For the monitoring of specific platelet ADP receptor antagonists
For In Vitro Diagnostic Use

For good results exercise great care in the pipeting of small reagent volumes (10 µL) by depositing them at the bottom of the test tubes. All reagents must be at room temperature.

We recommend to run a normal sample in parallel of each series as control.
8.1 Reagent tube and sample setup
On a rack, per sample, setup 3 plastic tubes labeled T1, T2 and T3:
- In tube T1: pipette 10 µL of reagent 2a.
- In tubes T2 and T3: pipette 10 µL of reagent 2b.
- Homogenize the tubes for 1 to 2 seconds using a vortex set on low speed.
- Incubate the tubes at room temperature for 10 minutes at room temperature.

8.2 Fixation
- In tubes T1, T2 and T3: pipette 10 µL of reagent 3.
- Homogenize the tubes for 1 to 2 seconds using a vortex set on low speed.
- Incubate the tubes at room temperature for 5 minutes.

8.3 Cell permeabilization and immunolabeling
- In tubes T1 and T2: pipette 8.4 Fluorescent staining and platelet counter-staining
- Homogenize the tubes for 1 to 2 seconds using a vortex set on low speed.
- Incubate the tubes at room temperature for 5 minutes.

8.4 Fluorescent staining and platelet counter-staining
- In tubes T1, T2 and T3: pipette 10 µL of reagent 4a.
- In tube T3: pipette 10 µL of reagent 4b.
- Homogenize the tubes for 1 to 2 seconds using a vortex set on low speed.
- Incubate the tubes at room temperature for 5 minutes.
- Add 2 mL of reagent 1 in each of the 3 tubes.
- Homogenize the tubes for 1 to 2 seconds using a vortex set on high speed and place them immediately at 2-8°C until the platelet analysis.

Thus treated, the contents of the tubes may be stored for 2 hours at 2-8°C before platelet analysis.

8.5 Cytometric analysis
Refer to the Operator’s Manual of the cytometer for instructions on how to perform cytometric readings.
The selected Mean Fluorescence Intensity (MFI) statistics is the geometric mean (Mn (y) or GeoMean depending upon the cytometer).
Vortex each tube for 1 to 2 seconds before analysis.
Analyze at least 5,000 platelet events in "B" region.

For the protocol, one FS LOG x SS LOG and one FL1 LOG x FL2 LOG cytograms are necessary.

- Analysis of tube T1:
  - On the FS LOG x SS LOG cytogram isolate the cellular cloud including platelets by the "A" region. Leukocytes (located at the arrow) are excluded from this "A" region (Fig. 1).
  - The FL1 LOG x FL2 LOG cytogram must be gated by the "A" region.
  - Adjust the PMT FL2 in order to get the FL2* cloud at the beginning of the 3rd decade.
  - Adjust the PMT FL1 in order to get the lower part of the FL1* / FL2* cloud at the beginning of the 2nd decade.
  - Add a discriminator threshold on FL2 LOG to minimize FL2- events (background of the instrument and cellular debris).
  - On the FL1 LOG x FL2 LOG cytogram, create a "B" region to isolate the platelets FL2* from the debris FL2-

- Analysis of tubes T2 and T3:
  - Do not change the position of the "B" region and the settings of SS, FS, FL1 and FL2 PMT voltages.
  - Analyze tubes T2 and T3 and note the MFI on the Y-axis (Figs. 3 and 4).

Note: some samples present a population of debris (comet aspect) located at the left of the cloud of interest (located at the arrow). Set the "B" region during tube T1 analysis in order to include all the platelet cloud and exclude the maximum of debris.

Note: the setting of fluorescence compensation is not required due to the use of the negative isotypic control.
However the setting of fluorescence compensation does not modify the test result.
8.6 Analysis of the results

After cytometric analysis, determine the value of the "corrected" MFI (MFIc) for tubes T1 and T2.

\[
\text{MFIc (PGE1)} = \text{MFI (T1)} - \text{MFI (T3)}
\]

\[
\text{MFIc (PGE1 + ADP)} = \text{MFI (T2)} - \text{MFI (T3)}
\]

9 RESULT INTERPRETATION

A platelet reactivity index (PRI) is calculated using corrected mean fluorescence intensities (MFIc), in the presence of PGE1 alone (PGE1) or PGE1 and ADP simultaneously (PGE1 + ADP) according to the following calculation:

\[
\text{Platelet reactivity index (PRI)} = \frac{\left[\text{MFIc (PGE1)} - \text{MFIc (PGE1 + ADP)}\right]}{\text{MFIc (PGE1)}} \times 100
\]

Each laboratory must establish its own interpretation values specific from the P2Y12 antagonist to evaluate.

Treatment with the clopidogrel:

Inter-individual variability in the response to clopidogrel has been demonstrated using PLT VASP/P2Y12 kit (1).

The PRI of the patients with ischemic cardiovascular disease (n=33) treated with clopidogrel for more than 1 week, vary from 6.6% to 85.8%.

Bad responders to clopidogrel 85.8% Good responders to clopidogrel 6.6%

In order to measure the efficiency of a P2Y12 antagonist such as clopidogrel, apply the following recommendation:

1- Determine the basal PRI range (expressed as mean +/- 2 SD (standard deviation)) on a group of patients relevant of the disease of interest and not receiving the P2Y12 antagonist to evaluate.

As a guide, according to the publication of Aleil B. et al. (1), the PRI of the patients (n=34) with ischemic cardiovascular disease, not receiving clopidogrel, is 79.0 +/- 4.1% (expressed as mean +/- SD).

2- Determine the basal PRI value of the patient to be tested before the treatment (PRI0) and confirm that this value is included in the basal PRI range pre-established. Otherwise, refer to the paragraph limitations (§11) and repeat the test if necessary.

3- Determine the PRI value at a time point T (PRI1) according to the pharmacodynamic properties of the P2Y12 antagonist evaluated. A PRI1 value which is still included in the basal PRI range signifies that the patient has not responded to the drug.

10 PERFORMANCES

PLT VASP/P2Y12 has been validated on Becton Dickinson instrument type FACSCalibur and Beckman Coulter instrument type XL and XL MCL.

• Repeatability:

Samples (n = 8) presenting different level of VASP response (PRI) are tested 5 times with the same kit. Variations are represented below (mean +/- SD):

• Inter lot reproducibility:

Samples (n = 30) presenting different level of VASP response (PRI) are tested with 1 kit from 3 different lots. Variations are represented on the following graph (mean +/- SD):

9.6 Analysis of the results

After cytometric analysis, determine the value of the "corrected" MFI (MFIc) for tubes T1 and T2.

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- Working range:

The working range for this method is from 0 up to 100 % of PRI.

- Interferences:

- Aspirin:

According to the publication from Aleil B. et al. (1), Aspirin has no significant interference on PLT VASP/P2Y12 assay (n=67) : p=0.328.

- Platelet count:

On non treated samples from 50,000 up to 300,000 platelets/µL blood the platelet count has no significant interference on PLT VASP/P2Y12 assay.

- Abciximab:

According to the publication from Van Werkum J. et al (2), abciximab has no significant interference on PLT VASP/P2Y12 assay (n=11) : p=0.89.

- Aggregation correlation:

According to the publication from Aleil B. et al (1), PLT VASP/P2Y12 test is strongly correlated with the inhibition of ADP-induced platelet aggregation due to in vitro specific P2Y12 blockade : r = 0.72 ; p < 0.0001.

11 LIMITATIONS

- PLT VASP/P2Y12 kit cannot be used on hemolyzed blood samples.

- PLT VASP/P2Y12 kit should not be used on samples presenting a red blood cell count lower than the normal values. In this case it is recommended to repeat PLT VASP/P2Y12 assay on a new sample collected at least 24 hours later.

- The immediate platelet analysis right after the final addition of the 2 mL of reagent may reveal an incomplete red cell lysis for some samples. An overlap between the platelet cloud and the red blood cell cloud then occurs.

In order to get a complete red cell lysis store the sample for 5 minutes at room temperature before vortexing the tubes and performing the cytometric analysis again.

12 LIABILITY

The in vitro diagnostic use is only valid within the strict application of the package insert. Any modification of the protocol can influence the result of the tests. Do not switch vials from different lots.

In these cases no contestation or replacement of the product will be accepted.

13 REFERENCES


14 NOTICE TO PURCHASER

PLT VASP/P2Y12 kit is covered by patent WO 99/24473.