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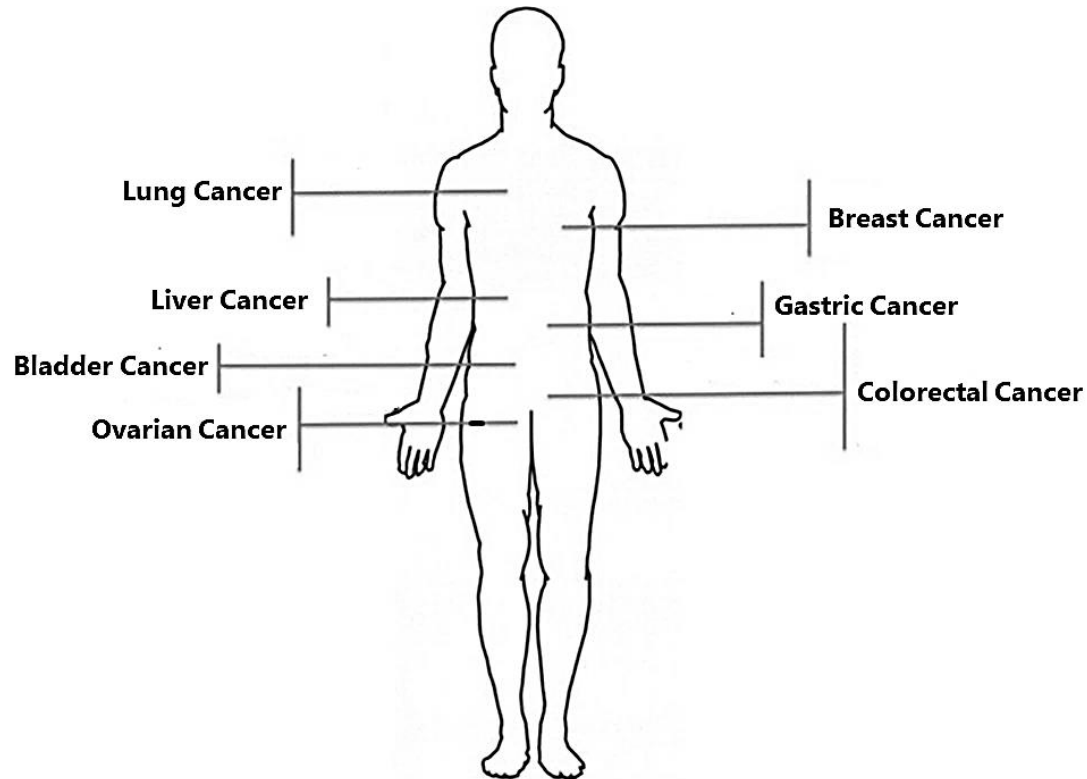
Folding paper-based analytical device for simultaneous quantification of cancer biomarkers exploiting quantum dots as ultrasensitive electrochemical labels

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Carcinoembryonic antigen (CEA)

- CEA is a human glycoprotein that is involved in cell adhesion.
- Normally produced during human fetal development.
- Over-expressed in many human cancers



One of the most widely used tumor biomarkers and prognostic indicators in clinical assays

	CEA concentration
Normal Level Healthy adults	2,5 - 5 ng/mL
Abnormal Level Sign of cancer	> 20 ng/mL

Cancer antigen 125 (CA 125)

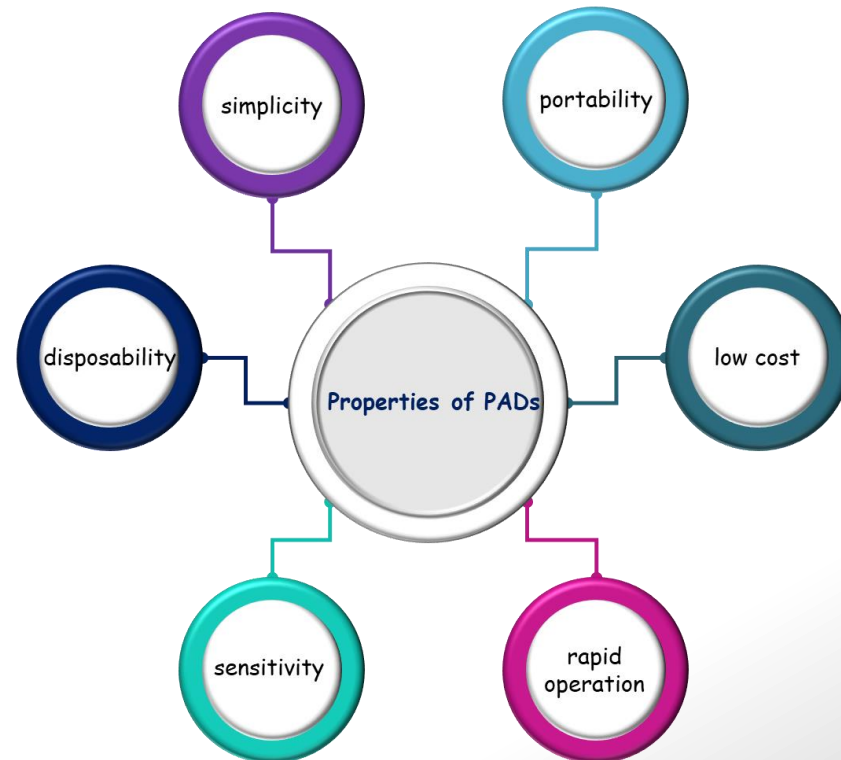
- A high molecular weight **glycoprotein** that has served as the main ovarian cancer biomarker for almost four decades.
- **Increased CA125** values have been reported in subjects with **non-ovarian malignancies** including breast, lung, liver, pancreatic, colon, stomach, cervical, uterine and endometrial carcinomas.

- ✓ CA125 has played an important role in the **screening, treatment, and follow-up phases** of ovarian cancer management
- ✓ **Early diagnosis** of ovarian cancer through screening with CA125 can have a considerable impact on **improving survival rates**.
- ✓ **Rising and falling values** of CA125 are **predictors** of disease progression and regression in 90% of cases.

CA 125 cut-off value: 35 U mL⁻¹

Paper-based analytical devices

- **Paper based analytical devices** (PADs) have attracted increasing attention and interest during recent years.
- A promising technology for **point-of-care testing** (POCT), **public health** and **environmental monitoring applications** in developing countries and remote regions.
- These systems combine the **properties of paper strip tests** with the capability of **multiplex analysis** and the complex function of conventional **lab on a chip devices** for analyte detection.



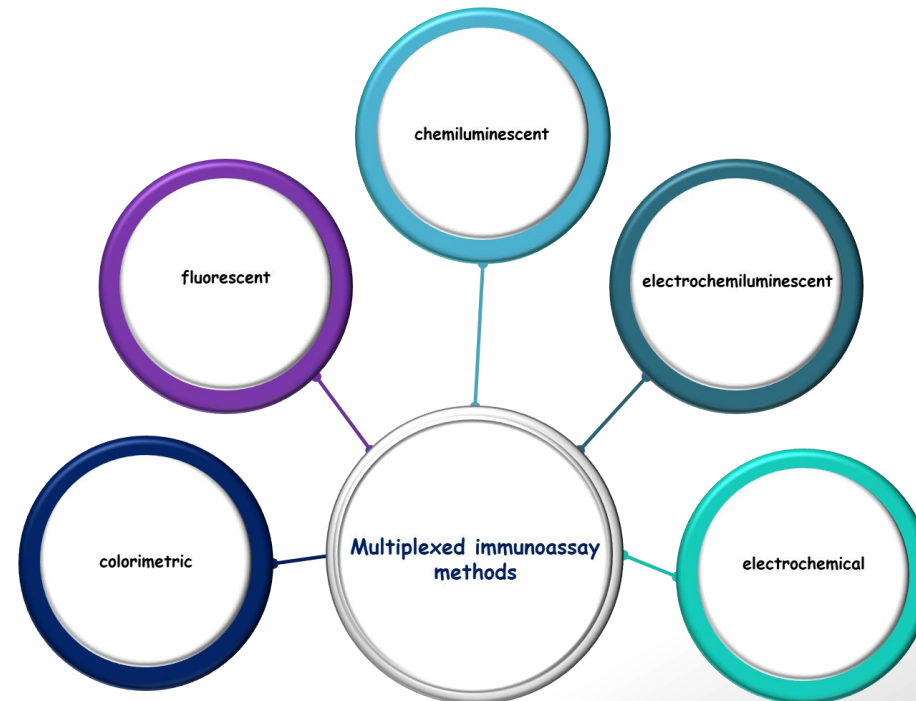
Multiplexed immunoassays

- **Multiplexed immunoassays** of biomarkers have recently attracted considerable interest due to their advantages in *early screening of diseases*, *evaluating the extent of diseases* and *monitoring the response of diseases to therapy*.

A single target biomarker detection may lead to misdiagnose



Simultaneous quantitation of biomarkers in serum improves **diagnostic power**



Paper - based electrochemical devices (ePADs)

- ePADs consist of three electrodes: a **working electrode (WE)**; a **reference electrode (RE)**; and a **counter electrode (CE)**
- The three-electrode system is formed by depositing conductive ink on a paper matrix using **hand drawing, manual brushing or screen printing**

Quantum Dots

- QDs are semiconductor nanoparticles made of heavy metals selenide and sulphide salts (CdSe, PbSe, CdS).
- They are used as fluorescent or electrochemical labels for the detection of various **biological analytes**, such as *DNA, proteins and cells*,
- QDs can be functionalized with **antibodies, DNA**, or **aptamers** specific to the target analyte.
- **Acid dissolution** promotes the release of metal ions from QDs by stripping voltammetry.

Sensing of multiple analytes is possible by the use of QDs with different compositions, for example CdS and PbS QDs

Advantages of ePADs

- **high sensitivity and selectivity**
- **cost-effectiveness**
- **high detection speed**
- **user-friendly operation**
- **stability**

Aim of The Study

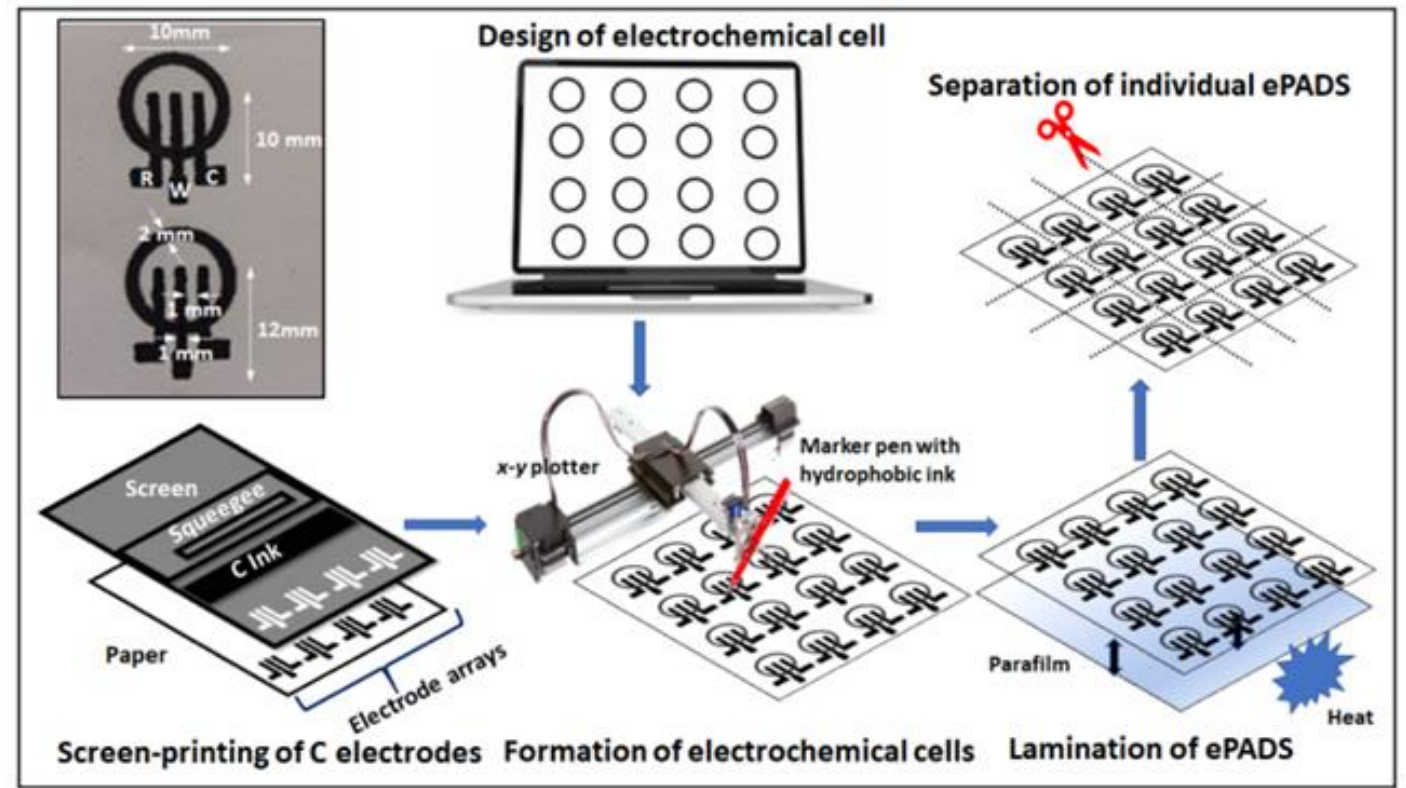
Simultaneous detection of CEA & CA 125 in human serum samples with a paper-based quantum dot-linked electrochemical assay

- **Fast**
- **Cost-effective**
- **Sensitive**
- **Selective**

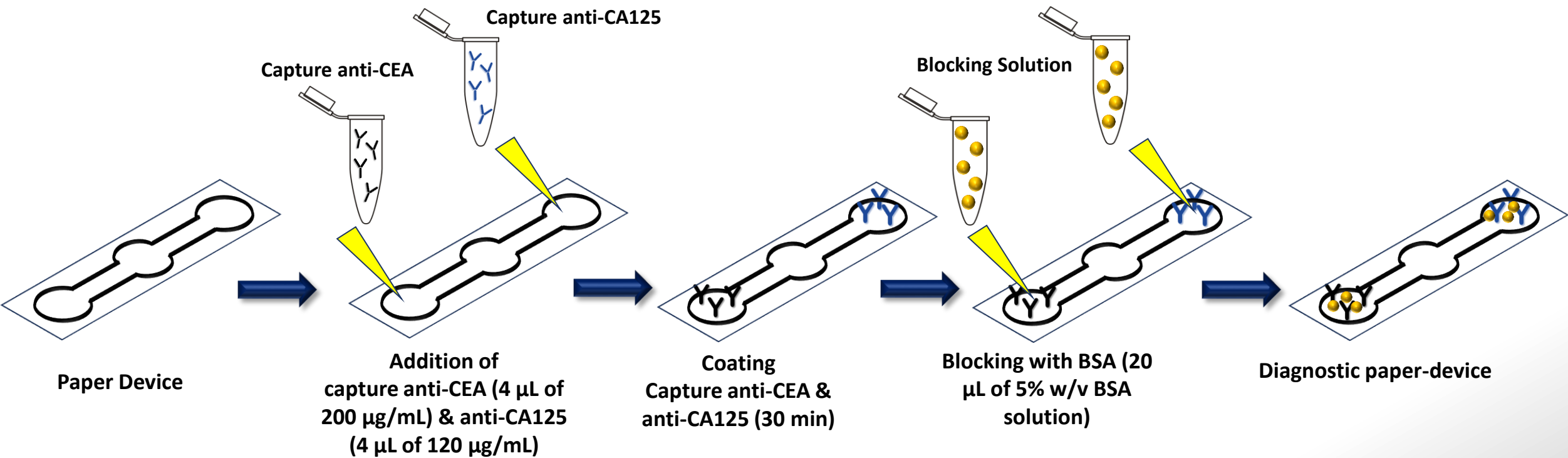
Fabrication of the Screen-Printed Electrodes

Fabrication of the ePADs:

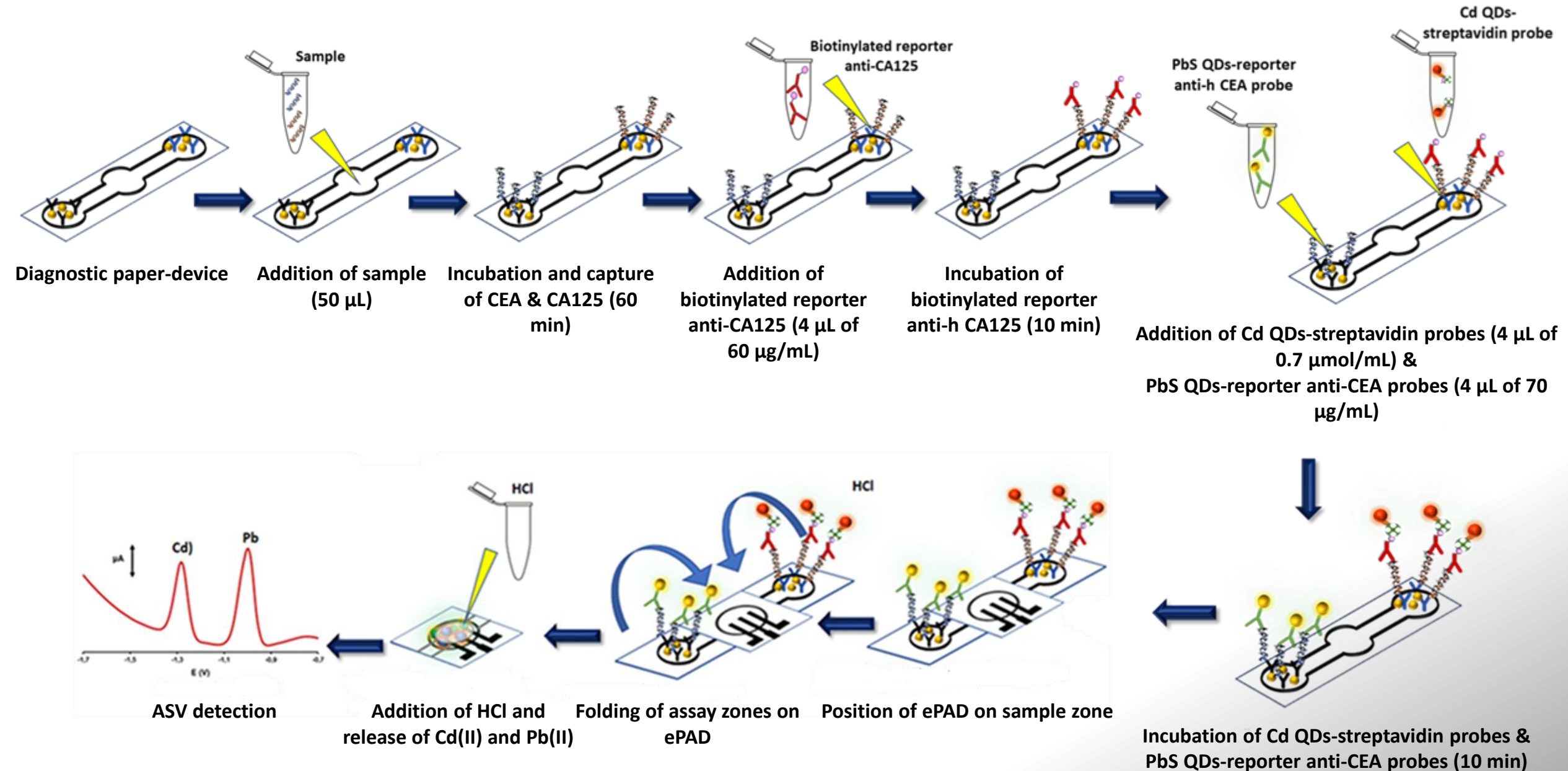
1. Screen-printing of the three-electrodes arrays.
2. Design and patterning of the electrochemical cells.
3. Lamination of the ePADs
4. Separation of individual ePADs



Fabrication of paper-based immunosensor



Working Principle of the Assay for CEA & CA125 detection

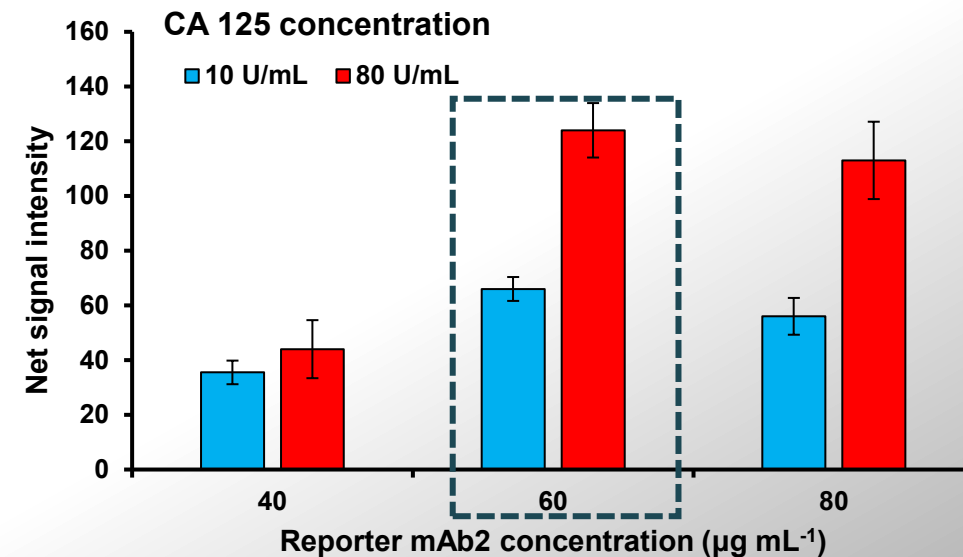
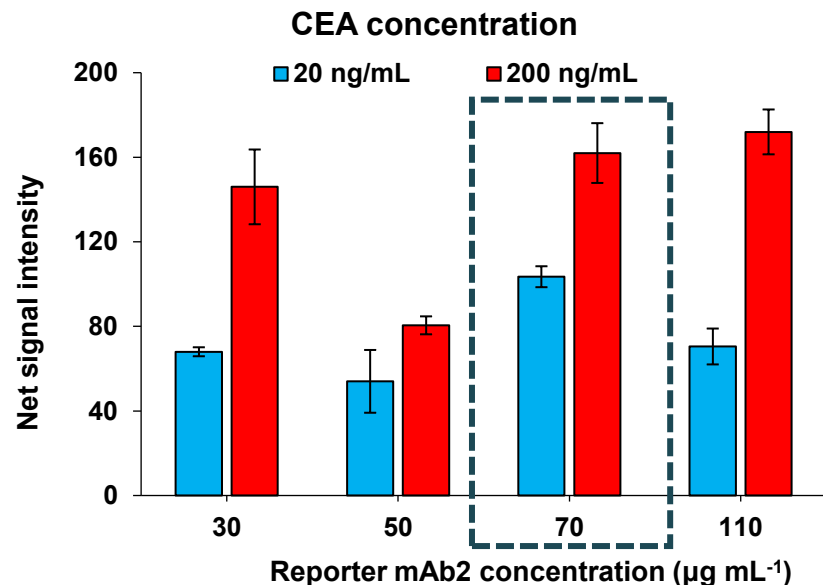
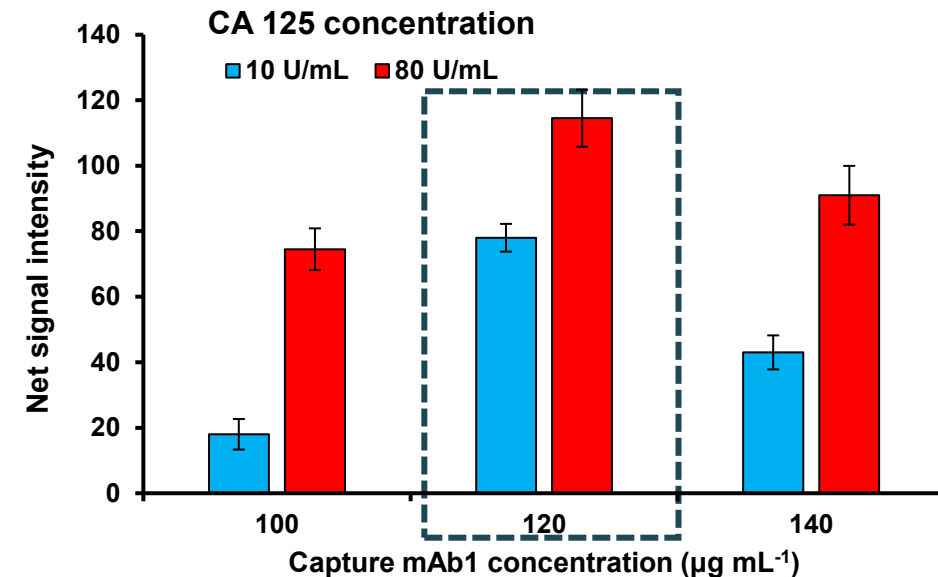
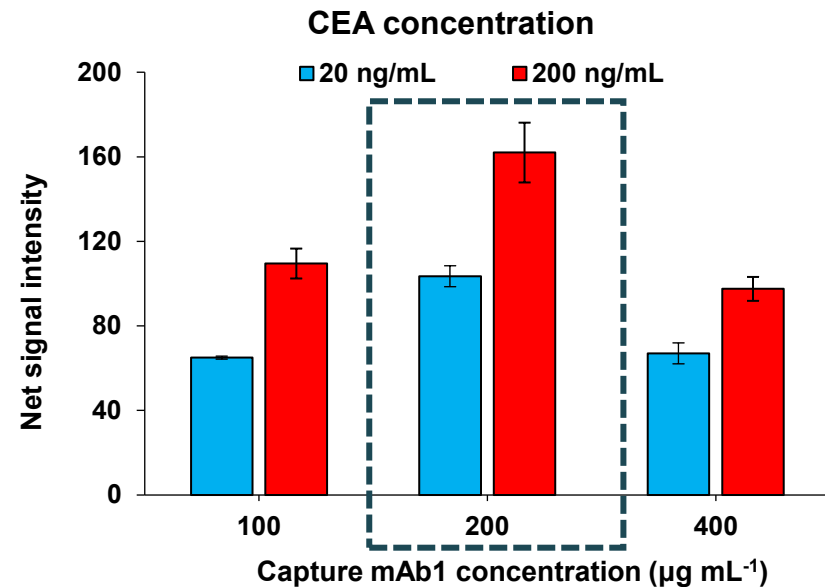


Optimization of Experimental Parameters

Optimized selected parameters
1. Concentration of reagents
2. Incubation times
3. Conditions for the electrochemical detection of Cd(II) and Pb(II) by ASV

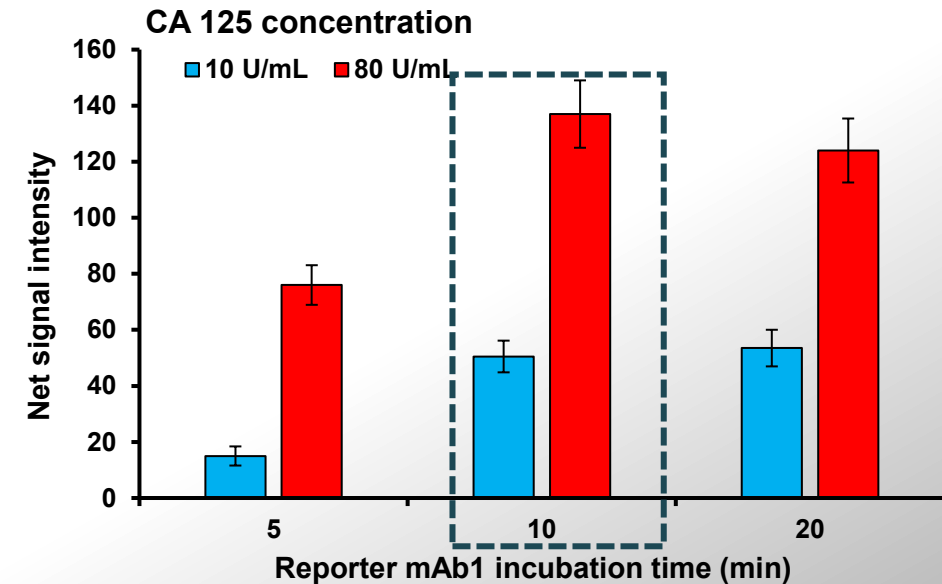
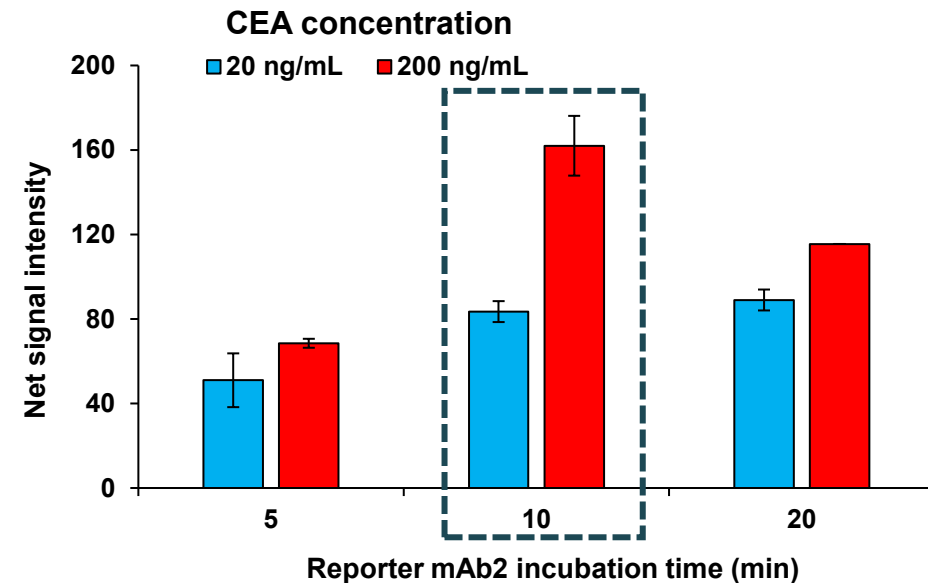
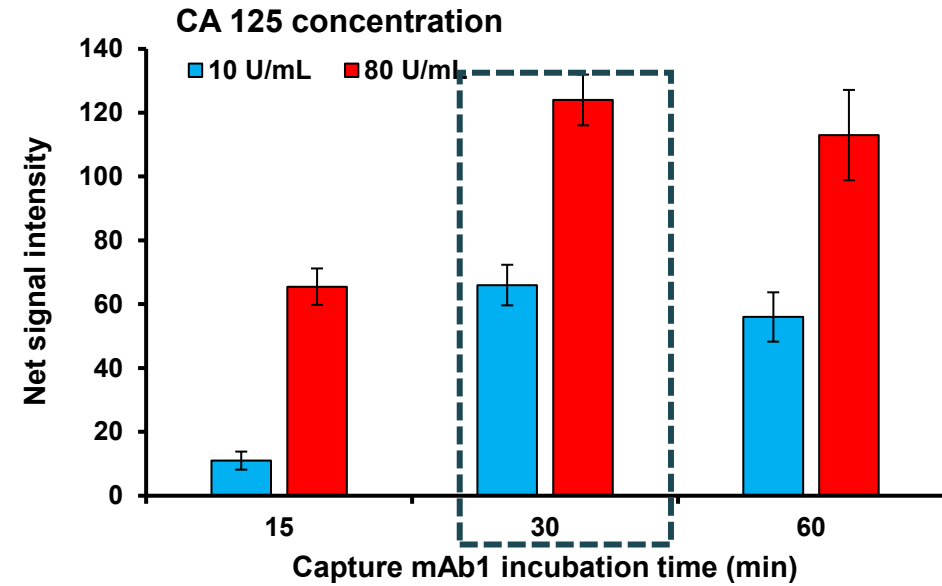
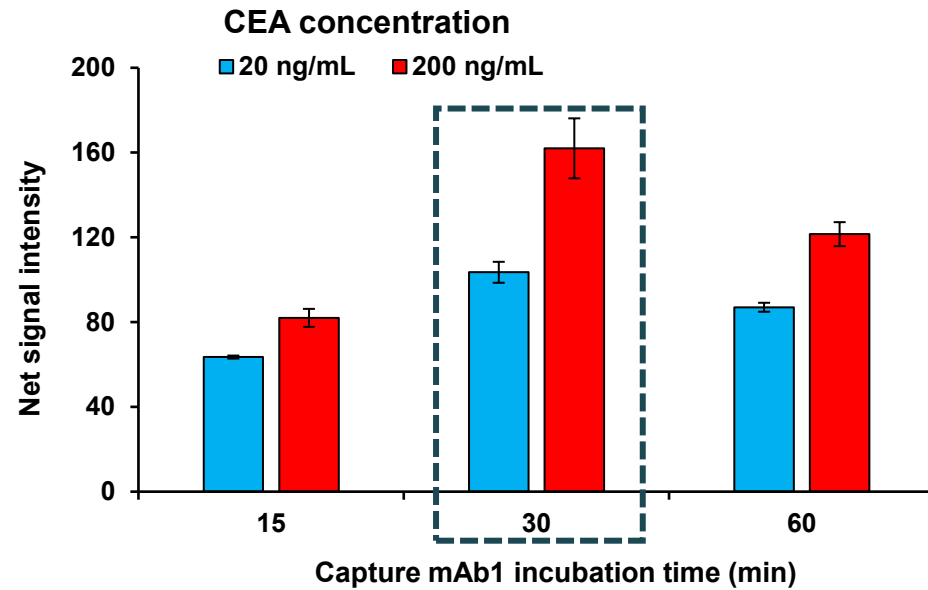
Optimization of Experimental Parameters

Concentration of reagents



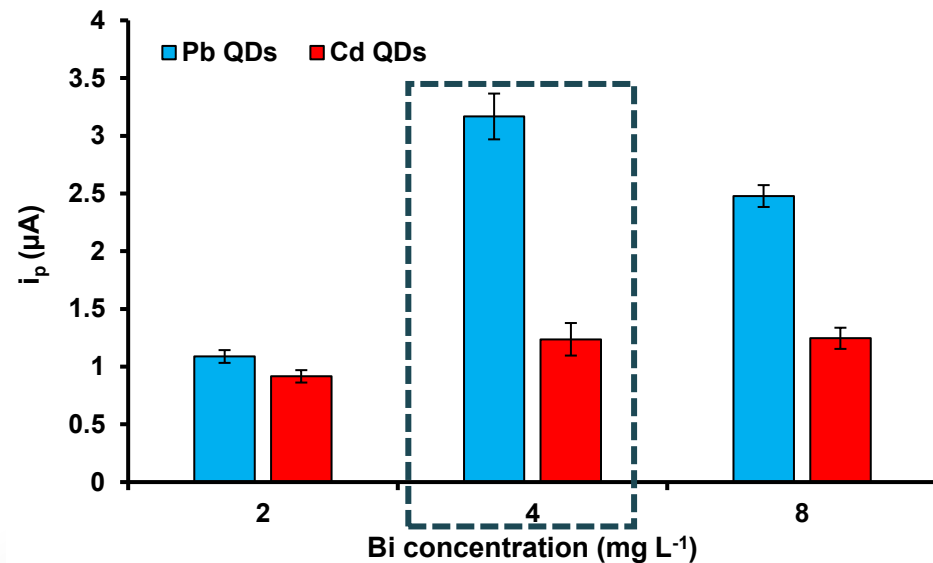
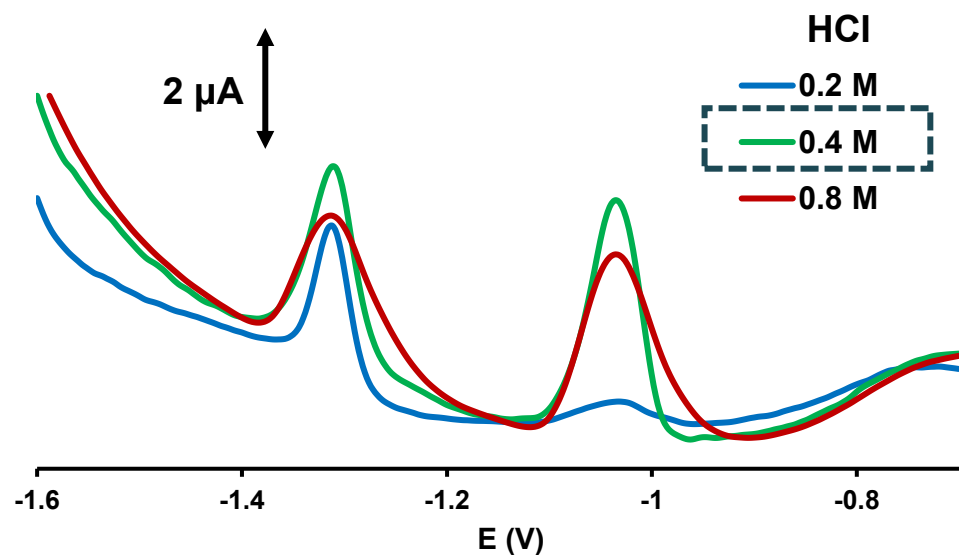
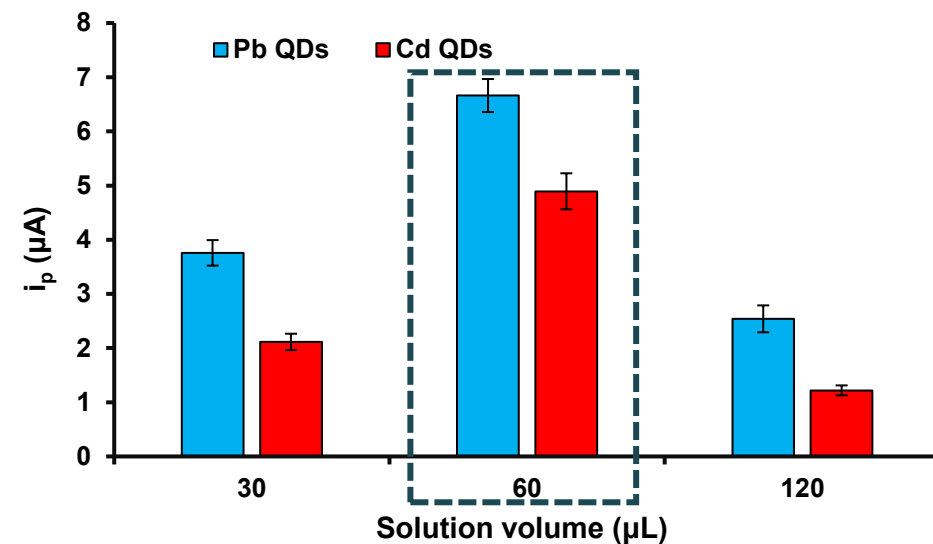
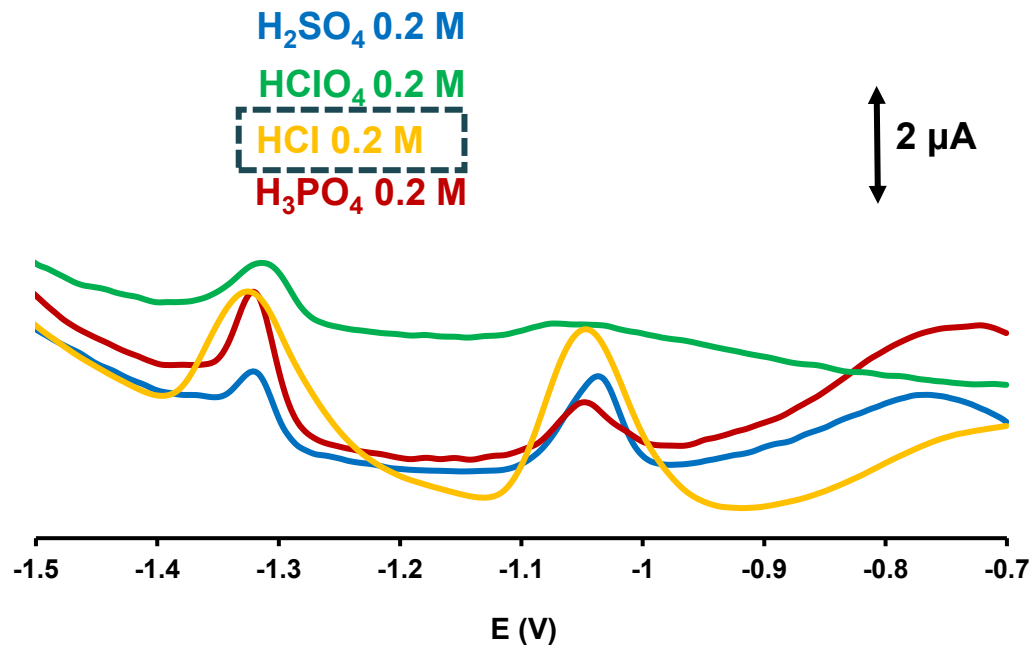
Optimization of Experimental Parameters

Incubation times

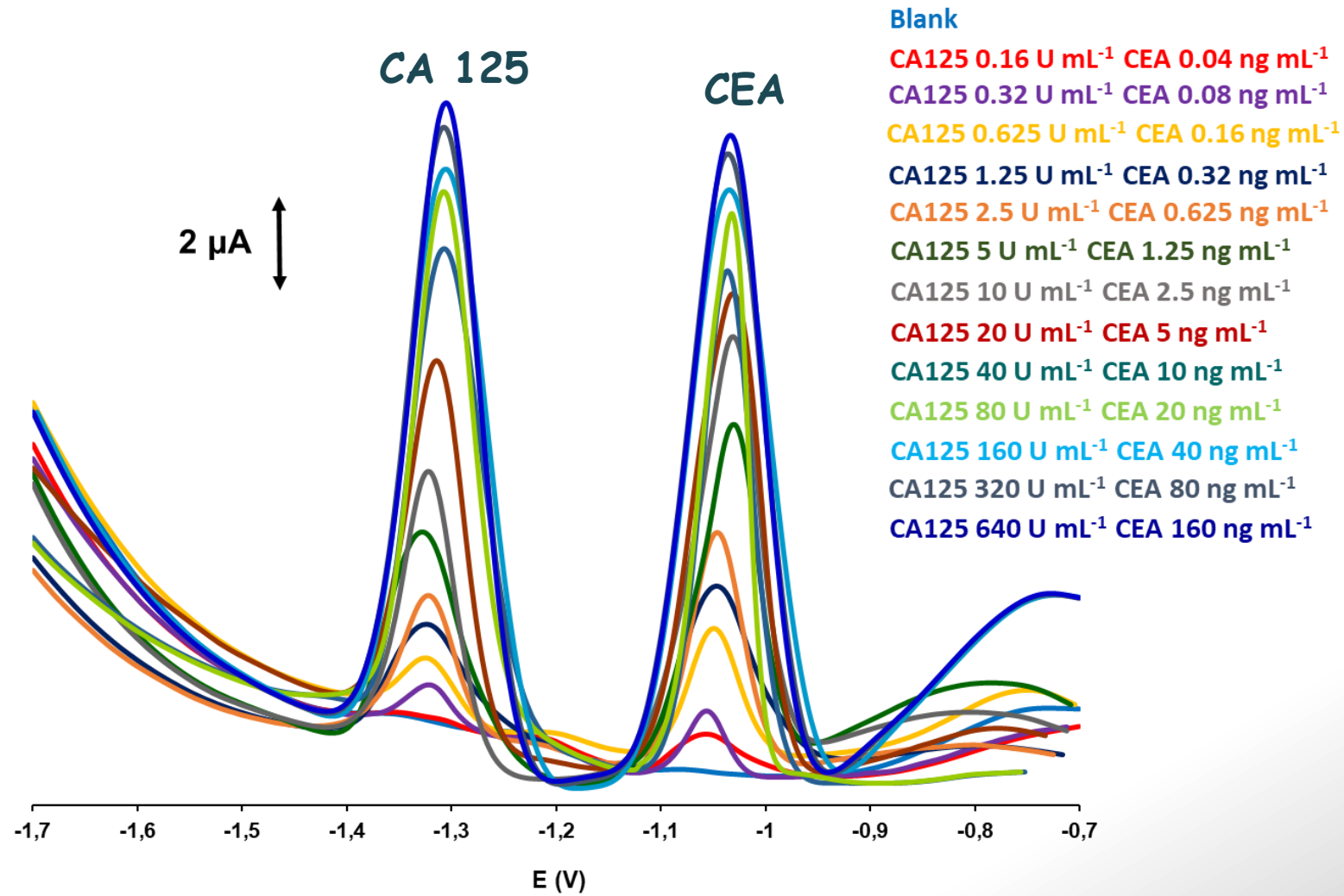


Optimization of Experimental Parameters

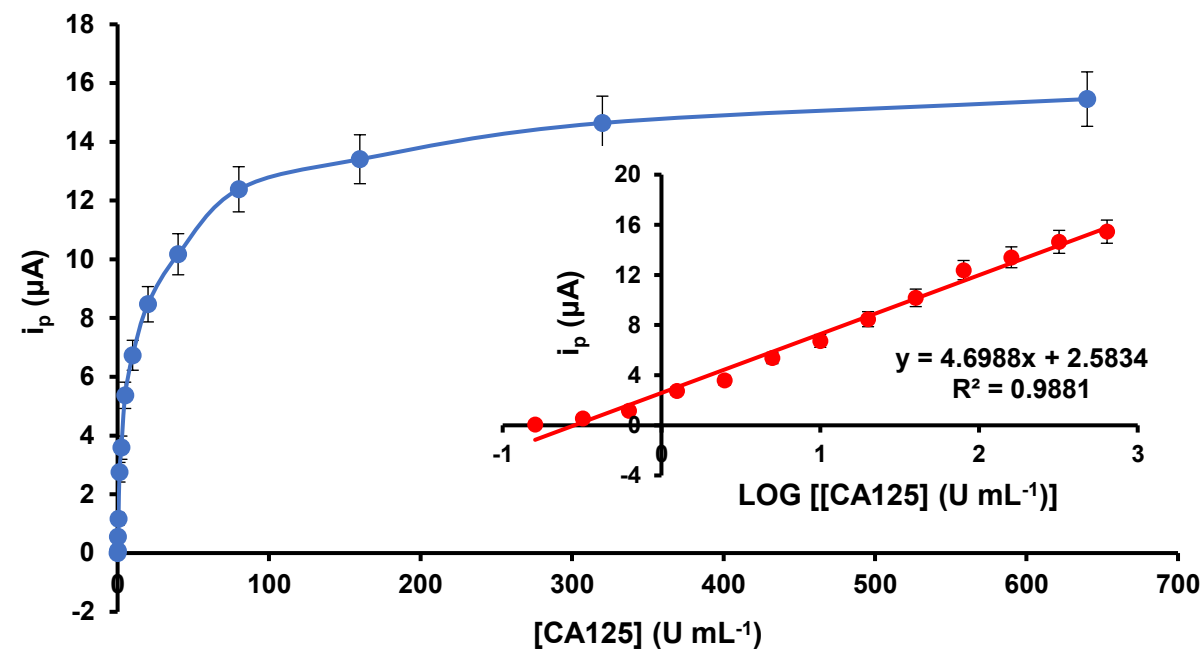
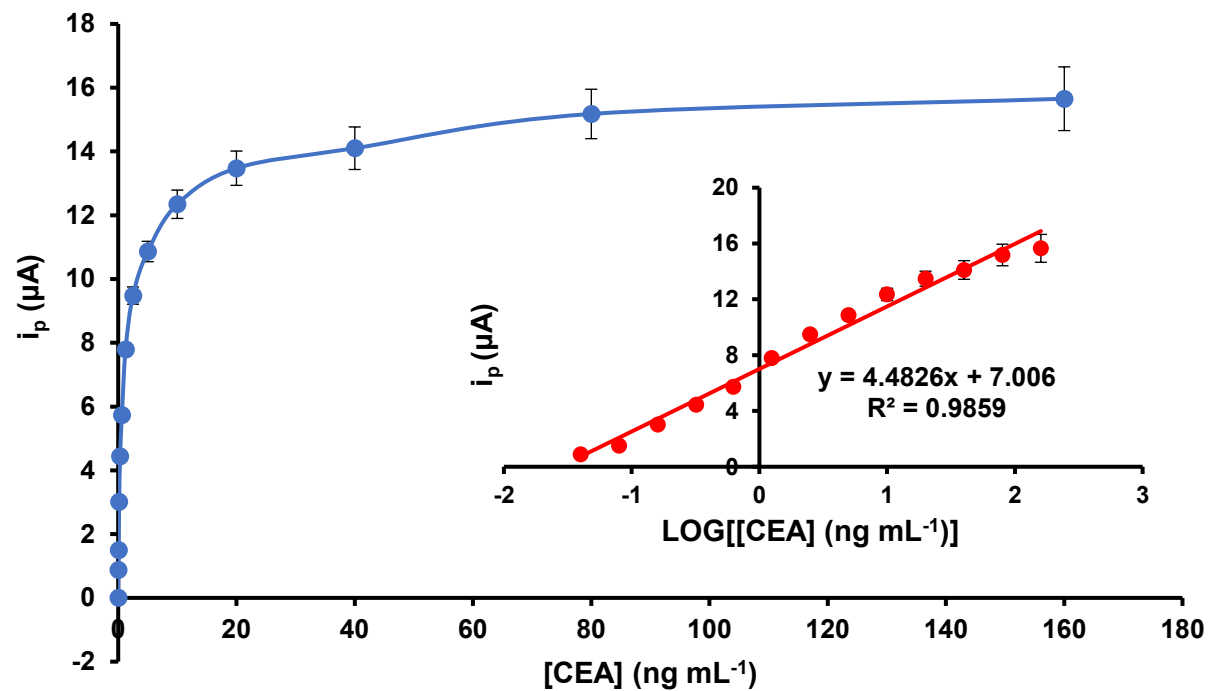
Determination of Cd(II) and Pb(II) by ASV



Analytical Performance (1/2)



Analytical Performance (2/2)

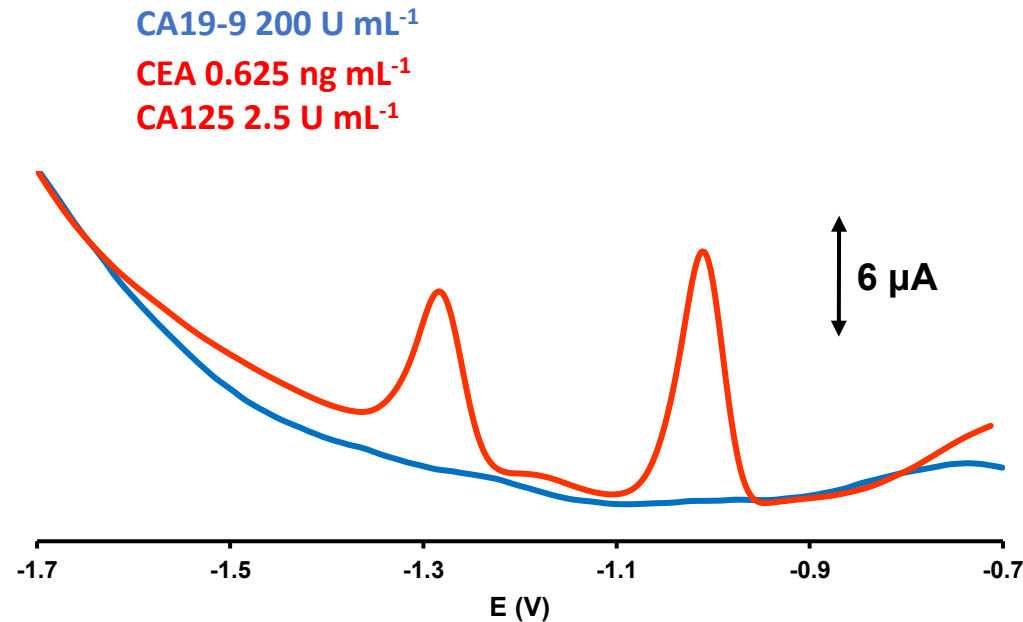


	Linear range	LOD	Within-day %RSD	Between-days %RSD
CEA	0.04 - 160 ng/mL	0.01 ng/mL	5 %	8 %
CA125	0.16 - 640 U/mL	0.1 U/mL	4 %	8 %

Evaluation of assay specificity

The possible cross-reaction of the developed immunosensor was tested by choosing carbohydrate antigen 19-9 (CA 19-9) as interference.

Carbohydrate antigen 19-9 (CA19-9): usually coexist in patients' serum with CEA and CA 125



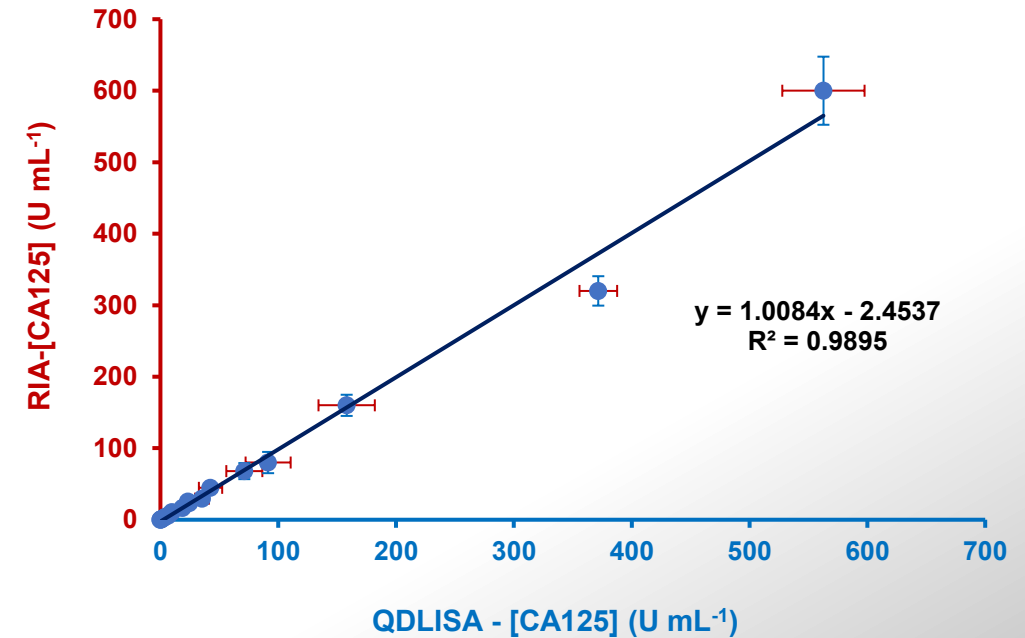
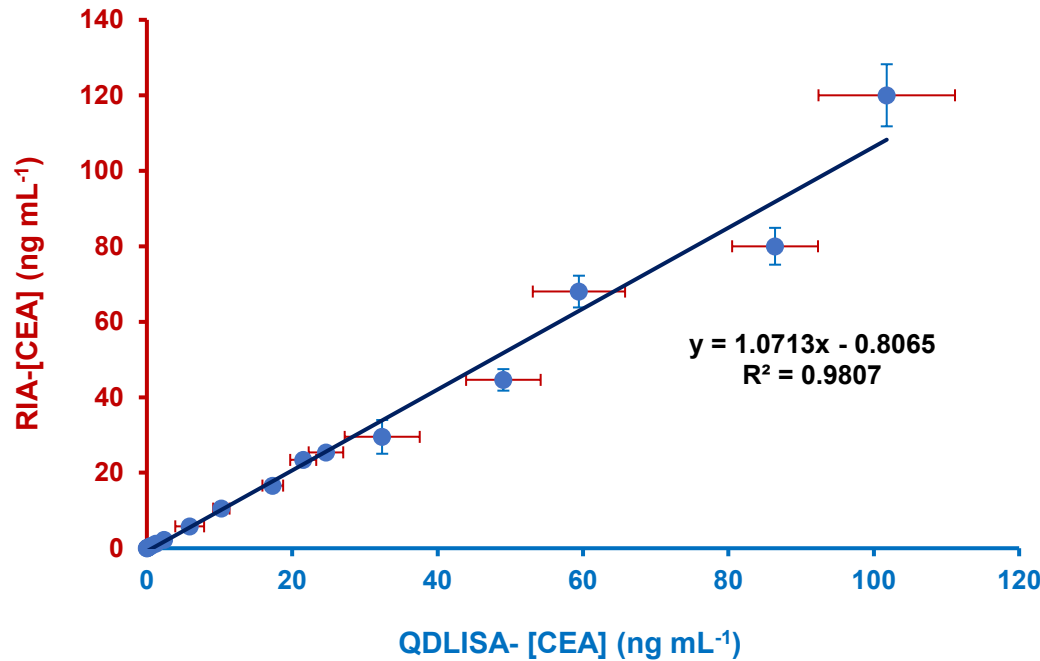
The absence of peak indicates excellent selectivity of immunosensor with no cross-reactivity between CEA-specific mAbs or CA 125-specific mAbs and CA 19-9

Evaluation of assay applicability (1/2)

Amount added	Amount determined	% Recovery	% RSD (n = 3)
CEA (ng mL ⁻¹)			
0.16	0.18	110	9.2
2.5	2.4	99	8.5
40	44	110	9.6
CA 125 (U mL ⁻¹)			
0.625	0.66	105	8.3
10	9.7	97	9.4
160	158	99	10.1

Evaluation of assay applicability (2/2)

- Samples spiked with CEA and CA 125 at specific concentrations in the range **0.15 – 68 ng mL⁻¹** and **1.2 – 160 U mL⁻¹** respectively, were analyzed using the **proposed paper-based sensor** and the **conventional RIA assay** which served as a reference method.



Comparison of the present multiplexed immunosensor for CEA & CA 125 compared to existing paper-based immunosensors

Electrochemical probe	Electrode type/fabrication/modification	Biorecognition conjugate	Electrochemical technique	CEA LOD	Linear range	CA125 LOD	Linear range	Complex fabrication of sensor	Complex preparation of biorecognition conjugate
o-phenylenediamine	Carbon/screen printing	HRP-labeled antibody	DPV	0.01 ng mL ⁻¹	0.05-50 ng mL ⁻¹	0.2 mU mL ⁻¹	0.001-75U mL ⁻¹	Yes (3D wax printed microfluidic)	No
o-phenylenediamine/ H ₂ O ₂	Carbon/manual Graphene modified Ag/screen printing	HRP-labeled antibody	DPV	0.001 ng mL ⁻¹	0.001-100 ng mL ⁻¹	0.001 ng mL ⁻¹	0.001-100 ng mL ⁻¹	Yes (daisy-shaped with photolithography)	No
o-phenylenediamine/ H ₂ O ₂	Carbon/ screen printing /GO-chitosan	Antibody labeled with HRP on PGMA	DPV	0.01 ng mL ⁻¹	0.01-100 ng mL ⁻¹	0.05 ng mL ⁻¹	0.05-100 ng mL ⁻¹	Yes (daisy-shaped with photolithography)	Yes
Cd(II) or Pb(II)	Au/ screen printing	Au/BSA-Pb ²⁺ or Au/BSA-Cd ²⁺ antibody	DPV	0.08 pg mL ⁻¹	0.1pgmL ⁻¹ - 50 ng mL ⁻¹	0.06 mU mL ⁻¹	0.1mUmL ⁻¹ - 50 UmL ⁻¹	Yes (3D origami wax printed microfluidic)	Yes
o-phenylenediamine/ H ₂ O ₂	Carbon/ MWCNTs-chitosan- glutaraldehyde/ screen printing	HRP-labeled antibody	DPV	5 pg mL ⁻¹	0.05 -105 ng mL ⁻¹	6 mU mL ⁻¹	0.02 - 85U mL ⁻¹	Yes (3D wax printed microfluidic)	No
Cd(II) and Pb(II)	Carbon/screen printing/Bi	PbS and Cd QDs-labeled antibody	DPASV	0.01 ng mL ⁻¹	0.04 - 160 ng mL ⁻¹	0.1 U mL ⁻¹	0.16 - 640 U mL ⁻¹	No (pen plotted microfluidic)	No

Conclusions

A **simple, rapid** and **cost-effective electrochemical** paper-based immunosensor was developed for simultaneous CEA & CA 125 detection in human serum samples

Operational features of immunosensor	
CEA	CA 125
linear range: 0.04 - 160 ng/mL	linear range: 0.16 - 640 U/mL
limit of detection: 0.01 ng/mL	limit of detection: 0.1 U/mL
assay time: 70 min	assay time: 80 min
No cross-reactivity between CEA-specific or CA 125-specific mAbs and CA19-9	

Future prospects

The application of these electrochemical PADs has the potential to be implemented as a substrate to for the detection of different analytes

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Thank you for your attention!