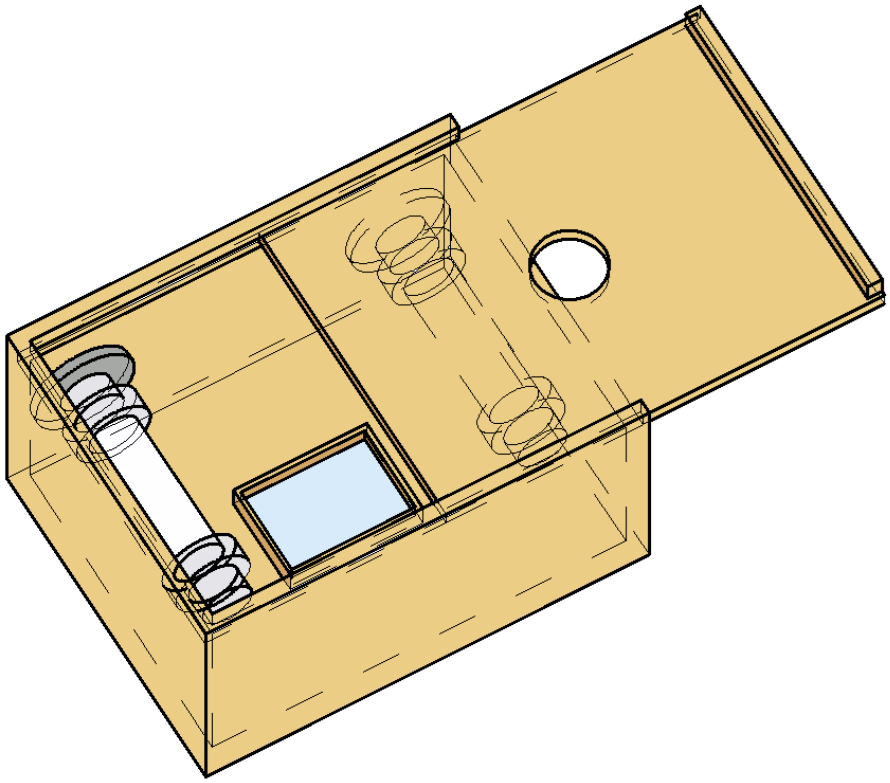
Sense Life with Color



2018
Team Results Document

Team Name: TruSense
University: Zhejiang University (ZJU)
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Date of Submission: 2018 / 8 / 29

TRUSENSE 2018

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Summary for the SensUs Website

Our team name '**TruSense'** demonstrates our serious attitude towards the SensUs 2018. Inspired by the principle of competitive enzyme-linked immunosorbent assay (ELISA), we designed and established our special biosensor system. With this, 8 samples are able to be detected at a time in 5 minutes, making doctors work more efficiently, which means a hot cake for hospitals. Furthermore, users can easily handle our biosensor and receive statistics just from the mobile application attached to the system, which offers a promising perspective. Besides, we are able to test the concentration of vancomycin not only in human plasma but in dairy like milk, signifying a broad, potential market in modern society. What we try is to utilize the color to make detection. In a word, the world is trying to change one's fate with technology and we are doing our best to sense life with color.

1. Biosensor System and Assay

1.1 Our Biosensor System

Our biosensor system is composed of test strips and a cartridge with a stable light in it (See **Figure 1**). The test strip is composed of a sample pad, a conjugate pad, an adhesive backing and an absorbent pad (See **Figure 2**). The cartridge of our biosensor is made of wood with two rechargeable lamps to provide stable light for taking photos (See **Figure 3**).

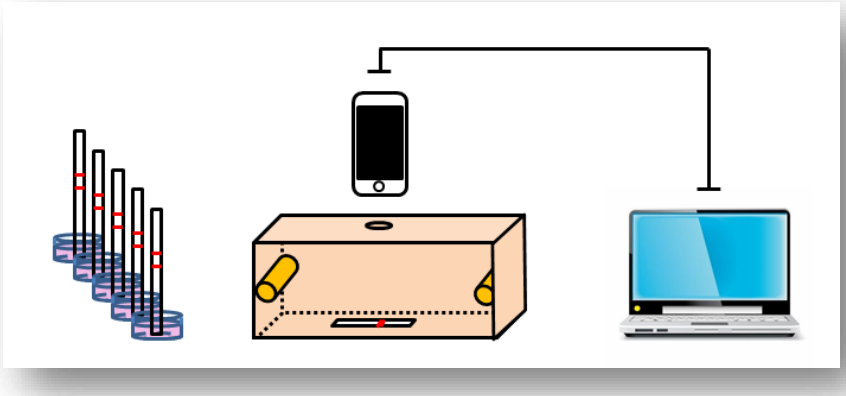

Figure 1 Structure of Our Biosensor System

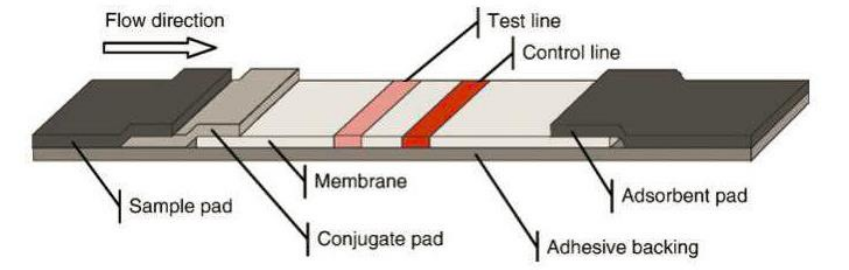

Figure 1 Structure of the Strip

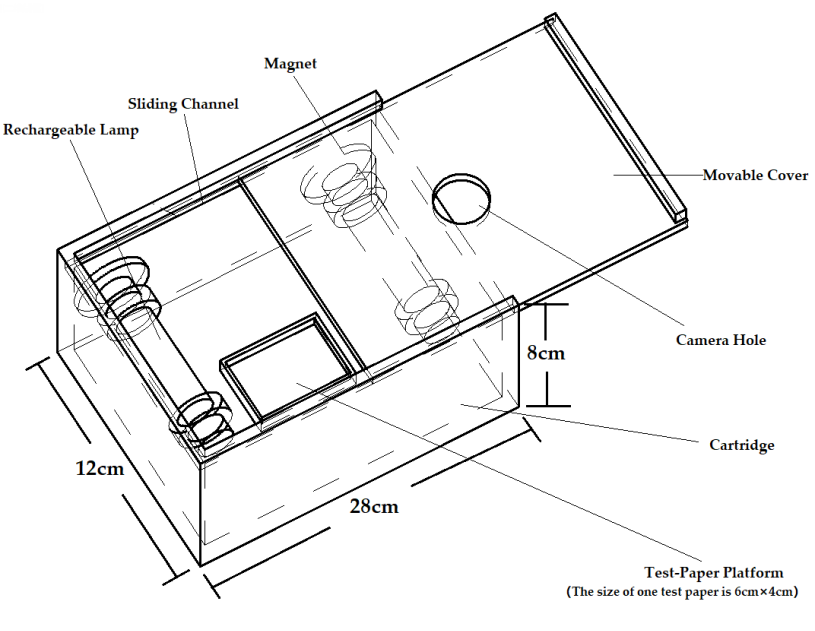

Figure 2 Prototype of Our Biosensor

1.2 Molecular Assay Principle

The detection of vancomycin by our biosensor is based on a competitive inhibition immunochromatography mechanism. In the process of chromatography, vancomycin in the sample binds to the specific monoclonal antibody labeled by colloidal gold, which inhibits the binding of the antibody to the vancomycin antigen conjugate on the detection line (T line), thus causing the detection line (T line) to display a band in light red or no color. In a certain range, the grayscale of the band has a linear relation with

the concentration of vancomycin and that is the fundamental for quantitative detection. Regardless of the presence or absence of vancomycin in the sample, the quality control line (C line) will be colored to show that the strip is effective.

1.3 Reagents

Test materials Reagents and solutions

HAuCL4, bovine serum albumin (BSA) (import, BASF or sigma), various common chemicals, surfactants, and phosphate buffered saline (PBS).

Equipment

Membrane instrument (BIODOT XYZ3060); colloidal gold particles (Beijing Qinbang Biotechnology Co., Ltd.), electromagnetic heating stirring sleeve thermostat with reflux round flask, snake type condenser tube, stirrer (burning gold); Spectrophotometer (measurement of gold particle quality); high speed refrigerated centrifuge (for marking process); oven (25 degrees to 65 degrees, membrane and gold drying); slitting machine (BIODOT CM4000); spray meter (IsoFlow Dispenser) Dehumidifier (dryness 20%); sealing machine (test paper storage); NC film (Sedolis 140), gold standard pad (glass fiber, Shanghai Jiening Biotechnology Co., Ltd.); sample pad (non-woven fabric); absorbent paper, Backboard (both domestically produced).

Ingredients

Antibody D-10, Antigen: 5055

2. Analytical Performance

First drop the sample solution of vancomycin into the 96-well plate and insert the strip. After that wait the T line to display the band for 3 minutes and put the strip into the cartridge. Since there are stable lights in the cartridge, we are able to take photos of the strip with mobile phone.

After collecting the photos of the strip, use ImageJ (Java-based image-processing and analysis software) to measure the grayscale of the T line. The specific steps are as follows:

1.Open ImageJ and read the picture (see **Figure 4A**);

2.Select ‘Process’ in the menu, then click ‘Subtract Background’, set the parameters (see **Figure 4B**);

3.Use mouse to select the measurement area, that is, the T line, then select 'Measure' and get the 'Results' box, use Mean as the final data (see **Figure 4C** and **4D**).

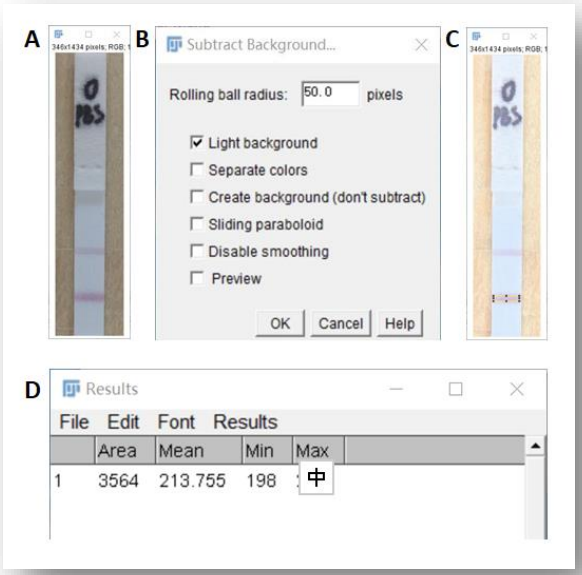

Figure 3 Analytical Performance

(A: Read the picture; B: Set the parameters; C: Select the T line; D: Measure)

We prepared standard vancomycin solutions with the concentrations ranging from 5-99 mg/ml (in 2mg/ml increments) and tested them using the experimental method described above. We've gotten the dose-response curve and designed a user-friendly interface for all users by Matlab. (see **Figure 5**)

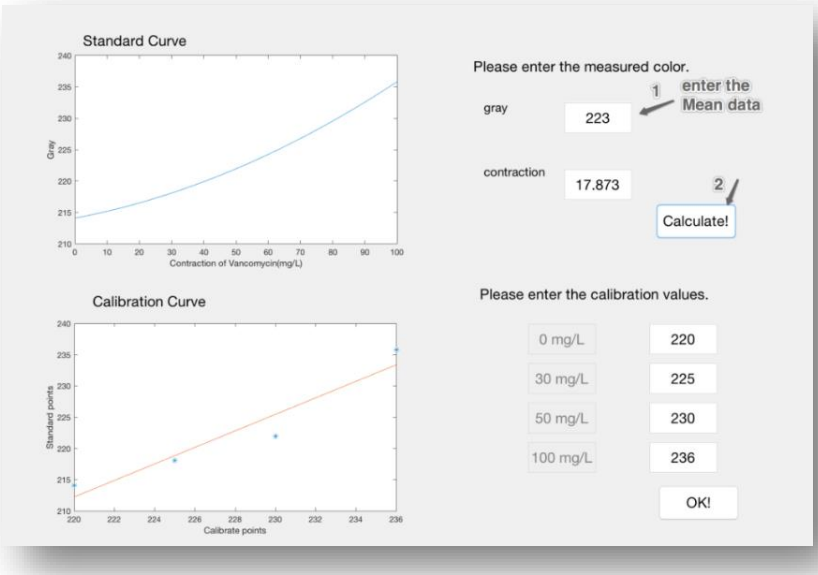

Figure 4 Interface

3. Novelty and Creativity

3.1 Already available

The assay is based on the principle of colloidal gold immunochromatography, which was considered to be a qualitative detection method. Our strips can be stored at 2-8℃ for 12 months in good quality. And the method for preparing the strips was optimized mostly by our sponsor, Beijing Kwinbon Biotechnology Co., Ltd.

ImageJ[1] is used to analyze the picture, and especially to get the grayscale of the strip (T line). In our detecting environment, ImageJ reads the original picture, then subtracts the background [2] to reduce the impact of imaging conditions such as brightness and device settings, and finally measures the average gray of the T line.

The idea of acquiring the concentration of vancomycin by measuring the grayscale of the testing strip was inspired by 'Study on Identification Model of Color and Material Concentration' [3]. As the material concentration increases, the value of each color reading tends to increase or decrease, indicating that there must be some correspondence between the concentration and color reading, which can be used to define the concentration by establishing the multivariate linear regression model.

3.2 New Developments

Quantitative detection： The test strips on the market are generally used for qualitative detection, while our biosensor can conduct quantitative detection of vancomycin, realizing the transformation from qualitative to quantitative detection.

Wide range of application: Our biosensor can not only determine the concentration of vancomycin in plasma, but also dairy products.

Simplified incubation process: The principle is similar to ELISA. The gold-labeled vancomycin antibody is sprayed directly on the conjugation pad as part of the test strip, so that the incubation can be carried out directly in the chromatography process.

4. Translation Potential

4.1 Stakeholder desirability

In the attempt to design this innovative product, we tried our best to meet the demands of potential customers from different perspectives. For medical staff, our vancomycin detection device has the capability of processing multiple serum samples with one single sample collection and detection task. This increases the work efficiency by releasing doctors and nurses from complex sample collection and processing procedures. For patients, on the other hand, our device provides a convenient home-based alternative to detecting vancomycin at a hospital. Our device has the advantages of being low cost, light weighted, and highly mobile. It achieves maximum convenience with a minimum pain in the sense that it only requires a small amount of serum to perform all the detection tasks. In the future, we plan to develop a cell phone application to automate the detection and data collection processes and to upload the data to internet for professional analysis and treatment suggestions. We believe that, our vancomycin detection device can help doctors to significantly improve the efficiency of serum sample collection, data analysis and monitoring. Our device achieves a high measurement accuracy as well as a wide measurement range. This is not only good for doctors and patients, but also good for medical insurance companies to reduce the potential legal cost due to erroneous measurements of vancomycin.

It is not difficult to measure the concentration of a medicine using a hospital facility. However, patients usually prefer a convenient home-based alternative to help them monitor the impact of antibiotics. While current devices like the HPLC can provide accurate measurements, their high price tag and large size do not permit the adoption by a normal household. The original intention of our design is to meet the demand of a low cost and convenient vancomycin measurement option suitable for a regular household.

Existing mobile antibiotic drug detecting devices can’t measure the concentration of vancomycin. Via collaboration with biotech corporations to adopt professional test strips, our device can provide complementary measurement data that is compatible with data from the hospital facilities. Therefore, its data can be integrated with measurements from hospital facilities to provide more comprehensive monitoring statistics, which can significantly improve the efficiency and accuracy of treatment of the patient.

4.2 Technical feasibility

Our device measures the vancomycin concentration based on color identification of the test strip. Therefore its main source of measurement error comes from the color identification capability of the cell phone. The detection procedure of our device consists of four steps: sample collection, colorization, color sensing, and data analysis. Experiments showed that, the same test procedure can be used to measure vancomycin concentration in blood, as well as in other liquids such as milk. The main body of our biosensor takes the shape of a wooden cuboid, with a size of 28 cm × 12 cm × 8 cm, and a weight of 2 kg. It is small but sturdy, and therefore easy to carry around. When the device is being used at home, a patient only needs to use a proper device to collect one or two drops of blood, drop them on the test strip, wait for several minutes, and then put the strip into the detection box for color identification. After taking photos of the test strip, our computer progress will compare the color of the test strip to our standard curve to obtain the concentration measurement of vancomycin in the blood sample. With the mobile app to be developed, the measurement data will be further uploaded to an online database to obtain professional analysis and feedback such as monitoring and treatment suggestions.

Currently, our vancomycin detection device achieved the key design objectives of small in size but reasonably accurate in measurement. However, its measurement accuracy is still inferior to professional facilities such as the HPLC. We plan to improve the measurement accuracy and the measurement range of our device particularly in the step of coloration. We will also make the detection box more user-friendly, for example, by adding a handle, a test strip storage slot and by integrating the coloration and the photography steps of the test procedure. In the current design, the shadowless lamp of the detection box is consists of two rechargeable and detachable diodes. Its design and positioning still need careful optimization to minimize its impact onto test results and to improve the usability of the detection box. The manufacturing process of our device is not complex. Its cost can be further reduced via mass production.

4.3 Business viability

The total cost of our detection device is within 200RMB. The cost of a single vancomycin measurement can be controlled to be within 5RMB. Comparing to other antibiotic sensing devices in the market, our device can attract both individual and hospital customers with its unique features such as being small and portable, being quick, accurate, and suitable for home use, and its low price tag proximate to other portable devices such as the glucose device. In addition to selling the hardware, we

can also provide service to our mobile app, data sharing and its integration with hospital facilities. Furthermore, because our device can be used to detect vancomycin concentration in other liquids such as milk, it can fit to a much wider market than classical single-function devices. Overall, we believe that our device should be highly competitive in the market. Its potential influence and profitability should be very attractive to business partners.

5. Team and Support

5.1. Contributions of the team members

In the team, ***Zhenwei Zhou*** is one of the leaders of the team, mainly responsible for the structure of vancomycin strip and specific improvement program. In addition, he was responsible for further communication and contact with instructors and sponsors, and made important contributions to the final results of the team.

Yixin Pan and ***Yadan Li*** are responsible for designing method of translating the information from color to concentration. To be specific, they used some color detecting app to get the color or gray of the detecting line (T line) on the test paper and then, calculated the corresponding concentration of the vancomycin. Li Yadan designed the specific algorithm to make the standard curve with gray as x-axis and concentration as y-axis.

Qixin Zhang is mostly responsible for writing the team result document. She was also in charge of the designing and construction of the cartridge with a stable light source.

Jiarui Huang helped explore the feasibility of RNA adapter to detect vancomycin, and also played a role in developing the color sensor. He is also in charge of commercializing the team's biosensor.

Yue Sun is the manager and responsible for financial management. Taking part in the experiment and writing documents.

Yuxiang Zhu tried to use the quantum dot method to combine vancomycin specifically for measurement, participated in the follow-up test, provided logistical support including designing the team logo and preparing the team attire.

Zhuonan Zhang participated in part of the experiment and publicization work.

Zeping Li proposed a few ideas in the early stage of biosensor design and made some contributions to poster production.

Chen Lu is in charge of experimental training and final presentation. Also, she led the early stage of experiment for the entire team.

Shitong Zhong discussed possible options for designing biosensors, participated in the early exploration of the different detection strategies, and ruled out impossible options for subsequent trials.

The work ***Heng Ni*** did was searching information and using ELISA to detect the concentration of vancomycin.

5.2 Support

This work is financially supported by the College of Life Sciences, Zhejiang University. The experimental platform is provided by the Integrated Open Laboratory in the Biological Experiment Center of Zhejiang University. We thank Prof. Liquan Huang for firstly recruiting the team members. He technically and mentally led the team heading for the right direction. We also thank our Associate Dean Prof. Luyang Yu for his support.

5.3 Sponsors

This work is sponsed by Beijing Kwinbon Biotechnology Co., Ltd. for its technical and financial support to our team. Specially, Kwinbon provided us with existing relatively mature technique for making the strips in our system.

6. Final Remarks

We sincerely thank Beijing Kwinbon Biotechnology Co., Ltd for its technical and financial support to our team. We also thank the Integrated Open Laboratory in the Biological Experiment Center of Zhejiang University for providing us with lab space and necessary equipment.

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Appendix

Preparation of test strips

1. Pretreatment of the colloidal gold pad and sample pad

(1) Prepare the buffer solution according to the recipe: buffer + NaCl + surfactant + protein (BSA, Casein).
(2) The sample pad is thoroughly soaked in the buffer solution for 5 minutes. After soaking, the pad is removed and dehydrated for 2 minutes. Then place the pad flat on the sieve.
(3) Dry the pad in a 37 ° C drying oven until the humidity is constant at about 20%.
(4) The dried material is sealed in an aluminum foil bag or a plastic bag with desiccant. Label the pad and record the batch number recorded.

2. Spotting

Colloidal gold drying preparation

(1) Prepare the finished colloidal gold and the finished colloidal gold pad.
(2) Use a gold detector, set the amount of gold to 0.3ul/ml.
(3) Put the gold-plated colloidal gold pad into an oven at 37 degrees (humidity less than 20%) for 2 hours, and then put the dried material in a bag with desiccant and store it with the label.

Antigen sprayed onto the NC membrane

(1) The NC membrane was taken out and equilibrated at room temperature and normal humidity for 0.5 hours.
(2) Dilute the antigen 1:4 with the buffer solution.
(3) Use a BIODOT instrument. Set the spray amount to 1 ul/cm and spot.
(4) Dried the membrane at 37 degrees overnight, and then sealed and packaged for use.