FINAL SCRIPT: APPROVED FOR FILMING

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Title: A Multiplex Serological Assay for the Detection of Antibody Responses to Arboviruses

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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? **Yes, Same building, two floors apart.**

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. **YES**

Current Protocol Length
Number of Steps: 13
Number of Shots: 35

Introduction

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

AUTHORS: Please note that only 2 introduction and 3 conclusion statements may be presented

INTRODUCTION:

~~What is the scope of your research? What questions are you trying to answer?~~

1.1. **Marie-Fabrice Gasasira:** In our lab, we measure immune responses to infectious diseases to better understand how they are transmitted in populations.

1.1.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B.roll:4.1*

~~What are the most recent developments in your field of research?~~

1.2. **Marie-Fabrice Gasasira:** Recent developments are multiplex assays, which allow us to measure many different antibodies in a single reaction.

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

CONCLUSION:

~~What significant findings have you established in your field~~?

1.3. **Marie-Fabrice Gasasira:** We have shown that our serological test can simultaneously measure antibodies to many arboviruses.

1.3.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B.roll:4.3*

~~What research gap are you addressing with your protocol~~?

1.4. **Marie-Fabrice Gasasira:** Our validated assay to measure multiple responses to different arboviruses will help in effort to understand the transmission of these viruses across Africa.

1.4.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B.roll:4.2*

~~What advantage does your protocol offer compared to other techniques~~?

1.5. **Marie-Fabrice Gasasira:** Our protocol provides huge time savings and reproducibility in the preparation of the reagents for multiplex assays.

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.

Testimonial Questions (OPTIONAL):

Videographer: Please capture all testimonial shots in a wide-angle format with sufficient headspace, as the final videos will be rendered in a 1:1 aspect ratio. Testimonial statements will be presented live by the authors, sharing their spontaneous perspectives.

- Testimonial statements will **not appear in the video** but may be featured in the journal's promotional materials.
- **Provide the full name and position** (e.g., Director of [Institute Name], Senior Researcher [University Name], etc.) of the author delivering the testimonial.
- Please **answer the testimonial question live during the shoot**, speaking naturally and in your own words in **complete sentences**.

How do you think publishing with JoVE will enhance the visibility and impact of your research?

1.6. **Michael White, Group leader, Infectious diseases epidemiology and analytics unit**: (authors will present their testimonial statements live)

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

Can you share a specific success story or benefit you've experienced—or expect to experience—after using or publishing with JoVE? (This could include increased collaborations, citations, funding opportunities, streamlined lab procedures, reduced training time, cost savings in the lab, or improved lab productivity.)

1.7. **Michael White, Group leader, Infectious diseases epidemiology and analytics unit**: (authors will present their testimonial statements live)

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

Ethics Title Card

This research has been approved by the National Ethics Board of Suriname, Ethics Board at The London School of Hygiene & Tropical Medicine, the Madagascar Comité d'Éthique de la Recherche Biomédicale and the Senegalese National Health Research Ethics Committee

Protocol

2. Automated Bead Coupling and Preparation for Downstream Assay

Demonstrators: Laura Garcia

2.1. To begin, obtain eight 96-deep well plates **[1].** Prepare each plate by adding tip combs, monobasic sodium phosphate, Triton solution, sulpho-NHS, EDC and antigen solution **[2]**.

2.1.1. WIDE: Talent looking at and labeling 8 deep well plates.

2.1.2. Talent pipetting a reagent into a labeled plate.

AUTHORS: Please perform any 1 representative action. Also keep labeled glassware containing the reagents in the background of this shot

AND

TEXT ON PLAIN BACKGROUND:

Plate 1 (Tip Combs): load tip combs.

Plate 2 (Beads): Add 8 µL of 0.125x Triton and 200 µL of the bead regions of interest.

Plate 3 (Wash1): Add 200 µL of 100 mM Monobasic sodium phosphate / 0.125x Triton.

Plate 4 (Activation): Add 80 µL of 100 mM Monobasic sodium phosphate / 0.125x Triton + 10 µL sulfo-NHS + 10 µL EDC.

Plate 5 (Wash2): Add 250 µL of 1x PBS /0.125x Triton.

Plate 6 (Coupling): Transfer 1 mL of antigen solution to the allocated well.

Plate 7 (Wash3): Add 500 µL of PBS-TBN/0.125x Triton.

Plate 8 (Final plate, contains coupled beads): Add 1 mL of PBS-TBN + 0.125x Triton.

Video Editor: Please play both shots side by side in a split screen

2.2. To set up the **coupling cycle** on the automated processor, switch on the machine **[1].** Set up the protocol parameters using the software **[2-TXT]**.

2.2.1. Talent switching on the automated processor.

2.2.2. SCREEN: JoVE_SCREEN_69259_step2.2.2_bis.mp4 00:10-00:40 *Video editor:*

Please speed up.

2.3. Load the plates into the allocated slots on the machine **[1]**, then select the protocol, and start the cycle **[2-TXT]**.

2.3.1. Talent loading Plates 1–8 into the processor slots.

2.3.2. ~~SCREEN: The protocol is being selected on the PC screen.~~ **NOTE**: This shot was not filmed, VO merged

2.3.3. SCREEN: The cycle is being started. **TXT: Estimated run time: 2 h 50 min** **NOTE**: Videographer filmed this shot

2.4. After completion, unload the plates **[1]** and switch off the machine as per manufacturer's instructions **[2].** Visually check that Plate 8 contains the beads visible as a brown pellet and export the run report from the instrument **[3]**.

2.4.2, Taleny unloading plates. **Author's NOTE**: Please move shot 2.4.2 (Talent unloading plates) before shot 2.4.1 (Talent switching off the machine)

2.4.1, Talent switching off the machine.

2.4.3, Shot of the Plate 8 well showing a brown pellet of beads.

~~2.4.4, SCREEN: PC display exporting the run report PDF~~. **Author's NOTE**: This shot was not filmed

2.5. Transfer the coupled beads from Plate 8 into individual 1.5-milliliter tubes for storage **[1]**. Store the coupled beads at 4 degree C until use **[2]**.

2.5.1. Talent transferring the beads into each 1.5 mL tube.

2.5.2. Shot of the tubes being placed into a 4 °C storage rack.

2.6. To count the beads, thoroughly vortex the coupled beads **[1]**. Transfer 10 microliters to a cell-counting chamber **[2]**, then follow the manufacturer's instructions to read the bead count per milliliter **[3]**.

2.6.1. Talent places the tube with beads on a vortex machine.

2.6.2. Talent transferring 10 µL from the tube to the counting chamber.

2.6.3. SCOPE/SCREEN: View through the cell counter screen showing the bead count result. **NOTE**: Videographer filmed this shot

2.7. Check aggregation by visualising the image on the cell counter **[1-TXT]**.

2.7.1. SCOPE/SCREEN: Show the aggregated beads image for comparison. **TXT: Compare to image of non-aggregated beads** **NOTE**: Videographer filmed this shot

3. Sample Preparation and Bead-Based Assay Setup for Imaging

Demonstrators: Laura Garcia, Marie-Fabrice Gasasira

3.1. Set up the plate layout **[1]**. Dilute samples and standard in PBT buffer in a separate non-binding plate **[2]**.

3.1.1. SCREEN : The layout plan for the microplate and dilution plate is being seen. **NOTE**: Videographer filmed this shot

3.1.2. Talent pipetting sample dilution into the non-binding plate.

3.2. Thoroughly vortex the coupled beads mix for 30 seconds **[1]**, then sonicate for at least 60 seconds **[2]**.

3.2.1. Talent places the coupled beads in a vortex.

3.2.2. Talent placing the beads tube into the sonicator.

3.3. Now transfer the adequate bead-antigen volumes to a centrifuge tube **[1]**, and adjust the volume with PBT buffer **[2]**. Thoroughly mix the premix **[3]**.

3.3.1. Talent transferring bead-antigen volume into a centrifuge tube.

3.3.2. Talent adding PBT buffer to adjust volume.

3.3.3. Talent vortexing or mixing the tube.

3.4. Distribute 50 microliters of the premix to the imaging microplate **[1]**. Then pipette 50 microliters of the diluted samples to the beads in the imaging microplate **[2]**, and mix on a plate shaker **[3-TXT].**

3.4.1. Talent dispensing 50 µL of premix into each well of the imaging plate.

3.4.2. Operator adding 50 µL sample to each well.

3.4.3. Shot of the plate being placed into the shaker. **TXT: Mixing: 30 min, RT, 700 rpm**

3.5. Wash the plates three times manually by placing the plate on a magnetic rack at room temperature for 60 seconds **[1]**. Then hold the plate tightly and discard the supernatant **[2]**. Add 100 microliters PBT buffer **[3]** and leave on the magnet rack for 60 seconds before discarding the supernatant **[4]**.

3.5.1. Talent placing the plate on a magnetic rack.

3.5.2. Talent discarding supernatant with plate held firmly.

3.5.3. Talent adding 100 µL PBT buffer.

3.5.4. Talent placing the plate on magnetic rack.

3.6. After the last wash, add 50 microliters of diluted secondary antibody and incubate on a shaker **[1-TXT]**. Perform three more washes again **[2]**, then resuspend the beads in 150 microliters of PBT buffer **[3]**. Incubate on a shaker for 5 minutes at room temperature at 700 rpm **[4]**. Insert the plate into the fluorescence reader **[5],** set up the protocol on the fluorescence reader, and save it **[6]**.

3.6.1. Talent pipetting 50 µL secondary antibody into each well. **TXT: Incubation: 15 min, RT, 700 rpm**

3.6.2. Talent performing 1 wash.

3.6.3. Shot of 150 µL PBT being added to the plate.

3.6.4. Talent placing the plate on a shaker.

Added shot: loading the plate on fluorescence reader

Added shot 3.6.6: SCREEN: JoVE_SCREEN_69259_step3.6.6_bis.mp4 00:25-00:33

Results

4. Results

4.1. Linearity of antibody response was confirmed for selected arbovirus antigens tested at optimal concentrations on the ORPAL *(or-pal)* standard pool **[1]**. Parallel linearity of antibody response was observed when comparing the manual and three automated coupling methods **[2]**.

4.1.1. LAB MEDIA: Figure 1. *Video editor: Highlight the three colored curves sequentially*

4.1.2. LAB MEDIA: Figure 2. *Video editor: Please highlight the curves of each panel.*

4.2. Median fluorescence intensity values obtained from 19 different plates for each antigen and control fell within the range of the mean **[1]**. A strong correlation was observed between the median fluorescence intensity values obtained from multiplex and single-plex assays using the ORPAL standard pool **[2]**.

4.2.1. LAB MEDIA: Figure 3. *Video editor: Sequentially highlight the blue curve for each panel*

4.2.2. LAB MEDIA: Figure 4. *Video editor: Please highlight the black dots and the diagonal line*

4.3. In the Senegal cohort, a clear bimodal distribution was observed in the antibody response to CHIKV VLP *(Chik-V-V-L-P)* **[1]**, and subtle bimodal tails were also seen for DENV1 NS1 *(Den-V-one-N-S-one)* **[2]** and RVFV NP *(R-V-F-V-N-P)* **[3]**.

4.3.1. LAB MEDIA: Figure 5. *Video editor: Highlight the Senegal (blue) density curve under the CHIKV VLP panel, showing two distinct peaks.*

4.3.2. LAB MEDIA: Figure 5. *Video editor: Highlight the Senegal (blue) density curve under the DENV1 NS1 panel*

4.3.3. LAB MEDIA: Figure 5. *Video editor: Highlight the Senegal (blue) density curve under the RVFV NP panel*

1. Triton
   Pronunciation link: https://www.merriam-webster.com/dictionary/Triton

IPA: /ˈtrɪtɑn/
Phonetic Spelling: tri·ton

2. Monobasic
Pronunciation link: https://www.merriam-webster.com/dictionary/monobasic
IPA: /ˌmɑːnoʊˈbeɪsɪk/
Phonetic Spelling: mah·noh·bay·sik
3. Sulfo-NHS
Pronunciation link: https://www.howtopronounce.com/sulfo-nhs
IPA: /ˈsʌlfoʊ ˌɛn eɪtʃ ɛs/
Phonetic Spelling: sul·foh en·aych·ess
4. EDC
Pronunciation link: https://www.howtopronounce.com/edc
IPA: /ˌiː diː ˈsiː/
Phonetic Spelling: ee·dee·see
5. Antigen
Pronunciation link: https://www.merriam-webster.com/dictionary/antigen
IPA: /ˈæn.tɪ.dʒən/
Phonetic Spelling: an·tuh·jin
6. Pipetting
Pronunciation link: https://www.merriam-webster.com/dictionary/pipette
IPA: /pɪˈpɛt/
Phonetic Spelling: pih·pet
7. Phosphate
Pronunciation link: https://www.merriam-webster.com/dictionary/phosphate
IPA: /ˈfɑsˌfeɪt/
Phonetic Spelling: fahs·fayt
8. PBS
Pronunciation link: https://www.howtopronounce.com/pbs
IPA: /ˌpiː biː ˈɛs/
Phonetic Spelling: pee·bee·ess
9. PBS-TBN
Pronunciation link: https://www.howtopronounce.com/pbs-tbn
IPA: /ˌpiː biː ˌɛs tiː biː ˈɛn/
Phonetic Spelling: pee·bee·ess tee·bee·en
10. Vortex
Pronunciation link: https://www.merriam-webster.com/dictionary/vortex
IPA: /ˈvɔːr.tɛks/
Phonetic Spelling: vor·teks
11. Sonicate
Pronunciation link: https://www.merriam-webster.com/dictionary/sonicate
IPA: /ˈsɑːnɪˌkeɪt/
Phonetic Spelling: sah·nih·kayt
12. Centrifuge
Pronunciation link: https://www.merriam-webster.com/dictionary/centrifuge

IPA: /ˈsɛntrɪˌfjuːdʒ/
Phonetic Spelling: sen·truh·fyooj

13. Microliters
Pronunciation link: https://www.merriam-webster.com/dictionary/microliter
IPA: /ˈmaɪ.kroʊˌliː.tər/
Phonetic Spelling: my·kroh·lee·ter
14. Supernatant
Pronunciation link: https://www.merriam-webster.com/dictionary/supernatant
IPA: /ˌsuːpərˈneɪtənt/
Phonetic Spelling: soo·per·nay·tuhnt
15. Fluorescence
Pronunciation link: https://www.merriam-webster.com/dictionary/fluorescence
IPA: /ˌflʊrˈɛsəns/
Phonetic Spelling: floor·ess·uhns
16. Linearity
Pronunciation link: https://www.merriam-webster.com/dictionary/linearity
IPA: /ˌlɪniˈærɪti/
Phonetic Spelling: lih·nee·air·ih·tee
17. Arbovirus
Pronunciation link: https://www.merriam-webster.com/dictionary/arbovirus
IPA: /ˈɑːrbəˌvaɪrəs/
Phonetic Spelling: ar·buh·vy·ruhs
18. ORPAL
Pronunciation link: https://www.howtopronounce.com/orpal
IPA: /ˈɔːrˌpæl/
Phonetic Spelling: or·pal
19. Multiplex
Pronunciation link: https://www.merriam-webster.com/dictionary/multiplex
IPA: /ˈmʌltɪˌplɛks/
Phonetic Spelling: mul·tih·pleks
20. Bimodal
Pronunciation link: https://www.merriam-webster.com/dictionary/bimodal
IPA: /ˌbaɪˈmoʊdəl/
Phonetic Spelling: bye·moh·duhl
21. CHIKV
Pronunciation link: https://www.howtopronounce.com/chikv
IPA: /ˈtʃɪk viː/
Phonetic Spelling: chik·vee
22. VLP
Pronunciation link: https://www.howtopronounce.com/vlp
IPA: /ˌviː ɛl ˈpiː/
Phonetic Spelling: vee·el·pee
23. DENV1
Pronunciation link: https://www.howtopronounce.com/denv1

IPA: /ˈdɛn viː wʌn/
Phonetic Spelling: den·vee·wun

24. NS1
Pronunciation link: https://www.howtopronounce.com/ns1
IPA: /ˌɛn ɛs ˈwʌn/
Phonetic Spelling: en·ess·wun
25. RVFV
Pronunciation link: https://www.howtopronounce.com/rvfv
IPA: /ˌɑːr viː ɛf ˈviː/
Phonetic Spelling: ar·vee·ef·vee
26. Cohort
Pronunciation link: https://www.merriam-webster.com/dictionary/cohort
IPA: /ˈkoʊˌhɔːrt/
Phonetic Spelling: koh·hort