A Portable Impedance Aptasensing System for the Rapid Detection of *Salmonella* Typhimurium in Poultry Products

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Background

- *Salmonella* Typhimurium is a pathogenic Gram-negative, rod-shaped bacterium (Sheikhzadeh,2016).
- People infected with Salmonella develop salmonellosis. The symptoms for salmonellosis include diarrhea, fever, and abdominal cramps, which can last between four to seven days (CDC, 2012).
- In the US, Salmonellosis causes an estimated 1.2 million cases each year, resulting in 23,000 hospitalization and 450 deaths. (CDC, 2012).
- The most recent outbreak (December 9, 2012 to February 20, 2013) involved 22 infected people and 7 hospitalizations in six different states (CDC, 2013).
- Although the current conventional methods used to detect *Salmonella* have high affinity and are reliable, they have many disadvantages which prevent them from being used for in-field and real time detection.

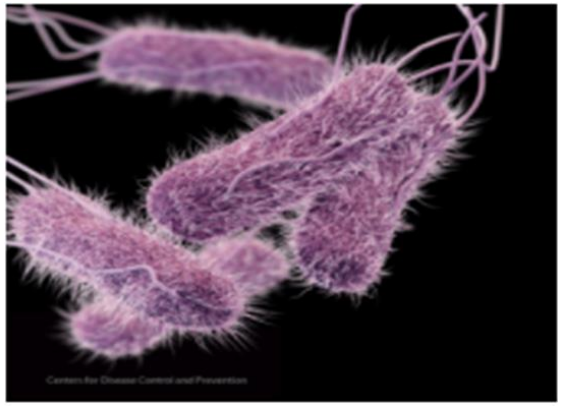

Fig. (1) © Copyright 2014 Discovery Scientific Solutions

Conventional detection methods

- Depend on microbiological techniques such as: pre-enrichment steps, cultivation of bacteria, and validation of suspicious colonies (Velusamy et al. 2010).
- The major disadvantages to these methods are that they are labor-intensive and time-consuming. It can take 2-3 days for preliminary results and 7-10 days for confirmation (Velusamy et al., 2010).
- Common methods include:
  - Enzyme linked immunosorbent assay (ELISA), (Thermal Fisher Scientific)
  - Polymerase Chain Reaction (PCR)

Velusamy, V., Arshak, K., Korostynska, O., Oliwa, K., Adley C. "An overview of foodborne pathogen detection: In the perspective of biosensors." *Biotechnology advances* 28.2 (2010): 232-254.
https://www.thermofisher.com/us/en/home/life-science/protein-biology/protein-biology-learning-center/protein-biology-resource-library/pierce-protein-methods/overview-elisa.html

Research objective

- Due to the drawbacks of traditional detection methods and the threat *Salmonella* poses on human health, there is an urgent need for the development of a rapid, reliable, and sensitive method to detect the presence of *Salmonella* in poultry products.
- The goal of this project was to develop a portable impedance aptasensing system using an interdigitated microarray electrode (IDME) for the rapid and sensitive detection of *S.* Typhimurium in poultry products.

Set up of the system

A Portable Immunosensing System for Rapid Detection of *Salmonella* Typhimurium

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- Published in Sensors journal (vol. 17, page 1973) in 2017.
- System included a laptop with LabVIEW software, a data acquisition card (DAQ), and antibody immobilized electrode.

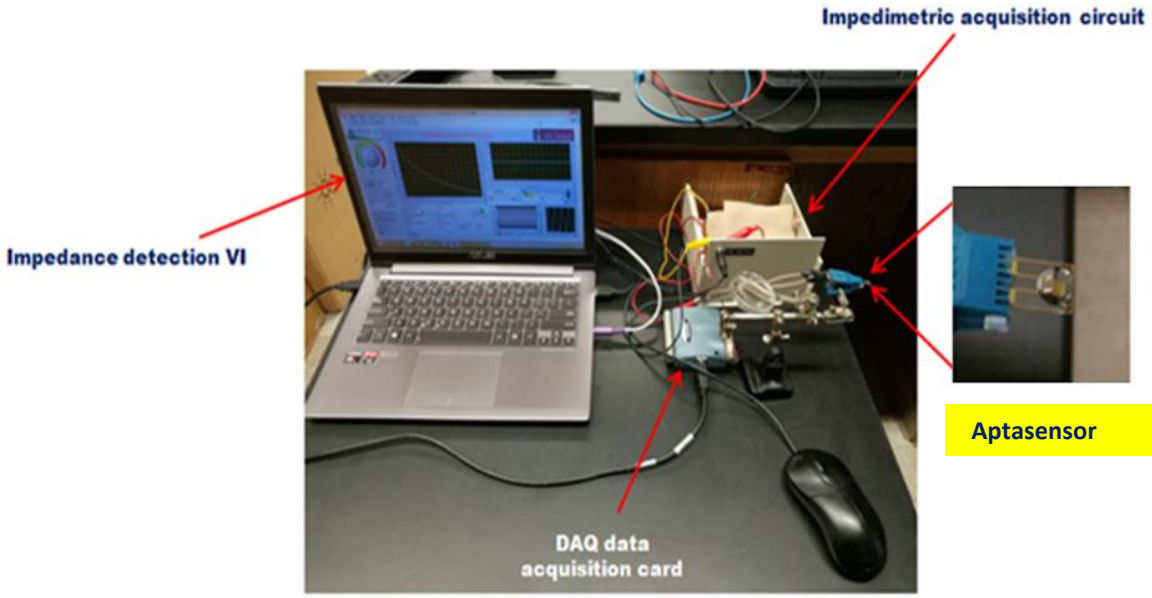
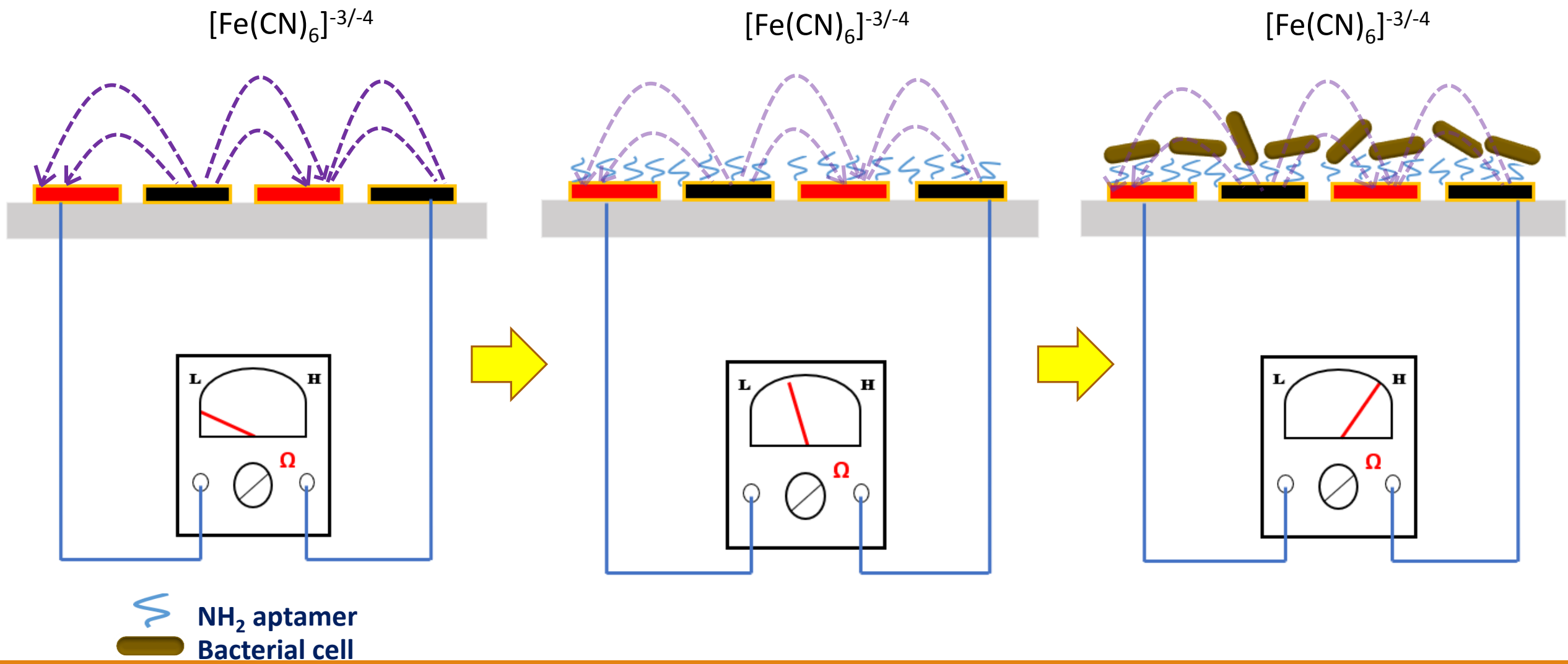


Fig. (2) Set up of the system

Principle of the aptasensor



Materials

Reagents

- 16-mecaptohexadecanoic acid 20 mM **(MHDA)**
- N-(3-dimehylaminoporpyl)-N'-ethylcarbodiimide hydrochloride, N-hydroxysuccinimide solution, 75 mM/30 mM, v/v, 1:1 **(EDC/NHS)**
- NH_2-aptamer (Integrated DNA Technologies)
- Poly (ethylene glycol) methyl ether thiol, 0.1 mg/ml in PBS **(PEG)**
- Redox probe, $[Fe(CN)_6]^{3-/4-}$

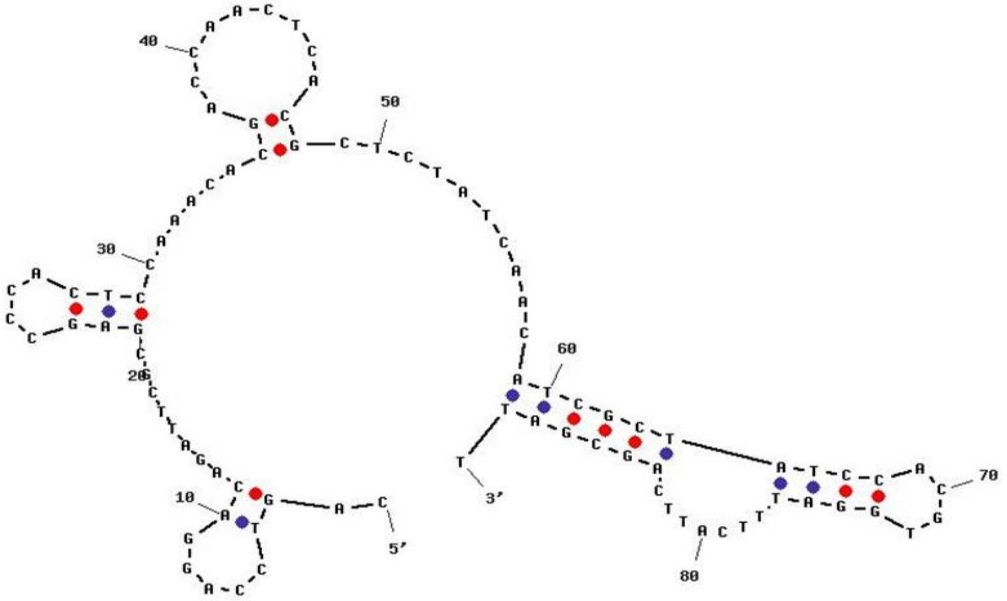


Fig. (3) Predicted secondary structure of the aptamer

Method

1. Electrode cleaning with 1 M **NaOH** (30 min) and 1 M **HCl** (5 min) to remove surface oxide.
2. Functionalization of electrode surface with **MHDA** to form self-assembled monolayer (24 - 48 h).
3. Electrode surface activation with **EDC/NHS** (10 min).
4. Aptamer immobilization on electrode surface with 50 µL **NH_2-aptamer** (40 min).
5. Background noise blocking with 50 µl **PEG** (30 min).
6. Rest period at room temperature (48 h).
7. Bacterial detection (40 min).

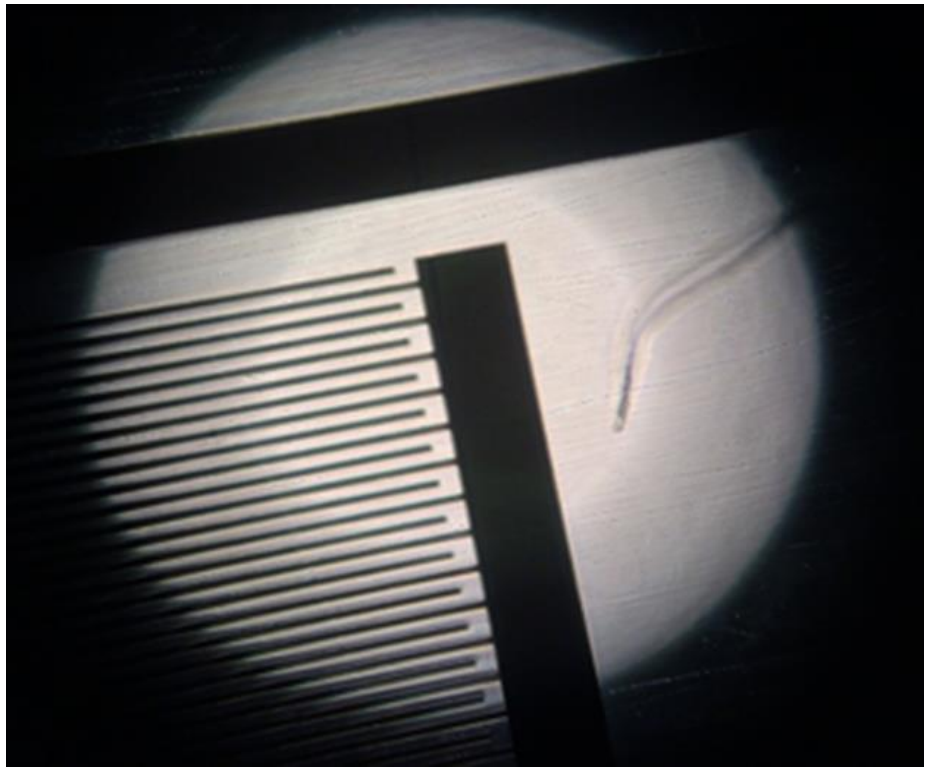


Fig. (4) Interdigitated microarray electrode

Results

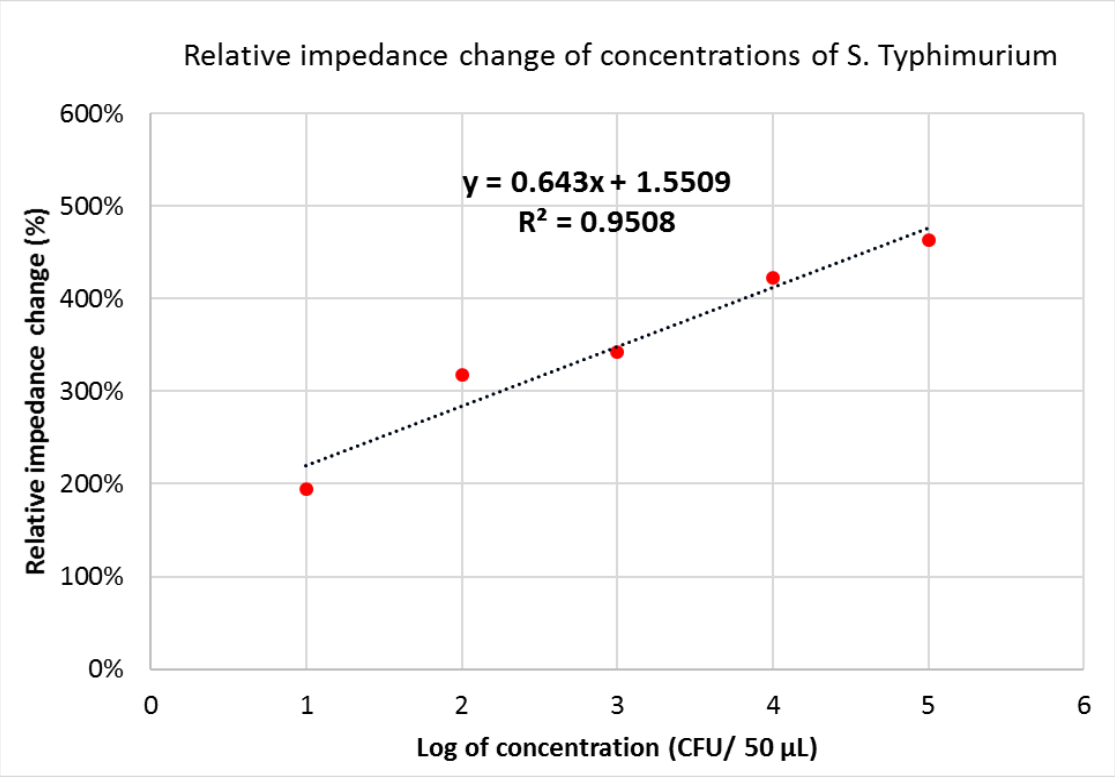


Fig. (6) The relationship between the logarithmic value of the concentration of *S*. Typhimurium and impedance change at a frequency of 101 Hz.

$$Z_R = \frac{Z_T - Z_B}{Z_B} * 100\%$$

Where,
Z_R = relative impedance change in percent
Z_T = impedance values caused by target detection
Z_B = impedance value of the baseline

Table 1. Impedance change caused by each concentration of *S*. Typhimurium at 101 Hz

Log of concentration (CFU/ 50 µL)	Relative impedance change
1	194%
2	318%
3	342%
4	422%
5	463%

Results

Table 2. Average relative impedance change at 101 Hz caused by non-specific targets using a concentration of 10^5 (CFU/ 50mL)

Target	Average relative impedance change
E. coli K12	-16%
E. coli O157:H7	-3%
C. jejuni	-6%
L. innocua	-2%
L. monocytogenes	1%

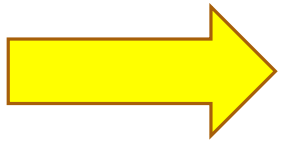


Table 3. Average relative impedance change at 101 Hz caused by non-specific targets compared to *S.* Typhimurium using a concentration of 10^5 (CFU/ 50mL)

Target	Average relative impedance change
S. Typhimurium	463%
E. coli K12	0%
E. coli O157:H7	0%
C. jejuni	0%
L. innocua	0%
L. monocytogenes	1%

Conclusion

- The results showed that there was a linear relationship with a correlation coefficient of 0.95 between the impedance change and log values of *S*. Typhimurium in a range of concentrations from 1.41×10^{1} to 1.41×10^{5} CFU $(50\ \mu L)^{-1}$ of pure culture samples.
- The aptasensor also showed a high specificity for *S*. Typhimurium with an average relative impedance change of 463% compared to 0% - 1% relative impedance change of non-target bacteria (*C. jejuni, E. coli* K12, *E. coli* O157:H7, *L. innocua, and L. monocytogenes*) at a concentration of 10^{5} CFU $(\mu L)^{-1}$.
- The developed impedance aptasensor has the potential to increase the sensitivity of detection, shorten detection time, lower costs per test, and allow for portability for in-field detection of pathogens.
- On-going research is focused on optimization of aptamer concentration and detection of *S*. Typhimurium in poultry products using a flow cell imbedded with the aptamer immobilized IDME.

Acknowledgments

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Thank you!

Questions?