Bio-Plex-200 Best Practice and maintenance

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Components of Bioplex 200

1. PC and monitor — controls the Bio-Plex system via Bio-Plex Manager software

2. Array reader — combines 2 lasers, fluidics, and real-time digital signal processing for beads identification and analytes signal

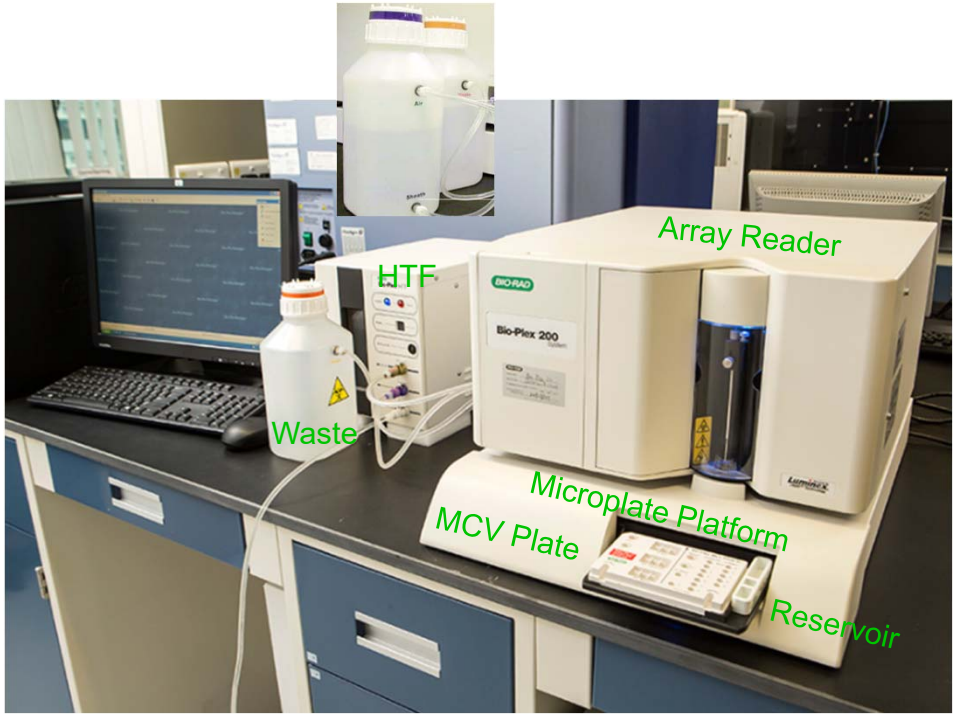
3. Microplate platform — automates the reading of 96-well plates

4. MCV plate — automates the maintenance, calibration, and validation functions of the array reader

5. Bio-Plex reservoir — hold water, 70% IPA and 10% bleach for maintenance functions eg. wash before and after a run

6. Optional HTF — delivers up to 20 L of sheath fluid without user intervention

7. Waste bottle — collect the waste during run



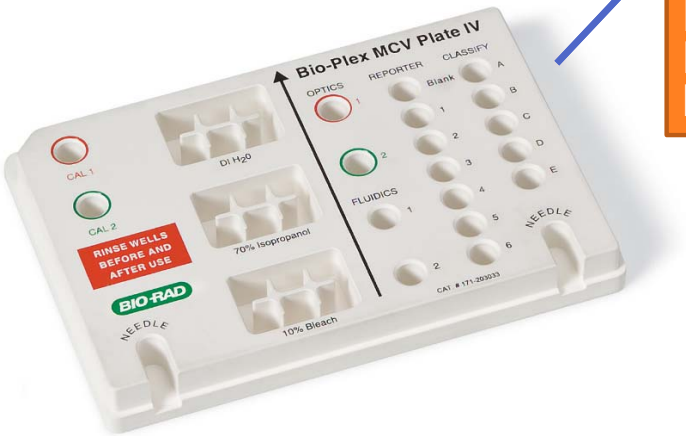
TIPS: Loosen the waste cap before run

The sheath fluid and waste bottle must be placed at the same level

The MCV plate

Calibration requires the Bio-Plex MCV Plate

- MCV Plate (Maintenance, Calibration, Validation)
  - Is required for most of the actions you will do on the system
    - Start Up
    - Calibration
    - Shut Down
  - Wash between plates
  - Needle height adjustment
  - Validation



Don't lose the plate! Keep it in the "Bio-Plex" logo compartment.

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Bio-Plex Manager Software v6.2

- The BPM v6.2
  - Win 7 and win 10
  - Controls operation of Bio-Plex protein array system
  - Performs automated instrument Validation and Calibration
  - Performs automated analysis of Bio-Plex assay data

- Comes with 3 types of dongle/license.
  - Instrument Control (full operations)
  - Desktop (protocol development and data analysis only)
  - Network (multiple concurrent users)

Bio-Plex Manager 6.2

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Readying your Bioplex (45min)

How many switches are there to turn on in the system?

- Calibration and Validation beads (if required) to bring to rtp.
- Switching on your machines
  - Reader
  - Platform
  - HTF
  - PC/laptop
- 30mins for the laser to warm up
- LOOSEN WASTE BOTTLE CAP
- Turn on BPM

If you have a plate ready to read and the system is NOT ready, keep the beads plate in suspension until it is.

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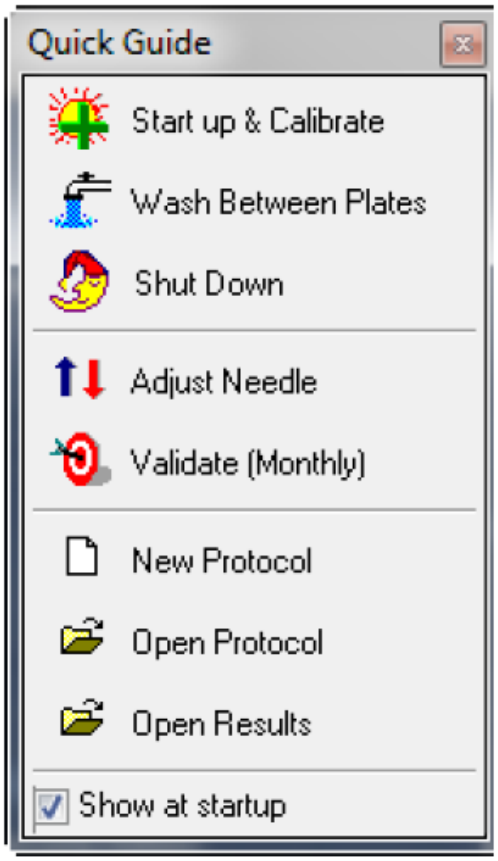
Startup + calibration



Once you click on startup, the system will prime the lines, regulate pressure for the sheath fluid and calibrate in sequence.

To open the Quick Guide after closing, select Quick Guide from the View menu

Start Up, Warm Up, and Calibration functions must be performed prior to running an assay with Bio-Plex Manager

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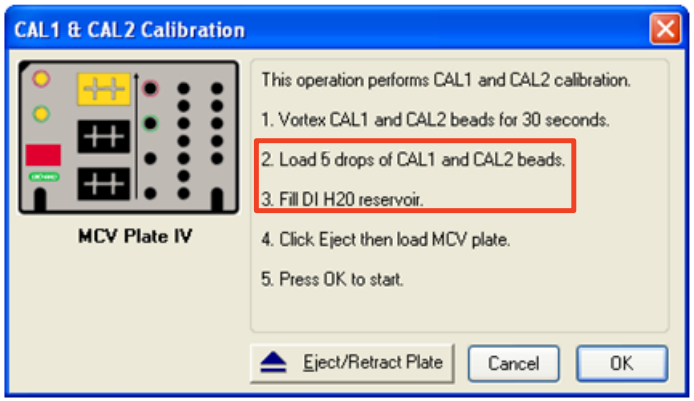
Readying your Bioplex- Calibration

- Essential for optimal performance and day to-day reproducibility of results.
- Perform after optics are warmed up (~30min)

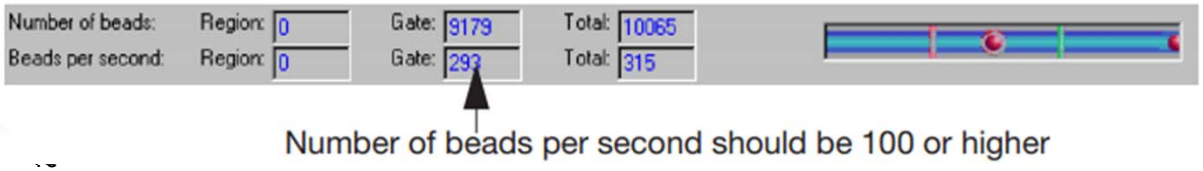


Calibration kit — contains CAL1 and CAL2 beads with stable fluorescent intensities

TIPS: Thaw the calibration beads to room temperature before used. Vortex to suspend the beads for efficient calibration

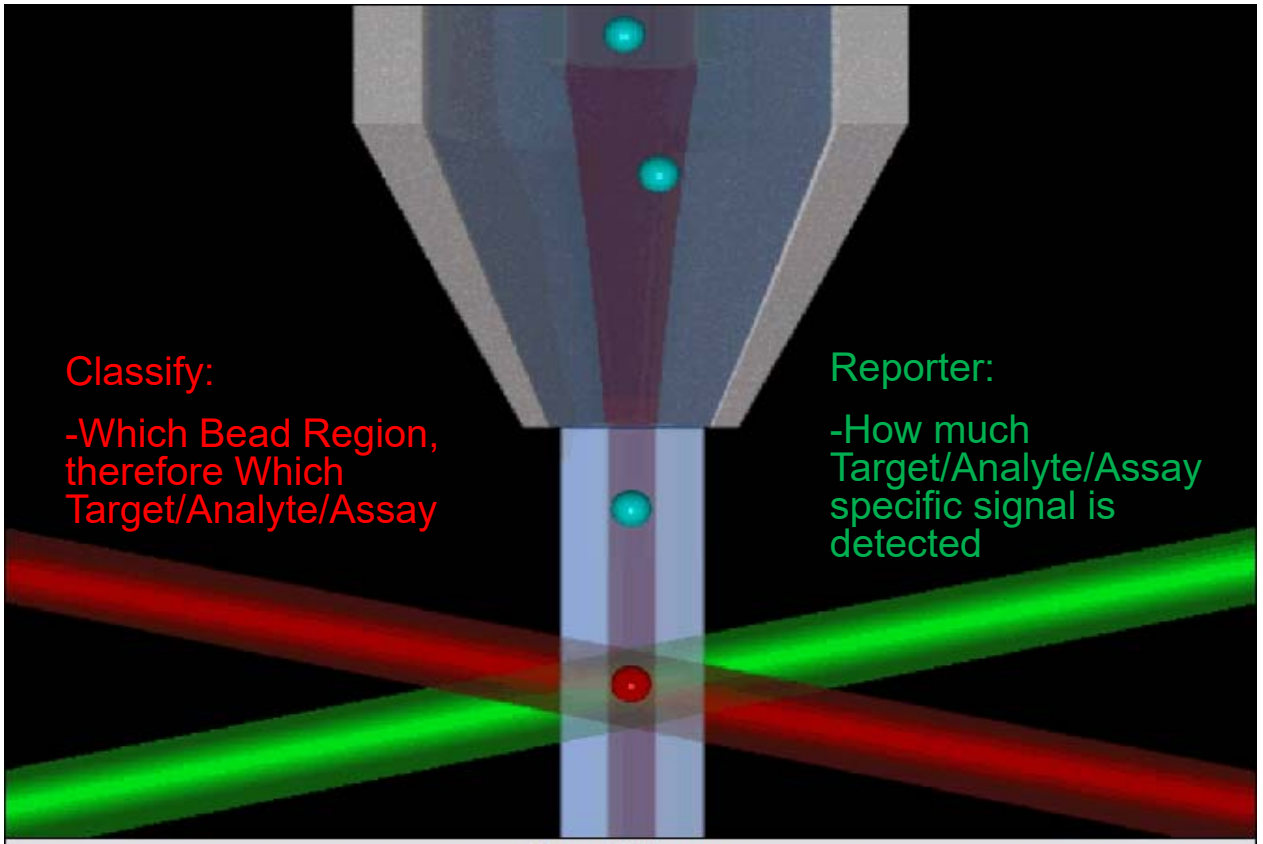
Optics will off automatically if the array reader is idle for more than four hours. Full 30-minute warm up period may be required.

Do the calibration the day before the important experiment as well



What should we do when the beads fall below 100?
1. Vortex the beads and redo calibration
2. Check the sample probe height
3. Clean the sample probe

Flow Cell



flowcell fullscreen

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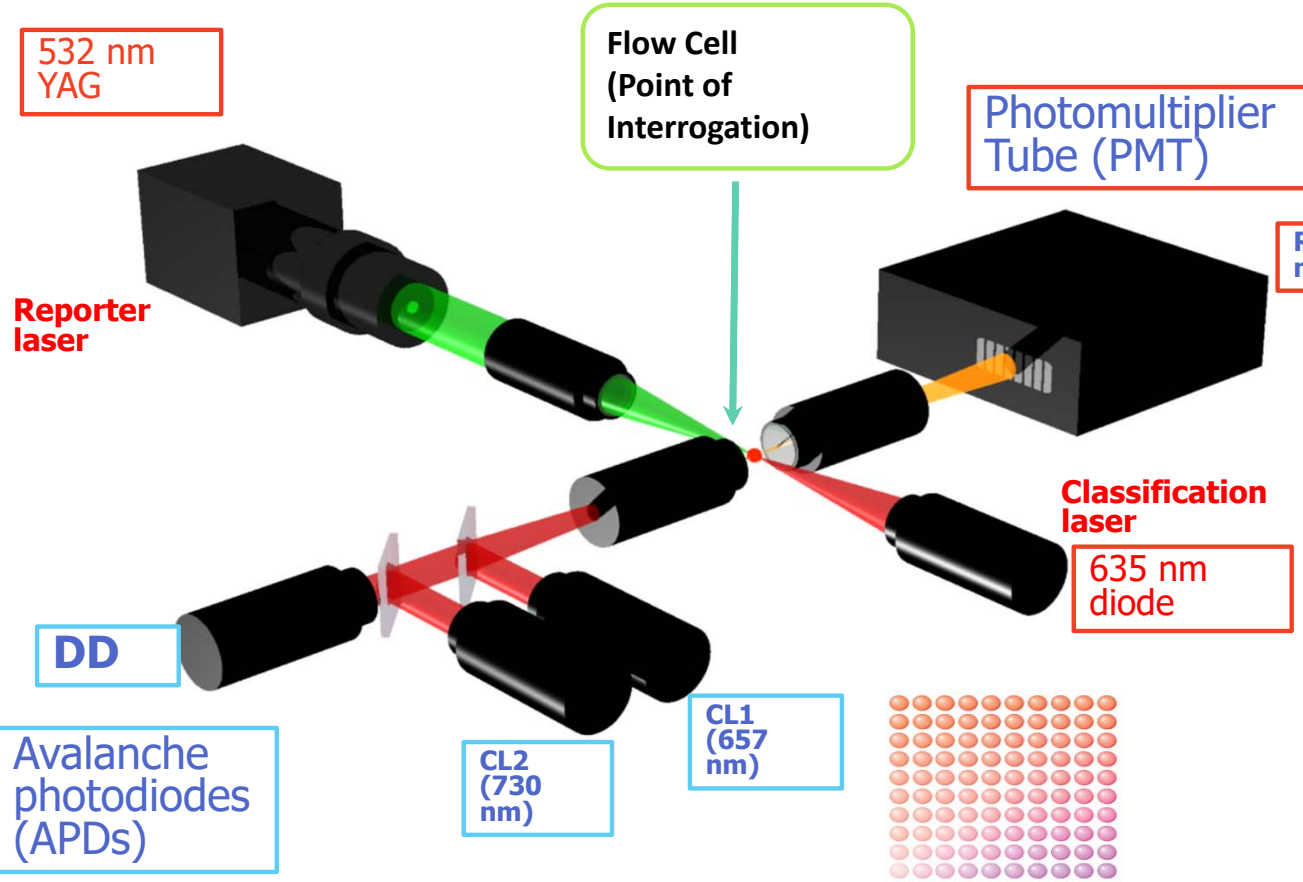
Where does calibration measure?



Calibration adjusts voltage settings for optimal and consistent readings over time and across different instruments.

CAL1 calibrate the array reader's doublet discriminator and classification channels

CAL2 beads calibrate the array reader's reporter channel for reporter fluorescence detection

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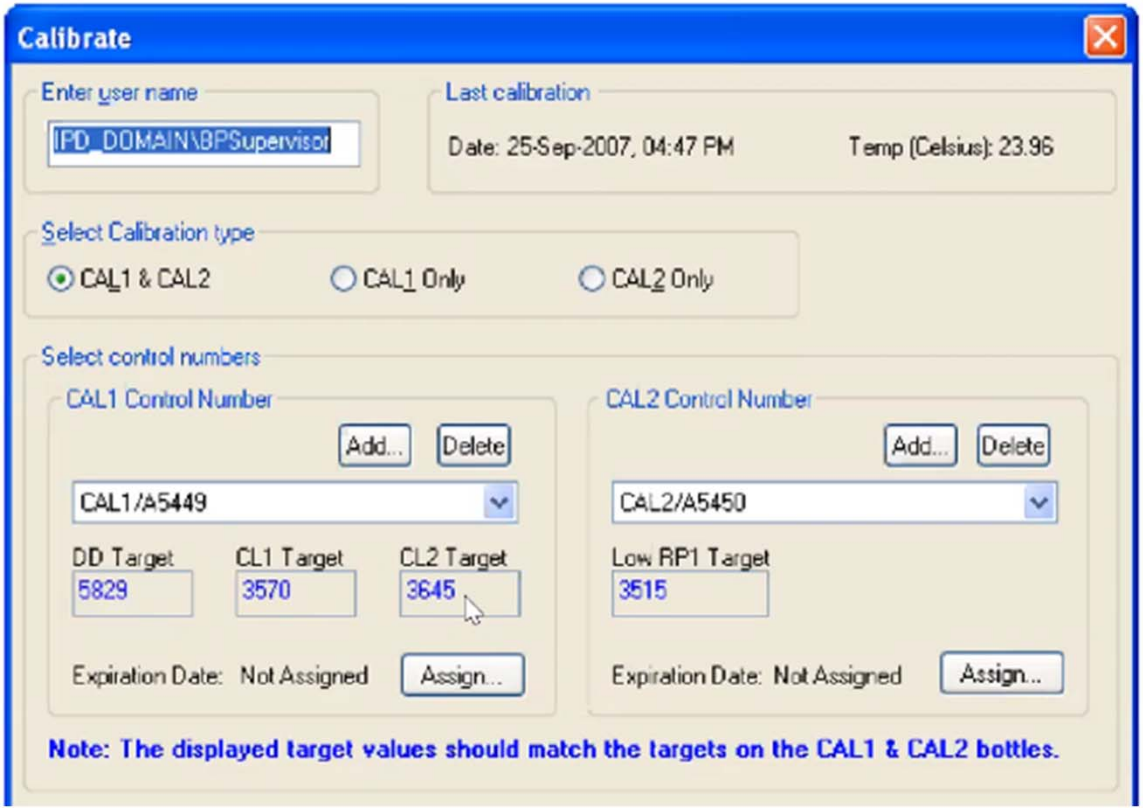
Where does calibration measure?



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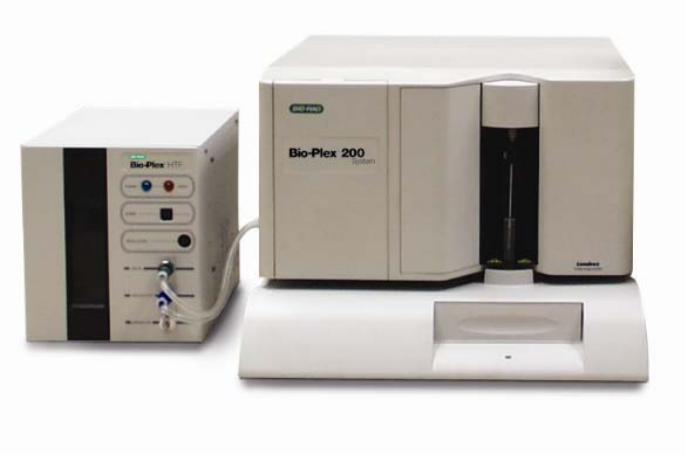
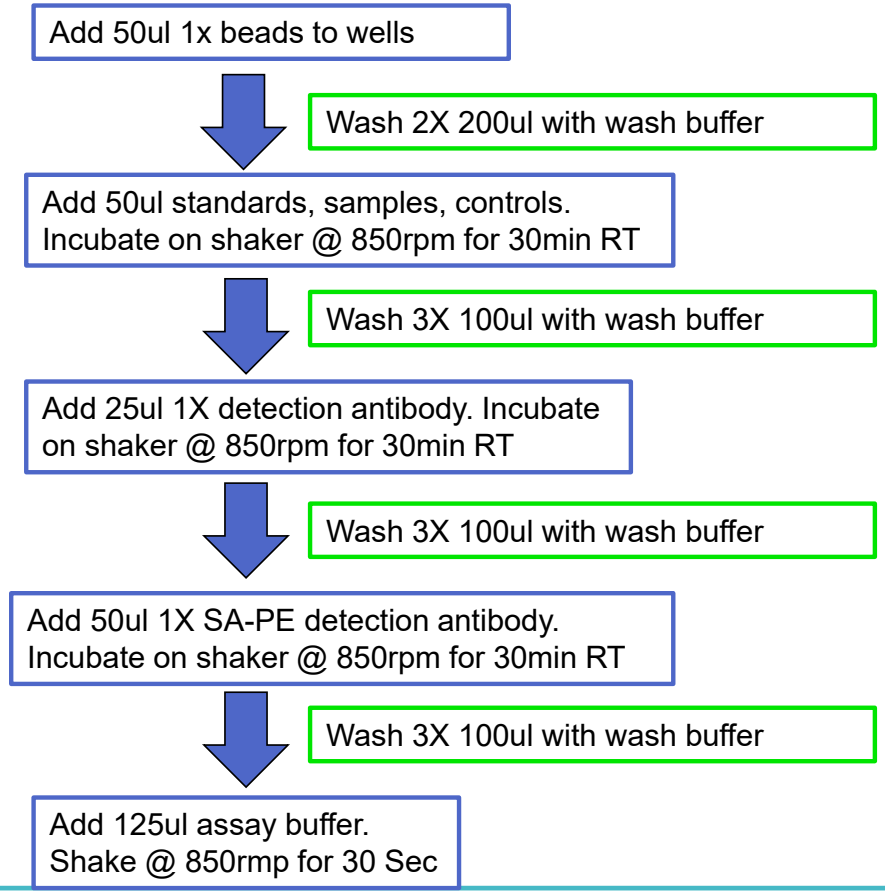
RECOMMENDED GOOD PRACTICES



Cytokine Assay workflow (~3 hours)



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Sample matrices used with Bio-Plex assays

Human, Mouse, Rat, Canine, NHP Samples

- Serum*
- Plasma*
- Tissue/Cell Culture Supernatant*
- Tumor biopsies
- Urine
- Tissue lysate - Colon, Kidney, Lung, Nervous System & Spleen
- Newborn blood spots
- Cerebrospinal Fluid
- Bronchoalveolar Lavage Fluid
- Fat (Adipose) Interstitial Fluid
- Nasal Lavage Fluid
- Peritoneal Fluid
- Blister Fluid
- Synovial Fluid

* Validated internally by Bio-Rad

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Cytokine Levels in Plasma and Serum

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Cytokine. 2012 November ; 60(2): 438–446. doi:10.1016/j.cyto.2012.05.019.

Effect of anticoagulants on multiplexed measurement of cytokine/chemokines in healthy subjects

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- Evaluated the performance characteristics of 72 analytes
- Differences of analytes measured in serum compared to plasma
- Impact of anticoagulants on the cytokine measurement in plasma

Cytokine Levels in Plasma and Serum

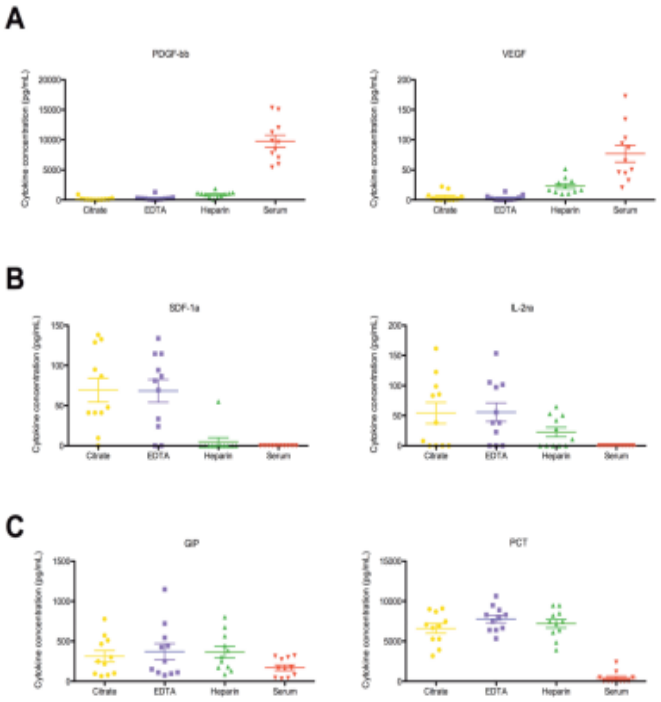

Figure 1. Comparison of measured concentrations for cytokines in serum specimens to all plasma specimens

Results show concentrations of analytes for all paired individuals (n=11) with the middle bar showing mean concentrations and bars the standard error of the mean.

A) Example of cytokines that yield higher measurements in serum than plasma

B) Example of cytokines that are not detectable in serum compared to plasma

C) Example of cytokines that yield lower measurements in serum than plasma

- **19 analytes**, including PDGF-bb, IL-4, IL-8, IL-9, FGF-b, PAI-1, CXCL-5, CCL-5, CD40L, EGF, VEGF, IL-2ra, IL-3, SDF-1a, PCT, MCP-3, GIP, IL-16 and fibrinogen, **showed significant differences** between measurements in serum and all types of plasma, regardless of anticoagulant
- Among plasma samples, **10 analytes** (eotaxin, SCGF-b, MCP-1, SCF, MIP-1b, VEGF, RANTES, PDGF-b, PAI-1 and ITAC) **showed significantly higher concentrations in heparinized plasma compared to citrated and EDTA plasma**.

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Serum or Plasma?

- Sample measurements can differ in both matrices, depending on analytes
- Plasma has anticoagulant
  - The process of coagulation makes serum qualitatively different from plasma
  - Protein concentration of serum is ~ 3 – 4% less than plasma
  - Keep consistency in sample collection, preparation and storage
- Choose matrix that results in more samples in linear range of curve
- Conduct a study with only one type of matrix to ensure data continuity

TIPS: Centrifugation to separate the serum from the red blood cells (10 mins at 1000g).

Aliquot the serum into 50-100ul aliquots and freeze at -70oC.

Avoid multiple freeze thaw cycles as cytokines degrade significantly after 2-3 freeze/thaws.

haemolysis.

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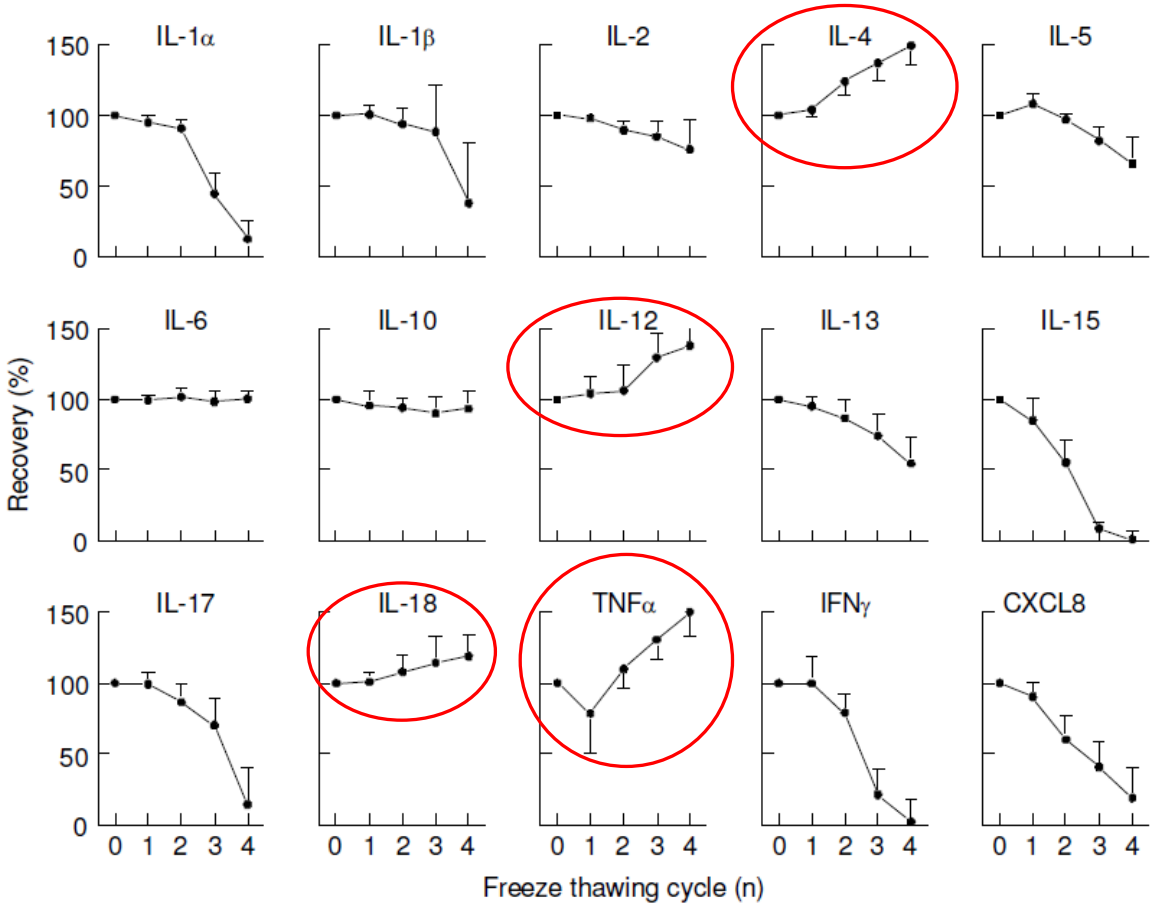
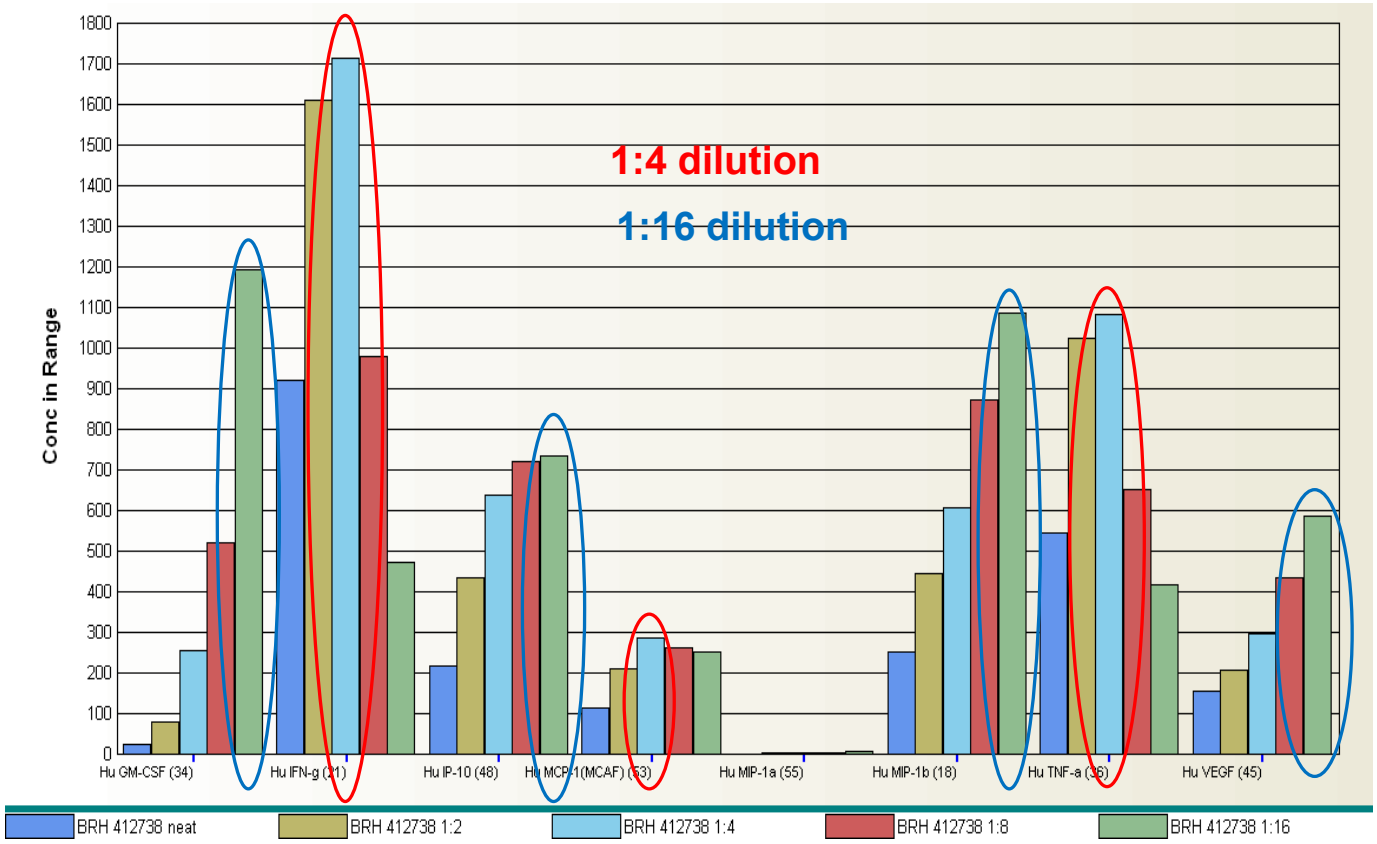
Effect of freeze thaw cycles on cytokine detection



Effect of sample dilution on cytokine detection



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Cytokine Assay workflow (~3 hours)

Add 50ul 1x beads to wells

Wash 2X 200ul with wash buffer

Kit reagents should be used at room temperature.

- The association and dissociation rates of an Ab:Ag complex are influenced by the temperature of the buffers.
- In cold buffers, the association rate constant will be reduced. The data might no be reproducible between assays.

Vortex the stock 10x beads for 30 seconds prior to dilution for use.

Add the required volume to the tube containing assay buffer

protect this from light until needed to avoid photo-bleaching

Cytokine Assay workflow (~3 hours)

Add 50ul 1x beads to wells

Wash 2X 200ul with wash buffer

Add 50ul standards, samples, controls.
Incubate on shaker @ 850rpm for 30min RT

Wash 3X 100ul with wash buffer

resuspend the lyophilized vial in **500ul** of standard buffer (or suitable alternative), **vortex gently** and then **incubate on ice for 30 minutes**.

Table 6. Summary of recommended diluents for standards and controls.

Sample Type	Diluent for Standards and Controls*	Addition of BSA
Serum and plasma	Standard diluent HB	None
Culture media, with serum	Culture medium	None
Culture media, serum free	Culture medium	To 0.5% final**
Lavage, sputum, other fluids	Sample diluent HB	To 0.5% final**
Cell/tissue lysate	Standard diluent HB	None

* If using diluents other than standard diluent, users must establish their own control ranges.

** At least 0.5% final BSA is recommended to stabilize analytes and reduce adsorption to labware.

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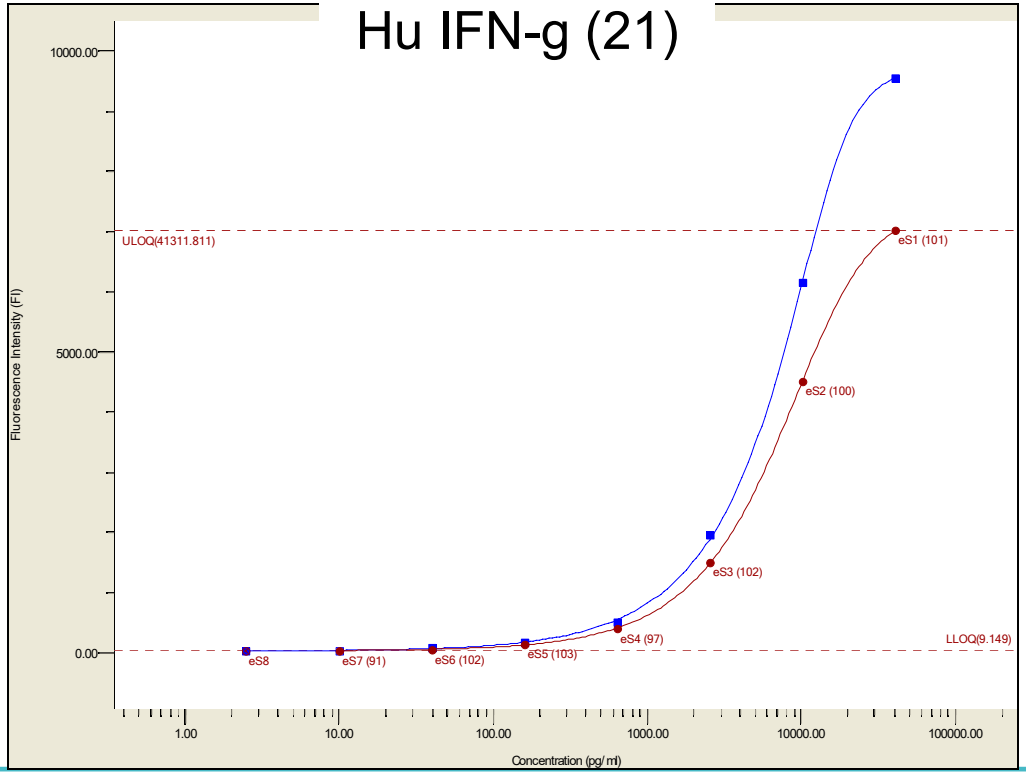
IFN-g resuspended on ice for 15 min (red graph) and for 30 min (blue graph)

When resuspended for 15 minutes on ice IFN-g standard curve showed a LLOQ of **9.15pg/ml** and a ULOQ of 41311 pg/ml

When resuspended for 30 minutes on ice the LLOQ was **2.75pg/ml** and ULOQ was 41205 pg/ml

This change in incubation time lead to a **>3x** reduction in the LLOQ for this target analyte.



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Cytokine Assay workflow (~3 hours)

Add 50ul 1x beads to wells

↓

Wash 2X 200ul with wash buffer

Add 50ul standards, samples, controls. Incubate on shaker @ 850rpm for 30min RT

↓

Wash 3X 100ul with wash buffer

Add 25ul 1X detection antibody. Incubate on shaker @ 850rpm for 30min RT

↓

Wash 3X 100ul with wash buffer

Add 50ul 1X SA-PE. Incubate on shaker @ 850rpm for 30min RT

↓

Wash 3X 100ul with wash buffer

Add 125ul assay buffer. Shake @ 850rmp for 30 Sec

Detection Antibodies,and 1X SA-PE :

Add using a **multichannel pipettor** in all steps, to minimize differences in incubation times between wells.

Detection antibodies, depending on level of multiplex, are supplied as **25x, 50x, or 100x**.

There is a specific **diluent**.

Use aluminum foil. Incubation in the dark.

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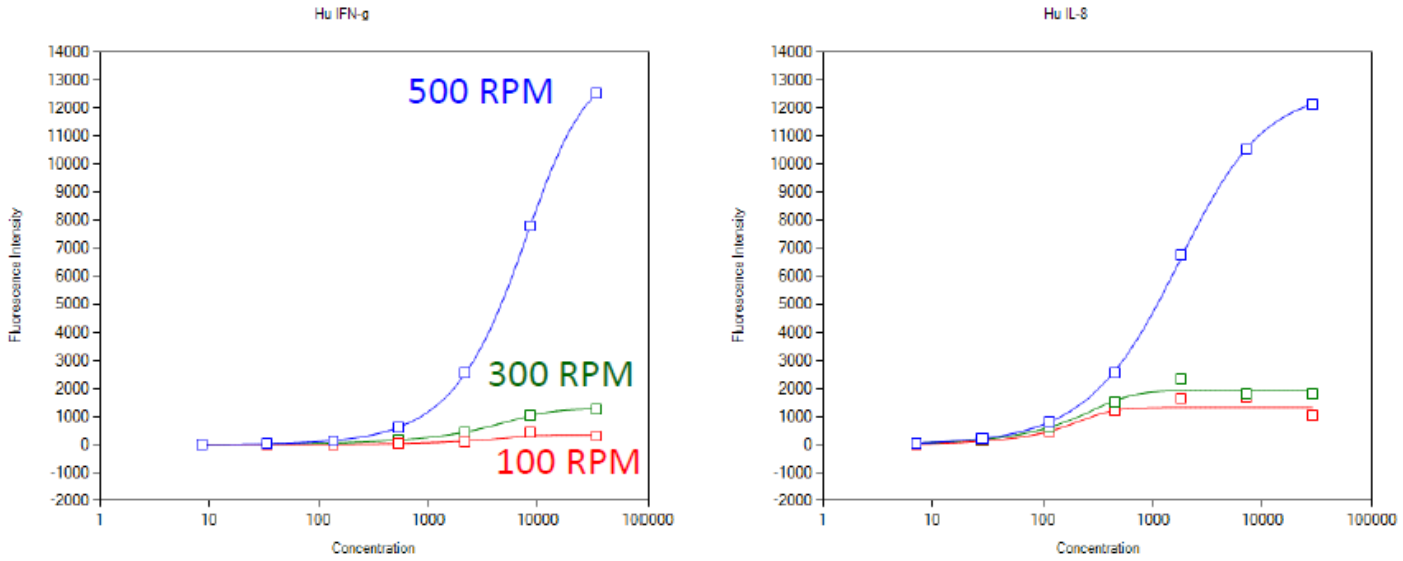
Impact of plate shaking speed



1. Optimal shaking speed is critical for complete re-suspension of beads.
2. Recommended setting 850 ±50 RPM
3. Keep assay settings consistent throughout studies

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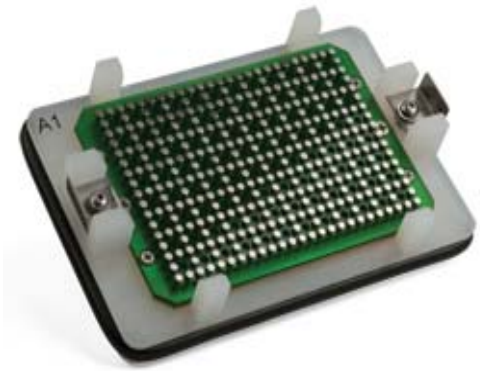
Consistent washing

- For our assay wash steps three 100ul washes are **not the same** as one 300ul wash!



TIPS: Make sure the plate is fitted nicely. You might loss the beads if the plate is not placed properly

TIPS: Check the dispensing probes before important run. Salt crystallization might clog the dispensing probe

Pipetting Tips

- Use calibrated pipettes and do not set them beyond their working volume range.
- **Pre-wet the pipette tip**: Aspirate and expel the required volume of sample 3-5 times before aspirating a sample for delivery.
- **Work at temperature equilibrium**: The viscosity of buffers and samples is affected by temperature. The volume of sample delivered by a pipette will vary with temperature.
- **Examine the tip before dispensing sample**: Check there are no drops of liquid on the outside of the tip before transferring to avoid variations in the volume transferred.
- **Pause after aspiration**: Pause with the tip in the liquid for 1-2 seconds after aspirating as the amount of liquid in the tip 'bounces' when the plunger stops.
- **Hold the pipette straight not at an angle**: Surface tension effects within the tip cause the sample volumes to very if the pipette is held at an angle, touching the tip on the side of the tube results in sample loss.
- **Use consistent plunger pressure and speed**: Depress and release the plunger smoothly and with consistent pressure and speed for each and every sample.

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Before you start sample prep

Map out your plate on paper. Do I want **duplicate or triplicate** data for samples and standards?

Review the protocol to be used. Gather materials needed.

Clean the work surface to avoid contamination of reagents.

Include **blanks (buffer only) in at least replicates.**

Bring all reagents to **room temperature**, including calibration kit bottles. Cold reagents warming during a plate read can form bubbles in the fluidics.

Document any changes to protocol both in the software and on paper.

https://www.bio-rad.com/webroot/web/html/lsr/products/bioplex_suspension_array/bio-plex-user-manuals.html

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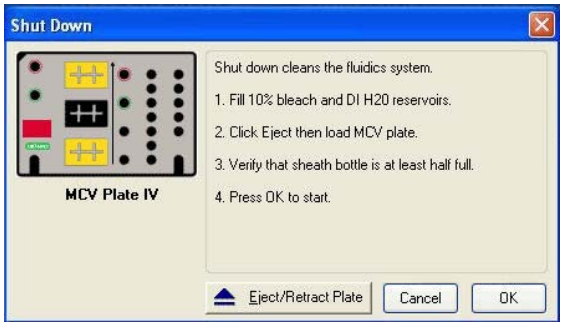
MAINTENANCE & UPKEEP

Recommended care and maintenance

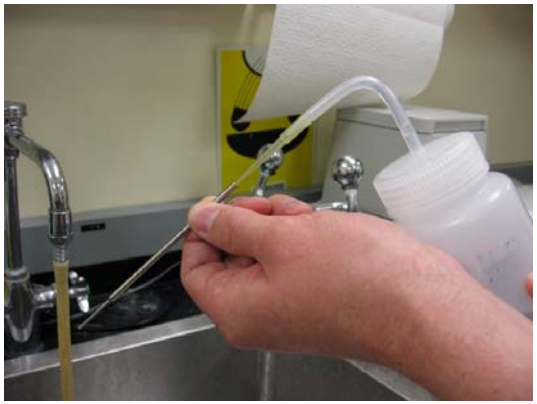
Daily	Startup, calibrate, wash between plates, shut down
Weekly	Sonicate needle, unclog, check for leaks
Monthly	Validation, clean exterior surface
6 months	Replace syringe seal, clean ventilation filter
Yearly	Replace sheath filter, replace air intake filter

Always run the shutdown step at the end of the day before turning off the Bio-Plex

- Perform **proper shutdown** procedure.
  - This **sanitizes** the fluidics system with 10% bleach and then flushes the needle and sample tubing with DI H_20.
  - Failure to do this will lead to **salt precipitation** in the sample probe and tubing.



- Once a week, remove and **clean** the sample **needle**.



How to remove probe (youtube):
https://www.youtube.com/watch?v=o5jWPfVjYuk

Probe Cleaning (youtube)
https://www.youtube.com/watch?v=RrzpYkRO568

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Set the needle height correctly for the type of assay plate you are running.

cut out for flat bottom or filter plates

cut out for **PCR plate** compatible with BPM 6.0

- If the sample needle is set **too low**,
  - Damaging the sample needle
  - Low bead counts
- If the sample needle is set **too high**,
  - Air gets drawn into the system during reading

TIPS: Get the tip to ~2mm gap between tip and MCV well bottom

Adjusting Probe height (youtube):
https://www.youtube.com/watch?v=W-ckfD025jw

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Validation

- Perform **validation** test once **every 30 days**
  - Validates operation of all the primary components of the array system
- Ensure the kit is at **RT** before use.
- Each Bio-Plex Validation Kit consists of reagents and procedures to evaluate:
  - Optical alignment
  - Integrity of fluidics
  - Reporter channel performance
  - Efficiency of multiplexing
- If one part of the validation test fail, then vortex the bottles again and re-run that test.

TIPS: Run before an important run/demo.

Template: D001227 Rev. C

Bio-Plex Suspension Array System Validation Report

Test Performed by: GLOBAL\djethwa
Validation Kit Control #: 310008797 (Low)
RP1 Target Value: 3855
Reader Serial #: LX10007005403
Date/Time: 04-Aug-2011 10:25:10 AM
Expiration Date: 12-Nov-2011
RP1 PMT Voltage: 619.54
Access Level: Unrestricted

I.Optics Validation Result: Passed

Parameter	Specification	Measured Value	Pass/Fail
DD Median	4774 - 6593	6401	Pass
CL1 Median	3134 - 4067	3885	Pass
CL1 CV%	2.00 - 7.00	6.36%	Pass
CL2 Median	3190 - 4142	3767	Pass
CL2 CV%	3.00 - 8.00	7.68%	Pass
RP1 Median	3260 - 4231	3702	Pass
RP1 CV%	4.00 - 10.00	9.35%	Pass

II. Fluidics Validation Result: Passed

Parameter	Specification	Measured Value	Pass/Fail
% Carryover	< or = 4.0%	2.0%	Pass

III. Reporter Validation Result: Passed

A. Calculated Values

Parameter	Specification	Measured Value	Pass/Fail
Dynamic Range	4.32 - 4.43	4.38	Pass
Linearity	> 0.995	1.000	Pass
Slope	34.83 - 43.82	40.08	Pass
Accuracy	> 90.00%	99.22%	Pass
Threshold	< 6 MFI	0	Pass

B. Raw Values

Bead	Median FI
Blank	0
1	15
2	56
3	576
4	1752
5	4023
6	24083

IV. Classify Validation Result: Passed

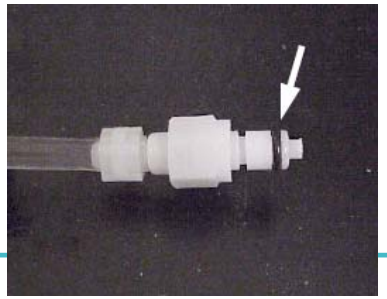
A. Classify Efficiency

Classify Bead	Specification	Measured Value	Pass/Fail
Bead A (1)	> 80.0%	98.2%	Pass
Bead B (4)	> 80.0%	98.4%	Pass
Bead C (40)	> 80.0%	97.7%	Pass
Bead D (54)	> 80.0%	97.8%	Pass

Report

System Maintenance

- **As Required**
  - Check sheath fluid and waste fluid levels
  - Adjust sample arm (needle) vertical height
  - Replace fuse
  - Check and replace O-rings on quick disconnect fittings



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Troubleshooting: Communication

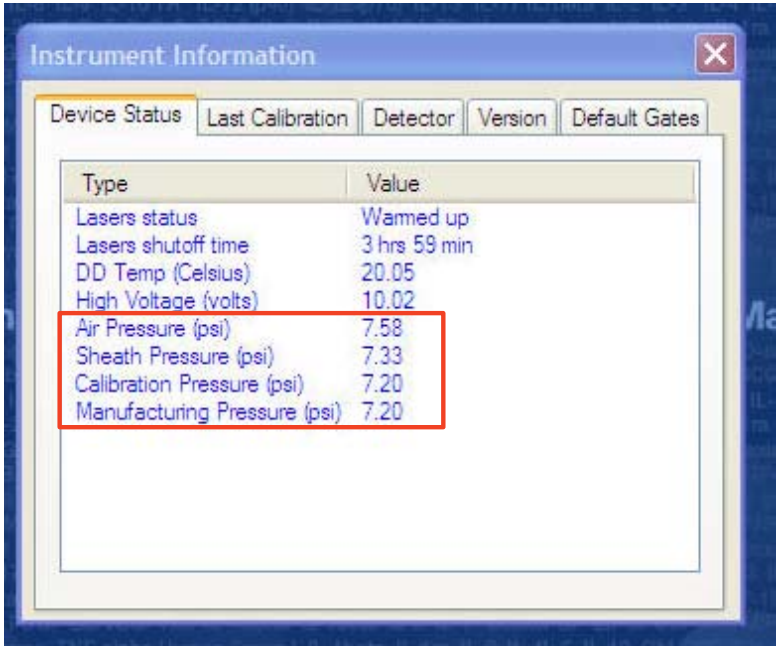
- **Connection Error**
  - Ensure that the outlets have power.
  - Check the power cord and communication cable connections.
  - Click the Reconnect button.
  - Shutdown the computer and instruments. Turn on the Reader, XY Platform and HTF and then restart the computer.

Troubleshooting: Pressurization

- **System is not pressurizing properly**
  - Check for Leaks.
  - Disconnect and reconnect the sheath and waste lines.
  - Inspect fittings for leaks or cracks.

Troubleshooting: Pressurization

- Click on the “I” icon (information) at the top left hand corner of the BPM software.
- Is the Calibration Pressure ± 5% and the Manufacturing Pressure ±10% of the Air/Sheath Pressure?



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Troubleshooting: Sample probe

- **Sample probe is leaking**
  - Sample probe may be clogged.
  - Sample valve may be faulty
- **Sample arm is stuck in the up position**
  - System may not be properly pressurized.
- **Sample arm fails to go all the way down (error during movement)**
  - Tubing above cheminert fitting is trapped in light housing.

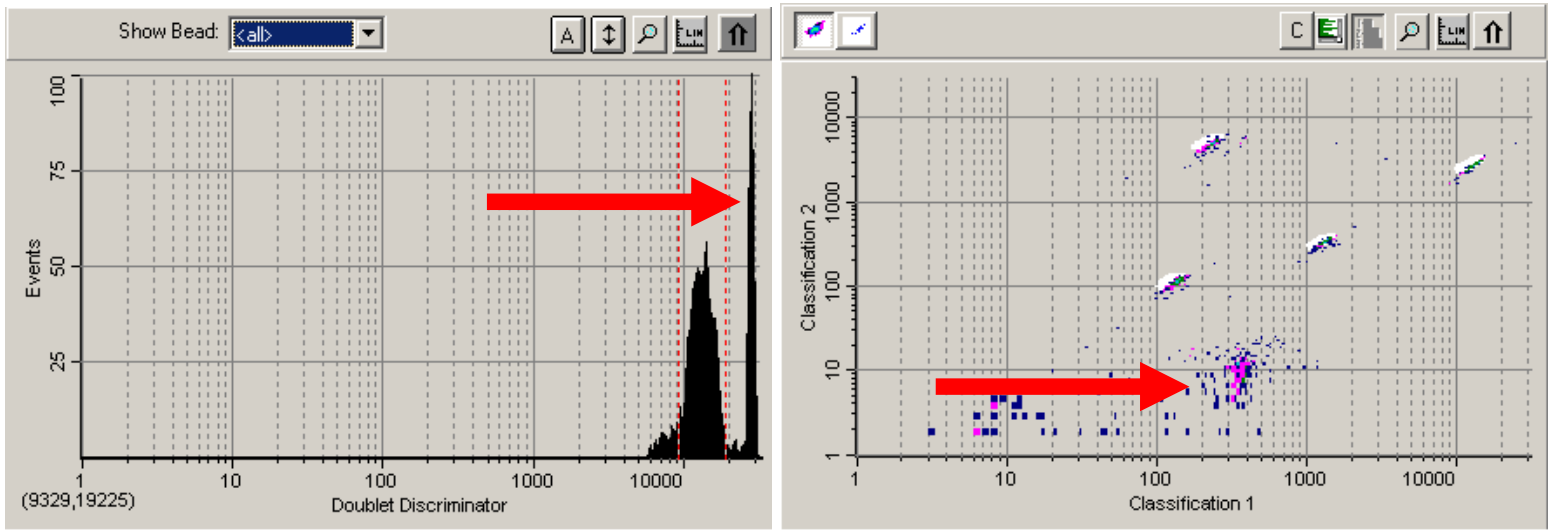
Troubleshooting: Sample probe

- **Sample arm does not go down smoothly**
  - 96-well plate may be warped or misaligned.
  - Sample probe may be bent.
- **Sample arm is stuck in the down position**
  - Sample probe height may be too low.
  - Path to the well may be blocked.

Troubleshooting: Calibration

- **If calibration fails, verify the following:**
  - Sample probe height was correct.
  - Correct lot numbers and target values were selected.
  - Vials were properly mixed prior to use (30 seconds)
  - Cal 1 and 2 beads added to correct wells of MCV plate.
  - At least 5 drops of the calibration beads were used.
  - At least 200 events/sec were collected.
  - Air and sheath pressure between 6-9 psi.
  - Repeat calibration with fresh reagents after checking the above.

What is this?



- Air in the System
  - Probe height needs adjusting
  - Leak in tubing

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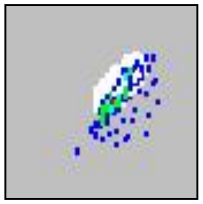
Bead irregularities

- Photobleached beads.



- Air in the system OR sheath fluid may be empty. Waste Bottle Cap is closed tight causing back pressure. Loosen the cap.

- Bead agglutination, incompatible solvent OR incompatible sheath fluid.

Thank you ☺

QUESTIONS?