

# ΑΝΑΠΤΥΞΗ ΒΙΟΤΡΑΠΕΖΑΣ ΜΕ ΣΚΟΠΟ ΤΗΝ ΑΝΙΧΝΕΥΣΗ ΠΡΩΙΜΩΝ ΒΙΟΔΕΙΚΤΩΝ ΣΕ ΑΣΘΕΝΕΙΣ ΜΕ ΧΡΟΝΙΑ ΝΕΦΡΙΚΗ ΝΟΣΟ

ΔΗΜΗΤΡΑ ΚΑΛΑΒΡΙΖΙΩΤΗ

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# ΣΚΟΠΟΣ

Δημιουργία Βιοτράπεζας για τη Χρόνια Νεφρική Νόσο στο Νεφρολογικό Κέντρο του Πανεπιστημιακού Νοσοκομείου Πατρών

- ⌚ Εντόπιση πρωτεϊνικών δεικτών που συνδέονται με διαφόρους τύπους ΧΝΝ.
- ⌚ Έλεγχος αποτελεσματικότητας δυνητικών βιοδεικτών που εμπλέκονται με τη νόσο ή σχετίζονται με την εξέλιξή της.
- ⌚ Παροχή ενός στρωματοποιημένου και καλά ταξινομημένου πληθυσμού ασθενών με ΧΝΝ για την ανίχνευση νέων βιοδεικτών.



ΠΑΝΕΠΙΣΤΗΜΙΟ  
ΠΑΤΡΩΝ  
UNIVERSITY OF PATRAS



The  
University  
Of  
Sheffield.



maine  
standards

# ΕΝΤΑΞΗ ΑΣΘΕΝΩΝ

Συλλογή επαναλαμβανόμενων δειγμάτων αίματος και ούρων (ανά 4 μήνες περίπου) από τουλάχιστον 450 ασθενείς με ΧΝΝ, 50 ασθενείς με διαβήτη χωρίς νεφρική συμμετοχή τη στιγμή της συλλογής και 50 υγιείς εθελοντές δότες.

- ⊕ Ένταξη ανοιχτή σε όλους τους τύπους ΧΝΝ
- ⊕ 25% διαβητική νεφροπάθεια και 2,5% FSGS
- ⊕ Διάρκεια: 3 έτη

# ΣΥΛΛΟΓΗ ΔΕΙΓΜΑΤΩΝ

☉ ΟΥΡΑ: 120-200 ml 2<sup>α</sup> πρωϊνά, απευθείας τοποθέτηση σε πάγο

1. 100 ml Complete Urine
2. 100 ml Cell Free Urine: 3.000 g για 10 min στους 4°C
3. Ίζημα

☉ ΑΙΜΑ: 10-15 ml

1. Πλάσμα : 1.000 g για 15 min στους 4°C
2. Ορός: 2.000 g για 10 min στους 4°C

☉ ΒΙΟΨΙΑ

Καταγραφή και φύλαξη των δειγμάτων με κωδικοποιημένο τρόπο



# Καταγραφή δειγμάτων και κλινικών δεδομένων

File Home Create External Data Database Tools

Αρχική
Ενέργειες
Προβολή
Επικοινωνία
Αρχεία & Έξτρα

View Paste Copy Format Painter Clipboard

Filter
Ascending
Descending
Remove Sort
Selection
Advanced
Toggle Filter
Sort & Filter
Refresh All
New
Save
Delete
More
Totals
Spelling
Find
Go To
Select
Replace
Find
Size to Fit Form
Switch Windows

Department of Nephrology and Renal Transplantation, University Hospital of Patras

Find a Patient Record




Patient Entered By

Study ID	PDN/ 227	Surname	[Redacted]	Ethnicity	Caucasian	CKD Diagnosis /	Chronic Glomerulonephritis
NHS Patient Number	[Redacted]	Forenames	[Redacted]	Employment Type	Manual	co-morbidity 1	Hypertension
DOB	09/10/1957	Gender	Male	Job Title		CKD Diagnosis /	
Is this a control_patient?	<input type="checkbox"/>	Address1	[Redacted]	Patient Height (m)	1.81	co-morbidity 2	
Is this a none CKD diabetic patient ?	<input type="checkbox"/>	Address2	[Redacted]	Smoker?	Smoker	CKD Diagnosis /	
Has this patient died ?	<input type="checkbox"/>	Address3	[Redacted]	Current Medications	medrol s. neoral zestril lepur omeprazol calcium d3	co-morbidity 3	
Date of death		Postal Town	patras	PAR code 1	1100:Minimal change nephropathy - hist	co-morbidity 4	
Has this patient had a kidney transplant ?	<input type="checkbox"/>	Postcode		PAR code 2			
Kidney transplant date		Telephone	[Redacted]				
Date Patient Joined Study	27/09/2016						

Sample Details
Sample Diabetic Data
Historic Data For This Patient

Sample Date 27/09/2016 Sample ID 288

Patient Weight (kg)	75.00	BMI	22.89	Blood Pressure: Systolic	140	Diastolic	80	Pulse (bpm)	70
BSA (Body Surface Area) (m2)	1.94	Waist Circum (cm)		Hip Circum (cm)		Mid Arm Circum (cm)		Triceps Skinfold (mm)	

urine? <input checked="" type="checkbox"/>	20ml Aliquots Complete Urine?	2	EGFR (ml/min/1.73m <sup>2</sup> )	105.00	serum_cholesterol (mg/dl)	194.00	Diabetic?	<input type="checkbox"/>
	1ml Aliquots Complete Urine?	10	Rate of Decline of EGFR (ml/min/year)	15.99	serum_triglycerides (mg/dl)	50.00	ACE Inhibitors?	<input checked="" type="checkbox"/>
	20ml Aliquots Cell Free Urine?	2	Urine Albumin (mg/lt)	0.00	serum_calcium (mg/dl)	9.10	ARBs?	<input type="checkbox"/>
	1ml Aliquots Cell Free Urine?	10	Urine Protein /Creatinine Ratio	0.00	serum_phosphorus (mg/dl)	3.60	Statins?	<input checked="" type="checkbox"/>
	Aliquots Urine Derived Cells?	1	Urine Albumin /Creatinine Ratio	0.00	serum_ca_p (mg2 x dl2)	32.76	CKD Stage? (1-5)	1
	Total Cells Recovered	1	Proteinuria (mg/dl)	0.00	serum_PTH (pg/ml)	42.00	AKI since last visit?	<input type="checkbox"/>
	Cell Conc in Urine (cells/ml)		Serum urea	36.00	serum_creatinine (mg/dl)	0.800		
	24 Hour Proteinuria	0.00			1/Creatinine Slope (current)	0.17		

Started dialysis ?

blood?  Serum Aliquots? 10 Plasma Aliquots? 10

biopsy?  SE - Glomerulosclerosis (From MT) 0 SE - Tubulointerstitial (From MT) 0 SE - Vascular Sclerosis (From MT) 0

urine creatinine (mg/dL) 33.92

**Sample Locations**

Complete Urine 20ml	1.47.1.9	+	?	-
Complete Urine 1ml	2.92.1.26	+	?	-
Cell Free Urine 20ml	1.47.3.9	+	?	-
Cell Free Urine 1ml	2.92.1.27	+	?	-
Serum	2.97.1.11	+	?	-
Plasma	2.97.1.12	+	?	-
Urine Cells	1.11.4.33	+	?	-
Biopsy Sample		+	?	-

The sample location is in format:  
Freezer.Draw.column.row  
e.g. 1.1.5.6

Navigation Pane

# Καταγραφή δειγμάτων και κλινικών δεδομένων

The image shows a software application window titled "Available holes on a draw" overlaid on a photograph of a laboratory freezer. The software interface includes a menu bar with options like "File", "Home", "Create", "External Data", and "Database Tools". Below the menu is a toolbar with various icons for file operations and data management. The main area of the software is divided into several sections:

- Form Fields:** Includes "Freezer:" (set to 2), "Draw:" (set to -92), "Function:" (set to LOCATE), "Sample:" (set to 288), and "Sample type:" (set to Complete Urine 1ml).
- Grid:** A grid with columns labeled "Box 1" through "Box 4" and rows labeled "col. 1" through "col. 10". The grid cells are color-coded: green for available locations, red for the current sample location, blue for selected locations, and yellow for used locations (other samples).
- Legend:** A legend at the bottom left explains the color coding: green for "Available location", red for "Current sample location", blue for "Selected location", and yellow for "Used location (other samples)".
- Buttons:** A "Return" button is located at the bottom of the grid area.


The background photograph shows a white Panasonic freezer with a digital display and control panel. A person's hand is visible near the open freezer door, which is filled with sample containers.

# Καταγραφή δειγμάτων και κλινικών δεδομένων

Αρχική
Ενέργειες
Προβολή
Επικοινωνία
Αρχεία & Έξτρα


File Home Create External Data Database Tools

View Paste Cut Copy Format Painter Filter Ascending Descending Remove Sort Advanced Toggle Filter Refresh All Delete More Records Find Replace Go To Select Find Size to Fit Form Switch Windows Text Formatting



## Department of Nephrology and Renal Transplantation, University Hospital of Patras

Filter/search data Close Form

Export to CSV file 

Find a Patient Record Add Patient Print Patient Record Save Patient Record Patient Entered By

Study ID: PON/ 227

NHS Patient Number: [Redacted]

Is this a control\_patient?

Is this a none CKD diabetic patient?

Has this patient died?

Has this patient had a kidney transplant?

Date Patient Joined Study: 27/09/2016

Surname: [Redacted]

Forenames: [Redacted]

DOB: 09/10/1957

Gender: Male

Address1: [Redacted]

Address2: [Redacted]

Address3: [Redacted]

Postal Town: patras

Postcode: [Redacted]

Telephone: [Redacted]

Ethnicity: Caucasian

Employment Type: Manual

Job Title: [Redacted]

Patient Height (m): 1.81

Smoker?: Smoker

Current Medications: medrol, s. neoral, zestril, lepur, omeprazol, calcium d3

CKD Diagnosis / Chronic Glomerulonephritis

co-morbidity 1: Hypertension

CKD Diagnosis / [Redacted]

co-morbidity 2: [Redacted]

CKD Diagnosis / [Redacted]

co-morbidity 3: [Redacted]

CKD Diagnosis / [Redacted]

co-morbidity 4: [Redacted]

PAK code 1: 1100:Minimal change nephropathy - hist

PAK code 2: [Redacted]

Sample Details Sample Diabetic Data Historic Data For This Patient

Sample Date: 27/09/2016 sample ID: 288

Patient Weight (kg)	75.00	BMI	22.89	Blood Pressure: Systolic	140	Diastolic	80	Pulse (bpm)	70
BSA (Body Surface Area) (m2)	1.94	Waist Circum (cm)		Hip Circum (cm)		Mid Arm Circum (cm)		Triceps Skinfold (mm)	

urine?

20ml Aliquots Complete Urine? 2

1ml Aliquots Complete Urine? 10

20ml Aliquots Cell Free Urine? 2

1ml Aliquots Cell Free Urine? 10

Aliquots Urine Derived Cells? 1

Total Cells Recovered 1

Cell Conc in Urine (cells/ml)

24 Hour Proteinuria 0.00

Started dialysis?

blood?  Serum Aliquots? 10 Plasma Aliquots? 10

biopsy?  SE - Serum albumin (From diff) 0 IE - Total protein (From diff) 0 VC - Vascular color (From diff) 0

urine creatinine (mg/dL) 53.92

Rate of Decline of EGFR (ml/min/year) 15.99

Urine Albumin (mg/l) 0.00

Urine Protein /Creatinine Ratio 0.00

Urine Albumin /Creatinine Ratio 0.00

Proteinuria (mg/dl) 0.00

Serum urea 86.00

serum\_cholesterol (mg/dl) 194.00

serum\_triglycerides (mg/dl) 50.00

serum\_calcium (mg/dl) 9.10

serum\_phosphorus (mg/dl) 3.60

serum\_ca\_p (mg<sup>2</sup> x dl<sup>2</sup>) 32.76

serum\_PTH (pg/ml) 42.00

serum\_creatinine (mg/dl) 0.800

1/Creatinine Slope (current) 0.17

Diabetic?

ACE Inhibitors?

ARBs?

Statins?

CKD Stage? 1 (1-5)

AKI since last visit?

### Sample Locations

Complete Urine 20ml	1.47.1.9	+	?	-
Complete Urine 1ml	1.92.1.26	+	?	-
Cell Free Urine 20ml	1.47.3.9	+	?	-
Cell Free Urine 1ml	2.92.1.27	+	?	-
Serum	2.97.1.11	+	?	-
Plasma	2.97.1.12	+	?	-
Urine Cells	1.11.4.33	+	?	-
Biopsy Swab		+	?	-

The sample location is in format: Freezer.Draw.column.row e.g. 1.1-5-6

Print Sample Labels Print Laminated Sample Labels

Add New Sample Save Sample Record

Navigation Pane



# Καταγραφή δειγμάτων και κλινικών δεδομένων

Navigation

Sample Details | **Sample Diabetic Data** | Historic Data For This Patient

Serum Glucose (mg/dl)	43.00
Haematuria (RBC/hpf)	
Micro Albuminuria (mg/g)	
HbA1c (%)	8.60
C Reactive Protein (mg/dl)	0.15
Retinopathy	
Neuropathy	
Haemoglobin (g/dl)	11.20
WBC (K/ $\mu$ L)	5.87
Platelets (K/ $\mu$ L)	173.00
Vitamin B12 (pg/ml)	234.00
Folate (ng/ml)	21.00
Serum Iron ( $\mu$ g%)	96.00
TIBC ( $\mu$ g/dl)	269.00

Navigation

Sample Details | Sample Diabetic Data | **Historic Data For This Patient**

Consultation Date	EGFR (ml/min/1.73m <sup>2</sup> )	Blood Pressure		Serum Creatinin (mg/dl)	Serum urea (mg/dl)	PCR
		Systolic	Diastolic			
22/01/2016	54	170	80	1.5	64.00	0.00
26/11/2015	50	180	85	1.6	64.00	0.00
30/04/2015	54	120	80	1.5	59.00	0.00
02/12/2014	62	130	90	1.3	67.00	0.00
02/10/2014	50	125	90	1.6	98.00	#####
02/06/2014	64	145	80	1.3	54.00	0.00
04/11/2013	64	145	80	1.3	69.00	#####
02/07/2013	64	0	0	1.3	69.00	#####
04/02/2013	55	150	90	1.5	78.00	0.00
29/10/2012	65	110	70	1.3	52.00	0.00

Rate of decline of eGFR/time

1/creatinine slope

# FIBROTIC SIGNATURE

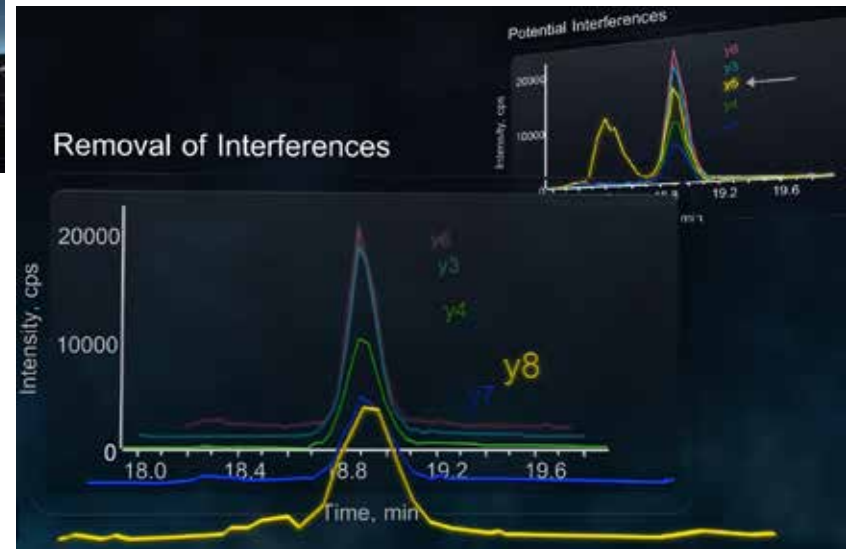
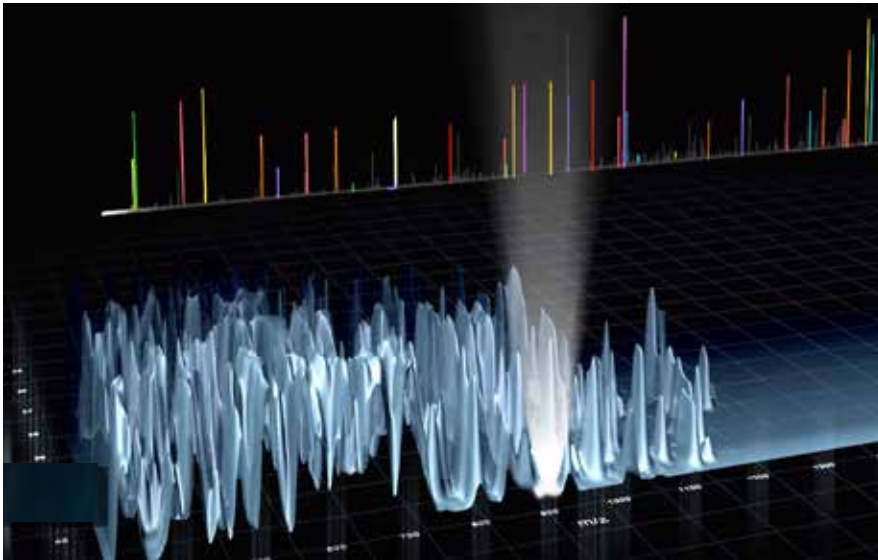
Πρωτεΐνη	Βιολογικό Υλικό
KIM-1	Urine
TIMP-1	Urine
EGF	Urine
pro-COL1A1	Urine
Clusterin	Urine
MCP-1	Urine
MMP-9	Urine
NGAL	Serum
uPAR	Serum



# ΑΝΕΥΡΕΣΗ ΝΕΩΝ ΒΙΟΔΕΙΚΤΩΝ

- ⌚ SWATH proteomics – Nottingham UK (CFU & plasma)
- ⌚ SomaScan – SomaLogic USA (CFU & plasma)
- ⌚ SnFN analysis – Tufts USA (plasma)
- ⌚ miRNA profiling – Cardiff UK (CFU & plasma)
- ⌚ Neoepitopes – Nordic Bioscience (CFU & serum)

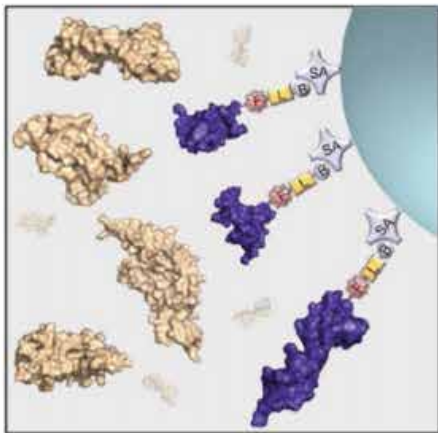
# SWATH proteomics (CFU & PLASMA)



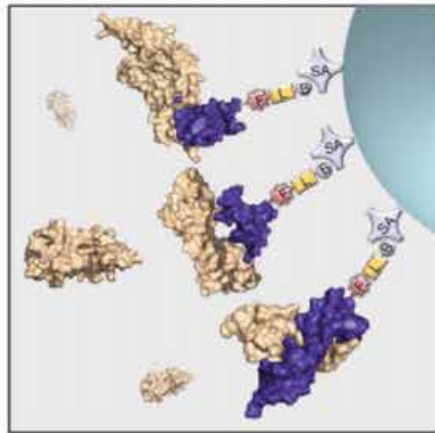
# ΑΝΕΥΡΕΣΗ ΝΕΩΝ ΒΙΟΔΕΙΚΤΩΝ

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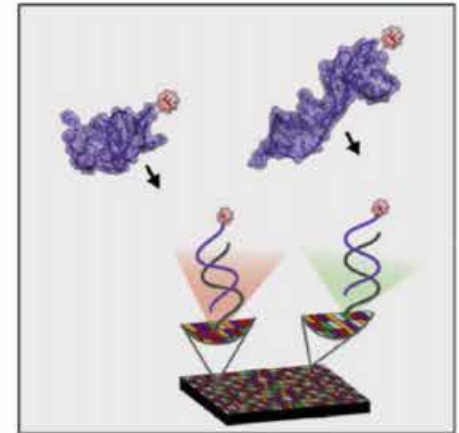
# SomaScan (CFU & PLASMA)



Mix plasma and immobilized SOMAmer® reagents



Capture specific proteins, remove unbound proteins and SOMAmer reagents

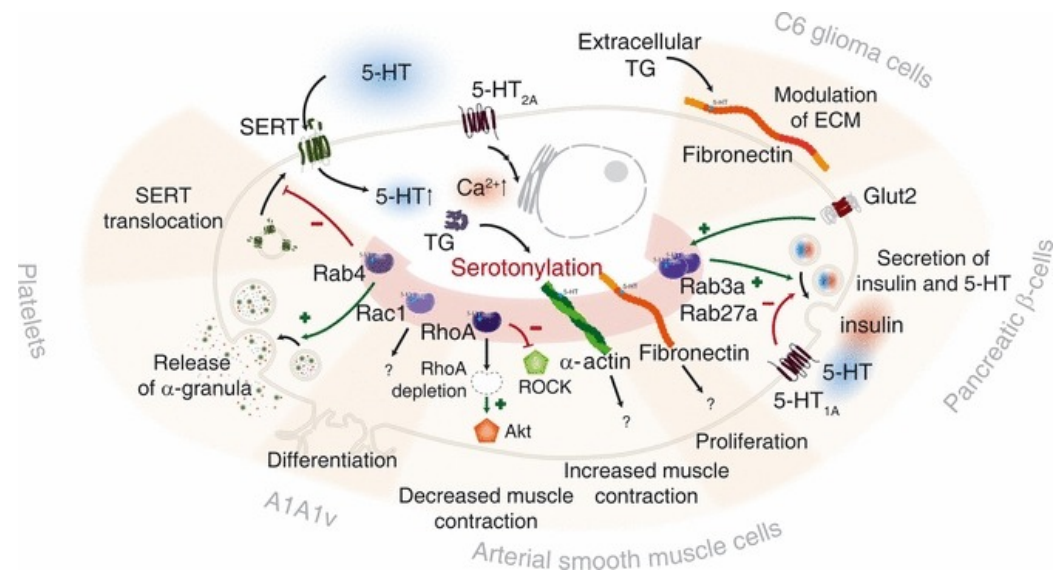
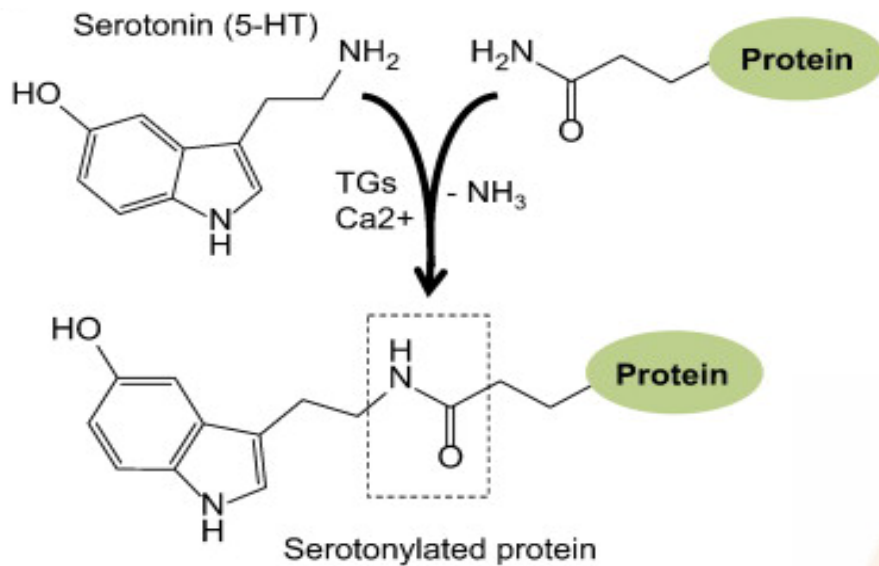


Quantify SOMAmer reagents by hybridization

# ΑΝΕΥΡΕΣΗ ΝΕΩΝ ΒΙΟΔΕΙΚΤΩΝ

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- ⌚ Neoepitopes – Nordic Bioscience (CFU & serum)

# SnFN (Serotonylated Fibronectin) analysis (PLASMA)

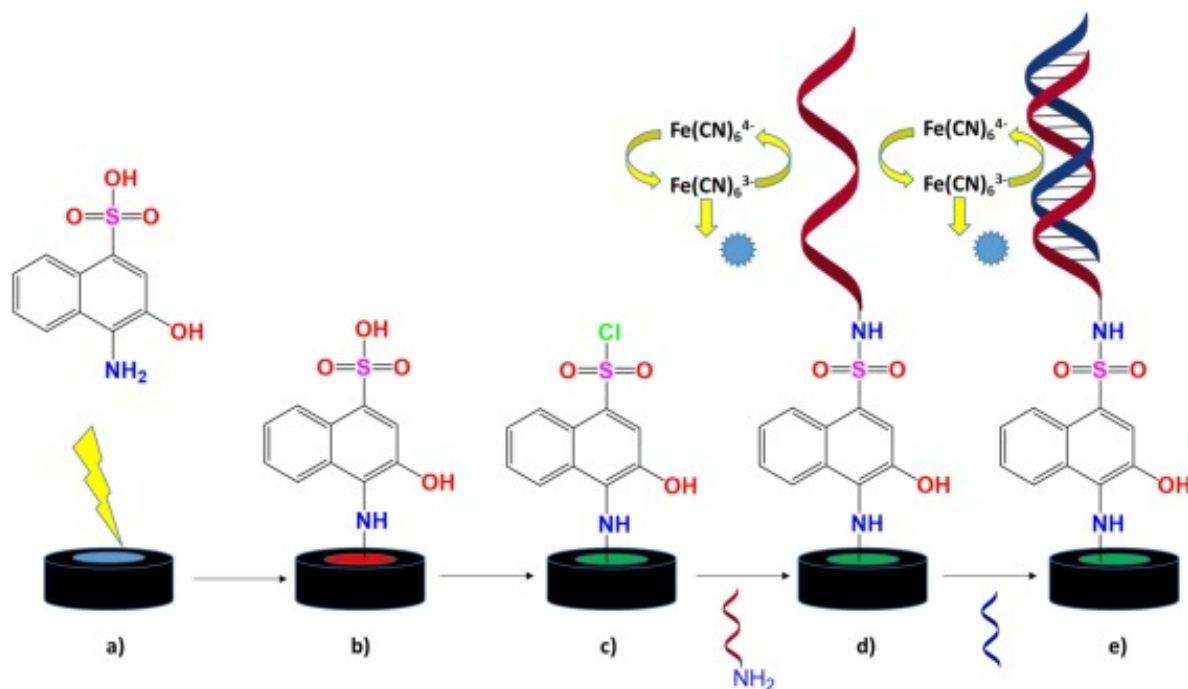




# ΑΝΕΥΡΕΣΗ ΝΕΩΝ ΒΙΟΔΕΙΚΤΩΝ

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# miRNA profiling (CFU & PLASMA)



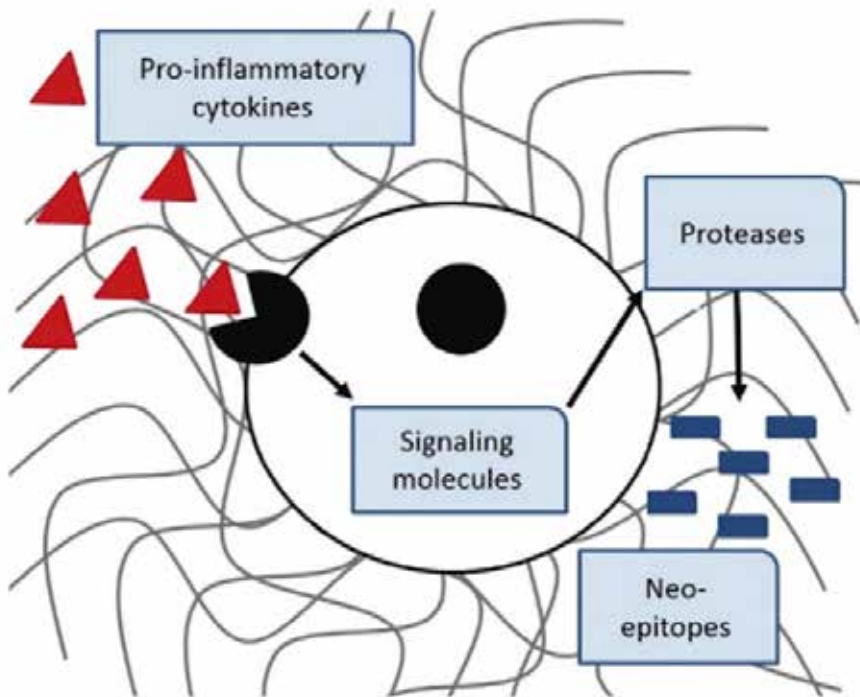
Schematic representation of the fabrication and operation procedure of the biosensor: a, b) A naphthalene sulfonic acid is electrochemically deposited via cyclic voltammetry onto a glassy carbon electrode surface and c) subsequently chlorinated using  $\text{PCl}_5$ . d) A DNA oligonucleotide with complementary sequence to the miRNA target (shown in red) is then added and an electrochemical analysis in ferri/ferrocyanide performed. e) Finally, the electrode is hybridised with the target miRNA (blue) and a second comparative electrochemical analysis performed.

*Smith D.A. et al Sensors and Actuators B: Chemical Vol. 253, Dec 2017, P 335-341.*

# ΑΝΕΥΡΕΣΗ ΝΕΩΝ ΒΙΟΔΕΙΚΤΩΝ

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- ⌚ Neoepitopes – Nordic Bioscience (CFU & serum)

# Neoepitopes (CFU & SERUM)



## Nordic Bioscience Fingerprint™ Technology - Assay Portfolio

FORMATION BIOMARKERS

DEGRADATION BIOMARKERS

ECM RELATED BIOMARKERS

WOUND HEALING BIOMARKERS

EXTERNALLY SOURCED BIOMARKERS

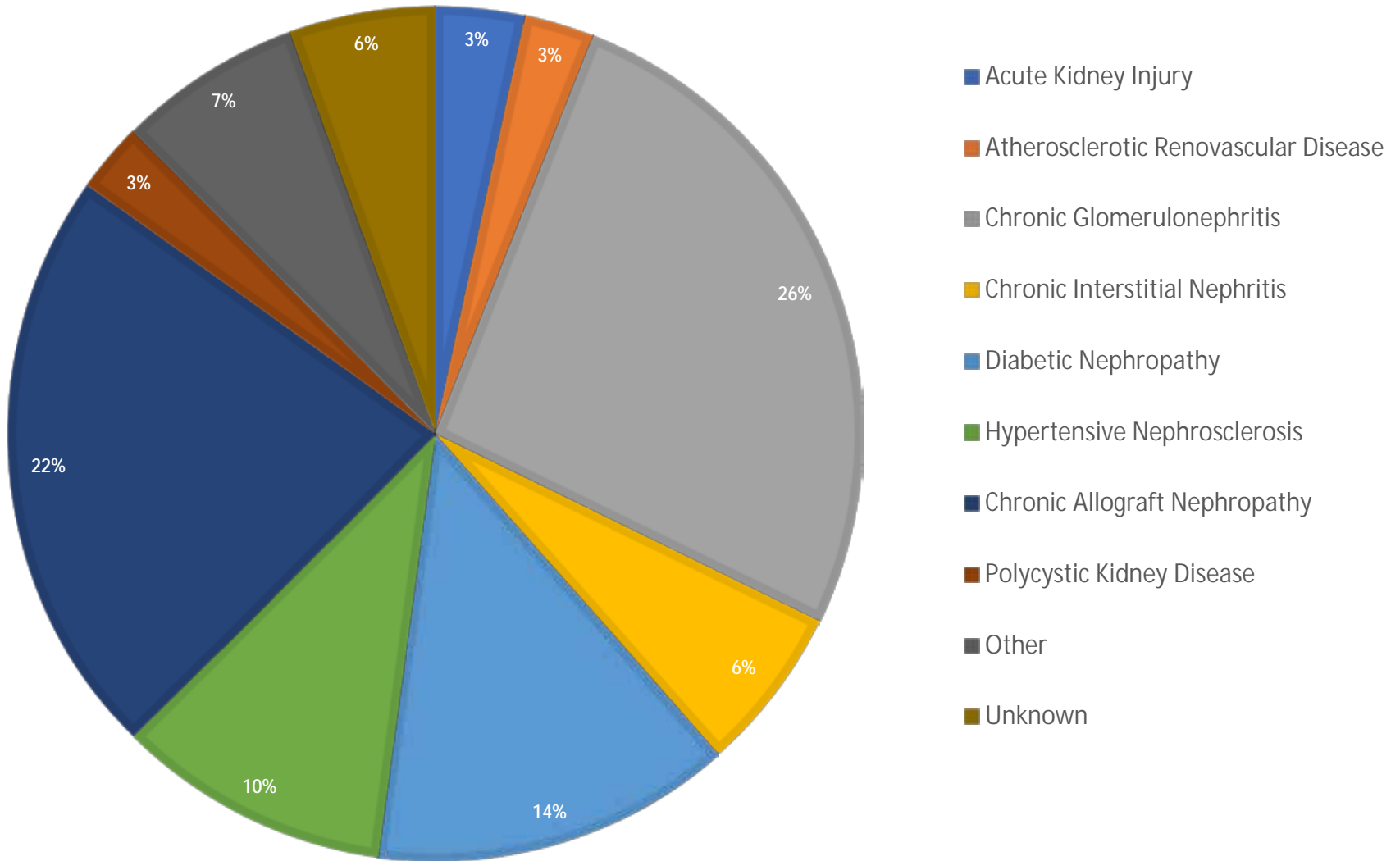
## 1.277 Συλλογές Δειγμάτων

Ασθενείς με ΧΝΝ	
1 <sup>η</sup>	N=419
2 <sup>η</sup>	N=294
3 <sup>η</sup>	N=214
4 <sup>η</sup>	N=153
5 <sup>η</sup>	N=95
6 <sup>η</sup>	N=40
Βιοψία	N=34
Υγιείς εθελοντές δότες	N=20
Ασθενείς με διαβήτη χωρίς νεφρική συμμετοχή	N=42

## ΔΗΜΟΓΡΑΦΙΚΑ ΣΤΟΙΧΕΙΑ

Ασθενείς με ΧΝΝ (N=419)	Φύλο (Α/Γ)	296/123	(70,67% - 29,33%)
	Ηλικία (έτη)	57,50 ± 16,07	
Υγιείς εθελοντές δότες (N=20)	Φύλο (Α/Γ)	13/7	(65% - 35%)
	Ηλικία (έτη)	41,3 ± 15,04	
Ασθενείς με Σ.Δ. χωρίς νεφρική προσβολή (N=42)	Φύλο (Α/Γ)	24/18	(57,5% - 42,5%)
	Ηλικία (έτη)	61,64 ± 10,72	

## ΑΣΘΕΝΕΙΣ (N=419)



## ΑΣΘΕΝΕΙΣ (N=419)

### Ταξινόμηση ασθενών βάσει του ρυθμού πτώσης σπειραματικής διήθησης

Σταθερή (ρυθμός πτώσης eGFR < 1 ml/min/year)	N=128
Μέτρια (1 < ρυθμός πτώσης eGFR < 4 ml/min/year)	N=252
Επιδείνωση (ρυθμός πτώσης eGFR > 4 ml/min/year)	N=39



## ΑΣΘΕΝΕΙΣ (N=419)

Συνοσηρότητα	Αρ. Ασθενών (N)
Υπέρταση	340
Οστική Νόσος	147
Αναιμία	117
Διαβήτης	116
Στεφανιαία Νόσος	72
Συμφορητική Καρδιακή Ανεπάρκεια	42
Περιφερική Αγγειοπάθεια	39
Καρκίνος	30
Αγγειακό Εγκεφαλικό Επεισόδιο	17

# ELISA – ΚΡΙΤΗΡΙΑ ΑΠΟΔΟΧΗΣ ΠΕΙΡΑΜΑΤΟΣ

	1	2	3	4	5	6	7	8	9	10	11	12
A	STD-07	STD-07	QC-L	QC-L								
B	STD-06	STD-06	QC-M	QC-M								
C	STD-05	STD-05	QC-H	QC-H								
D	STD-04	STD-04										
E	STD-03	STD-03										
F	STD-02	STD-02									QC-L	QC-L
G	STD-01	STD-01									QC-M	QC-M
H	Blank	Blank									QC-H	QC-H

$$CV (\%) = \frac{\text{Standard deviation (n replicates)}}{\text{Mean calculated concentration (n replicates)}} \times 100 \quad (\leq 20 \%)$$

$$RE (\%) = \frac{(\text{mean calculated concentration} - \text{nominal concentration})}{\text{Nominal concentration}} \times 100 \quad (\pm 20 \%)$$

# ΑΝΑΠΤΥΞΗ ΒΙΟΤΡΑΠΕΖΑΣ ΜΕ ΣΚΟΠΟ ΤΗΝ ΑΝΙΧΝΕΥΣΗ ΠΡΩΙΜΩΝ ΒΙΟΔΕΙΚΤΩΝ ΣΕ ΑΣΘΕΝΕΙΣ ΜΕ ΧΡΟΝΙΑ ΝΕΦΡΙΚΗ ΝΟΣΟ

ΔΗΜΗΤΡΑ ΚΑΛΑΒΡΙΖΙΩΤΗ

Βιοχημικός MSc, PhD

Υπεύθυνη Ερευνητικού Εργαστηρίου Νεφρολογικού Κέντρου ΠΓΝΠ

