Agence française de sécurité sanitaire des produits de santé
Drugs and Biologicals Evaluation Division
Haemovigilance Unit



# Investigation of Grade ≥ 2 allergic Recipient Adverse Reactions (RAEs)

This Standard Operating Procedure cancels and replaces the version of May 2009.
**It concerns all Labile Blood Products (LBPs)**

The purpose is to describe the causality work-up which proceeds in two phases:

- ✓ whatever the LBP in question, histamine and tryptase assays are to be carried out on samples drawn in the minutes or hours following a RAE of grade 2 or over (e-fit 2),

- ✓ plus tests carried out later for a **serious** RAE **(grade 3)**, i.e. at least 4 weeks after the reaction, possibly including both *in vitro* and *in vivo* tests. The purpose of these tests is to detect sensitisation to a medicinal product or substance administered at the time of the allergic reaction and which may have caused it. For a Methylene blue treated plasma (FFP MB), this work-up is intended to investigate the role of its constituents, notably the Methylene Blue.

## FFP-MB

After a first allergic reaction associated with a FFP-MB, the AFSSAPS "Allergy" Working Group recommends that **this product should not be transfused again until complementary tests have ruled out sensitisation to the constituents of the FFP-MB**, notably the Methylene Blue (MB, the residual concentration of which is fixed by the regulations at under 30µg/L).

## I. Immediate tests

These tests should be performed **whatever the BLP transfused.** for any accident of **grade 2 or worse** which is suspected of being allergic (e-Fit 2),
How to investigate grade 1 RAEs with only skin symptoms is left to the discretion of the medical team but histamine and tryptase assays are recommended in grade 2 reactions (Ring and Messmer system) as described in the "Allergy" Technical Sheet, including isolated hypotension.

***Blood samples from the recipient for histamine and tryptase assays:***
3 samples are needed:

| Sampling time | < 30 min | 30 min to 2 h | > 24 h |
|---|---|---|---|
| Test | Histamine | Tryptase | Tryptase (baseline) |
| Type of tube | EDTA | EDTA or dry | EDTA or dry |

These tests can be carried out post mortem before intensive care is withdrawn or in the minutes immediately after (there is a risk of false positive results if samples are taken at autopsy).

***Treatment of samples***
Each tube is taken to the local laboratory, within two hours if it is being kept at room temperature or within 16 hours if it is at 4°C. The local laboratory centrifuges the tubes at a temperature of 6 - 15°C. Then the plasma is **gently drawn off—without approaching the pellet** (do not aspire the blood cells)—and divided into a series of aliquots: two aliquots of 300-500 µL per sample and per test (histamine, tryptase) with the sampling time clearly marked on each one. The aliquots are frozen at -20°C and then one aliquot per test is sent to the laboratory responsible for the testing (preferably transported frozen). The other aliquot is kept frozen.

***Interpretation of the results***
After a severe allergic reaction, the histamine concentration in the blood peaks straight away and then drops with a half-life of about 15-20 minutes. The first sample should be taken as soon as possible after the beginning of the reaction. The normal concentration is below 6nmol/L and a concentration of over 9nmol/L is considered as positive.
Tryptase peaks later, 30 minutes to two hours after the beginning of the reaction. Samples should not be drawn in the first 15 minutes to prevent false positives. Its half-life is 90 minutes. For very severe reactions, the result can remain positive for 6 hours or longer. The normal concentration is below 12.5µg/L and a concentration of over 25µg/L is considered as pathological. Since normal levels vary significantly between individuals, it is recommended to establish a baseline measurement later (24 hours or more) for the sake of interpretation.

## II. Later tests

These tests are **specially performed for immediately life-threatening allergic RAEs** (grade 3 of severity).
Ideally, they are carried out 4-6 weeks after the initial reaction even if the blood histamine and tryptase results were normal or these tests were not carried out. The aim is to investigate sensitisation to a drug or a substance administered at the time of the hypersensitivity reaction and which might have caused it. Which tests will be performed will be decided at an allergy consultation on the basis of timing and clinical history.

**For a FFP-MB**, this work-up is intended to establish the responsibility of the constituents of

the FFP-MB, notably the Methylene Blue.
It is important to keep the FFP-MB bag(s) in question at -20°C, after disconnection according to the procedure described in Appendix B of the Technical Sheet on "Transfusion-transmitted bacterial infections" (March 2010). These products are intended for use in *in vitro* tests but not skin tests because of non-sterility.
If no samples of native plasma or no other bags of FFP-MB from the donor(s) are available, a new donation should be sought from the donor(s) to obtain native plasma and a new FFP-MB (with Informed Consent). For ethical reasons, the donor's native plasma will not be used for skin tests.

On request, Macopharma will kindly provide a bottle containing 5 mL of the Methylene Blue used to prepare the FFP-MB, diluted to 1% in Water for Injection. When submitting the request, it is essential to **specify the batch number of the FFP-MB bag** so that the Methylene Blue tested will be the same one as that used to prepare the FFP-MB that may have caused the allergic reaction. A new Methylene Blue (Proveblue from Provepharm) is being brought into use and there will inevitably be a period during which there will be FFP-MB prepared with the two different dyes, both the new and the old.

**Address and contact persons:**


- Jacqueline Fauquet: +33.3.20.11.85.24 (06.50.19.33.85)
  jacqueline.fauquet@macopharma.com

- Béatrice Carvalho: +33.3.20.11.85.45 (06.63.30.55.65)
  beatrice.carvalho@macopharma.com

- Typhaine Labouré: +33.3.20.11.86.78
  typhaine.laboure@macopharma.com
  Fax: 03.20.11.84.90

## a. Skin tests: for any Grade 3 RAE, whatever the BLP

DIAGNOSTIC SKIN TESTS

**Patient's name:** **Date of the RAE:**
**Date of the testing:**

Protocol:
◊ Grade 3 RAE occurring in the course of or after a BLP transfusion other than FFP-MB, and according to the opinion of an allergy specialist, prick-tests followed by an IDR with latex and medications used in the course of the transfusion will be carried out according to the protocol below.
◊ Grade 3 RAE occurring during FFP-MB use, prick-tests followed by an IDR with latex, FFP-MB, the MB and possibly other products used in the course of the transfusion according to the opinion of an allergy specialist will be carried out according to the protocol below.
If the prick-test is positive, still carry out the IDR (to define the reactivity threshold): *Mertes PM, Malinovsky JM, Mouton-Faivre C, et al. Anaphylaxis to dyes during the perioperative period: Reports of 14 clinical cases. J Allergy Clin Immunol 2008 ; 122: 348-352.*
The diluent used for skin tests is the same as that used for the negative control, i.e. normal saline or phenolated water.

| | | IDR | | | | |
|---|---|---|---|---|---|---|
| | Prick-test | 1/10000 | 1/1000 | 1/100 | 1/10 | |
| | | IP/OP* | IP/OP* | IP/OP* | IP/OP* | |
| Codeine phosphate 9% or Histamine 10 mg/mL | | | | | | |
| Latex | | | | | | |
| Forlax (PEG) | | | | | | FFP-MB |
| Donor's FFP-MB [1] | | | | | | FFP-MB |
| Methylene Blue provided by Macopharma | | | | | | FFP-MB |
| Other : …….. | | | | | | |
| Negative control | | | | | | |

PI/PO*: injection papule (IP) (mm) / oedematous papule (OP) after 20 min (mm). *Pure IDRs may give non-specific reactions*
Positive reactions: *Trace prick and IDR positive in the following cases.*
**prick: diameter of the oedema > half the diameter of oedema for the codeine control or ≥3 mm with erythema**
**IDR: diameter of the OP ≥3 mm of the diameter of the IP**
(1) If possible, a sample from the same donation (sterile closed segment of the FFP-MB tubing, another FFP-MB derived from the same donor); otherwise a new FFP-MB derived from the same donor.

**CONCLUSIONS FROM SKIN TESTS**

Signature:

## b. in vitro TESTS: for any Grade 3 RAE with FFP-MB

## BASOPHIL ACTIVATION TEST BY FLOW CYTOMETRY TO TEST FOR HYPERSENSITIVITY TO METHYLENE BLUE

The Methylene Blue provided by Macopharma will be tested. For the CAST® Flow2 method (Bühlman Laboratories AG), whole blood is drawn on EDTA.

Samples are prepared in the following way:

- Methylene Blue:

*Test tube:* 50 µL whole blood + 100 µL activation buffer + 50 µL Methylene Blue diluted (in activation buffer) to 5, 1, 0.2, 0.04, 0.008 µg/mL + 20 µL antibody against CCR3/CD63.

- FFP-MB:

*Test tube:* 50 µL whole blood + 100 µL activation buffer + 50 µL pure FFP-MB diluted to 1/5, 1/25, 1/125 + 20 µL antibody against CCR3/CD63.
Run a control with native plasma from the donor diluted in the same way.
If the donor's plasma cannot be obtained, the alternative protocol uses a FFP-MB from another donor.

- Control tubes

*Negative control tube:* 50 µL whole blood + 150 µL activation buffer + 20 µL antibody against CCR3/CD63.

*Positive control tube:* 50 µL whole blood + 100 µL activation buffer + 50 µL antibody against the IgE receptor (or 50 µL fMLP) + 20 µL antibody against CCR3/CD63.

For all tubes (tests and controls), the expression of CD63 is measured in percentage and MFI. For more information, refer to the manufacturer's instructions.

Any laboratory that carries out basophil activation tests will be capable of carrying out this test to detect hypersensitivity to Methylene Blue. A sample drawn on EDTA can be kept for 24 hours at 4°C before testing. The model protocol provided here may be adapted according to the procedures of the laboratory that is carrying out the test.