DiaMed Impact-R Test Kit For the determination of platelet functions in anticoagulated whole blood with the DiaMed Impact-R Product-Identification: 47600

Introduction / Intented use:

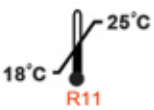
The Impact-R Test Kit is a reagent kit for platelet function testing on the DiaMed Impact-R device.
The device is testing platelet adhesion in anticoagulated whole blood under arterial flow conditions (1800 s^{-1}; for 2 min.) . The laminar flow oveer polystyrene surface of the well is achieved according to the Cone and Plate principle. The Impact-R test method is aimed at the study of platelet function, screening of primary haemostasis abnormalities (e.g. Von Willebrand disease) including thrombocytopenia and monitoring therapies. It can be used for testing both hypo- and hyperfunction of pplatelets. Furthermore, it provides a quick method for monitoring the response to various anti-platelet drugs.

Reagents:

Impact-R Test Kit, containing:
- 1 x 50 pcs. cones
- 5 x 10 pcs. wells
- 1 x 55 pcs. pipette tips
- 1 x 100 mL "May Grünwald" staining solution to be bought separately from www.VWR.com (contains Methanol ≥ 50%)

Chemicals must be disposed of in compliance with the respective national regulations.

R11, R23, R24, R25, R39 *
S7, S16, S36, S37, S45 *



Stability: see expiry date on label.

R11	Highly flammable
R23	Toxic by inhalation
R24	Toxic in contact with skin
R25	Toxic if swallowed
R39	Danger of very serious irreversible effects
S7	Keep container tightly closed
S16	Keep away from sources of ignition. No smoking.
S36	Wear suitable protective clothing
S37	Wear suitable gloves
S45	In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible)

Further materials required:

- Matis Medical Impact-R
- PC (requirements see User Manual Diamed Impact-R)
- Matis Medical EP-3 pipette or equivalent.
- Sodium citrate vials 0, 105 M (Becton Dickinson 4,5 ml., 3,2%)

Sample material:

Whole blood samples to be tested should be collected in 0, 105 mol/L (BD 3,2%) sodium citrate. Store the sample at room temperarture (18-25·C).

The samples should be tested not earlier then 45 minutes and not later then 3 hours after collection.

For platelet testing never use the first tube from a blood collection.

Caution:

There is a risk of infection from skin contact with blood samples. Always wear protective gloves when working, in accordance with laboratory safety regulations.

Controls:

Known samples should be included in accordance with the relevant guidelines of quality assurance.

Test procedure for whole blood assay (Standard test)

For detailed instructions, please consuit the User Manual Impact-R Chapter 6.2
For routine platelet testing , the following parameters must be defined:
Set 1: 0 RPM for 15 sec. (pre-incubation)
Set 2: 720 RPM for 120 sec.

1. Define the blood test parameters (motor 1).
2. Place up to 4 kits (well and cone) in their places. Mark the date and running number on each well.
3. Place the bell-housing with the collet over each well and cone: Press the head of the collet to verify that the cone and the collet are attached. Remove the bell-housing with the engaged cone from each well.
4. Mix the blood test tubes on the tube rotator at 10 RPM 1 minute. When the cycle is complete, immediately open the blood tubes.
5. Important : if the tube is used for the first time after the blood was taken or if the tube has not touched for more than 10 minutes, repeat point 4.
6. Attach a tip to the pipetor and calibrate to 130 µL. Push the button on top of the pipetor and insert the tip into the first test tube about half way into the blood; then press the pipetor button to suck the blood into the tip.
7. Insert the tip into the first well and empty by pressing the pipetor button again. Release the blood over the center of the well.
8. Remove the tip and **immediately** put the bell-housing with the engaged cone in place.
   Recommendation: *Make sure that you perform this step in less than 4-5 seconds after you take the tip out of the well.*
9. Dispose of the used tip into a biological waste container by pressing the release button of the piptor.
10. Immediately repeat steps 4 through to 9 for the other blood test tubes.
    Recommendation: *4 samples must be completed in less than 45 seconds.*
11. Push the "A" key to start the rotation process according to the pre-defined parameters.
12. When the rotation is finished, remove the bell-housing with the cone on the collet; pull the collet stick to release the cone above the biological waste container.
13. Using a Pasteur Pipette with a Diamed tip attached to, dispose off the excess blood, which remained in the well by washing the well with regular tap water or saline solution.
    Hold the well gently with your fingers. In any case, do not touch the well inner surface. Wash the well by directing a gentle water stream from the Pipette on the inner side wall of the well.
14. Add about 1 mL " May Grünwald" staining solution to the empty (washed) well. Wait for about 1 minute and completely suck the stain out using a suction pipette; leave the well to dry for about 1 minute until there are no visible residues of liquid on the surface.
15. Cnntinue the test by analyzing the result.

Interpretation of the value:

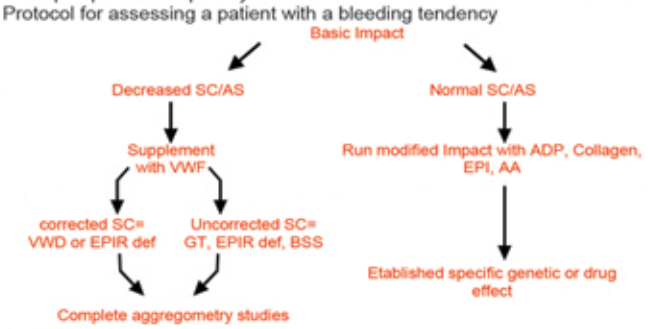
1. General diagnostic criteria:
   Normal range:
   Surface Coverage: ≥ 7,5 % and Average Size ≥ 25 µm²
2. Screening for von Willebrand Disease (vWD):
   Surface Coverage: < 7,5 % or Average Size < 25 µm²
   (with normal platelet count)
   Sensitivity : 76%
   Sensitivity : 91%
3. vWD Confirmation:

Blood sample supplemented with von willebrand Factor (0,25 IU/mL) is tested by the Impact-R. If the results indicated above are increased to the following values the diagnosis of vWD is confirmed.
Surface Coverage: ≥ 7,5 % and Average Size ≥ 25 µm²

4. vWD Confirmation Notes:

4.1. Low SC and/or AS , which are corrected byt the supplemented vWF, may indicate an Epinephrine receptor dysfunction as well !
4.2. In a case where the SC/AS values are not corrected by vWF check for Glanzmann's thrombocytopeinia, Bernard Soulier disease and Epinephrine receptor dysfunction.

Protocol for assessing a patient with a bleeding tendency



Remarks:

For detailed remarks refer to the User Manual DiaMed Impact-R.

Limitations:

a) Too fast or too slow agitation of the cone may cause wrong results! Control before every run the speed and duration of the agitation.This must be in a normal testing procedure: 720 rpm (= 1800 s-1) for 2 minutes.
b) Over-agitation when mixing the blood sample after collection and before use may activate the platelets and cause incorrect results.
c) Strict adherence to the procedures and recommended equipment is essential.
d) Use only the pipette tips from the Impact-R test kit. The use of other tips may cause incorrect results.
e) The adhesion properties of paltelets is affected by both haematocrit and platelet count. Blood samples must have a minimum platelet count of 25'000. Therefore , testing blood samples with haematocrit < 30 or platelet count < 100'000 may result in a SC < 7,5 % and AS < 25 µm² that can be increased by correcting the haematocrit or platelet count (indicating normal platelet function).
f) In the case of thrombocytopenia an AS > 25 µm² in spite of low SC may represent a lower number but with normal function.
g) The AS parameter requires a certain level of surface coverage in order to provide diagnostic information. SC values of 1,5 % or lower do not allow an interpretation of the AS parameter.

Bibliography:

D. Varn, No. Savion: Cone and Platele(let) Analyzer, Platelets (A.D. Michelson,ed.), Academic Press,p. 337-345, 2002.