SSIEM 2014 Academy,
Paris, 1st - 2nd April 2014
Laboratory Scientist Course

Optimisation of Keratan Sulfate Separation by using Peltier System

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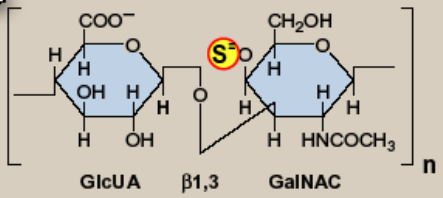
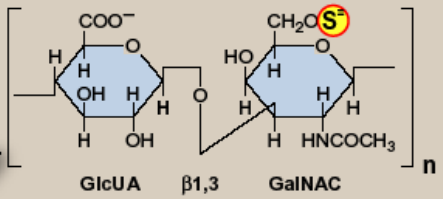
CHONDROITIN 4- AND 6-SULFATES

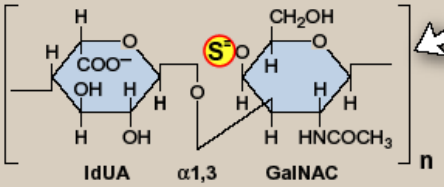
- Disaccharide unit: N-acetylgalactosamine with sulfate on either C-4 or C-6, and glucuronic acid.
- Most abundant GAG in the body.
- Found in cartilage, tendons, ligaments, and aorta.
- Form proteoglycan aggregates, often aggregating noncovalently with hyaluronic acid.
- In cartilage, they bind collagen and hold fibers in a tight, strong network. They are also found in aorta, tendons, and ligaments.





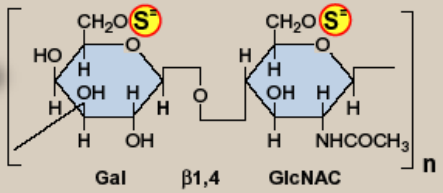
DERMATAN SULFATE

- Disaccharide unit: N-acetylgalactosamine and L-iduronic acid (with variable amounts of glucuronic acid).
- Found in skin, blood vessels, and heart valves.



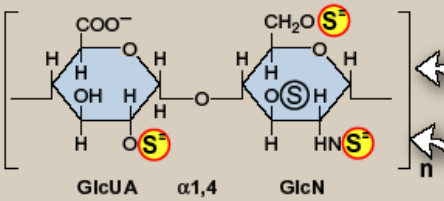
KERATAN SULFATES I and II

- Disaccharide unit: N-acetylglucosamine and galactose (no uronic acid). Sulfate content is variable and may be present on C-6 of either sugar.
- Most heterogeneous glycosaminoglycans because they contain additional monosaccharides such as L-fucose, N-acetylneuraminic acid, and mannose.
- KS II is found in loose connective tissue proteoglycan aggregates with chondroitin sulfate. KS I is found in cornea.



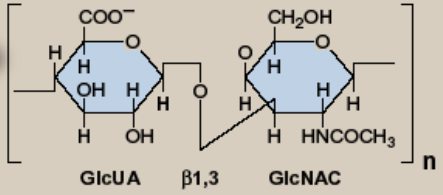
HEPARIN

- Disaccharide unit: Glucosamine and glucuronic or iduronic acid. Most glucosamine residues are bound in sulfamide linkages. Sulfate is also found on C-3 or C-6 of glucosamine and C-2 of uronic acid (an average of 2.5 S per disaccharide unit).
- Unlike other GAGs that are extracellular compounds, heparin is an intracellular component of mast cells that line arteries, especially in liver, lungs, and skin.
- Serves as an anticoagulant.



HYALURONIC ACID

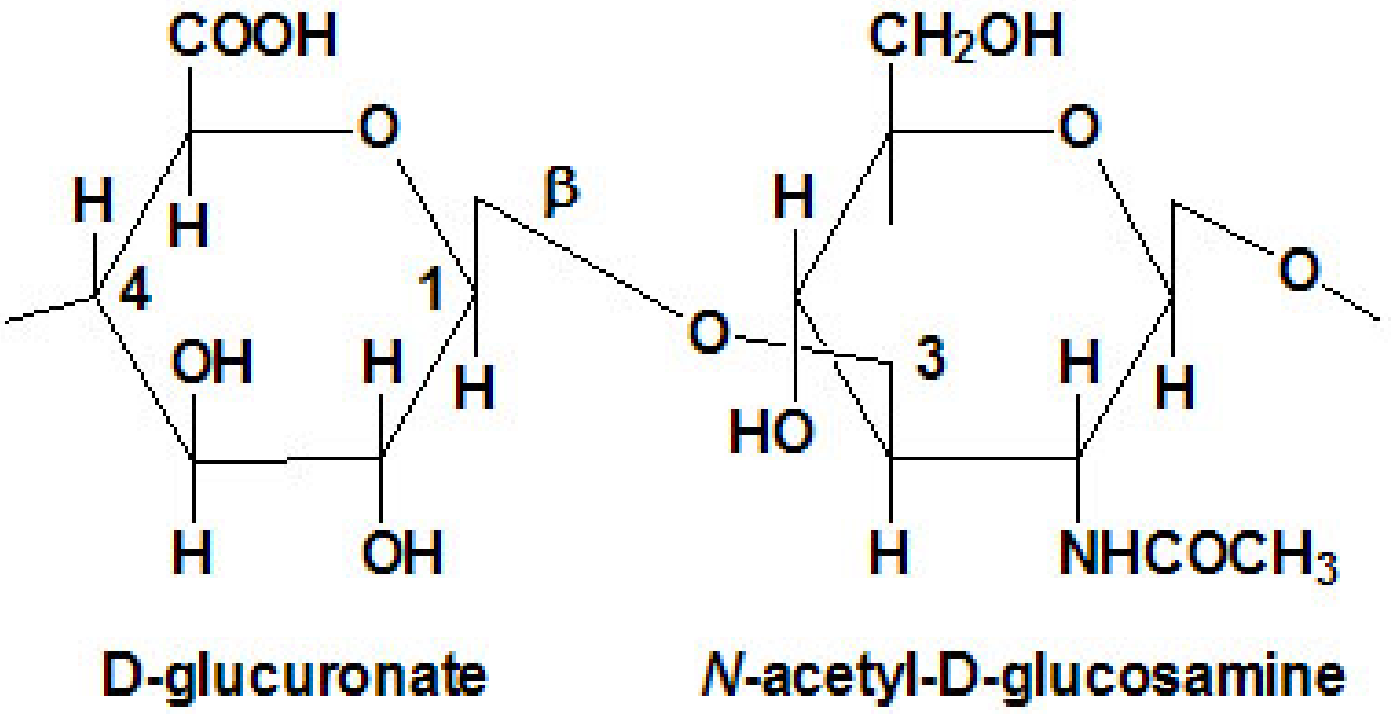
- Disaccharide unit: N-acetylglucosamine and glucuronic acid.
- Different from other GAGs: Unsulfated, not covalently attached to protein, and only GAG not limited to animal tissue, but also found in bacteria.
- Serves as a lubricant and shock absorber.
- Found in synovial fluid of joints, vitreous humor of the eye, the umbilical cord, loose connective tissue and cartilage.



HEPARAN SULFATE

- Disaccharide unit: Same as heparin except some glucosamines are acetylated and there are fewer sulfate groups.
- Extracellular GAG, found in basement membrane and as a ubiquitous component of cell surfaces.

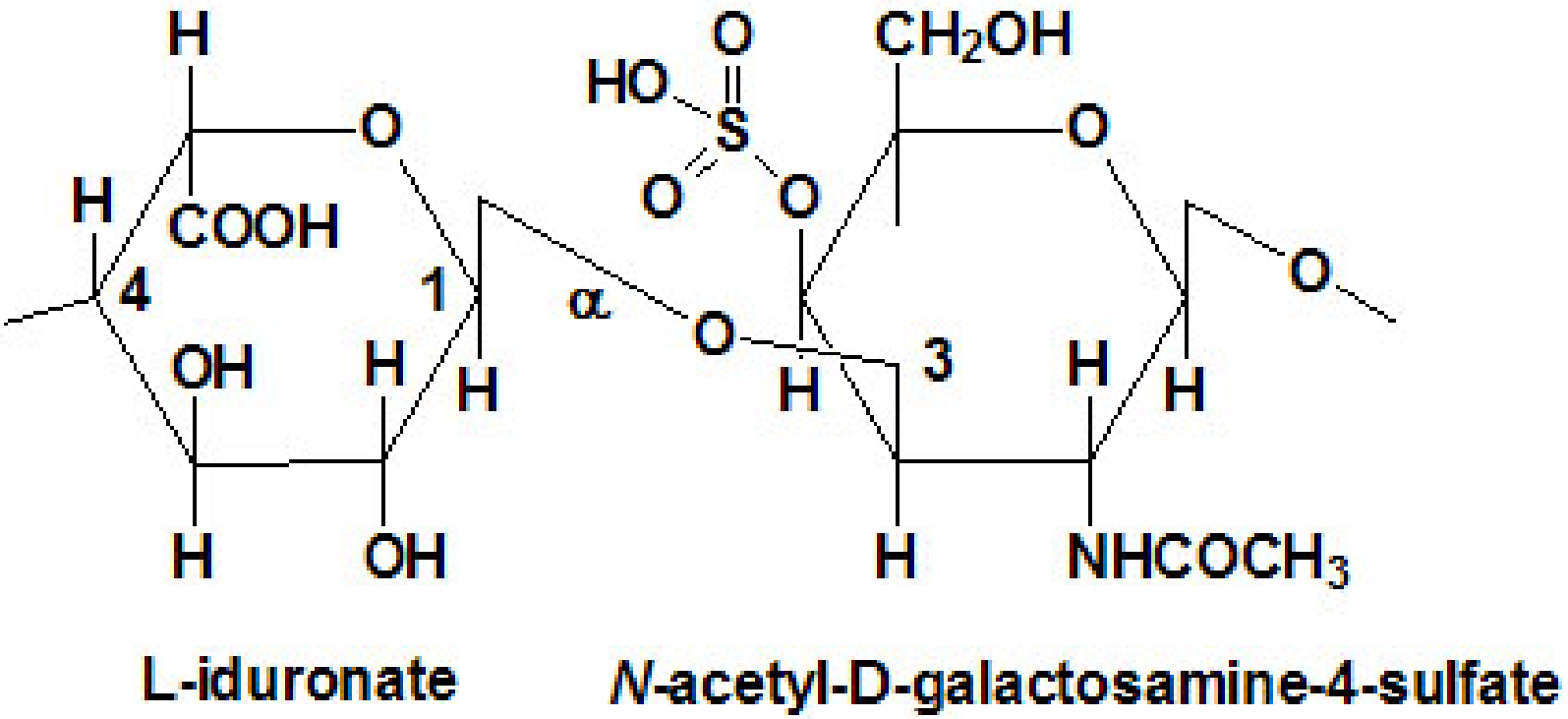
Hyaluronates:
composed of D-glucuronate + GlcNAc
linkage is β(1, 3)



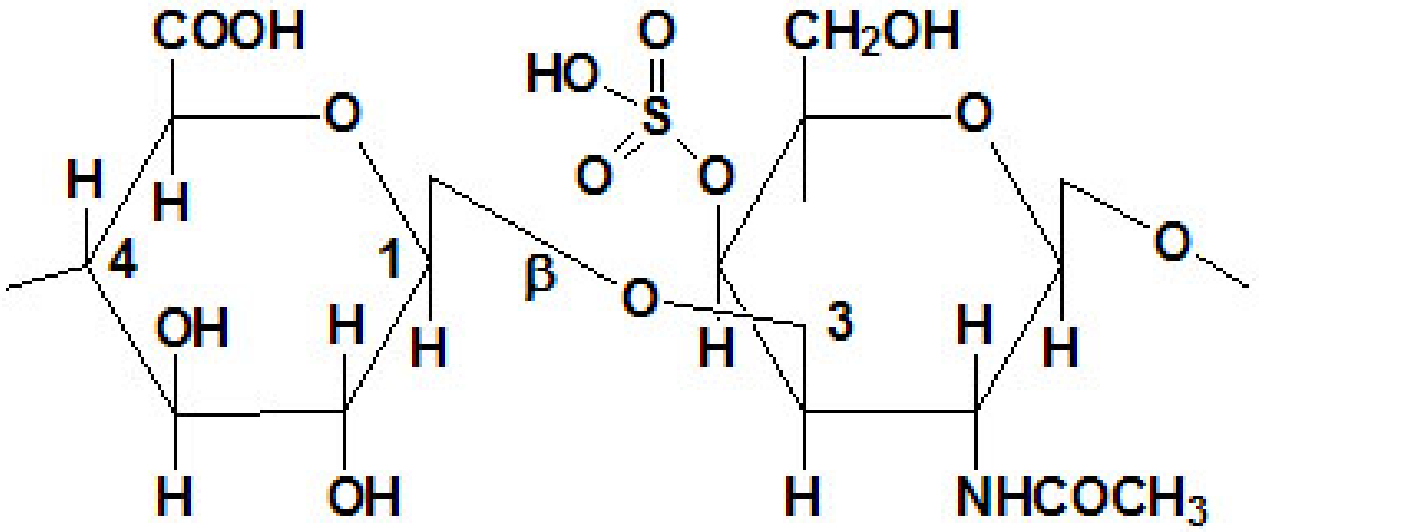
D-glucuronate

N-acetyl-D-glucosamine

Dermatan sulfates:
composed of L-iduronate (many are sulfated)
+ GalNAc-4-sulfate
linkages is α(1, 3)

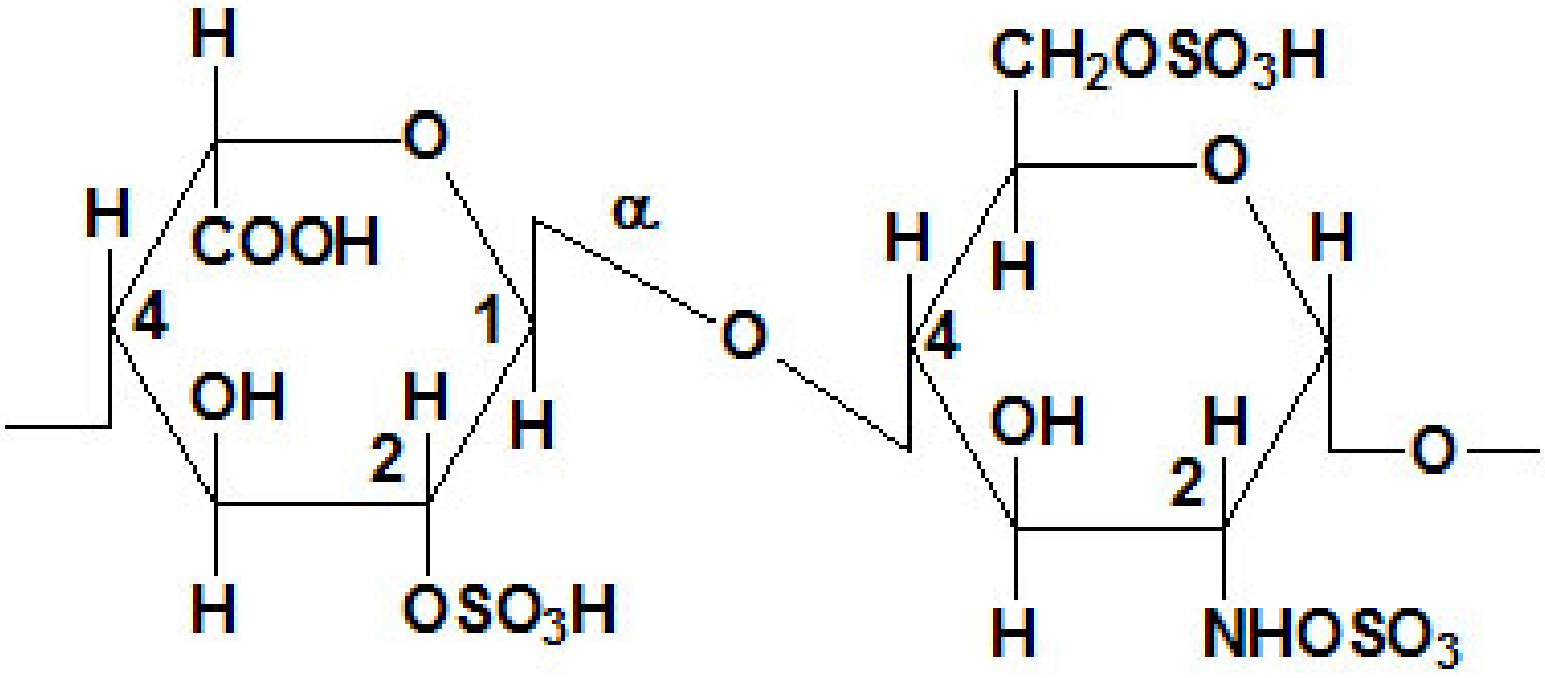


Chondroitin 4- and 6-sulfates :
composed of D-glucuronate
and GalNAc-4- or 6-sulfate
linkage is β(1, 3)
(the figure contains GalNAc 4-sulfate)



D-glucuronate *N*-acetyl-D-glalactosamine-4-sulfate

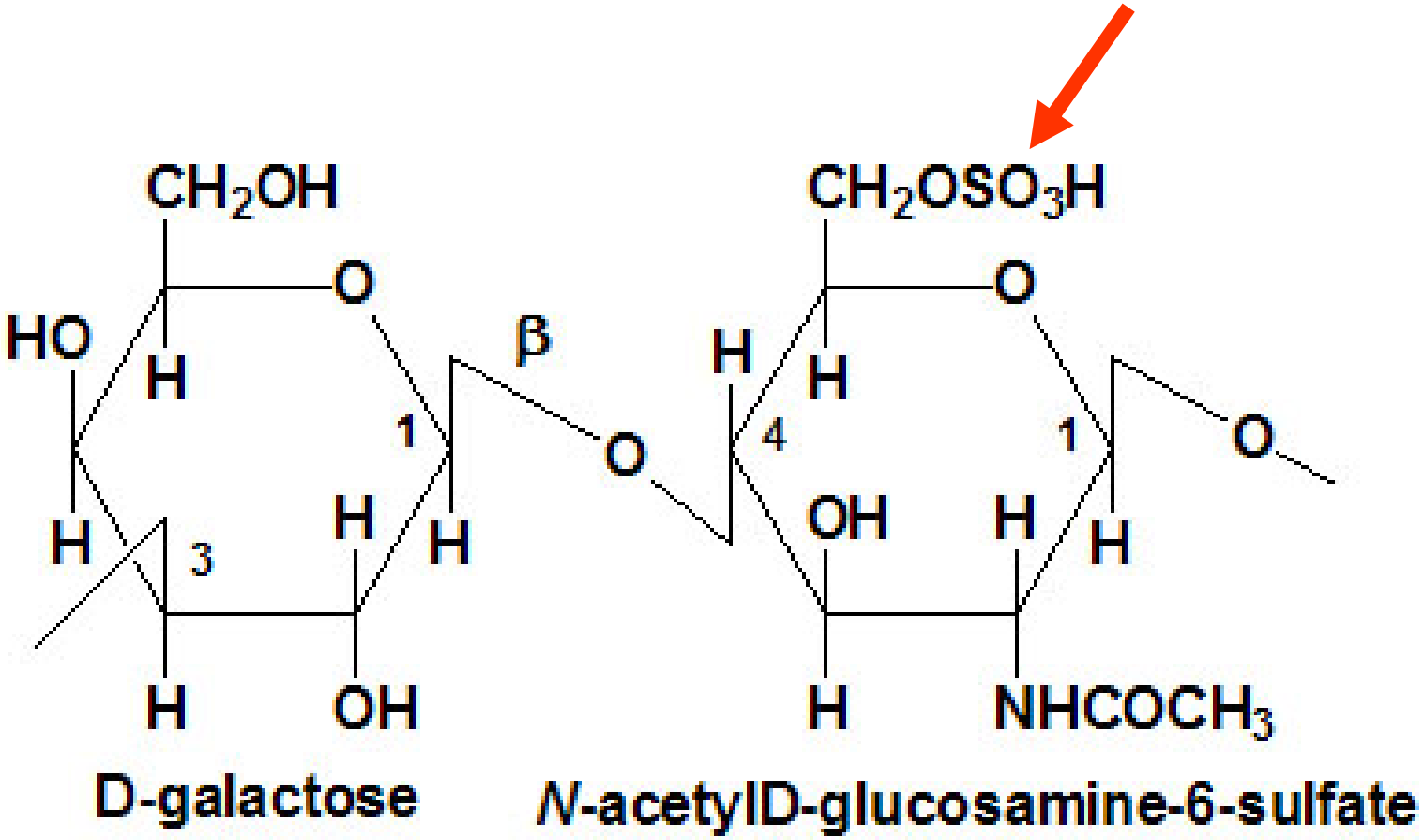
Heparin and Heparan sulfates:
composed of iduronate-2-sulfate (D-glucuronate-2-sulfate)
and *N*-sulfo-D-glucosamine-6-sulfate
linkage is α(1, 4)
(heparans have less sulfate than heparins)



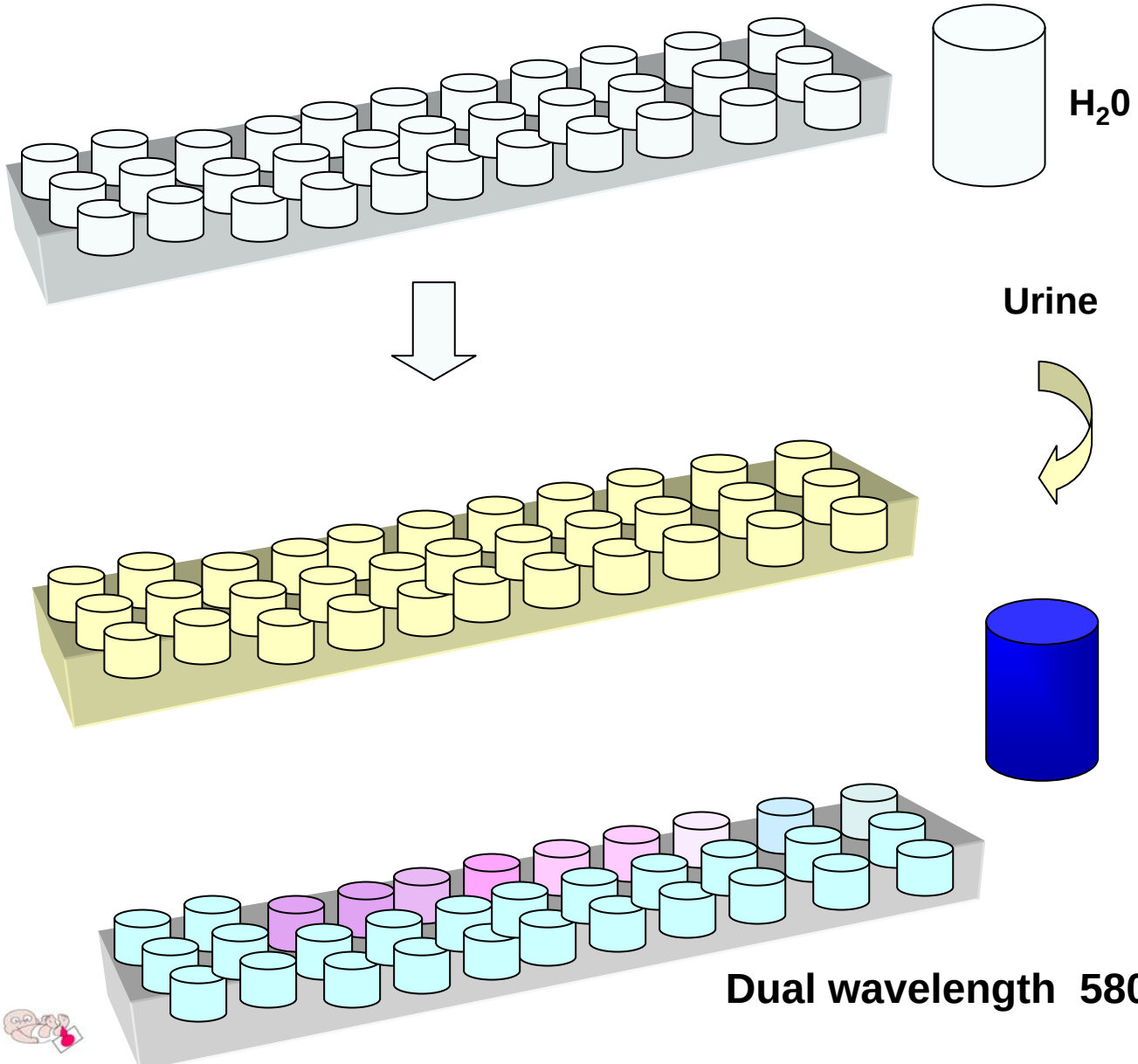
L-iduronate-2-sulfate

N-sulfo-D-glucosamine-6-sulfate

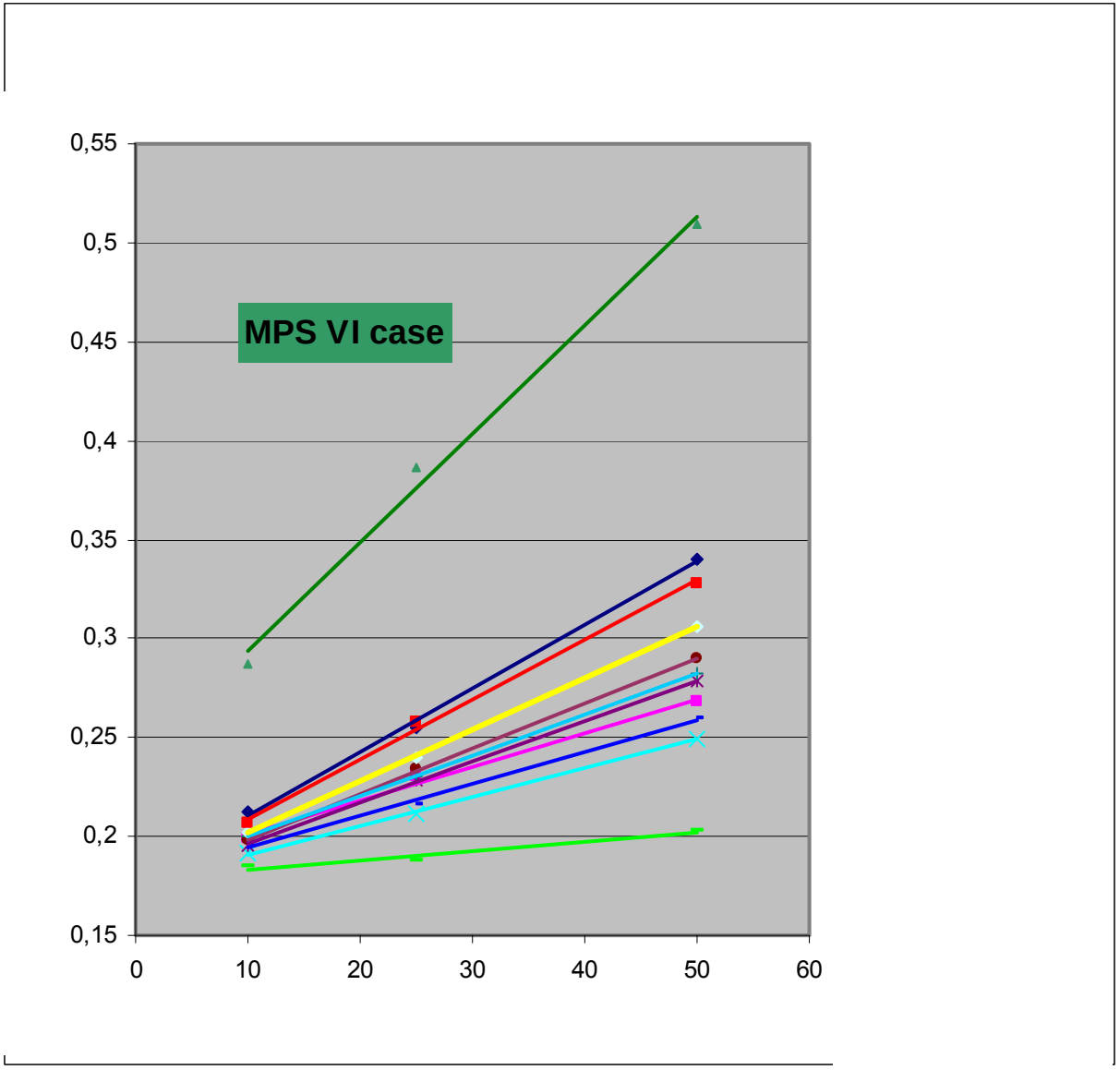
Keratan sulfates:
composed of galactose + GlcNAc-6-sulfate
linkage is β(1, 4)



TOTAL GAG METHOD



•Absorbance 580/690 nm



mg/L GAG excretion

Assay Calibration Report

Assay Information

Assay Name: GAG Ctrl Dual Polyn 2nd
Units: mg/L
Assay Description:
Assay Substances:
Blank used:
Valid Interval: 7 days 0 hours
Controls:
Blankk
Normal Control
Abnormal Control
Standards:
Std1, Concentration = 3.00, Minimum number to use: 1
Std2, Concentration = 6.00, Minimum number to use: 1
Std3, Concentration = 12.00, Minimum number to use: 1
Std4, Concentration = 24.00, Minimum number to use: 1
Std5, Concentration = 30.00, Minimum number to use: 1
Std6, Concentration = 36.00, Minimum number to use: 1
Std7, Concentration = 42.00, Minimum number to use: 1
Std8, Concentration = 48.00, Minimum number to use: 1
Std9, Concentration = 54.00, Minimum number to use: 1
Curve valid Interval: 7 days 0 hours
Axis Mode: Y = Abs, X = Ln(Conc)

Assay Mode: Polynomial 2nd order
# of decimals: 2

Assay Calibration

Current Calibration Status: " Expired "

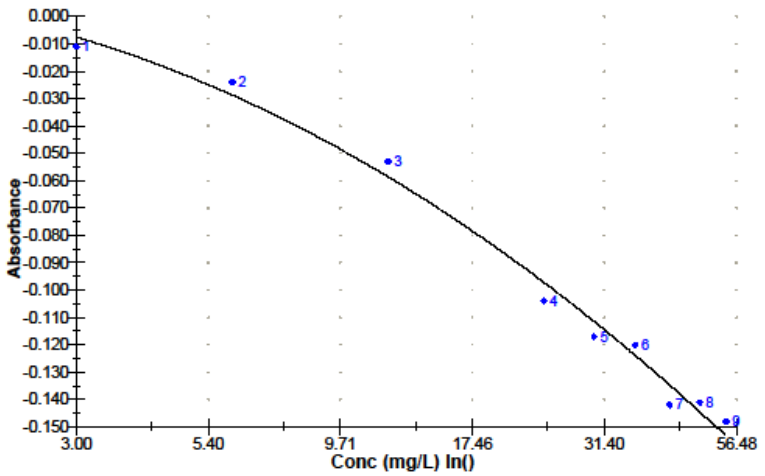
Name	Absorbance	Concentration	Interpretation	Position
03.08.2012 17:09:45				
Blank	0.223 Abs			A01
Std1	-0.011 Abs	3.43 mg/L		B01
Std2	-0.024 Abs	5.26 mg/L		C01
Std3	-0.053 Abs	10.70 mg/L		D01
Std4	-0.104 Abs	26.75 mg/L		E01
Std5	-0.117 Abs	32.56 mg/L		F01
Std6	-0.120 Abs	34.01 mg/L		G01
Std7	-0.142 Abs	46.33 mg/L		H01
Std8	-0.141 Abs	45.80 mg/L		A02
Std9	-0.148 Abs	50.24 mg/L		B02
03.08.2012 17:09:45				
Blankk	0.002 Abs	< 3.00 mg/L	Out(LR)	C02
Blankk	0.002 Abs	< 3.00 mg/L	Out(LR)	D02
Normal Control	-0.110 Abs	29.34 mg/L		E02
Normal Control	-0.109 Abs	28.92 mg/L		F02
Abnormal Control	-0.153 Abs	53.69 mg/L		G02
Abnormal Control	-0.157 Abs	> 54.00 mg/L	Out(LR)	H02
Statistic				
Blank [MEAN]	0.223			
Std1 [MEAN]	-0.011	3.430		
Std1 [%DIFF]		14.33		
Std2 [MEAN]	-0.024	5.260		
Std2 [%DIFF]		-12.33		
Std3 [MEAN]	-0.053	10.700		
Std3 [%DIFF]		-10.83		
Std4 [MEAN]	-0.104	26.750		
Std4 [%DIFF]		11.46		
Std5 [MEAN]	-0.117	32.560		
Std5 [%DIFF]		8.53		
Std6 [MEAN]	-0.120	34.010		
Std6 [%DIFF]		-5.53		

Assay Calibration Report

Name	Absorbance	Concentration	Interpretation	Position
Std7 [MEAN]	-0.142	46.330		
Std7 [%DIFF]		10.31		
Std8 [MEAN]	-0.141	45.800		
Std8 [%DIFF]		-4.58		
Std9 [MEAN]	-0.148	50.240		
Std9 [%DIFF]		-6.96		
Blankk [MEAN]	0.002			
Blankk [SD]	0.000			
Blankk [%CV]	0.00			
Normal Control [MEAN]	-0.109	29.130		
Normal Control [SD]	0.001	0.297		
Normal Control [%CV]	0.65	1.02		
Abnormal Control [MEAN]	-0.155			
Abnormal Control [SD]	0.003			
Abnormal Control [%CV]	1.82			

Assay Curve

Y = A + B*X + C*X^2
A = 0.00814387
B = -0.0042814
C = -0.00908206
R2 coef = 0.98866



Assay Information

Assay Name: GAG Ctrl Dual Polyn 2nd
Units: mg/L
Assay Description:
Assay Substances:

Assay Mode: Polynomial 2nd order
# of decimals: 2

Blank used:
Valid interval: 7 days 0 hours
Controls:
Blankk
Normal Control
Abnormal Control
Standards:
Std1, Concentration = 3.00, Minimum number to use: 1
Std2, Concentration = 6.00, Minimum number to use: 1
Std3, Concentration = 12.00, Minimum number to use: 1
Std4, Concentration = 24.00, Minimum number to use: 1
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Std7, Concentration = 42.00, Minimum number to use: 1
Std8, Concentration = 48.00, Minimum number to use: 1
Std9, Concentration = 54.00, Minimum number to use: 1
Curve valid interval: 7 days 0 hours
Axis Mode: Y = Abs, X = Ln(Conc)

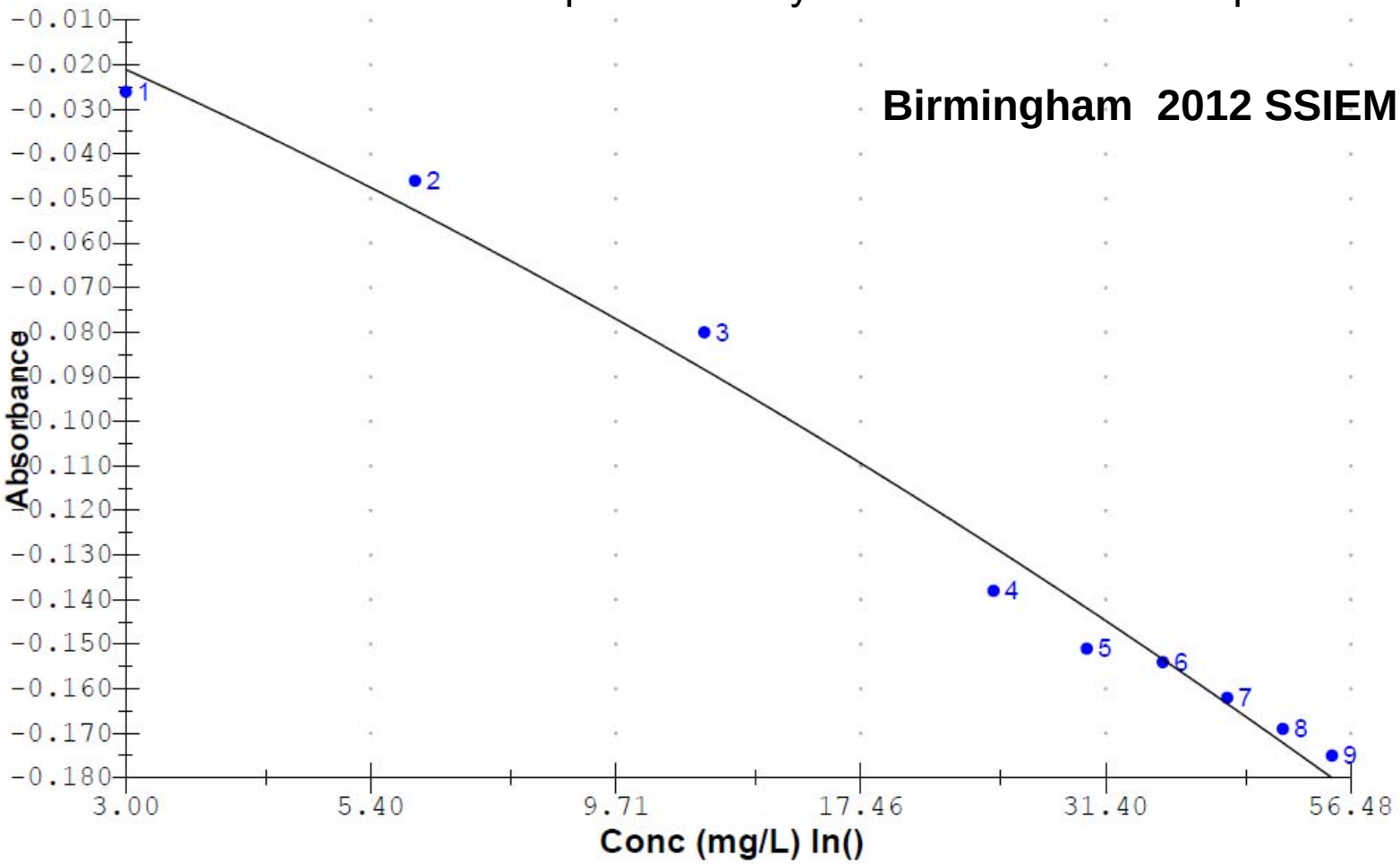
Assay Curve

Y = A + B*X + C*X^2
A = 0.0206451
B = -0.0332446
C = -0.00427568
R2 coef = 0.98608

300 ug/mL GAG containing urine is target concentration for CPC precipitation

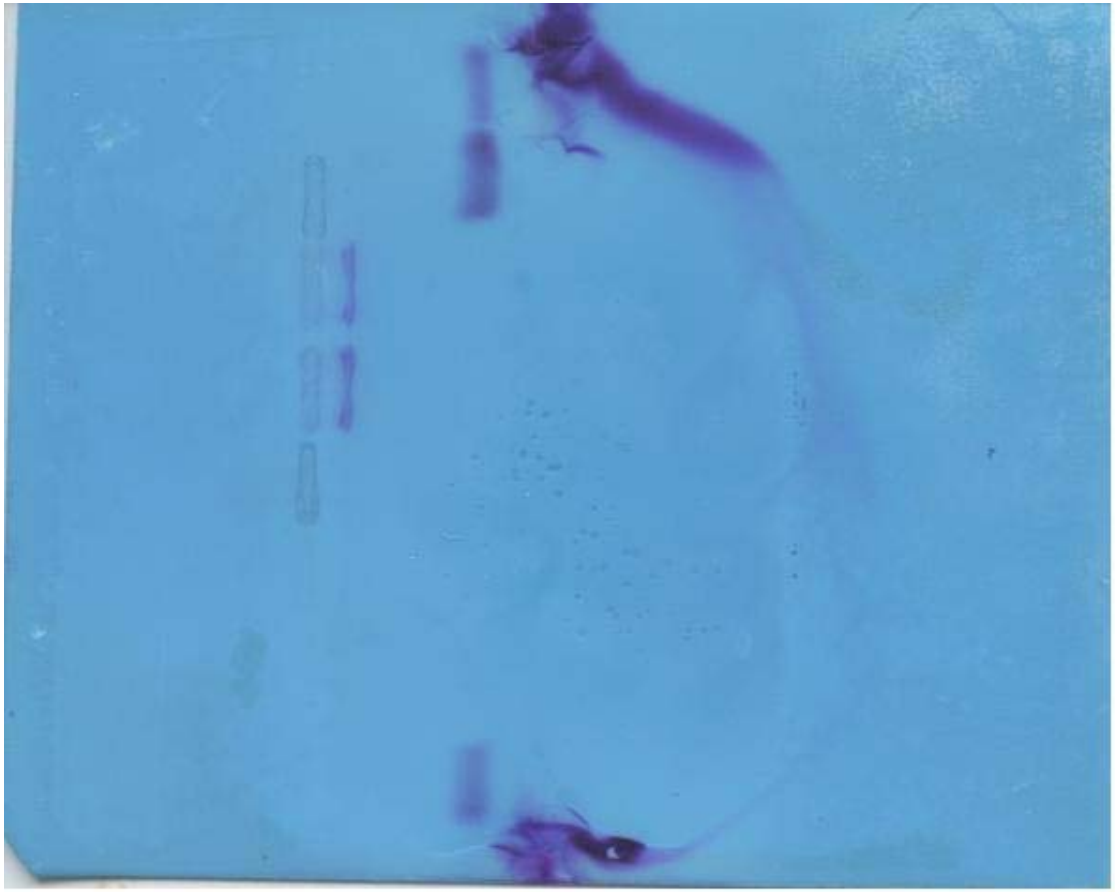
http://www.tanyalcin.com/Data/P-208.pdf

Birmingham 2012 SSIEM



-

+



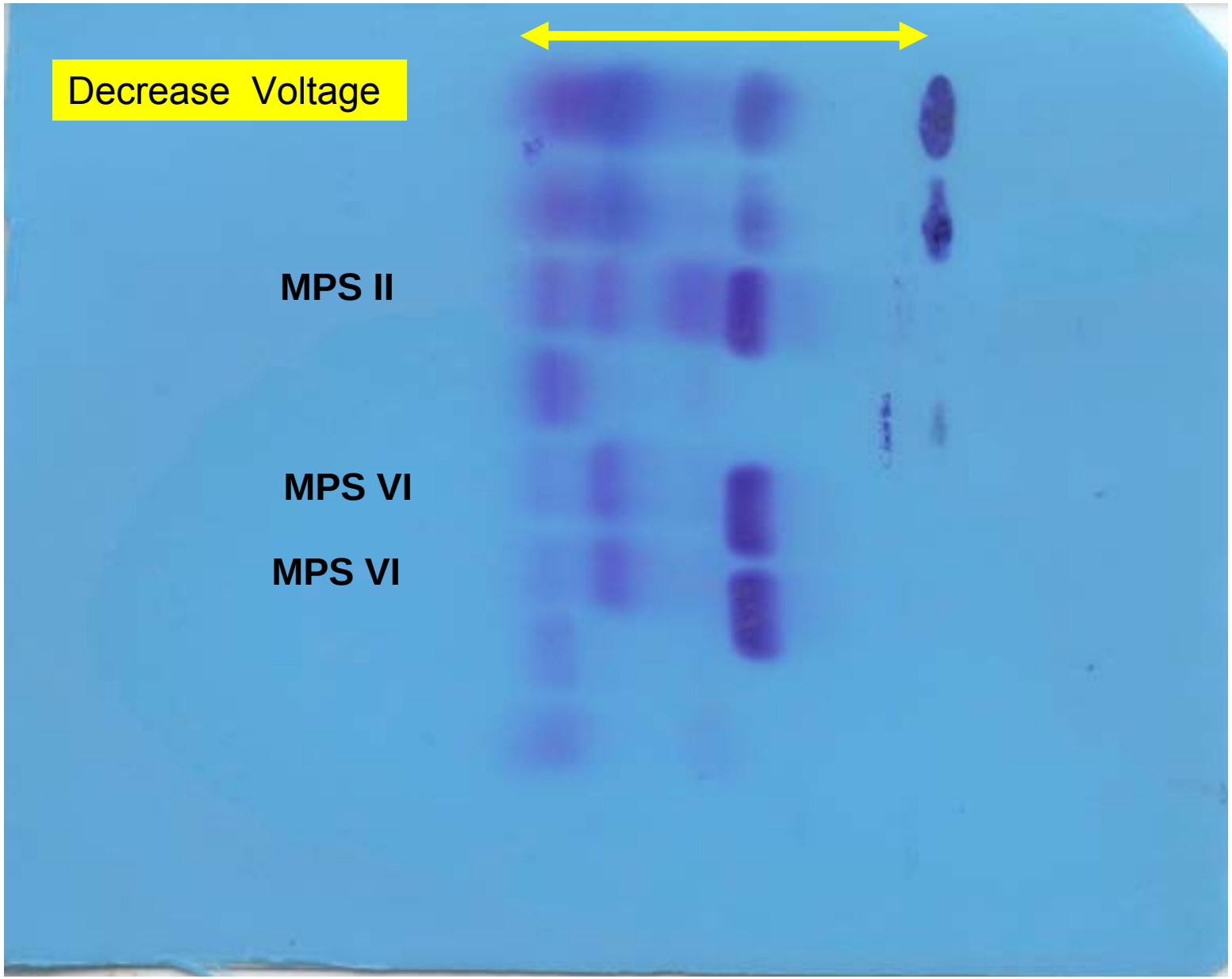
pH = 5.0

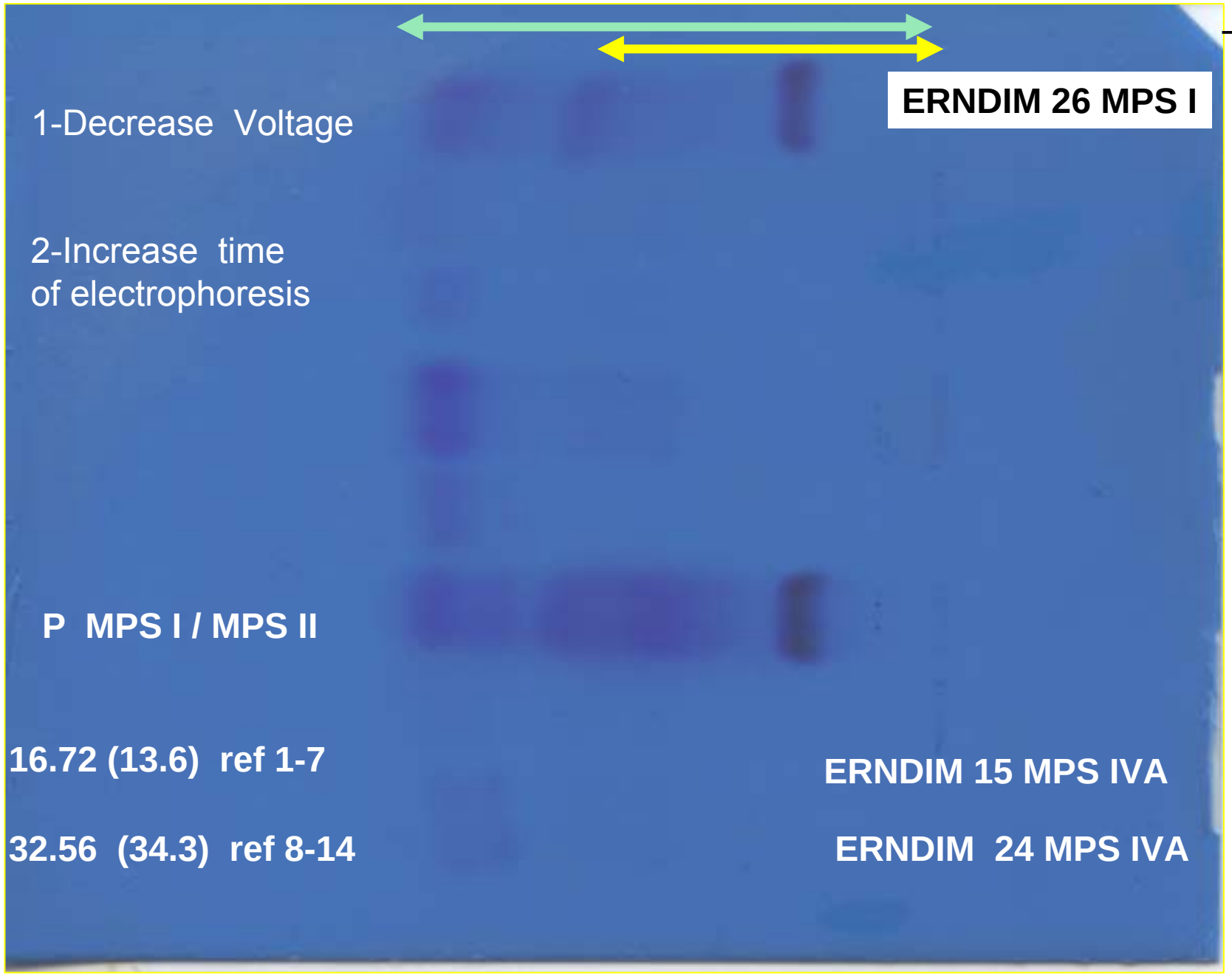
BaAc = 1M - 0,05 M

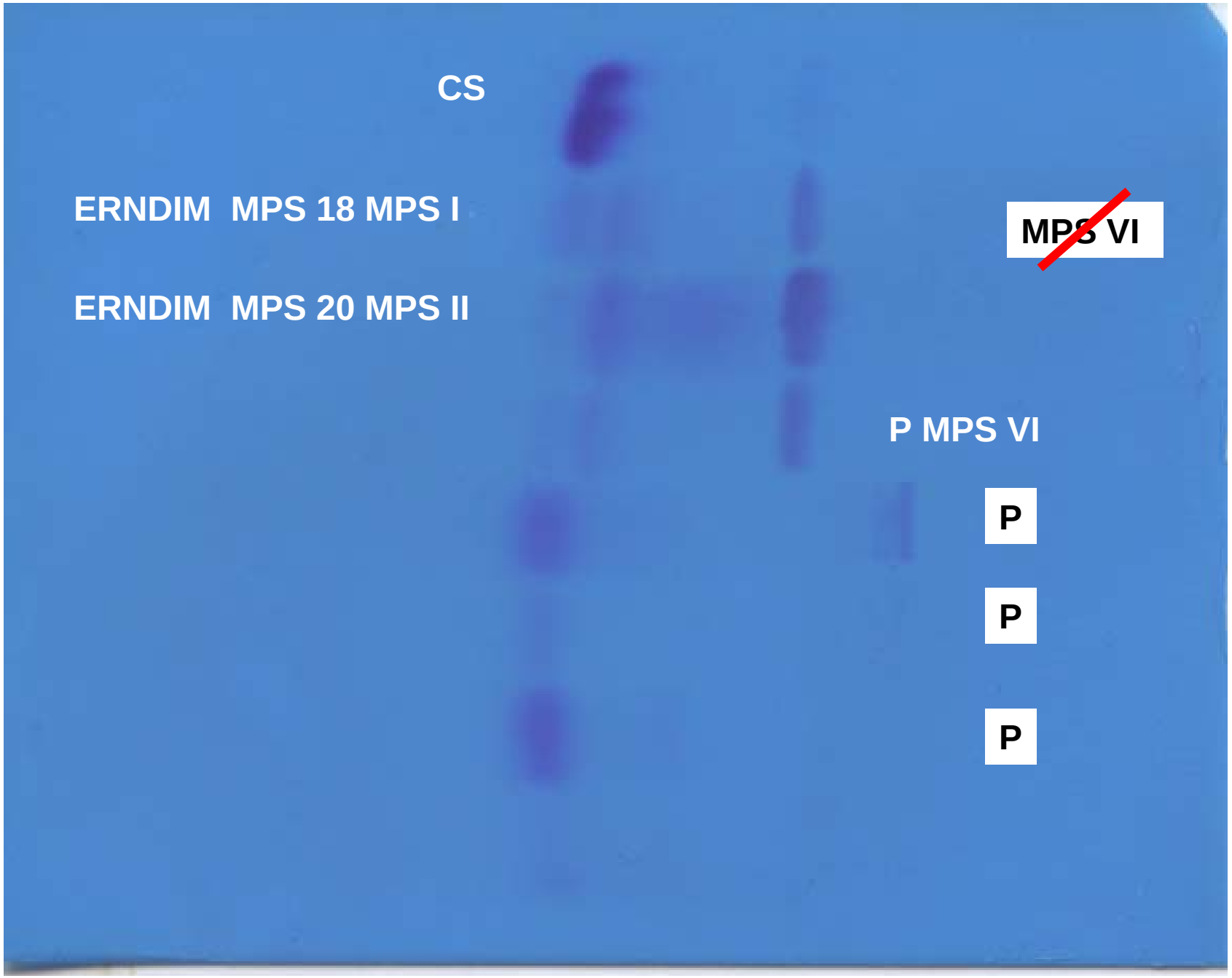
180- 200 V

+

–



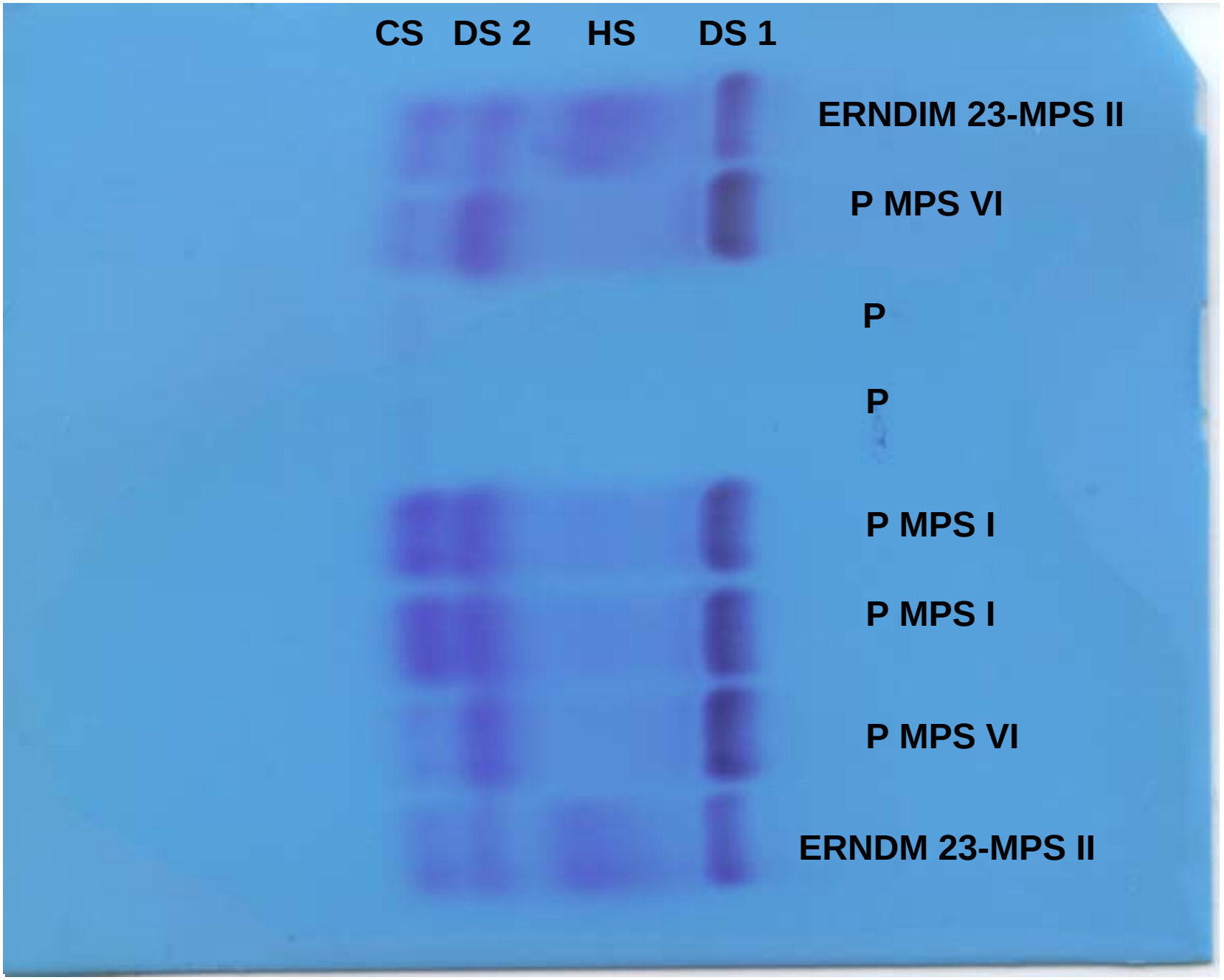


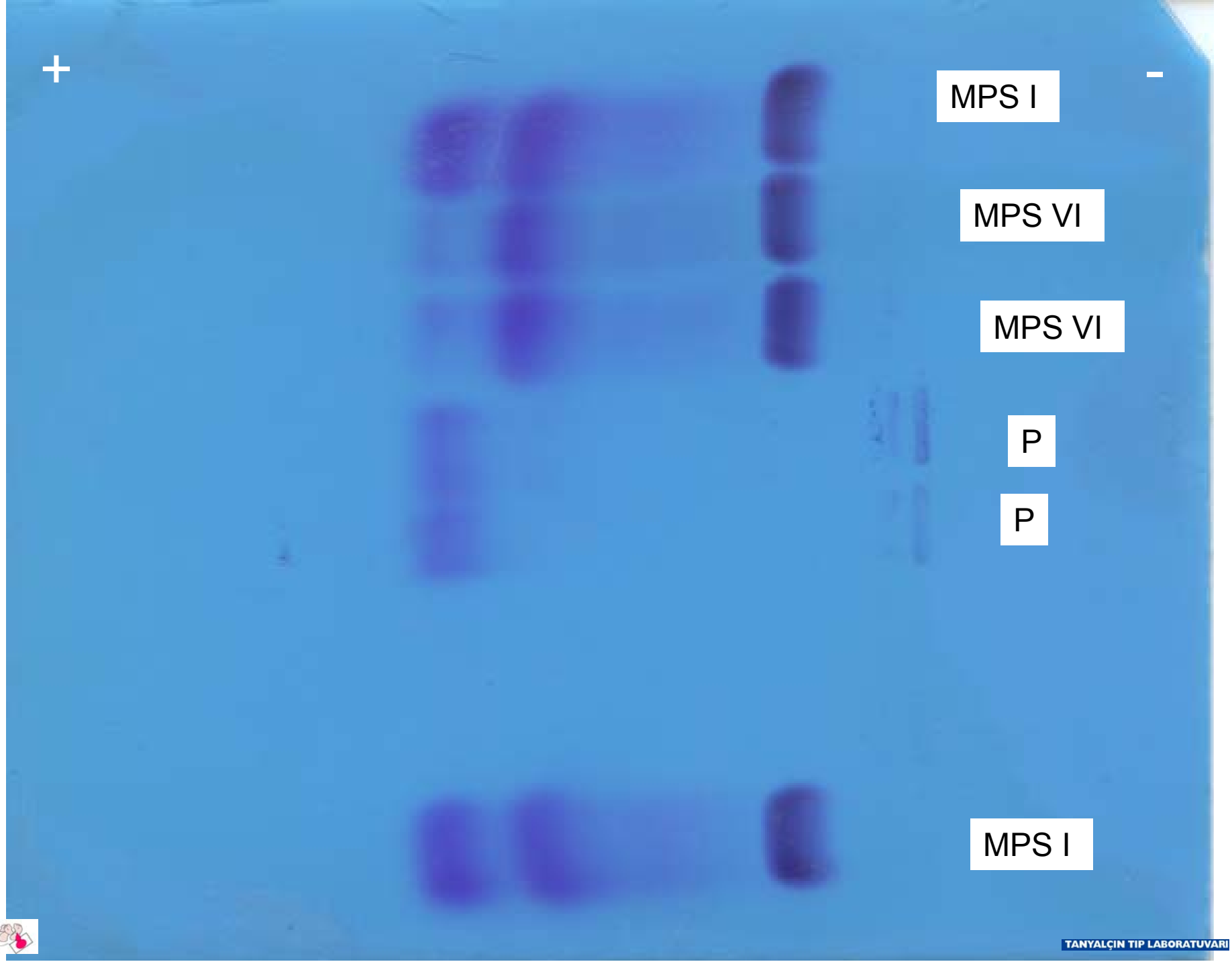


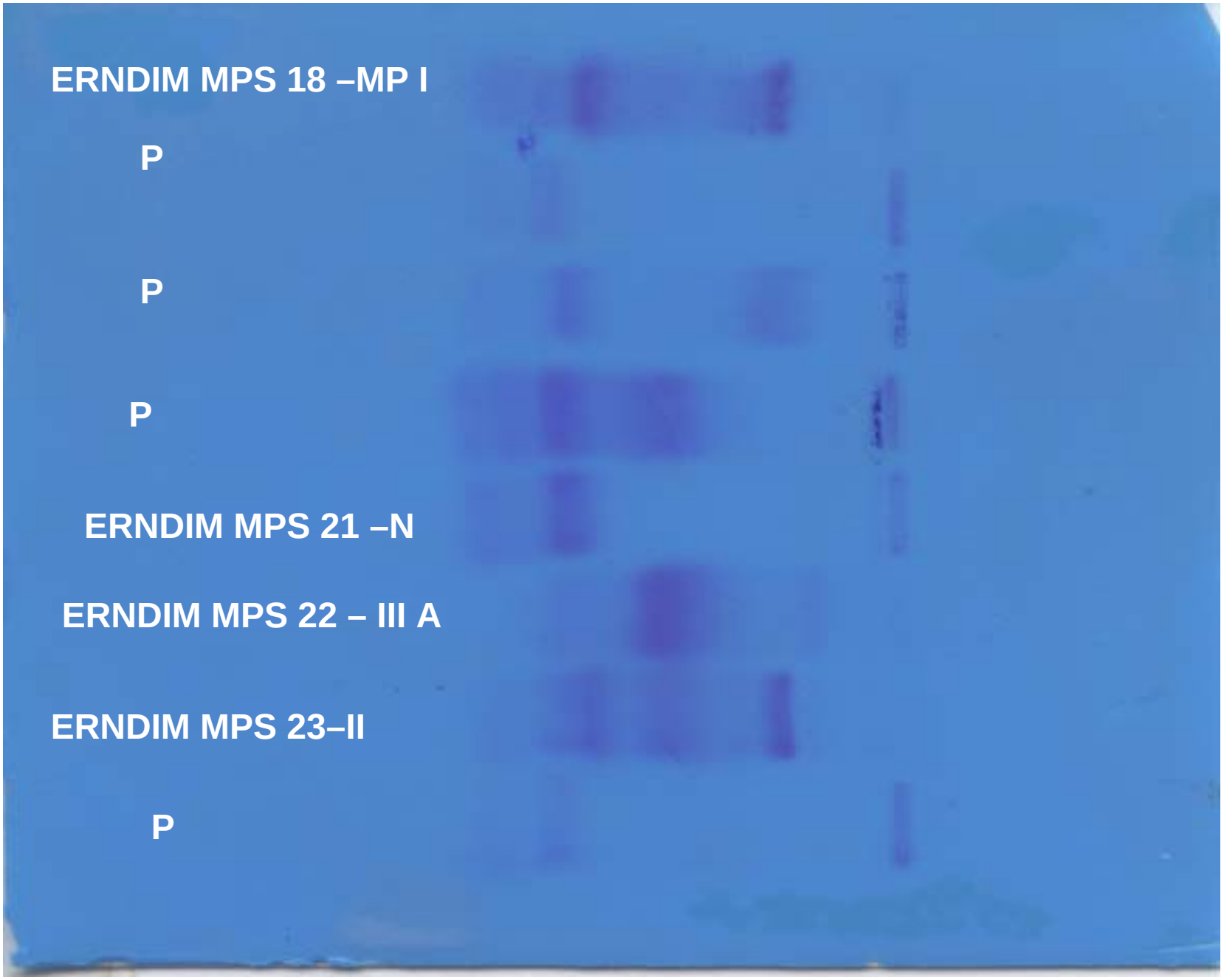
1 D electrophoresis High Resolution
Discontinuous electrophoresis
= electrophorese multiple times

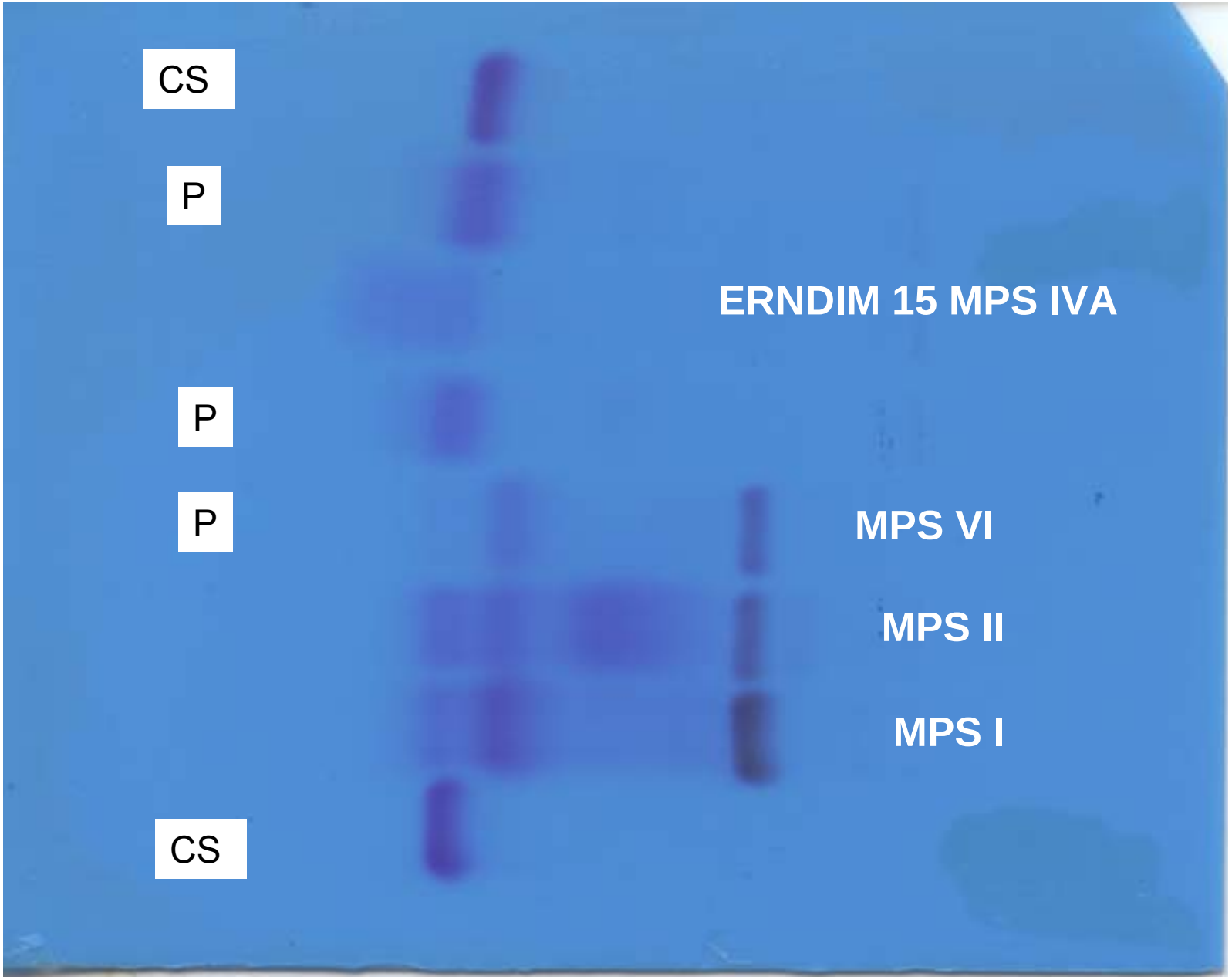
- 0.025 M BaAc cellulose acetate plates
- No pH adjustment ; pH ~ 7.0
- %15 EtOH + BaAc
- % 50 EtOH + BaAc
- DMB staining
- Densitometric interpretation

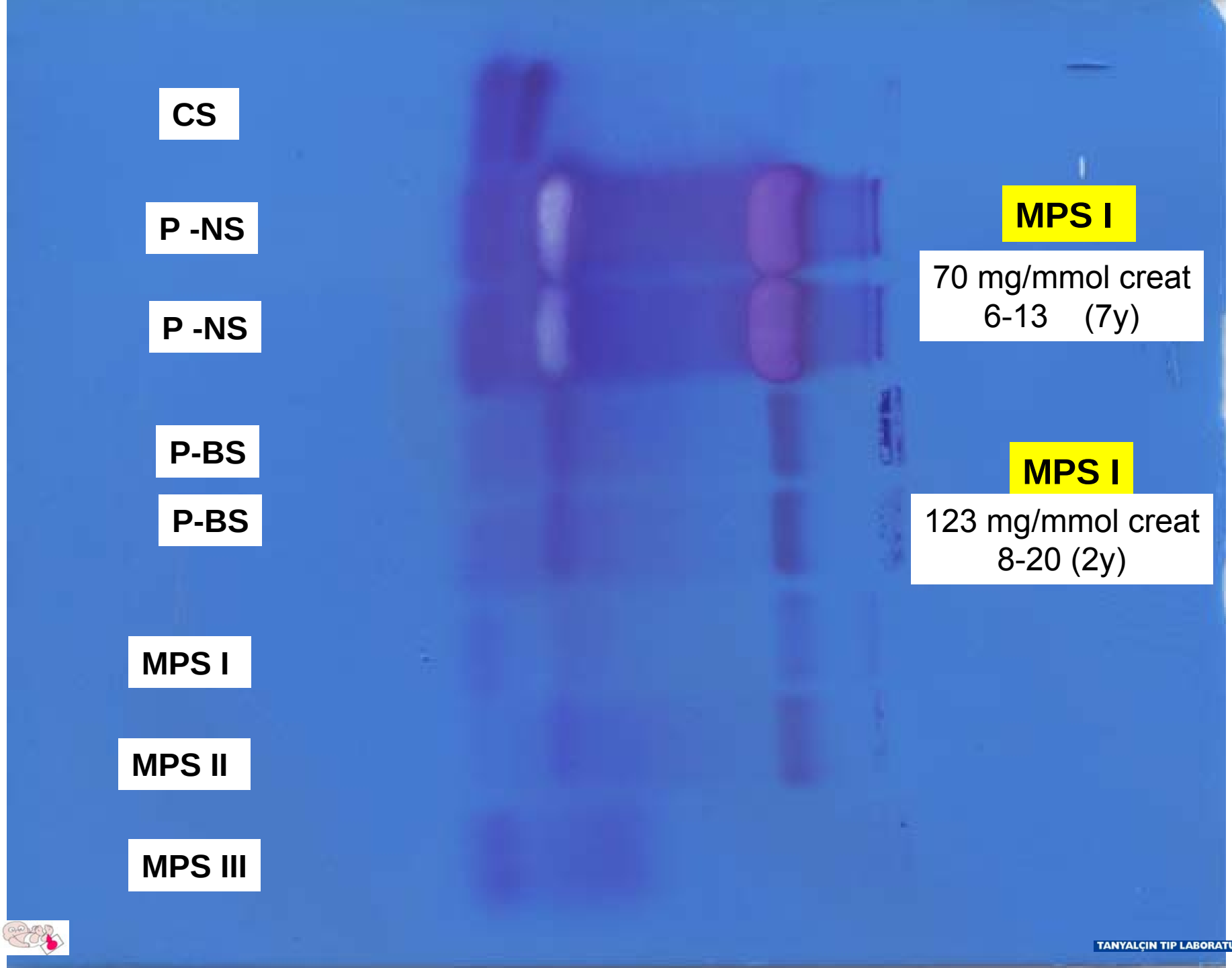
Modified Hopwood &Harrison 1982 Anal Biochem &
Nor Azimah et al.2010 Malaysian J Pathol





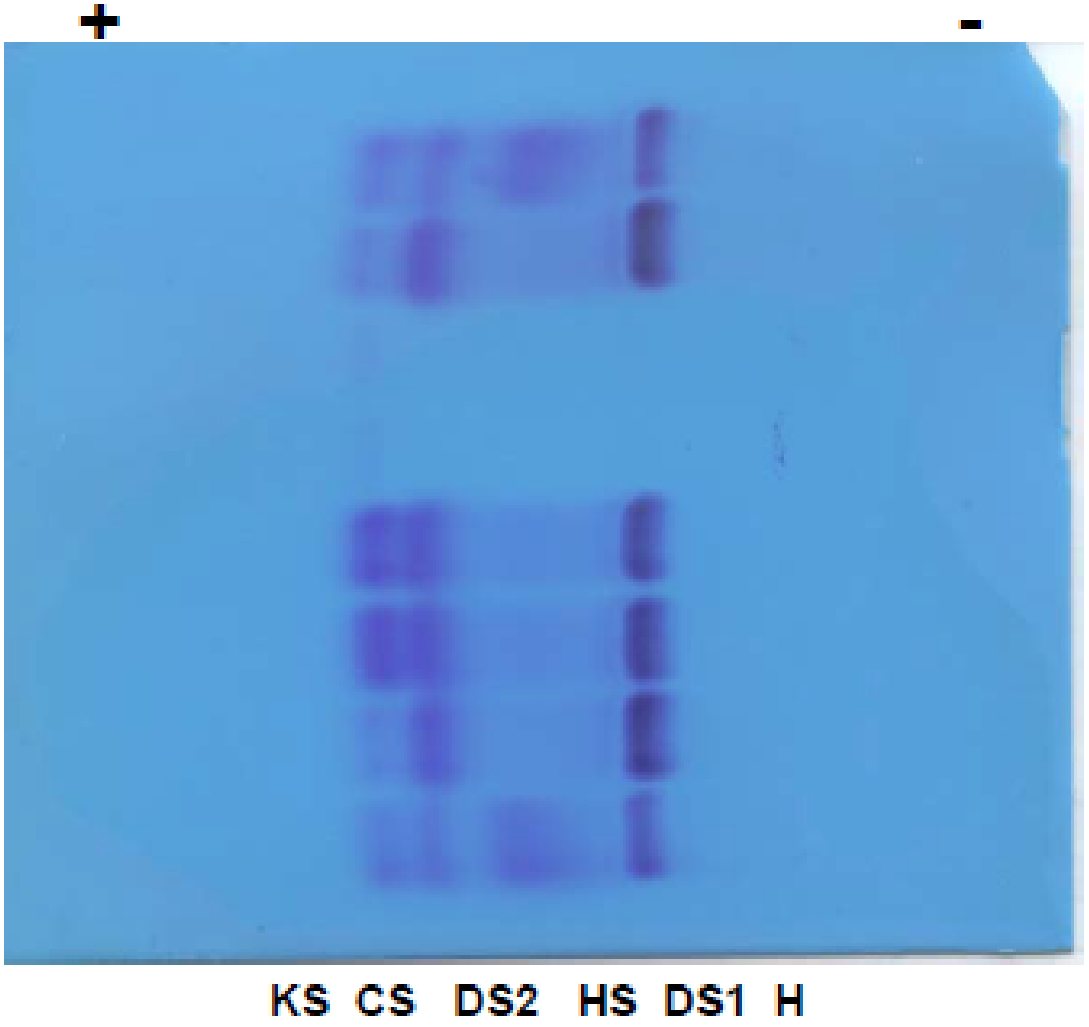





CS
P -NS
P -NS
P-BS
P-BS
MPS I
MPS II
MPS III
MPS I
70 mg/mmol creat
6-13 (7y)
MPS I
123 mg/mmol creat
8-20 (2y)
TANYALÇIN TIP LABORATUVARI

Tüm Hastalara Ait Asetat

ERNDIM MPS 23
MPS VI
40223 GB
40223 GB
38096 B. Ertas
38096 B.Ertas
MPS VI
ERNDIM MPS 23



TANYALCIN TIP LABORATUVARI

Idrar GAG Elektroforez Raporu

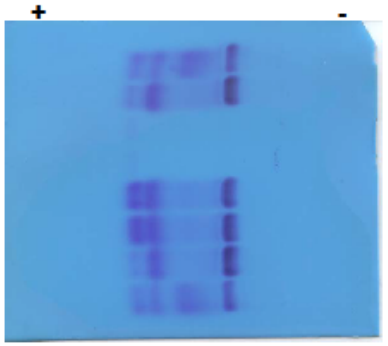
Item	Value
	13.05.2013 15:06:07
KODU / ADI SOYADI	
YAS / CINSIYETI	4 ay 10 gun
KLINIK BULGULAR	Mukopolisakkaridoz ?
ORNEK ALIM TARIHI	02.05.2013
ORNEK TESLIM TARIHI	03.05.2013
ISTEK YAPAN	
Total GAG mg/L	1453.9
KREATININ: mg/dL / mmol/L	79.6 / 7.04
REFERANS ARALIK: mg/mmol creat	15 - 52
Total Mukopolissakkarit (GAG) (mg/mmol creat)	206.61

DB Status

TIBBI YORUM:
Idrarda MASSIF GAG (Glikozaminoglikan ekskresyonu mevcut) .Dermatan sulfat 1 +++++, Heparan sulfat +/- , dermatan sulfat 2 ++++, Kondroitin sulfat ++++, olumlu. Keratan sulfat gozlenmedi. Profil daha cok MPS I ile uyumlu ancak MPS II de olabilir. Enzimatik konformasyonu.

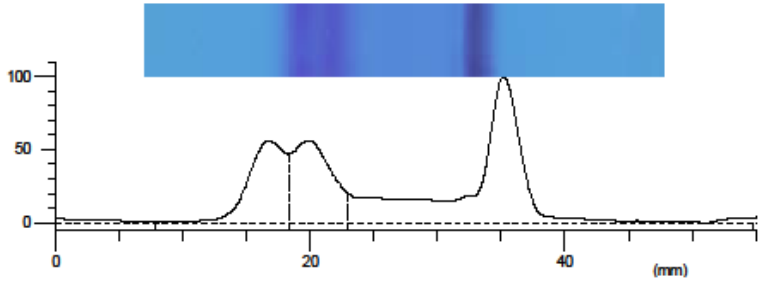
Prof. Dr Tijen Tanyalcin MD.PhD

Tüm Hastalara Ait Asetat



Index	Band	Rel.Area	Conc. (mg/mmol creat)
1	Kondroitin Sülfat	21.97%	45.39
2	Dermatan Sülfat 2	23.80%	49.18
3	Dermatan Sülfat 1	54.23%	112.04 H
Total			206.61
Ratio		0.00	

Hastaya Ait Bant



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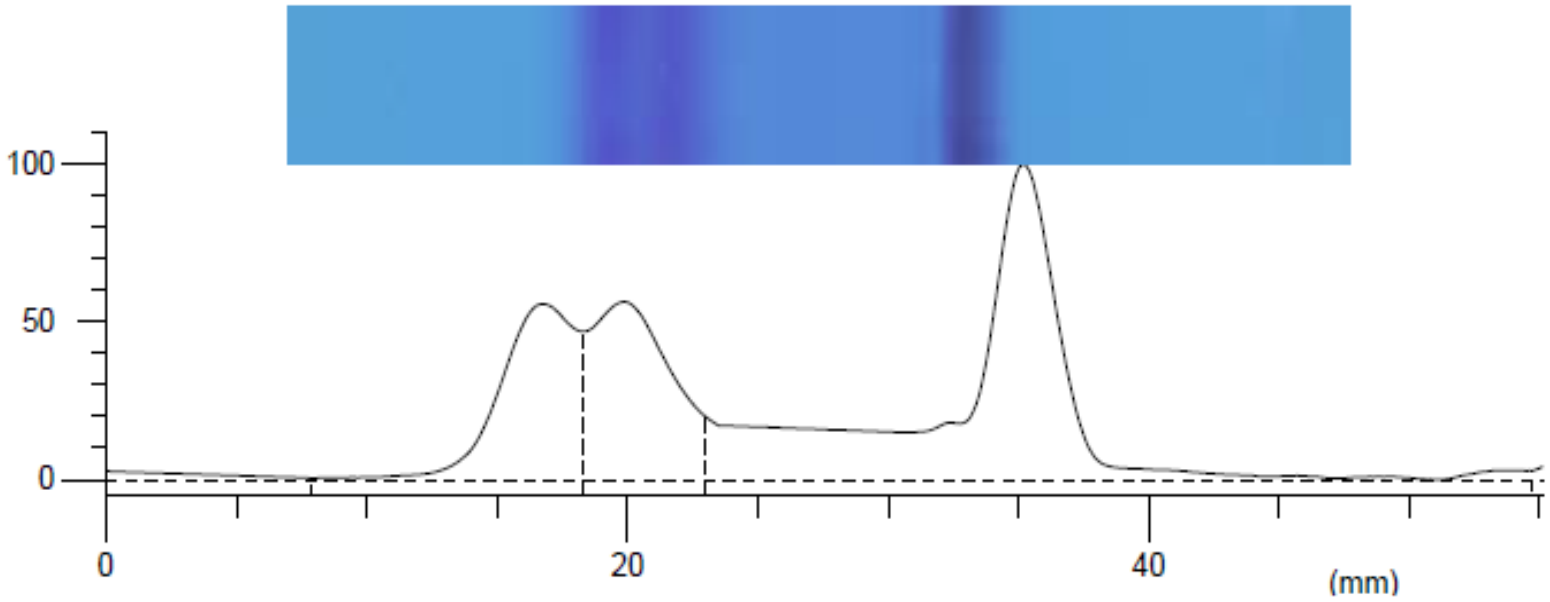
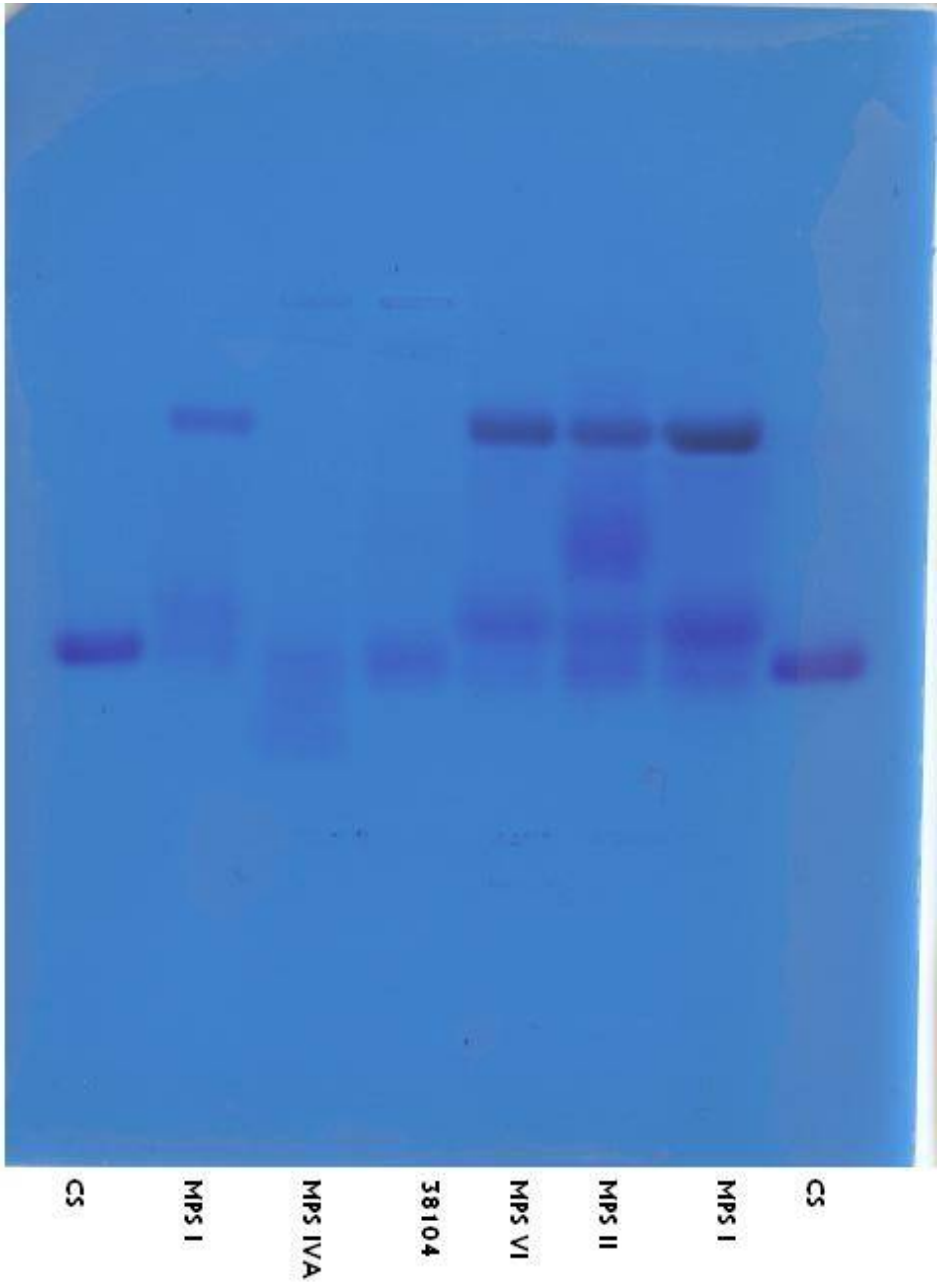




- Cathode

+Anode

BaAc 0,05 M pH = 5.0

BaAc 0,025 M
Buffer 1 pH = 7.3

✓

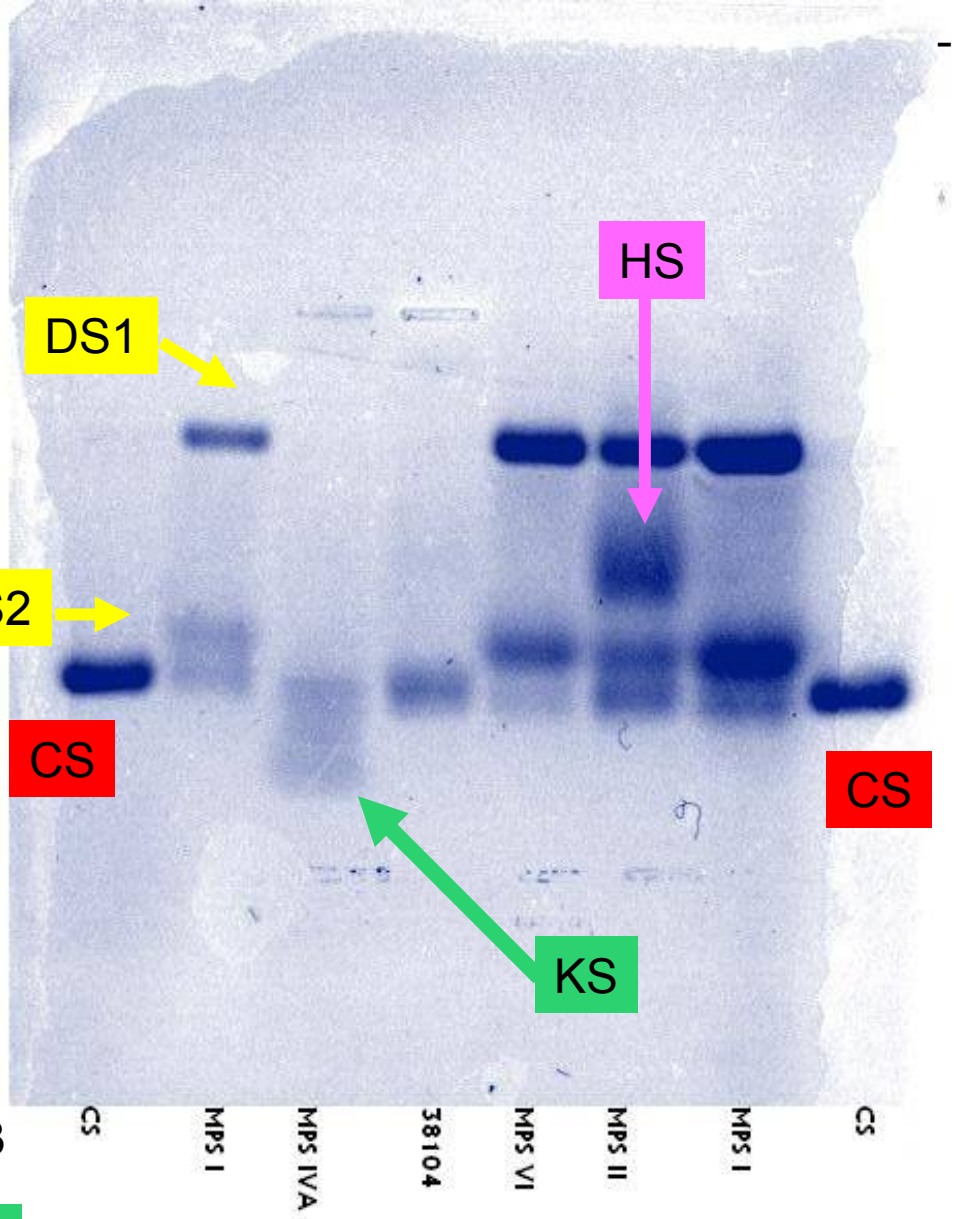
Soaked in very COLD Buffer 2
BaAc 0.025 M, 15% ethanol

✓

Soaked in very COLD Buffer 3
BaAc 0.025 M, 50% ethanol

✓

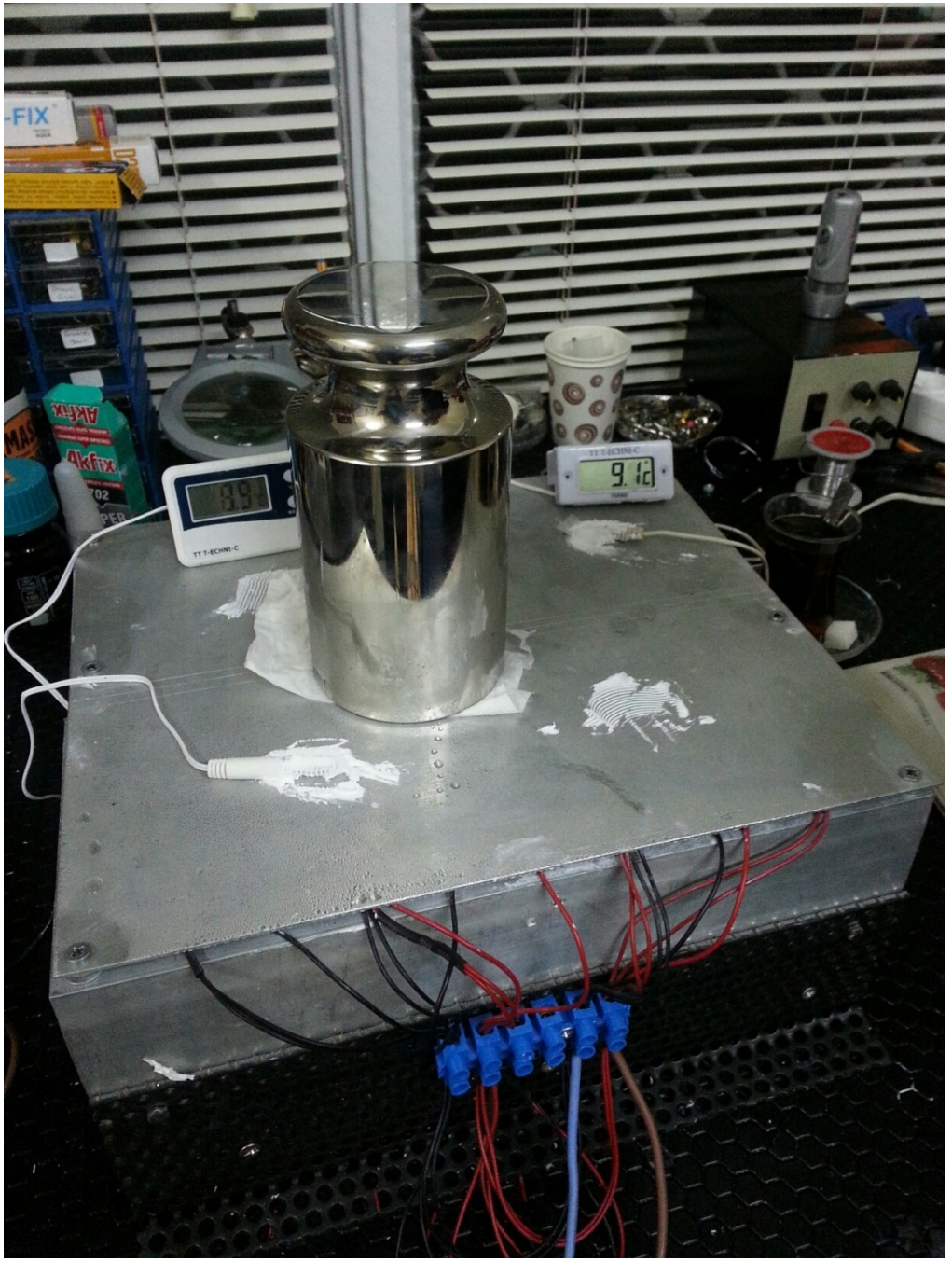
Voltage 130V

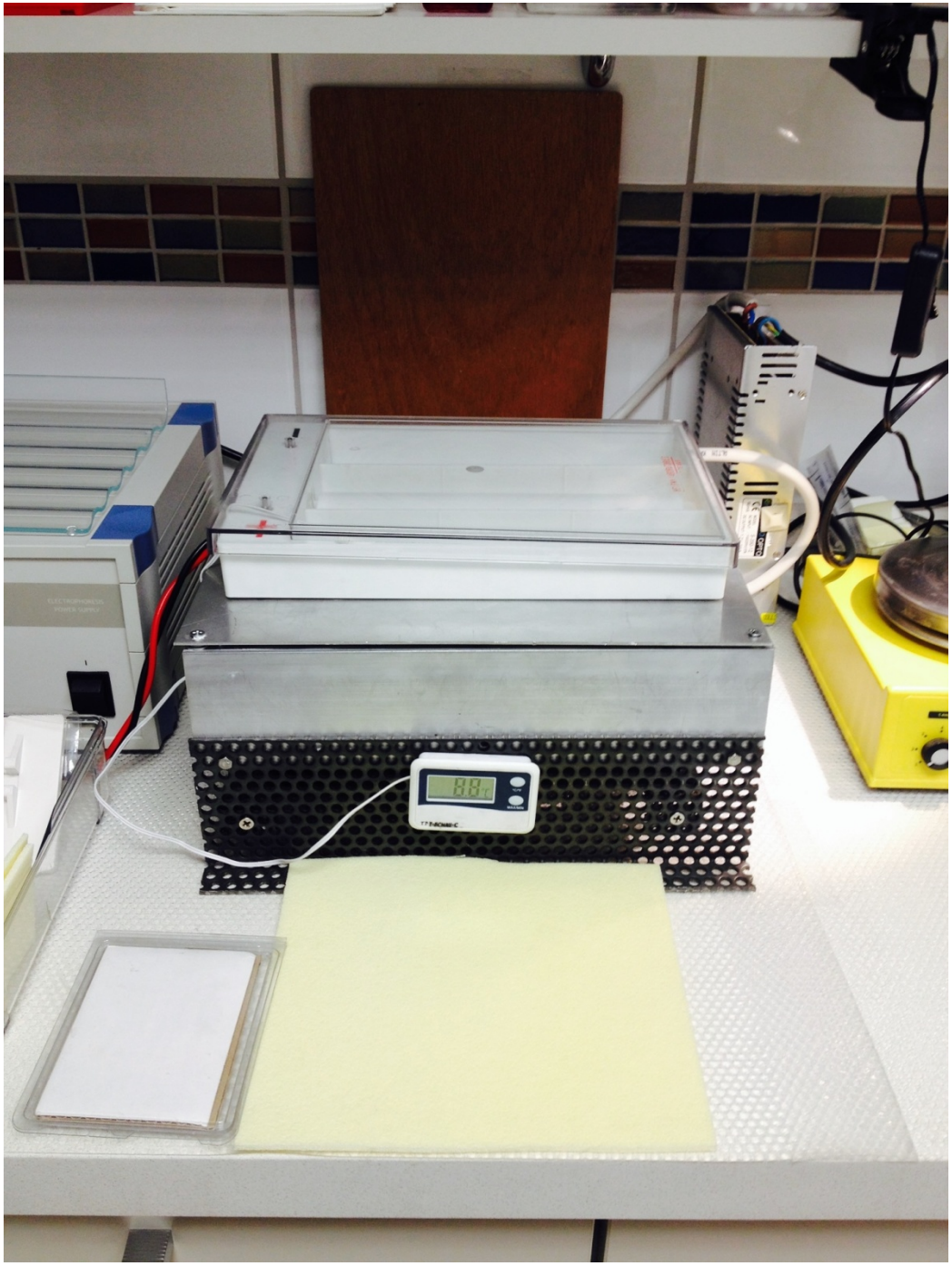


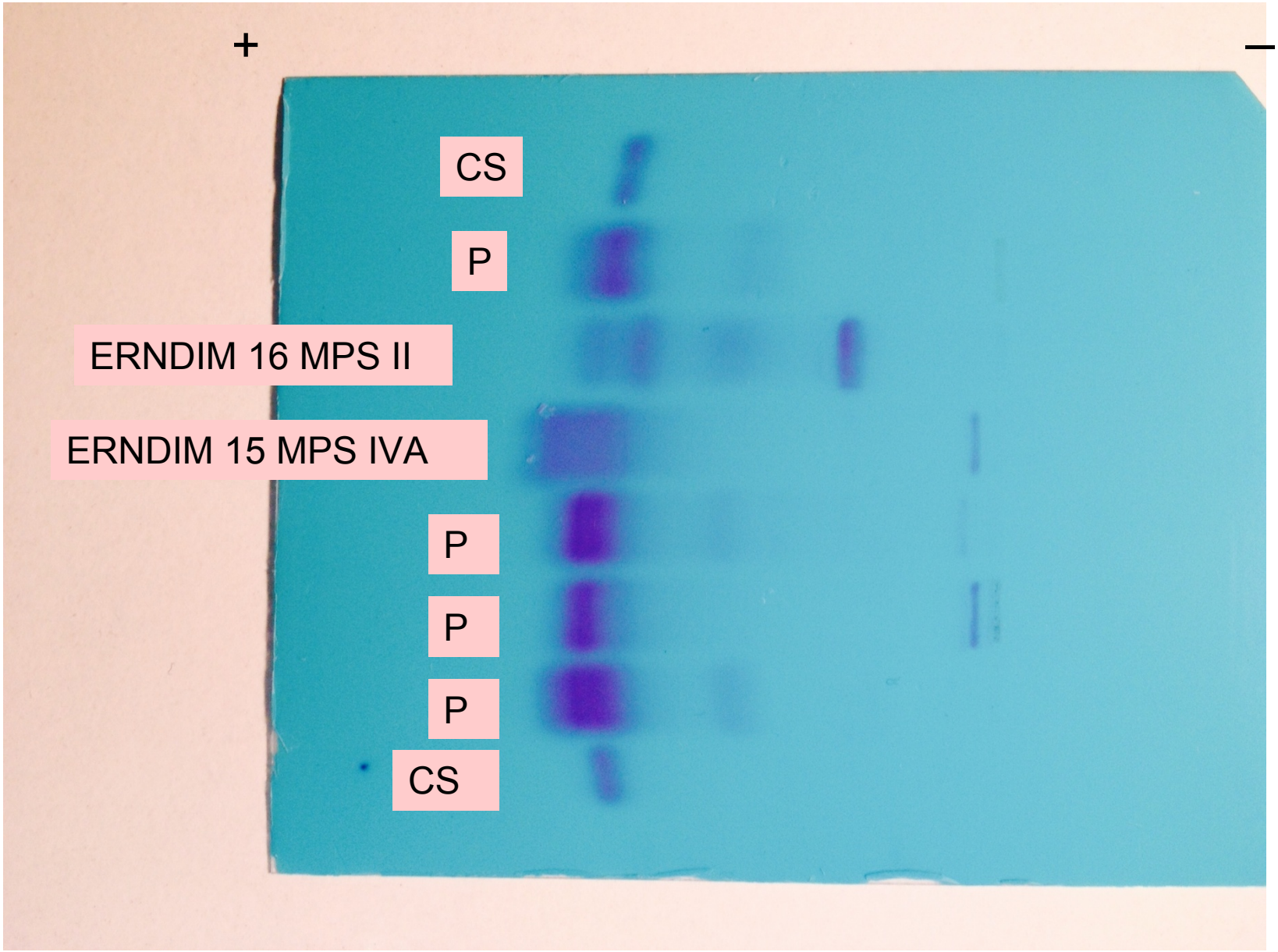
aliminium plate 20X30 cm

6 peltiers / 30 watt
supported by the 380 watt
power supply

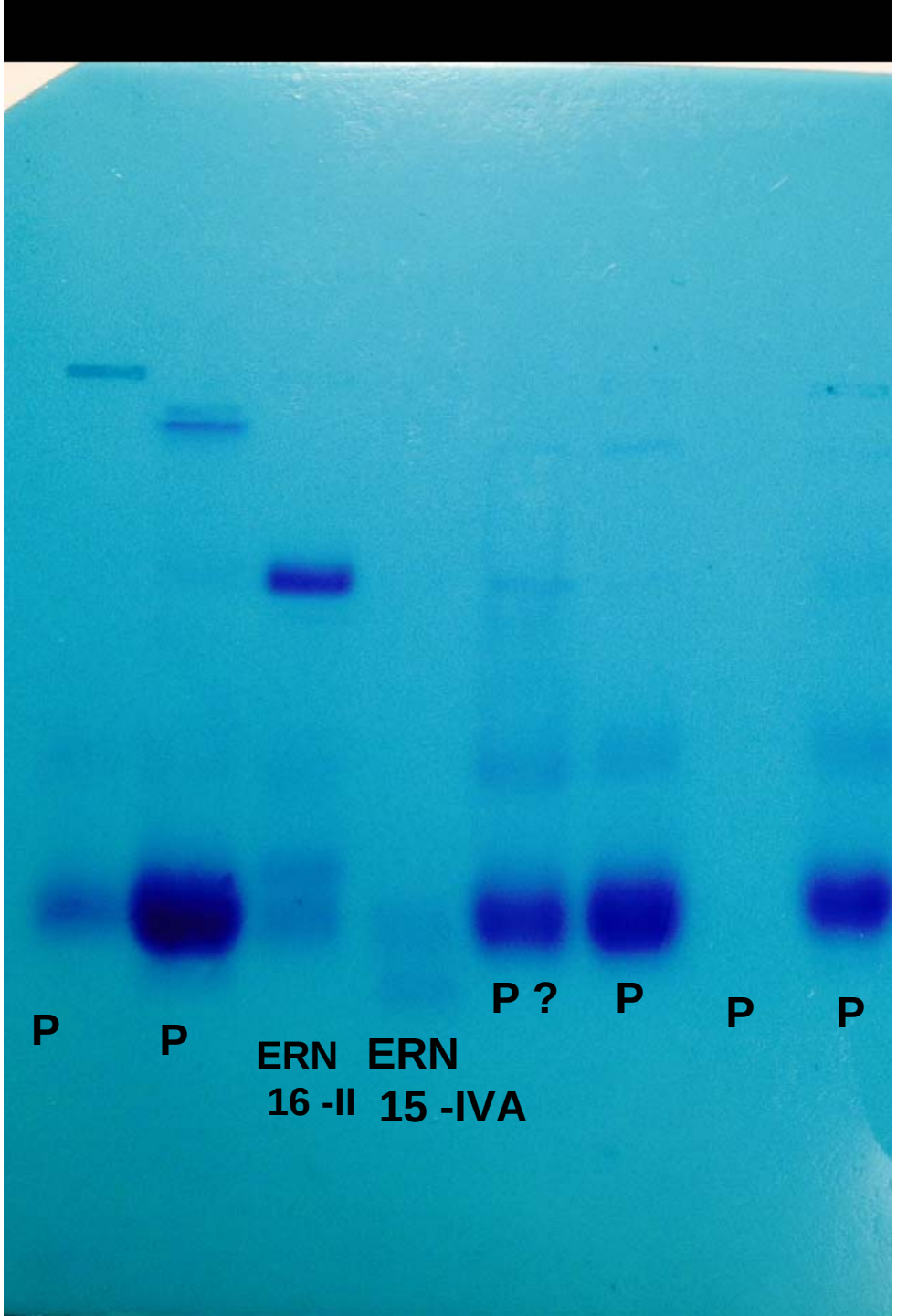
8 - 10 degree ° C



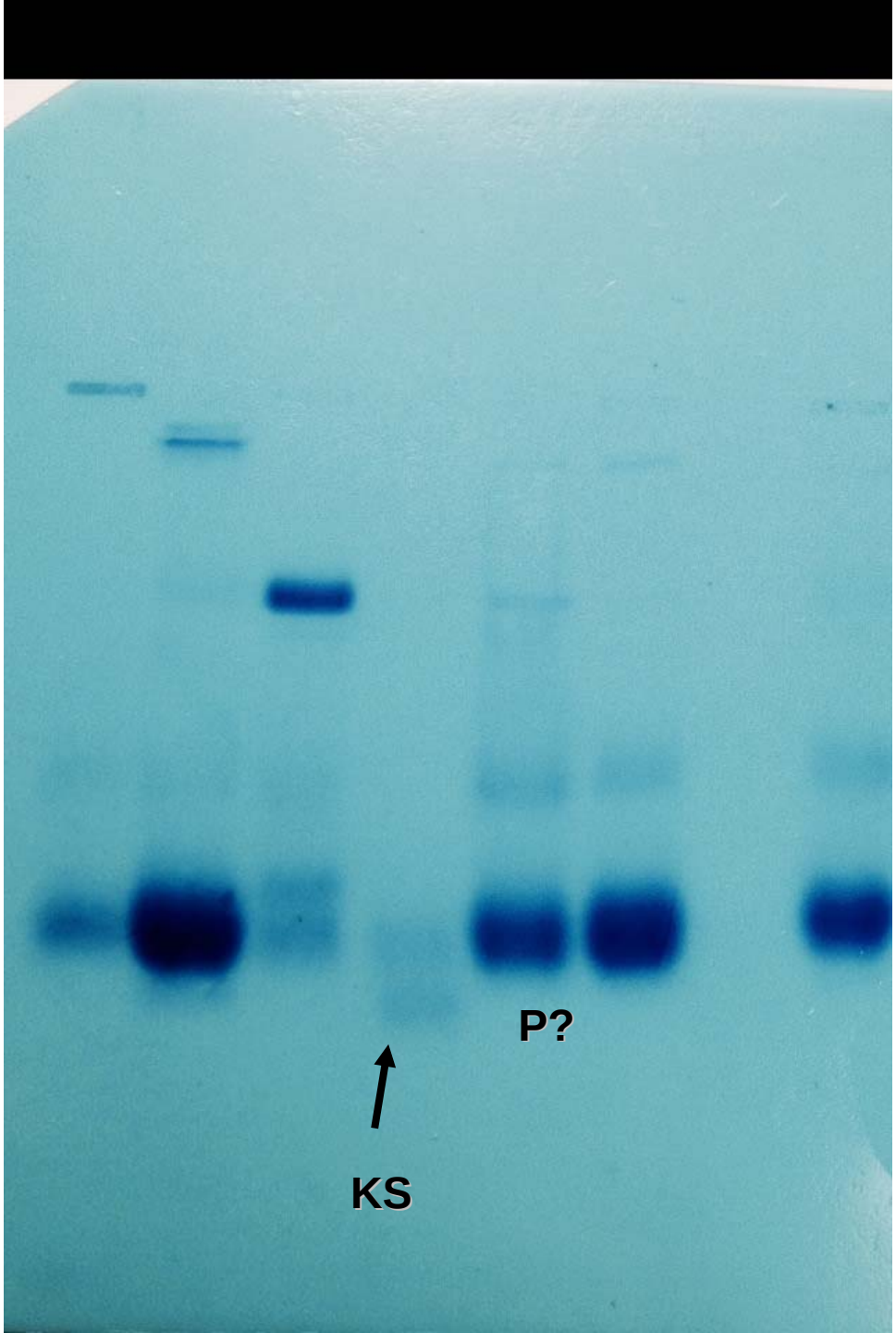




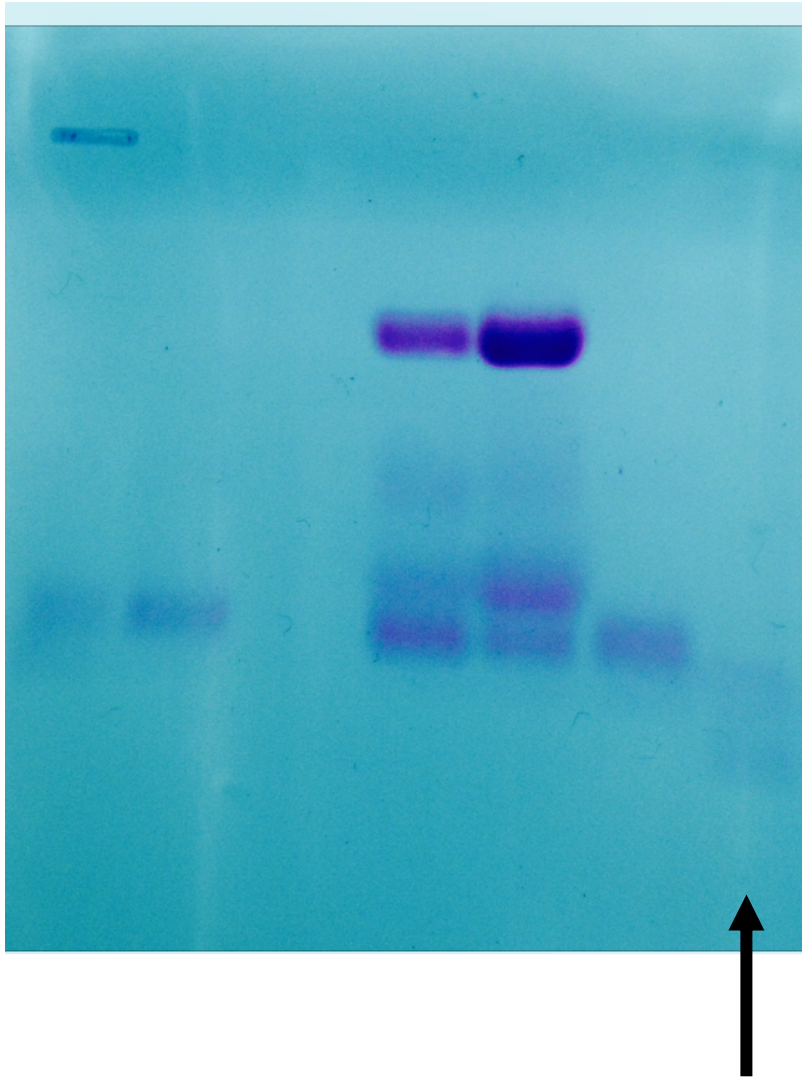
12.02.2014

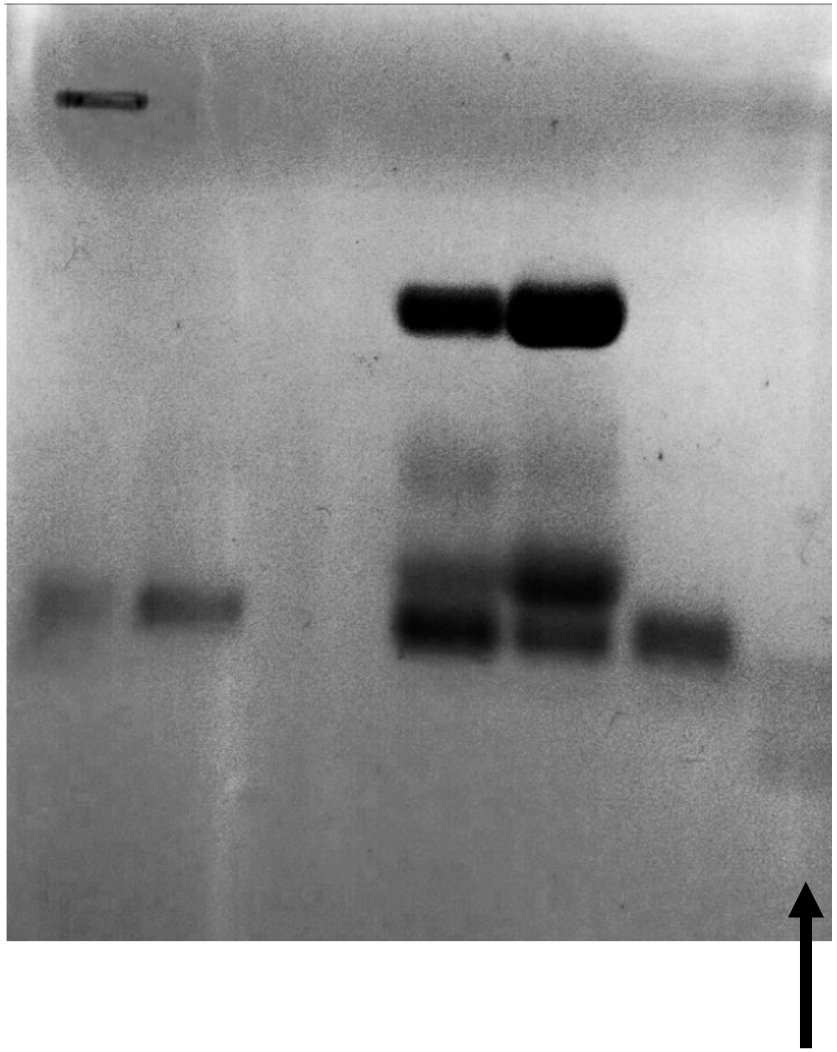


20.03.2014



ERNDIM SAMPLES





CONCLUSION

YOU make our own rules

For optimisation for the GAGE (GAG electrophoresis)

1-Accurate measurement of total GAG

2-Try to be realistic with the real life samples
Small volume of urine samples
Standardisation CPC – GAG precipitation
300 ug/mL GAG – 1000 uL CPC

3- Buffer molarity , pH , Voltage , Temperature must be optimised according to the system used : COLD medium for Keratan Sulfate better separation

4-Make accurate interpretation of lab results (less false positives and NO!!! false negatives)

Thank you !

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