

Luminex Control Microsphere (Con1 and Con2) Overview.

The Control beads (L100-CON1 and L100-CON2) are used on the Luminex 100 IS system only (not on 1.7 version Luminex instruments) to verify calibration and optical integrity of the system.

xMap Classification Control Microspheres (Con1) are used to verify the calibration and optical integrity for both the classification channels and doublet discrimination channel for the Luminex® 100™ IS system.

xMAP Reporter Control Microspheres (Con2) are used to verify the calibration and optical integrity for the reporter channel for the Luminex 100 IS system.

STarStation Version 1.1 software does not currently possess the functionality to perform CON1 or CON2 calibration validation steps. STarStation Version 2.0, does allow Con1 and Con2 calibration validation steps to be performed on IS version instruments.

Please Note that version 1.7 Luminex LX100 instruments do not possess the necessary firmware to allow the control verification procedure to be performed.

Classification Control Beads (CON1) are used to check the bead classification function. Con1 reagent is a mixture of beads with five different color-codes used to check that the bead classification channel is functioning correctly. The beads are selected to be on the periphery of the beadmap, the bead numbers used are 6,21,73,81 and 96.

Reporter Control microspheres (Con2) are a mixture containing roughly equal concentrations of 4 beads with different concentrations of proprietary reporter fluorophores. Con2 beads are used to check reporter channel response over the dynamic range of the Reader. The Con2 beads have no classifier dye but have four levels of RP1 proprietary dye giving a 3 decade dynamic range and sensitivity check. These beads are not strictly a 4-plex since they cannot be individually identified or "de-multiplexed" by gating.

Examples of the target data sheets for both CON1 and CON2 microspheres are shown below (Figures 1 and 2).

Examples of data displays indicating how CON1 and CON2 microspheres are detected via the Luminex detectors are shown in figures 3 through 6 below.

Certificate of Quality

xMAP™ Classification Control Microspheres, CON1

Product Number L100-CON1
 Lot Number A3635
 Expiration Date 27 FEB 2004

	<u>Parameter A1 Target Value</u>	<u>Parameter B1 Target Value</u>	<u>Parameter C1 Target Value</u>
Reference 01	10145	765	220
Reference 02	9501	73.3	64.8
Reference 03	10036	23159	5636
Reference 04	8553	2522	17738
Reference 05	9048	230	5748

Figure 1.

Certificate of Quality

xMAP™ Reporter Control Microspheres, CON2

Product Number L100-CON2
 Lot Number A3689
 Expiration Date 27 FEB 2004

	<u>Parameter A1 Target Value</u>
Reference 01	29.1
Reference 02	197
Reference 03	1910
Reference 04	17429

Figure 2.

The reporter (RP1) channel is the fluorescence channel used for assay quantitation. R-phycoerythrin (R-PE) is the primary reporter molecule used in Luminex assays. A series of beads dyed with varying intensities of a fluorochrome spectrally matched to R-phycoerythrin are used for this procedure. Each of the reporter beads has been assigned a specific intensity value corresponding to the number of fluorescent R-PE molecules. These units of fluorescent measure are known as molecules of equivalent soluble fluorescence (MESF). MESF units allow direct correlation of instrument performance to a typical assay using R-PE as the indicator molecule. The available range of channels on the Luminex is 4.5 log amp decades or 32,767 relative linear channels.

Always run system controls after calibration and as needed for troubleshooting.

To minimize carryover, Luminex recommends a wash with distilled water or sheath fluid between instrument calibration and instrument control steps.

The target values from the data sheet are entered in StarStation version 2.0 via the reagent manager.

Control verification is carried out in two phases. The first phase of control verification verifies the side-scatter, classification one, and classification two channels. The second phase of control verification control verifies the reporter channel.

What procedures should I follow if Calibration and/or Controls fail in the Luminex IS Software?

1. Verify that the correct Lot numbers and Target Values are selected.
2. Vortex the microsphere vials.
3. Allow the microspheres to reach room temperature before performing calibration/validation.
4. Verify that the correct wells have been selected if using the XY Platform.
5. Remove the sample probe and sonicate the narrow end for 2-3 minutes.
6. Using a syringe, flush the sample probe with distilled water from the narrow end out through the larger end.
7. Readjust the sample probe height upon replacement in the arm.
8. Complete 3 Backflush, 3 Drain, 2 Alcohol Flush and 3 Washes with water.
9. Repeat calibration. Verify that the pressure readings are between 6 and 9 psi and the Events/Second rate reads around 200 (note the event rate indicator is not available in STarStation version 1.1).

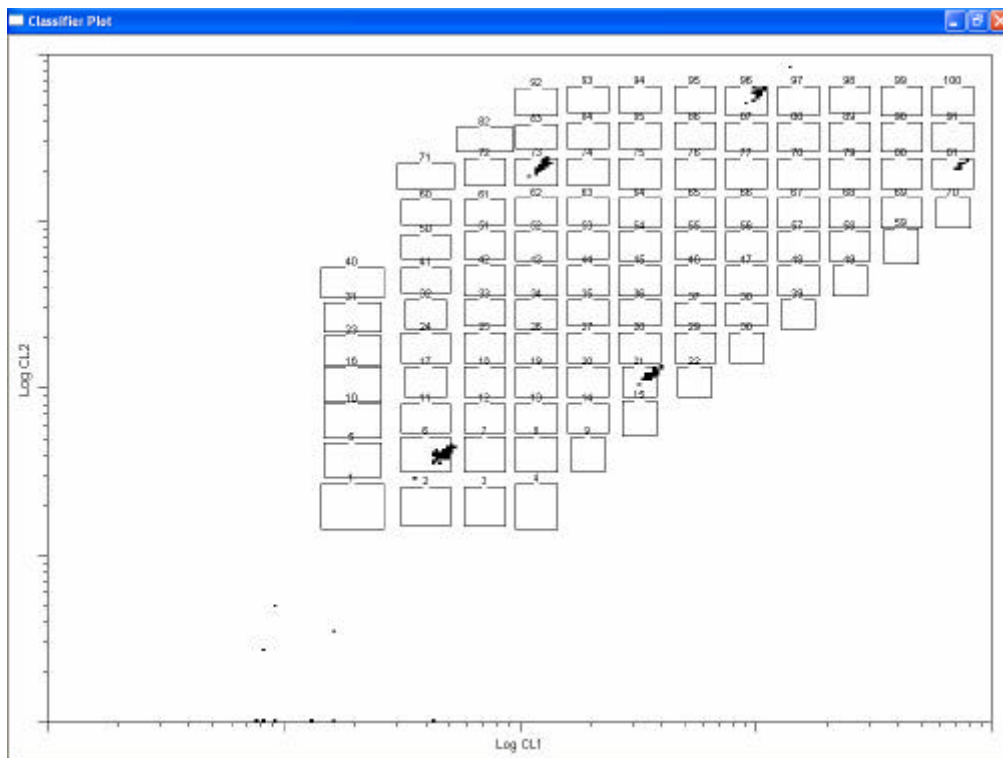


Figure 3.
CON1 Classification Microspheres on the 100 region bead map (STarStation Classifier Plot).

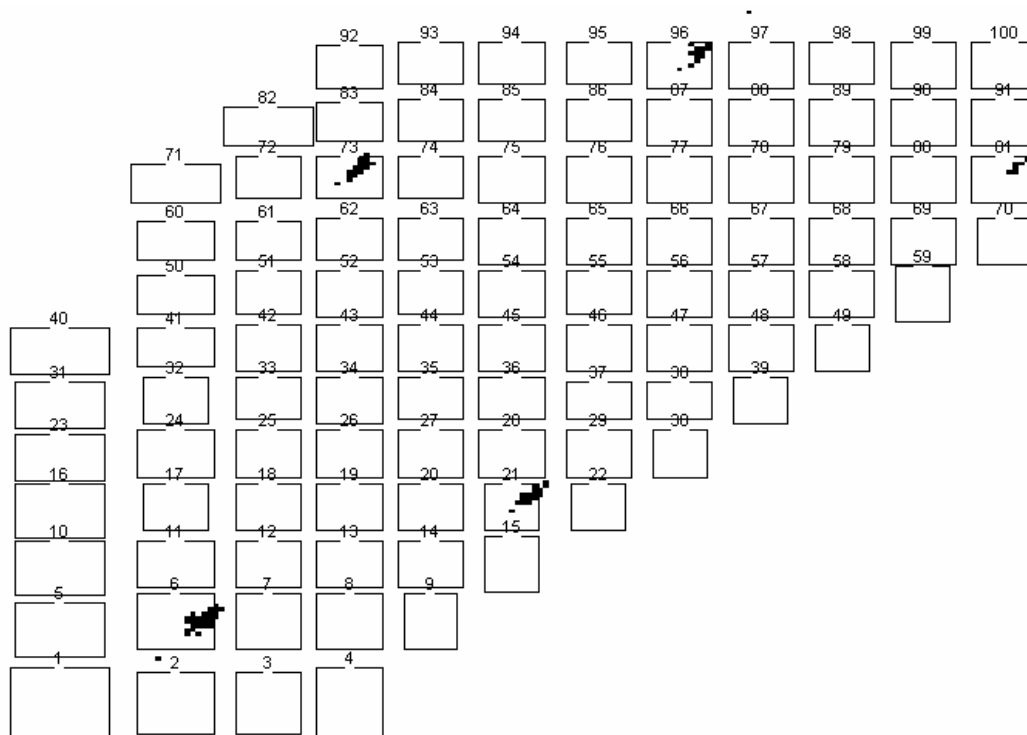


Figure4.

Luminex Control Microsphere (Con1 and Con2) Overview.

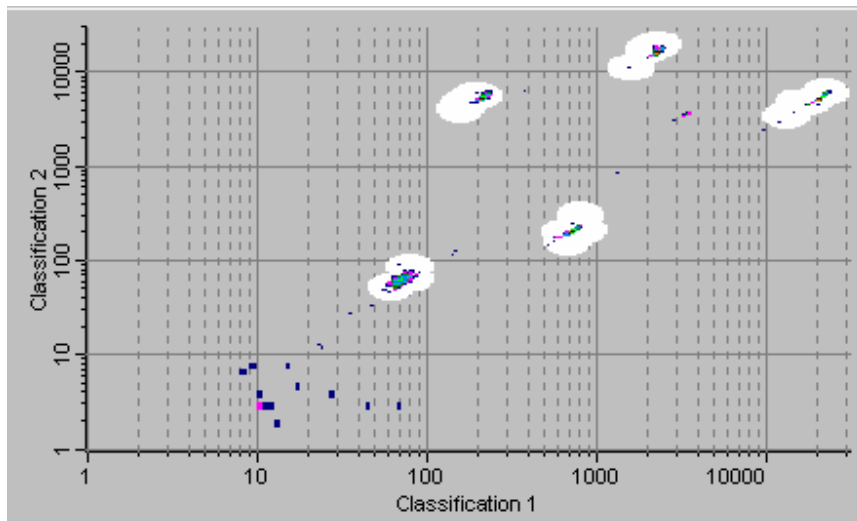


Figure 5. CON1 Classification Control Microspheres (Luminex Simplex Mode Plot).

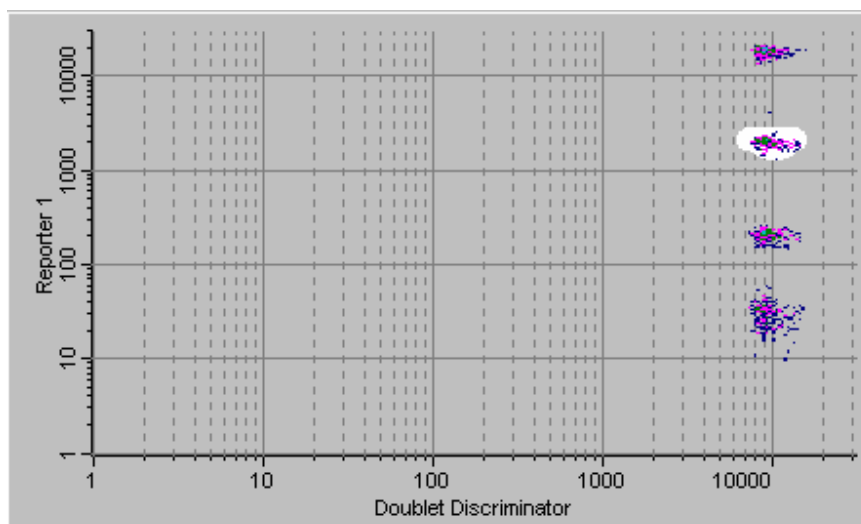
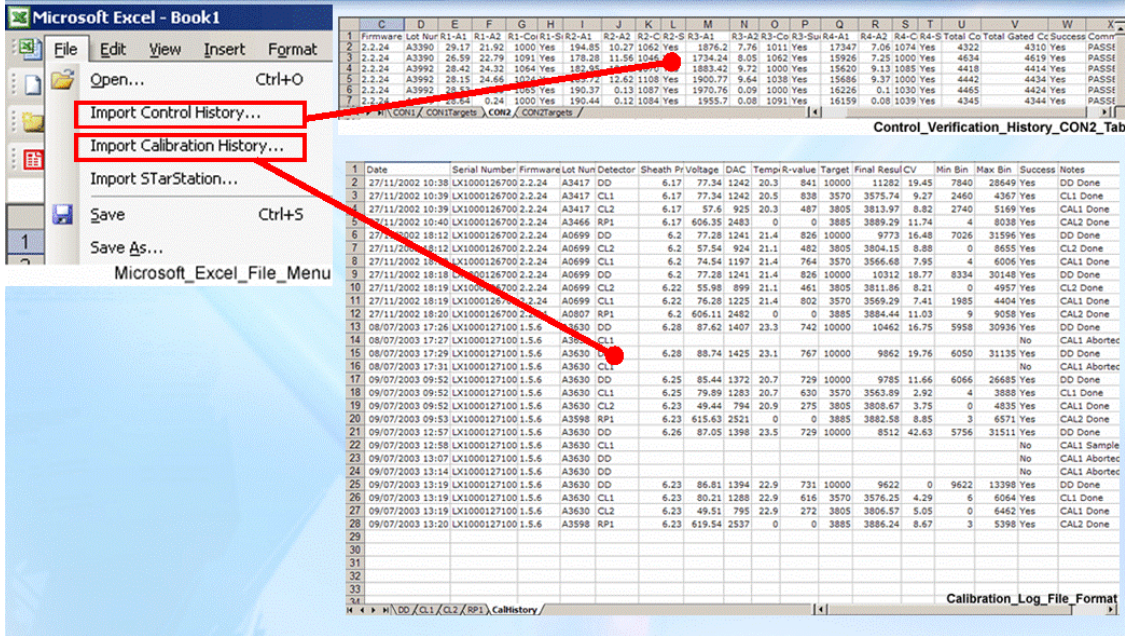


Figure 6. CON2 Reporter Control Microspheres (Luminex Simplex Mode Plot).

The Control verification history for your Luminex system can be reviewed via the **Import Control History...** option in the STarStation Data Import Excel Add-In (see below)

CON and CAL History .CSV File

Applied Cytometry Systems



Microsoft Excel - Book1

File Edit View Insert Format

Open... Ctrl+O

Import History...

Import Calibration History...

Import SStarStation...

Save Ctrl+S

Save As...

Microsoft_Excel_File_Menu

Control_Verification_History_CON2_Tab

1	Date	Serial Number	Firmware Lot	Nur	Detector	Sheath P	Voltage	DAC	Temp	R-value	Target	Final Res	CV	Mn Bin	Max Bin	Success	Notes
2	27/11/2002 10:38	LX1000126700 2.2.24	A3417	DD	6.17	77.34	1242	20.3	841	10000	11282	19.45	7840	28649	Yes	DD Done	
3	27/11/2002 10:39	LX1000126700 2.2.24	A3417	CL1	6.17	77.34	1242	20.5	838	3570	3575.74	9.27	2460	4367	Yes	CL1 Done	
4	27/11/2002 10:39	LX1000126700 2.2.24	A3417	CL2	6.17	57.6	925	20.3	487	3805	3813.97	8.82	2740	5169	Yes	CL1 Done	
5	27/11/2002 10:40	LX1000126700 2.2.24	A3466	RP1	6.17	606.35	2483	0	0	3885	3889.29	11.74	4	8038	Yes	CL2 Done	
6	27/11/2002 18:12	LX1000126700 2.2.24	A0699	DD	6.2	77.28	1241	21.4	826	10000	9773	16.48	7026	31596	Yes	DD Done	
7	27/11/2002 18:12	LX1000126700 2.2.24	A0699	CL2	6.2	57.54	924	21.1	482	3805	3804.15	8.88	0	8655	Yes	CL2 Done	
8	27/11/2002 18:18	LX1000126700 2.2.24	A0699	CL1	6.2	74.54	1197	21.4	764	3570	3566.68	7.95	4	6006	Yes	CL1 Done	
9	27/11/2002 18:18	LX1000126700 2.2.24	A0699	DD	6.2	77.28	1241	21.4	826	10000	10312	18.77	8334	30148	Yes	DD Done	
10	27/11/2002 18:19	LX1000126700 2.2.24	A0699	CL2	6.22	55.98	899	21.1	461	3805	3811.86	8.21	0	4957	Yes	CL2 Done	
11	27/11/2002 18:19	LX1000126700 2.2.24	A0699	CL1	6.22	76.28	1225	21.4	802	3570	3569.29	7.41	1985	4404	Yes	CL1 Done	
12	27/11/2002 18:20	LX1000126700 2.2.24	A0807	RP1	6.2	606.11	2482	0	0	3885	3884.44	11.03	9	9058	Yes	CL2 Done	
13	08/07/2003 17:26	LX1000127100 1.5.6	A3630	DD	6.28	87.62	1407	23.3	742	10000	10462	16.75	5958	30936	Yes	DD Done	
14	08/07/2003 17:27	LX1000127100 1.5.6	A3630	CL1	6.28	88.74	1425	23.1	767	10000	9862	19.76	6050	31135	Yes	DD Done	
15	08/07/2003 17:29	LX1000127100 1.5.6	A3630	CL2	6.28	88.74	1425	23.1	767	10000	9862	19.76	6050	31135	Yes	DD Done	
16	08/07/2003 17:31	LX1000127100 1.5.6	A3630	CL1	6.25	85.44	1372	20.7	729	10000	9785	11.56	6066	26685	Yes	DD Done	
17	09/07/2003 09:52	LX1000127100 1.5.6	A3630	DD	6.25	85.44	1372	20.7	729	10000	9785	11.56	6066	26685	Yes	DD Done	
18	09/07/2003 09:52	LX1000127100 1.5.6	A3630	CL1	6.25	79.89	1283	20.7	630	3570	3565.89	9.92	4	3888	Yes	CL1 Done	
19	09/07/2003 09:52	LX1000127100 1.5.6	A3630	CL2	6.23	49.44	794	20.9	275	3805	3808.67	3.75	0	4835	Yes	CL1 Done	
20	09/07/2003 09:53	LX1000127100 1.5.6	A3598	RP1	6.23	615.63	2521	0	0	3885	3882.58	8.85	3	6571	Yes	CL2 Done	
21	09/07/2003 12:57	LX1000127100 1.5.6	A3630	DD	6.26	87.05	1398	23.5	729	10000	8512	42.63	5756	31511	Yes	DD Done	
22	09/07/2003 12:58	LX1000127100 1.5.6	A3630	CL1	6.26	87.05	1398	23.5	729	10000	8512	42.63	5756	31511	Yes	DD Done	
23	09/07/2003 13:07	LX1000127100 1.5.6	A3630	DD	6.23	87.05	1398	23.5	729	10000	8512	42.63	5756	31511	Yes	DD Done	
24	09/07/2003 13:14	LX1000127100 1.5.6	A3630	DD	6.23	87.05	1398	23.5	729	10000	8512	42.63	5756	31511	Yes	DD Done	
25	09/07/2003 13:19	LX1000127100 1.5.6	A3630	DD	6.23	86.81	1394	22.9	731	10000	9622	0	9622	13398	Yes	DD Done	
26	09/07/2003 13:19	LX1000127100 1.5.6	A3630	CL1	6.23	80.21	1288	22.9	616	3570	3576.25	4.29	6	6064	Yes	CL1 Done	
27	09/07/2003 13:19	LX1000127100 1.5.6	A3630	CL2	6.23	49.51	795	22.9	272	3805	3806.57	5.05	0	4642	Yes	CL1 Done	
28	09/07/2003 13:20	LX1000127100 1.5.6	A3598	RP1	6.23	619.54	2537	0	0	3885	3886.24	8.67	3	5398	Yes	CL2 Done	

Calibration_Log_File_Format

A table outlining the CON microsphere parameter definitions is provided below.

CON History File

Applied Cytometry Systems

Masking Term	CON1 Definition	CON2 Definition
Count	The number of classified beads collected for a reference	The number of classified beads collected for a reference
Target	The target value for a reference	The target value for a reference
Tolerance	The allowed tolerance expressed as a percentage of the corresponding target value.	The allowed tolerance expressed as a percentage of the corresponding target value.
A1	The resulting DD peak for the reference	The resulting RP1 mean for the reference
A2	The resulting DD CVs for the reference	The resulting RP1 CVs for the reference
B1	The resulting CL1 mean for the reference	Not used.
B2	The resulting CL1 CVs for the reference	Not used.
C1	The resulting CL2 mean for the reference	Not used.
C2	The resulting CL2 CVs for the reference	Not used.
D1	The resulting RP1 mean for the reference	Not used.

Control_Verification_Parameter_Definitions



	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q
1	Date Entered	Lot Number	Expiration Date	R1-A1	R1-A2	R1-Count	R2-A1	R2-A2	R2-Count	R3-A1	R3-A2	R3-Count	R4-A1	R4-A2	R4-Count		
2	10/04/2003 16:35	A3390		29	23.1	1000	197	11.6	1000	1910	9.4	1000	17429	9.7	1000		
3	05/08/2004 12:06	A3992		28	23.1	1000	187	11.6	1000	1936	9.4	1000	15988	9.7	1000		
4	20/01/2005 12:17	A4249		28.4	23.1	1000	187	11.6	1000	1936	9.4	1000	15988	9.7	1000		

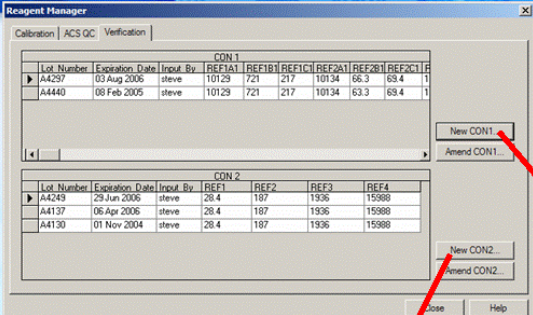
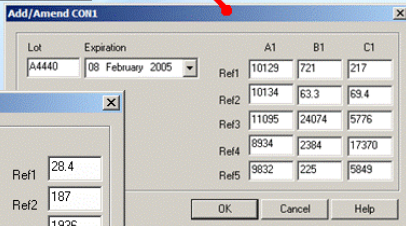
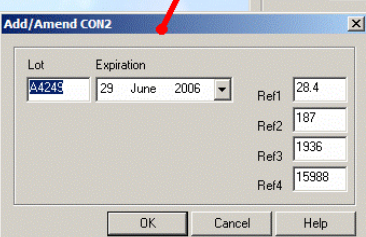
Control_Verification_History_File_CON2Targets_Tab

Luminex Control Microsphere (Con1 and Con2) Overview.

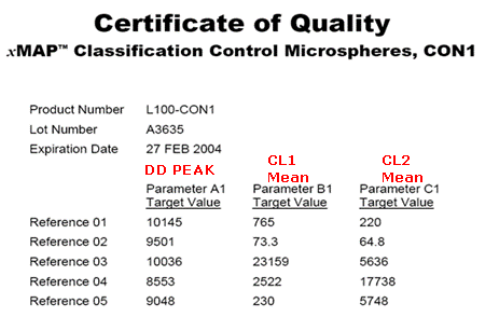
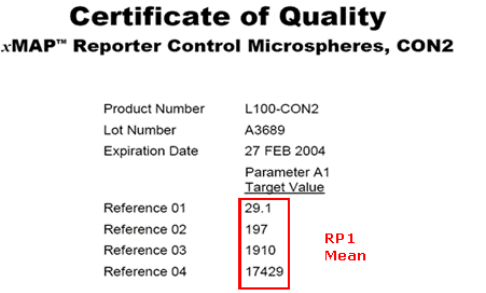
To perform Control verification of your system, the target values for the CON1 and CON2 microspheres must first be entered into the STarStation Reagent Manager.

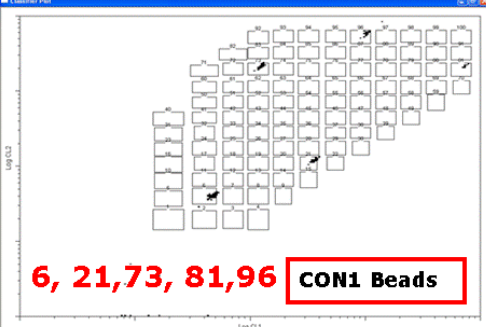
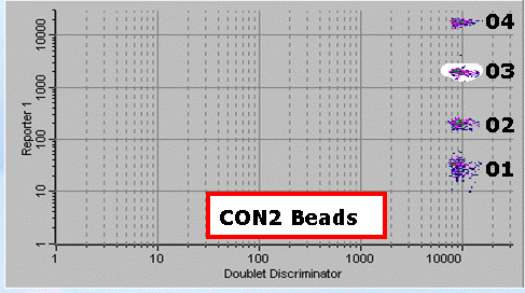
Reagent Manager Control Verification Reagents

Control Verification Script

Luminex Control Microsphere (Con1 and Con2) Overview.

CON2

Applied Cytometry Systems

CON2 Microspheres Reporter Controls No Colour Code, 4 levels of Reporter Fluorescence

