T-SPOT.COVID

Oxford Immunotec

PACKAGE INSERT

For Research Use Only

Not for use in diagnostic procedures

This Package Insert covers use of:

COV.435/300 RUO

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1. INTENDED USE

The T-SPOT.*COVID* assay is a standardised ELISPOT (Enzyme Linked ImmunoSpot) based technique intended for qualitative detection of a cell mediated (T cell) immune response to SARS-CoV-2 in human whole blood (sodium or lithium heparin). The T-SPOT.*COVID* assay is intended for research use as an aid in identifying an adaptive immune response to SARS-CoV-2, specifically the T cell response. This assay is for research use only (RUO), not for use in diagnostic procedures.

2. SUMMARY & EXPLANATION

The T-SPOT.*COVID* assay is a simplified, standardised variant of the ELISPOT assay technique. ELISPOT assays detect and measure T cell responses by enumerating the number of T cells that are secreting cytokine in response to stimulation with antigens. ELISPOT assays are exceptionally sensitive since the target cytokine is captured directly around the secreting cell, before it is diluted in the supernatant, bound by receptors of adjacent cells or degraded. This makes ELISPOT assays much more sensitive than conventional ELISA assays[1,2,3,4].

The test enumerates effector T cells responding to stimulation using two separate peptide pools derived from the SARS-CoV-2 Spike and Nucleocapsid proteins. The T cell response to each protein is measured in parallel in individual wells. T-SPOT.*COVID* antigen panels are designed as overlapping peptides spanning sequences of the Spike (COV-A) and Nucleocapsid (COV-B) proteins. This peptide design offers maximum epitope coverage for enhanced detection of T cell reactivity and no HLA restrictions. Antigenic formulations of 253 peptides covering the most immunogenic regions of the virus genome allows measurement of the breadth of immunity and ensures the impact of point mutations is minimised. Specificity to SARS-CoV-2 has been enhanced by removing potentially cross-reactive peptide sequences with high homology to other coronaviruses.

PRINCIPLE OF TEST

The immune response to infection with SARS-CoV-2 is mediated through both B cell and T cell activation. As part of the T cell response, T cells are sensitised to SARS-CoV-2 antigens designed to activate both CD4 and CD8 effector T cells, which then produce the cytokine interferon-gamma (IFN-γ) when stimulated by these antigens[5,6]. The T-SPOT.*COVID* assay uses the enzyme-linked immunospot (ELISPOT) methodology to enumerate SARS-CoV-2-sensitised T cells by capturing interferon-gamma (IFN-γ) in the vicinity of T cells from which it was secreted[7].

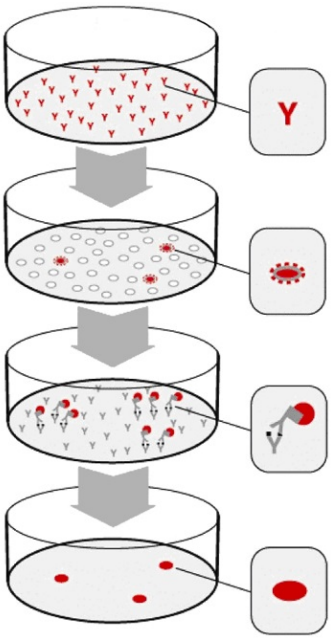
Peripheral blood mononuclear cells (PBMCs) are separated from a whole blood sample, washed and then counted before being added into the assay.

Isolated PBMCs (white blood cells) are placed into microtiter wells where they are exposed to a phytohemagglutinin (PHA) control (a mitogenic stimulator indicating cell functionality), nil control, or two separate panels of SARS-CoV-2 antigens derived from Spike and Nucleocapsid proteins respectively. The PBMCs are incubated with the antigens to allow stimulation of any sensitised T cells present.

Secreted cytokine is captured by specific antibodies on the surface of the membrane, which forms the base of the well, and the cells and other unwanted materials are removed by washing. A second antibody, conjugated to alkaline phosphatase and directed to a different epitope on the cytokine molecule, is added and binds to the cytokine captured on the membrane surface. Any unbound conjugate is removed by washing. A soluble substrate is added to each well; this is cleaved by bound enzyme to form a (dark blue) spot of insoluble precipitate at the site of the reaction.

Evaluating the number of spots obtained provides a measurement of the abundance of effector T cells in the peripheral blood primed against SARS-CoV-2. These principles behind the T-SPOT technology platform are described in Figure 1 below.

***Figure 1**: Principles of the T-SPOT assay system. For illustration only, refer to Section 6, Instructions for Use for detailed procedural instructions.*



First a blood sample is collected from the subject. The white blood cells are separated and then put into the wells of a 96-well microtiter plate. The plates are pre-coated with high affinity antibodies (Y) to Interferon-gamma (IFN-γ), a cytokine released by effector T cells when fighting SARS-CoV-2 infection.

Antigens from the SARS-CoV-2 virus are then added to the wells with the white blood cells to provoke interferon-gamma (IFN-γ) secretion from any effector T cells primed against SARS-CoV-2.

Antigen specific responding T cells ( ) release the cytokine ( ), which is captured in the immediate vicinity of the T cells, by the antibodies lining the bottom of the well.

After a short incubation time the wells are washed, removing the antigens and cells from the wells. A conjugated second antibody ( ) is then added which binds to the interferon-gamma (IFN-γ) secreted by the T cells (and captured by the primary antibody).

A substrate is then added which produces spots ( ) where the interferon-gamma (IFN-γ) was secreted by T cells. The number of spots is counted.

3. REAGENTS & STORAGE

MATERIALS PROVIDED

T-SPOT.*COVID* COV.435/300 RUO kit contains:

1. 1 microtiter plate: 96 wells, supplied as 12x 8-well individual strips in a separate frame, coated with a mouse monoclonal antibody to the cytokine interferon-gamma (IFN-γ).
2. 2 vials (0.8 mL each) Panel A (COV-A): contains Spike antigens, bovine serum albumin and antimicrobial agents.
3. 2 vials (0.8 mL each) Panel B (COV-B): contains Nucleocapsid antigens, bovine serum albumin and antimicrobial agents.
4. 2 vials (0.8 mL each) Positive Control: contains phytohemagglutinin (PHA), for use as a cell functionality control, bovine serum albumin and antimicrobial agents.
5. 1 vial (50 µL) 200x concentrated Conjugate Reagent: mouse monoclonal antibody to the cytokine interferon-gamma (IFN-γ) conjugated to alkaline phosphatase.
6. 1 bottle (25 mL) Substrate Solution: ready-to-use BCIP/NBT^{plus} solution.
7. Package insert

Note: 8 well strips used in the COV.435/300 RUO kit are single use items and should be used immediately once opened and not reused. Do not mix components between different kits.

STORAGE & STABILITY

Store the unopened kit at 2-8 °C. The components of the kit are stable up to the expiration date printed on the kit box, when stored and handled under the recommended conditions. The kit must not be used beyond the expiration date on the kit label. If a component has an expiration date later than that on the (outer) kit box, do not retain and do not use that component with another kit; do not use any component in the kit, after the expiration date on the outer kit box.

Store opened kit components at 2-8 °C. Opened components must be used within 8 weeks of opening, such period ending no later than the expiration date on the kit label. **Avoid prolonged exposure of the Substrate Solution to light.**

EQUIPMENT AND MATERIALS REQUIRED BUT NOT PROVIDED

1. 8-well strip plate frame (available from Oxford Immunotec).
2. BLII cabinet (recommended).
3. Blood collection tubes, such as Vacutainer® CPT™ or heparinised tubes.
4. T-Cell *Xtend*® reagent - whole blood samples stored at room temperature (18 – 25 ºC) between 0 and 32 hours post venipuncture, can be processed with the use of T-Cell *Xtend* reagent.
5. Ficoll® (if not using CPT tubes).

6. A centrifuge for preparation of PBMCs (capable of at least 1800 RCF (g) and able to maintain the samples at room temperature (18-25 °C) if using density centrifugation methods to separate the PBMCs.
7. Equipment and reagents to enable counting of PBMCs; either manually using Trypan Blue (or other appropriate stain) and a hemocytometer on a microscope or automatically using a suitable hematology analyser.
8. A humidified incubator capable of 37 ± 1 °C with a 5 % CO_2 supply.
9. An automatic microtiter plate washer or an 8 channel or stepper pipette to manually wash plates.
10. Adjustable pipettes to cover a range of volumes from 1-1000 µL (such as four pipettes capable of delivering volumes of 1-10 µL, 2-20 µL, 20-200 µL and 100-1000 µL) and sterile pipette tips.
11. Sterile PBS solution: such as GIBCO® 1x D-PBS (Life Technologies; catalogue number 14040-133).
12. Distilled or deionised water.
13. A means of visualising the wells, or capturing a digital image of the well, such as a stereomicroscope, magnifying glass or plate imager to allow counting of spots.
14. Sterile cell culture medium such as GIBCO AIM V® (Life Technologies; catalogue number 31035-025 research grade). (Note: AIM V media is available from Oxford Immunotec). **The use of this serum free medium for the incubation step is strongly recommended**. RPMI 1640 (Invitrogen; catalogue number 11875-093) may be used in the initial sample preparation steps only. It is recommended that cell culture media are stored in appropriate aliquots and excess material is discarded after use. **Cell culture media should be pre-warmed to 37 °C before use with the T-SPOT.*COVID* assay**. To avoid problems with contaminated media, it can be helpful to dispense bottles of AIM-V or RPMI 1640 into smaller aliquots.

4. WARNINGS & PRECAUTIONS

- For research use only. Not for use in diagnostic procedures.
- For professional use only.
- Operators should be trained in the assay procedure and be sure they understand the instructions for use before performing the assay.
- Read the assay instructions carefully before use. Deviations from the instructions for use in this package insert may yield erroneous results.
- Care should be taken when handling material of human origin. All blood samples should be considered potentially infectious. Handling of blood samples and assay components, their use, storage and disposal should be in accordance with procedures defined in appropriate national, state or local biohazard and safety guidelines or regulations.
- Care should be taken when working with chemicals. All chemicals should be considered potentially hazardous. A material safety data sheet for the kit is available from Oxford Immunotec.
- Discard unused reagents and biological samples in accordance with Local, State and Federal regulations.
- The correct number of PBMCs must be added to each well. Failure to do so may lead to an incorrect interpretation of the result.
- Do not mix components from different kit lots.
- Observe aseptic technique to avoid contaminating the reagents, assay wells, cell suspensions and cell culture media.
- Variation to the stated pipetting and washing techniques, incubation times and/or temperatures may influence the actual results obtained and should be avoided.
- Blood should be collected and processed as soon as possible.
- Store and transport blood samples to the laboratory at room temperature (18-25 °C). Do not refrigerate or freeze whole blood samples.
- Failure to adhere to the recommended incubation times and temperatures may lead to an incorrect interpretation of the results.
- Indentations in the membrane caused by pipette or well washer tips may develop as artifacts in the wells which could confuse spot counting.

5. SPECIMEN COLLECTION & HANDLING

Individual laboratories should validate their procedures for collection and separation of PBMCs to obtain sufficient numbers. It is recommended that:

1. Whole blood samples should be maintained between 18 °C and 25 °C until processed.
2. Collect a blood sample according to the instructions supplied with the collection device. The tube contents must be inverted (8-10 times) to ensure that the whole blood is mixed thoroughly with the anticoagulant. Store collected blood at room temperature (18-25 °C). **Do not refrigerate or freeze.**
3. Typically, for an immunocompetent subject, sufficient PBMCs to run the assay can be obtained from venous blood samples according to the following guidelines:

   One 8 mL or two 4 mL tubes (CPT) or one lithium heparin 6 mL tube.

   A subject's PBMCs can be pooled, if necessary to obtain sufficient PBMCs from multiple tubes of blood which

were collected and processed concurrently.

4. When using the T-SPOT.*COVID* assay **without the use of T-Cell *Xtend* reagent** blood samples should be processed within 8 hours of collection. Samples may be collected into either sodium citrate or sodium heparin Vacutainer CPT tubes (Becton Dickinson) with PBMCs separated in the tube using the manufacturer's instructions. Alternatively, blood samples may be collected into lithium heparin tubes with PBMCs being subsequently separated using standard separation techniques such as Ficoll-Paque® or alternative methods to purify the PBMC fraction. Blood collection tubes containing the anticoagulant EDTA should not be used.

a. For CPT blood collection tubes, centrifuge 8 mL CPT tubes at 1600 RCF(g) for 28 minutes or 4 mL CPT tubes at 1800 RCF (g) for 30 minutes at room temperature (18-25 °C).

b. If using Ficoll-Paque Plus, dilute the blood with an equal volume of RPMI 1640 medium (1 part blood to 1 part RPMI). Layer carefully the diluted blood onto Ficoll-Paque Plus (2-3 parts diluted blood to 1 part Ficoll-Paque) and centrifuge at 1000 RCF (g) for 22 minutes at room temperature (18-25 °C).

***Note**: Review the manufacturer's instructions before using either CPT tubes or Ficoll-Paque. Ensure the tubes are centrifuged at the correct speed. The speeds given above are in g or Relative Centrifugal Force (RCF). This is not the same as Revolutions Per Minute (RPM). If the centrifuge will only measure speed in RPM, then convert to the recommended RCF value by measuring the rotor radius and using a conversion table. Leucosep tubes (Greiner Bio-One) offer a time-saving approach to density gradient separation. The tubes contain a porous barrier that enables the blood sample to be poured onto the density gradient separation medium, thereby eliminating the need to gently layer on the sample.*

5. When using the T-SPOT.*COVID* assay **with T-Cell *Xtend* reagent** blood samples should be collected into lithium heparin tubes. Vacutainer CPT tubes and blood collection tubes containing the anticoagulant EDTA should not be used. The T-Cell *Xtend* reagent should be added prior to PBMC separation using standard separation techniques. Whole blood samples should be stored at room temperature (18-25 ºC) between 0 and 32 hours post venipuncture with the use of T-Cell *Xtend* reagent.

   If the T-Cell *Xtend* reagent is to be used, immediately before cell separation remove the cap from the blood collection tube and add 25 µL of the T-Cell *Xtend* reagent solution per mL of blood sample. Replace the cap and invert blood collection tube gently 8 to 10 times to mix. Incubate for 20 ± 5 minutes at room temperature (18-25 °C) and then proceed to isolate the PBMC layer using Ficoll density gradient centrifugation as presented in sections 4b, and 6 - 9. See the T-Cell *Xtend* reagent package insert for further details.

6. Collect the white, cloudy band of PBMCs using a pipette and transfer to a 15 mL conical centrifuge tube. Bring the volume to 10 mL with cell culture medium. **Cell culture media for the washing steps should be pre-warmed to 37 °C before contact with PBMCs.**

   Circulating factors in whole blood samples are known to interfere with whole blood interferon-gamma tests, e.g., rheumatoid factor, heterophilic antibodies, and pre-existing amounts of interferon-gamma. The separation and washing of the PBMCs enables removal of these potentially interfering substances prior to performing the assay.

   Note: After centrifugation, PBMCs should be extracted using a large bore (e.g. 1 mL) pipette tip, by immersing the pipette tip into the PBMC layer. This cloudy layer should be carefully aspirated and transferred to a sterile conical tube for the wash steps. Ensure that all of the cloudy PBMC layer is collected. It is better to take more of the plasma layer than to leave any of the PBMCs in the blood collection tube. However, if using CPTs avoid transferring any of the separation gel, which can block the tip. If this happens transfer the cells already in the tip into a centrifuge tube and then use a new tip to transfer the remaining PBMCs. A variety of media can be used for washing the cells during these steps 3-5; both AIM V and RPMI 1640 have been used successfully and are recommended.

7. Centrifuge at 600 RCF (g) for 7 minutes. Pour off the supernatant and resuspend the pellet in 1 mL medium.

8. Bring the volume to 10 mL with fresh medium and centrifuge at 350 RCF (g) for 7 minutes.

9. Pour off the supernatant and resuspend the pellet in 0.7 mL cell culture medium. **The serum-free medium AIM V has been used successfully and is strongly recommended.**

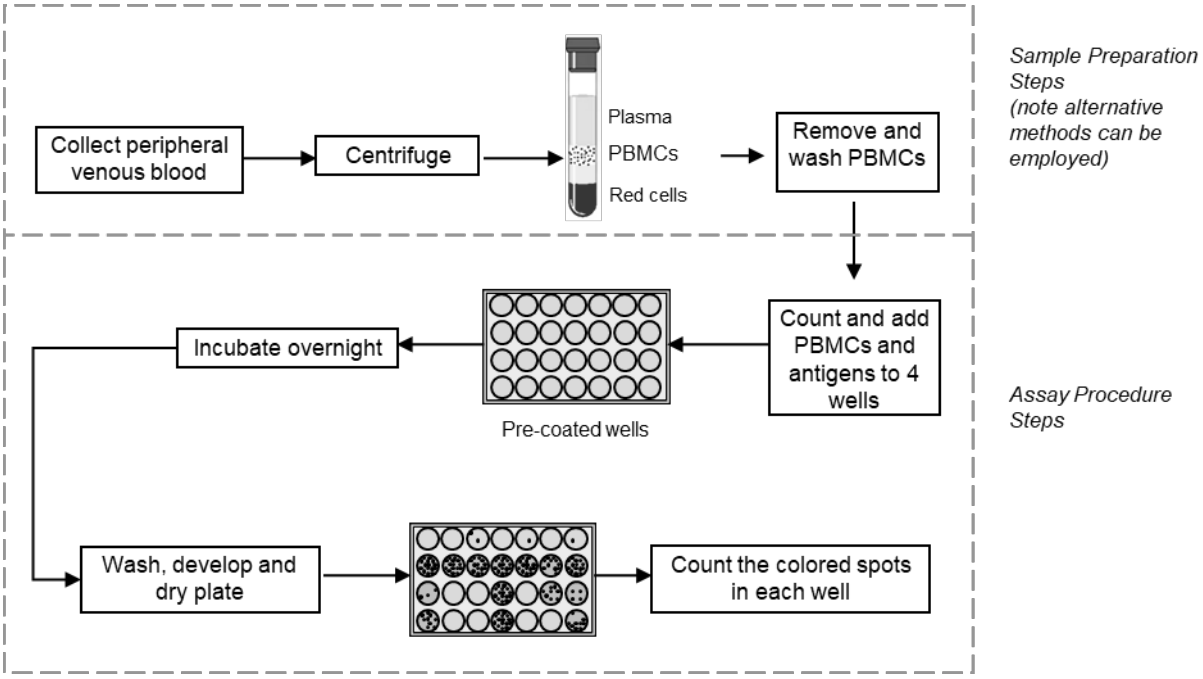
   ***Note:** Steps 2-7 should be performed in a BLII cabinet to protect the user and prevent contamination of samples.*

6. INSTRUCTIONS FOR USE

A full T-SPOT.*COVID* assay plate will process 24 samples. The assay is typically carried out on the afternoon of one day and the morning of the following day, to allow the 16-20 hr incubation step to take place overnight. If this timing is used, then on the afternoon of day 1 the blood samples are processed to prepare the PBMCs for the assay and the assay is initiated by adding the PBMCs and antigens to the assay plate and placing the plate into the incubator. On day 2, the plate is removed from the incubator and the development steps are performed and the plate is read. The time to process a full plate is approximately 3 hours of elapsed time (actual hands-on labor time will be less due to the centrifugation steps) on day 1 and 30 minutes of labor time (not including the 1 hour incubation of the secondary

antibody and time for plate drying) on day 2. The procedure for conducting the test is summarised in Figure 2 and further described below:

***Figure 2**: Diagram illustrating the main steps required to perform the T-SPOT.COVID* assay. *Note that not all 96 wells of the plates are shown in the illustration.*



REAGENT PREPARATION

1. The vials of SARS-CoV-2 Spike antigens (Panel A), SARS-CoV-2 Nucleocapsid antigens (Panel B) and the Positive Control are supplied ready to use.
2. Prepare a 1:200 dilution working Conjugate Reagent solution. Calculate the volume of working Conjugate Reagent solution required. Conjugate Reagent can be made to working strength and stored at 2-8 °C up to six weeks before using in the assay.

   Note: Each sample uses 4 wells. 50 µL diluted Conjugate Reagent will be added to each well. Thus, for one strip (2 samples, 8 wells), prepare 500 µL of working strength solution by adding 2.5 µL of concentrated Conjugate Reagent to 497.5 µL PBS. For one 96-well plate (24 samples), prepare 5 mL of working strength solution by adding 25 µL of concentrated Conjugate Reagent to 497 5µL PBS.

3. The Substrate Solution is supplied ready to use. Prior to removing the plate from the incubator (day 2) remove the substrate solution from storage and allow to equilibrate to room temperature.

CELL COUNTING AND DILUTION

The T-SPOT.*COVID* assay requires 250,000 ± 50,000 PBMCs per well. A total of four wells are required for each sample; thus 1 x 10^6 PBMCs are required per samplet. The number of SARS-CoV-2 T cells in the specimen is normalised to a fixed number of PBMCs.

1. Perform a PBMC count. Cells can be counted by a variety of methods, including manual counting using Trypan Blue (or other appropriate stain) and a hemocytometer, or using an automated hematology analyser.
2. Briefly, for manual counting with a Neubauer hemocytometer using Trypan Blue, add 10 µL of the final cell suspension to 40 µL 0.4 % (w/v) Trypan Blue solution. Place an appropriate aliquot onto the hemocytometer and count the cells in the grid. For other types of hemocytometer and for automated devices, follow the manufacturers' instructions.

   Note: Care should be taken to ensure that the cell suspension is well mixed immediately prior to removal of aliquots for counting. Cells can settle towards the bottom of the tube leading to a misinterpretation of the true cell number. Mixing should be done by either gentle swirling of the tube by hand, or by gently agitating the suspension by pipetting the suspension up and down several times.
3. Calculate the concentration of PBMCs present in the stock cell suspension.

   Note: Ensure the calculation is correct for the cell counting system used as the use of either insufficient or excess cells may lead to an incorrect interpretation of the result.
4. Prepare 500 µL of the final cell suspension at a concentration of 2.5 x 10^5 cells/100 µL (giving 1.25 x 10^6 PBMCs in total).

Note: Ensure cells are thoroughly mixed, by gently agitating the suspension by pipetting the suspension up and down several times, before removing an aliquot for dilution. PBMC numbers between 200,000 and 300,000 per well have been shown to give consistent T-SPOT.COVID assay results.

PLATE SET UP AND INCUBATION

The T-SPOT.*COVID* assay requires four wells to be used for each sample. A Nil Control and a Positive Control should be run with each individual sample. It is recommended that the samples be arranged vertically on the plate as illustrated below.

- Nil Control
- Panel A (COV-A) (Spike)
- Panel B (COV-B) (Nucleocapsid)
- Positive

Each 96-well plate can process up to 24 samples. Use the numbers of plates required for the numbers of samples that you wish to process. Each strip will process 2 samples. Use only the numbers of strips that you require. Seal remaining strips in the foil pouch along with the silica gel pouch. The remaining strips must be used within eight weeks of first opening the pouch provided the strips are stored at 2-8 °C during the period.

T-SPOT.*COVID* is an assay that measures T cell function; no standard curves are required. Therefore the assay will only require 4 wells to be used for each sample. The recommended plate layout for 24 samples is shown below:

Row	1	2	3	4	5	6	7	8	9	10	11	12
A	1N	3N	5N	7N	9N	11N	13N	15N	17N	19N	21N	23N
B	1A	3A	5A	7A	9A	11A	13A	15A	17A	19A	21A	23A
C	1B	3B	5B	7B	9B	11B	13B	15B	17B	19B	21B	23B
D	1M	3M	5M	7M	9M	11M	13M	15M	17M	19M	21M	23M
E	2N	4N	6N	8N	10N	12N	14N	16N	18N	20N	22N	24N
F	2A	4A	6A	8A	10A	12A	14A	16A	18A	20A	22A	24A
G	2B	4B	6B	8B	10B	12B	14B	16B	18B	20B	22B	24B
H	2M	4M	6M	8M	10M	12M	14M	16M	18M	20M	22M	24M

Key: N=Nil control, A=Panel A, B=Panel B, M=Mitogen Positive Control

1. For COV.435/300 remove the required, pre-coated 8-well strips from the packaging, clip into a plate frame and allow to equilibrate to room temperature. Remove the required number of strips only, reseal any remaining unused strips and the desiccant pouch in the outer foil packaging and return to storage at 2-8 ºC.

   Note: Clip the strips to be used into an empty plate frame fitted with an undercover and lid. Frames, covers and lids should be retained and reused.

2. Add in the Panels and the Controls;

   i. Add 50 µL AIM-V cell culture medium to each Nil Control well.
   ii. Add 50 µL Panel A solution to each well required.
   iii. Add 50 µL Panel B solution to each well required.
   iv. Add 50 µL Positive Control solution to each cell functionality control well.

   Do not allow the pipette tip to touch the membrane. Indentations in the membrane caused by pipette tips may cause artifacts in the wells.

3. To each of the 4 wells to be used for a sample, add 100 µL of the final cell suspension (containing 250,000 PBMCs). Use a new tip for the addition of each individual sample's cells to avoid cross-contamination between wells. Take care not to contaminate adjacent wells, by passing liquid from one well to another if pipette tips are reused for multiple wells.

   Note: Ensure mixing (as in the Cell Counting & Dilution steps) before removal of each 100 µL aliquot.

4. Incubate the plate with the lid on in a humidified incubator at 37 °C with 5 % CO_2 for 16-20 hours. Avoid disturbing the plate once in the incubator. Do not stack plates as this may lead to uneven temperature distribution and ventilation.

   Note: The CO_2 incubator must be humidified. Check the water dish has sufficient water to ensure a humid atmosphere is achieved.

SPOT DEVELOPMENT AND COUNTING

1. Remove the plate from the incubator and discard the cell culture medium by flicking the contents into an appropriate container.

   Note: At this time remove the Substrate Solution from the kit and allow to equilibrate to room temperature.

2. Add 200 µL PBS solution to each well. **Do not use PBS containing Tween® or other detergents, as this causes high background counts**.

   Note: Use freshly prepared or sterile PBS.

3. Discard the PBS solution. Repeat the well washing an additional 3 times with fresh PBS solution for each wash. An automated washer can be used for the washing steps.

   Note: For washing, a multi-channel pipette or a plate washer may be used. Discard PBS into a suitable container after each wash. Do not use pipettes to remove the PBS as this risks damaging the membrane. If using a plate washer, ensure the manifold is adjusted so that the tips do not touch the membrane. After the final wash, tap the plate on lint-free towel to ensure all PBS is removed – any excess left will further dilute the Conjugate Reagent.

4. If not already prepared during the reagent preparation step; dilute concentrated Conjugate Reagent 200x in PBS to create the working strength solution.

5. Add 50 µL working strength Conjugate Reagent solution to each well and incubate at 2-8 °C for 1 hour.

   Note: Use of a multi-channel pipette or stepper pipette is recommended. Care should be taken to ensure that the Conjugate Reagent is added to every well as the solution is clear and uncolored - therefore, it may be difficult to see into which wells it has been added.

6. Discard the conjugate and perform the four PBS washes as described in steps 2. and 3. above.

7. Add 50 µL Substrate Solution to each well and incubate at room temperature for 7 minutes.

8. Wash the plate thoroughly with distilled or deionised water to stop the detection reaction.

9. Allow the plate to dry by standing the plate in a well ventilated area or in an oven at up to 37 °C.

   Note: Spots become more visible as the plate dries; therefore ensure that the plate is thoroughly dry before reading. Allow 4 hours drying time at 37 °C or at least 16 hours at room temperature.

10. Count and record the number of distinct, dark blue spots on the membrane of each well. Apply the Results Interpretation and Assay Criteria (see below) to determine whether a sample is 'Reactive' or 'Non-Reactive'. **The spots produced as a result of antigen-stimulation should appear as large, round and dark spots. Often a gradient effect can be observed with a darker center and a more diffuse periphery. Non-specific artifacts that can occur are smaller, less intense and irregular in shape.**

    Note: Spots can be counted directly from the well using a magnifying glass or stereomicroscope or from a digital image captured from a microscope, or plate imager.

    Once developed, the completed assay plates remain stable and they do not therefore need to be read immediately. The plates may be archived for retrospective quality control or re-examination for up to 12 months if kept in a dry, dark environment at room temperature.

QUALITY CONTROL

A typical result would be expected to have few or no spots in the Nil Control and 20 or more spots in the Positive Control.

A Nil Control spot count in excess of 10 spots should be considered as 'Invalid'.

Typically, the cell functionality Positive Control spot count should be ≥ 20 or show saturation (too many spots to count). A small proportion of samples may have T cells which show only a limited response to PHA. Where the Positive Control spot count is < 20 spots, it should be considered as 'Invalid', unless either Panel A or Panel B are 'reactive or 'Borderline (equivocal)' as described in the Results Interpretation and Assay Criteria (see below), in which case the result is valid.

RESULTS INTERPRETATION AND ASSAY CRITERIA

Refer to the Quality Control section before applying the following criteria.

Results for the T-SPOT.*COVID* assay are interpreted by subtracting the spot count in the Nil control well from the spot count in each of the Panels, according to the following algorithm:

- The test result is Reactive if (Panel A-Nil) and/or (Panel B-Nil) ≥ 8 spots.
- The test result is Non-Reactive if both (Panel A-Nil) and (Panel B-Nil) ≤ 4 spots. This includes values less than zero.
- Results where the highest of the Panel A or Panel B spot count is such that the (Panel minus Nil) spot count is 5, 6 or 7 spots should be considered Borderline (equivocal).

- **A 'Reactive' result indicates that the sample contains effector T cells sensitised to SARS-CoV-2.**
- **A 'Non-Reactive' result indicates that no effector T cells sensitised to SARS-CoV-2 were detected.**

The interpretation algorithm is described in the following Flow Diagram (Figure 3) and Tables 1-3. This algorithm also includes quality control criteria.

Figure 3 *– Algorithm Flow Diagram*

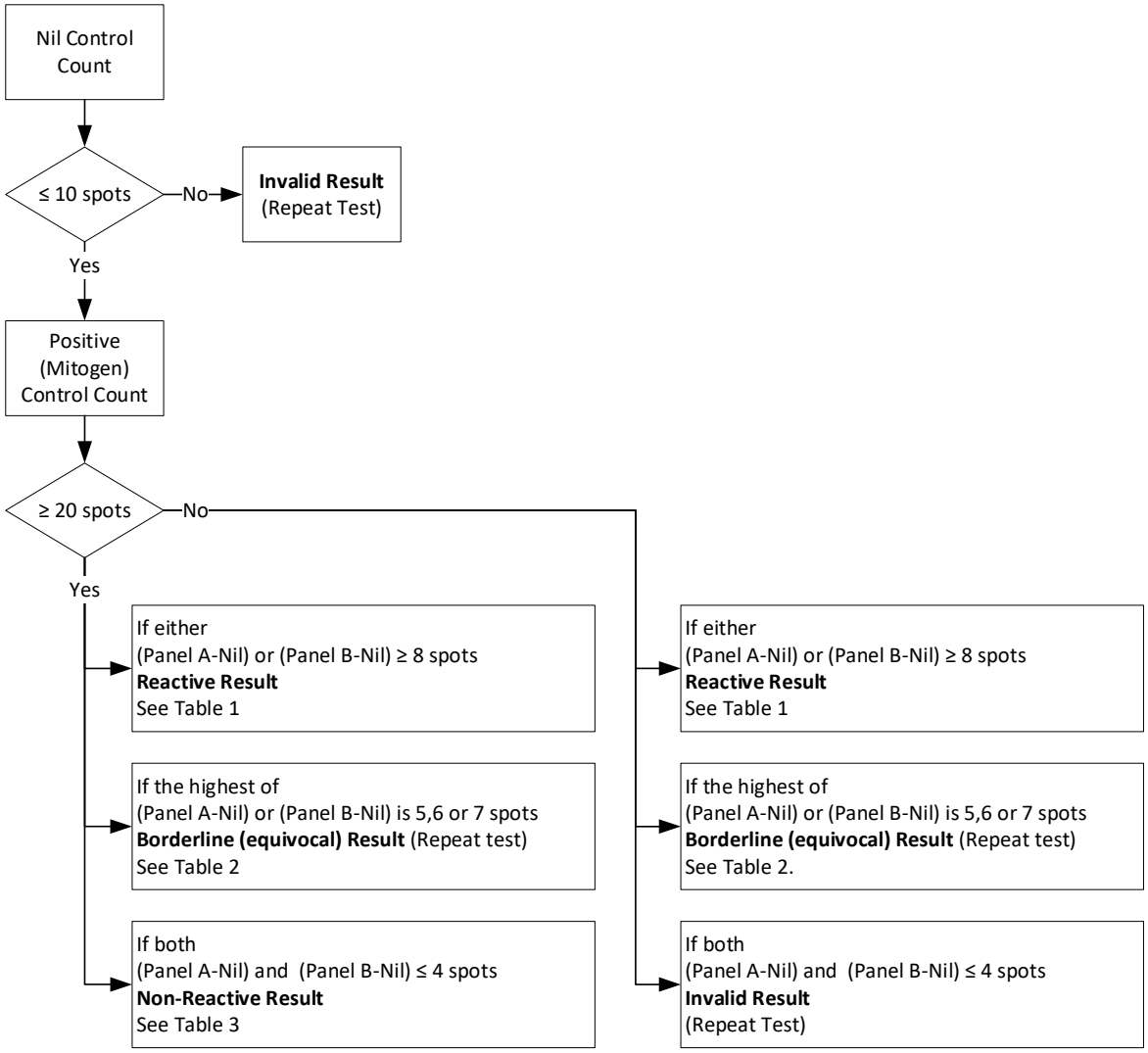


Table 1: *Reactive Interpretation: Either (Panel A minus Nil) or (Panel B minus Nil) ≥8 spots*

Nil Control Well Count	Either Panel A **or** Panel B has the following number of spots†	Result Interpretation
0	≥8	**Reactive**
1	≥9	**Reactive**
2	≥10	**Reactive**
3	≥11	**Reactive**
4	≥12	**Reactive**
5	≥13	**Reactive**
6	≥14	**Reactive**
7	≥15	**Reactive**
8	≥16	**Reactive**
9	≥17	**Reactive**
10	≥18	**Reactive**
>10 spots	n/a	**Invalid**

†*Note: The highest Panel-Nil spot count is to be used to determine the test outcome.*

Table 2: *Borderline (equivocal) Interpretation: The highest of (Panel A minus Nil) or (Panel B minus Nil) is 5, 6 or 7 spots*

Nil Control Well Count	The highest of Panel A **or** Panel B has the following number of spots	Result Interpretation
0	5, 6, or 7	**Borderline (equivocal)***
1	6, 7, or 8	**Borderline (equivocal)***
2	7, 8, or 9	**Borderline (equivocal)***
3	8, 9, or 10	**Borderline (equivocal)***
4	9, 10, or 11	**Borderline (equivocal)***
5	10, 11, or 12	**Borderline (equivocal)***
6	11, 12, or 13	**Borderline (equivocal)***
7	12, 13, or 14	**Borderline (equivocal)***
8	13, 14, or 15	**Borderline (equivocal)***
9	14, 15, or 16	**Borderline (equivocal)***
10	15, 16, or 17	**Borderline (equivocal)***
>10 spots	n/a	**Invalid**

Table 3: *Negative Interpretation: Both (Panel A minus Nil) and (Panel B minus Nil) ≤4 spots*

Nil Control Well Count	Both Panel A and Panel B has the following number of spots	Result Interpretation
0	≤4	**Non-Reactive**
1	≤5	**Non-Reactive**
2	≤6	**Non-Reactive**
3	≤7	**Non-Reactive**
4	≤8	**Non-Reactive**
5	≤9	**Non-Reactive**
6	≤10	**Non-Reactive**
7	≤11	**Non-Reactive**
8	≤12	**Non-Reactive**
9	≤13	**Non-Reactive**
10	≤14	**Non-Reactive**
>10 spots	n/a	**Invalid**

** Results where the highest of the Panel A or Panel B spot count is such that the (Panel minus Nil) spot count is 5,6 or 7 spots should be considered Borderline (equivocal)*

7. ABBREVIATIONS & GLOSSARY OF SYMBOLS

Abbreviations

BCIP/NBT	5-bromo, 4-chloro, 3-indoylphosphate/nitroblue tetrazolium
CPT	Cell Preparation Tubes
EDTA	Ethylenediamine tetraacetic acid
ELISA	Enzyme-Linked Immunosorbent Assay
ELISPOT	Enzyme-Linked Immnunospot Assay
IFN-γ	Interferon-gamma
IL	Interleukin
PBMC	Peripheral Blood Mononuclear Cells
PBS	Phosphate Buffered Saline
PHA	Phytohemagglutinin
RCF	Relative Centrifugal Force
RPM	Revolutions per minute
TNF	Tumor Necrosis Factor

Glossary of Symbols

- Use by/Expiration date (Year-Month-Day)
- LOT Lot number
- REF Catalogue number
- Attention, see instructions for use
- Date of manufacture
- Manufacturer
- Temperature limitation/Store between
- Consult instructions for use

8. REFERENCES

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The use of the T-Cell *Xtend* reagent is protected by the following patents and patents pending:
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