PLATELET SOLUTIONS

Customer Information Sheet

Platelet Function Testing Kits

(catalogue numbers PSK-001, PSK-002, PSK-003)

For remote testing of platelet function and the inhibitory effects of antiplatelet agents such as aspirin, clopidogrel and other $P2Y_{12}$ antagonists.

PSK-001 is a platelet function testing kit specifically designed to assess the inhibitory effects of the cyclooxygenase inhibitor aspirin on platelet function measured using P-selectin as a marker of the level of platelet activation. PSK-002 is a platelet function testing kit specifically designed to assess the inhibitory effects of a $P2Y_{12}$ antagonist, such as clopidogrel, on platelet function also measured using P-selectin as a marker of the level of platelet activation. PSK-003 is dual kit that allows measurement of the effects of both drug types.

INTENDED USE

The Platelet Function Testing Kits were developed to give information on the state of activation of platelets by measuring platelet activation markers such as P-selectin (CD62P). The agonists used to activate the platelets were chosen with a view to determining the effectiveness of antiplatelet agents such as aspirin and $P2Y_{12}$ receptor antagonists such as clopidogrel. The fixation and stabilisation of the activated platelets allows for remote platelet testing and subsequent analysis in a central laboratory between 24 hours and 9 days following fixation. P-selectin is quantified using immunofluorescence staining and flow cytometric analysis.

SUMMARY AND EXPLANATION

When platelets are activated, P-selectin which is normally present in α-granules, appears on the platelet surface and the amount present provides a measure of the degree of activation. Antiplatelet drugs are used to reduce the ability of platelets to form clots or thrombus by interfering with the platelet activation process. However, the effectiveness of the antiplatelet agents may not be optimum. Our easy-to-use platelet function testing kits provide a means of monitoring the effectiveness of these agents.

PRINCIPLES OF THE PROCEDURE

Activation of platelets with the agonists leads to exposure of activation markers on the platelet surface. The markers are then fixed by using a fixing solution (PAMFix), so that the expression of the platelet activation markers such as P-selectin can be measured by flow cytometry at any time point between 24hrs and 9 days after fixation. Platelet agonists are selected to stimulate platelets via the cyclooxygenase pathway and via the adenosine diphosphate (ADP) pathway. The dual kit is used to measure the effects of aspirin and a $P2Y_{12}$ antagonist simultaneously.

To process samples, 1 ml of anticoagulated whole blood or PRP is dispensed into each of the test vials containing lyophilised platelet agonists. Vials are then placed into the insulation pouch with a heating gel pack to keep blood at body temperature, and are incubated for 5 minutes. After incubation, when platelets have been stimulated with relevant agonists, the blood samples are transferred into tubes containing fixing solution. The fixing solution stabilises the exposure of the activation marker at the time of activation.

USING A PLATELET FUNCTION TESTING KIT

Kit Components

1. Luer lock syringe (1ml)
2. Purple vial adaptor
3. Activation vial (containing relevant stimulatory reagents in lyophilised form)
4. White luer lock adaptor
5. Fixing solution tube
6. Blood collection tube (see 6a) – Becton Dickinson Vacutainer ® 4.5ml 9NC 0.109M (citrate). [Check the use-by date and replace if necessary.]

6a. Alternatively, a Sarstedt S-Monovette® 5ml 9NC (citrate) may be used and no special adaptor is needed, (the purple vial adaptor is used to pierce the septum).

7. BD vacutainer blood transfer device
8. Insulation pouch (reusable)
9. Heating gel pack (reusable)
10. Instruction sheet and product information

Postage and packaging may be provided to return samples to a central laboratory for analysis (if this is required).

The number and type of components 1-5 will vary depending on the type of kit purchased.

REAGENT STORAGE AND HANDLING

Kits may be stored in a dry place at ambient temperature for up to 6 months. However, for extended vial stability (component 3), the vials should be stored separately at 4°C. Do not use past the expiry date stated on the vial or other components.

SAMPLE COLLECTION AND HANDLING

- Collect venous blood into an appropriate anticoagulant blood collection tube. Fill the blood collection tube to correct level to ensure the appropriate ratio of blood to anticoagulant, and gently invert three times to ensure complete mixing of the contents. **Do not freeze or refrigerate blood samples**
- Take care to avoid haemolysis and do not use if the blood sample is clotted
- Sample handling precautions should be undertaken following local rules for working with biohazardous material

PRECAUTIONS

- PSL test kits are for in vitro diagnostic use only
- The kits should be used strictly according to the manufacturer's instructions
- Fixing tube contains between formaldehyde. [R45 – may cause cancer; R43 – may cause sensitization by skin contact; S36/37 – wear suitable protective clothing and gloves; R20/21/22 – harmful by inhalation and if swallowed]
- Do not use the kits past the expiry date
- All patient samples should be handled as if capable of transmitting disease
- Follow the appropriate directions for contaminated and sharp waste disposal

TEST INFORMATION

Check that all components are present before starting the test. Allow at least 7 minutes to complete the procedure (which should not be halted before completion). Follow the step by step instructions below and the graphic instructions provided with each kit.

Briefly, 1 ml of anticoagulated blood or PRP is dispensed using a 1ml syringe and a purple vial adaptor into each of the labelled test vials containing lyophilised reagents. The syringe should be left attached to the vial, which is then gently mixed and placed into the insulation pouch with an activated heating gel pack to keep blood at body temperature of around 37°C. (Alternatively a heating block set at 37°C and capable of accommodating the blood tubes and vials may be used in place of the pouch and gel pack).

After incubating with the relevant stimulatory agonists, the blood samples are transferred into the labelled corresponding tubes containing fixing solution (PAMFix).

PAMFix stabilises the exposure of P-selectin at the time of activation and this exposure is then measured using flow cytometry.

All transfers (from blood tube to vial and from vial to fixing tube) are performed with specific adaptors thus making the test a closed system, with no blood handling required by the user.

NOTE. If, for any reason the procedure is not to be completed immediately after the blood has been taken, it must be completed within three hours of blood collection. The blood should be stored at ambient temperature in the interim, but requires at least 20 minutes to rewarm.

Only proceed to Step 1 when ready to begin the test.

STEP BY STEP GUIDE

Step 1
- Activate the heating gel pack just before it is to be used, and place it in one of the pockets of the insulation pouch.
- Place the activation vials into the second pocket of the insulation pouch to warm the contents.

Step 2
- Take the blood into the blood collection tube and fill to the correct level, mix by gently inverting three times and place immediately into the same pocket of the insulation pouch as the activation vials. Wait for 5 minutes until proceeding to the next step. (Wait 20 minutes if the blood is to be re-warmed).

Step 3
- Attach the BD blood transfer device (if using a citrate Vacutainer® blood collection tube) to the 1ml luer lock syringe. (Alternatively attach a purple vial adaptor if using a Monovette® system).
- Remove the blood tube from the insulation pouch and mix gently by inverting the tube three times.
- Keeping the tube upright, use the blood transfer device that is attached to the 1ml luer lock syringe to pierce the Vacutainer® membrane (if using the Monovette® system use the purple vial adaptor to pierce the tube). Ensure the connection is secure. Invert the tube and withdraw 1ml of blood into the syringe. Make sure the syringe is correctly filled without air bubbles (gently use a small push and pull motion).
- When filled, disconnect the syringe and attach a purple vial adaptor (if not already attached). After filling up all the required syringes discard the residual blood sample.

Step 4
- Remove the activation vial from the insulation pouch.
- Holding the body of the syringe which now contains the 1ml of blood sample, attach by means of the purple vial adaptor to the top of the activation vial piercing the rubber top. A vacuum should draw the blood into the vial. If this does not occur push the rest of the blood into the vial using the plunger of the syringe.

Step 5
- Mix the vial gently and return it, with the syringe still attached, back into the insulation pouch (into the same pocket with other vials, NOT the one with heating gel pack).
- Repeat for all the vials in the test kit.
- Wait 5 minutes for the activation to take place.
- After 5 minutes remove the vial and syringe from the pouch.
- Unscrew the 1ml syringe from the purple vial adaptor and discard the syringe.
- Attach the white luer lock adaptor to the purple vial adaptor that is on the vial.

Step 6
- Immediately attach the fixing tube (with matching label) to the luer lock adaptor and push the fixing solution into the vial.
- If you accidentally attach the wrong fixing tube to a vial it is vital to indicate it on the fixing tube – clearly write the letter of vial used with the fixing tube.
- Mix, then invert (with the vial upside down) and using a push-pull technique, finish by pulling the contents of th vial into the fixing tube.

Step 7
- After transferring the contents of the vial into the fixing tube, pull back the plunger fully, snap it off and discard.

- Remove the white luer lock adaptor and vial from the fixing tube and discard.
- The fixing tube containing the activated and fixed sample is then ready to be sent for analysis.

Step 8

- If the samples need to be posted for analysis, use suitable UN 3373 packaging. [These are supplied only if the kit is purchased with analytical services.]

It is recommended to perform the test straight away after venepuncture while the blood is still warm. If the test is to be delayed re-warm the blood sample for at least 20 minutes. Even then, the test should be completed within 3 hours of blood collection. Make sure the sample is incubated with the agonists for 5 minutes.

Failure to follow instructions will invalidate the test. In this case, repeat the test with a new kit. The following are some cases where the test needs to be repeated:

- Venepuncture/blood collection has complications
- Incorrect blood volume is used
- Failure to follow temperature control
- Blood is not mixed properly with vial content and fixing tube content

PRESENTATION OF DATA

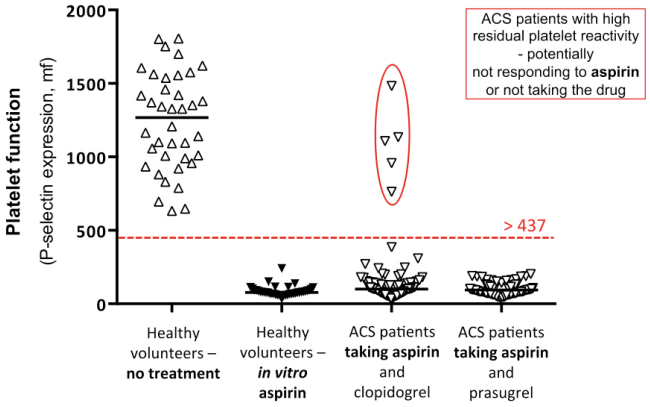
Flow cytometric analysis of the processed samples can be performed in any preferred validated way. **If analysis is performed locally, obtain normal ranges in the usual way and follow local Quality Control procedures for flow cytometry.**

Below is an example of the results of a flow cytometric analysis using PSK-003, all performed in a single laboratory.

The kit contains an A vial to measure platelet response to arachidonic acid and a C vial to measure platelet response to ADP. The performance of the vials was evaluated in blood samples obtained from healthy volunteers and treated with $P2Y_{12}$ inhibitors or aspirin in vitro, and also in blood samples obtained from cardiac patients receiving dual antiplatelet therapy with aspirin and clopidogrel or aspirin and prasugrel.

Performance of the A vial in healthy volunteers and patients on dual antiplatelet therapy

Platelet function is represented by the level of P-selectin expression (expressed as median fluorescence values, mf).



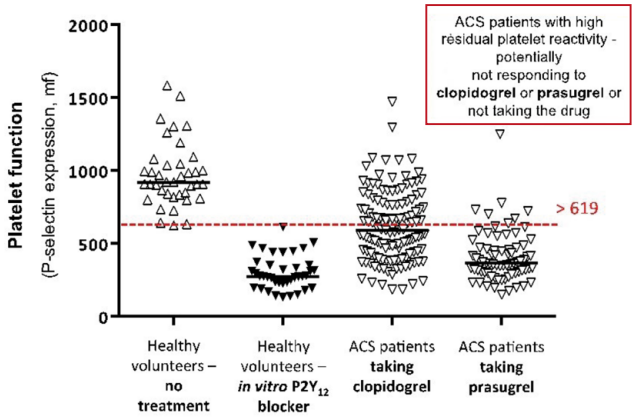
In these experiments performed in healthy volunteers the P-selectin expression induced by AA ranges between 633 and 1805 with the median at 1268 MF values. The in vitro addition of aspirin brings the values down significantly, with the range from 32 to 240 and the median at 78 MF values. The cut-off level for appropriate/desired platelet inhibition was determined using receiver operator curve (ROC) analysis. ROC curves were generated using the values obtained from healthy volunteers without and with, in vitro aspirin. The cut-off for the A vial at 437 MF values provides 100% sensitivity and 100% specificity in determining the presence and absence of aspirin effect.

The majority of patients on dual antiplatelet therapy, receiving either aspirin and clopidogrel or aspirin and prasugrel, show good platelet inhibition by the aspirin. The P-selectin expression stimulated by AA is similar to the levels seen with in vitro aspirin and is below the threshold level of 437 MF. However, several tested patients demonstrated low inhibition of platelet response to AA represented by high P-selectin values well above the cut-off level of 437 MF values.

There is clear definition between platelet responsiveness with and without the inhibitory effect of aspirin, both in vitro and ex vivo, using the A vial.

Performance of the C vial in healthy volunteers and patients on dual antiplatelet therapy

Platelet function is represented by the level of P-selectin expression (expressed as median fluorescence, mf).



In healthy volunteers the P-selectin expression induced by ADP in the C vial ranges between 624 and 1585 with the median at 920 MF values. The in vitro addition of cangrelor, a direct acting $P2Y_{12}$ receptor antagonist, brings the values down significantly, with the range from 133 to 613 and the median at 276 MF values. The cut-off level was determined using receiver operator curve (ROC) analysis. ROC curves were generated using the values obtained from healthy volunteers without and with, in vitro cangrelor. The chosen cut-off level provides 100% sensitivity and 100% specificity in determining the presence of $P2Y_{12}$ receptor inhibition.

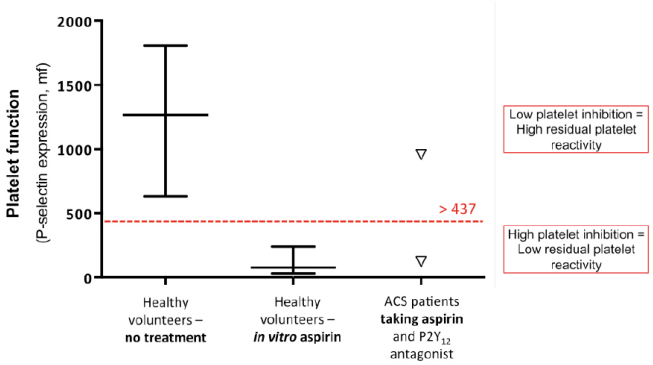
The ex vivo inhibition of platelet response to ADP by $P2Y_{12}$ receptor antagonists in patients is much more variable than the inhibition of platelet response to AA by aspirin. However, still most patients on dual antiplatelet therapy, receiving either aspirin and clopidogrel or aspirin and prasugrel, show significant platelet inhibition by the $P2Y_{12}$ receptor blocker seen as the P-selectin expression stimulated by ADP is below the threshold level of 619 MF. In agreement with the existing evidence generated using other assays for platelet function a significant proportion of the tested patients treated with aspirin and clopidogrel demonstrated insufficient platelet inhibition by clopidogrel as P-selectin expression induced by ADP was above the cut-off level. Among patients treated with aspirin and prasugrel only few demonstrated insufficient platelet inhibition by prasugrel. It could be concluded that in such patients, with P-selectin expression above the cut-off level, platelet inhibition with either of the two $P2Y_{12}$ receptor antagonists was less than that observed with in vitro addition of the $P2Y_{12}$ antagonist.

A published study suggests that such patients have higher risk of recurrent thrombosis [Thomas et al, Platelets 2014], however, it is yet to be determined if the alteration of antiplatelet therapy based on P-selectin measurements is associated with improved clinical outcomes.

The results of the test are generated by the central laboratory where the flow cytometric analysis is performed according to routine well established analytical procedures and are then sent to the clinician/researcher in the format of a standard report.

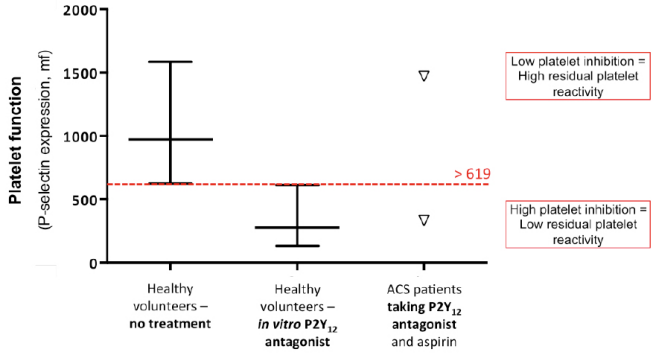
The level of platelet inhibition by aspirin could be labelled as "low level of platelet inhibition = high level of residual platelet reactivity" or "high level of platelet inhibition = low level of residual platelet reactivity" depending on the result of the AA-induced P-selectin expression.

The example of reported result for aspirin effect is shown for two representative patients. The range of values and the median seen in healthy volunteers without any therapy and with the in vitro addition of aspirin are given as a reference:



The level of platelet inhibition by a $P2Y_{12}$ antagonist could also be labelled as "low level of platelet inhibition = high level of residual platelet reactivity" or "high level of platelet inhibition = low level of residual platelet reactivity" depending on the result of the ADP-induced P-selectin expression.

The example of reported result for clopidogrel effect is shown for two representative patients. The range of values and the median seen in healthy volunteers without any therapy and with the in vitro addition of cangrelor, a direct acting $P2Y_{12}$ antagonist, are given as a reference:



The results obtained using PSK-003 should be interpreted in relation to other clinical and laboratory data available on a patient.

ASSAY LIMITATIONS

Patients with inherited platelet function disorders, such as Glanzmann thrombasthenia, Bernard-Soulier syndrome and von Willebrand disease have not been tested using PSK-001, PSK-002 or PSK-003 as these kits are not intended for use to diagnose these disorders.

The effect of platelet count on the results are unknown as patients with abnormally low (<100 x 103/µl) or abnormally high (>500 x 103/µl) platelet count have not been studied using this test kit.

Other drugs known to inhibit platelet function, may affect the results of the test.

EXAMPLES OF STUDIES USING PSK-001/002/003

Fox SC et al (2009). Measurement of platelet P-selectin for remote testing of platelet function during treatment with clopidogrel and/or aspirin. Platelets, 20(4):250-9.

Fox SC et al (2013). Effects on platelet function of an EP3 receptor antagonist used alone and in combination with a $P2Y_{12}$ antagonist both in-vitro and ex-vivo in human volunteers. Platelets, 24(5):392-400.

Thomas MR et al (2014). A platelet P-selectin test predicts adverse cardiovascular events in patients with acute coronary syndromes treated with aspirin and clopidogrel. Platelets, 25(8):612-8.

Bath PM et al (2017). Remote assessment of platelet function in patients with acute stroke or transient ischaemic attack. Stroke Research and Treatment; https://doi.org/10/1155/2017/7365684

TECHNICAL SUPPORT

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