

## BD Celesta Automated QC Guide-CS&T

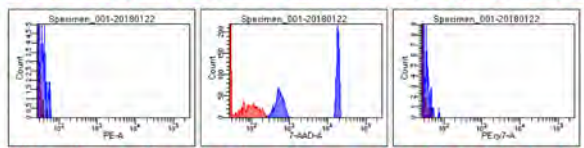
CS&T beads are used to characterize, track, and report performance measurements of the cytometer and should be ran **daily**.

1. Startup system: Lasers should be on for at least 20mins.
2. While lasers are warming, switch from Standby to Run (MEDIUM SPEED) to clean the sample line.
  - \*Add water to the tube if needed to prevent the tube from running dry
3. Make up CS&T beads: add **1 drop of beads** from the open vial (should be dated) to CS&T PBS tubes in green rack.
  - \*These tubes have enough PBS to get you the right dilution for QC.

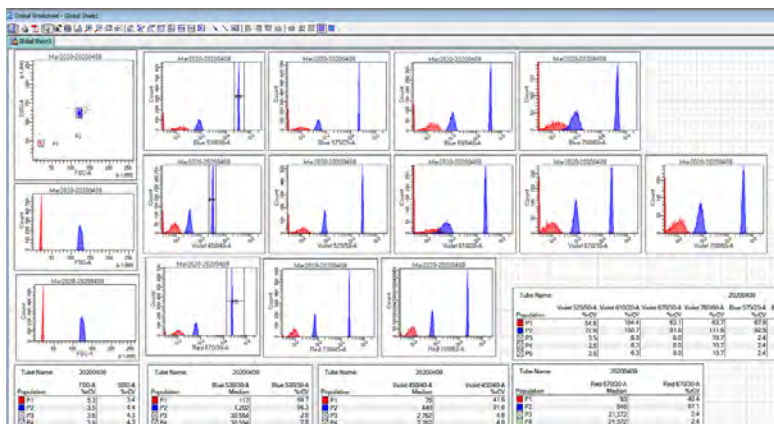


4. Open QC template in SHARED view (bottom of Browser).
5. Open current Specimen, click next tube and rename the tube the date and your initials. Do not make a new specimen.

\*No signal in FCS,SSC, or fluorescent channel at intial start up-> Software bug



Click voltage value up or down by one and back to original value  
This will reset the software



6. Run beads on **LOW SPEED**, click acquire and, after 10 seconds, click refresh and record 5000 events.
7. Leave the beads running and select Cytometer>CS&T
8. The cytometer will disconnect from DIVA and connects to the CS&T platform (this takes a few seconds). Once connected you can select **RUN**.

9. Click ok to verify the following information:

- Waste has been emptied
- LotID is correct
- Configuration is ok

7. Plots show data and the tasks list will displays progress: should take around 5mins to complete.
8. Verify that the performance check passes
  - \*if performance check passes with warnings or fails refer to CS&T troubleshooting guide (pg3)
9. When you're done, date the beads and put them in the CS&T box. Beads can be used 2 days in a row. If you're using beads from the previous day you can discard remaining beads in the biohazard box.



**Cytometer Performance Report**

|                          |   |                     |                     |
|--------------------------|---|---------------------|---------------------|
| Cytometer:               | FACSCelesta                                       | User:               | Administrator       |
| Cytometer Name:          | FACSCelesta                                       | Institution:        | N/A                 |
| Serial Number:           | 1   | Software:           | BD FACSDiva 8.0.1.1 |
| Input Device:            | HTS   | Date:               | 03/05/2018 12:07 PM |
| Tube Loaded Manually:    | Yes   | Cytometer Baseline: | 01/30/2018 08:34 PM |
| Cytometer Configuration: | Copy of PEcy7 Configuration 4-Blue 3-Red 5-Violet | P/F:                | Pass                |

**Setup Beads**

|                       |                 |                  |            |
|-----------------------|-----------------|------------------|------------|
| Bead Product:         | CST Setup Beads | Part #:          | 910858     |
| Lot ID:               | 71597           | Expiration Date: | 07/31/2019 |
| Bead Lot Information: | Available       |                  |            |

**Detector Settings**

| Laser  | Detector | Parameter       | Target Value | Actual Target Value | % Difference Target Value | Bright Bead % Robust CV | Mid Bead Median Channel | Mid Bead % Robust CV |
|--------|----------|-----------------|--------------|---------------------|---------------------------|-------------------------|-------------------------|----------------------|
| Blue   | FSC      | FSC             | 125000       | 123744              | -2                        | 3.99                    | 124173                  | 4.15                 |
| Blue   | E        | SSC             | 125000       | 123345              | -2                        | 5.18                    | 124781                  | 5.39                 |
| Blue   | D        | Alexa Fluor 488 | 7760         | 7449                | -5                        | 4.09                    | 162                     | 20.49                |
| Blue   | C        | PE              | 13140        | 12663               | -4                        | 4.02                    | 280                     | 16.54                |
| Blue   | B        | 7-AAD           | 20392        | 19635               | -4                        | 4.6                     | 554                     | 20.53                |
| Blue   | A        | PEcy7           | 13966        | 13936               | -1                        | 6.39                    | 267                     | 39.5                 |
| Red    | C        | Alexa Fluor 647 | 24555        | 23873               | -3                        | 2.67                    | 671                     | 14.98                |
| Red    | B        | APC-R700        | 16596        | 16314               | -2                        | 2.43                    | 471                     | 9.41                 |
| Red    | A        | APC-Cy7         | 15538        | 15023               | -4                        | 2.7                     | 405                     | 11.53                |
| Violet | E        | BV421           | 2962         | 2884                | -3                        | 4.62                    | 325                     | 14.46                |
| Violet | D        | BV510           | 29472        | 28799               | -3                        | 3.71                    | 664                     | 11                   |
| Violet | C        | BV605           | 32871        | 33277               | 1                         | 5.28                    | 700                     | 35.77                |
| Violet | B        | BV650           | 31274        | 30831               | -2                        | 5.22                    | 1284                    | 15.93                |
| Violet | A        | BV786           | 25118        | 25084               | -1                        | 8.87                    | 476                     | 22.48                |

**Detector Settings (Continued)**

| Laser  | Detector | Parameter       | Dim Bead Median Channel | Dim Bead % Robust CV | PMTV | Δ PMTV | Qr     | Br   | P/F  |
|--------|----------|-----------------|-------------------------|----------------------|------|--------|--------|------|------|
| Blue   | FSC      | FSC             | 14458                   | 5.68                 | 417  | 30     | N/A    | N/A  | Pass |
| Blue   | E        | SSC             | 51170                   | 4.1                  | 280  | 6      | N/A    | N/A  | Pass |
| Blue   | D        | Alexa Fluor 488 | 23                      | 86.86                | 469  | 3      | 0.0224 | 323  | Pass |
| Blue   | C        | PE              | 52                      | 59.06                | 471  | 1      | 0.1275 | 222  | Pass |
| Blue   | B        | 7-AAD           | 89                      | 57.53                | 599  | 1      | 0.0090 | 160  | Pass |
| Blue   | A        | PEcy7           | 42                      | 112                  | 639  | 8      | 0.0122 | 25   | Pass |
| Red    | C        | Alexa Fluor 647 | 127                     | 34.99                | 566  | -6     | 0.0168 | 47   | Pass |
| Red    | B        | APC-R700        | 89                      | 27.55                | 452  | -2     | 0.0093 | 4088 | Pass |
| Red    | A        | APC-Cy7         | 77                      | 31.35                | 471  | -3     | 0.0062 | 1491 | Pass |
| Violet | E        | BV421           | 79                      | 36.33                | 416  | -4     | 0.0159 | 878  | Pass |
| Violet | D        | BV510           | 101                     | 41.13                | 460  | -3     | 0.0211 | 2189 | Pass |
| Violet | C        | BV605           | 111                     | 143.63               | 667  | -2     | 0.1430 | 34   | Pass |
| Violet | B        | BV650           | 142                     | 48.92                | 613  | -5     | 0.0558 | 14   | Pass |

**KEY COLUMNS**

- 1 Bright Bead rCV=**Test of laser alignment
- 2 PMTV=**PMT voltages from current performance check
- 3 ΔPMTV=**Difference between PMT voltage value for baseline check and current performance check

**System Summary:** OK

Cytometer Performance:

Cytometer Performance Results: Passed

**System Summary:** Requires Attention

Cytometer Performance: (Completed with warnings)

Cytometer Performance Results: Passed

**Detector Settings**

| Laser | Detector | Parameter       | Target Value | Actual Target Value | % Difference Target Value | Bright Bead % Robust CV | Mid Bead Median Channel | Mid Bead % Robust CV |
|-------|----------|-----------------|--------------|---------------------|---------------------------|-------------------------|-------------------------|----------------------|
| Blue  | FSC      | FSC             | 125000       | 124439              | -1                        | 4.5                     | 124579                  | 4.52                 |
| Blue  | E        | SSC             | 125000       | 125285              | 0                         | 7.06                    | 126489                  | 6.97                 |
| Blue  | D        | Alexa Fluor 488 | 7760         | 7584                | -3                        | 6.45 (U)                | 165                     | 20.9                 |

Cytometer Performance Results: Failed

**Detector Settings (Continued)**

| Laser | Detector | Parameter       | Dim Bead Median Channel | Dim Bead % Robust CV | PMTV | Δ PMTV  | Qr     | Br  | P/F  |
|-------|----------|-----------------|-------------------------|----------------------|------|---------|--------|-----|------|
| Blue  | FSC      | FSC             | 14616                   | 5.92                 | 418  | 31      | N/A    | N/A | Pass |
| Blue  | E        | SSC             | 51688                   | 4.26                 | 280  | 6       | N/A    | N/A | Pass |
| Blue  | D        | Alexa Fluor 488 | 23                      | 87.48                | 470  | 4       | 0.0244 | 354 | Pass |
| Blue  | C        | PE              | 53                      | 58.41                | 627  | 157 (U) | 0.1376 | 247 | Fail |

## CS&T Troubleshooting

### -No beads detected

Beads not mixed well

Shake don't vortex

Beads too dilute

1 drop+500ul= $\sim$ 400ul/min **MUST RUN IN LOW SPEED**

Clogged sample line

Run 3ml bleach w/ arm open, run 7 mins with arm closed

Run 3ml water w/ arm open, run 15 mins with arm closed

### -Sample rate too low to complete analysis

Beads not mixed well

Shake don't vortex

Beads too dilute

1 drop+500ul= $\sim$ 400ul/min **MUST RUN IN LOW SPEED**

### -Unable to set laser delays

Beads not mixed well

Shake don't vortex

Beads too dilute

1 drop+500ul= $\sim$ 400ul **MUST RUN IN LOW SPEED**

Air bubbles in the flow cell or sheath filter

Sheath filter-bleed filter (roller clamp connected to blue tubing)

Unstable sheath pressure

Check for leaks and bubbles (\*plenum)

### -Alignment warning: Bright bead % rCV for primary channel is greater than 6%

### -Linearity warning: Unable to reach maximum channel with maximum PMTV

Beads exposed to direct light

Prepare fresh beads

Insufficient warm-up time of lasers

Let lasers warm up for at least 20mins

Dirty flow cell

\*Requires a monthly clean

Instrument alignment changed

\*Requires engineer

### -PMT settings change >50 volts (or user specified value) between performance checks

Beads exposed to direct light

Prepare fresh beads

Insufficient warm-up time of lasers

Let lasers warm up for at least 20mins

Dirty flow cell

\*Requires a monthly clean

Laser Issues

\*Requires engineer