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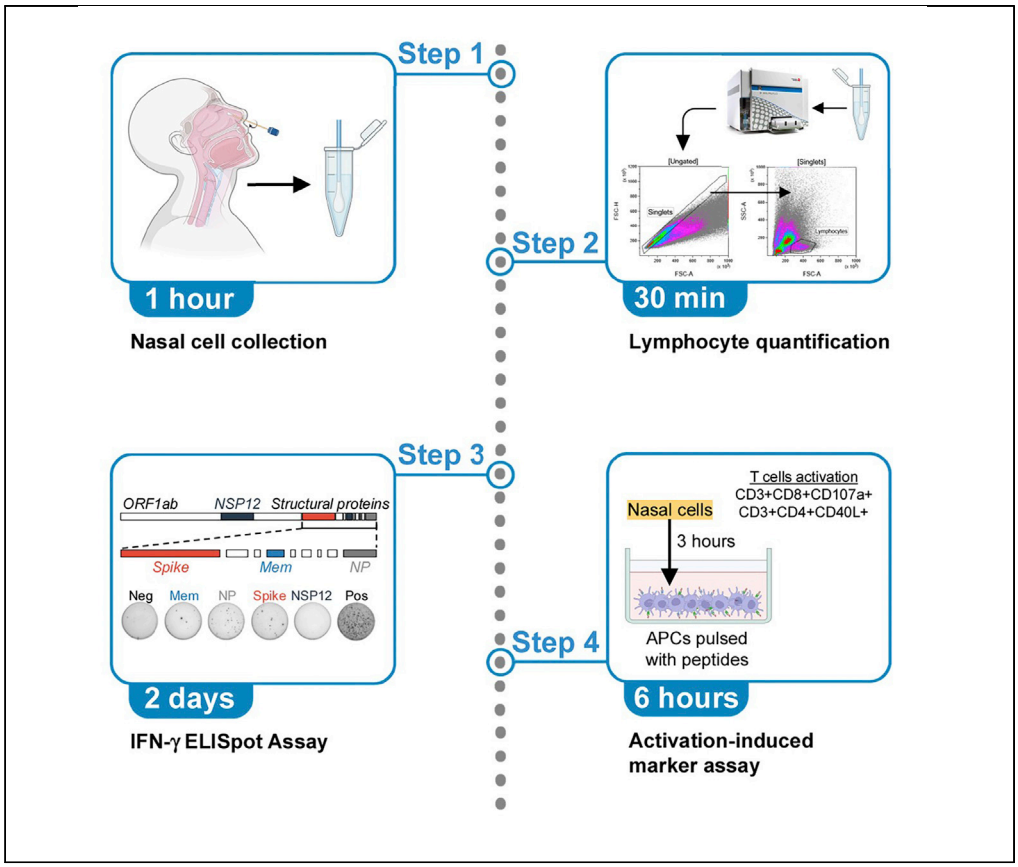
Protocol to detect antigen-specific nasal-resident T cells in humans



Specialized T cells are located in the nasal cavity and act as the first line of defense against respiratory viral infection. Here, we present a protocol for the detection and characterization of antigen-specific nasal-resident T cells. We detail steps for localized nasal swabbing to collect the nasal samples. We then describe IFN-γ ELISpot and an activation-induced marker assay to detect and characterize antigen-specific nasal-resident T cells.

Publisher's note: Undertaking any experimental protocol requires adherence to local institutional guidelines for laboratory safety and ethics.

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Highlights

Nasal T cells can be isolated with a simple nasal swab

Perform IFN-γ ELISpot to quantify SARS-CoV-2-specific nasal T cells

Characterize SARS-CoV-2-specific nasal T cells with flow cytometry

Protocol can be adapted for detection of other antigen-specific nasal T cells

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Protocol to detect antigen-specific nasal-resident T cells in humans

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SUMMARY

Specialized T cells are located in the nasal cavity and act as the first line of defense against respiratory viral infection. Here, we present a protocol for the detection and characterization of antigen-specific nasal-resident T cells. We detail steps for localized nasal swabbing to collect the nasal samples. We then describe IFN-γ ELISpot and an activation-induced marker assay to detect and characterize antigen-specific nasal-resident T cells.

For complete details on the use and execution of this protocol, please refer to Lim et al. (2022).[1]

BEFORE YOU BEGIN

This protocol outlines the detection of SARS-CoV-2-specific T cell isolated from the nasal cavity of SARS-CoV-2-infected individuals as described in Lim et al.[1] This protocol includes handling of human samples and all experiments should be performed in appropriate biosafety cabinets.

Institutional permissions

This protocol requires nasal cells obtained from patients. Ethical approvals are required before starting this procedure. The study performed here was approved by SingHealth Centralized Institutional Review Board (CIRB/F 2021/2014).

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Anti-human IFN-γ coating antibody (for ELISpot) dil. 1:200	Mabtech	Cat# 3420-3-1000; RRID: AB_907282
Anti-human IFN-γ biotin (for ELISpot) dil. 1:2000	Mabtech	Cat# 3420-6-1000; RRID: AB_907272
Brilliant Violet 605 Anti-human CD3 Antibody dil. 2.5:100	Biolegend	Cat# 317322; RRID: AB_2561911
Brilliant Violet 650 Mouse Anti-human CD4 Antibody dil. 2.5:200	BD Horizon™	Cat# 563875; RRID: AB_2744425
PE/Cyanine7 Mouse Anti-human CD8 Antibody dil. 1:500	BD Pharmingen™	Cat# 557746; RRID: AB_396852
Alexa Fluor® 700 Anti-human CD69 Antibody dil. 1:50	Biolegend	Cat# 310922; RRID: AB_493775
CD103 (Integrin alpha E) Monoclonal Antibody (B-Ly7), FITC dil. 8:50	eBioscience™	Cat# 11-1038-42; RRID: AB_465176
APC Mouse Anti-human CD107a Antibody dil. 1:50	BD FastImmune™	Cat# 641581; RRID: AB_1645722
PE Anti-human CD154 Antibody dil. 1:50	Biolegend	Cat# 310806; RRID: AB_314829

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REAGENT or RESOURCE	SOURCE	IDENTIFIER
Biological samples		
Nasal cells	Singapore General Hospital	N/A
Peripheral blood mononuclear cells (PBMCs)	Singapore General Hospital	N/A
Chemicals, peptides, and recombinant proteins		
15-mer SARS-CoV-2 overlapping Spike, Nucleoprotein, Membrane and Non-structural protein 12 (NSP12) peptide pools (Lim et al.[1])	Genscript	N/A
AIM-V media (1×)	Gibco	Cat# 12055-091
Bovine serum albumin (BSA)	Gold Biotechnology	Cat# A-420-250
Dynabeads™ Human T-Activator CD3/CD28	Gibco	Cat# 11131D
Dithiothreitol (DTT)	Thermo Fisher	Cat# R0861
Ethylenediaminetetraacetic acid solution (EDTA)	Sigma-Aldrich	Cat# 03690
Ethyl alcohol (EtOH)	Sigma-Aldrich	Cat# E7023
Heat-inactivated fetal bovine serum (FBS)	Gibco	Cat# 10082-147
Human AB serum	Sigma-Aldrich	Cat# 6914
KPL BCIP/NBT phosphatase substrate (for ELISpot)	SeraCare	Cat# 5420-0038
Phosphate buffered saline, pH 7.4 (PBS)	Gibco	Cat# 10010023
Streptavidin-ALP (for ELISpot)	Mabtech	Cat# 3310-10-1000
Zombie NIR Fixable Viability Kit	Biolegend	Cat# 423105
Critical commercial assays		
EasySep Human CD3 Positive Selection Kits II	Stemcell Technologies	Cat# 17851
Software and algorithms		
Immunospot software	Cellular Technology Limited	http://www.immunospot.com/ImmunoSpot-analyzers-software
Kaluza Analysis Software	Beckman Coulter	https://www.beckman.com/flow-cytometry/software/kaluza
Other		
FLOQSwabs ® (Flexible Minitip Flocked Swab with 100 mm Breakpoint)	COPAN Diagnostics Inc	#503CS01

MATERIALS AND EQUIPMENT

Culture media		
Reagent	Final concentration	Amount
AIM-V media	N/A	49 mL
Human AB serum	2%	1 mL
Total	**N/A**	**50 mL**

Note: Culture media can be stored at 4°C for up to 1 month and should be warmed to 37°C before use.

Nasal isolation media		
Reagent	Final concentration	Amount
Culture media	N/A	998.5 µL
DTT (1 M)	1.5 mM	1.5 µL
Total	**N/A**	**1 mL**

Note: Nasal isolation media should be prepared fresh right before use. Do not store left over nasal isolation media.

Depletion buffer		
Reagent	Final concentration	Amount
PBS	1×	48.5 mL
FBS	2%	1 mL
EDTA	1 mM	0.5 mL
Total	N/A	50 mL

Note: Depletion buffer can be stored at 4°C for up to 3 months.

ELISpot blocking media		
Reagent	Final concentration	Amount
AIM-V media	N/A	225 mL
FBS	10%	25 mL
Total	N/A	250 mL

Note: ELISpot blocking media can be stored at 4°C for up to 3 months.

ELISpot development media		
Reagent	Final concentration	Amount
PBS	1×	225 mL
FBS	10%	25 mL
Total	N/A	250 mL

Note: ELISpot development media can be stored at 4°C for up to 3 months.

Staining buffer		
Reagent	Final concentration	Amount
PBS	1×	49.5 mL
BSA	1%	0.5 mL
Total	N/A	50 mL

Note: Staining buffer can be stored at 4°C for up to 3 months.

ELISpot antibody		
Antibodies	Dilution factor	Final concentration
Anti-human IFN-γ coating antibody	1:200 (diluted in PBS)	5 μg/mL
Anti-human IFN-γ biotin	1:2000 (diluted in ELISpot development media)	0.5 μg/mL
Streptavidin-ALP	1:2000 (diluted in ELISpot development media)	N/A (not provided by manufacturer)

Note: ELISpot antibodies should be prepared on the same day as experiment and stored at 4°C until used.

Surface staining antibody	
Antibodies	Dilution factor (from manufacturer's stock concentration)
Brilliant Violet 605 Anti-human CD3 Antibody	1.25:50 (diluted in staining buffer)
Brilliant Violet 650 Mouse Anti-human CD4 Antibody	0.625:50 (diluted in staining buffer)
PE/Cyanine7 Mouse Anti-human CD8 Antibody	0.1:50 (diluted in staining buffer)
Alexa Fluor® 700 Anti-human CD69 Antibody	1:50 (diluted in staining buffer)

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Antibodies	Dilution factor (from manufacturer's stock concentration)
CD103 (Integrin alpha E) Monoclonal Antibody (B-Ly7), FITC	8:50 (diluted in staining buffer)
APC Mouse Anti-human CD107a Antibody	2:100 (diluted in culture media)
PE Anti-human CD154 Antibody	2:100 (diluted in culture media)

Note: Surface staining antibodies should be prepared right before using.

STEP-BY-STEP METHOD DETAILS

Nasal sample collection

Timing: 1 h

Nasal samples are obtained by a simple localized sampling using FLOQSwab.

1. Nasal swabbing of subject.
   a. Insert flocked swab into the inferior turbinate of the subject through the nostril at around 4 cm in.
   b. Twirl the swab 10–20 times.

   Note: Some minor irritation of the nasal cavity should occur resulting in tearing and mucus secretion.

   c. Insert the same swab to the other nostril at around 4 cm in.
   d. Twirl the swab 10–20 times again.

   Note: Some minor irritation of the nasal cavity should occur resulting in tearing and mucus secretion.

   e. Place swab into 1 mL of nasal isolation media in a 1.8 mL Eppendorf tube and cut the excess length of the swab in order to close the cap of the Eppendorf tube.
   f. Vortex tube vigorously for 10 s.
   g. Incubate tube at 37°C for 30 min.
2. Remove the swab from the Eppendorf tube carefully and minimize the amount of nasal isolation media trapped by the swab by gently pressing the swab on the wall of the tube.
3. Spin down the cells at 1,000 × *g* for 10 min.
4. Remove the supernatant without disturbing the pellet.
5. Resuspend the pellet in 1 mL of culture media.
6. Repeat steps 3 and 4 to wash the cells again.
7. Resuspend the cells in culture media to the desired volume (1 mL of culture media is recommended).

Quantification of the nasal lymphocytes collected

Timing: 10 min

Nasal samples are highly heterogenous, as it includes epithelial cell, ciliated cell and more. Thus, it is difficult to quantify using conventional cell counting method. We employed flow cytometry to quantify the number of lymphocytes retrieved from the nasal swabs.

8. Aliquot 5% of the total volume of nasal sample obtained to a new Eppendorf tube and adjust the volume with PBS such that the total volume in the Eppendorf tube is 50 μL.

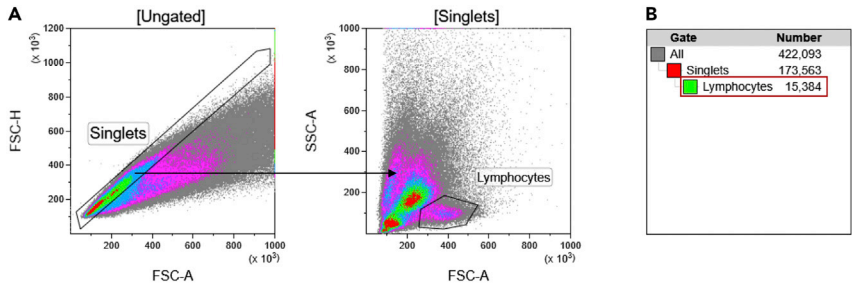

Figure 1. Lymphocyte quantification by flow cytometry

(A) Gating strategy for quantification of lymphocyte.

(B) Number of lymphocytes gated (*y*) from the corresponding flow plots, to be used in the calculation for the number of lymphocytes isolated for further experiment.

9. Dilute the cells 2× by adding 50 μL of PBS (total volume 100 μL).
10. Acquire the sample using a flow cytometer.
    a. Set the volume to acquire to 50 μL.
    b. Gate according to Figure 1.
    c. Note down the total number of events acquired in the lymphocyte gate (*y*).
11. Calculate the number of cells isolated.
    a. $\left(\frac{y\times2\times2}{5}\right)\times95$ = Number of lymphocytes isolated for further experiment.

Note: A 2 times dilution were done before acquisition at step 9 and half (50 μL out of 100 μL) of the 2 times diluted sample were acquired at step 10a, thus the number of lymphocytes gated should be multiplied by 2 twice. That will represent the total lymphocytes aliquoted at step 8 (5% of total lymphocytes obtained). Therefore, to derive the number of lymphocytes present in the remaining 95% of the sample, 4*y* were divided by 5 and then multiplied by 95.

Note: The number of lymphocytes isolated from different donors are highly variable. It is not recommended to continue with other experiments (IFN-γ ELISpot assay and activation induced markers assay) if less than 15,000 lymphocytes were isolated. Up to 200,000 lymphocytes could be isolated from each donor.

IFN-γ ELISpot assay

Timing: 2 days

Nasal cells will be stimulated overnight with different peptide pools specific for different SARS-CoV-2 proteins. Peptide pools designed based on other pathogens need to be re-validated for this assay. Antigen-specific T cells will produce IFN-γ and will be detected as spot forming units.

12. Ethanol (EtOH) activation of Millipore ELISpot plate.
    a. Prepare 35% EtOH diluted with sterile water.
    b. Aliquot 25 μL of 35% EtOH into each well.
    c. Ensure that the membranes are completely coated by the 35% EtOH by tapping the plate gently.
    d. Allow the 35% EtOH to activate the membrane for 30 s.
    e. Add 200 μL of sterile water into each well to stop the activation.
    f. Wash the plate five times with 200 μL/well of sterile water.
13. Coating Millipore ELISpot plate with IFN-γ coating antibody.
    a. Dilute IFN-γ coating antibody in PBS according to concentration stated above.
    b. Flick the plate to remove sterile water and tap it dry.
    c. Aliquot 100 μL/well of diluted IFN-γ coating antibody.

d. Incubate at 4°C in the dark for at least 24 h.

14. Blocking ELISpot plate.
    a. Flick the plate to remove coating antibody and tap it dry.
    b. Wash the plate 6 times with 200 μL/well of PBS.
    c. Aliquot 100 μL/well of blocking media.
    d. Incubate at room temperature (RT; approximately 25°C) for at least 30 min.
15. Dilute peptide pools to a working concentration of 6 μg/mL (per peptide) in culture media.
16. Dilute nasal cells to desired concentration to 100 μL/well (at least 5000 lymphocytes/well).
17. Remove the blocking media by flicking the plate.
18. Aliquot 50 μL of peptides into each well (final concentration of 2 μg/mL (per peptide)) followed by 100 μL of nasal cells into each well (total volume: 150 μL).
    a. For negative control, aliquot 50 μL of culture media without peptides into each well.
    b. For positive control, instead of peptides, aliquot 5 μL of α-CD3/28 dynabeads (2×10^5 beads/well) with 45 μL of culture media into each well.

Note: Negative and positive controls need to be run for all individual samples to determine the validity of the result.

19. Incubate at 37°C, 5% CO_2 overnight.
20. Develop ELISpot plate.
    a. Dilute anti-human IFN-γ biotin and streptavidin-ALP in ELISpot development media according to the concentrations stated above.
    b. Flick the plate to remove the cells.
    c. Wash the plate 6 times with 200 μL/well of PBS and tap it dry.
    d. Aliquot 100 μL/well of anti-human IFN-γ biotin.
    e. Incubate the plate for 2 h at RT in the dark.
    f. Wash the plate 6 times with 200 μL/well of PBS and tap it dry.
    g. Aliquot 100 μL/well of streptavidin-ALP.
    h. Incubate the plate for 1 h at RT in the dark.
    i. Wash the plate 6 times with 200 μL/well of PBS and tap it dry.
    j. Aliquot 50 μL/well of KPL BCIP/NBT phosphatase substrate.
    k. Incubate the plate for 10–20 min at RT in the dark and wash the plate under running tap water when spots are developed and before the negative control well turns purple.

    Note: After 5 min of incubation, ELISpot plate should be checked consistently every two minutes by looking at the negative and positive control wells.

    l. Allow the plate to dry completely.

    Note: The membrane will take around 30–60 min to completely dry and the membrane will change from translucent to completely opaque white when dry.

    m. Capture the image of each well with Immunospot reader.

    Note: Test should only be considered successful for each sample if positive control contains more than 100 spots (Figure 2).

Activation-induced markers assay

Timing: 6 h

Nasal cells will be stimulated with peptide-pulsed autologous CD3-depleted PBMC for 3 h and the activation status will be analyzed with flow cytometry to better characterize the antigen-specific nasal T cells.

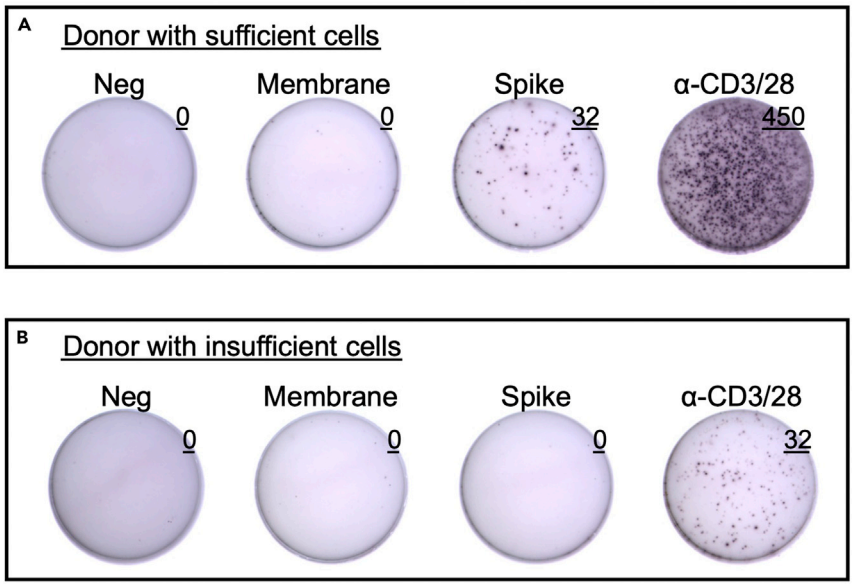

Figure 2. Results of IFN-γ ELISpot assay from donors with or without sufficient nasal T cells isolated

(A) Results of IFN-γ ELISpot assay from a donor with sufficient nasal T cells isolated as demonstrated by the positive control (α-CD3/28 stimulated well) with more than 100 spots counted. Top right of each well reflects the number of spots counted by the Immunospot reader. The donor has Spike-specific T cells detected but not Membrane-specific T cell.

(B) Results of IFN-γ ELISpot assay from a donor with insufficient nasal T cells isolated as demonstrated by the positive control (α-CD3/28 stimulated well) with less than 100 spots counted. Top right of each well reflects the number of spots counted by the Immunospot reader. The test for the donor should be excluded from the result.

21. CD3 depletion of autologous PBMC using EasySep Human CD3 Positive Selection Kits II.
    a. Resuspend PBMC at 100 × 10^6 PBMC/mL with depletion buffer in a 5 mL round-bottom polystyrene tube.
    b. Aliquot 100 μL/mL of human CD3 positive selection cocktail to the PBMC and mix well.
    c. Incubate at RT for 5 min.
    d. Vortex RapidSpheres vigorously for 1 min.
    e. Aliquot 60 μL/mL of RapidSpheres to the PBMC and mix well.
    f. Incubate at RT for 5 min.
    g. Top up sample to 2.5 mL with depletion buffer.
    h. Place the tube (without the lid) into Easysep magnet.
    i. Incubate at RT for 5 min.
    j. After 5 min, pick up the magnet and in one continuous motion pour the supernatant into another 5 mL round-bottom polystyrene tube (Supernatant contains the CD3-depleted cells).
    k. Repeat steps h–j two more times.
    l. Spin down the cells at 400 × *g* for 5 min and remove supernatant.
    m. Resuspend CD3-depleted cells in 1 mL of culture media and count the number CD3-depleted cells isolated.

Note: We recommend using trypan blue staining and counting in a hemocytometer to determine the viability and cell count.

Optional: A simple flow cytometry staining including Live/Dead, CD3, CD4 and CD8 antibodies could be performed to check the purity of the CD3-depleted cells isolated. This will allow user to be sure that the antigen-specific response detected in the following steps are not contributed by circulatory cells.

Pause point: CD3-depleted cells could be isolated in bulk and frozen down for storage. For the same donor, CD3-depleted cells could then be thawed and used for the following steps in the future.

22. Peptide pulsing with CD3-depleted PBMC.
    a. Dilute peptide pool to 20 μg/mL (final concentration of 10 μg/mL (per peptide)) followed) in 50 μL of culture media.
    b. Resuspend 1 × 10^6 CD3-depleted cells in 50 μL of culture media.
    c. Aliquot 50 μL of diluted peptide pool and 50 μL of resuspended CD3-depleted cells into a 1.8 mL Eppendorf tube.
    d. Incubate at 37°C, 5% CO_2 for 1 h.
    e. After 1 h, spin down the cells at 400 × *g* for 5 min.
    f. Remove supernatant and resuspend in 50 μL of culture media.

23. Stimulation of nasal cells with peptide-pulsed autologous CD3-depleted PBMC.
    a. Add 2 μL of APC anti-CD107a antibody and 2 μL of FITC anti-CD40L antibody to the peptide pulsed CD3-depleted cells.
        i. For negative control, un-pulsed CD3-depleted PBMC should be added, instead of peptide-pulsed autologous CD3-depleted PBMC.
        ii. For positive control, add 2 μL of APC anti-CD107a antibody and 2 μL of FITC anti-CD40L antibody to 5 μL of α-CD3/28 dynabeads with 41 μL of culture media.

    Note: Negative and positive controls need to be run for all individual samples to determine the validity of the result.

    Note: APC anti-CD107a and FITC anti-CD40L antibodies are added during stimulation of nasal cells with peptide-pulsed autologous CD3-depleted PBMC, instead of during the cell surface markers staining step later, to better capture the expression of CD107a and CD40L during activation.

    b. Add 50 μL of nasal cells (containing at least 5,000–10,000 lymphocytes).
    c. Incubate at 37°C, 5% CO_2 for 3 h.
        i. After 3 h, add 500 μL of PBS to the cells, spin it down at 400 × *g* for 5 min and remove supernatant carefully.
24. Cell surface markers staining.
    a. Stain the cells in the same 1.8 mL Eppendorf tube with 100 μL/sample of NIR (1:1000 in PBS) in the dark at RT for 10 min.
    b. After 10 min, add 100 μL of staining buffer to the cells, spin it down at 400 × *g* for 5 min and remove supernatant carefully.
    c. Prepare antibody mix by diluting BV605 anti-CD3, BV650 anti-CD4, PE-Cy7 anti-CD8, AF700 anti-CD69 and FITC anti-CD103 antibodies in staining buffer to the concentrations stated above, final volume of antibody mix for each sample should be 50 μL.
    d. Stain the cells with 50 μL/sample of antibody mix for 30 min in the dark on ice.
    e. After 30 min, wash the cells twice with 200 μL staining buffer each time.
    f. Resuspend the cells in 100–200 μL of staining buffer for acquisition.
25. Acquire samples with flow cytometer immediately.
    a. Gating strategy is shown in Figure 3.

EXPECTED OUTCOMES

Following the steps described in this protocol, you will be able to determine whether there are SARS-CoV-2-specific T cells in the nasal cavity of the subject. These SARS-CoV-2-specific T cells can be further characterized by flow cytometry to determine if they are tissue-resident memory CD4 or

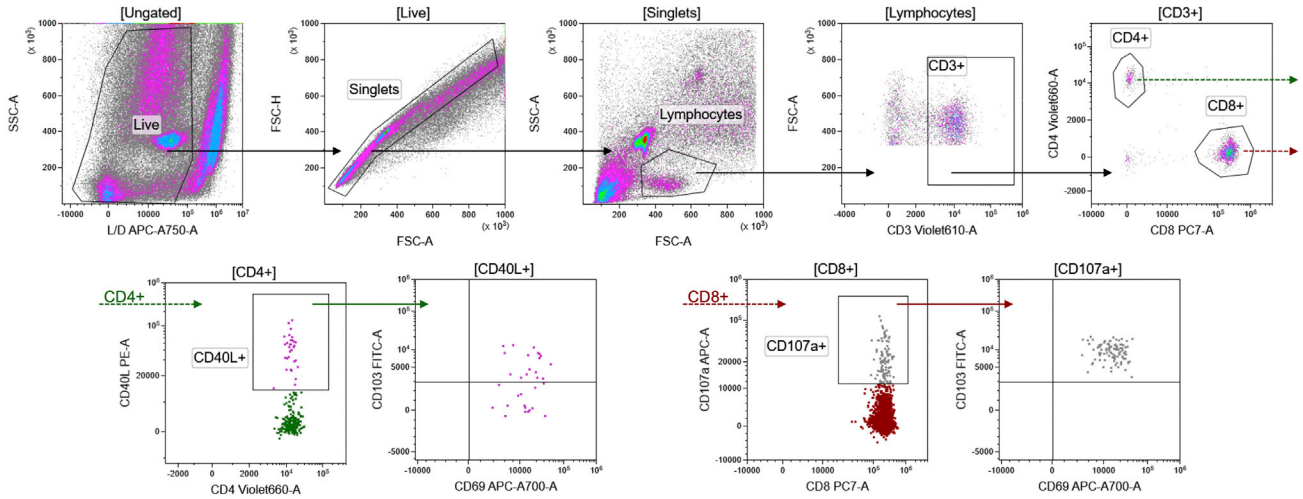

Figure 3. Gating strategy for the activation induced marker assay performed with nasal cells stimulated by peptide-pulsed autologous antigen presenting cells

CD8 T cells. Some practical examples of the application of this protocol can be found in our recent publication.[1]

LIMITATIONS

The protocol described here is for direct *ex vivo* detection of antigen-specific T cells found in the fluid lining the nasal cavity. Nasal T cells are collected using nasal swabs of the inferior turbinate region. The samples collected with the swab will be very heterogenous, consisting of many other cell types and foreign contaminants which cause it to be difficult to quantify using conventional cell counting method. We used flow cytometry here without any staining to quantify the lymphocytes count, however, this method can only differentiate between dead and live lymphocytes based on their FSC and SSC profile. Optionally, live/dead discrimination dyes could be incorporated in the analysis to determine the live lymphocytes more precisely. Moreover, since the samples will be collected directly from the nose, it will not be sterile and thus, not favorable to be used in longer term *in vitro* culture experiments. Furthermore, due to the low number of nasal T cells isolated from each swab, it is not recommended to cryopreserve the nasal sample. Experiment should be done immediately after collection of nasal cells.

TROUBLESHOOTING

Problem 1

Low purity and yield of sample. The nasal sample collected from the swab might contain too much mucus and the number of lymphocytes retrieved might be very low. This will lead to poor results for the positive controls in the ELISpot.

Potential solution

Ensure that the swabbing process is done correctly. The swab inserted cannot be too shallow as it will fail to reach the inferior turbinate. The swab has to be rotated sufficiently in order to collect the cells. This will usually result in a minor irritation of the nasal cavity that resolves in less than 1 h. Vortex the cells vigorously before and after incubation with DTT to breakdown the mucus and dislodge the cells. To ensure that functioning nasal T cells are collected and sufficient number of cells were used for each experiment, positive control should be run for every sample.

Problem 2

Contamination of nasal cells. Since the nasal cells are collected directly from the nasal inferior turbinate, which is exposed to the environment, the sample collected might be contaminated by foreign

pathogen or particles. It might result in the T cells being very activated and thus high background reading (apparent from negative control) in all experiments.

Potential solution

Sterility of the sample cannot be achieved as the sample is obtained directly from the nasal cavity. To reduce the chances of having a high background due to contamination, general cell culture aseptic techniques should be followed. If all else fails, the sample will have to be collected again.

Problem 3

The nasal swabbing might cause minor lesions in the nasal cavity which result in bleeding. The blood will contaminate the nasal cells.

Potential solution

Avoid the nostril that have bled and swab the other nostril. Swab gentler but still ensure that the swabbing process is done correctly. Ideally, perform the swabbing the next day to make sure that enough cells could be collected.

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Antonio Bertoletti (antonio@duke-nus.edu.sg).

Materials availability

This study did not generate any new reagent.

Data and code availability

This study did not generate any new data or code.

ACKNOWLEDGMENTS

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AUTHOR CONTRIBUTIONS

Conceptualization, J.M.E.L., A.T.T., A.B.; methodology, J.M.E.L., A.T.T., A.B.; investigation, J.M.E.L., A.T.T., A.B.; data curation and writing, J.M.E.L., A.T.T., A.B.; funding acquisition, A.B.; supervision, A.B.

DECLARATION OF INTERESTS

A.B. and A.T.T reported a patent for a method to monitor SARS-CoV-2–specific T cells in biological samples pending and licensed (Hyris).

REFERENCES

1. Lim, J.M.E., Tan, A.T., Le Bert, N., Hang, S.K., Low, J.G.H., and Bertoletti, A. (2022). SARS-CoV-2 breakthrough infection in vaccinees induces virus-specific nasal-resident CD8+ and CD4+ T cells of broad specificity. J. Exp. Med. *219*, e20220780. https://doi.org/10.1084/jem.20220780.