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Title: Rapid Detection of *Helicobacter pylori* Virulence and Typing Using Quantum Dot Labeling Technology

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Author Questionnaire

1. **Microscopy**: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **No**

2. **Software:** Does the part of your protocol being filmed include step-by-step descriptions of software usage? **Yes**

Videographer: Please record the computer screen for the shots labeled as SCREEN

3. **Filming location:** Will the filming need to take place in multiple locations? **No**

Current Protocol Length
Number of Steps: 12
Number of Shots: 28 (8 SC)

Introduction

Videographer: Obtain headshots for all authors available at the filming location.

1.1. **Hui-Quan Gan**: Our research focuses on developing diagnostic reagents for in vitro immunochromatography.We aim to use quantum dot labeling to enable specific antigen-antibody binding for precise *Helicobacter pylori* detection **[1]**.

1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 2.2.1*

What significant findings have you established in your field?

1.2. **Hui-Quan Gan**: We've discovered that using quantum dot technology for *Helicobacter pylori* typing detection can really help with the precise treatment of this bacteria **[1]**.

1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 2.8.2*

What research gap are you addressing with your protocol?

1.3. **Ya-Nan Yao**: We've solved the problems of traditional technology being unable to distinguish the virulence of Helicobacter pylori and having complicated operations through quantum dot technology **[1]**.

1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 2.12.1*

What advantage does your protocol offer compared to other techniques?

1.4. **Ya-Nan Yao**: While judging whether *Helicobacter pylori* is infected, we can distinguish the strength of virulence and provide individualized treatment plans **[1]**.

1.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 3.1.1*

Videographer: Obtain headshots for all authors available at the filming location.

Ethics Title Card

This research has been approved by the human research ethics committee at the Guangdong Provincial People's Hospital (Guangdong Academy of Medical Sciences), Southern Medical University

Protocol

2. Sample Processing and Testing for *Helicobacter pylori* Detection

Demonstrator: Lu-Si Wu

2.1. To begin, collect blood in a separation gel tube while taking care to avoid hemolysis **[1]**.

2.1.1. WIDE: Talent picking up the separation gel tube with blood from the workbench.

2.2. If testing the serum within 4 hours is not possible **[1]**, store the sample at a temperature between 2 and 8 degrees Celsius for up to 3 days **[2]**. For long-term storage, seal and store the sample below minus 20 degrees Celsius for up to 1 year **[3]**.

2.2.1. Talent examining the tube with blood.

2.2.2. Talent placing the serum sample in a refrigerator set between 2 and 8 degrees Celsius.

2.2.3. Talent sealing and placing the serum tube into a freezer tempered below minus 20 degrees Celsius.

2.3. Restore the samples to room temperature and thoroughly mix them before use **[1]**.

2.3.1. Talent removing samples from refrigerator.

2.4. Take out the reagent kit and quality control materials and allow them to equilibrate to room temperature **[1]**.

2.4.1. Talent placing reagent kit and QC materials on the bench at room temperature.

2.5. Now centrifuge the whole blood samples at 1467 *g* for 10 minutes **[1]**.

2.5.1. Talent placing whole blood tube in the centrifuge.

2.6. Now, power on the instrument and the operating computer **[1].** Launch the corresponding software and log in to the software.**[2]**

2.6.1. Talent pressing the power buttons on both the instrument and the computer.

2.6.2. SCREEN: Click on the software icon to launch the application.

Videographer: Please record the computer screen for the shots labeled as SCREEN

2.7. Verify that the Secure Digital or SD card lot number matches that of the reagent kit **[1]**. Insert the SD card into the instrument **[2]** and select **Read ID Chip** in the application for automatic matching **[3]**.

2.7.1. Talent examining the lot number on the SD card.

2.7.2. Talent inserting the SD card into the instrument slot.

2.7.3. SCREEN: Click Read ID Chip.

2.8. In the application, select the **Test** module **[1]**. Choose **Initialization Settings** to initialize the instrument and wait for the process to complete **[2]**.

2.8.1. SCREEN: Click on the software icon to launch the application and navigate to **Test** module.

2.8.2. SCREEN: Select **Initialization Settings** and wait until the initialization is complete.

2.9. Access the instrument's reagent area and remove the test card slot **[1]**. Now, open the reagent kit and select the required number of test cards **[2]**.

2.9.1. Talent opening the instrument’s reagent area and sliding out the test card slot.

2.9.2. Talent opening the reagent kit and selecting the correct number of test cards.

2.10. Then, open the foil pouches of the test cards **[1]** and place them in the slot according to the indicated orientation **[2]**. Reinsert the card slot into the instrument's reagent area **[3]**.

2.10.1. Talent unsealing test card foil pouch.

2.10.2. Talent placing the card in the slot with correct orientation.

2.10.3. Talent reinserting the slot back into the instrument.

2.11. Within the **Test** module, select bidirectional LIS **[1]**. Enter the Manual Entry Module, click **Information Entry,** and input the specific quality control material information **[2]**. Click **Generate [3]** and place the quality control materials in the designated sample

rack, ensuring correct order and orientation **[4]**. Click **Start Test** to initiate QC testing **[5]**.

2.11.1. SCREEN: Select bidirectional LIS in the Test module.

2.11.2. SCREEN: Click on **Information Entry** and type in the QC material information. Note: 2.11.2 and 2.11.3 are shot together.

2.11.3. SCREEN: Click **Generate** to finalize the entry.

2.11.4. Talent placing QC materials in the sample rack with correct orientation.

2.11.5. SCREEN: Click **Start Test** to begin QC testing.

2.12. Now, in the **Test** module, select Manual Entry to display **Bidirectional LIS [1]**. Place the test samples in the designated sample rack, ensuring proper order and orientation **[2]**. Click **Start Test [3]**.

2.12.1. SCREEN: Navigate to and click on Manual Entry to display Bidirectional LIS in the Test module.

2.12.2. Talent placing patient samples in the sample rack in correct order and orientation.

2.12.3. SCREEN: Click **Start Test** to initiate testing.

2.13. Once testing is completed, the instrument automatically ejects the used test cards **[1]** and the operator disposes of them as medical waste **[2]**.

2.13.1. The operator handles the used test cards ejected from the instrument. Note: 2.13.1 and 2.13.2 are shot together

2.13.2. Talent discarding cards in a medical waste bin.

Results

3. Results

3.1. The peak diagrams of two negative samples showed no significant signals at X1 Urease, X2 CagA *(C-A-G-A)*, or X3 VacA *(V-A-C-A)*, confirming *Helicobacter pylori*-negative results **[1]**, while the strong peak at X4 C line indicated valid test results **[2]**.

3.1.1. LAB MEDIA: Figure 4. *Video editor: Highlight the flat lines at X1, X2, and X3*.

3.1.2. LAB MEDIA: Figure 4. *Video editor: Highlight the tall peak at X4.*

3.2. Two samples positive for Urease showed strong signal peaks at X1, confirming *Helicobacter pylori* type II *(2)* infection **[1]**, while X2 and X3 remained at baseline or low signal, indicating negative CagA and VacA antibodies **[2]**.

3.2.1. LAB MEDIA: Figure 5. *Video editor: Highlight the tall peak at X1.*

3.2.2. LAB MEDIA: Figure 5. *Video editor: Mark the flat lines at X2 and X3*.

3.3. Three samples positive for Urease, and also positive for CagA and or VacA, showed high peaks at X1, X2, and X3, indicating *Helicobacter pylori* type I *(1)* infection **[1]**.

3.3.1. LAB MEDIA: Figure 6*. Video editor: Highlight the three separate high peaks for X1, X2, and X3*.

3.4. Receiver Operating Characteristic or ROC curves for individual biomarkers and combined indicators showed that combining five indicators with G17, PGI, PCI, PGR, and *Helicobacter pylori* antibody typing enhanced diagnostic performance, with the highest sensitivity and specificity **[1]**.

3.4.1. LAB MEDIA: Figure 7, 8, 9 *Video editor: Highlight the "5 indicators combined" line in all 3 figures*

Pronunciation Guide:

1. Hemolysis
   Pronunciation link: https://www.merriam-webster.com/dictionary/hemolysis
   merriam-webster.com
   IPA: /ˌhiːˈmɑːləsɪs/
   Phonetic: hee-MAH-luh-sis

2. Centrifuge
   Pronunciation link: https://www.merriam-webster.com/dictionary/centrifuge
   merriam-webster.com
   IPA: /ˈsɛntrəˌfjuːʤ/
   Phonetic: SEN-truh-fyooj

3. Urease
   Pronunciation link: https://www.merriam-webster.com/dictionary/urease
   merriam-webster.com
   IPA: /jʊˈriːs/
   Phonetic: yoo-REESE

4. Peristalsis
   Pronunciation link: https://www.merriam-webster.com/dictionary/peristalsis
   merriam-webster.com
   IPA: /ˌpɛrəˈstɒlsɪs/
   Phonetic: per-uh-STOL-sis

5. Equilibrate
   Pronunciation link: https://www.merriam-webster.com/dictionary/equilibrate
   forvo.com
   IPA: /ɪˈkwɪlɪˌbreɪt/
   Phonetic: ih-KWIL-ih-brayt

6. Specificity
   Pronunciation link: https://www.merriam-webster.com/dictionary/specificity
   howtopronounce.com
   IPA: /ˌspɛsəˈfɪsəti/
   Phonetic: spes-uh-FISS-ih-tee

7. Sensitivity
   Pronunciation link: https://www.merriam-webster.com/dictionary/sensitivity
   shabdkosh.com
   IPA: /ˌsɛnsəˈtɪvəti/
   Phonetic: sens-uh-TIV-uh-tee

8. Biomarker
   Pronunciation link: https://www.merriam-webster.com/dictionary/biomarker
   howtopronounce.com
   IPA: /ˈbaɪ.oʊˌmɑrkɚ/
   Phonetic: BY-oh-MAR-ker

9. Receiver Operating Characteristic (ROC)
   Pronunciation link: No confirmed link found
   IPA: /ɑr oʊ siː/
   Phonetic: ar-oh-see

10. LIS (Laboratory Information System)
    Pronunciation link: No confirmed link found
    IPA: /ˌɛl aɪ ˈɛs/
    Phonetic: ell-eye-ESS

11. CagA
    Pronunciation link: No confirmed link found
    IPA: /ˌsiː eɪ dʒiː eɪ/
    Phonetic: SEE-AY-JEE-AY

12. VacA
    Pronunciation link: No confirmed link found
    IPA: /ˌviː eɪ siː eɪ/
    Phonetic: VEE-AY-SEE-AY

13. Helicobacter
    Pronunciation link: https://www.merriam-webster.com/dictionary/helicobacter
    spanishdict.com
    IPA: /ˌhɛlɪkoʊˈbæktər/
    Phonetic: HEL-ih-co-BACK-ter

14. Pylori
    Pronunciation link: https://www.howtopronounce.com/pylori
    howtopronounce.com
    IPA: /paɪˈlɔːraɪ/
    Phonetic: py-LOR-y

15. Foil
    Pronunciation link: https://www.merriam-webster.com/dictionary/foil
    howtopronounce.com
    IPA: /fɔɪl/
    Phonetic: foyl

16. Foil pouch (compound phrase; "pouch" link)
    Pronunciation link: https://www.merriam-webster.com/dictionary/pouch
    howjsay.com
    IPA: /paʊʧ/
    Phonetic: powch

17. Thermocouple (common in temp context)
    Pronunciation link: https://www.merriam-webster.com/dictionary/thermocouple
    IPA: /ˈθɜːrmoʊˌkʌpəl/
    Phonetic: THER-mo-kuh-pul

18. Aliquot (lab sampling term)
    Pronunciation link: https://www.merriam-webster.com/dictionary/aliquot
    IPA: /ˈælɪkwɒt,ˈælɪkwət/
    Phonetic: AL-ih-kwot

19. Anaerobic (storage context)
    Pronunciation link: https://www.merriam-webster.com/dictionary/anaerobic
    IPA: /ˌænəˈroʊbɪk/
    Phonetic: an-uh-ROH-bik