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Project Page Link: <https://review.jove.com/account/file-uploader?src=21817918>

Title: Rapid Point-of-Care Detection of Pathogens for Lower Respiratory Tract Infections in ICU Patients

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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**
- 3. Filming location:** Will the filming need to take place in multiple locations? **No**
- 4. Testimonials (optional):** Would you be open to filming two short testimonial statements **live during your JoVE shoot**? These will **not appear in your JoVE video** but may be used in JoVE's promotional materials. **No**

Current Protocol Length

Number of Steps: 16

Number of Shots: 34

Interviews

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

INTRODUCTION:

~~REQUIRED: Summarize your research focus and the main questions it aims to answer.~~

- 1.1. **Xudong Liu:** Our research focuses on developing rapid, integrated detection of lower respiratory pathogens to support timely antimicrobial decisions in critically ill patients.
 - 1.1.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

~~Describe the research gap or limitation in existing methods and how this protocol addresses them.~~

- 1.2. **Xudong Liu:** Conventional methods are slow and labor-intensive; whereas our closed microfluidic LAMP workflow delivers multiplexed results in about one hour.
 - 1.2.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

CONCLUSION:

~~What does this protocol allow researchers to study or measure?~~

- 1.3. **Liang Bai:** This protocol allows researchers to measure qualitative detection and time-to-positivity for 15 respiratory pathogens in a single integrated run.
 - 1.3.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

~~Describe the most important consideration or challenge when performing this protocol.~~

- 1.4. **Liang Bai:** The most important consideration is complete specimen liquefaction and accurate loading, because viscous samples or incomplete transfer can cause channel failures.
 - 1.4.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

~~How can future studies build on this work? Describe the questions or research directions that can be investigated next.~~

1.5. **Qianlin Wang:** Future studies can build on this work by prospectively validating diagnostic performance, expanding resistance targets, and assessing clinical impact across multiple centers.

1.5.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

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

Ethics Title Card

The samples were collected under a protocol approved by the Ethics Review Committee of Peking Union Medical College Hospital, Chinese Academy of Medical Sciences. Written informed consent was obtained from all the participants or their legally authorized representatives

Protocol

***Videographer:** Please always record an audio slate while filming each shot mentioned in the shotlist.*

NOTE: All SCREEN Shots are filmed by the videographer

2. Sample Preprocessing

Demonstrator: Liang Bai

- 2.1. To begin, obtain the pre-collected lower respiratory tract specimens, including sputum, bronchial aspirates, and bronchoalveolar lavage fluid [1].
 - 2.1.1. WIDE: Talent arranging pre-collected sputum cup, a specimen trap containing a bronchial aspirate, and a container containing bronchoalveolar lavage fluid, on the laboratory bench. ***Videographer:** Please obtain CU shot as well for this shot.*
- 2.2. Using the number 2 disposable pipette, aspirate the specimen to the marked line [1] and dispense it into the liquefaction tube [2]. Squeeze the pipette bulb repeatedly to transfer all of the viscous material, then cap the tube [3].
 - 2.2.1. Talent aspirating the specimen to the marked line using the number 2 disposable pipette.
 - 2.2.2. Talent dispensing the specimen into the liquefaction tube.
 - 2.2.3. Talent squeezing the pipette bulb repeatedly to transfer all viscous material and capping the liquefaction tube.
- 2.3. Shake the liquefaction tube vigorously by hand for 1 minute [1]. Inspect the sample visually and continue shaking until no visible mucus clumps remain and the specimen flows freely through the number 1 disposable pipette without resistance [2].
 - 2.3.1. Talent vigorously shaking the liquefaction tube by hand.
 - 2.3.2. Shot of the uniform, water-like consistency specimen flowing freely through the number 1 disposable pipette without resistance.
- 2.4. Open the sealing cap of the lysis tube [1]. Using the number 1 disposable pipette, aspirate approximately 0.5 milliliters of the homogenized sample [2] and dispense it into the lysis tube [3].
 - 2.4.1. Talent opening the sealing cap of the lysis tube.
 - 2.4.2. Talent aspirating approximately 0.5 milliliters of the homogenized sample using the number 1 disposable pipette.
 - 2.4.3. Talent dispensing the sample into the lysis tube.

2.5. Then, close the sealing cap and verify that the tip cap is securely tightened [1]. Place the lysis tube into the Sample Preprocessing or SP (S-P) device and press down until it is secure [2]. After that, press **Start** [3].

2.5.1. Talent closing the sealing cap and checking that the tip cap is securely tightened.

2.5.2. Talent placing the lysis tube into the Sample Preprocessing device and pressing it down until secure.

2.5.3. SCREEN: Press **Start** on the Sample Preprocessing device interface.

Videographer: Please film the computer screen as a back up

2.6. Allow the SP device to complete the automated liquefaction-lysis cycle for approximately 15 minutes [1].

2.6.1. Shot of the Sample Preprocessing device running the automated liquefaction-lysis cycle with the lysis tube in place.

3. Chip Preparation

Demonstrator: Liang Bai

3.1. Remove the CD chip from 4 degrees Celsius storage and equilibrate it at room temperature for 10 minutes [1].

3.1.1. Talent placing the CD chip on the benchtop for equilibration at room temperature.

3.2. Open the foil pouch and place the chip on a flat surface [1]. Rotate the cap counterclockwise to open the chip [2].

3.2.1. Talent opening the foil pouch and placing the chip on a flat surface.

3.2.2. Talent rotating the chip cap counterclockwise to open the chip.

3.3. Flick the vial O to collect the liquid at the bottom of the vial [1]. Then, uncap vial O [2], insert the nozzle into the O port, and dispense the entire contents into the port [3]. Discard vial O [4] and repeat the procedure using vial B and the B port [5].

3.3.1. Talent flicking the vial O to collect the liquid at the bottom.

3.3.2. Talent uncapping the vial O

3.3.3. Talent inserting the nozzle into the O port and dispensing the contents of vial O into the port.

3.3.4. Talent discarding vial O.

3.3.5. Talent dispensing the contents of vial B into the B port.

3.4. Then, remove the processed lysis tube from the SP device and remove the tip cap [1]. Align the tube tip with the S port and dispense the entire lysate volume into the chip

[2]. Discard the used lysis tube [3].

3.4.1. Talent removing the processed lysis tube from the Sample Preprocessing device and removing the tip cap.

3.4.2. Talent aligning the tube tip with the S port and dispensing the entire lysate into the chip.

3.4.3. Talent discarding the used lysis tube.

3.5. Twist the chip cap clockwise until two audible clicks confirm a complete seal [1].

3.5.1. Talent twisting the chip cap clockwise until it is fully sealed.

4. Automated Nucleic Acid Analysis

Demonstrator: Qianlin Wang

4.1. Power on the automated nucleic acid analyzer [1]. Tap **Detect**, scan the barcode on the chip foil pouch, and enter the patient demographics and sample identification [2].

4.1.1. Talent powering on the automated nucleic acid analyzer.

4.1.2. SCREEN: Tap **Detect**, scan the barcode on the chip foil pouch, and enter the patient demographics and sample identification.

4.2. Mount the chip on the rotational spindle and press down until a click confirms engagement [1]. Then, press **Start** [2].

4.2.1. Talent mounting the chip on the rotational spindle and pressing it down until it clicks into place.

4.2.2. SCREEN: Press **Start**.

4.3. Allow the automated nucleic acid analyzer to complete the automated analysis in approximately 45 minutes [1].

4.3.1. Shot of the automated nucleic acid analyzer running with the chip mounted.

4.4. Review the amplification results and record the time to positivity or TP (*T-P*) values reported by the software [1]. Compare each TP value with the corresponding threshold to classify each channel as positive or negative [2].

4.4.1. SCREEN: Display the amplification curves with the corresponding time to positivity values.

4.4.2. SCREEN: Show the reported time to positivity values and the threshold values and display the corresponding positive or negative results.

4.5. Verify that the internal control channel exhibits an S-shaped amplification curve with a TP value below 12 minutes [1]. Reject the run if the internal control fails and repeat the test using a fresh chip or recollect the specimen [2].

- 4.5.1. SCREEN: Display the internal control amplification curve for well 16 with a time to positivity value below 12 minutes.
- 4.5.2. SCREEN: Display an invalid run result indicating internal control failure.

Results

5. Results

- 5.1. On completion of a valid run, positive channels exhibited the expected S-shaped amplification curves with automatically annotated TP values [1], whereas negative channels remained at baseline [2]. The internal control channel yielded an S-shaped curve with TP below 12 minutes in all valid runs [3].
 - 5.1.1. LAB MEDIA: Figure 4. *Video editor: In S3 panel, highlight the magenta-coloured curve that rises from the lower left to the upper right.*
 - 5.1.2. LAB MEDIA: Figure 4. *Video editor: In S3 panel, highlight the coloured lines that remain flat along the bottom of the graph.*
 - 5.1.3. LAB MEDIA: Figure 4. *Video editor: In S3 panel, highlight the dark blue curve labelled "IC".*
- 5.2. Sample S1 produced a single positive signal for *Aspergillus* spp. [1], whereas Sample S10 produced a single positive signal for *Streptococcus pneumoniae* [2].
 - 5.2.1. LAB MEDIA: Figure 4. *Video editor: In panel "S1" highlight the purple coloured amplification curve labelled Asp.*
 - 5.2.2. LAB MEDIA: Figure 4. *Video editor: In panel "S10" and highlight the amplification curve labeled Spn.*
- 5.3. Samples S2 to S5 contained *Aspergillus* spp. together with 1 or more bacterial pathogens [1], and 2 specimens also contained *Candida albicans* [2]. Sample S5 generated 7 simultaneous positive signals for *Aspergillus* spp., *Burkholderia cepacia*, *Corynebacterium striatum*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Stenotrophomonas maltophilia* [3].
 - 5.3.1. LAB MEDIA: Table 5. *Video editor: Highlight the rows for specimens S2 to S5 containing "Aspergillus spp."*
 - 5.3.2. LAB MEDIA: Table 5. *Video editor: In specimens S3 and S4, highlight the rows containing "Candida albicans."*
 - 5.3.3. LAB MEDIA: Figure 4 and Table 5. *Video editor: In figure 4, panel "S5", highlight the curves labelled as Asp", "Bcc", "Cst", "Eco", "Pae", "Sau", and "Sma". In table 5, highlight the S5 row listing the seven detected pathogens.*
- 5.4. Samples S6 to S9 contained bacterial co-infections without fungal involvement, ranging from dual-pathogen detection in S6 [1] to quadruple-pathogen detection in S8 [2].
 - 5.4.1. LAB MEDIA: Table 5. *Video editor: Highlight the S6 row showing two detected*

pathogens.

5.4.2. LAB MEDIA: Table 5. *Video editor: Highlight the S8 row showing four detected pathogens.*

5.5. Relative to targeted metagenomic next-generation sequencing, the assay showed 100% positive percent agreement and 100% negative percent agreement across all targets [1]. Relative to sputum culture, positive percent agreement was 100% for every culture-positive target [2], whereas negative percent agreement ranged from 44.4% to 100% [3].

5.5.1. LAB MEDIA: Table 6. *Video editor: Highlight the "PPA" and "NPA" columns on the right side of the table.*

5.5.2. LAB MEDIA: Table 6. *Video editor: Highlight the first percentage in the "(SC) PPA/NPA" column.*

5.5.3. LAB MEDIA: Table 6. *Video editor: Highlight the second percentage in the "(SC) PPA/NPA" column.*

1. Bronchoalveolar
Pronunciation link: <https://www.merriam-webster.com/medical/bronchoalveolar>
IPA: /ˌbrɑːŋ.koʊ.əlˈviː.ə.lə/
Phonetic Spelling: brong·koh·al·vee·uh·ler
2. Bronchial aspirate
Pronunciation link: No confirmed link found
IPA: /ˌbrɑːŋ.ki.əl ˈæs.pəˌreɪt/
Phonetic Spelling: brong·kee·uhl as·puh·rayt
3. Liquefaction
Pronunciation link: <https://www.merriam-webster.com/dictionary/liquefaction>
IPA: /ˌlɪk.wəˈfæk.ʃən/
Phonetic Spelling: lik·wuh·fak·shuhn
4. Homogenized
Pronunciation link: <https://www.merriam-webster.com/dictionary/homogenize>
IPA: /həˈmɑː.dʒəˌnaɪzd/
Phonetic Spelling: huh·mah·juh·nyzd
5. Lysate
Pronunciation link: <https://www.merriam-webster.com/medical/lysate>
IPA: /ˈlaɪ.seɪt/
Phonetic Spelling: ly·sayt

6. Nucleic acid
Pronunciation link: <https://www.merriam-webster.com/dictionary/nucleic%20acid>
IPA: /nuːˈkliː.ɪk ˈæs.ɪd/
Phonetic Spelling: noo·klee·ik as·id
7. Amplification
Pronunciation link: <https://www.merriam-webster.com/dictionary/amplification>
IPA: /,æm.plə.fəˈkeɪ.fən/
Phonetic Spelling: am·pluh·fuh·kay·shuhn
8. Aspergillus
Pronunciation link: <https://www.merriam-webster.com/medical/Aspergillus>
IPA: /,æs.pəˈdʒɪl.əs/
Phonetic Spelling: as·per·jil·uhs
9. Streptococcus pneumoniae
Pronunciation link: <https://www.merriam-webster.com/medical/Streptococcus>
IPA: /,strep.təˈkɑː.kəs nuːˈmoʊ.ni.i/
Phonetic Spelling: strep·tuh·kok·uhs noo·moh·nee·ee
10. Burkholderia cepacia
Pronunciation link: <https://www.merriam-webster.com/medical/Burkholderia>
IPA: /,bɜːk.hoʊlˈdɪr.i.ə səˈpeɪ.fɪ.ə/
Phonetic Spelling: berk·hol·deer·ee·uh suh·pay·shee·uh
11. Corynebacterium striatum
Pronunciation link: <https://www.merriam-webster.com/medical/Corynebacterium>
IPA: /,kɔːr.ɪ.ni.bækˈtɪr.i.əm straɪˈeɪ.təm/
Phonetic Spelling: kor·ih·nee·bak·teer·ee·uhm stry·ay·tum
12. Escherichia coli
Pronunciation link: <https://www.merriam-webster.com/dictionary/Escherichia%20coli>
IPA: /,ɛʃ.əˈrɪk.i.ə ˈkoʊ.laɪ/
Phonetic Spelling: esh·uh·rik·ee·uh koh·ly
13. Pseudomonas aeruginosa
Pronunciation link: <https://www.merriam-webster.com/medical/Pseudomonas>
IPA: /,suː.dəˈmoʊ.nəs erˈuː.dʒəˈnoʊ.sə/
Phonetic Spelling: soo·duh·moh·nuhs air·oo·juh·noh·suh
14. Staphylococcus aureus
Pronunciation link: <https://www.merriam-webster.com/medical/Staphylococcus>
IPA: /,stæf.ə.ləˈkɑː.kəs ˈɔːr.i.əs/
Phonetic Spelling: staf·uh·luh·kok·uhs or·ee·uhs
15. Stenotrophomonas maltophilia
Pronunciation link: <https://www.merriam-webster.com/medical/Stenotrophomonas>
IPA: /,sten.oʊ.trəˈfəˈmoʊ.nəs ,mæl.toʊˈfɪl.i.ə/
Phonetic Spelling: sten·oh·trof·uh·moh·nuhs mal·toh·fil·ee·uh
16. Metagenomic
Pronunciation link: <https://www.merriam-webster.com/medical/metagenomics>
IPA: /,met.ə.dʒəˈnoʊ.mɪk/
Phonetic Spelling: met·uh·juh·noh·mik

17. Sequencing
Pronunciation link: <https://www.merriam-webster.com/dictionary/sequencing>
IPA: /'si:.kwən.sɪŋ/
Phonetic Spelling: see·kwuhn·sing
18. Pathogen
Pronunciation link: <https://www.merriam-webster.com/dictionary/pathogen>
IPA: /'pæθ.ə.dʒən/
Phonetic Spelling: path·uh·jun
19. Demographics
Pronunciation link: <https://www.merriam-webster.com/dictionary/demographics>
IPA: /,dem.ə'græf.ɪks/
Phonetic Spelling: dem·uh·graf·iks
20. Bronchoalveolar lavage fluid
Pronunciation link: No confirmed link found
IPA: /,brɑ:ŋ.koʊ.æl'vi:.ə.lə lə'vɑ:ʒ 'flu:.ɪd/
Phonetic Spelling: brong·koh·al·vee·uh·ler luh·vahzh floo·id