Multiplate® Platelet Function Analyzer: An Overview

The Multiplate® is for research use only in the US and Canada

Scope: This is a summary of most aspects of platelet function testing using the Multiplate® 5.0 analyzer. It does not include the operation of Multiplate® instrument which is covered extensively during installation and user training. This summary was designed to help the new user comprehend the integration of platelet function testing into the laboratory environment.

1. **Instrument.** The instrument, monitor, and keyboard are the size of a desk top computer and along with the printer require a space of 3' (L) X 2.5' (D) X 2' (H). Hospital grade cords are supplied with the instrument and monitor. The Multiplate system will require 3 standard, grounded outlets: instrument, monitor, and printer. A four inch clearance (minimum) around the instrument is required for air circulation and ventilation.[1]

   (Note: A extended electronic pipette cord (MP0385) is available for left-handed individuals. Pipette tips are specifically manufactured for the electronic pipette and are available boxed (MP0050-10, 10 X 96 tips) or in a bulk configuration (MP0040, 1000 tips).)

2. **Instrument Operating Conditions**[1]

   a. Room Temperature: 18 to 30° C
   b. Humidity: 20 to 80%
   c. Elevation: 0 to 2000 meter above sea level

3. **Test Cells.** The test cells have a storage temperature of 4-25° C and are typically stored at room temperature. Note that once the PET packaging is opened, the cells must be used within one month after opening. Verify that the PFTE stir bar is present when using each cell. (See test cell insert for specific details.)

4. **Controls.** Electronic Control (EC) is an instrument operation and is run on each day of testing at the beginning of sample testing.[2] The sensor cables are required to be in the park position and the instrument at its nominal 37° C temperature range before performing the EC check. The software has to run 20 minutes before EC.

   Liquid Controls (08115 966 001), Level 1 for all five channels and Level 2 for all five channels, is performed on approximately a monthly interval when testing daily. (There is no frequency of Liquid Control use provided by the manufacturer.) The key measurement here is aggregation (in AU) and acceptability of results is to lot-specific ranges. (Note: There is a specific pipetting process for Liquid Controls. Solution 2 must be pipetted **onto** the surface of Solution 1 rather than **into** Solution 1! See assay insert for specific details.)

   No control is commercially available which can be used to test platelet activation and aggregation. To examine *in vitro* platelet function, draw a normal individual (no ASA/NSAIDs, antiplatelet drugs, herbal supplements, and no excessive alcohol consumption) and test with Multiplate®. Compare result to assay ranges determined in studies by the Multiplate® manufacturer.[3]

5. **Diluent**. The diluent is either 0.9% NaCl (provided by user) or 0.9% NaCl with 3mM $CaCl_2$ (08115 974 001D). The 0.9% NaCl must be preservative free. Diluents are pipetted into a diluent tube (06675 654 001) and are warmed to 37° C in the instrument heating block. Add 5 mL or less of 0.9% NaCl to the diluent tube so the solution level is BELOW the top of the instrument heating block. (Note: **DO NOT** warm whole blood nor agonists in the instrument heating block!)

   The diluent for the assay depends on the anticoagulant and the agonist. **ONLY when using citrated whole blood is 0.9% NaCl with 3 mM $CaCl_2$ used .....**

Citrated Whole Blood[4]	
Test	**Diluent**
ADPtest	0.9% NaCl with 3 mM $CaCl_2$
COLtest	0.9% NaCl with 3 mM $CaCl_2$
TRAPtest	0.9% NaCl with 3 mM $CaCl_2$

Citrated Whole Blood[4]	
Test	**Diluent**
ASPItest	0.9% NaCl
RISTOlow	0.9% NaCl
RISTOhigh	0.9% NaCl

   Note: The citrated vacutainer tube must be filled to a specific volume of whole blood to maintain the correct ratio of anticoagulant to blood. Incomplete blood draws can lead to erroneous results!

6. **Reagents.** The Multiplate® reagents are lyophilized materials stored at 2-8° C with expiration dating on the vial. (Each reagent has a unique color code that is called for in the assay software. For example, the assay software will prompt the user to add 20 µL of ADP (green) which is the color of the vial label.)

   All reagents are reconstituted with 1 mL of high purity water. The reagents are typically stable for 7 days when stored at 2-8° C or for 4 weeks when stored at < -20° C. (See individual agonist assay inserts for specific details.)

   The agonists can be aliquoted into smaller volumes. Micro vials (color-coded to each agonist) are available and have a secure locking lid which is important to minimize sublimination. The manufacturer recommends 100 µL is the minimum volume for frozen storage.[5] Freshly thawed aliquots are stable at room temperature for 24 hours after one freeze/thaw cycle. (See individual agonist assay inserts for specific details.)

7. Multiplate Agonist Concentrations

The ADP, ASPI, COL, and TRAP tests all use 20 µL of agonist in the nominal assay protocols along with 300 µL of diluent and 300 µL of anticoagulated whole blood. The RISTOlow assay and the RISTOhigh test uses 12 µL and 50 µL of ristocetin, respectively.

Test	Agonist	Reagent Concentration	[Agonist] in Test Cell
ADPtest	ADP	200 µM	6.5 µM
ADPtest HS	ADP + PGE1	200 µM + 300 nM	6.25 µM + 9.4 nM
ASPItest	arachidonic aid	15 mM	0.5 mM
COL test	collagen	50 µg/mL	1.6 µg/mL
RISTOtest	ristocetin	10 mg/mL	
		RISTOlow	0.2 mg/mL
		RISTOhigh	0.77 mg/mL
TRAPtest	TRAP-6 peptide	1000 µM	32 µM
Controls			
ASA Control	acetylsalicylic acid	30 mg/mL	1 mg/mL
GPIIb/IIIa Antagonist	synthetic inhibitor	50 µg/mL	1.6 µg/mL

The calculation of agonist concentration in the test cell uses whole blood (WB) volume without consideration that approximately half of WB is red cell volume. Values from Multiplate® product literature.

8. Maintenance

a. Sensor cable. Replace biannually when using the instrument every day (06675 204 001).[6] (Note: All sensor cables must be the same lot number.)
b. Pipette tip filter. No replacement schedule. Replace regularly or if contaminated (06675 620 001).[7]
c. It is necessary to verify that the electronic pipette is performing within specification after routine service or maintenance.[8]
d. The frequency of electronic pipette volume verification is defined by the user Quality System requirements.
e. The manufacturer recommends an annual technical service of the instrument done by a Service Engineer.[6] This consists of sensor cable replacement, electronic control measurement, liquid control measurement, QC check of temperature, QC check of stirring, and verification of electronic pipette volume delivery.

9. Proficiency Testing

a. Ex vivo controls. ASA Control, as an ex vivo control, will inhibit the ASPItest and the COLtest. The GPIIb/IIIa Antagonist inhibits platelet aggregation and will inhibit aggregation in the ADPtest, ASPItest, COLtest, and TRAPtest; the RISTOtest shows partial inhibition of aggregation.

b. College of American Pathologists. CAP provides two testing events in Mar and Oct as the Platelet Function Survey, PF-Platelet Aggregation. The survey kit contains four, 3.2% sodium citrate vacuum tubes (4.5 mL draw) and two capped polypropylene tubes (10 mL) containing inhibitors or no inhibitors. A normal individual is drawn in the citrate vacutainer tubes and then 6 mL of citrated whole blood is added to each of the capped polypropylene tubes (10 mL). The two samples can then tested by Multiplate using the nominal tests for ADP, ASPI, COL, or TRAP. Multiplate is not listed as a test method for this survey but is planned for the future test events under the methodology of "Whole Blood Aggregation [without luminescence]".

10. Printer

a. A HP LaserJet M12w black and white printer is the standard printer and requires a separate, standard power plug.

11. Data Output

a. Data files should be saved to an external data storage device on a regular basis.
b. Individual channel results, screen shots, and print results are automatically saved to specific folders found in the "DATA" folder on the instrument desktop:

- Sensor 1 and sensor 2 individual channel data are found in the "XML" folder
- mean channel data are in the "EXPORT" folder
- collective results of a series of results are in the "SCREENSHOTS" folder
- individual channel results are in the "GRAPHICS" folder

c. To transfer data, it is easiest to export the data for a specific period and then copy the "EXPORT" file to a flash drive. Then create a program to extract the cell specific data such as ID, test date/time, result (AUC in Units), velocity (in AU/min), aggregation (in AU), CC, and DIF and /or other parameters of specific interest.

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1. User Manual I, V.2.0-US-RUO, section 3.2.
2. Short Instruction Manual, V4.0-RUO MP0921-US, page 4.
3. Reference Ranges for Multiplate® Analysis, V.2.0/10.10.2011
4. Short Instruction Manual, V4.0-RUO MP0921-US, page 15.
5. Short Instruction Manual, V4.0-RUO MP0921-US, page 5.
6. User Manual I, V.2.0-US-RUO, section 9.1.
7. Short Instruction Manual, V4.0-RUO MP0921-US, page 17.
8. User Manual I, V.2.0-US-RUO, section 8.3.

DOCUMENT REVISION HISTORY

NOTE: When using form, discard this page of form.

Revision	Effective Date	DCN Number	Description of Document Revision	Revision Author
03	12/XX/2012	2012-00220	Updated pipette tip/pipette filter catalogue numbers and information of test cell storage.	S. Delaney
04	02/12/2018	2018-00015	Updated to Roche product number, collagen stability, and standard printer.	M. Sobo

Note: For approval signatures see Document Change Notice noted in table above.