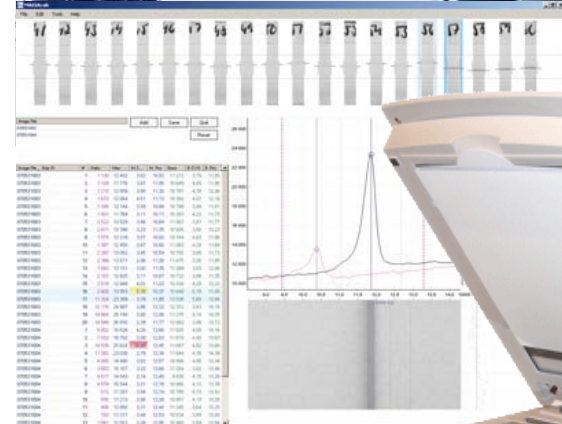
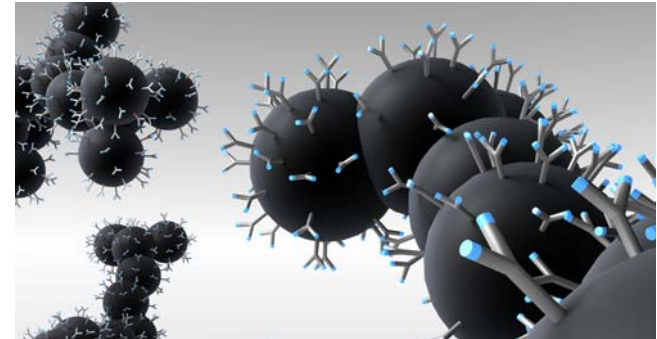
- LECTIN AFFINITY
- WGA MAIIA

# EPO WGA MAIIA test in < 1 hour

## Procedure



## Detection system

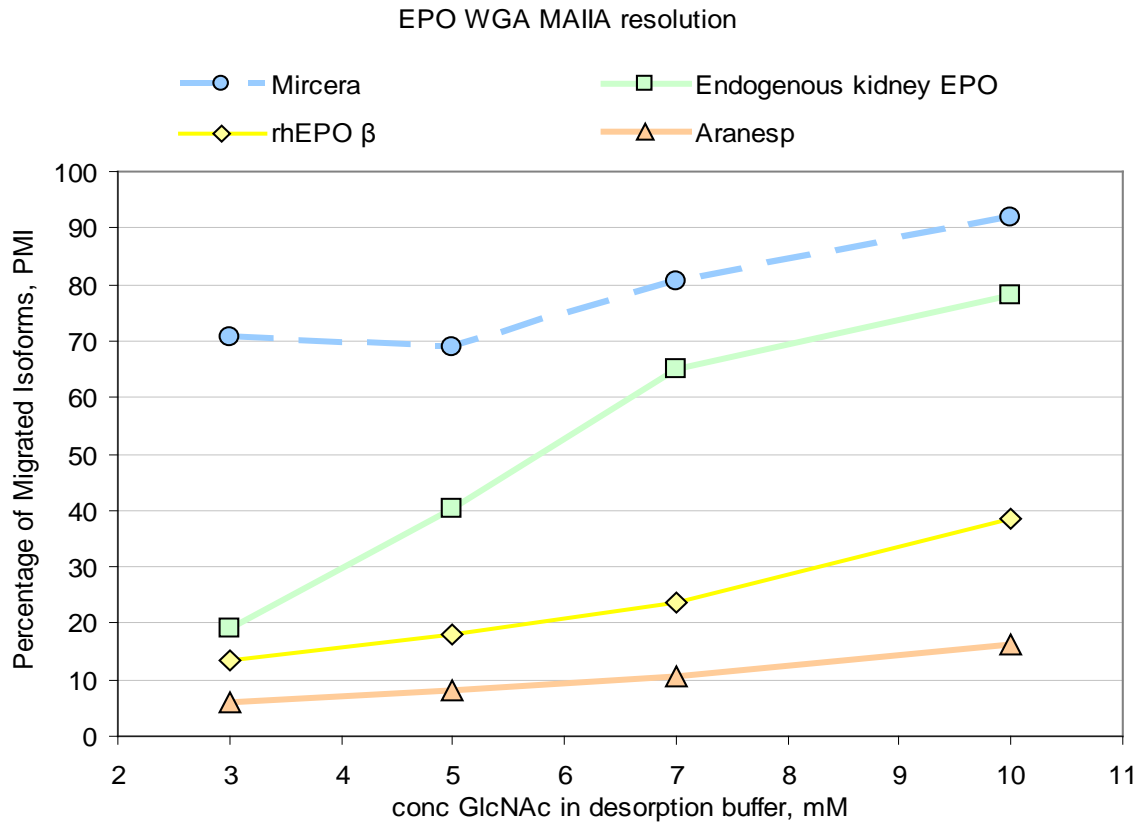


## Calculation - Percentage Migrated Isoforms, PMI

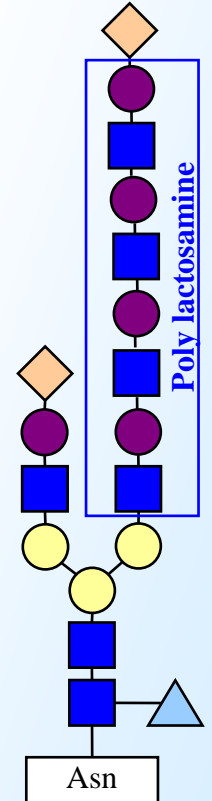
Two different WGA desorption buffers				Desorption buffer GlcNAc, Low		Desorption buffer GlcNAc, High		PMI	Result
		MAIIA low ng/L	MAIIA high ng/L						
Sample 1	Replicate 1	30,2	44,3					= 68,1	: Endo EPO
	Replicate 2	29,8	43,7						
	Average	30,0	44,0						
	CV%	0,98	0,93						
Sample 2	Replicate 1	42,4	179,0					= 25,0	: rhEPO
	Replicate 2	46,7	177,8						
	Average	44,5	178,4						
	CV%	6,89	0,48						

# EPO WGA MAIIA

## chromatographic resolution in the WGA zone



- △ Fucose (Fuc)
- Galactose (Gal)
- Mannose (Man)
- N-acetylglucosamine (GlcNAc)
- ◇ Sialic Acid (Sia)



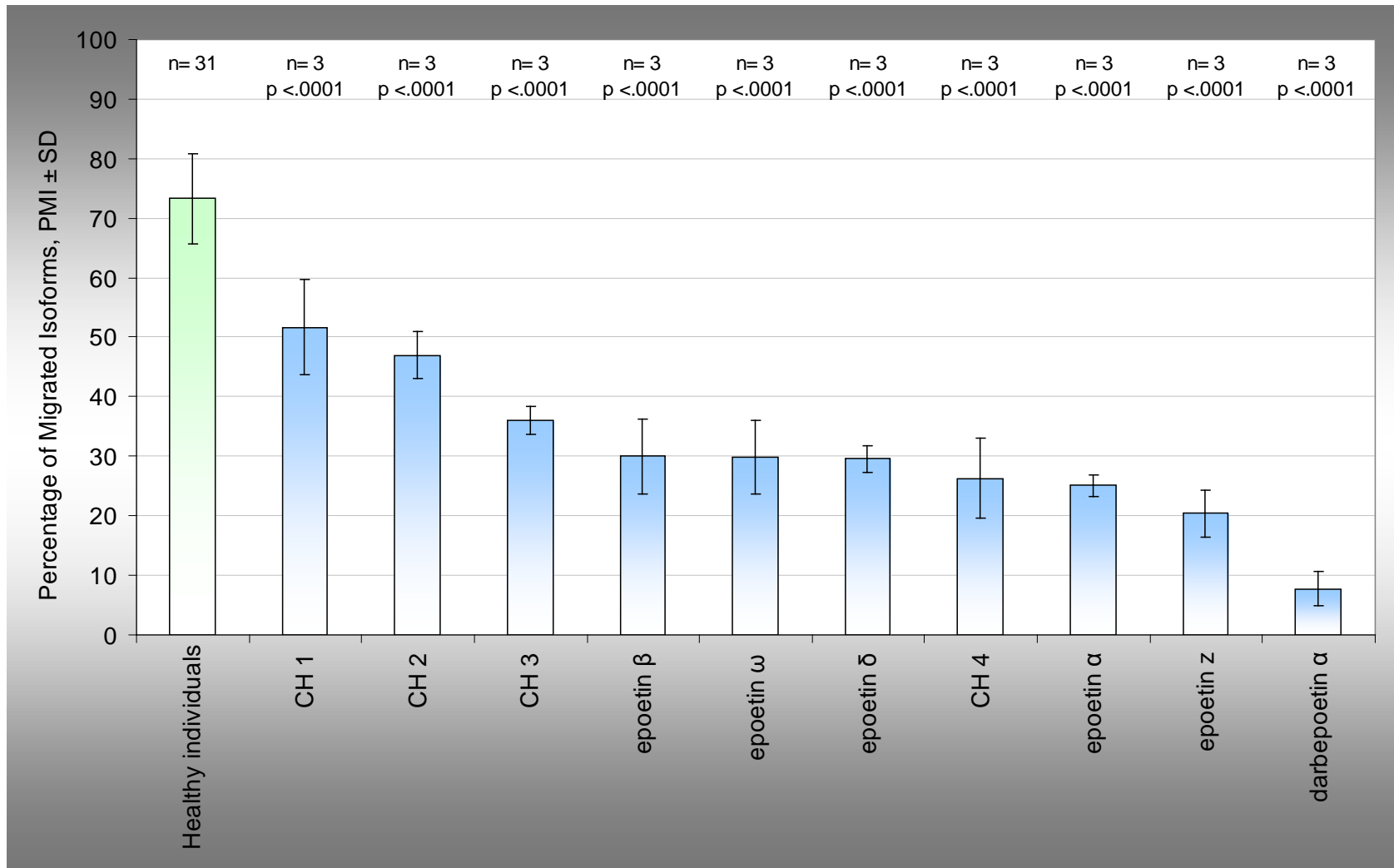
# EPO WGA MAIA results

## test performance

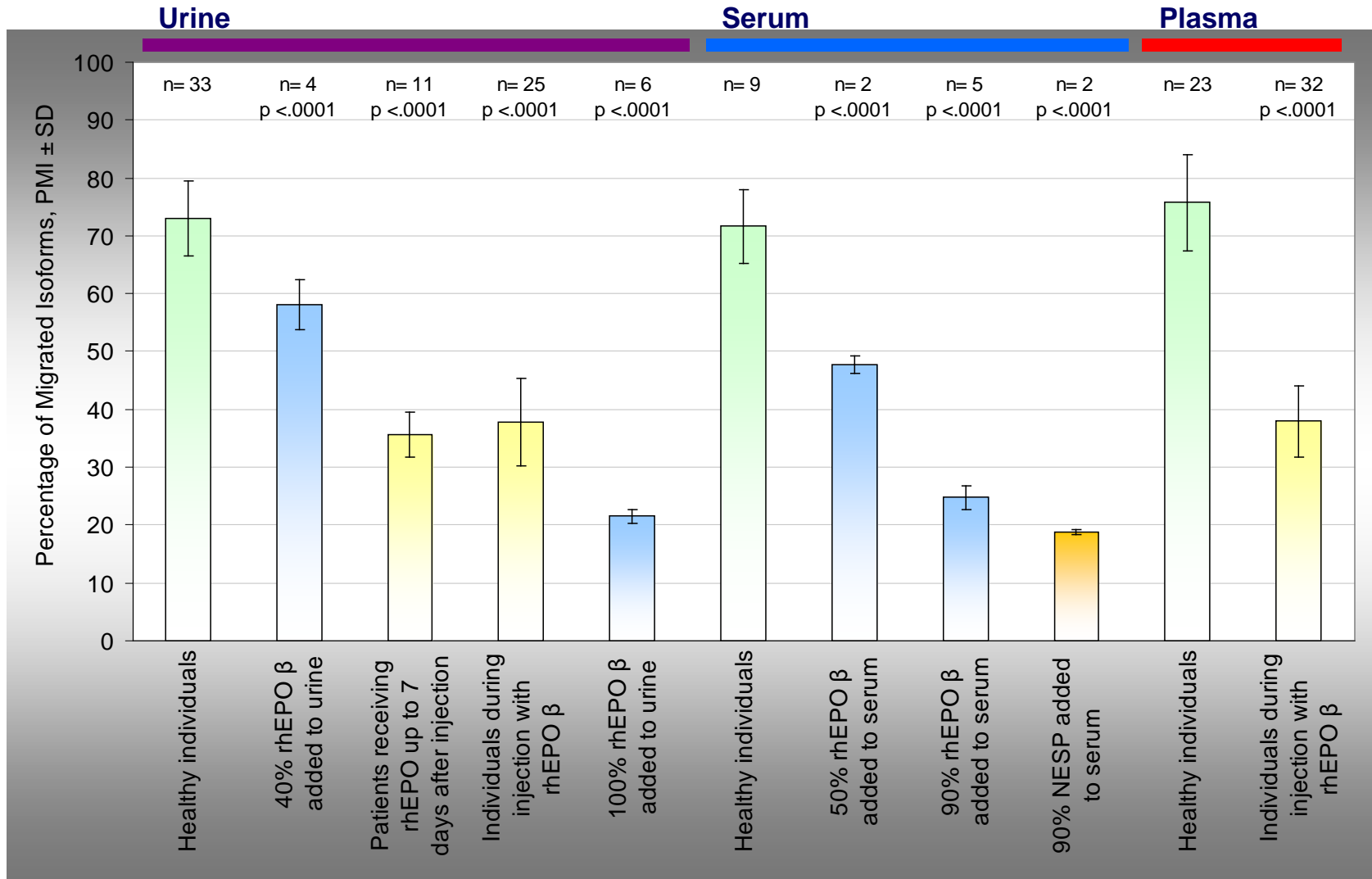
- Intra-assay imprecision for immunoassay (ng/L)
  - CV = 4.9 %, n = 442 duplicates
- Intra-assay imprecision for the MAIA value (PMI)
  - CV = 6.3 %, n = 157
- **Inter**-assay imprecision for the MAIA value (PMI)
  - CV = 7.0 %, 15 different test occasions (mean of 3 controls)
  
- Amount required in biol. sample for quantification of PMI:
  - **> 2 pg EPO**

# EPO WGA MAIA results

## different epoetins and analogues

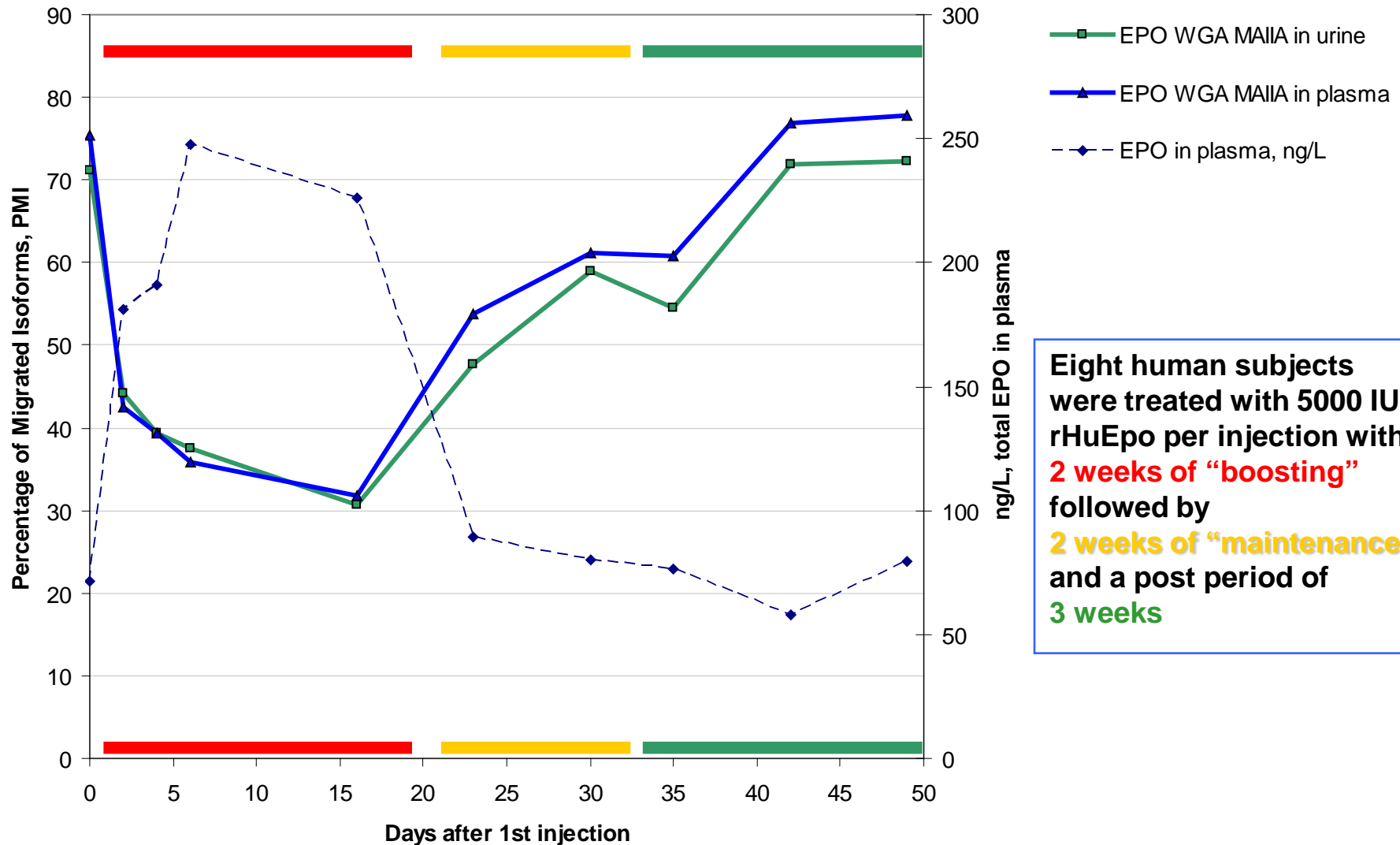


# EPO WGA MAIA results for human urine, serum and plasma



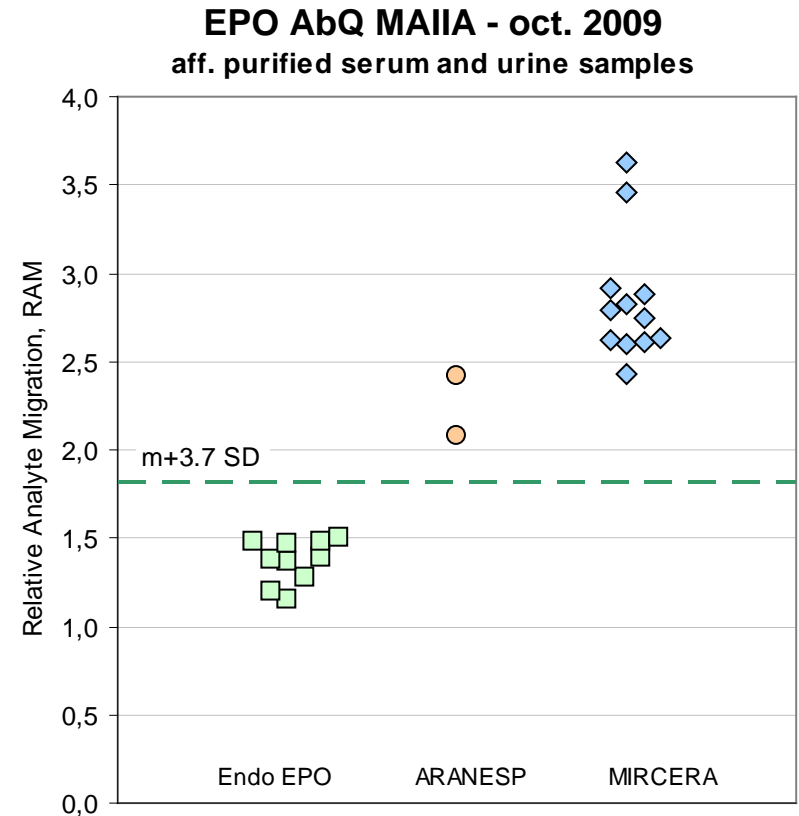
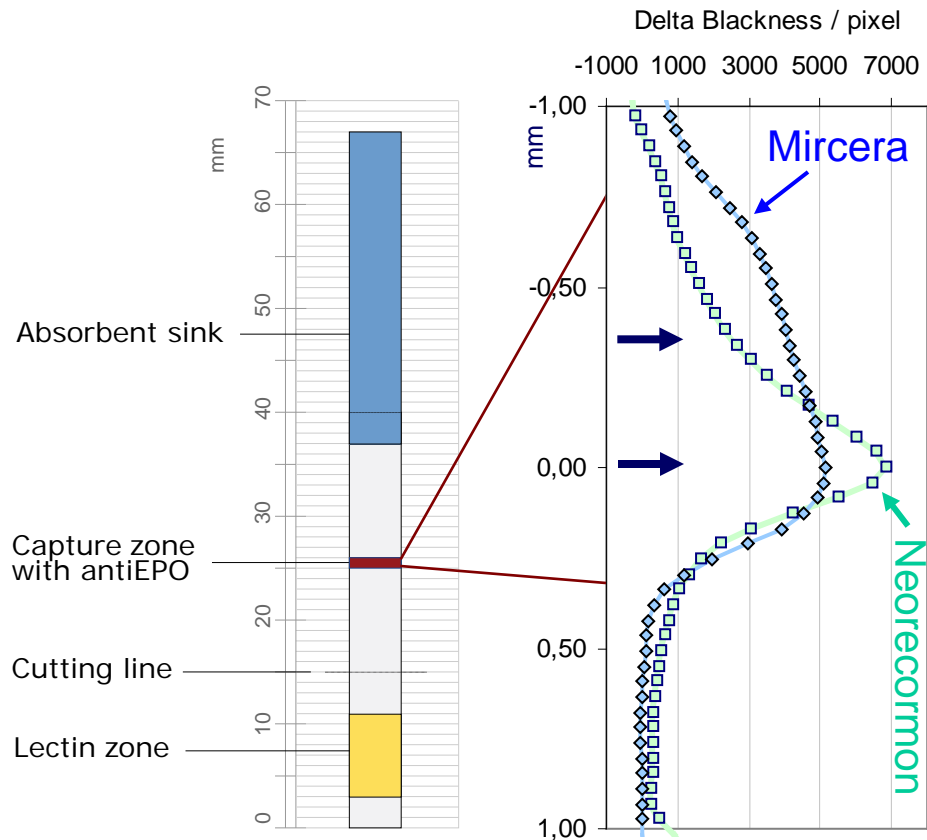
# Injection of Neorecormon / Carsten Lundby 2007

## EPO WGA MAIA in plasma and urine, total EPO in plasma



Eight human subjects were treated with 5000 IU rHuEpo per injection with 2 weeks of "boosting" followed by 2 weeks of "maintenance" and a post period of 3 weeks

# EPO AbQ MAIIA – for EPO analogues utilizing antibody affinity chromatography



Required amount of Mircera in biological sample: > 10 pg

Imprecision for RAM duplicate, CV = 6.1% (n=40)

# MAIIA compared to other methods

Both obtained from one MAIIA

## EPO, epoetins and analogues

		EPO WGA MAIIA <sup>(1)</sup>	EPO AbQ MAIIA	IEF- PAGE	SDS- PAGE	ELISA for Mircera
		PMI	RAM	band- position	rel. mobility	ng M/L
hEPO	Renal endogenous EPO in urine	75	1	endo	0,536	
hEPO	Renal endogenous EPO in serum/plasma	75	1	$\alpha$ , 1 - 3	neg	<100
hEPO	Liver endogenous EPO	~ 90	1	4 - 6 <sup>(2)</sup>	neg <sup>(2)</sup>	
hEPO	Tumour EPO					
rhEPO	CH 1	51,7	1	4 - 5		neg
rhEPO	CH 2	46,9	1	5 - 7		neg
rhEPO	CH 3	36,0	1	5 - 7		neg
rhEPO	Epoetin beta, Neorecormon	30,0	1	2 - 3	0,628	neg
rhEPO	Epoetin omega, Epomax, (SDS: Repotin)	29,8	1	4 - 5	neg	neg
rhEPO	Epoetin delta, DynEPO	29,6	1	1 - 2	0,605	neg
rhEPO	CH 4	26,3	1	2 - 3		neg
rhEPO	Epoetin alpha, Eprex, (SDS: Erypro)	25,1	1	2 - 3	0,655	neg
analogue	Darbepoetin alpha, Aranesp	7,7	1,5	acidic	> 0,66	neg
analogue	Mircera	~ 90	3	4 - 6 <sup>(4)</sup>	>> 0,66 <sup>(3)</sup>	> 100
<b>QUANTIFICATION limit</b> , req. amount of EPO in biol. sample, <b>pg</b>		2		60	?	
<b>RESOLUTION</b> , Proportion of rhEPO beta/ endogenous EPO, %		40		75	?	n.app.
EPO beta detectable up to x days		~ 7		~ 5		
Testing time		1h	1h	2-3 days	2-3 days	1 day
Hands-on-time per sample		Medium		HIGH	HIGH	Low
Recommended type of biological sample		S and U	S and U	U (M-S)	S and U	S

(1) opt. res. for rhEPO

(2) cord sera (3) sarcosyl-PAGE

(4) spec pattern

# The advantages of the EPO WGA MAIIA test - practical aspects

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- All necessary reagents will be available in the kit
- The test is rapid, easy-to-use, can be implemented at every laboratory and no expensive equipment is needed
- Exactly the same method and quality controlled reagents can be used world wide
- Single sample as well as large numbers of samples can easily be tested without wasting excess reagents

# The challenge to reduce EPO doping

- Analyze 20 times more samples for EPO doping !!
- Apply a cost-effective strategy for testing them !!
- Collect the samples at strategic time-points
- Reduce the number of "false negative" samples
  
- Make sure that every-one knows:
  - High risk to be tested for EPO doping
  - All types of EPO:s can be identified
  - Several methods exist
  - Rapid development of new methods

# Future research activities

- Creation of an EPO binding lectin library
- Enhanced resolution rhEPO / endogenous EPO
- “Selection” MAIIA and “Confirmation” MAIIA
- 2D MAIIA

