Protocol Number 175151

UK DEVICE MANUAL:

Effective Date: 19 December 2025

Randox Evidence MultiSTAT

Prepared by: **Name:**	**Title:**	**Signature & Date:**
Michael Hetherington	Clinical Trial Monitor	Digitally signed by Michael Hetherington Date: 2025.12.19 11:45:06 Z
Approved by: **Name:**	**Title:**	**Signature & Date:**
Janis Best-Lane	International Trial Manager	Digitally signed by Janis Best-Lane Date: 2025.12.19 11:53:05 Z

This document explains the different functionalities of the Randox Evidence MultiSTAT Device. This document will guide you through all the options from start to finish to ensure the optimum use of the device. Please read this manual carefully before using the device for the first time, and as a reference thereafter as needed.

ABBREVIATIONS

IL-6	Interleukin 6
sTNFR1	soluble Tumour Necrosis Factor Receptor 1
IFU	Instructions for Use
RB	Reconstitution Buffer
QC	Quality Control

TABLE OF CONTENTS

1. CONTACT DETAILS

Technical Assistance
Contact Randox Laboratories Technical Services, Northern Ireland, telephone +44 (0) 28 9445 1070 or email technical.services@randox.com

2. EXPLANATION OF APPLICABLE SYMBOLS

Randox Laboratories uses the following symbols which may appear on the product labelling:

Symbol	Description
REF	Catalogue number
LOT	Batch code
CONTROL	Control
	Use-by date
	Manufacturer
EC REP	Authorized representative in the European Community
	Consult IFU
IVD	*In vitro* diagnostic medical device
CE	CE Marking
CE XXXX	CE Marking with Notified Body

Symbol	Description
RxOnly	Prescription device (US only)
	Temperature limit
	Do not re-use
YY/MM/DD	Date format (year/month/day)
UK CA	UK CA mark
	Keep away from sunlight
	Biological risks
	This way up
	Caution
	Do not use if package is damaged and consult instructions for use

3. MULTISTAT DEVICE SPECIFICATIONS

Dimensions	585 (H) x 535 (D) x 570 (W) mm
Weight	48kg (106lbs)
Sample Volume	250µl (0.25mL)
Time to Result	36mins

4. RECEIPT OF THE DEVICE/KITS

Randox and the PANTHER study team will arrange for the order and delivery of your MultiSTAT device and separate kits:- an InflamiSTRAT kit and an accessory kit. Details of each kit is listed below:-

The **InflamiSTRAT kit** contains the following:-

12 x InflamiSTRAT Assay Test Cartridge

4 x 1mL InflamiSTRAT Adjuster 1

4 x 1mL InflamiSTRAT Adjuster 2

4 x 1mL InflamiSTRAT Control 1

4 x 1mL InflamiSTRAT Control 2

2 x 10ml Reconstitution buffer

1 x Barcode sheet

1 x Instructions For Use (IFU)

The **Accessory Kit** contains the following:-

12 x Tip Cartridges

A scanner is required (and will be delivered as part of the MultiSTAT device), pipettes/syringes are also required and will be either supplied by the site or the PANTHER study team.

The PANTHER team will arrange for the order and delivery of the separate kits. The entire InflamiSTRAT kit should be stored in a fridge 2-8°C upon delivery. Unreconstituted the contents are stable until the expiry of the kit. When reconstituted the vials are stable for 7 days when stored at 2-8°C.

5. THE MULTISTAT DEVICE & INFLAMISTRAT ASSAY

The Evidence MultiSTAT InflamiSTRAT assay measures Interleukin 6 (IL-6), and soluble Tumour Necrosis Factor Receptor 1 (sTNFR-1) in plasma samples.

These values combined with sodium bicarbonate levels and an algorithm determine the subphenotypes of patients into hypo- and hyper-inflammatory subgroups.

Please ensure you have completed your Randox MultiSTAT device training before following these instructions.

Before the patient sample is collected the device should be prepared. Please ensure you have the following equipment available and ready:-

1 x Randox MultiSTAT device with accompanying barcode scanner

1 x InflamiSTRAT Assay Kit

1 x Accessory kit

Pippettes/Syringes

1 x Barcode sheet

1 x USB will be provided to sites by the PANTHER study team

a) Ensure the USB has been updated to match the assay kit batch – this step will be completed by the PANTHER team before despatch to site.
b) Ensure the device has been calibrated – to be completed every month (28 days)
c) Ensure the QC process is complete – to be completed every 2 weeks.

Before a patient sample can be analysed, the following steps are required to be completed on the device:-

6. OVERVIEW

1. Batch Update

Each time a new manufacture batch of kit is received an updated USB with the required batch software will be provided by the PANTHER study team. The PANTHER team will inform the site when a new manufacturer batch is available. The USB is updated from the Randox website. Please see section 7.1 for further details.

2. Device Calibration

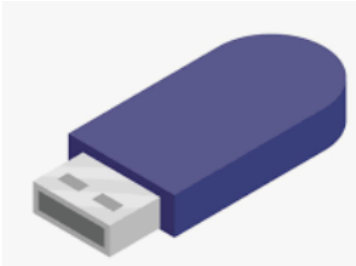
When the first InflamiSTRAT kit is first received a calibration on the device should be completed. Calibrations will then continue monthly (every 28days) or on receipt of a kit from a new batch. Adjuster materials (Adjuster 1 and Adjuster 2) are provided with each kit. These are required to complete the 3-step calibration process. This process will then be completed monthly; the device will provide a prompt when required. See section 6.3 for further detail.

3. Device Quality Control (QC) Check

Once the calibration steps are completed a QC check is required using the material provided with each kit (Control 1 and Control 2). Detailed instructions on these steps are provided below.

7. DEVICE INSTRUCTIONS

7.1 Batch update - USB



When a new manufacturer kit batch is received, the USB should be updated with the corresponding batch files. This will be completed by the site or the study team depending on restrictions. To do this follow the instructions below:-

1. On your computer enter www.randox.com into a search engine

2. Select **Support & Documentation** ⟶ **Reagent Product Inserts**

Note:- an account is required at this point, to do this please select **Request Access** and complete the online form.

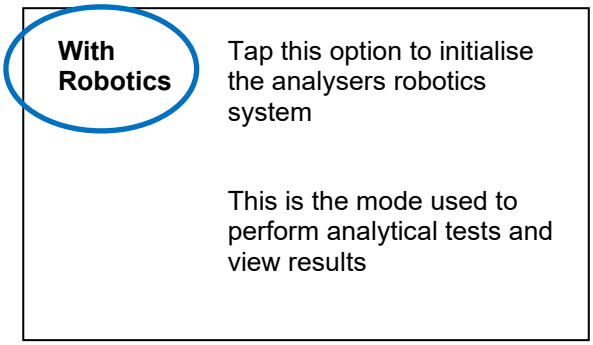
3. Once logged in enter the catalogue number in the **Search** field. The catalogue number may change on each batch. The study team/Randox will provide the catalogue number. Plug the provided USB into the computer and copy these files on to the USB.

7.2 Getting the Device Ready

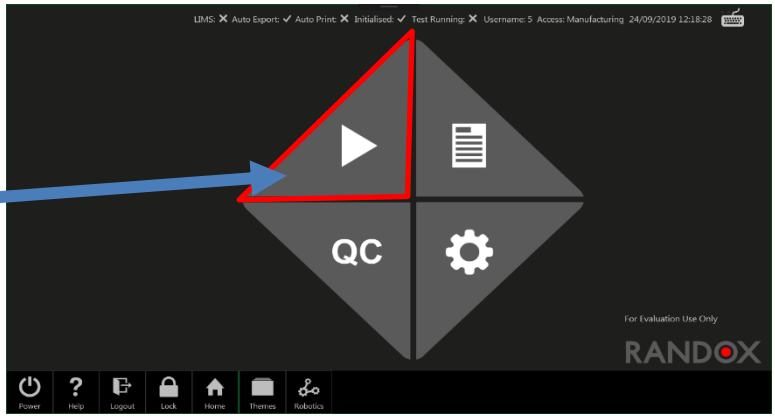
1. Switch the analyser on using Power Button located on right hand side of the device and insert USB.

2. Login with the user details you will have set up with Randox Technical Support during training

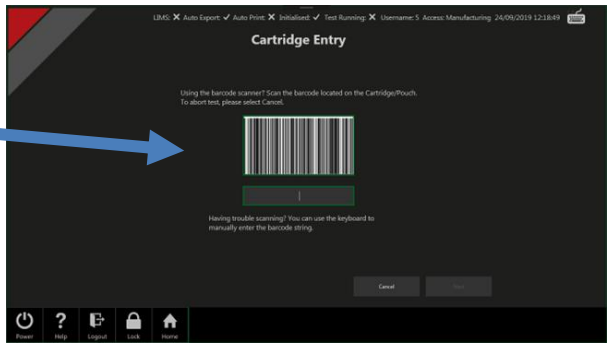
3. Following login select the 'With Robotics' option to bring the user to the Home Screen



4. From the Home Screen select the **Run Test** (Play) Button– the Cartridge Entry Screen will be displayed

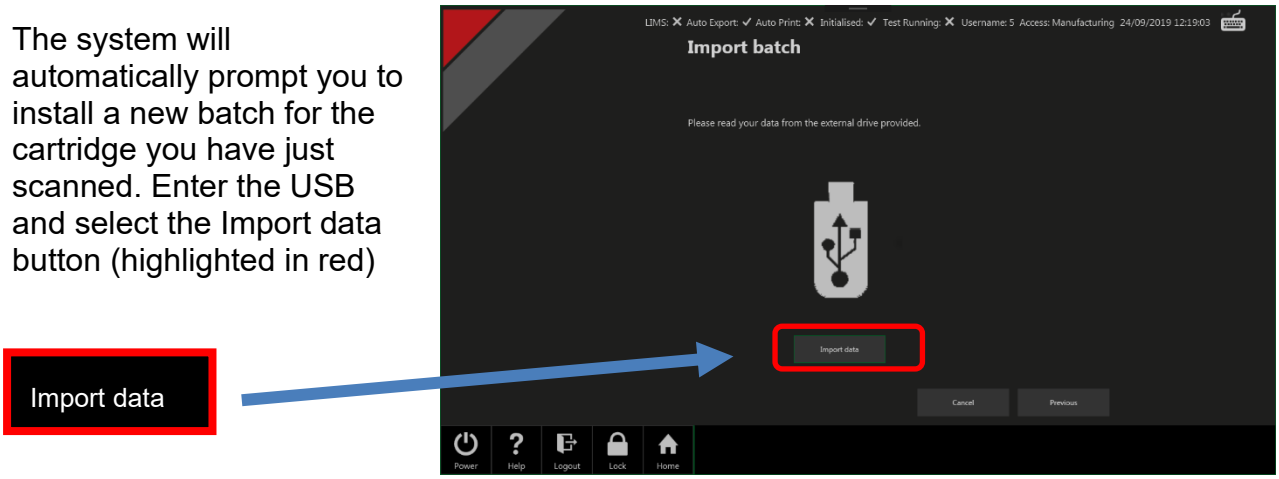


5. A prompt will appear to scan the barcode

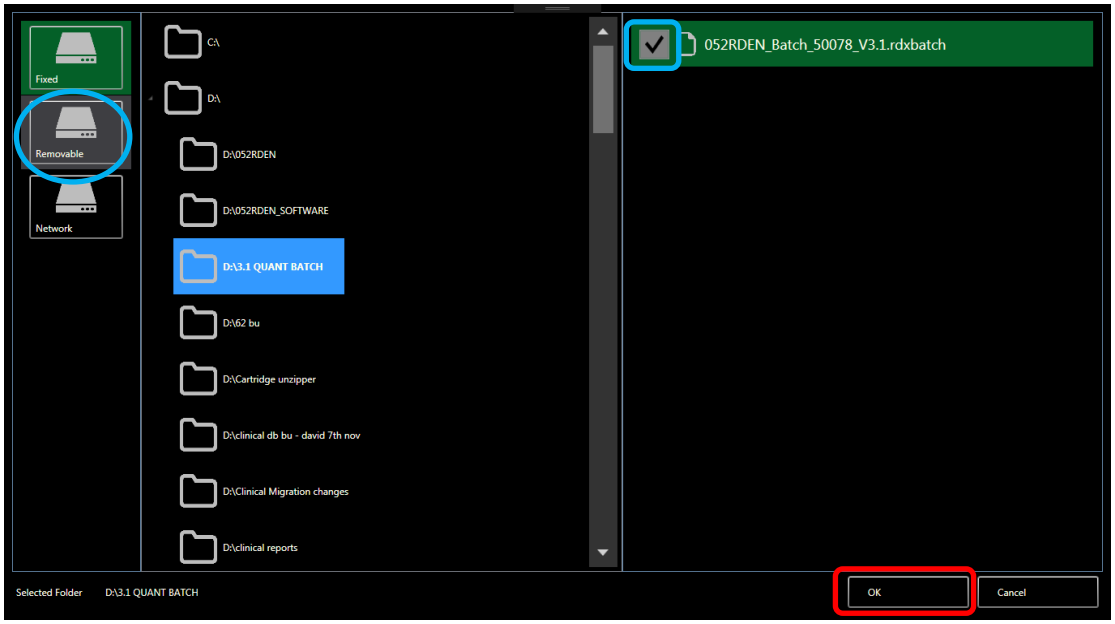


6. Scan the **Cartridge** Barcode from the Barcode sheet from the InflamiSTRAT kit.

7. The system will automatically prompt you to install a new batch for the cartridge you have just scanned. Enter the USB and select the Import data button (highlighted in red)



8. Double tap **Removable**, followed by **E:** and finally double tap the file you have downloaded on to the USB.
   Ensure the checkbox beside the filename is selected (highlighted in blue)
   Press the Ok button (highlighted in red)



7.3 Device Calibration

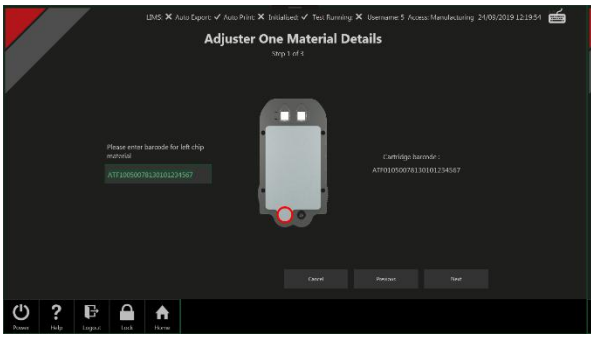
To be completed on the receipt of a new batch of kit and each month, the device will prompt when due.

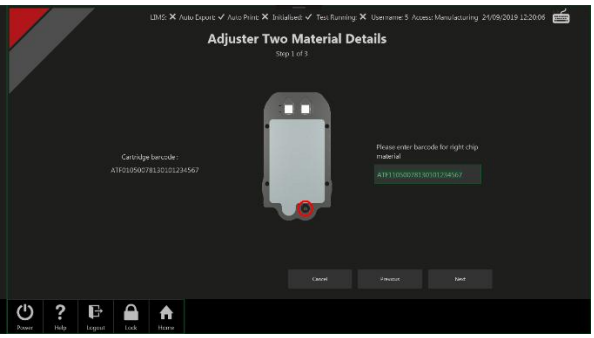
Step 1 – Preparing the device

1. Remove the InflamiSTRAT Assay Cartridge from the kit but leave sealed in the foil bag for 30 minutes at +15°C to +25°C.

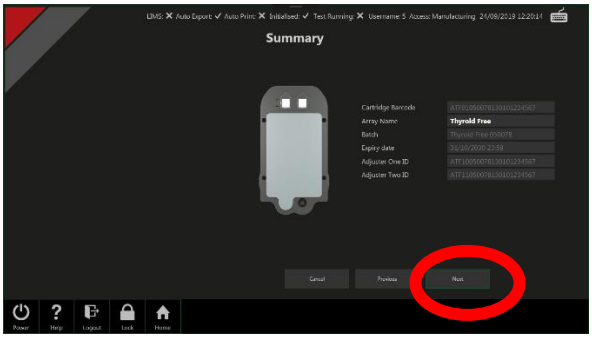
2. Scan the **Adjuster 1** Barcode from the Barcode sheet from the InflamiSTRAT kit. This will now become the barcode for the Adjusters. The system will then prompt to scan the **Adjuster 2** barcode.

First scan adjuster 1 then adjuster 2 when prompted.

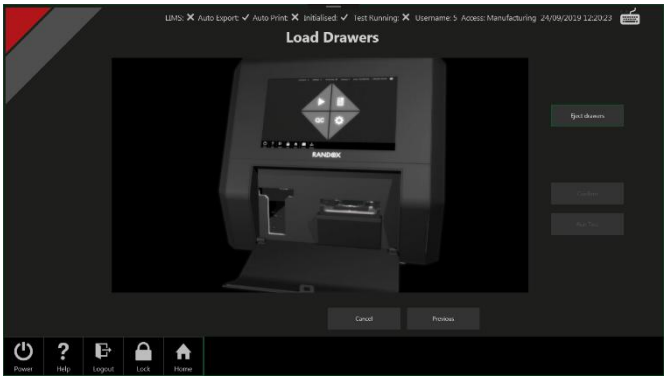




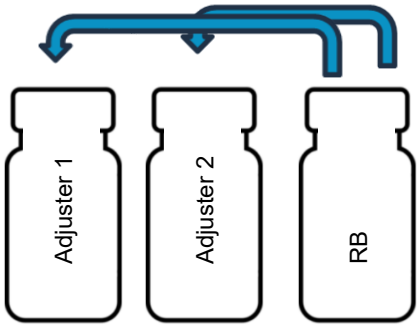
3. A summary screen will then appear, then click next



4. Select next and tap the screen when prompted to eject the drawers. The door will then open automatically followed by the cartridge and tip drawers.



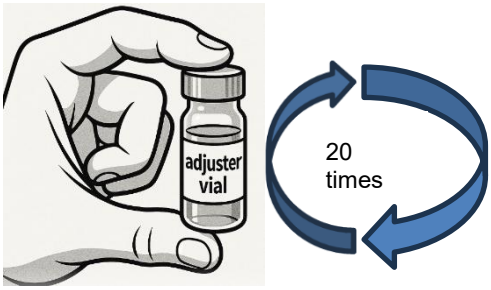
Step 2 – Adjuster Run #1



1. From the InflamiSTRAT Assay Kit take one vial each of the freeze-dried Adjusters 1 & 2 and one vial each of Reconstitution Buffer (RB)

2. Transfer 1ml of RB into each of the Adjuster vials using a 1ml syringe or pipette.

3. Invert the Adjusters gently 20 times, allow to stand for 30min at room temperature and reinvert gently 20 times



4. Remove a pipette tip from the tip cartridge (accessory kit), pierce the sealed sample well on the left. Do not dispose of the tip, replace this back in the tip cartridge.

5. Using a pipette/syringe transfer 250µl (or 0.25ml) of reconstituted adjuster 1 to the left well

6. Using a fresh pipette/syringe transfer 250µl (or 0.25ml) of reconstituted adjuster 2 to the right well

7. Load the InflamiSTRAT assay test cartridge (right hand drawer) and tip cartridge (left hand drawer) into the device. Push both drawers into place and close the door.

Destroy the reconstituted adjusters used for this run as new adjusters will be reconstituted for the next run.

8. Select confirm, wait for the device to perform checks and select run test. The InflamiSTRAT Assay takes 36 minutes. In this time, you can remove the Test Cartridge from the kit but leave sealed in the foil bag for 30 minutes at +15°C to +25°C. Please also remove 1 vial of adjuster 1, adjuster 2 and reconstitution buffer from the InflamiSTRAT Assay Kit. Transfer 1ml of Reconstitution buffer into both Adjuster 1 and Adjuster 2, using a different pipette tip/syringe each time. Gently invert each vial 20 times and let them sit for 30mins at room temperature and then invert again 20 times. This will be used for adjuster run 2.

9. Once the run is complete, select Eject Drawers and remove the used cartridges and discard into biohazardous waste

10. Push empty drawers back into place, close the door and select confirm

11. The device will then prompt to perform 'Adjuster Run 2' see step below.

Step 3 - Adjuster Run #2 – repeat steps from Step 2 above

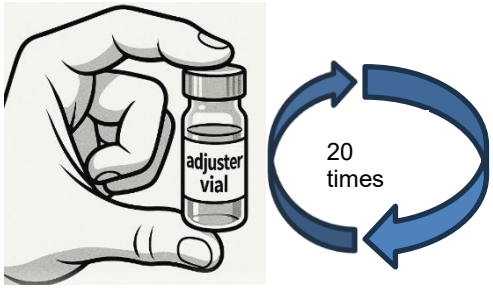
1. From point 8 above the second set of adjusters should be reconstituted, inverted and ready to use.

1. Scan the adjuster 1 and 2 barcodes from the sheet.

2. On the device check the summary, select next, then select eject drawers

3. Open the foil bag carefully remove the cartridge

4. Using a pipette tip, pierce the foil covering the left well. Do not dispose of the tip, replace this back in the tip cartridge



5. Using a 1ml syringe transfer 250µl (or 0.25ml) of reconstituted adjuster 1 into the left well

6. Using a fresh 1ml syringe transfer 250µl (or 0.25ml) of reconstituted adjuster 2 into the right well

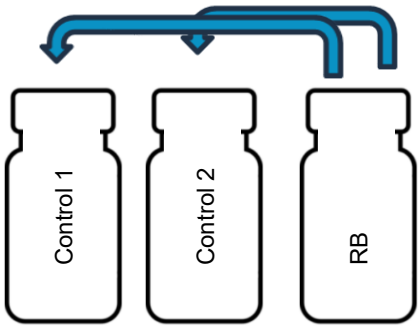
7. Eject drawers and load anaylser

8. Press confirm (wait for analyser to perform checks and select run test)

9. Once run is complete, select Eject Drawers, and remove the used cartridges to discard into biohazardous waste

10. The device will then generate a prompt to perform ‘Control Run’ see step below.

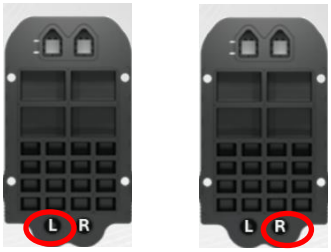
7.4 Device Quality Control (QC) Check



To be completed immediately after the calibration (Adjuster Runs 1 and 2 as above) and every 2 weeks after calibration.

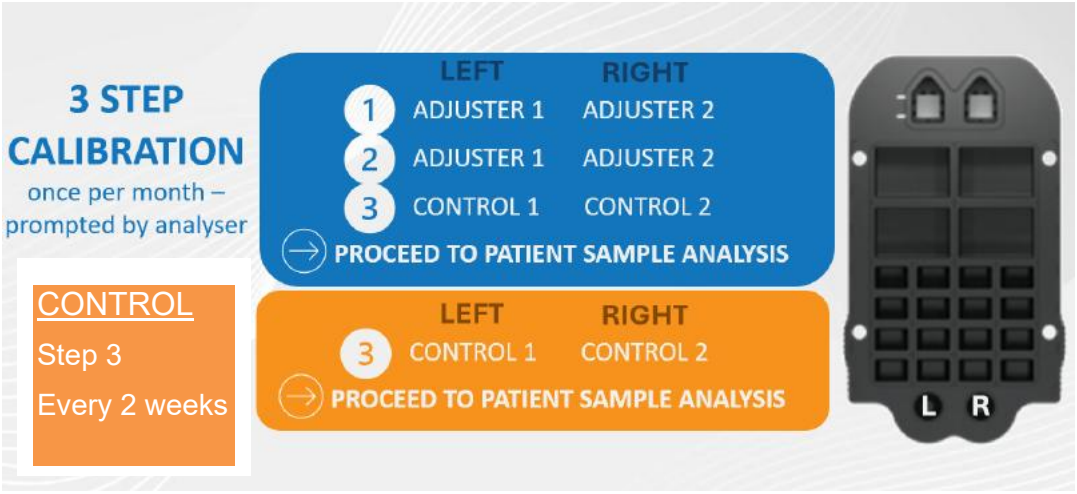
1. Transfer 1ml of reconstitution buffer into each control vial. Use a fresh syringe for reconstituting each control vial
2. Remove the InflamiSTRAT Assay Test Cartridge from the kit but leave sealed in the foil bag for 30 minutes at +15°C to +25°C
3. Invert vials 20x leave to stand with the cartridge at room temperature for 30 minutes, then invert 20x further times
4. Scan **Control 1** barcode from the sheet

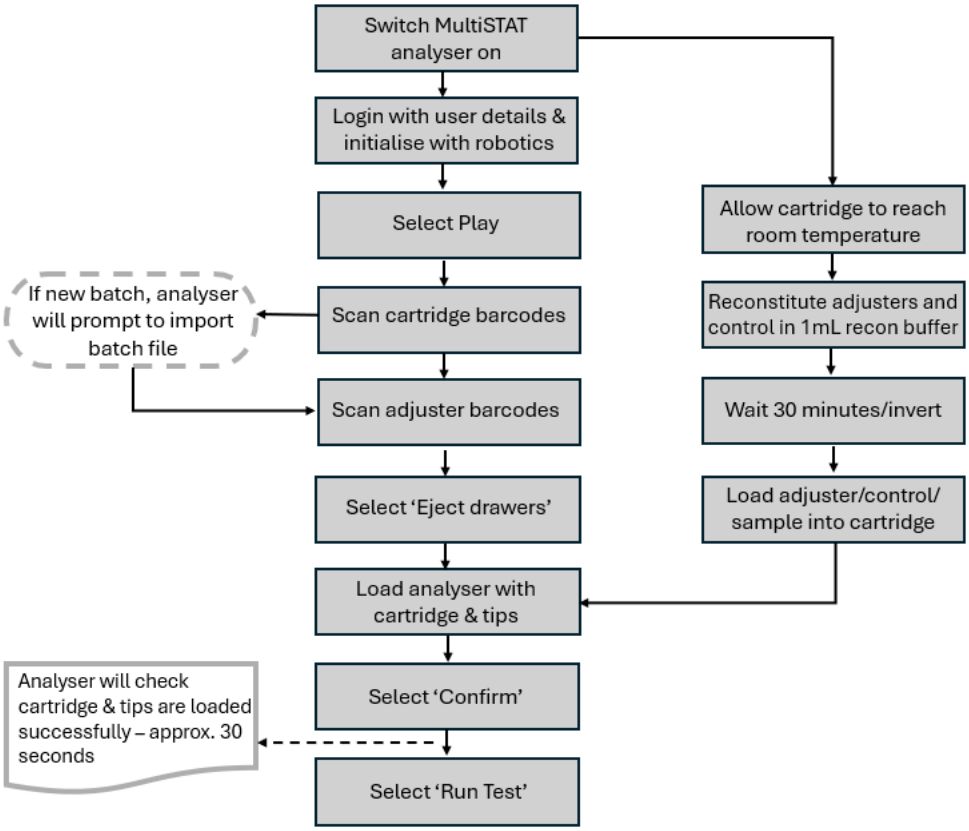
5. Import the batch details from the USB provided, select import data, navigate to the file and select ok
   Note – if this step has already been completed for this batch, this step can be missed.
6. Scan the **Control 2** barcode, check the summary and select next
7. Select eject drawers
8. Using a pipette tip pierce the foil covering the left well. Do not destroy the tip, replace this in the tip cartridge
9. Using a 1ml syringe transfer 250µl (or 0.25ml) of reconstituted internal Control 1 into the left well
10. Using a fresh 1ml syringe transfer 250µl (or 0.25ml) of reconstituted Control 2 into the right well



11. Eject drawers and load analyser
12. Press confirm, wait for analyser to perform checks and select run test
13. Once run is complete, select Eject Drawers and remove the used cartridges and discard into biohazardous waste
14. Push empty drawers back into place, close the door and select confirm

Summary of calibration and QC checks





8. COLLECTING THE SAMPLE FROM THE PATIENT

Personnel must follow 'Universal Precautions' when handling blood products as well as site-specific requirements for handling each type of specimen. Processing of blood specimens should be initiated as soon as possible (~ 30 minutes) following collection with all plasma and serum samples ideally processed and frozen within one hour.

8.1 Equipment preparation

Before the sample is collected, ensure you have the following equipment available and ready:-

- 1 x 4ml Lithium Heparin Tube
- Ice for temporary storage
- 1 x centrifuge
- 1 x Pasteur pipette
- 1 x 1ml cryovial tube
- 1 x red topped tube
- 1ml syringes (2 for each run, adjuster 1, adjuster 2, control, and patient sample)

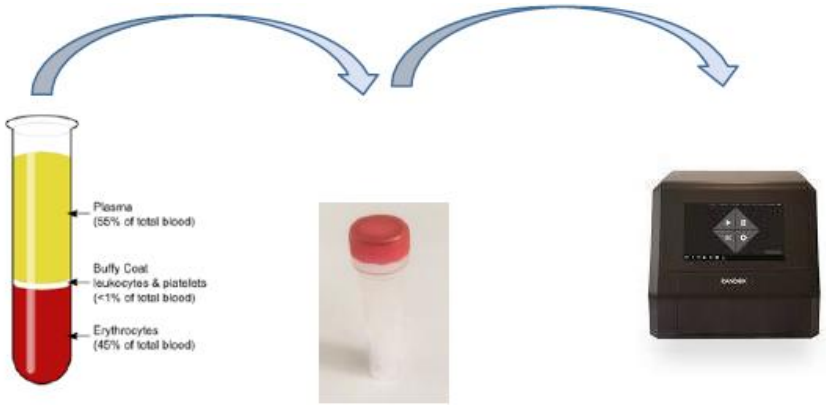
8.2 Collecting the sample for the subphenotype determination

Before the patient is randomised we need to determine the subphenotype, this sample will determine if the patient is hyper or hypo inflammatory.

- Collect 4ml of whole blood from the patient in a lithium heparin tube, invert the tube several times, then place on ice.

4ml Lithium Heparin Tube

- Centrifuge the blood at 2000g for 10 minutes.
- Pipette the plasma into the 1ml cryovial tube.
- Keep on ice until sample analysed in MultiSTAT. Keep any residual plasma in the red-topped tube on ice, until assay is complete (see below).
- Please refer to Step 5 below to run the samples. After sample has been successfully analysed by the MultiSTAT, discard this sample only.



Note - please see Sample Manual for details on the other tiers.

Step 5 - Running samples on MultiSTAT

1. Scan the cartridge barcode and enter sample details
2. Select next and eject drawers
3. Using a pipette tip pierce the foil covering the left well. Do not destroy the tip, replace this back in the tip cartridge.
4. Using a syringe transfer 250µl (or 0.25ml) of the patient sample into both cartridge wells
5. Load the analyser
6. Press confirm (wait for analyser to perform checks) and select run test
7. Once run is complete, select Eject Drawers, and remove the used cartridges to discard into biohazardous waste
8. Push empty drawers back into place, close the door and select confirm

Concentration values for IL-6 (pg/mL) and sTNFR1 (ng/mL) will be shown on the screen. Make a note of these to enter in the eCRF to allow the patient to be randomised.

Example results

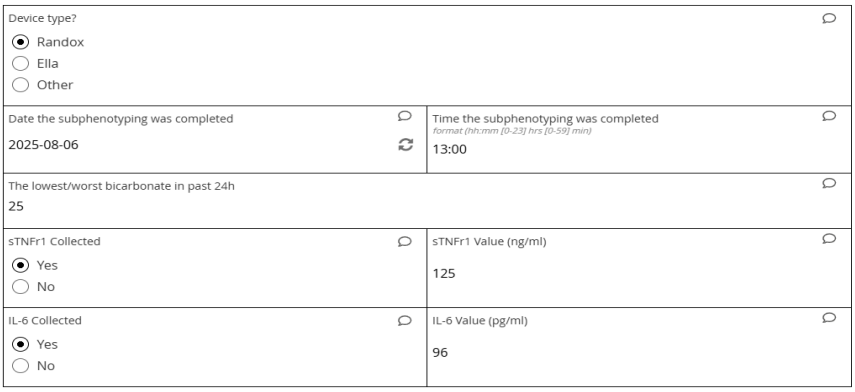
Analyte	Concentration	Units
IL-6	253.09	pg/mL
sTNFR1	7.18	ng/mL

Note - Only one patient sample can be run per cartridge.

8.3 Entering on the database

Once the MultiSTAT has provided the required concentration values (IL-6 and sTNFR1) these can now be entered into the CRF and the patient can be randomised.

UA0-003: Phenotyping



9. DEVICE TROUBLESHOOTING

- If a run should fail Randox will require the Support File in order to identify the issue
- If you experience a QC fail select the QC button on the Home Screen
    - Select InflamiSTRAT assay and at the top right select Display Calibration
    - Select the run file(s) corresponding to the failed QC run(s)
    - Select Support Files and navigate to the desired export location.
    - Select Ok
- If you experience a Sample fail select the Results button on the Home Screen
    - Select InflamiSTRAT assay and the run file corresponding to the failed sample run
    - Select Support Files and navigate to the desired export location
    - Select Ok

10. ON-SITE EQUIPMENT

All sites will be required to process the initial blood sample for centrifugation to separate plasma and be able to measure sTNFr-1 and IL-6 for stratification via the MultiSTAT device.

All participating centres must be equipped with the following minimum equipment:

- Centrifuge with 2000g force capability
- Evidence MultiSTAT point of-care analyzer (Randox laboratories Inc., Antrim, Northern Ireland)
- InflamiSTRAT assay kits

11. ORDERING ASSAY KITS

The MultiSTAT device uses specific kits for PANTHER, sites will first inform the PANTHER team when new kits are required, the team will then order the kits directly from RANDOX. These will be delivered to the site FAO the preferred contact and site address.
The kits that are being used are:

- **InflamiSTRAT Assay Kit (EV4578)**
  - Kit contains 12 tests, Adjuster material, QC material, Instructions for Use (IFU), Barcode Sheet
  - Assay contained in a sealed cartridge
  - QC runs must be completed before patient samples can be assayed
  - Requires addition of a plasma sample separated by centrifugation from whole blood

- **Accessory Kit (EV4452)**

Please refer to the document InflamiSTRAT EV4578 IFU for full details on the following:

- Reagent Compositions
- Safety Precautions and Warnings
- Stability and Preparation of Reagents
- New Batch Updates
- Preparation of Tip Cartridge
- Preparation of Adjusters 1&2
- Preparation of Controls 1&2
- Cartridge Analysis
- Quality Control
- Instrument Settings
- Results Processing
- Quantitative Analysis
- Result Interpretation
- Interleukin-6 (IL-6)
- Soluble Tumour Necrosis Factor Receptor-I (sTNFRI)

12. REVISION HISTORY

Version	Date	Summary of changes
1.0	08 AUG 2025	First version
2.0	19 DEC 2025	Amended QC to every 2 weeks rather than before each patient