THE IMPORTANCE OF SERUM HYALURONIDASE MEASUREMENT IN DISCRIMINATION OF PATIENTS WITH PROSTATE CANCER AND BENIGN PROSTATIC HYPERPLASIA

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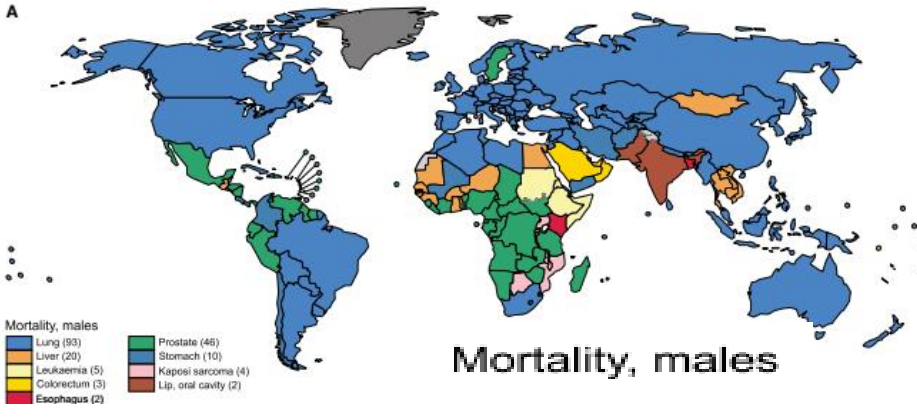
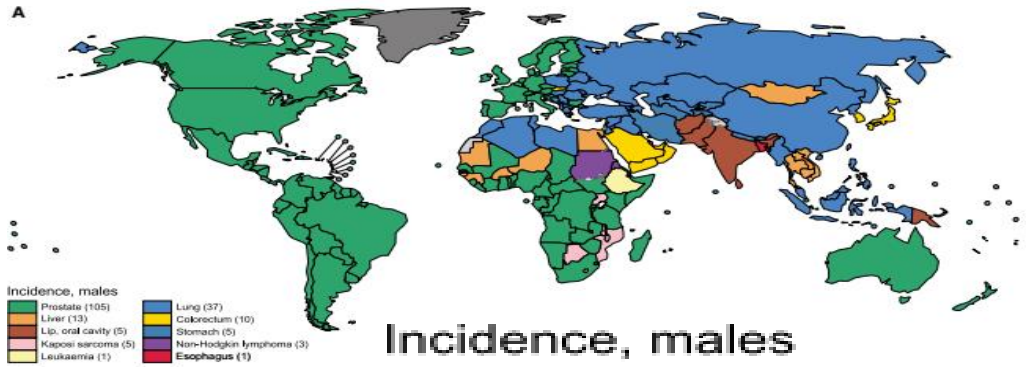
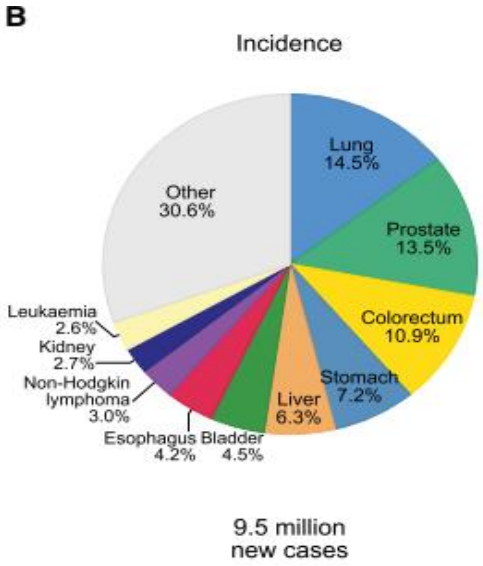
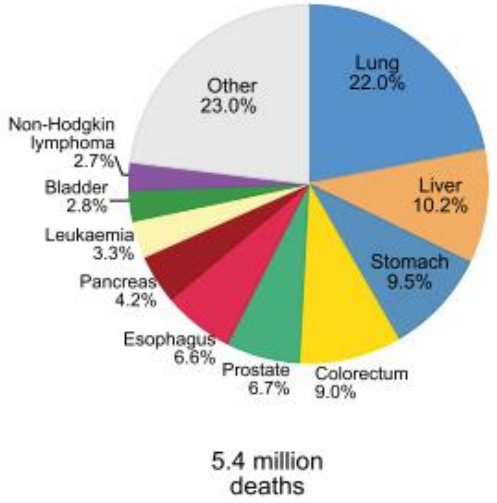
PROSTATE CANCER

- **Second most common cancer in men**
- **5th leading cause of cancer-related death worldwide**









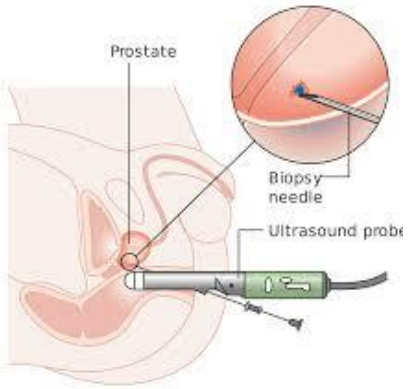
Males

Prostate

World Health Organizations - GLOBOCAN, 2018.

PROSTATE CANCER

- ❑ Screening
  - ✓ Serum total prostate specific antigen (PSA)
  - ✓ Digital rectal examination
- ❑ Diagnosis
  - ✓ Most commonly used marker: PSA
- ❑ Definitive diagnosis
  - ✓ Biopsy



PROSTATE CANCER

PSA:

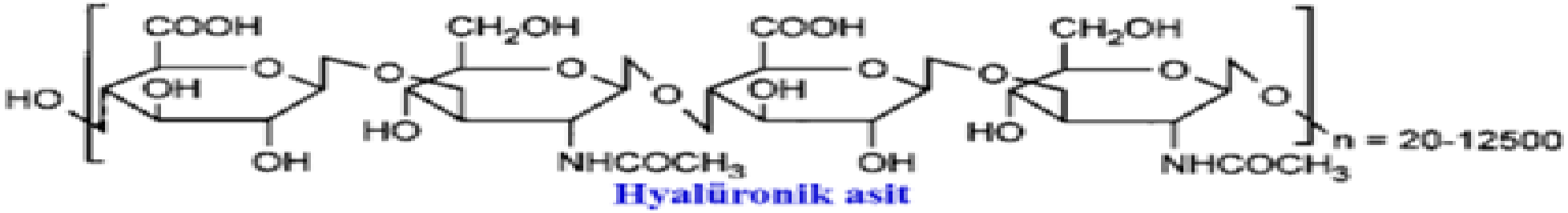
- Specificity and positive predictive value are *low.*
- *Absolute lower limit for cancer* diagnosis has not been defined yet.

❑ ***Parameters used as secondary support:***

- ✓ PSA derivatives *(PSA density and age-specific PSA)*
- ✓ Molecular forms of PSA *(percentage of free PSA and proPSA)*
- ✓ PSA kinetics *(PSA increase rate and doubling time)*
- ✓ Imaging of the prostate

HYALURONIC ACID (HA)

- Structural component of the extracellular matrix
- High molecular weight, non-sulfated, linear, unbranched glycosaminoglycan
- Consists of a repetition of β-1,3-N-acetyl-glucosamine and β-1,4-D-glucuronic acid molecules bound by glycosidic bonds



- It is an active signaling molecule
  - *provides an ideal environment for cell proliferation, apoptosis, migration, differentiation and morphogenesis*

HYALURONIDASE (HYAL)

- Endoglycosidase (EC 3.2.1.35)
- Degrades hyaluronic acid (HA) → small fragments with angiogenic properties are formed
- HYAL has been detected at different levels in tissue, serum and urine in many diseases and cancers.

Objective;

- Investigate the ability of serum **HYAL activity and mass concentration to** distinguish prostate cancer from benign conditions and compare it with PSA.

- Evaluate the clinical and analytical performance of serum HYAL activity measurement method.

MATERIALS and METHODS

- Colorimetric HYAL activity in serum and plasma was first measured by Morgan and Elson (1933).
- Morgan-Elson colorimetric determination method was modified in 1955 by Reissig et al.
- Takahashi et al. optimized the Reissig method in 2003.

- We used the following methods in our study,

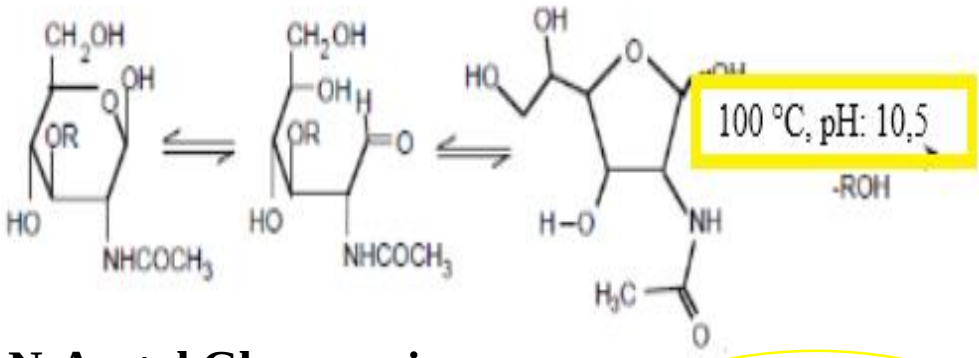
➢ The method **optimized by Takahashi et al** in the measurement of serum **HYAL activity (HYALa)**

➢The **sandwich ELISA method** for the determination of **serum HYAL concentration (HYALc)**

Anal. Biochem. 2003, 322, 257-263.

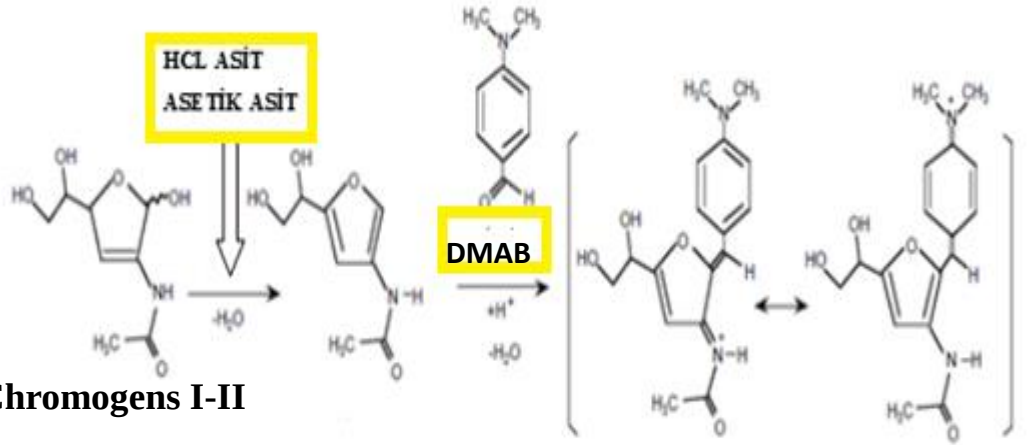
HYAL ACTIVITY PROCEDURE

	Sample (µL)	Blank (µL)	Standard (µL)
Sample (µL)	50	-	-
Standard (µL)	-	-	50
Deionized water	-	50	-
Substrate solution	250	250	250
Incubation	37 °C, 15 min		
Water bath	95 – 100 °C, 5 min		
Tetraborate Reagent	100	100	100
Water bath	95 – 100 °C, 3 min		
Dimethylaminobenzaldehyde Reagent	1500	1500	1500
Incubation	37 °C, 20 min		
Centrifugation at 1500 g	4 °C, 10 min		
Reading against blank at 585 nm			



N-Acetyl Glucosamine (NAG)

Morgan-Elson Reaction

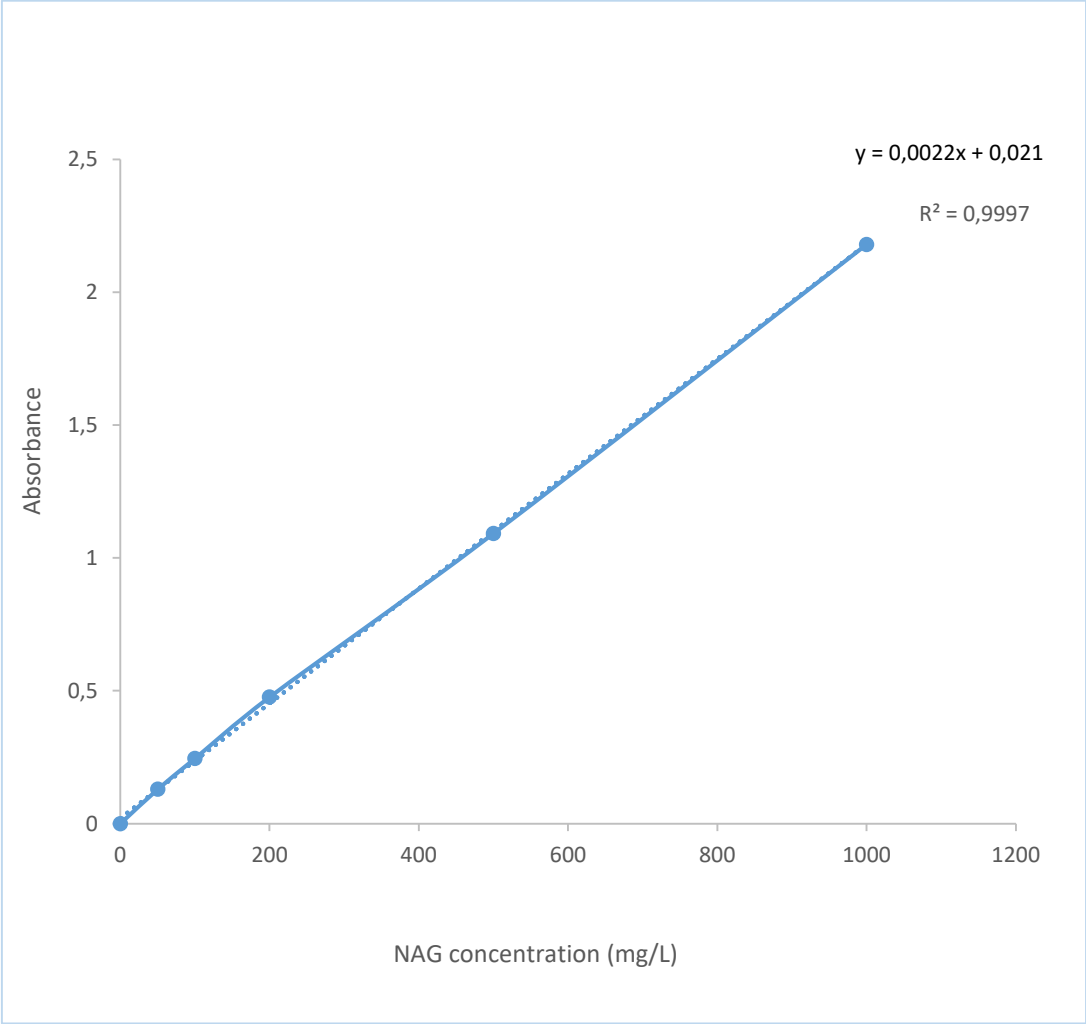


Chromogens I-II

Chromogen III

Red-colored product

ε: Calculation of the molar absorptivity coefficient of NAG at 585 nm:



NAG concentration-absorbance linear regression graph and equation

A=a x b x c

A: Absorbance

a: Molar absorptivity coefficient (ε)

b: Light Path

c: Concentration

Absorbance of 100 mg/L NAG (221.2 g/mol) standard = 0.245

Molar Absorptivity Coefficient=20598 L.mol-1.cm-1

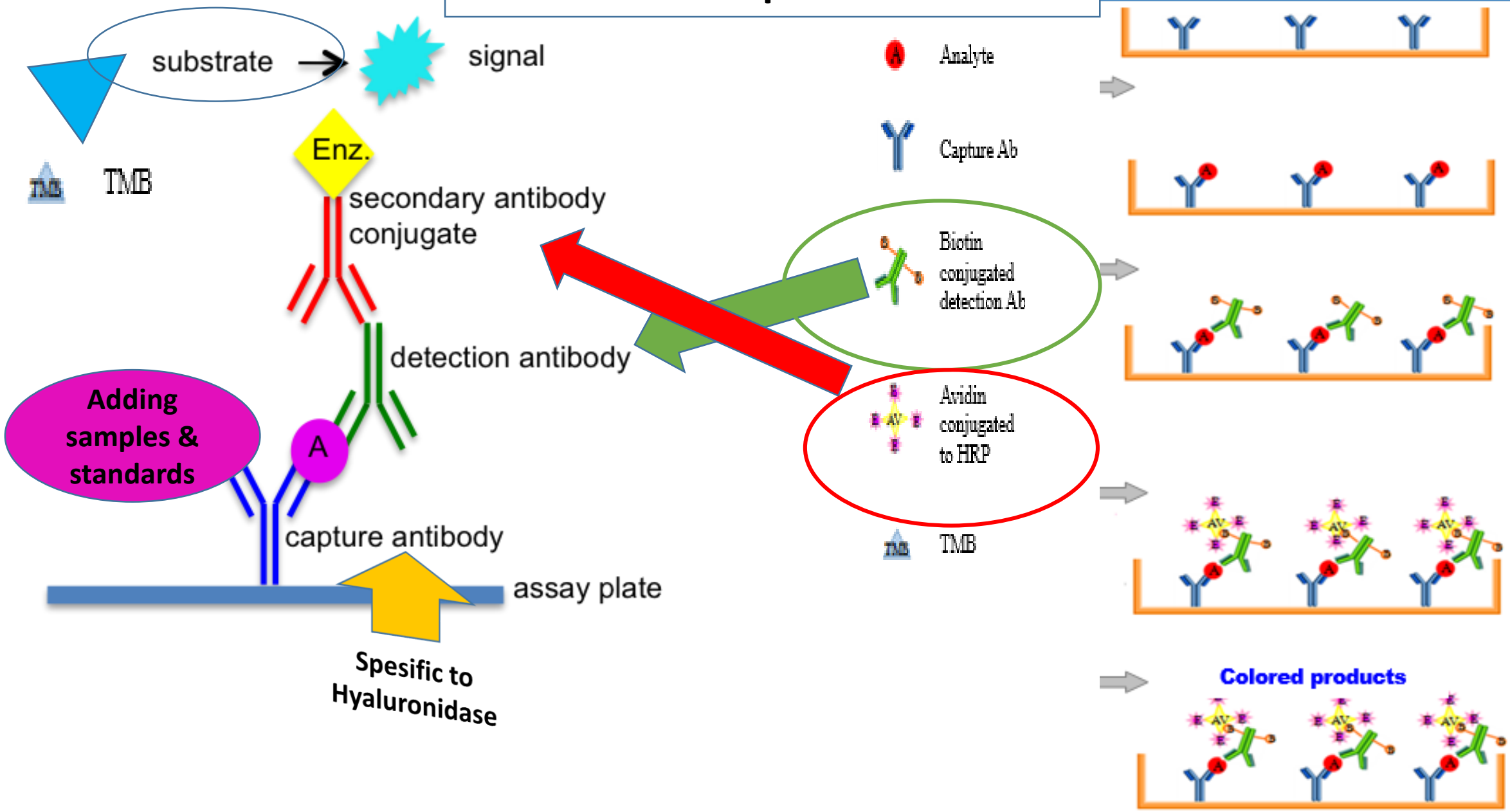
With the present procedure the molar extinction coefficient of the chromogen (based on acetylhexosamine concentration) is about 21,000 for *N*-acetylglucosamine.

J. L. REISSIG, J. L. STROMINGER, AND L. F. LELOIR

J. Biol. Chem. 1955, 217, 959-966.

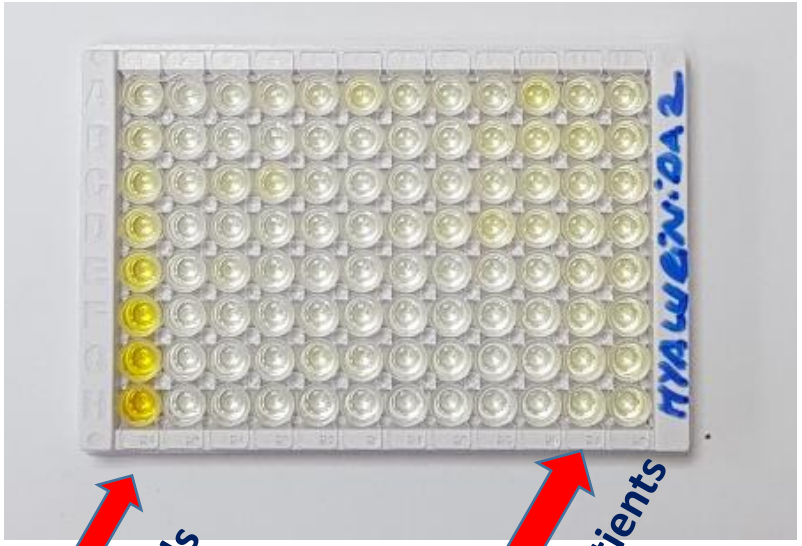
Sandwich ELISA

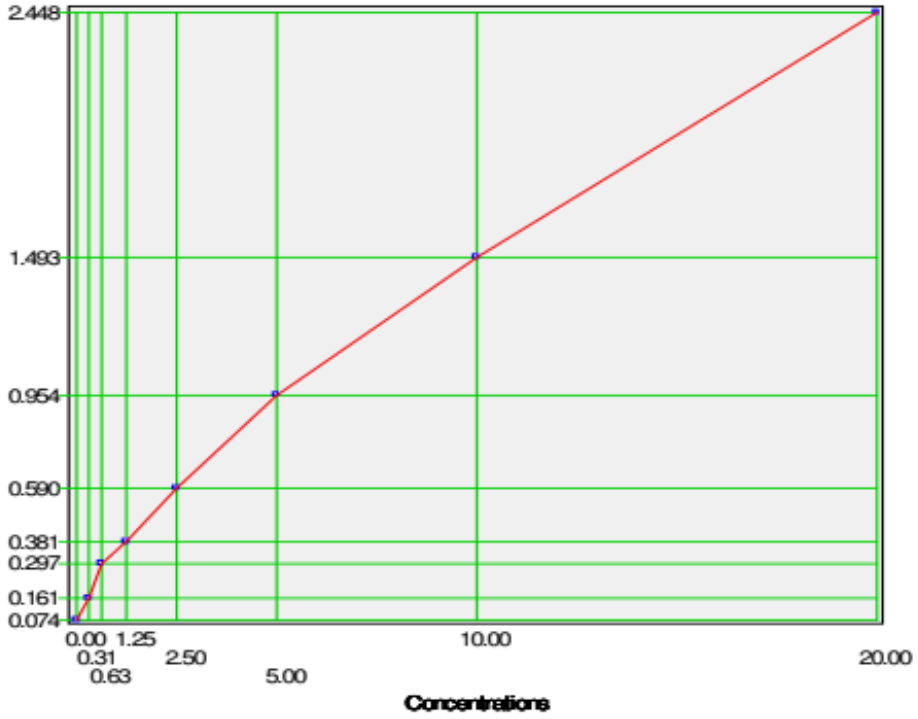
Then read the plate at 450 nm



Sandwich ELISA HYAL CONCENTRATİON

- The concentration of hyaluronidase in the samples is determined by comparing the O.D. of the samples to the standard curve.





PATIENT AND CONTROL GROUPS

September 2018-July 2019

- **Our study included age-matched 37 newly diagnosed PC, 72 benign prostatic hyperplasia (BPH), 53 chronic prostatitis (CrP) patients according to biopsy results and 49 control patients.**

Hemolysed, lipemic, icteric sera; other cancers; liver, rheumatologic, collagen tissue diseases and dermatological disorders that could increase serum HYAL levels were excluded.

GROUPS (HYALa)	n
BPH	63
CrP	45
PC	33
Control	30

PATIENT AND CONTROL GROUPS

Patient, HYALa (n)	BPH n = 63	CrP n = 45	PC n = 33	Control n=30
Age, year; x ± s	63 ± 7 year	64 ± 7 year	65 ± 9 year	60 ± 8 year
DM (n)	11	10	6	-
HT (n)	11	23	9	-
CAD (n)	2	10	5	-
Family history of cancer (n)	0	1	1	-

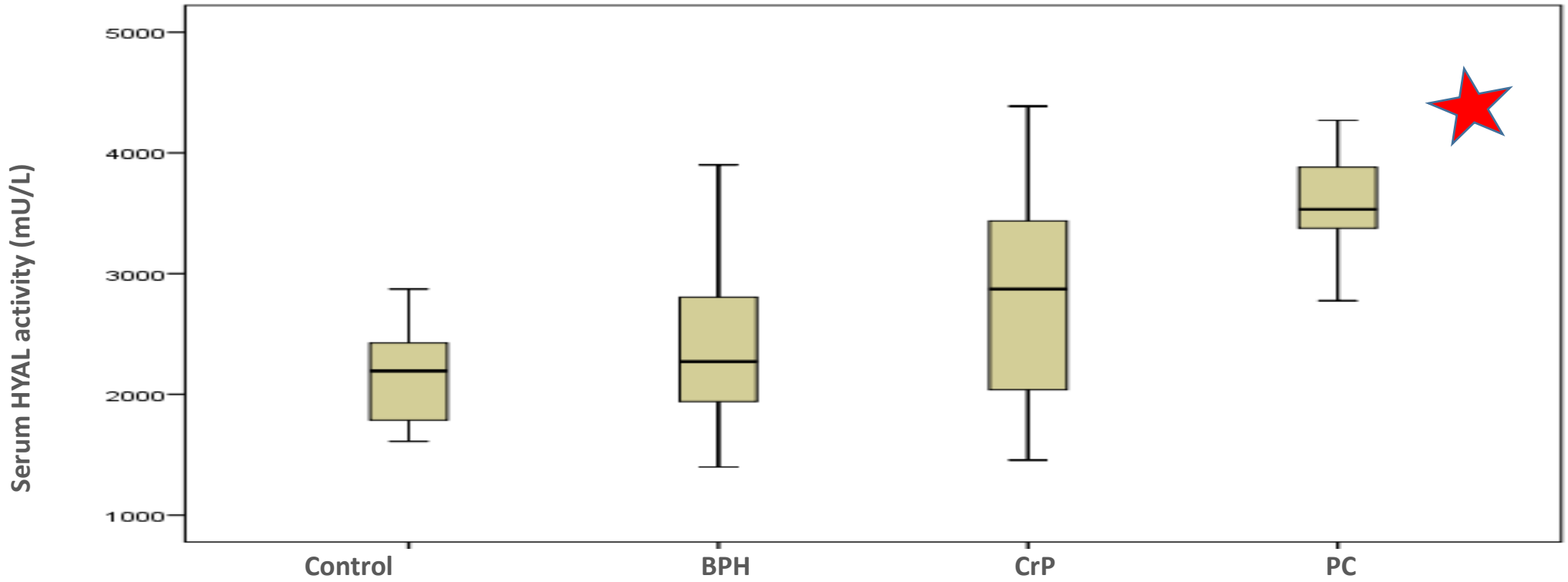
- **DM, HT, CAD which could increase serum HYAL levels, were not excluded at the beginning as it would decrease the number of patients.**
  - ***At the end of the study, these three diseases were excluded and evaluated separately.**
  - ***BPH (n)=46, *CrP (n)=20, *PC(n) =19**

HYALc = 22 patients from each group were studied.

RESULTS

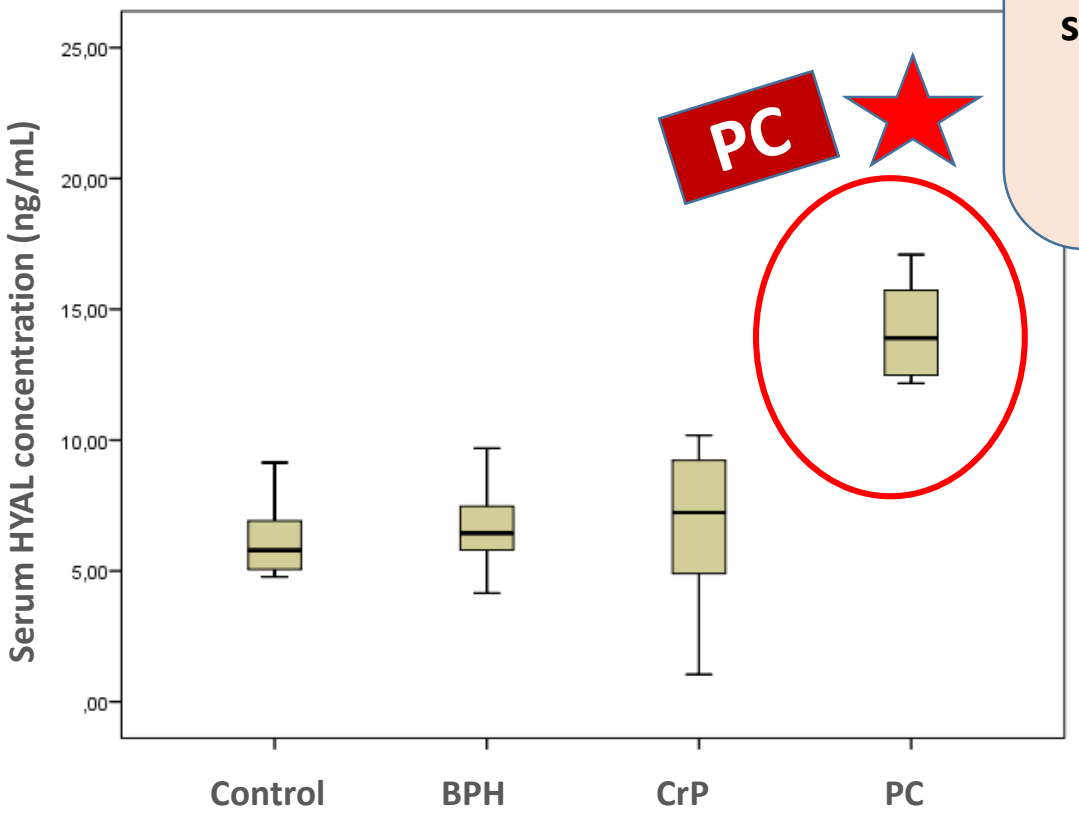
Groups	Control x ± s (n=30)	BPH x ± s (n=63)	CrP x ± s (n=45)	PC x ± s (n=33)	p
HYALa (mU/L)	2176 ± 387	2387 ± 584	2816 ± 809	**3559 ± 441**	<0.01
PSA (µg/L)	1,17 ± 0,91	7,24 ± 4,72	6,96 ± 3,11	**47,04 ± 91,45**	<0.01

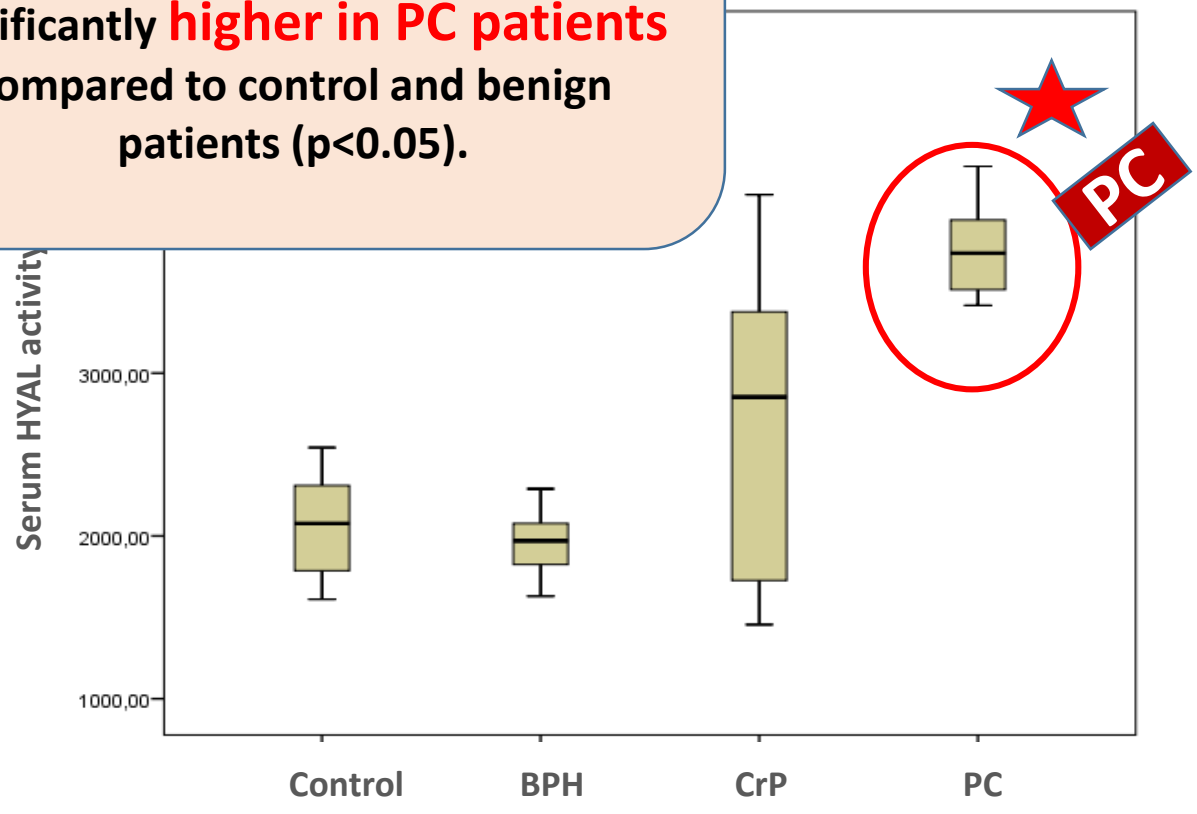
x: average, s: standard deviation



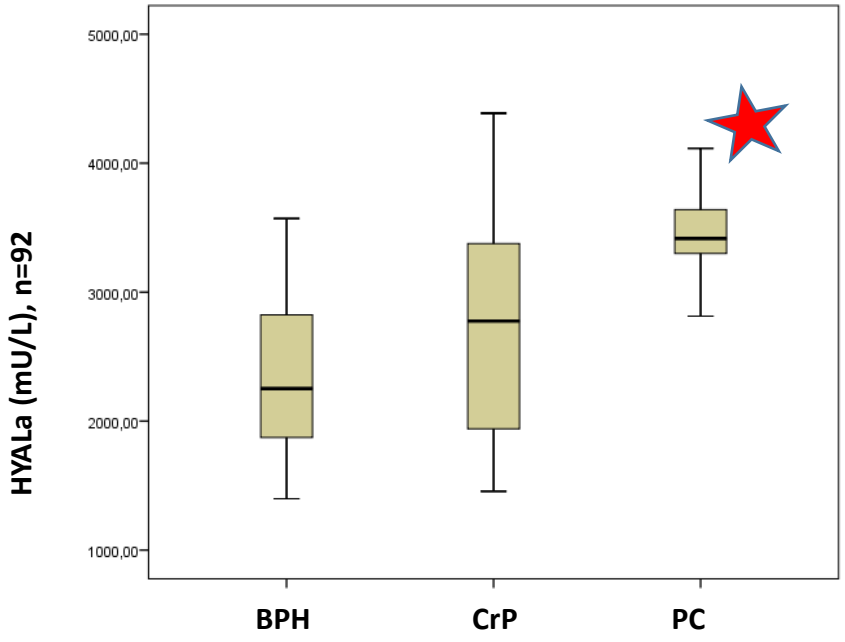
Groups	Control x ± s (n=22)	BPH x ± s (n=22)	CrP x ± s (n=22)	PC x ± s (n=22)	p
HYALc (ng/mL)	7,00 ± 3,2	7,30 ± 3,4	8,60 ± 5,4	**13,42 ± 3,7**	<0.01
HYALa (mU/L)	2052 ± 65	1945 ± 55	2697 ± 177	**3775 ± 58**	<0.01

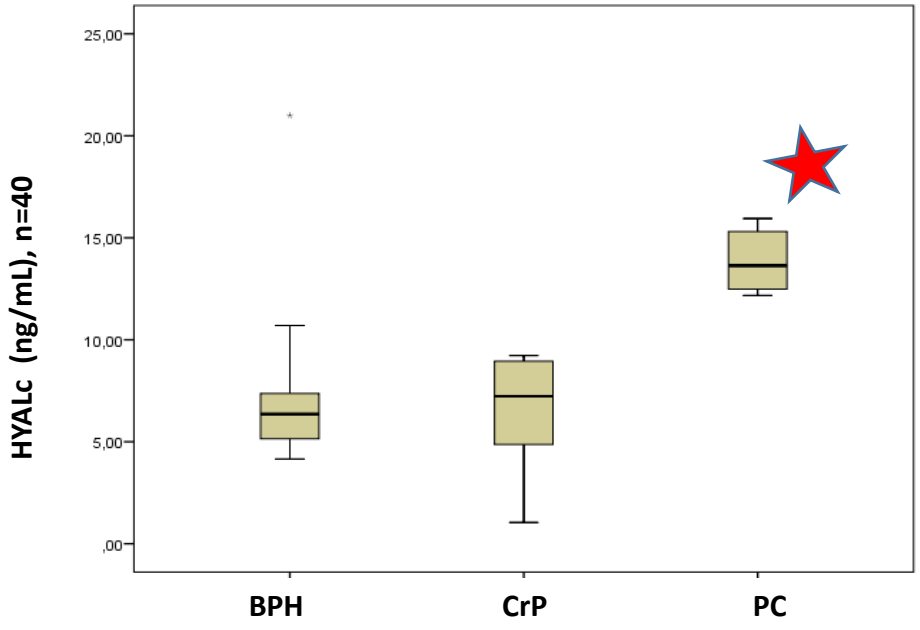
Serum **HYALa, HYALc and total PSA** levels were found to be significantly **higher in PC patients** compared to control and benign patients (p<0.05).

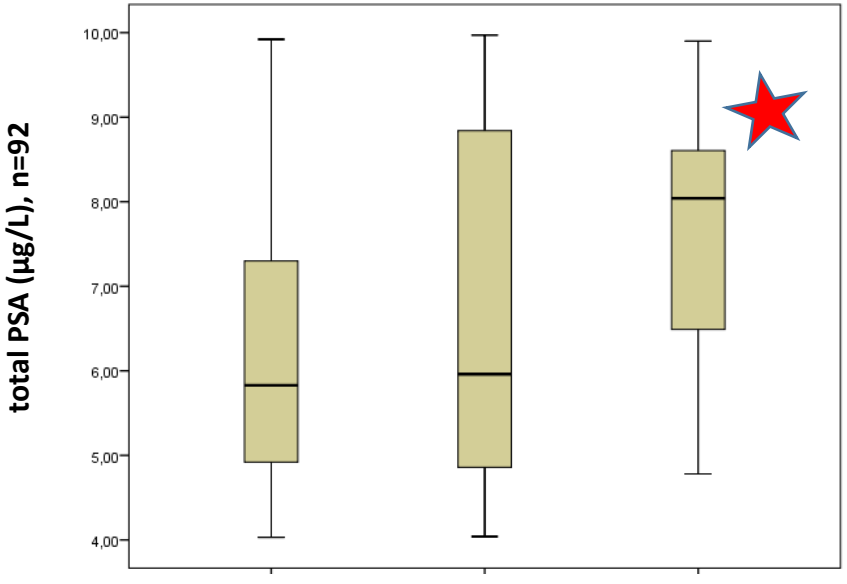


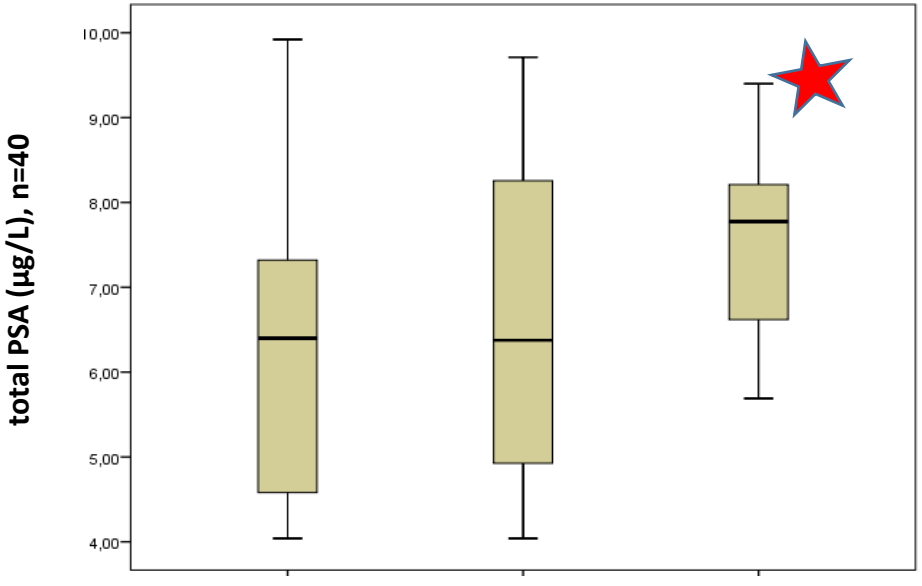


HYALa, HYALc and total PSA levels were found to be significantly **higher in PC patients** with PSA values in **GRAY ZONE** (4-10 µg/L) compared to other benign patient groups ($p<0.05$).





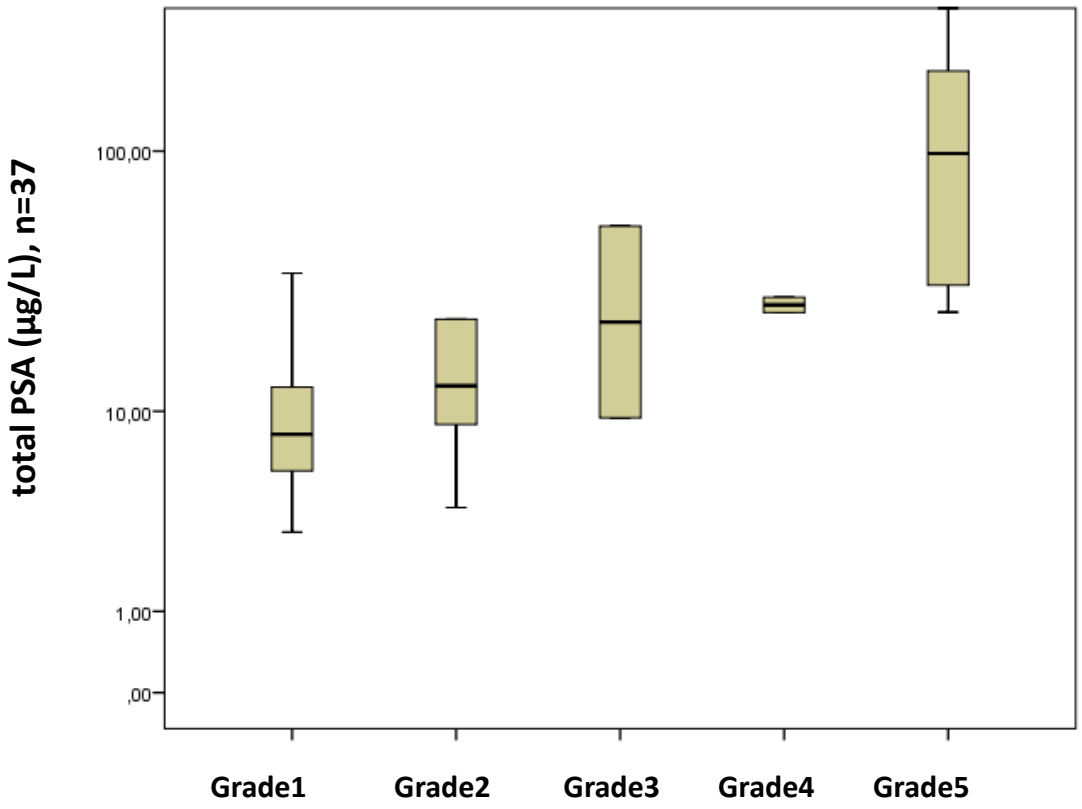




PC Gleason rating system

- **PSA : Grade Group 5 was significantly higher** than the other groups ($p<0.05$)
- **HYALa and HYALc : no significant** difference between the groups ($p>0.05$)

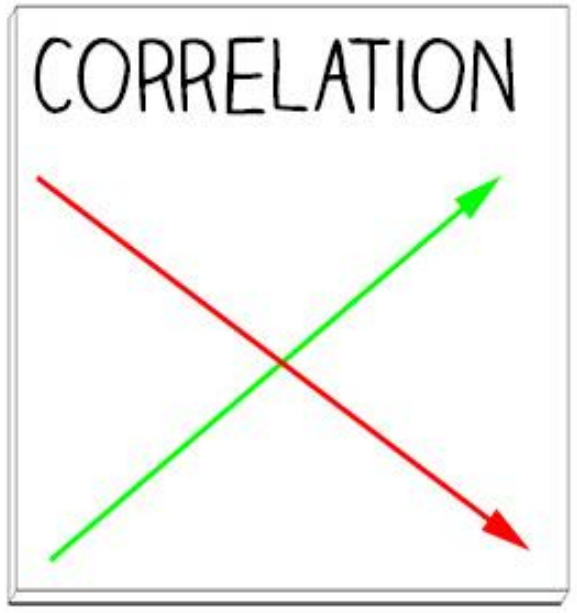
WHO GRADE GRUP	total PSA (µg/L) x ± s	n
Grade grup 1 (3+3=6)	10,44 ± 7,27	23
Grade grup 2 (3+4=7)	49,28 ± 83,43	5
Grade grup 3 (4+3=7)	30,83 ± 30,31	2
Grade grup 4 (4+4=8)	26,29 ± 2,51	2
Grade grup 5 (9-10)	**166,72 ± 189,12**	5



- Serum HYALa and serum total PSA
- Serum HYALc and serum total PSA

r=0.405 p<0.05 n=171

r=0.344 p<0.05 n=88



Serum HYAL activity and mass concentration

r=0.743 p<0.05 n=88

METHOD PERFORMANCE STUDIES

ANALYTIC

- Linearity
- Precision
- Recovery
- Detection Limits

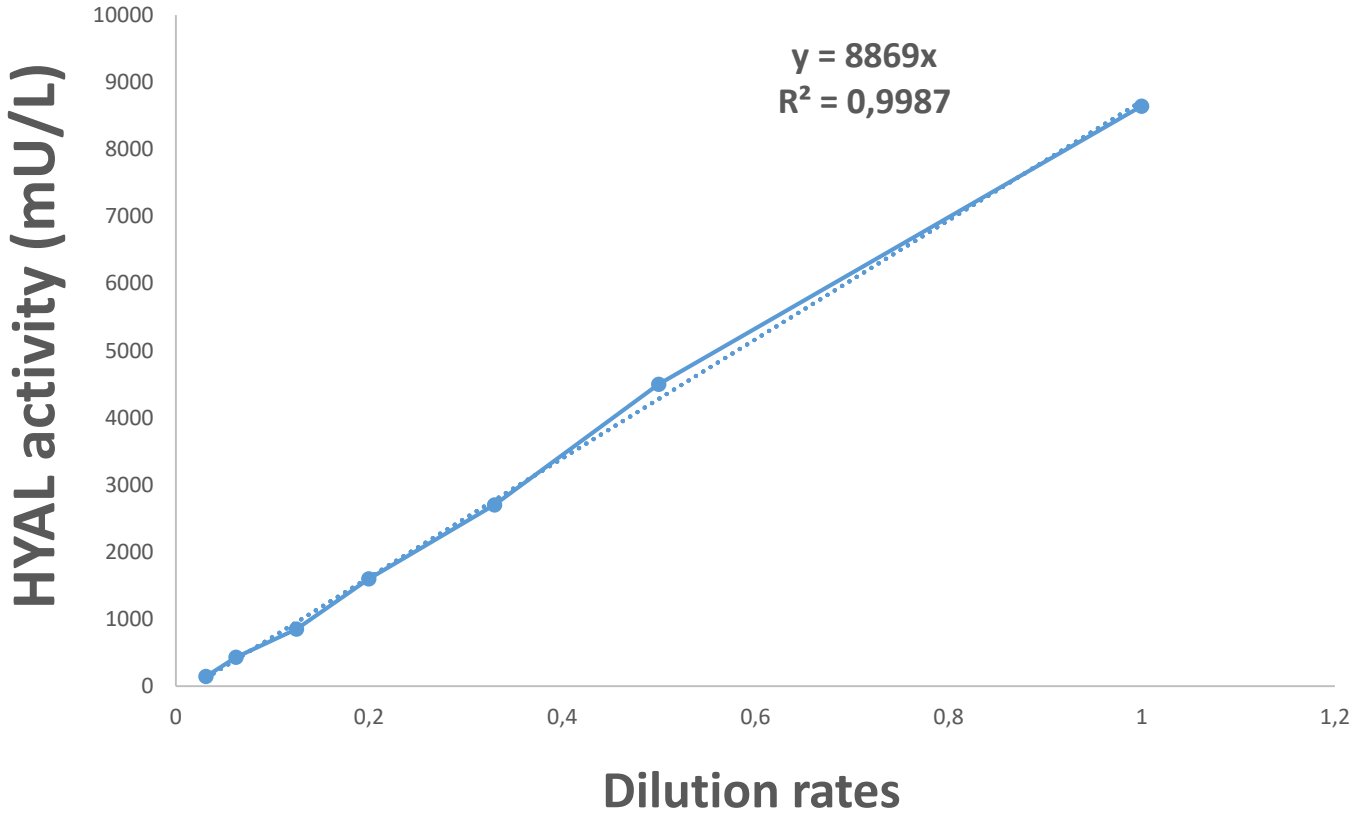
  (LOB, LOD, LOQ)

CLINIC

- Receiver Operating Curve (ROC) Analysis
- Cut-off Value
- Sensitivity
- Specificity
- Likelihood Ratio (Likelihood Ratio)

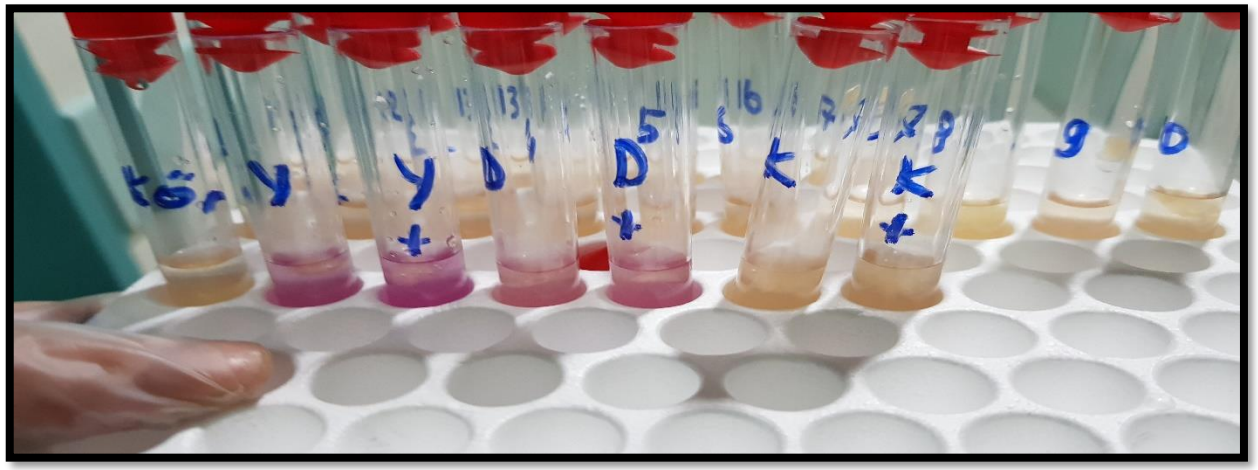
➢ Linearity

HYAL aktivity (mU/L)	Dilution Rates
8645	-
4505	1/1
2702	1/2
1615	1/4
856	1/8
430	1/16
145	1/32



➢ Precision

- Intra-assay and inter-assay precision study
- In accordance with **CLSI EP-5A** recommendations
- Serum pool containing three different concentrations of analyte (low, medium, high)
- Serum pools were separated into 80 identical samples and stored at -80 °C.
- The pair was studied by repeating twice daily for 20 days.



LOW LEVEL	
Intra-assay %CV	7,59
Between-day %CV	7,37
Inter-assay %CV	4,56
Total %CV	10,21

MEDIUM LEVEL	
Intra-assay %CV	11,53
Between-day %CV	5,66
Inter-assay %CV	10,68
Total %CV	9,86

HIGH LEVEL	
Intra-assay %CV	8,59
Between-day %CV	5,80
Inter-assay %CV	7,29
Total %CV	8,51

when 3.5–15 μl serum was used in a 4 h incubation (data not shov. **Intra assay variability** ssed by assaying two normal control sam..., one with high activity, five times in the same run. The mean and s... for the average activity sample were 3926 mU/l and 180 m... (CV 4.6%), the mean and s... on for the high activity sa... U/l and 768 mU/l (CV 7.4%) spectively. **nter-assay variabilit**... amined by analyzing the same control specimen ... ndard deviation in that analysis were 3541 mU/l and 296 (CV 8.4%).

Marvin R. Natowicz*[a,b], Yu Wang[a]

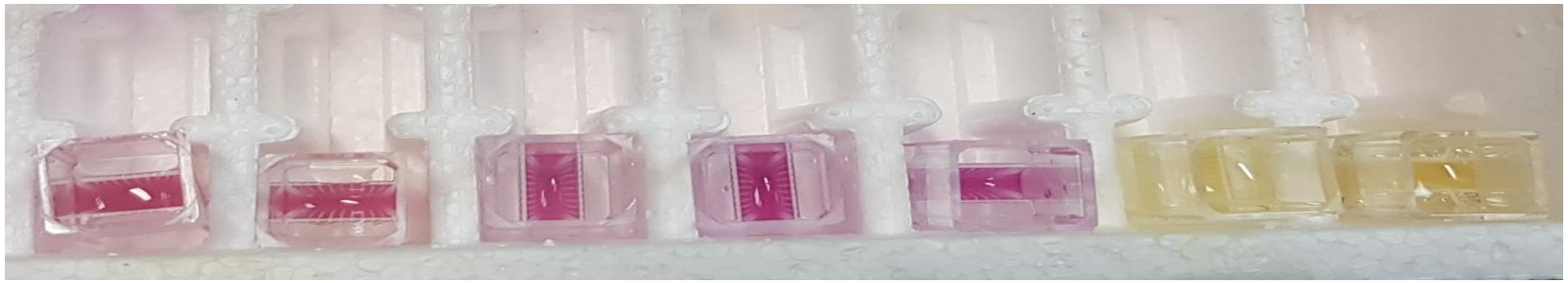
Clinica Chimica Acta 245 (1996) 1–6

These data which we found for a manual and enzymatic test; **show that** the accuracy of **the method is acceptable**.

Kesinlik çalışmasında çalışma içi, günler arası, çalışmalar arası ve toplam % varyasyon katsayılarını düşük seviye için ... %4.71, %8.09, %4.34 ve %10.32; ...rta seviye %4.26, %5.76, %4.93 ve %8.69; seviye için ... %5.63, %6.85, %4.54 ve %9.96 bulduk.

Serum hyaluronidase activity in patients with bladder cancer. Tuba ÖZGÜN, ANKARA, 2018

➢ Recovery



Rate	Expected value (mU/L)	Measured value (mU/L)	R (%)
H		12054	
3H/1L	9263	9278	100,16
2H/1L	8333	8579	102,95
1H/1L	6473	6425	99,25
1H/2L	4613	4348	94,25
1H/3L	3683	3843	104,35
L		893	
Mean			100,19

H: high, L: low

➢ Detection Limits (LOB, LOD, LOQ)

20 times absorbance values of blank samples

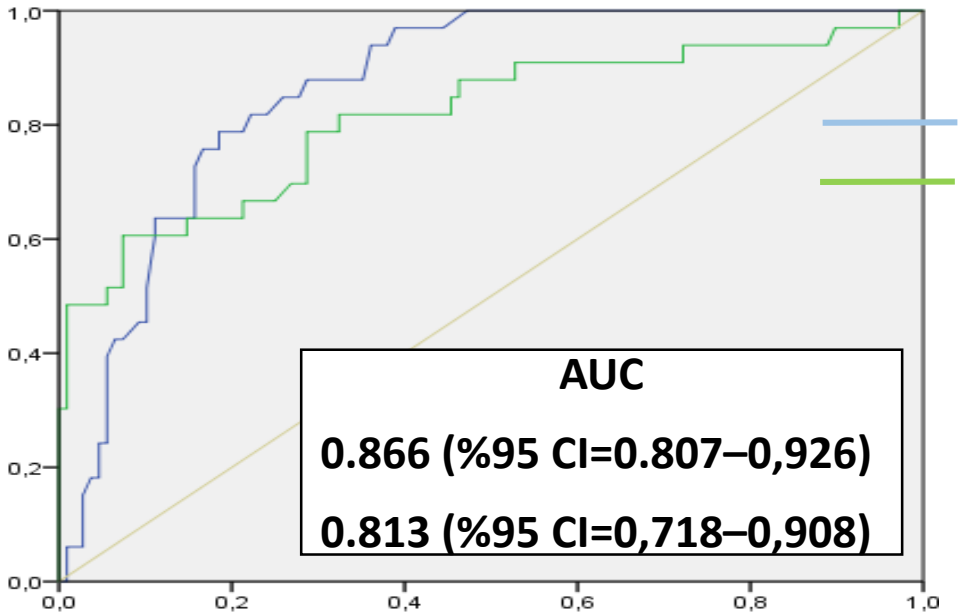
- ➢ mean (xblank) = 0,049
- ➢ standart deviation (sblank) = 0,004

LOB = xblank + 1.645 * sblank → 1087 mU/L

LOD = xblank + 3.3 * sblank → 1217 mU/L

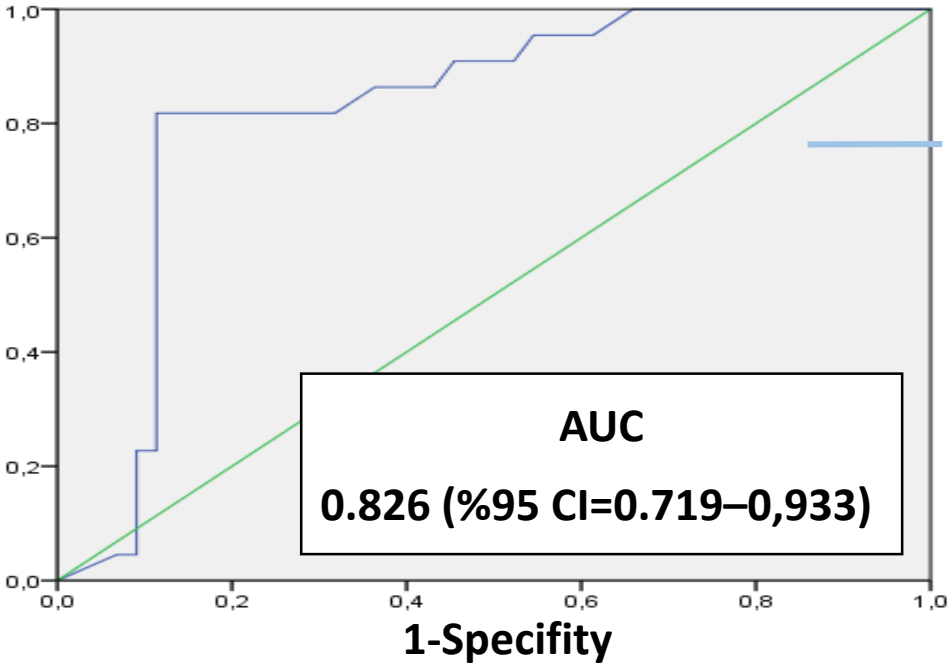
LOQ = xblank + 10 * sblank → 1745 mU/L

➢ ROC Analysis, Cut-off Value, Sensitivity, Specificity and Likelihood Ratios



n=141; 33 PC

108 biopsy (-) patient



n=66; 22 PC

44 biopsy (-) patient

	HYALa	HYALc	PSA
Cut-off Value	3047 (mU/L)	11,43 (ng/mL)	8,03 (µg/L)
Sensitivity	%88	%82	%79
Specifity	%71	%89	%71
LR (+)	3	8	3
LR (-)	0,1	0,2	0,2

DISCUSSION

➢**In the literature, different serum HYAL activity values for healthy population**

Journal of Pharmaceutical and Biomedical Analysis
Volume 14, Issue 6, April 1996, Pages 707-712

Measurement of hyaluronidase activity in normal human serum

Christine R. Wilkinson, Lynne M. Bower, Christine Warren

4476 ±1144 mU/L

Volume 245, Issue 1, 9 February 1996, Pages 1-6

Research communication

Human serum [illegible] assay

Marvin R. Natowicz

17.1 ± 3.6 U/L

Clinica Chimica Acta
Volume 301, Issues 1–2, November 2000, Pages 159-167

Hyaluronidase activity in serum of patients with monoclon[illegible] gammapathy

Antoine Laudat, Jérôme Guechot, Karine Lecourbe, Richard Damade, Anne-Marie

1.4-1.8 U/L

2838 ± 417.67 mU/L

Bratisl Lek Listy. 2009;110(1):

Plasma hyaluronidase activity as an indicator of atherosclerosis in patients [illegible] disease.

Kucur M[1], Karadag B, Isman FK, Ataev Y, Duman D, Karadag N, Ongen Z, Vural VA.

Author information

9.3 ± 1.7 U/L

of tumor patients and healthy volunteers

Ingo Muckenschnabel[a], Günther Bernhardt[a], Thilo Spruss[a], Barbara Dietl[b], Armin Buschauer[a,*]

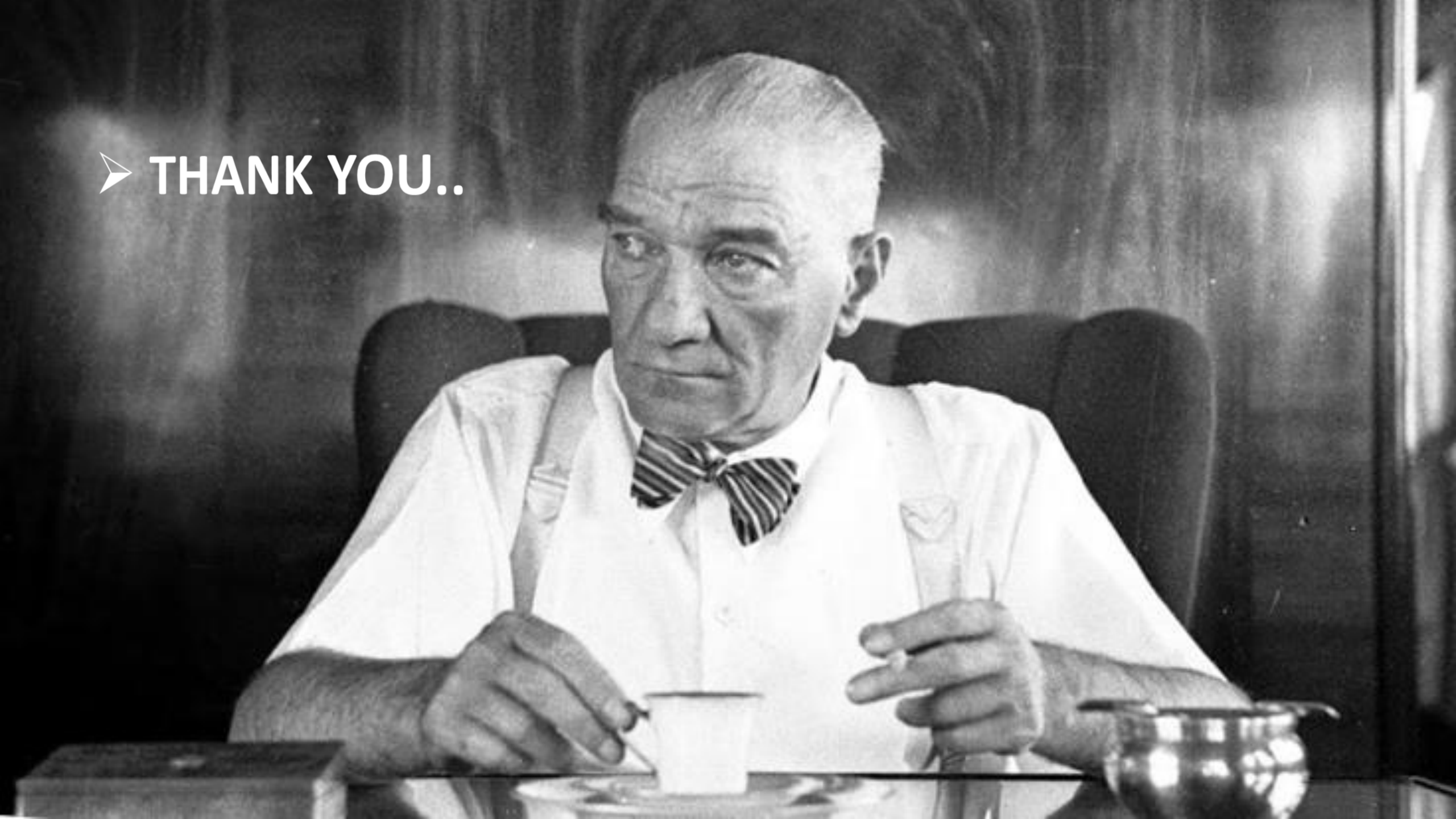
Causes of Differences

- Characteristics of control groups
- Amount of sample used
- Substrate concentration
- Incubation time and incubation temperature
- Arbitrary units used in unit calculation
- The pH of the environment
- Centrifugal speed and time

Conclusion and Suggestions;

- In our study, **it is advantageous** to compare HYAL activity with mass measurement.
- The ability to measure HYAL activity under laboratory conditions and to have good analytical and clinical performance characteristics make the **method suitable for routine studies.**
- However, it is **not possible** to automate the colorimetric method due to the large number of processing steps and the high incubation time and temperature.
- In larger patient groups of HYAL levels, further studies with new data on disease recurrence and progression will be more valuable.

- **Combining the measurements of HYALa or HYALc with PSA, examination and USG information may be useful in the evaluation of PC patients.**


THANK YOU..