

Hemagglutination Test (HAT) to detect antibodies against the RBD domain of the SARS2-Covid19 virus

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Method Article

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Abstract

Serological detection of antibodies to SARS-CoV-2 is essential for establishing rates of seroconversion in populations, and for seeking evidence for a level of antibody that may be protective against COVID-19 disease. Several high-performance commercial tests have been described, but these require centralised laboratory facilities that are comparatively expensive, and therefore not available universally. Red cell agglutination tests do not require special equipment, are read by eye, have short development times, low cost and can be applied at the Point of Care. We describe a quantitative Haemagglutination test (HAT) for the detection of antibodies to the receptor binding domain of the SARS-CoV-2 spike protein. The HAT has a sensitivity of 90% and specificity of 99% for detection of antibodies after a PCR diagnosed infection. We will supply aliquots of the test reagent sufficient for ten thousand test wells free of charge to qualified research groups anywhere in the world.

Introduction

Reagents

Equipment and Reagents for HAT

- O-ve blood as a source of red cells collected in K2EDTA tube, diluted in PBS to 1:20 or 1:40 as needed. Resuspend by inverting gently ~12 times.

- BD Contact Activated Lancet Cat. No. 366594 (2 mm x 1.5 mm)

100 µL, 20 µL pipettes, Multichannel pipettes

V-bottomed 96-well plates (Greiner Bio-One, Cat. No. 651101, Microplate 96-well, PS, V-bottom, Clear, 10 pieces/bag)

Eppendorf Tubes

K2EDTA solution (add 5 mL PBS to 10 mL K2EDTA blood collection tube = 3.6 mg K2EDTA/mL, store at 4 °C)

Phosphate Buffered Saline Tablets (OXOID Cat. No. BR0014G)

- IH4-RBD Reagent diluted 2 µg/mL in PBS. This remains active for at least 1-2 weeks stored at 4 °C
- V-bottomed 96-well plates, numbered, dated, timed (helps when timing many plates)
- Positive control monoclonal antibody CR3022 diluted to 2 µg/mL in PBS

Other Reagents

Monoclonal antibody to human IgG (Gamma chain specific) Clone GG-5 Sigma Cat. No. I5885

Equipment

Procedure

1. Spot test on Stored Serum/Plasma samples (Figure 4).

1. Plate out 50 µL of 1:20 serum/plasma in alternate columns 1,3,5,7,9,11 (add 2.5 µL sample to 47.5 µL PBS).
2. Add 50 µL 1:20 O-ve blood collected in (so that now sample is diluted to 1:40 and red cells at ~1% v/v)
3. Mix and transfer 50/100 µL to neighbouring columns 2,4,6,8,10,12 for -ve controls. The negative control is important because in rare cases, particularly in donors who have received blood transfusions, the sample in principle may contain antibodies to non-ABO or Rhesus D antigens.
4. Add 50 µL IH4-RBD reagent (2 µg/mL in PBS = 100 ng/well) to Columns 1,3,5,7,9,11
5. Add 50 µL PBS to columns 2,4,6,8,10,12.
6. Inc 1 hr RT
7. Tilt for 30 seconds
8. Photograph: with mobile phone use the zoom function to obtain a complete field
9. Read as Positive = No teardrop, Negative <1:40 = partial teardrop, Neg = complete teardrop.
10. Two readers should read the plates independently, and disagreements resolved by taking the lesser reading.

11. For each batch of samples set up positive control wells containing 20-100 ng monoclonal antibody CR3022 (as in Finger-Prick test below). This establishes that all of the reagents are working.

2. Titration of Stored Serum/Plasma Samples.

1. Dilute samples to 1:20 in 50 μ L PBS (2.5 μ L to 47.5 μ L) in V-bottomed plate in Rows A-H, column 1. Prepare WHO standard serum 20/130 as above at 1:20, or CR3022 50 μ L at 20 μ g/mL, for calibration for each batch of titrations. WHO standard 20/130 should titrate to \sim 1:1280, and 20 μ g/mL CR3022 to \sim 1:512.
2. Prepare doubling dilutions with PBS across the plate columns 1-11 (1:40 to 1:40,960), PBS control in column 12. Eight samples can be titrated per 96-well plate.
3. Add 50 μ L 1:40 O-ve red cells (1% v/v or 1:40 fresh EDTA O-ve blood sample) to all wells
4. Add 50 μ L IH4-RBD (2 μ g/mL, = 100 ng/well). [Note: the red cells and IH4-RBD can be pre-mixed and added together in either 50 μ L or 100 μ L volume, to save a step. This variation in technique does not alter the measured titres.]
5. Allow red cells to settle for 1 hr
6. Tilt plate for at least 30 s and photograph. The titre is defined by the last well in which the tear drop fails to form. Partial teardrop regarded as negative.

3. Finger-prick test on capillary blood as a Point of Care Test

Preparation: Clean Hands, warm digit. Prepare a plate (96-well V-bottomed) labelled with Date and Time.

Prick skin on outer finger pulp with disposable, single use BD or another Lancet.

Wipe away first drop of blood with sterile towel/swab

Massage second drop

Take a minimum of 5 μ L blood with 20 μ L pipette, mix immediately into 20 μ L K2EDTA (3.6 mg/mL/PBS) in Eppendorf. If possible, take 25 μ L of blood and mix into 100 μ L K2EDTA solution. Another approach is collection of blood drops into a BD Microtainer K2E EDTA lavender vials REF 365975 that take 250-500 μ L.

For 5 μL sample dilute to 200 μL with PBS (add 175 μL PBS), for 25 μL sample dilute to 1ml (add 975 μL PBS). Sample is now at 1:40, and the red cells are at the correct density ($\sim 1\%$ v/v assuming a haematocrit of 40%) to give a clear tear drop.

Plate 50 μL x 3 in V bottomed microtitre wells labelled T (Test), + (PC, positive Control), - (NC, negative control).

Add 10 μL of control anti RBD Mab CR3022 (2 $\mu\text{g}/\text{mL}$ stock in PBS, 20 ng/well) to “+” well

Add 50 μL IH4-RBD (2 $\mu\text{g}/\text{mL}$ in PBS) to “T” (Test) and “+ve” wells, 50 μL PBS to “-ve” well.

Incubate 1 hour at RT for Red Cells to form a pellet in the “-ve” well

Tilt plate against a well-lit white background for ~ 30 seconds to allow Tear drop to form in “-ve” well.

The presence of antibodies to RBD is shown by loss of Tear Drop formation in the “T” and “+ve” wells. Occasionally a partial tear drop forms – these wells are counted as Negative.

Photograph the plate to record the results with the date and time. Results can be reviewed and tabulated later. Taking picture from a distance and using the zoom function helps to take a clear picture of all wells in a 96-well plate.

The negative (PBS) control should be done on every sample for comparison. The Positive control induced by CR3022 is used to check that all the reagents are working, and that the glycoprotein epitope recognised by VHH(IH4) is present on the red cells. Absence of the IH4 epitope should be very rare (Habib et al., 2013). For setting up cohorts a positive control on every sample is therefore not necessary but should be included in every *batch* of samples.

15. If a 25 μL sample of blood was taken from the finger prick there should be 850 μL of the 1:40 diluted blood left. The red cells can be removed and a preparation of 1:40 O-ve red cells used as above to titrate the sample. In principle the autologous red cells could be washed x3, resuspended in the same volume of PBS, and used as indicators for the titration, however we have not attempted to do this. The supernatant is 1:40 plasma that can be used in confirmatory ELISA or other tests.

Troubleshooting

Time Taken

15 minutes manipulation, 60 minutes incubation.

Anticipated Results

References

<https://www.medrxiv.org/content/10.1101/2020.10.02.20205831v3>

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