PURPOSE

The intent of this SOP is to describe the procedures for use and maintenance of the GUAVA HTS flow cytometer

# **SCOPE**

This SOP applies to the GUAVA HTS system located in the CBE laboratories.

# **RESPONSIBILITES**

## Responsible Person

## Daily cleaning and quality checks are carried out by the responsible person. If the responsible person is unable to carry out daily maintenance it is their responsibility to find a replacement. The trend graph showing the results of the quality check must be assessed on a weekly basis. If there are any issues these should be addressed by the responsible person. Any reported issues with the GUAVA should be addressed by the responsible person.

## CBE Laboratory Users

All personnel who operate the GUAVA are responsible for the proper operation and maintenance of the instrument as outlined in this document. Users must ensure that the working area is kept clean during and after use of the GUAVA. All actions carried out with the machine must be recorded in the user log. If a problem should occur perform as much troubleshooting as you are confident to do so, seek advice from a more experience user and then report to the responsible person.

# **EQUIPMENT AND MATERIALS**

All specific equipment for use can be found in the GUAVA manual.

96 well plates – code DIS-984-030H (Fisher)

Short, freestanding 0.5mL tubes – code FB74141 (Fisher)

1.5mL tubes – code TUL-918-068G (Fisher)

ICF solution – code 4200-0140 (Luminex)

Bleach (sodium hypochlorite solution) – code 11448842 (Fisher)

GUAVA easyCheck Kit – part number: 4500-0025 (Luminex)

* Kit contains Guava easyCheck Bead Reagent (part number: 4200-0075) and Guava Check Diluent (part number: 4200-0050)

# **PROCEDURE**

**Important Information**

1. Tubes must be filled between each cleaning cycle run in the cleaning module. Air entering the system is a major cause of machine failure.
2. Approximately three Quick Clean cycles can be performed from a single well in a plate containing 250ul and seven Quick Cleans from a 1.5-mL tube containing 1.5 mL of fluid before well or tube runs dry and reach dead volume. Ensure cleaning tube is filled up during long runs (pause run, eject tray, top up cleaning tube, load tray, restart run).
3. Record all actions in the user log.
4. Flow cell removal is NOT RECOMMENDED. If there are issues with the machine which you think require the flow cell to be removed please speak to the responsible person.
5. Before starting to use the GUAVA check that there is no air in the ministack tubing connecting the machine and the capillary flow cell. If there is air present, please consult the responsible person.

**5.1 GUAVA START UP**

**5.1.1 System Start-up**

1. Turn on the laptop computer.
2. When the computer is finished booting up, turn on the guava easyCyte HT System. The power switch is located half-way up on the right side at the back of the instrument.
3. Start guavaSoft Software by double-clicking the guavaSoft 1.1 application icon on the desktop. You can also click the Start button, point to Programs, point to Millipore, point to guavaSoft 1.1, then click guavaSoft.
4. Check there is no air in the ministack tubing connecting the machine to the capillary flow cell. If there is air present please consult the responsible person.

**5.1.2 System prime**

1. Prime the system by performing a complete cleaning procedure.
2. Click on the “Essential Tools” option and open the “Cleaning” module. Click on “Start clean”: the tray will eject.
3. The tubes from the previous procedures will be on the tray
4. If tubes are one day old-refill the tubes with MilliQ water or ICF in the proper locations. If more than one day old, replace tubes that appear to be contaminated or dirty. Use “short” Microtubes to spare reagents.



**W**  Wash tubes- 1400µL water in 1.5mL tubes

**B**  Backflush - empty 1.5mL tube

**C**  Clean/Rinse 750µL ICF – short 0.5ml free standing tube for this to save on ICF.

**CS** Capillary Shutdown 1400 µL water in 1.5mL tubes

1. Run the “Clean” procedure to prime/wash the system **TWICE** (it lasts for 15mins each). Do this at least once if not enough time or Guava is in regular use. Do at least TWICE after weekends, or long period of not in use.
2. Select the CS tube (position 9) as the “Capillary shutdown” tube, as system won’t be in use immediately.

**5.1.3 Capillary Shutdown**

1. The “Capillary Shutdown” procedure is automatically done at the end of the “Clean” procedure.
2. The capillary is placed into a tube of water and can stay like this until acquisition without any risk of drying particles on the capillary glass wall.

**5.1.4 Backflushig the Fluid System**

1. If the fluid system is suspected as being blocked, click “Backflush” on the easyCheck screen, or any Guava assay screen. A message should appear on the screen with a prompt to select the well/tube to be used for backflushing – the default position is w1, conaining 100ul of bleach



NOTE: If the system is acquiring samples,

click “Pause”, then click “Backflush”

1. Leave the default position for Backflushing as w1 and click “OK” (w1 must be containing 100ul bleach solution before clicking “OK”).
2. Once the Backflush procedure is complete, click “Quick Clean” to rinse any bleach from the capillary.
3. If a run has been paused in order to perform the the Backflush procedure, click “Resume”.

**5.1.5 System preparation**

1. Open the “InCyte” module. Open the tray by clicking on the EJECT button.
2. Remove the old tubes corresponding to the “Cleaning & shutting down” procedure & replace them with new ones.
3. Place the “acquisition” cleaning tubes on the tray (prepare a new set every day). Use “short” 500uL microtubes to spare reagents especially ICF.

**S** Spin/dry empty (use regular 1.5mL tubes)

**W** Mixer wash 1400µL water in 1.5mL tubes

**Q** Quick clean 1400µL water in 1.5mL tubes

**B**  Backflush - empty 1.5mL tubes

**C** Clean/Rinse 750µL ICF – short 500uL free standing tube for this to save on ICF.

**CS** Capillary Shutdown 1400µL water in 1.5mL tubes

1. Before doing a Guava easyCheck with beads- perform one “Clean & Rinse” or “Quick clean” in InCyte to clean the system. The system is now ready for sample acquisition.

NOTE: If you do not plan to use the Guava immediately, perform a “Capillary shutdown” procedure (found in cleaning mode).

**5.1.6 easyCheck**

At the start of each day (when in use), once the system has been cleaned, run the easyCheck procedure to ensure the system is performing properly before starting sample acquisition. easyCheck averages the results from three acquisitions of a guava easyCheck Bead sample to determine if the results are within the expected range.

1. Prepare a 1:20 dilution of the guava® easyCheck bead reagent. Refer to the *guava easyCheck Kit* package insert for information: 10 uL beads + 190 uL diluent minimum
2. Click easyCheck from the main menu. Allow the instrument to warm up for 10 minutes before acquiring the beads (also allow bead and diluent to warm to RT).
3. Make sure the correct Bead Lot # and Bead Expiration Date (found on the guava easyCheck Bead Reagent vial label) and Expected Particles/mL are entered in the appropriate fields. Optionally, you may enter the guava® easyCheck Kit lot number and expiration date (found on the side of the guava easyCheck Kit box). The particles/mL corresponds to the concentration of beads in your prepared sample where the guava easyCheck Bead Reagent is diluted 1:20 with diluent.
4. Click Start.

**WARNING:** Keep the area in front of the tray clear as the tray ejects.

# The sample tray opens and a dialog box appears prompting you to load DI water, and empty tubes, and select the tube/well containing the beads.

# Load tubes filled with fresh DI water in w2, w4, and w5 (make sure they are all full).

# Load empty tubes in w3 and w6.

# Load a tube containing 100 μL of bleach in position w1 (for performing a backflush)

# Load the easyCheck sample into the chosen well (make sure you mark the chosen well).

# Click on the tube/plate map to select the tube/well with beads.

# Click **OK**. The system will run easy check

**WARNING:** If you click **Cancel** in the dialog box, the tray will automatically load. Keep the area clear as the tray loads.

**NOTE:** If you are using a microplate, make sure well A1 of the plate is in the top-right corner of the tray.

**5.1.7 easyCheck Results**

The software displays %CVs and averages for particles/mL (bead count), FSC and SSC intensities, and GRN, YLW, RED, NIR, RED2, and NIR2 (if applicable) mean fluorescence intensity (MFI) for three replicates.

1. If any result for Particles/mL falls outside ±10% of the expected value, the result is outside the acceptable range and appears in red. For example, if the actual particle count is 50,000, the acceptable Particles/mL range (±10%) is 45,000 to 55,000.
2. If the %CV for Particles/mL is >10%, it appears in red.
3. The %CV for FSC and SSC Intensities, and GRN/YLW/RED/NIR/RED2/NIR2 MFI for the three replicates should be <5%.
4. Refer to the information card that comes with the guava easyCheck Kit for the acceptable intensity ranges for each parameter.
5. If the Particles/mL (count) for a replicate or the average falls outside the acceptance range, or if an intensity value is outside the acceptable range, run Quick Clean or Clean & Rinse. Rerun the easyCheck Procedure with new bead dilution. If values continue to fall outside the acceptance range, refer to “easyCheck Procedure Troubleshooting” on page 2-8 of the manual, for more information.
6. If the signal intensity for any of these parameters shows significant drift over time beyond the range listed, and this change is not correlated to a change in the bead lot, a new flow cell, or instrument service, contact LUMINEX technical support Asha Lad: [alad@luminexcorp.com](mailto:priya.santhalingam@merckgroup.com)

**5.2 SAMPLE ACQUISITION**

1. Once the worklist is created, the tray will open: you can now place your plate.
2. Make sure that the tubes Q (DI water) and C (ICF) are filled up before starting the acquisition. Check these tubes every time you put a new plate, since a cleaning procedure is automatically performed at the end of a plate acquisition.
3. Settings can only be saved after settings have been adjusted, before running the worklist.
4. For optimal acquisition, use cells at a concentration of 500 cells/µL.
5. Beware of dead volumes: round bottom w96: 50µL; flat-bottom w96: 75µL; 0.5mL Microtubes: 70µL; 1.5mL tubes: >1mL.
6. If the acquisition rate slows dramatically, and there is sufficient sample volume, the fluid pathway may be blocked. Click “Stop”, wait for the system to stop, and then click “Backflush”. Once the Backflush procedure is complete, click “Quick Clean” to rinse any bleach from the capillary.
7. Empty the waste, wash it and add bleach to the vial when more than half full.
8. Notify Asha Lad: [alad@luminexcorp.com](mailto:priya.santhalingam@merckgroup.com), (+44(0)795518329) of any acquisition problems

**5.2.1 Quick Clean**

1. Whenever leaving a system ON and NOT IN USE - do quick clean then Capillary Shutdown.
2. Before beginning a sample run do a “quick clean”, automatically select quick clean after 12 sample wells in work list edit.
3. Approximately three Quick Clean cycles can be performed from a single well in a plate containing 250ul and seven Quick Cleans from a 1.5-mL tube containing 1.5 mL of fluid before well or tube runs dry and reach dead volume. Ensure cleaning tube is filled up during long runs (pause run, eject tray, top up cleaning tube, load tray, restart run).

**5.2.2 Capillary Shutdown**

**IMPORTANT:**

1. At the end of your sample acquisition, close the “InCyte” module, open the “Cleaning” module and perform a **“Capillary Shutdown”** procedure, to make sure that the capillary is not getting dry.
2. Always use the CS tube in position 9.
3. Never let the Guava stand overnight without making sure that the capillary has been cleaned and that “Capillary shutdown” procedure has been performed!

**5.3 GUAVA SHUT DOWN**

1. Quit “InCyte” module and go to the “Cleaning” module. Click on “Start clean”: the tray will eject.
2. Remove the tubes corresponding to the ‘acquisition clean’ procedure.
3. Place the cleaning tubes on the tray (prepare a new set every day). Use “short” 0.5mL, free standing Microtubes to spare reagents.

**W**  Wash tubes- 1400µL water in 1.5mL tubes

**B**  Backflush - empty 1.5mL tube

**C**  clean/rinse 750µL ICF – short 0.5ml free standing tube for this to save on ICF.

**CS** Capillary Shutdown 1400 µL water in 1.5mL tubes

1. Run the “Clean” procedure to wash the system **TWICE** (lasts 15min each run), do this AT LEAST ONCE if no time.
2. Select the CS tube (position 9) as the “Capillary shutdown” tube. The “Capillary shutdown” procedure is automatically done at the end of the “Clean” procedure.

**Never let the Guava stand overnight without making sure that the capillary has been cleaned and that “Capillary shutdown” procedure has been performed!**

1. Once the “Clean” procedure is over, quit GuavaSoft; switch off laptop and Guava system.

**5.4 MAINTENANCE**

**5.4.1 Daily**

1. If necessary, refill the cleaning solution vial with ICF up to half. 
2. At end of the day, empty waste vial, wash it & add 10ml bleach to the vial. 

* If waste vial does not contain any cytotoxic chemicals, the contents of the vial can be put down the sink with copious amounts of water.
* With the nature of the assays being performed, if the waste vial contains residues of cytotoxic reagents, empty the contents of the vial into a beaker and dispose the waste using the cytotoxic waste route.

**5.5 FLOW CELL**

**5.5.1 Cleaning**

**NOTE:** When cleaning the flow cell, always use the syringe cleaning tool. Handle flow cell with care and avoid touching the capillary unnecessarily. When handling the flow cell assembly, always grasp it at the top of the shuttle avoiding getting fingerprints on the optics window.

**WARNING:** To avoid exposure to laser radiation, turn off the power to the guava easyCyte HT System before attempting to remove the flow cell

1. Remove the metal plate at the top of the instrument.
2. Remove minstac tubing from the clamp and disconnect from the instrument:



1. Remove the flow cell by grasping at the top and pulling it straight up. Do not pull up on the tubing.
2. Fill the syringe cleaning tool with water or Guava ICF. Connect the syringe to the minstac tubing on the flow cell. Ensure the fitting is tight:



1. Using a Kimwipe®, hold the flow cell at the top of the shuttle to capture any fluid that may leak. Apply gentle, steady pressure to the plunger and watch as the fluid flows from the tip of the capillary.

**IMPORTANT:**

* The fluid stream must be straight. If it is not straight, the tip of the flow cell may be chipped or there may be a partial clog in the flow cell in which case the flow cell might need to be replaced.
* Make sure there are no leaks along the length of the capillary.
* Make sure fluid is not leaking where the tubing is connected at the top of the flow cell.

1. Unscrew the syringe from the minstac tubing, leaving the minstac tubing attached to the flow cell.
2. If there are leaks at the tubing connection, tighten to ensure that fluid does not leak from the top of the flow cell.
3. Using a Kimwipe®, dry the end of the tubing previously connected to the syringe.
4. Providing the flow cell is clean and intact, reinstall back into the recepable and connect the tubing to the instrument. Then insert the tubing back into the clamp:

**1)**

**2)**



**IMPORTANT:**

* Avoid bumping the capillary when reinstalling the flow cell.
* Do not press down on the tubing at the top of the shuttle.

1. Ensure that the cleaning solution vial is full, then run Quick Clean to prime the system. If starting the instrument after it has been shut down, run Guava Clean to prime.
2. To ensure that the flow cell was correctly installed, run the easyCheck procedure. While the first replicate is being acquired, watch for bubbles in the minstac tubing. If bubbles or leaks are visible, the tubing may not be adequately tightened.

**5.5.2 Replacing**

If the flow cell becomes damaged or clogged,so severely that backflushing the system, running Guava Clean, or cleaning using the syringe tool do not fix the problem, the flow cell will need to be replaced.

**NOTE:** When replacing the flow cell, handle with care and do not force the flow cell into the receptable. The capillary is fragile; avoid touching unnecessarily.

**WARNING:** To avoid exposure to laser radiation, turn off the power to the guava easyCyte HT System before attempting to remove the flow cell

1. Remove the metal plate at the top of the instrument.
2. Remove minstac tubing from the clamp and disconnect from the instrument:



1. Remove the flow cell by grasping at the top and pulling it straight up. Do not pull up on the tubing.
2. Install a new flow cell by correctly positioning it above the instrument and carefully lowering it into the receptacle. Using fingers, press down on the top of the flow cell on either side of the tubing until the flow cell clicks into place:



**IMPORTANT:**

* Keep the flow cell completely vertical and avoid bumping the capillary against the instrument or sides of the receptacle.
* Do not press down on the tubing at the top of the shuttle.

1. Connect the tubing to the instrument, then insert the tubing into the clamp:



1. Ensure that the cleaning solution vial is full, then run Quick Clean to prime the system. If starting the instrument after it has been shut down, run Guava Clean to prime.
2. To ensure that the flow cell was correctly installed, run the easyCheck procedure. While the first replicate is being acquired, watch for bubbles in the minstac tubing. If bubbles or leaks are visible, the tubing may not be adequately tightened.

# **DOCUMENTATION**

The following records are outputs of this SOP:

* User Log Book available in CBE Laboratory
* Decontamination certificate (an example can be found on the LEARN)

## SOP Version History

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| --- | --- | --- | --- |
| **Version**  **Reviewed** | **Date Revised/**  **Reviewed** | **Revision Summary** | **New**  **Version**  **Number** |
| 001 | 11th June 2012  By Victoria Workman | Changes to responsibilities. Addition of important information section. Removal of section describing flow cell removal. Additional documentation provided. New version reviewed by P.Mitchell and approved by P.Hourd (FSOP001) | 002 |
| 002 | 15th February 2021  By Nishant Joglekar | * Changes to ‘Equipment and Materials’ section. * Addition of ‘Backflushing the Fluid System’ section. * Change to contact details of technical support team. * Addition to the ‘Sample Acquisition’ section. * Addition to the ‘Maintenance’ section. * Addition of the ‘Flow Cell’ section. * Changes to the ‘Documentation’ section. * Revision of version number * Revision of date reviewed | 003 |
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