PGE1 Reagent

HB-5515-FG PGE1 Reagent 1 x 1.0mL
HB-5544-FG PG E1 Reagent 2 x 1.0mL

INTENDED PURPOSE

GB Handling Instructions

Hart Biologicals Prostaglandin E1 (PGE1) Reagent is used in conjunction with ADP in tests to diagnose platelet dysfunction, or normal platelet activity in human platelet rich plasma or whole blood. It is used to enhance the sensitivity of ADP-induced platelet aggregation to the effects of the P_2Y_{12} antagonist drugs (such as Clopidogrel)

SUMMARY

PGE1 is a natural platelet inhibitor which triggers an increase in cAMP levels in the platelet. A decrease in platelet cAMP level in the platelet leads to platelet activation. An increase in platelet cAMP level counteracts platelet activation. The addition of PGE1 to the platelet aggregation tests with ADP induces a moderate inhibition of platelet activation in healthy normal blood samples, but a significant increase in sensitivity of the ADP test towards inhibition of platelet reactivity by P_2Y_{12} antagonist drugs (such as Clopidogrel). Additionally, the addition of higher concentrations of PGE1 into the ADP test normally induces a strong inhibition of ADP induced aggregation and can be used as a positive control for ADP test.

TEST PRINCIPLE

The platelet aggregation test measures the rate and degree to which dispersed platelets in a sample of platelet rich plasma (PRP) or anticoagulated whole blood forms clumps (aggregates) after the addition of a substance that normally stimulates platelet aggregation (agonist). In optical aggregometry, the clumping of the platelets causes the platelet rich plasma to become less turbid . This is measured on a platelet aggregometer, which plots the rate and maximum extent of the aggregation reaction. In whole blood aggregometry, platelets adhere to small wires suspended in the blood sample and the impedance between the wires as the platelets adhere and aggregate is measured and plotted. The inhibition of ADP-induced platelet aggregation by drugs such as Clopidogrel can be difficult to discriminate, particularly as sodium citrate is used as anticoagulant. The addition of PGE1 to the test cuvette prior to ADP increases the sensitivity of the ADP test for Clopidogrel, making the discrimination between responders and non-responders easier to see.

WARNINGS AND PRECAUTIONS

For *in-vitro* diagnostic use only.
Do not pipette by mouth. Do not smoke, eat or drink in areas where specimens or kit reagents are handled.
Wear disposable gloves when handling specimens and kit reagents, and wash hands thoroughly afterwards.

MATERIALS PROVIDED

PGE1 Reagent

Ingredients: The reagent contains a lyophilised preparation of Prostaglandin E1 with added buffer and stabilisers.
Preparation for use: Each vial of PGE1 Reagent should be reconstituted with exactly 1.0mL of purified water. Allow to stand for 10 minutes and swirl to mix – do not shake.
Storage and stability: The unopened product should be stored at 2…8°C and is stable until the expiry date printed on the vial label. After opening, the product is stable for 2 weeks at 2..8°C and 4 weeks at -20°C. If the vial shows any sign of contamination, please discard.

MATERIALS REQUIRED BUT NOT PROVIDED

Platelet aggregometry system – the Hart Biologicals GpIIbIIIa Antagonist Reagent will perform satisfactorily when used on any aggregometer system. Follow the manufacturer's instructions for the operation of the aggregometer in use.
Purified water
1.0mL pipette

SAMPLE COLLECTION AND PREPARATION[4]

Preparation of Platelet-Rich and Platelet- Poor Plasma for Optical Aggregation

Blood for platelet aggregation testing should be collected in to plastic syringes and transferred to plastic tubes, or collected in siliconised glass evacuated blood collection tubes.

Blood (9 parts) should be mixed with 0.11M or 0.13M sodium citrate anticoagulant (1 part). Invert gently to mix. Do not shake.

- Prepare platelet rich plasma by centrifuging the anticoagulated blood at 150-200 x g for 10-15 minutes at room temperature.
- Remove the platelet rich plasma with a plastic transfer pipette and place in a plastic container (with cap) labelled 'PRP'. Cap the container and keep at room temperature.
- Prepare platelet poor plasma by centrifuging the remaining blood specimen at 2000 x g for 20 minutes.
- Remove the platelet poor plasma with a plastic transfer pipette and place in a plastic container (with cap) labelled 'PPP'. Cap the container and keep at room temperature.
- Adjust the platelet concentration in the PRP to 200-300x10^9/L using PPP, cap and allow to stand at room temperature for 30 minutes prior to testing.
- Testing should be completed within 3 hours of blood collection.

Whole Blood Aggregation Samples

Refer to the aggregometer manufacturer recommendations for the preparation of samples for whole blood aggregometry.

TEST PROCEDURE

A) Optical Aggregometry

1. Set the 0% and 100% aggregation levels on the aggregometer using platelet poor plasma and platelet rich plasma according to the manufacturers instructions.
2. Pipette the required volume of platelet rich plasma in to an aggregation cuvette and add a stir bar.
3. Pre-warm to 37^{o}C for 120 seconds.
4. Add the required volume (10µL per 250µL of PRP) of PGE1 Reagent directly in to the cuvette. Do not allow reagent to run down the wall of the cuvette.
5. Add the required volume of Platelet Aggregation agonist (TRAP-6, ADP, Arachidonic Acid or Collagen) directly in to the cuvette. Do not allow reagent to run down the wall of the cuvette.
6. Allow the aggregation pattern to form for a minimum of 5 minutes.

B) Whole Blood Aggregometry

Refer to the manufacturers instructions for the correct performance of the test.

QUALITY CONTROL

The results of platelet aggregation studies should be interpreted against the results of aggregation profiles of a normal sample tested at the same time. The normal donor should not have ingested aspirin or aspirin containing compounds in the preceding 10 days.

EXPECTED VALUES [5,6]

The presence of GpIIbIIIa antagonists in the sample may lead to weak aggregation in the ADP test. The intake of clopidogrel or other thienopyridines as well as the presence of direct ADP receptor antagonists in the sample leads to weaker aggregation responses.

FURTHER TESTING

If the test results are abnormal, the test should be repeated on a separate occasion. If the results are consistently abnormal, and the patient is not taking any medication known to interfere with platelet function, additional tests should be considered [7].

LIMITATIONS

In optical aggregometry, the presence of red blood cells in the PRP will cause the total observed aggregation to be reduced. The presence of platelets in the PPP will cause the total observed aggregation to appear increased.

Spurious results can be observed when the total platelet count of the PRP is less than 75 x 10^9/L.

PRP tested less than 30 minutes after preparation may exhibit abnormal aggregation profiles.

Bibliography

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