PreVent-ACaLL

Laboratory Manual

For local central laboratories incubating TruCulture

Version 3.0

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1 Introduction

The purpose of this laboratory manual is to outline the procedures that will be undertaken at the central laboratories to handle, store and transport TruCulture samples for the PreVent-ACaLL trial.

This Laboratory Manual must be used alongside the current version of the PreVent-ACaLL trial protocol. This document ONLY covers the aspects of the protocol related to handling, storing and transporting of exclusively the TruCulture sample. The manual will be updated throughout the course of the trial if amendments become necessary.

The PreVent-ACaLL study collects biological material from CLL patients with increased risk of infections. Participants have consented to have biological material collected. Peripheral blood for TruCulture will be collected at baseline, 12 weeks after C1D1, 24 weeks after C1D1 and in case participant develops disease progression.

2 Participant Privacy Protection

The blood collection tubes will be labeled with the participant's study identification number (PID), thus no personal identification will be noted at the sample material when arriving to the laboratory.

3 Protect yourself

Follow all your institution's safety guidelines when collecting, processing or handling biological samples, including the use of personal protective equipment (PPE) gown or laboratory coat and gloves. The institution is responsible for providing all PPE.

4 Equipment and materials

4.1 Equipment needed by the central lab

- Access to an internet linked computer
- Barcode scanner
- Freezing capacity at -20 °C and -80 °C
- Manual Finn pipette (100-1000 µL)
- Finn pipette tips (1000 µL)
- Single-use pipettes
- Digital heat block incl. block
- Heatblock thermostat
- Vortex mixerTrizol Reagent 100 ml

4.2 Equipment provided by sponsor

The PreVent-ACaLL coordination center CHIP will assemble and ship study material to the central labs. First shipment will contain storage boxes for the TruCulture stimuli tubes and matrix tubes as well as matrix tubes, box labels, stimuli labels and Specimen & Shipping Logs (see next page). Future kits will be provided upon notification from your local central lab. Please notify sponsor in good time:

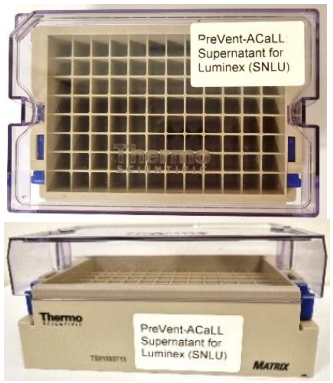
chip-prevent.rigshospitalet@regionh.dk

Equipment provided by sponsor

Components	Quantity per kit
TruCulture (TC) kit:	6
TC stimuli tubes	30 (5 per TC kit)
Seraplas (stick + membrane)	30 (5 per TC kit)
Matrix tubes	96 (one box)
Stimuli labels	10 per stimulus
Storage boxes:	-
Cryo box for TC tubes containing cell pellets	2
Matrix box for matrix tubes containing supernatant	3
Other:	-
Matrix work box	1
Specimen & Shipping Logs for central lab	6

Pre-labeled Matrix work box

Pre-labeled Matrix box:
Supernatant for Biobank



Pre-labeled Matrix box:
Supernatant for Luminex

Pre-labeled Cryo box:
Cell pellets for Biobank (CPBB)

Stimuli labels for
Luminex matrix tubes

Matrix tubes
(96 per matrix box)

TruCulture (TC) stimuli tubes

Seraplas
stick

Seraplas
membrane

5 TruCulture sample

The TruCulture sample is peripheral blood collected in a green 9 ml Li-Hep VATUETTE tube. The blood sample will be collected at site at baseline, 12 weeks after C1D1, 24 weeks after C1D1 and in case of disease progression. TruCulture samples must be delivered to the local central lab:

within 1 hour from sampling time in **Denmark**

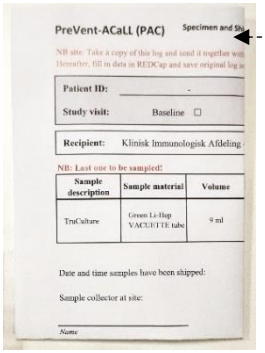
within 2 hours from sampling time in **Holland**

within 3 hours from sampling time in **Sweden**

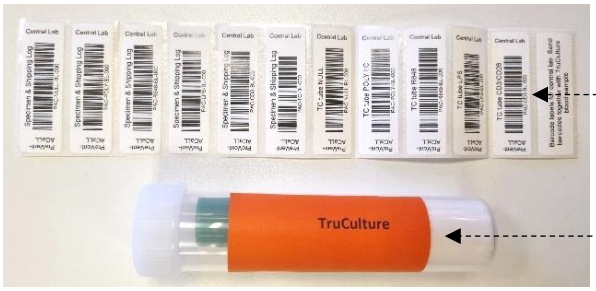
Site must notify the local central lab must when date of study visit is known. Below you can see all components received from site when the TruCulture sample is delivered at the lab.

TruCulture sample from site

Components	Quantity per kit
Blood sample	1
TruCulture transport tube	1
Barcode labels for central lab	1



Specimen & Shipping Log



Barcode labels

VACUETTE tube inside transport tube

5.1 Central lab barcode labels

A total of 11 barcode labels to use at central lab will be shipped together with the TruCulture blood sample from site. Five of these barcode labels must be placed on the TruCulture stimuli tubes (CD3/CD28, LPS, R848, POLY I:C and NULL) from the TruCulture kit. Rest of the barcode labels must be placed on the matching field at the Specimen & Shipping Log for central lab (Appendix 8.1). Explanations of the barcode label items can be found on the figure below (Figure 5-1).

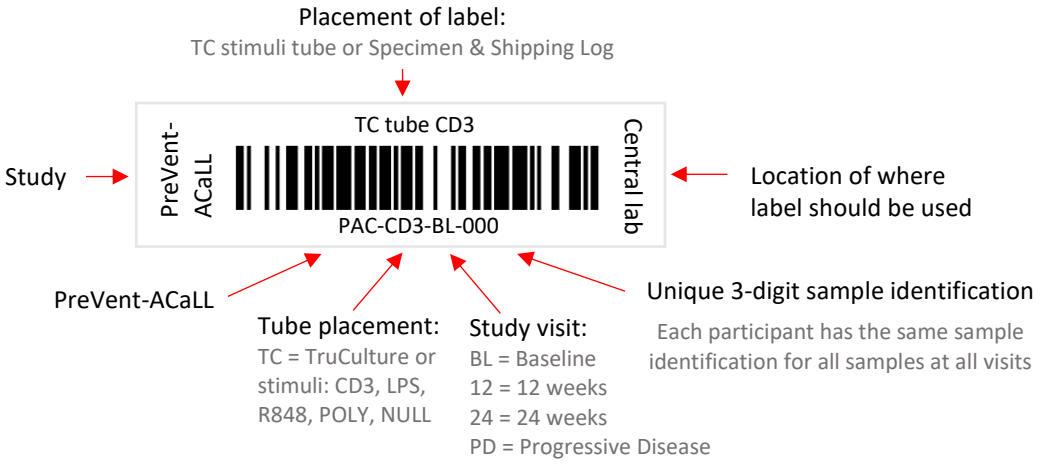


Figure 5-1: Explanation of barcode label items.

6 Instruction: TruCulture incubation, harvest and storage

This instruction describes the preparation, reception and incubation of the TruCulture blood sample as well as harvest and freezing of supernatant after the incubation. The subsequent TruCulture analysis (Luminex) will be performed at Rigshospitalet in Denmark after shipment.

TruCulture is a commercial developed and validated analysis of in vitro stimulation of whole blood in standardized tubes (TruCulture stimuli tubes) with the purpose of assessing the participant's induced immune response. The TruCulture (TC) stimuli tubes contain a standardized amount of different immunological stimuli to assess the induced immune response on five different stimuli: CD3/CD28, LPS, R848, Poly I:C and NULL (negative control).

The TruCulture stimuli tubes are shipped from sponsor to site. Each shipment will contain 6 TruCulture sets. Each TruCulture set consists of five different stimuli tubes of the above-mentioned stimuli. The tubes are shipped at -20˚C and must be stored at site at the same temperature (-20˚C).

IMPORTANT: Use gloves during the whole process.

Preparation before reception of the TruCulture blood sample

1. Switch on the heating block.
2. Thaw the appropriate number of TruCulture® stimuli tubes, either at room temperature for 1 hour or overnight at 4°C.

Do not leave the TC tubes at room temperature for longer than required to thaw them. To accelerate thawing, the tubes can be incubated at 37 °C. If the TC tubes are thaw in the refrigerator they should be taken out and left at room temperature 20 minutes before use.

3. After thawing, gently homogenize the TC tubes by inverting and remove the medium remaining in the lid by gently tapping the TC tube on the lab bench (cap on top).
4. Label each TC tube with barcode labels received from the site (the label should not cover the stimulus number for future controls).
5. Arrange the TC tubes in the rack in following stimuli order: CD3/CD28, LPS, R848, POLY I:C and NULL.
6. Leave the rack under the hood, loosening the caps from the TruCulture tubes without unscrewing.

Reception of the TruCulture blood sample

1. Staff member at central lab receives the blood sample (green 9 ml Li-Hep VATUETTE tube) from site together with a Specimen & Shipping Log stating PID and the sampling date and time.

Remember to fill out a Specimen & Shipping Log for central lab along the way. Appendix 8.1 shows how a completed Specimen & Shipping Log for central lab must be filled out.

Incubation of blood sample (whole blood)

7. Exactly **1 hour (DK)** / **2 hours (NL)** / **3 hours (SE)** after blood sampling time (± 10 min): Invert the Li-Hep blood sample 4-5 times. Aspirate and transfer exactly 1 ml Li-Hep blood from the VACUETTE tube to each of the five TC stimuli tubes with a pipette. The pipette must not touch the inside of the TC tubes. Note the exact time-point for blood transfer.

8. Recap, gently mix by inverting each TC tube 3 times (Figure 6-1, picture 1), then lightly tap the tube on the lab bench in such as a way that no drops of blood remain in the cap.
9. Place the TC tubes in the incubator (Figure 6-1, picture 2) in the defined order (CD3/CD28, LPS, R848, Poly I:C and NULL) with the lid upwards. Start the timer with an incubation time at 22 hours (± 30 minutes) at 37°C.

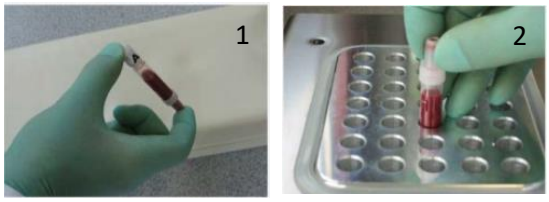


Figure 6-1: Mix by inverting each TC tube gently (1). Place TC tubes in the incubator (block) (2).

Data must be reported in REDCap and on the Specimen & Shipping Log for central lab:

10. Open REDCap and log-on your personal account. For further details, please see the REDCap Guide.
11. Open and fill out the eCRF "TC blood sample reception" and first part of the eCRF "TruCulture stimuli tubes" in REDCap. Second part "Storage of TC stimuli tubes" must be filled out when tubes are being stored the next day (point 34).

    IMPORTANT: The barcodes should be scanned into REDCap and not entered manually!
12. Fill out Specimen & Shipping Log for central Lab with following information:
    a. Site number, participant ID, study visit
    b. Sample recipient date and time
    c. Date and time for initiation of TruCulture incubation
    d. Place barcode labels in correct fields

Harvest and storage of supernatant after incubation

Preparation of matrix tubes

13. Place stimuli labels on five empty matrix tubes (Figure 6-2).
14. Place the five labeled matrix tubes in the matrix work box – in the Luminex row (Figure 6-2).
15. Place additionally 10 matrix tubes in the biobank row of the work box – these matrix tubes are not labeled with stimuli labels.

Figure 6-2: Matrix tubes labeled with stimuli labels and placed in the matrix work box (supernatant for Luminex).

Harvest of supernatant

IMPORTANT: keep the TC tubes in the vertical position during following process.

16. At the end of the 22 hours incubation (± 15min), retrieve the TC tubes and place them on a rack. Check for the absence of hemolysis in the TC tubes, indicate it a comment in REDCap if hemolyzed.

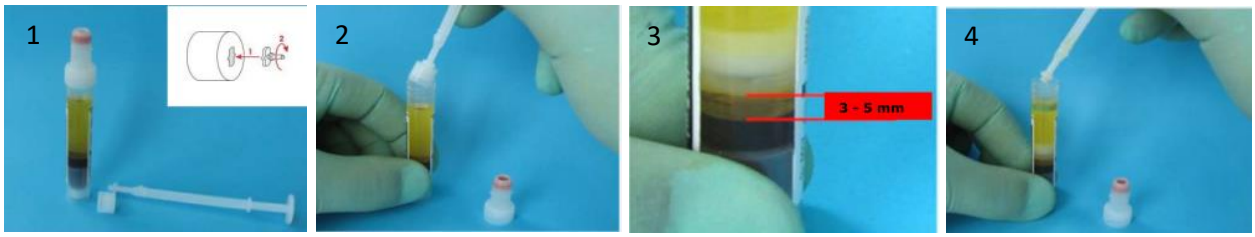


Figure 6-3: Harvest of supernatant from TruCulture stimuli tubes. 1: Connect stalk to Seraplas membrane. 2: Insert Seraplas membrane. 3: The Seraplas membrane should be placed up to base limit. 4: The Seraplas membrane is removed from the TruCulture stimuli tube after harvest.

17. Connect the stalks to the Seraplas membrane (Figure 6-3, picture 1): insert the stalks with their little "lip" in the slit in the separator and lock them (on clockwise turn).
18. Unscrew the caps of the TC tubes and gently insert the Seraplas up to the base limit (1 mm) (Figure 6-3, picture 2 and 3).
19. Unlock the Seraplus stalks and remove it. Put each TC tube back in its place in the rack.
20. Harvest in total 1200 µl of the supernatant from each TC stimuli tube into three matrix tubes placed in the matrix work box (Figure 6-2): Two matrix tubes for biobank and one matrix tube for Luminex (400 µL supernatant per tube).
21. Harvest remaining plasma left in the TC stimuli tubes and discard it with the pipette tips.

Freezing of matrix tubes containing supernatant

22. Open REDCap and log-on your personal account. For further details, please see the REDCap Guide.

Matrix tubes for Luminex:

23. Scan barcodes from the matrix tubes into the eCRF “Matrix tubes for Luminex” and note freezing date and time. Create a repeating instrument for each matrix tube in REDCap. Date and time of harvest and freezing will automatically be filled out if these fields are filled out in the first instrument.
24. Place the tubes in a temporary matrix box to freeze samples at -20 °C. Place matrix box in the freezer.

    Remember to also fill out the Specimen & Shipping Log (example in Figure 8-1, Appendix 8.1).
25. The day after harvest: Relocate the matrix tubes to the matrix box labeled “Supernatant for Luminex” and store the box in a -80°C freezer. Register each tube placement in the eCRF “Matrix tubes for Luminex”.

    The tubes should be placed in the “Supernatant for Luminex” matrix box as indicated in next page (Figure 6-4) – each color represents the tubes arrived per TruCulture blood sample incubated:

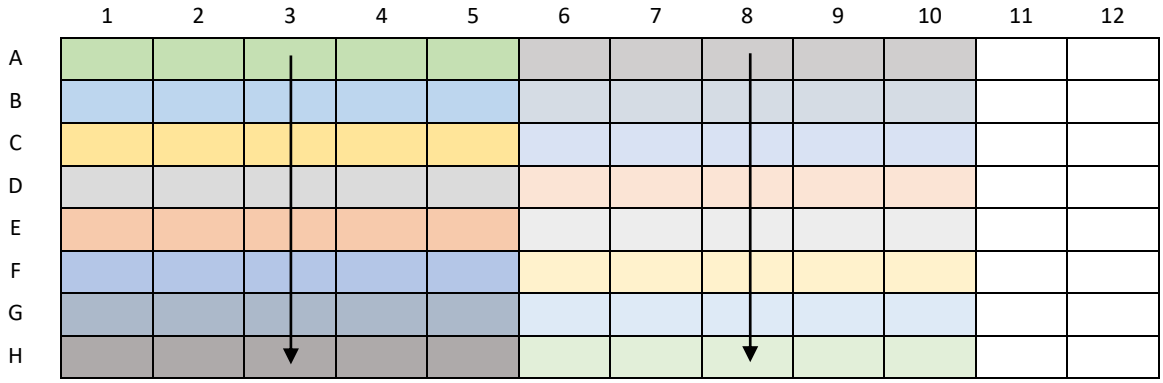


Figure 6-4: How to place matrix tubes in the matrix box labeled "Supernatant for Luminex".
Each color represents matrix tubes arrived per TruCulture blood sample (5 matrix tubes for Luminex).
No color signifies that no tubes should be placed here (row 11 and 12).

Matrix tubes for biobank:

26. Scan barcodes from the matrix tubes into the eCRF "Matrix tubes for Biobank" and note freezing date and time. Create a repeating instrument for each matrix tube in REDCap. Date and time of harvest and freezing will automatically be filled out if these fields are filled out in the first instrument.
27. Place the tubes in a temporary matrix box to freeze samples at -20 °C. Place matrix box in the freezer.

    Remember to also fill out the Specimen & Shipping Log (example in Figure 8-1, Appendix 8.1).
28. The day after harvest: Relocate the matrix tubes to the matrix box labeled "Supernatant for Biobank" and store the box in a -80°C freezer. Register each tube placement in the eCRF "Matrix tubes for Biobank".

    The tubes should be placed in the "Supernatant for Biobank" matrix box as indicated below (Figure 6-5) – each color represents the tubes arrived per TruCulture blood sample incubated:

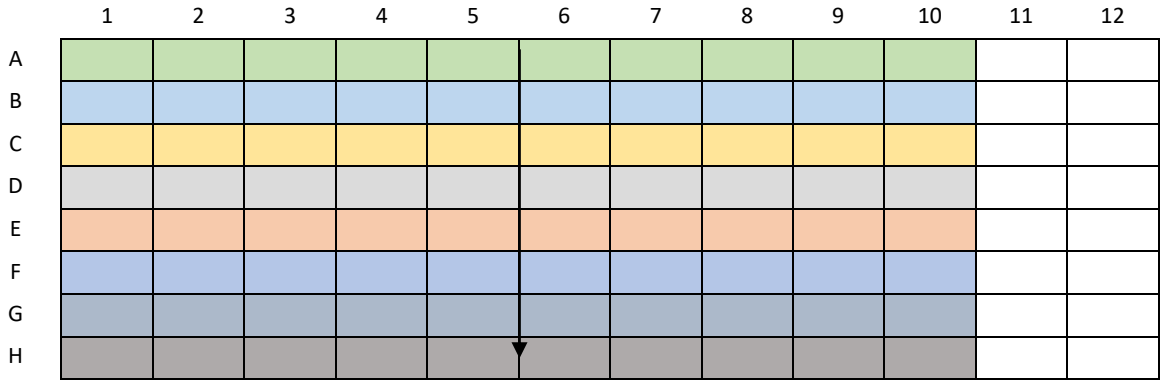


Figure 6-5: How to place matrix tubes in the matrix box labeled "Supernatant for Biobank".
Each color represents matrix tubes arrived per TruCulture blood sample (10 matrix tubes for biobank).
No color signifies that no tubes should be placed here (row 11 and 12).

Freezing of TruCulture stimuli tubes containing cell pellets

29. For each TC tube: Reinsert the stalks with their little "lip" into the slit of the separator and lock them (one clockwise turn) then pull the Serplas membranes from the TC tubes (Figure 6-3, picture 4). Discard the Seraplus with the stalk.
30. Lightly tap the tube to unstick the base.
31. Add 2 ml of Trizol and carefully recap the TC tubes.
32. Vortex TC tubes for 2 min at maximum speed.
33. Let the TC tubes rest at room temperature for 10 min, 20 minutes maximum if required (start timer).
34. Open REDCap and log-on your personal account. For further details, please see the REDCap Guide.
35. Report freezing date and time in the “Storage of TC stimuli tubes” part in the eCRF “TC stimuli tubes” for all five TC tubes.
36. Place the tubes in a temporary cryo box to freeze samples at -20 °C. Place cryo box in the freezer.

    Remember to also fill out the Specimen & Shipping Log (example in Figure 8-1, Appendix 8.1).
37. The day after harvest: Move the TC stimuli tubes to the cryo box labeled “Cell pellets for Biobank (CPBB)” and store the box in a -80°C freezer. Register each tube placement in the eCRF “TC stimuli tubes” part “Storage of TC stimuli tubes”.

    Remember to also fill out the Specimen & Shipping Log (example in Figure 8-1).

    The tubes should be placed as indicated below (Figure 6-6) – each color represents the tubes arrived per TruCulture blood sample incubated:

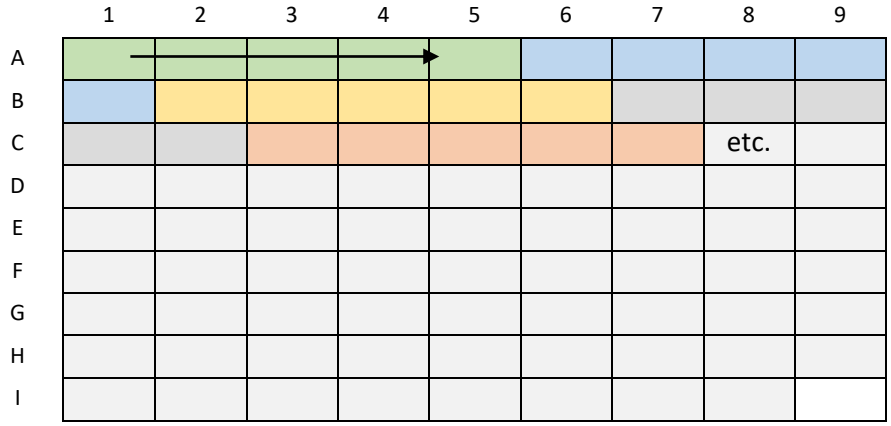


Figure 6-6: How to place TC stimuli tubes in the cryo box labeled “Cell pellets for Biobank”. Each color represents TC stimuli tubes arrived per TruCulture blood sample (5 stimuli tubes for biobank). Tubes should be placed right after each other. No color signifies that no tubes should be placed here.

7 Shipment of tubes to Rigshospitalet (Denmark)

If possible, all sample tubes must be stored at central lab until end of study. If lack of space, sample tubes can be shipped to Rigshospitalet when needed. Please contact CHIP if you wish to have sample tubes shipped to Denmark before end of study: chip-prevent.rigshospitalet@regionh.dk.

IMPORTANT: When boxes are shipped to Rigshospitalet in Denmark, the eCRF "Shipment of tubes to Rigshospitalet, Denmark" must be filled out.

The sample tubes are shipped with a transport company chosen by sponsor. Shipment must be coordinated with CHIP (use email above) before coordinated with courier.

The transport company will provide transport material prior to pick-up. A copy of each patient Specimen & Shipping Log must be included in the shipment.

8 Appendix

8.1 Specimen & Shipping Log for central lab: Example of completion

For each sample, a Specimen & Shipping log must be filled out at central lab. Figure 8-1 shows an example of a completed Specimen & Shipping Log.

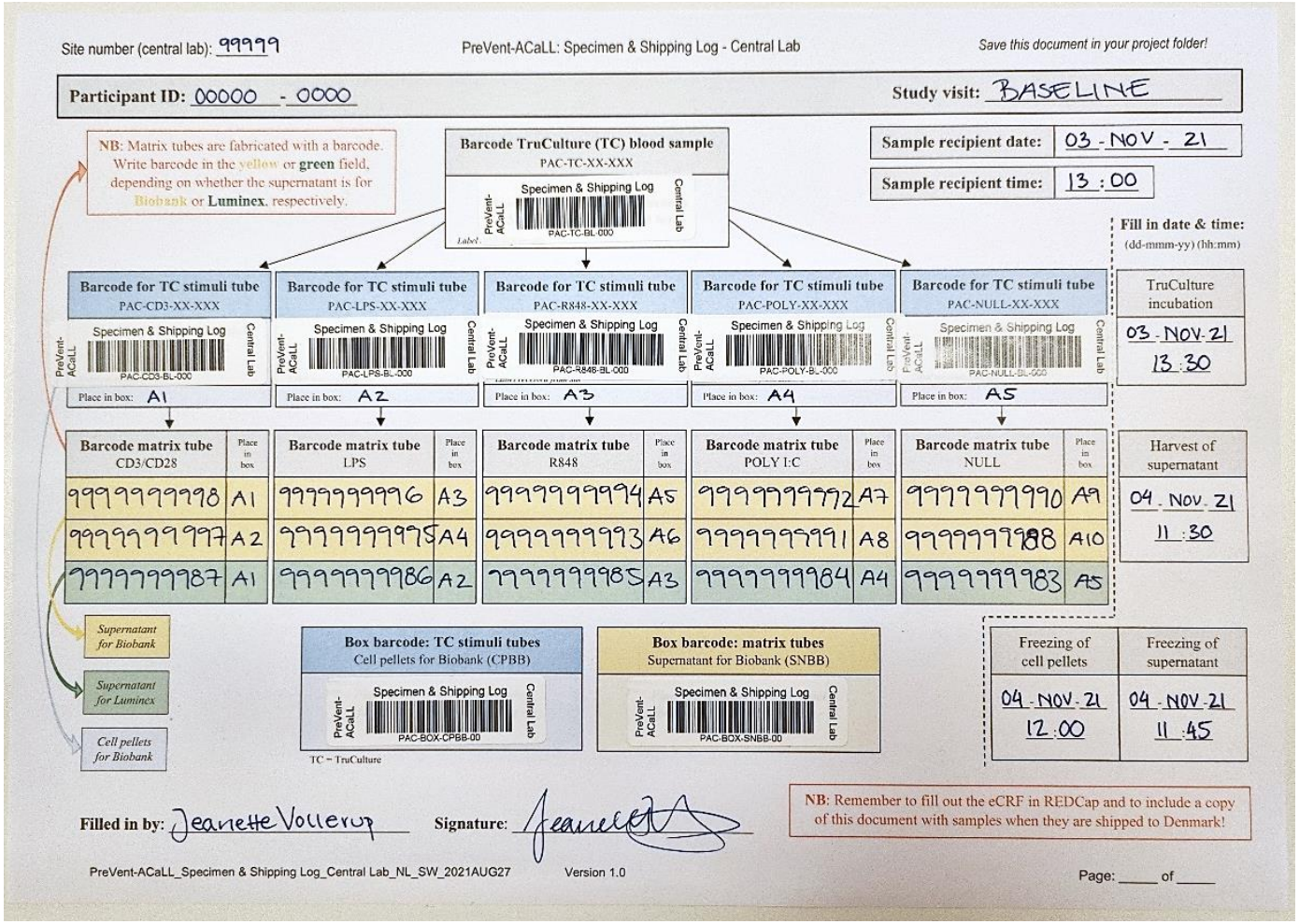


Figure 8-1: Specimen & Shipping Log for Central Lab. Example with data reported.