

Submission ID #: 67886

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Project Page Link: <https://review.jove.com/account/file-uploader?src=20705278>

Title: COVID-19 Seroprevalence Test for IgG Antibody Levels Among Healthy Donors Across Different Pandemic Phases in Jeddah

Authors and Affiliations:

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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? **No**

- 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

Introduction

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

Videographer's Note: Dr. Thamir A. Alandijany was not available at the time of filming.

- **As an alternative, the team provided Dr. Hessa A. Al-Sharif as a replacement.**
- **All interview shots originally planned for Dr. Thamir were filmed with Dr. Hessa A. Al-Sharif instead.**

INTRODUCTION:

- 1.1. **Abrar AL-Ghamdi**: This research examines IgG seroprevalence in healthy Jeddah donors across early COVID-19 phases, assessing immunity changes before, during, and after lockdown.

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

What technologies are currently used to advance research in your field?

- 1.2. **Hessa Al-Sharif**: Current research uses ELISA, CLIA, PCR, high-throughput serology platforms, and statistical modeling tools to measure antibody levels and track population immunity.

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

CONCLUSION:

What research gap are you addressing with your protocol?

- 1.3. **Abrar Al-Ghamdi**: To our knowledge, this is the first study in the country addressing COVID-19 seroprevalence Across Different Early Pandemic Phases.

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

What advantage does your protocol offer compared to other techniques?

- 1.4. **Hessa Al-Sharif**: Our in-house ELISA and micro-neutralization protocols enhance accuracy, reducing false results and providing more reliable detection of SARS-CoV-2-specific antibodies.

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

What new scientific questions have your results paved the way for?

- 1.5. **Abrar Al-Ghamdi**: Our results raise questions about asymptomatic transmission dynamics, long-term immunity patterns, and how similar surveillance can guide future epidemic and pandemic preparedness.

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

Videographer's Note:

- We also captured an interview segment with Dr. Esam I. Azhar, who spoke about his unit/department. This segment was requested on-site and was not originally part of the script.
- Additional B-roll shots were captured, including:
 - Department signage
 - Unit name visuals
 - Institutional logos

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

Ethics Title Card

This research has been approved by the Research Ethics Committee (REC) of the Unit of Biomedical Ethics at the Faculty of Medicine, King Abdulaziz University

Protocol

2. Sera Blood samples ELISA for SARS-CoV-2

Demonstrator: Abrar AL-Ghamdi

- 2.1. To begin, obtain blood samples from healthy patients who do not have any history of COVID-19 (*Koh-Vid-Nineteen*) [1]. Centrifuge the samples at 200 g for 10 minutes to obtain the serum supernatant [2]. Store at minus 20 degrees Celsius in a Biosafety Level 3 facility at the SIAU (*S-I-A-U*) until testing [3-TXT].
 - 2.1.1. Talent holding labelled sample bottles.
 - 2.1.2. Talent placing blood samples into the centrifuge.
 - 2.1.3. Shot of the vials being placed at – 20 °C. **TXT: SIAU: Special Infectious Agents Unit**
- 2.2. To screen for anti-SARS-CoV-2 (*Saard-Koh-V-Two*) IgG (*I-G-G*) antibodies, coat microtiter plates with 100 nanograms of SARS-CoV-2 spike recombinant protein diluted in PBS (*P-B-S*) [1]. Incubate the plates overnight at 4 degrees Celsius [2].
 - 2.2.1. Talent pipetting diluted spike protein solution into the wells of a microtiter plate.
 - 2.2.2. Talent placing the coated plate into a cold storage unit marked 4 degrees Celsius.
- 2.3. Wash the plates three times with PBST (*P-B-S-T*) [1]. Then add 5 percent skim milk to block the plates and incubate [2-TXT]. Wash the plates three more times with PBST after blocking [3].
 - 2.3.1. Talent adding PBST over the plates.
 - 2.3.2. Talent pipetting 5 percent skim milk solution into each well and setting the plate on the bench for incubation. **TXT: Incubation: RT, 1 h**
 - 2.3.3. Talent repeating the plate wash using PBST after blocking.
- 2.4. Now, dilute the sera samples at a ratio of 1 to 100 in 5 percent skim milk [1]. Add 100 microliters of diluted sera into each well [2]. Then incubate the plate at 37 degrees Celsius for 1 hour [3].
 - 2.4.1. Talent preparing diluted sera samples in 5 percent skim milk.
 - 2.4.2. Talent dispensing 100 microliters of diluted samples into each well.
 - 2.4.3. Talent placing the plate in a 37 degrees Celsius incubator.

- 2.5. After washing the plates three more times with PBST, pipette each well with 100 microliters of goat KPL (*K-P-L*) peroxidase-labeled antibodies to human IgG [1-TXT]. Incubate the plate for 1 hour at 37 degrees Celsius before washing the plates with PBST [2].
 - 2.5.1. Talent pipetting diluted peroxidase-labeled antibodies into each well. **TXT: Antibody dilution: 1: 64,000**
Video Editor: Please note that the on-screen text has a ratio
 - 2.5.2. Talent placing the plate back into the 37 degrees Celsius incubator.
- 2.6. Next, pipette 100 microliters of TMB (*T-M-B*) to each well [1-TXT]. Incubate for 5 minutes for color development [2]. Stop the reaction by adding 100 microliters of hydrochloric acid to each well [3].
 - 2.6.1. Talent adding TMB solution to each well. **TXT: TMB: 3,3',5,5'-Tetramethylbenzidine**
 - 2.6.2. Shot of the solutions in the wells changing color.
 - 2.6.3. Talent pipetting hydrochloric acid into each well.
- 2.7. Measure the absorbance at 450 nanometers using a microplate spectrophotometer [1-TXT]. Run all samples in duplicate and include positive controls from recovered COVID-19 patients [2].
 - 2.7.1. Talent placing the plate in a microplate reader.
TXT: If OD₄₅₀ is >= 0.27, consider reactive
If OD₄₅₀ is < 0.27, then specimen is non-reactive
 - 2.7.2. Shot of plate with labeled wells.

NOTE: Authors chose not to film section 3

~~3. Micro Neutralisation Assay for the Confirmation of Seropositivity of Blood Samples~~

~~Demonstrator: Ahmed M. Tolah~~

- ~~3.1. Place serum samples in a water bath set at 56 degrees Celsius for 30 minutes to heat-inactivate them [1]. Prepare serial dilutions of the heat inactivated samples at ratios of 1 to 10, 1 to 20, and 1 to 40 using DMEM [2-TXT].~~
 - ~~3.1.1. Talent placing serum sample tubes in a water bath labeled 56 degrees Celsius.~~
 - ~~3.1.2. Talent pipetting DMEM into labeled tubes to prepare 1:10, 1:20, and 1:40 dilutions of the heat inactivated serum samples. **TXT: DMEM contains 100 mL from 50% tissue culture infection dose (TCID₅₀) of SARS-CoV-2**~~

- ~~3.2. Next, culture Vero E6 (WE-Roh-E-Six) cells in DMEM [1-TXT]. Incubate the plates for 24 hours at 37 degrees Celsius under 5% carbon dioxide supplementation [2]. Once confluent, add 100 microliters of the diluted serum samples to the wells [3].~~
- ~~3.2.1. Talent pipetting Vero E6 cells into culture plates with DMEM. **TXT: Seeding density: $2-3 \times 10^5$ cells/well**~~
- ~~3.2.2. Shot of plates with confluent Vero E6 cultures.~~
- ~~3.2.3. Talent inspecting the confluent Vero E6 cells and pipetting diluted serum samples into the wells.~~
- ~~3.3. Now pipette 100 microliters of DMEM containing 50 percent tissue culture infection dose of SARS-CoV-2 to each well [1]. Incubate the plates at 37 degrees Celsius in a 5 percent carbon dioxide humidified incubator for 3 to 4 days [2].~~
- ~~3.3.1. Talent pipetting virus-containing DMEM into the wells.~~
- ~~3.3.2. Talent placing the plates inside a humidified incubator labeled 37 degrees Celsius and 5 percent CO₂.~~
- ~~3.4. With an inverted microscope, assess the cytopathic effect three days after infection to read the plate [1].~~
- ~~3.4.1. Talent inspecting the well plate under a microscope.~~
- ~~3.5. Include positive control wells with SARS-CoV-2 infected cells and no human serum [1]. Include negative control wells without virus or serum [2].~~
- ~~3.5.1. Talent labeling and pipetting into designated wells for positive controls.~~
- ~~3.5.2. Talent preparing and labeling wells for negative controls with no additions.~~

Results

4. Results

- 4.1. The number of blood donor varied before, during and after lockdown and were lower than usual due to COVID-19 pandemic [1].
 - 4.1.1. LAB MEDIA: Table 1 *Video Editor: Please sequentially highlight the rows corresponding to “Before lockdown”, “during lockdown” and “After lockdown”.*
- 4.2. Of the tested 3,825 serum samples, 173 tested positive for anti-SARS-CoV-2 IgG antibodies, while 3,652 tested negative [1].
 - 4.2.1. LAB MEDIA: Table 2 *Video Editor: Please sequentially highlight the “positive” column” and then the “negative” column*
- 4.3. Overall, among the three study periods, the highest seropositivity rate was observed after easing the lockdown, with 111 out of 848 samples testing positive [1]. In contrast, the highest seronegative rate was observed before the lockdown, with 1,487 out of 1,495 samples testing negative [2].
 - 4.3.1. LAB MEDIA: Figure 2 and Table 2 *Video Editor: Please highlight the dots corresponding to “after lockdown” in Figure 2. Please highlight positive value in the “after lockdown” row o f table 2*
 - 4.3.2. LAB MEDIA: Figure 2 and Table 2 *Video Editor: Please highlight the dots corresponding to “before lockdown” in Figure 2. Please highlight positive value in the “before lockdown” row o f table 2*
- 4.4. The Micro-neutralization assay confirmed seropositivity of 147 ELISA (*E-lie-sah*) positive cases for SARS-CoV-2 while 26 were seronegative [1].
 - 4.4.1. LAB MEDIA: Table 3 *Video Editor: Please highlight the “Positive” column*

Pronunciation Guide:

🔍 Seroprevalence

Pronunciation link: <https://www.merriam-webster.com/dictionary/seroprevalence>

IPA: /ˌsɪrəʊˈprɛvələns/

Phonetic Spelling: seer-oh-prev-uh-luhns

🔍 Immunoglobulin

Pronunciation link: <https://www.merriam-webster.com/dictionary/immunoglobulin>

IPA: /ˌɪm.jə.noʊˈglɑː.bjə.lɪn/

Phonetic Spelling: im-yuh-noh-glob-yuh-lin

🔍 IgG

Pronunciation link: <https://www.merriam-webster.com/dictionary/IgG>

IPA: /ˌaɪ.dʒiːˈdʒiː/

Phonetic Spelling: eye-jee-jee

🔍 SARS-CoV-2

Pronunciation link: <https://www.merriam-webster.com/dictionary/SARS-CoV-2>

IPA: /ˌsɑːrʒ koʊvˈtuː/

Phonetic Spelling: sahrz-kohv-too

🔍 COVID-19

Pronunciation link: <https://www.merriam-webster.com/dictionary/COVID-19>

IPA: /ˈkoʊvɪd naɪnˈtiːn/

Phonetic Spelling: koh-vid-nine-teen

🔍 ELISA

Pronunciation link: <https://www.merriam-webster.com/dictionary/ELISA>

IPA: /ˈɪˌlaɪzə/

Phonetic Spelling: ih-lye-zuh

🔍 CLIA

Pronunciation link: <https://www.howtopronounce.com/clia>

IPA: /ˈkliːə/

Phonetic Spelling: klee-uh

🔍 Polymerase

Pronunciation link: <https://www.merriam-webster.com/dictionary/polymerase>

IPA: /pəˈlɪm.ə.reɪs/

Phonetic Spelling: puh-lim-uh-rays

🔍 PCR

Pronunciation link: <https://www.merriam-webster.com/dictionary/PCR>

IPA: /ˌpiː siː ˈɑːr/

Phonetic Spelling: pee-see-are

🔍 Micro-neutralization

Pronunciation link: <https://www.howtopronounce.com/microneutralization>

IPA: /ˌmaɪ.kroʊˌnuː.trəˌlaɪˈzeɪ.jən/

Phonetic Spelling: my-kroh-noo-truh-lye-zay-shun

☐ Centrifuge

Pronunciation link: <https://www.merriam-webster.com/dictionary/centrifuge>

IPA: /ˈsɛn.trɪˌfjuːdʒ/

Phonetic Spelling: sen-trih-fyooj

☐ Supernatant

Pronunciation link: <https://www.merriam-webster.com/dictionary/supernatant>

IPA: /ˌsuː.pəˈneɪ.tənt/

Phonetic Spelling: soo-per-nay-tuhnt

☐ Biosafety

Pronunciation link: <https://www.merriam-webster.com/dictionary/biosafety>

IPA: /ˌbaɪ.əʊˈseɪf.ti/

Phonetic Spelling: bye-oh-sayf-tee

☐ Microtiter

Pronunciation link: <https://www.merriam-webster.com/dictionary/microtiter>

IPA: /ˈmaɪ.kroʊˌtaɪ.tər/

Phonetic Spelling: my-kroh-ty-ter

☐ Recombinant

Pronunciation link: <https://www.merriam-webster.com/dictionary/recombinant>

IPA: /ˌriː.kəmˈbaɪ.nənt/

Phonetic Spelling: ree-kuhm-bye-nuhnt

☐ Phosphate-buffered saline

Pronunciation link: <https://www.merriam-webster.com/dictionary/phosphate-buffered%20saline>

IPA: /ˈfɑːs.feɪt ˈbʌf.əd səˈliːn/

Phonetic Spelling: fahs-fayt buff-erd suh-leen

☐ PBST

Pronunciation link: <https://www.howtopronounce.com/pbst>

IPA: /ˌpiː biː ɛs ˈtiː/

Phonetic Spelling: pee-bee-ess-tee

☐ Peroxidase

Pronunciation link: <https://www.merriam-webster.com/dictionary/peroxidase>

IPA: /pəˈrɑːk.sɪ.deɪs/

Phonetic Spelling: puh-rok-sih-days

☐ Tetramethylbenzidine

Pronunciation link: <https://www.howtopronounce.com/tetramethylbenzidine>

IPA: /ˌtɛ.trəˌmɛθ.əlˌbɛn.zɪˈdiːn/

Phonetic Spelling: teh-truh-meth-uhl-ben-zuh-deen

☐ Spectrophotometer

Pronunciation link: <https://www.merriam-webster.com/dictionary/spectrophotometer>

IPA: /ˌspɛk.troʊˌfoʊˈtɑː.mɪ.tər/

Phonetic Spelling: spek-troh-foh-tah-mi-ter

☐ Absorbance

Pronunciation link: <https://www.merriam-webster.com/dictionary/absorbance>

IPA: /əb'zɔ:r.bəns/

Phonetic Spelling: uhb-zorb-uhns

🔊 Seropositivity

Pronunciation link: <https://www.howtopronounce.com/seropositivity>

IPA: /,sɪr.ɒs.pɑ:..zə'tɪv.ə.ti/

Phonetic Spelling: seer-oh-pah-zuh-tiv-uh-tee