HemoCue®
ISS Technology Demonstration

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Expanding the Boundaries of Space Medicine and Technology

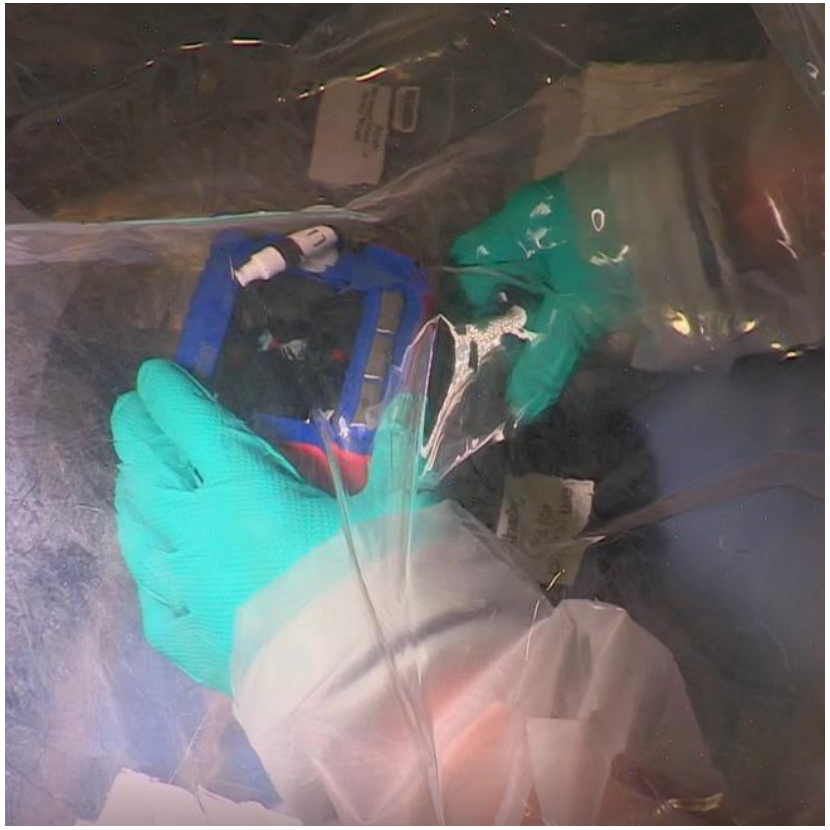
Introduction

- The HemoCue® White Blood Cell (WBC) with Differential (DIFF) Analyzer a commercial-off-the-shelf (COTS) device selected for this technology demonstration.

- HemoCue® measures total WBC count and 5-part differential (Neutrophils, Lymphocytes, Monocytes, Eosinophils & Basophils) in capillary or venous whole blood samples.

- WBC's are among the most common clinical biomarkers, by measuring them the management of numerous in-flight medical conditions become possible by providing the capability to assess severity of conditions and track responses to treatment. Examples include:
  - bacterial & viral infections (potential risk for sepsis)
  - acute radiation syndrome (potential risk for cancer)

- Ultimate goal is to validate HemoCue's ® performance in flight.



[HemoCue® Analyzer measuring a sample in the ISS Portable Glove Bag (IPGB) on-orbit]

Project Background

[Preparing a sample for ground testing with HemoCue® Analyzer]

- Prior to its demo on ISS, this HemoCue® Analyzer was successfully tested on a NASA parabolic flight aircraft in 2012. Further testing on ISS was warranted due to the ~25 sec. duration of each parabola was too short to capture the entire 3 to 5 min. analyzing run.
- During ExMC work on Exploration Lab Analysis (ELA) since 2017, WBC was deemed a required analyte (and differential was identified as a desirable analyte) for future exploration missions to the Moon & Mars.
- NASA does not currently have the operational capability to measure WBC counts in space.

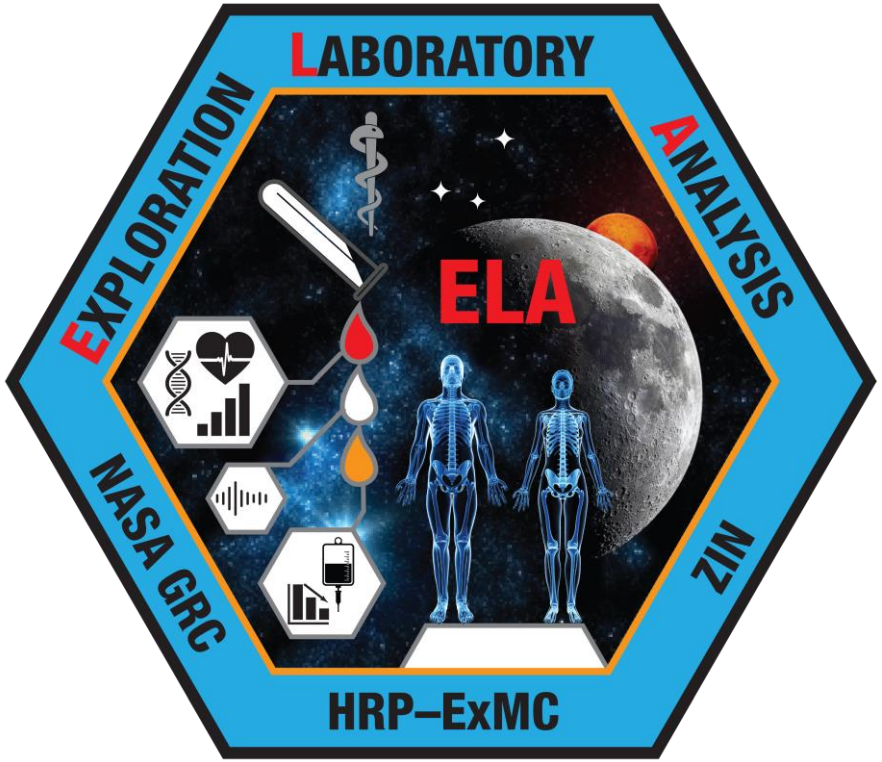
Relevant HRP Risk and Gaps

- This activity addresses the following Risk from the Program Requirements Document (PRD):
  - Risk of Adverse Health Outcomes & Decrements in Performance due to Inflight Medical Conditions.

- This activity addresses the following Gaps from the Integrated Research Plan (IRP):
  - Med11: We do not have the capability to minimize medical system resource utilization during exploration missions.
  - Med12: We do not have the capability to mitigate select medical conditions.
  - Med13: We do not have the capability to implement medical resources that enhance operational innovation for medical needs.

- NASA-STD-3001 requires clinical diagnostics at Medical Level of Care II, this also drove ExMC to identify WBC count as an important analyte.

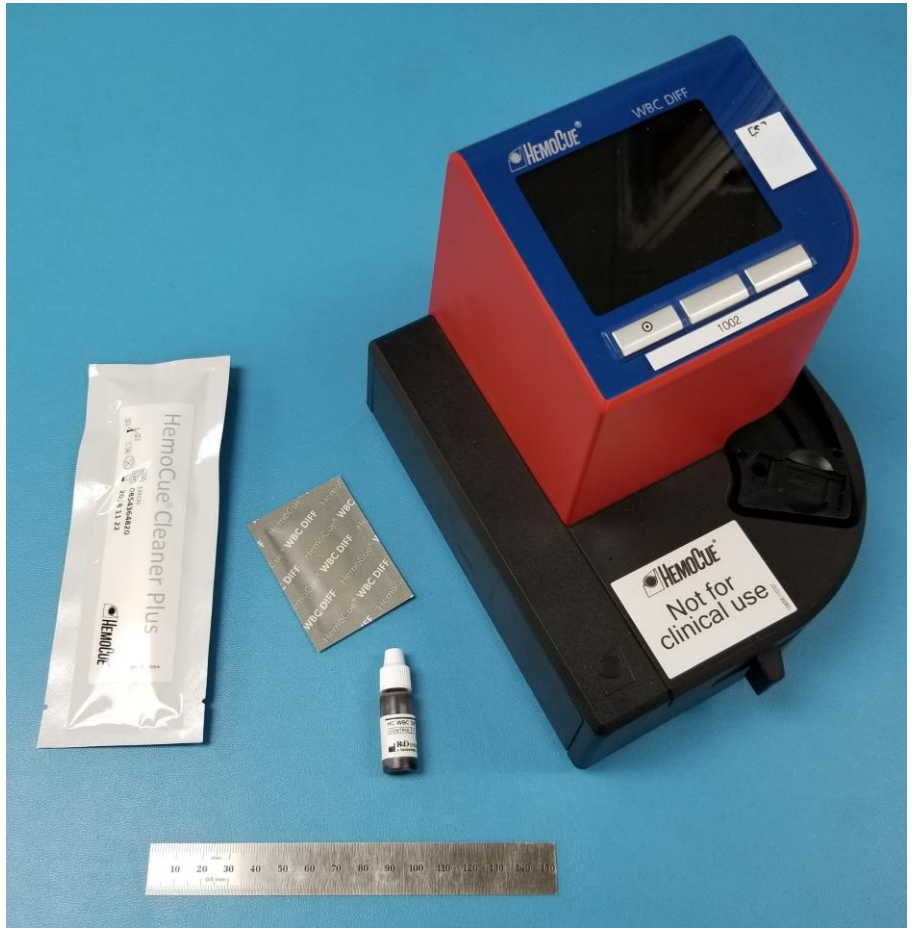
- The risk that this flight task supports is a **Red** Risk.



[Exploration Laboratory Analysis (ELA) Project Graphical Identifier]

Hardware List for Flight Operations on ISS

- Experiment hardware launched to ISS
  - HemoCue® Analyzer
  - HemoCue® Cuvette Kit
  - HemoCue® Cleaner Swab Kit
  - R&D Systems Control Solutions Kit
- Additional items needed already on ISS
  - ISS Portable Glove Bag (IPGB)
  - Kapton Tape
  - Ziploc bags
  - Gauze pads
  - BZK wipes
  - Alkaline C batteries
  - Camcorder
  - Hands Free Voice Headset



[The 4 individual hardware items for the HemoCue® experiment on ISS]

HemoCue® Analyzer

[HemoCue® Analyzer flight hardware prior to launch to the ISS]

- The HemoCue® Analyzer is battery operated, has a mass of 1.3 kg, and is smaller than the average toaster (19x16x16 cm).
- A 10 µl sample is transferred into a cuvette where red blood cells (RBC) are lysed and WBC's nuclei are stained with methylene blue, which preps the blood sample prior to the Analyzer performing its image analysis.
- The measurement principle utilized is an imaging method for which a stack of 37 images is taken through the depth of field in the region of interest of the probed area at the tip of the cuvette.
- No calibration or instrument adjustment needed

R&D Systems Control Solutions

- The HemoCue® Analyzer is tested with 3 different control solutions **(Low, Normal, and High)** from R&D Systems. Using control solutions allows for comparison of the flight data with ground test results using control solutions from the same lot. The controls solutions contain varying amounts of concentrations so that the full measurement range of the Analyzer could be tested (0.3 – 30.0 x 10^9/L).

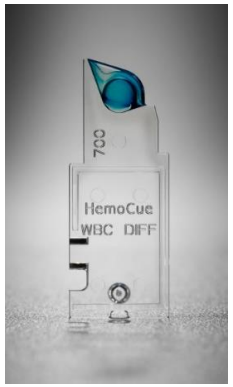
  - **Low** contains lower than normal amounts of WBC's and expected number of each differentials is lower.
  - **Normal** contains the number of WBC's and differential amounts that have been determined to be a representative of what is expected from an average healthy adult.
  - **High** contains higher than normal amounts of WBC's and expected number of each differential is higher.

Control Solutions
Low, Normal, High
2°C – 8°C Cold Stowage
3.5 month Closed Bottle Shelf Life
1 month Open Bottle Shelf Life

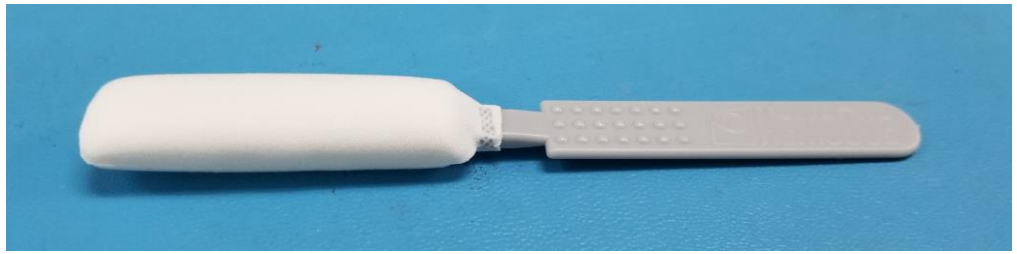
Control Solutions Kit
(Packaged for Flight)
[Rated BSL 2M]

HemoCue® Cuvettes and Cleaner Swabs

- HemoCue® Cuvettes
  (a.k.a. WBC DIFF Microcuvettes)
  [1 year shelf life, THL 1]



- HemoCue ® Cleaner Swabs
  [1 year shelf life, THL 0]



HemoCue® Operations Steps

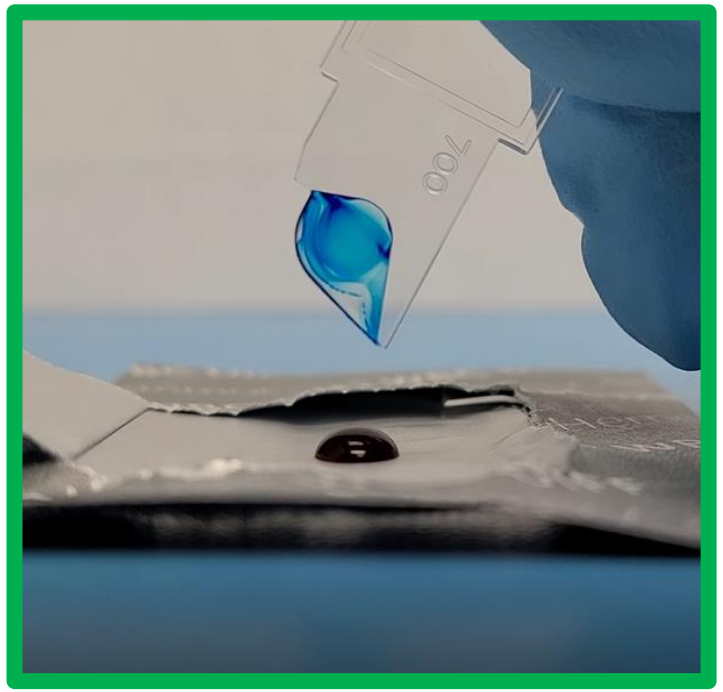
- The target for our Success Criteria is to complete a minimum 9 trials [3 per control solution (L, N, & H)], with the resulting 11 data values for each be in the manufacturer's Estimated Range:
  - > Prep control solution by performing a series of rolls and lateral motions to fully disperse the solution.
  - > Transfer droplet of solution to surface of cuvette packaging.
  - > Fill cuvette (must be analyzed within 1 minute of filling).
  - > Load cuvette into tray (see images to right).
  - > Wait for HemoCue analysis (~ 3 to 5 minutes depending on number of cells being counted).
  - > Record results via a screenshot for data analysis.

[Empty cuvette tray magnetically attached to the track of the analyzer base]

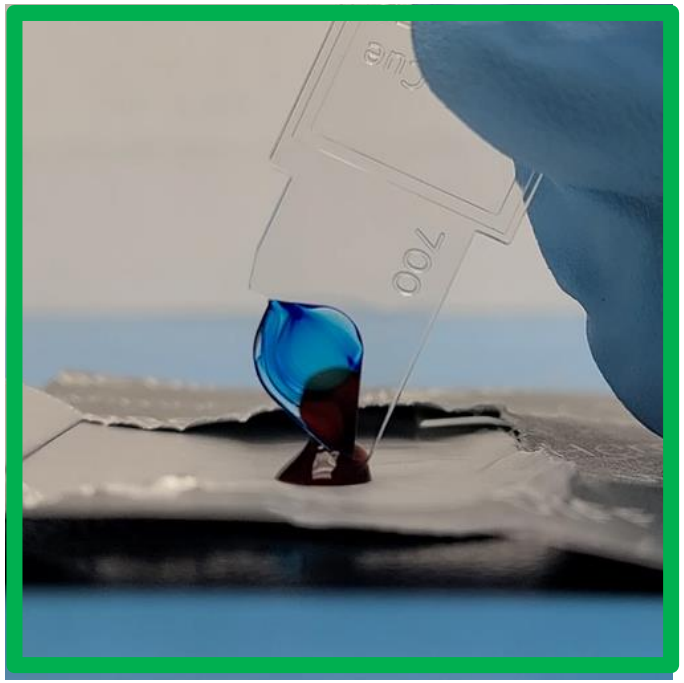
[Cuvette temporarily taped into cuvette tray with Kapton tape to avoid motion in the absence of gravity]

Proper Method to Fill a Cuvette

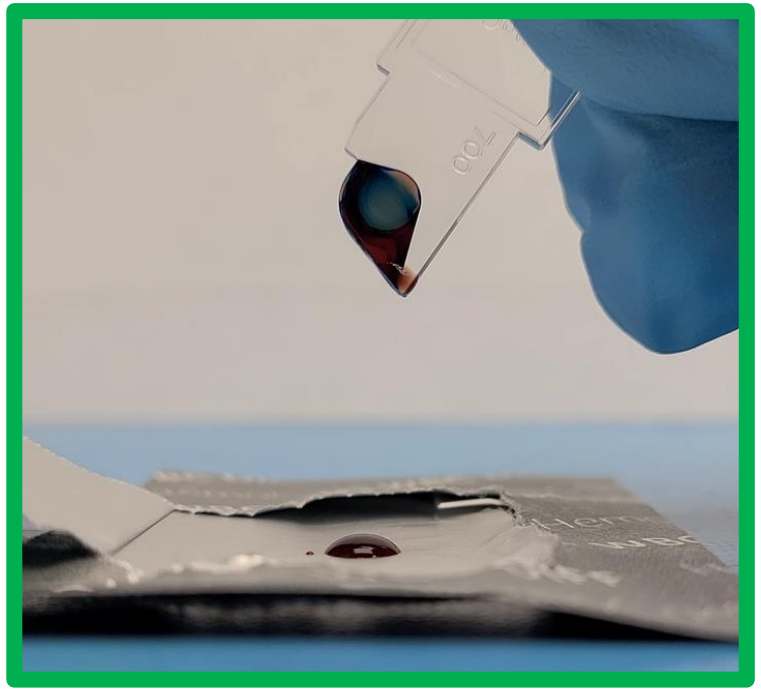
Correct Droplet Size



Proper Approach Angle



Good Fill



Variations in Technique Used to Fill the Cuvette

Optimal Fill
(High Probability of Success)

- 1 touch fill method
- Short fill time (~ 1 sec.)
- No bubbles
- Medium blue circle
- Dark maroon edge

Not Ideal Fill
(May Produce Error Message)

- Multi-touch fill method
- Extended fill time (> 10 sec.)
- No bubbles
- Half light pink circle
- Half medium blue circle
- Dark maroon edge

Not Acceptable Fill
(Skip Analysis, Discard Old Cuvette, & Use a New Cuvette)

- Multi-touch fill method
- Extended fill time (> 10 sec.)
- 1 Unwanted bubble
- Half light pink circle
- Half medium blue circle
- Dark maroon edge

Results Displayed After Each Run

Test run is completed
[Low Control Solution]

Results display on screen
[Normal Control Solution]

After test completes, analyzer performs a self test again

Summary of Test Results on ISS for Session #1

WBC	10^9/L	DIFF	10^9/L	%
9.2		NEU ⚠	6.1	66
		LYM ⚠	1.7	18
		MON ⚠	0.3	3
		EOS ⚠	1.2	13
		BAS ⚠	0.0	0
Test #:	Run: 5, Level: Normal, SN: 1001			
GMT:	2020 – 363 – 1043			

WBC	10^9/L	DIFF	10^9/L	%
8.6		NEU ⚠	5.5	65
		LYM ⚠	1.3	15
		MON ⚠	0.4	5
		EOS ⚠	1.3	16
		BAS ⚠	0.0	0
Test #:	Run: 11, Level: Normal, SN: 1001			
GMT:	2020 – 363 – 1154			

WBC	10^9/L	DIFF	10^9/L	%
9.5		NEU ⚠	6.2	65
		LYM ⚠	1.8	19
		MON ⚠	0.4	4
		EOS ⚠	1.0	11
		BAS ⚠	0.0	0
Test #:	Run: 13, Level: Normal, SN: 1001			
GMT:	2020 – 363 – 1211			

These 3 sets of data were for the Normal Control SN: 1001

Green is within range.
Orange is slightly outside of the range.

[R&D Systems expected mean and range as the manufacturer]

Cell Type	Units	Normal (Level 2) [HD11202]	
		Assay Mean	Expected Range
WBC	10^9/L	8.0	6.0 – 10.0
NEU	10^9/L	2.4	1.0 – 3.8
LYM	10^9/L	2.2	0.8 – 3.6
MON	10^9/L	0.3	0.0 – 0.6
EOS	10^9/L	2.6	1.3 – 3.9
BAS	10^9/L	0.4	0.0 – 0.8
NEU	%	30	12 – 48
LYM	%	28	10 – 46
MON	%	4	0 – 8
EOS	%	33	17 – 49
BAS	%	5	0 – 10

- Performed 13 test points:
  - Attempted to analyze samples for 5 Low, 7 Normal, & 1 High with Control SN: 1001.
- The analysis process of the Analyzer only completed and displayed data for 3 runs (all with the Normal control). The rest of the runs produced error codes with no data.
- An interesting trend was observed in these 3 test points. Total WBC counts were within the manufacturer's expected range, (LYM & MON & BAS) were within the proper ranges; but (NEU) were twice as high as expected, and (EOS) were half the expected value.

Summary of Test Results on ISS for Session #2

WBC	10^9/L	DIFF	10^9/L	%
3.1		NEU ⚠	0.8	25
		LYM ⚠	1.0	33
		MON ⚠	0.1	2
		EOS ⚠	1.1	36
		BAS ⚠	0.1	4
Test #:	Run: 4, Level: Low, SN: 1002			
GMT:	2021 – 018 – 0536			

WBC	10^9/L	DIFF	10^9/L	%
8.5		NEU ⚠	3.5	42
		LYM ⚠	2.3	28
		MON ⚠	0.2	3
		EOS ⚠	2.2	26
		BAS ⚠	0.1	2
Test #:	Run: 8, Level: Normal, SN: 1002			
GMT:	2021 – 018 – 0624			

WBC	10^9/L	DIFF	10^9/L	%
18.8		NEU ⚠	11.3	60
		LYM ⚠	5.6	30
		MON ⚠	0.4	2
		EOS ⚠	1.5	8
		BAS ⚠	0.0	0
Test #:	Run: 1, Level: High, SN: 1002			
GMT:	2021 – 018 – 0511			

One representative set of data from each of the 3 levels of Controls SN: 1002

Green color indicates all values were within manufacturer's range.

- Performed 14 test points:
  - Obtained data for 3 Low, 5 Normal, & 3 High with Control SN: 1002.
  - Repeated 1 Low, 1 Normal, & 1 High with Control SN: 1001.
- Data was successfully obtained for all 14 tests, only 2 error messages were displayed and were attributed to operational inconsistencies that were explainable and correctable in real-time.
- Although the majority of the data obtained during Session #2 were within the manufacturer's expected ranges, an interesting trend was noted whereby the NEU counts were trending higher and EOS were trending low overall.

[Results from a repeat of test with Normal Controls SN: 1001]

WBC	10^9/L	DIFF	10^9/L	%
8.0		NEU ⚠	3.3	41
		LYM ⚠	2.1	26
		MON ⚠	0.3	3
		EOS ⚠	2.2	27
		BAS ⚠	0.2	3
Test #:	Run: 12, Level: Normal, SN: 1001			
GMT:	2021 – 018 – 0704			

Observations & Lessons Learned During Session #1

- ISS Demo performed on December 28th, 2020 (Session #1):
  - The control solution didn't appear to wick the same into the cuvette in microgravity as in normal gravity, it seemed like it was much slower.
  - The control solution appeared to move around the circle (region of analysis) in the cuvette tip, and not into it as planned.
  - Decided to hold the cuvette in solution for a longer period of time, which was later found to cause imprecise mixing and potentially unintentionally redistribution of the reagent as the droplet was being transferred into the cuvette.
  - After careful reflection on the procedure and methodology, the team's original hypothesis for the root cause was holding the tip of the cuvette in the blood droplet too long. This either caused some EOS cells to be counted as NEU cells, or the ratio of the reagent to the cell types to truly be altered due to the extended liquid exchange.

Observations & Lessons Learned During Session #2

- ISS Demo performed on January 18th, 2021 (Session #2):
  - Plans for this follow-up ops session were based on troubleshooting during ground testing.
  - The new plan was to alter the technique and transfer control solution from the droplet into the cuvette very briefly (~ 1 sec) regardless of visual cues and run the test points as planned.
  - This plan was executed flawlessly by the crew member and provided the science data that was sought after.
  - Before data, results, and analysis are published, further discussion and consideration is be given to the interpretation of this data, so we can properly report the precision and accuracy of the analyzer appropriately.
- This experiment with the HemoCue® WBC DIFF Analyzer marks the first real-time hematology measurements have ever been performed in microgravity.