Calculating Cellular Mass Balance After Corning® X-SERIES® Processing

Protocol

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In order to ensure a Corning X-LAB® or X-WASH® protocol is optimized, it is recommended to perform a cellular mass balance to confirm cell recovery. By knowing the starting and final number of cells in the cartridge compartments there will be a better understanding of cell recovery and how to improve it. To differentiate between multiple cell types, a hematology analyzer, or similar method for quantifying the cell product of interest is required. Care must be taken to get the most accurate volume measurements possible as small inaccuracies in volume can have a large impact on yield. If unable to get accurate volume measurements, it is possible to use specific gravity to calculate volumes using information provided in Table 1. In this protocol, we will demonstrate how to perform mass balance using results from a typical mononuclear cell (MNC) isolation from whole blood as an example. It will be necessary to record values in a spreadsheet for analysis – a template can found at **www.corning.com/XseriesMassBalanceTemplate** or upon request by contacting your Corning Field Application Scientist.

Table 1. Specific Gravity of Blood Components

Blood Component	Specific Gravity
Whole Blood	1.05 g/mL
Mononuclear (MNC) Fraction	1.02 g/mL
Red Blood Cell (RBC) Fraction	1.09 g/mL
Plasma Fraction	1.02 g/mL

1. After whole blood has been added to the X-LAB cartridge, an initial sample can be taken via the needleless port. Sample should be taken post-clot filter. This sample is for the pre-sample cell counts. We recommend taking two counts of every sample and then using the average for calculations.

   Example:

Pre-Sample		Read 1	Read 2	Average
WBC	**10^6/mL**	7.06	7.2	**7.13**
RBC	**10^9/mL**	3.82	3.81	**3.815**
PLT	**10^6/mL**	162.00	156.00	**159**
NEUT	**10^6/mL**	4.97	5.12	**5.045**
LYMP	**10^6/mL**	1.78	1.71	**1.745**
MONO	**10^6/mL**	0.27	0.33	**0.3**

2. Determine the volume in the X-LAB cartridge (post-sample removal). This can be done by subtracting the pre-sample volume from what was added to the cartridge, or by weighing the cartridge with and without blood and using the specific gravity in Table 1 to calculate. An empty cartridge weighs about 208 g assuming the input tubing has been sealed and cut to about an inch from the top of the cartridge.

   Example:

   Starting whole blood volume = 120 mL

3. Once the X-LAB cartridge has been processed, volumes and cell counts need to be collected from the main, depletion, and harvest chambers. Depending on what is being processed it may be necessary to dilute samples from the depletion and harvest chambers to get accurate counts. We recommend a 1:5 or 1:8 dilution for the harvest and a 1:3 dilution for the depletion chamber with your desired resuspension media as a starting point. No dilution is necessary for the main chamber.

Example:

Depletion Chamber		Read 1	Read 2	Average	Volume (mL)
WBC	10^6/mL	2.26	2.21	**2.235**	45
RBC	10^9/mL	3.23	3.21	**3.22**	45
PLT	10^6/mL	7	6	**6.5**	45
NEUT	10^6/mL	2.18	2.11	**2.145**	45
LYMP	10^6/mL	0.08	0.08	**0.08**	45
MONO	10^6/mL	0	0.01	**0.005**	45

Harvest Chamber		Read 1	Read 2	Average	Volume (mL)
WBC	10^6/mL	3.81	3.88	**3.845**	17
RBC	10^9/mL	0.04	0.04	**0.04**	17
PLT	10^6/mL	105	107	**106**	17
NEUT	10^6/mL	2.11	2.23	**2.17**	17
LYMP	10^6/mL	1.39	1.38	**1.385**	17
MONO	10^6/mL	0.27	0.24	**0.255**	17

Main Chamber		Read 1	Read 2	Average	Volume (mL)
WBC	10^6/mL	0.05	0.05	**0.05**	54
RBC	10^9/mL	0	0	**0**	54
PLT	10^6/mL	25	24	**24.5**	54
NEUT	10^6/mL	0.01	0.02	**0.015**	54
LYMP	10^6/mL	0.03	0.03	**0.03**	54
MONO	10^6/mL	0.01	0	**0.005**	54

4. Determine total cells from the pre-sample and all post-sample compartments by multiplying the averaged cell density with the volume and dilution factor.

Example:

Pre-sample		Read 1	Read 2	Average	Volume (mL)	Cell Number	Dilution Factor	Total Cells 10^6
WBC	10^6/mL	7.06	7.2	**7.13**	120	**855.6**	1	**855.6**
RBC	10^9/mL	3.82	3.81	**3.815**	120	**457.8**	1	**457800**
PLT	10^6/mL	162.00	156.00	**159**	120	**19080**	1	**19080**
NEUT	10^6/mL	4.97	5.12	**5.045**	120	**605.4**	1	**605.4**
LYMP	10^6/mL	1.78	1.71	**1.745**	120	**209.4**	1	**209.4**
MONO	10^6/mL	0.27	0.33	**0.3**	120	**36**	1	**36**

Depletion Chamber		Read 1	Read 2	Average	Volume (mL)	Cell Number	Dilution Factor	Total Cells 10^6
WBC	10^6/mL	2.26	2.21	**2.235**	45	**100.575**	3	**301.725**
RBC	10^9/mL	3.23	3.21	**3.22**	45	**144.9**	3	**434700**
PLT	10^6/mL	7	6	**6.5**	45	**292.5**	3	**877.5**
NEUT	10^6/mL	2.18	2.11	**2.145**	45	**96.525**	3	**289.575**
LYMP	10^6/mL	0.08	0.08	**0.08**	45	**3.6**	3	**10.8**
MONO	10^6/mL	0	0.01	**0.005**	45	**0.225**	3	**0.675**

Harvest Chamber		Read 1	Read 2	Average	Volume (mL)	Cell Number	Dilution Factor	Total Cells 10^6
WBC	10^6/mL	3.81	3.88	**3.845**	17	**65.365**	8	**522.92**
RBC	10^9/mL	0.04	0.04	**0.04**	17	**0.68**	8	**5440**
PLT	10^6/mL	105	107	**106**	17	**1802**	8	**14416**
NEUT	10^6/mL	2.11	2.23	**2.17**	17	**36.89**	8	**295.12**
LYMP	10^6/mL	1.39	1.38	**1.385**	17	**23.545**	8	**188.36**
MONO	10^6/mL	0.27	0.24	**0.255**	17	**4.335**	8	**34.68**

Main Chamber		Read 1	Read 2	Average	Volume (mL)	Cell Number	Dilution Factor	Total Cells 10^6
WBC	10^6/mL	0.05	0.05	**0.05**	54	**2.7**	1	**2.7**
RBC	10^9/mL	0	0	**0**	54	**0**	1	**0**
PLT	10^6/mL	25	24	**24.5**	54	**1323**	1	**1323**
NEUT	10^6/mL	0.01	0.02	**0.015**	54	**0.81**	1	**0.81**
LYMP	10^6/mL	0.03	0.03	**0.03**	54	**1.62**	1	**1.62**
MONO	10^6/mL	0.01	0	**0.005**	54	**0.27**	1	**0.27**

5. Determine percent recovery of the cells from each chamber by dividing the total cells from each chamber by the total cells in the pre-sample.

   Example:

Depletion Chamber		Total Cells 10^6	% Recovered Cells
WBC	10^6/mL	301.73	35.26
RBC	10^9/mL	434700	94.95
PLT	10^6/mL	877.5	4.60
NEUT	10^6/mL	289.58	47.83
LYMP	10^6/mL	10.8	5.16
MONO	10^6/mL	0.675	1.88

Harvest Chamber		Total Cells 10^6	% Recovered Cells
WBC	10^6/mL	522.92	61.12
RBC	10^9/mL	5440	1.19
PLT	10^6/mL	14416	75.56
NEUT	10^6/mL	295.12	48.75
LYMP	10^6/mL	188.36	89.95
MONO	10^6/mL	34.68	96.33

Main Chamber		Total Cells 10^6	% Recovered Cells
WBC	10^6/mL	2.7	0.32
RBC	10^9/mL	0	0
PLT	10^6/mL	1323	7
NEUT	10^6/mL	0.81	0.13
LYMP	10^6/mL	1.62	0.77
MONO	10^6/mL	0.27	0.75

6. To determine the MNC percent recovery for each chamber, divide the total lymphocytes and monocytes by the pre-sample lymphocytes and monocytes.

	Sample Recovery			
	Total	Harvest Chamber	Depletion Chamber	Main Chamber
MNC	96.33%	90.89%	4.68%	0.77%
RBC	96.14%	1.19%	94.95%	0.00%
PLT	87.09%	75.56%	4.60%	6.93%

When determining the best protocol, it is important to understand what aspects of the harvest are most important for the downstream application. Increasing the % of a desired cell type may result in more undesired cells in the harvest chamber. Alternatively, a lower recovery of desired cells might occur if the purity of the harvest is more important.

Additional Helpful Tips

- In order to accurately assess mass balance, it is essential that all samples collected for cell counts are representative. We recommend thorough mixing prior to sampling.
- Make sure the cell pellet is fully resuspended prior to collecting from the harvest chamber.
- Pre-loading the harvest chamber with a small amount of buffer can help to prevent the harvest from sticking and being left behind.
- Compare the total volume recovered with the initial volume loaded to determine any missing volume that might be inadvertently left behind in one of the chambers.
- Ideally, mass balance should be between 95% to 105%.
- Make sure the cell counts are within the appropriate range for the analyzer. Optimization of the dilutions may be required.
- If cells in the harvest chamber are very concentrated, a wash step might help with recovery.
- Ensure samples are well mixed prior to dilution or counting.
- Results may vary depending on donor or sample quality. Try to ensure fresh samples whenever possible.

If cell recovery is still lower than desired, please reach out to your Corning Field Application Scientist for additional support.

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