



Novel Sensitive Electrochemical Immunosensor Development for the Selective Detection of HopQ *H.pylori* Bacteria Biomarker

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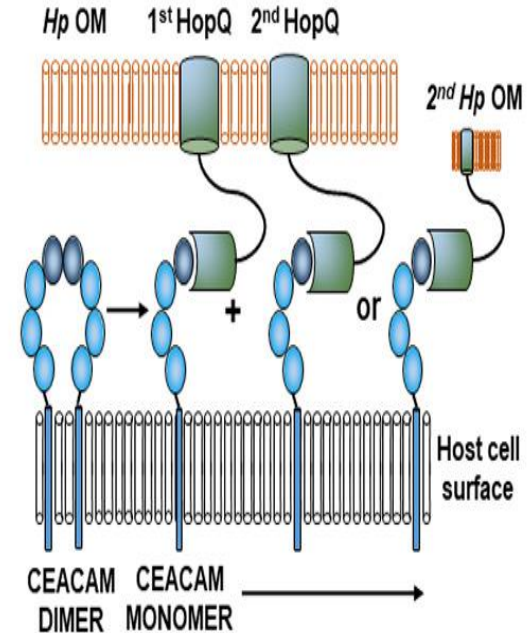
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Introduction

- ❖ *Helicobacter pylori* (*H. pylori*)
- ❖ HopQ
- ❖ *H. pylori* utilizes its outer membrane HopQ proteins to facilitate the mechanism of transfer of its pathogenic factor, such as CagA, to the host cells at early stages of *H. pylori* infection
- ❖ HopQ is considered an excellent biomarker for reliable, selective, and specific non-invasive direct detection of *H. pylori* bacteria

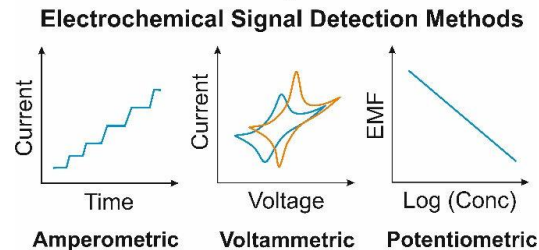
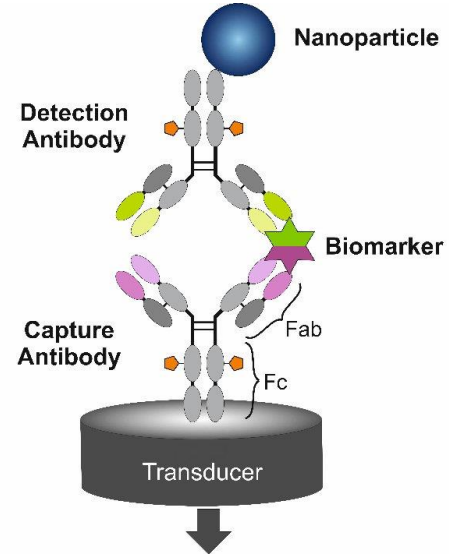




Introduction

biosensor platform

- ❖ Immunosensors based on electrochemical methods
- ❖ Biomarker-based biosensors need to be one-time use (disposable), cost-affordable, simple, accurate, reproducible and sensitive
- ❖ Nanomaterials used in electrochemical biosensors: carbon-based nanomaterials, metallic nanomaterials
- ❖ Multi-walled CNTs (MWCNT)
- ❖ AuNP

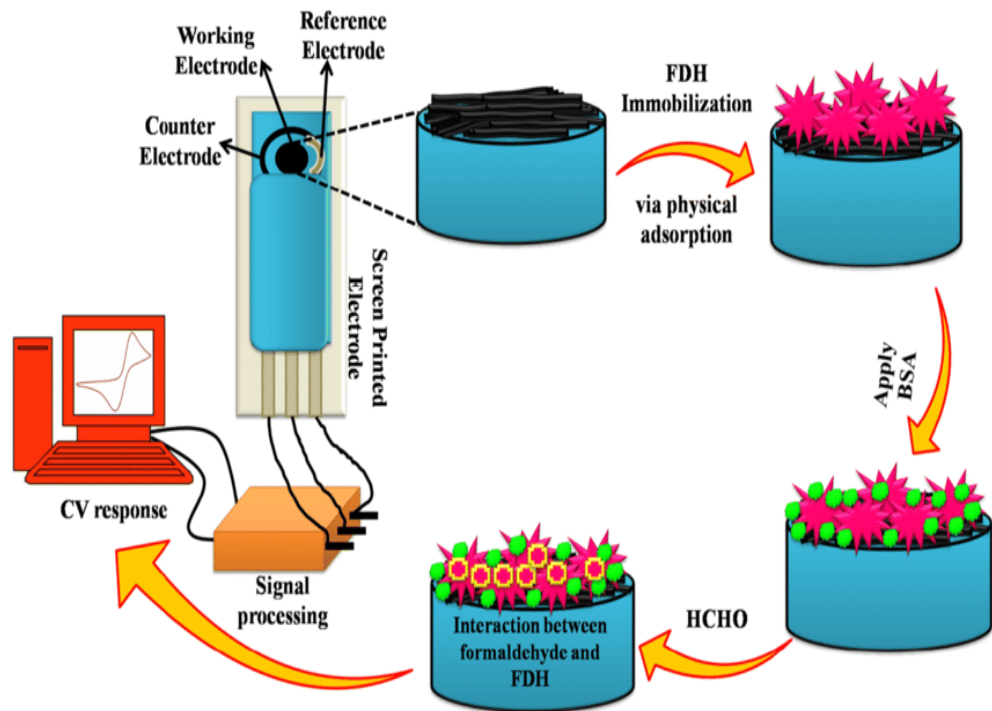




Introduction

Screen-printed carbon electrodes (SPCEs)

- ❖ working electrode (WE)
- ❖ counter electrode (CE)
- ❖ reference electrode (RE)





Materials and Methods

Immunosensor Preparation

- ❖ Activation and Pretreatment of SPCE
- ❖ Nanocomposite Preparation and Surface Modification
- ❖ WE Preparation and HopQ-Ab Immobilization

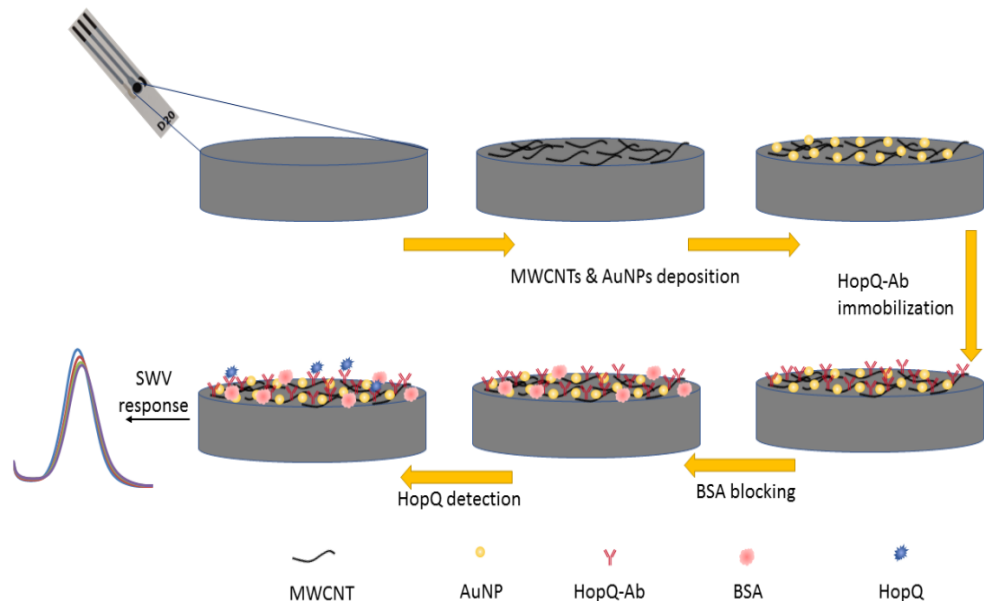


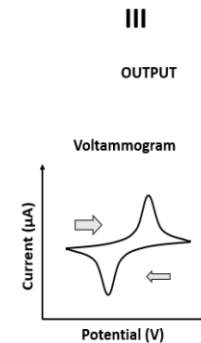
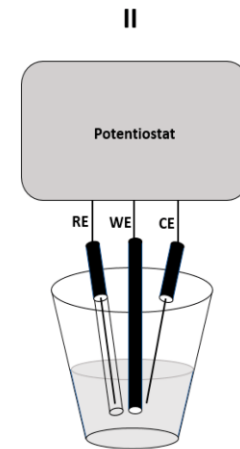
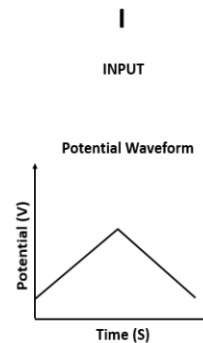
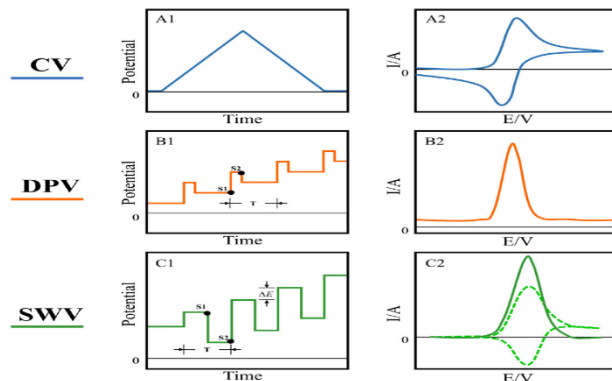
Figure 1. HopQ biosensor interface development process schematic diagram.



Materials and Methods

Electrode Characterization , Analytical Performance and Detection of HopQ

- ❖ Electrochemical Measurements
- ❖ Surface Characterization by Scanning Electron Microscopy
- ❖ Detection of HopQ and Calibration Curve
- ❖ Analytical Performance

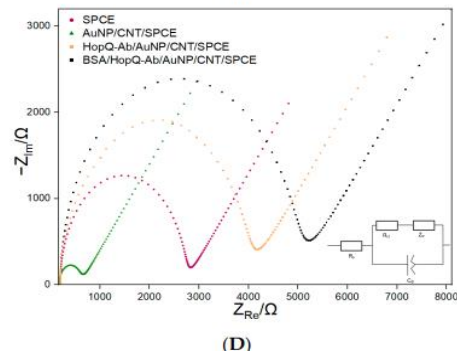
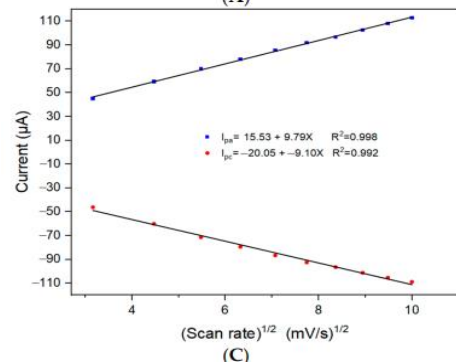
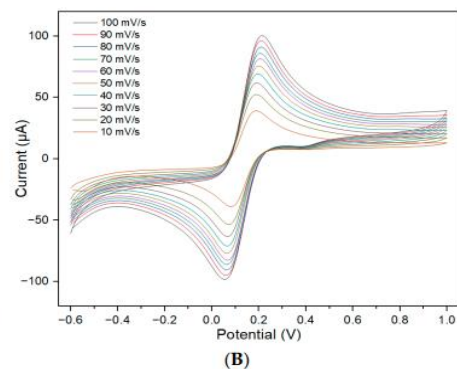
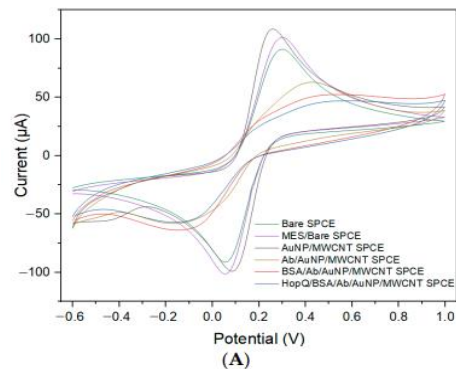




Results

Electrochemical Measurements and Detection of HopQ

- ❖ Electrochemical Measurements
- ❖ Electrochemical Impedance Studies
- ❖ Immunosensor Analytical Performance



$y=4.79+11.7x$

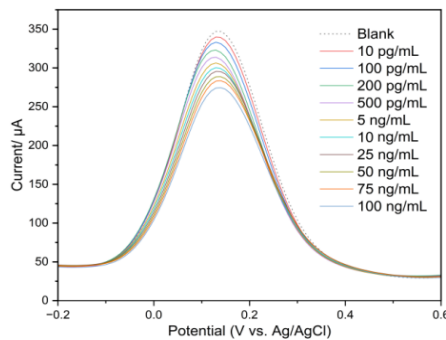


Results

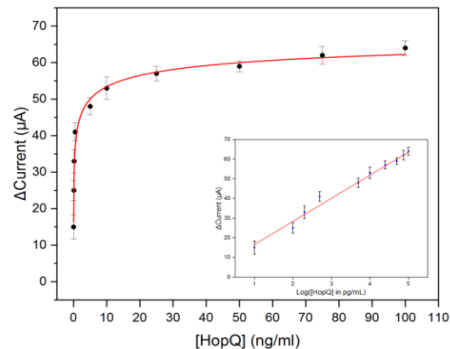
LOD & LOQ

$$LOD = 10 \frac{3.3 \times \delta}{\alpha}$$

$$LOQ = 10 \frac{10 \times \delta}{\alpha}$$



(A)



(B)

Figure 4. (A) SWV responses of BSA/HopQ-Ab/AuNP/MWCNT SPCE electrode with different HopQ concentrations, (B) variation in SWV peak current with respect to a blank solution vs. [HopQ] (and vs. Log[HopQ] for the inner plot). Error is the relative standard deviation for $n = 3$.

LOD = 2.0 pg/mL
LOQ = 8.6 pg/mL



Results

Comparison with other methods

Table 2. Limit of detection, detection range, and stability properties for different studies [61–65].

Ref.	Performance of Reported <i>H. pylori</i> Sensors						Stability at 4 °C (Weeks)	
	Interface	Detection Method	Biomarker	LOD (ng/mL)	Linear Range (ng/mL)			
[64]	CagA-Ab/ZnO*-T/SP-AuE	DPV	CagA	0.2	0.2–50	8–9	Up to 90%	
[62]	CagA-Ab/TiO ₂ -NPs/c-MWNT/Pin5COOH/AuE	SWV	CagA	0.1	0.1–8.0	~3	90%	
						~6	50%	
[61]	CagA-Ab/Pt _{nano} /PEDOT/rGO/AuE	EIS	CagA	0.1	0.1–30	~8	60–70%	
[65]	BabA-Ab/Pd _{nano} /rGO/PEDOT/AuE	EIS	BabA	0.2	0.2–20	8–9	70%	
[63]	VacA-Ab/g-C ₃ N ₄ /ZnO/AuE	DPV	VacA	0.1	0.1–12.8	~2	94%	
This work	BSA/HopQ-Ab/AuNP/CNT/SPCE	SWV	HopQ	0.002	0.01–100	4	~85%	
						8	~60%	

ZnO*-T: Irradiated Zinc Oxide Tetrapods, SP-AuE: screen printed gold electrode, AuE: gold electrode, TiO₂-NPs: Titanium oxide nanoparticles, c-MWCNT: carboxylated multi-walled carbon nanotubes, Pin5COOH: polyindole carboxylic acid, Pd/Pt_{nano}: palladium/platinum nanoparticles, PEDOT: poly(3,4-ethylenedioxythiophene), rGO: reduced graphene oxide, g-C₃N₄: graphitic carbon nitride.



Results

Accuracy and specificity

Table 1

Comparison of Analytical Parameters of Different Detection Methods with the Presented Immunosensor

<i>H. pylori</i> detection methods	accuracy	specificity	time consumption
histopathology	95.3%	77.8%	about 7 days
PCR	94.5%		24 h
serology	86%	60%	more than 3 h
stool antigen test	80.2%	86.7%	1–4 days
rapid urease test	73.6%	85%	40 min
presented immunosensor	96.2%	87.03%	10–15 min



Results

Selectivity and Cross-Reactivity

❖ Selectivity

❖ Cross-Reactivity

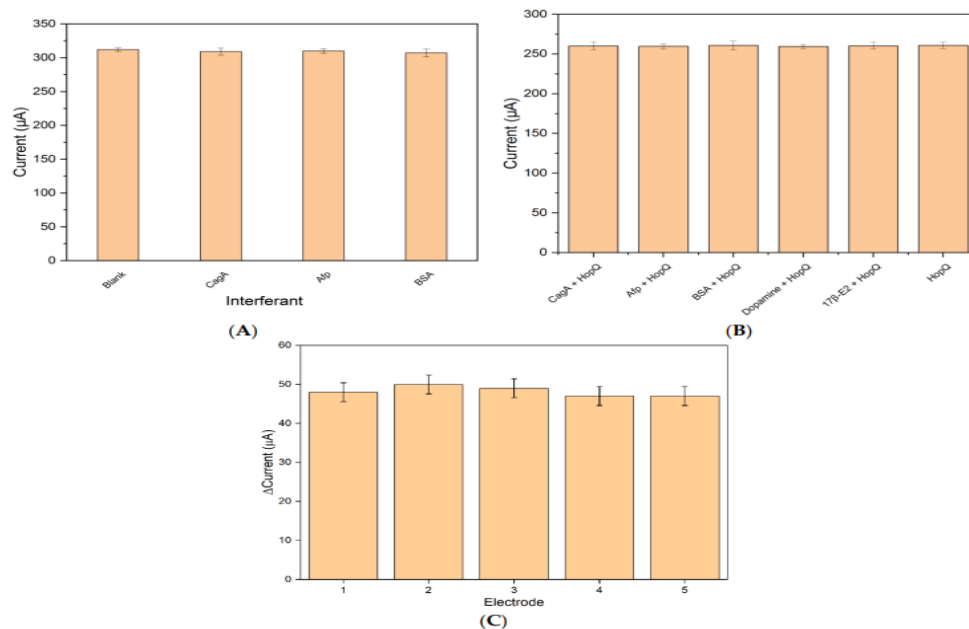


Figure 5. (A) Interferent study of BSA/HopQ-Ab/AuNP/MWCNT SPCE electrode with 5 ng/mL interferent concentration. (B) Interferent study of BSA/HopQ-Ab/AuNP/MWCNT SPCE electrode with 5 ng/mL HopQ antigen. (C) SWV peak current response of identically fabricated electrodes with the same criteria; the error bar is for $n = 5$.



Results

- ❖ Reproducibility
- ❖ Shelf-Life Studies and Comparison with Other Platforms

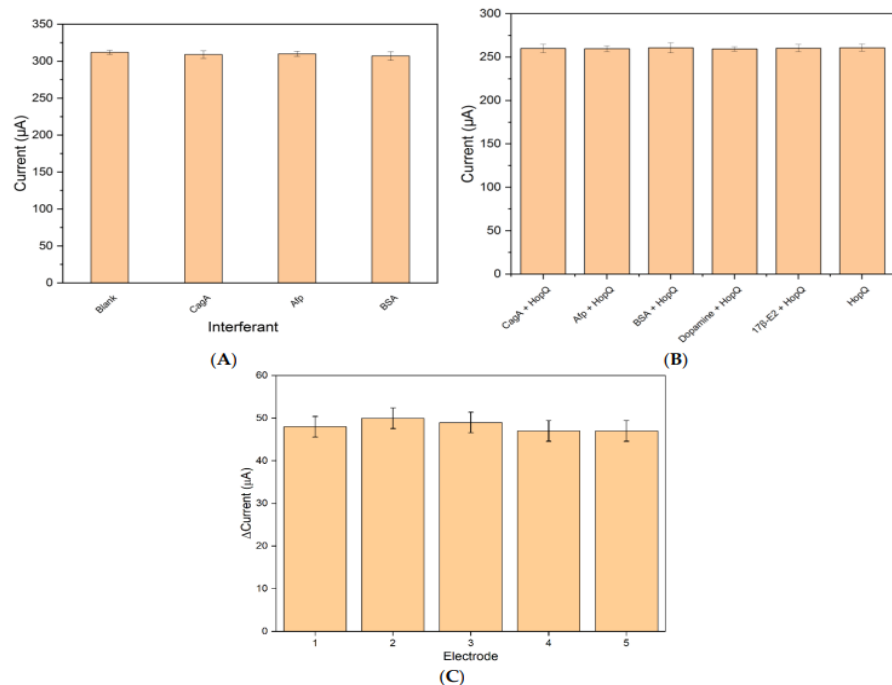
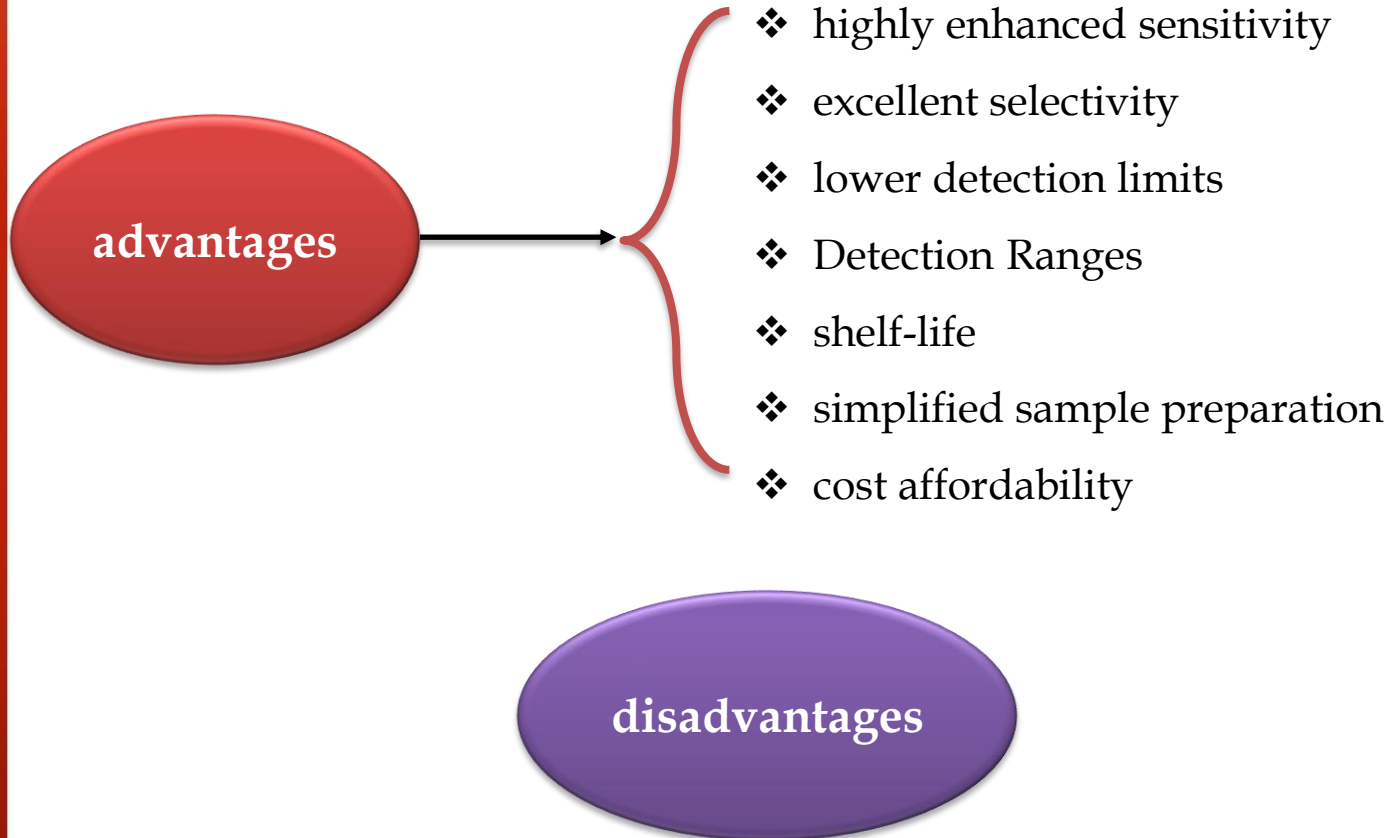


Figure 5. (A) Interferent study of BSA/HopQ-Ab/AuNP/MWCNT SPCE electrode with 5 ng/mL interferent concentration. (B) Interferent study of BSA/HopQ-Ab/AuNP/MWCNT SPCE electrode with 5 ng/mL HopQ antigen. (C) SWV peak current response of identically fabricated electrodes with the same criteria; the error bar is for $n = 5$.



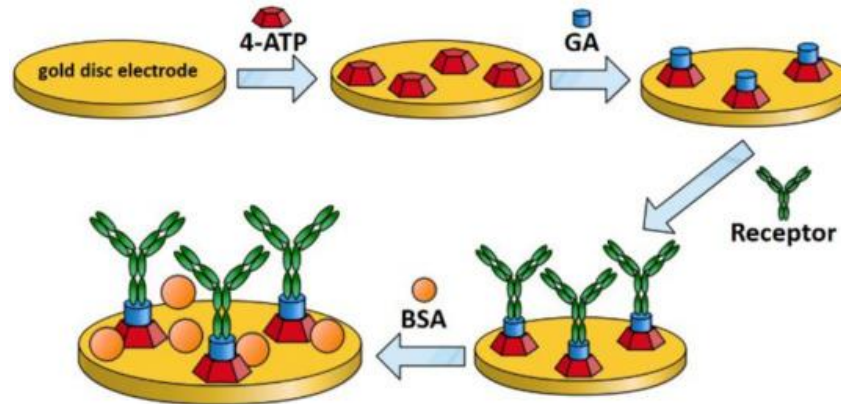
advantages and disadvantages





Other studies

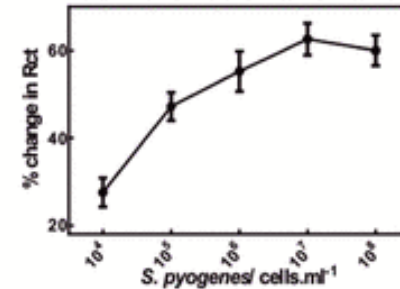
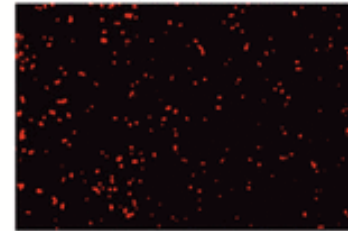
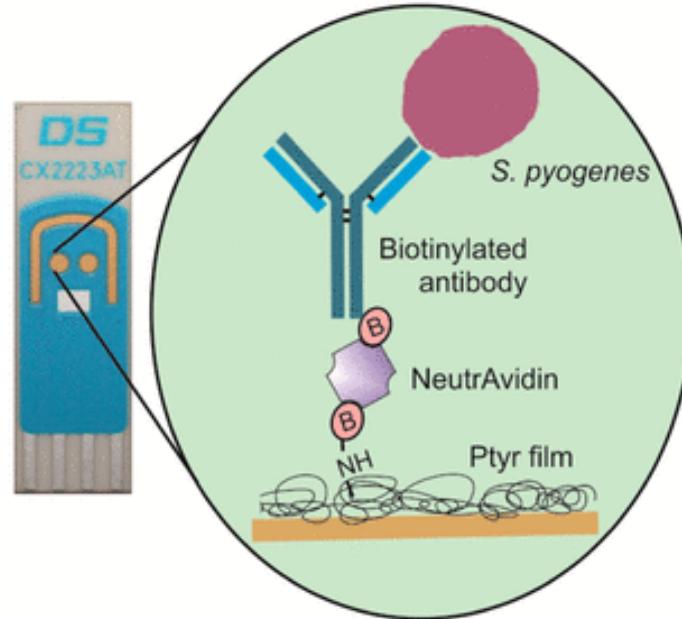
Methodology of Selecting the Optimal Receptor to Create an Electrochemical Immunosensor for Equine Arteritis Virus Protein Detection





Other studies

Novel Impedimetric Immunosensor for Detection of Pathogenic Bacteria *Streptococcus pyogenes* in Human Saliva





Conclusions

Why should we use immunosensors?



MWCNT/AuNP



References

- ❖ Jaradat H, Al-Hamry A, Ibbini M, Fourati N, Kanoun O. **Novel Sensitive Electrochemical Immunosensor Development for the Selective Detection of HopQ *H. pylori* Bacteria Biomarker.** Biosensors (Basel). 2023 May 8;13(5):527. doi: 10.3390/bios13050527. PMID: 37232889; PMCID
- ❖ Kurzawa, J.K.; et al. **Methodology of Selecting the Optimal Receptor to Create an Electrochemical Immunosensor for Equine Arteritis Virus Protein Detection.** Chemosensors 2021, 9, 265.
- ❖ Ahmed A, Rushworth JV, Wright JD, Millner PA. **Novel impedimetric immunosensor for detection of pathogenic bacteria *Streptococcus pyogenes* in human saliva.** Anal Chem. 2013 Dec 17;85(24):12118-25. doi: 10.1021/ac403253j. Epub 2013 Nov 27. PMID: 24256123.



**Thank you
for your attention**