Smart and Friendly to Use Functional Assay for Confirmation of Heparin-Induced Thrombocytopenia

Combination with Clinical Probability and Immunoassay for the Diagnosis of HIT
Diagnosis of Heparin-induced Thrombocytopenia (HIT) is still an important challenge for physicians, especially in cardiology, in intensive care and emergency units, when heparin remains the anticoagulant of choice for managing acute cardiovascular and thromboembolic diseases. In presence of a suspicion of HIT, heparin must be withdrawn, however continuation of this therapy can be of benefit for most of the patients if this iatrogenic complication is ruled out with a functional assay. Combination of clinical probability evaluation with laboratory testing is needed for patients’ risk classification. The conventional IgG-specific immunoassay is highly sensitive but lacks specificity, as it is positive in many patients who remain asymptomatic, and disease develops only in some of them. Using the magnitude of immunoassay positivity, with a cut-off value at a high Optical Density (OD), detects better heparin induced thrombocytopenia, but a functional, platelet activation, assay is required for confirming this complication. Whilst usual functional assays are restricted to few laboratories, and require highly skilled personnel for interpretation, a new method using Flow Cytometry becomes available on-demand, in all sites equipped with a flow cytometer. This Emosis HIT Confirm® assay is a one-step technology, requiring only 30 minutes, and it only needs platelet rich plasma from a healthy donor, without any need to use washed platelets. This assay is expected to greatly improve the rapid diagnosis and confirmation of HIT in critical clinical contexts, and to offer a better management of affected patients.
Heparin-induced Thrombocytopenia (HIT) remains a rare but severe and life-threatening complication of heparin therapy, presenting a higher incidence with Unfractionated (UFH) Heparin, than with Low Molecular Weight Heparin (LMWH) treatments (1-5). Risk to develop HIT is increased in some clinical situations, and with heparin exposure duration. This complication tends to occur more often in orthopedic patients, although the highest rate of antibody development is observed in Extra-Corporeal Circulation (ECC), Cardio Pulmonary Bypass (CPB) and ExtraCorporeal Membrane Oxygenation (ECMO), or in hemodialysis patients (6-8). However, recent big data analysis indicates a different incidence of HIT than previously reported, in the various clinical contexts where heparin is used (4, 5). This incidence is reported lower in orthopedic patients, and the highest one is in ECC. Development of heparin dependent antibodies means the presence of an immune response, heparin dependent, mediated by Platelet Factor 4 (PF4), however most of the generated antibodies remain asymptomatic (9-11). These antibodies can be of the IgG, IgM or IgA isotype (12, 13). They are targeted mainly to Heparin-Platelet Factor complexes (HPF4) in typical HIT, but some disease variants are associated with heparin dependent antibodies targeted to other antigens than PF4 (Interleukin-8/IL8, Neutrophil-Activating Peptide 2/NAP2, or Protamine Sulfate), as this has been reported (14-18). Symptomatic antibodies are mainly of the IgG isotype, although many of them can remain asymptomatic (13). Factors which contribute to render antibodies pathogenic are not fully understood, but antibodies with the highest affinity to HPF4 complexes or to PF4 are those with the highest capacity to activate platelets (19, 20). Antibody bind to platelets through the antigen site (onto platelet bound HPF4 complexes), and through their IgG-Fc fragment to platelet FCy-RIIA receptors (CD32) (3, 12, 21). This binding produces platelet activation and destruction, and thrombosis can develop in some cases. Heparin dependent antibodies are generally formed between 5 and 15 days from the onset of heparin therapy and they are usually of the IgG isotype (2, 3, 16). IgM or IgA antibodies can develop in many heparin treated patients but remain asymptomatic, excepted in rare cases (13). The direct and fast development of anti-HPF4 antibodies of the IgG
isotype in symptomatic patients (but never previously exposed to heparin) suggests a former immune stimulation with PF4 complexed with glyco-amino-glycans (polyanions), this protein exposing then an altered structure (10-12, 20). The HIT immune response, which develops during heparin therapy, could then be secondary (anamnestic) to a former immune stimulation (22). The primary stimulation could be consecutive to an infectious episode, PF4 binding to bacterial polysaccharides, and inducing a self-response (23). The higher incidence of HIT in patients with former periodontitis or gum diseases supports this hypothesis (4).

Laboratory diagnosis of HIT is of major importance as this complication becomes rapidly severe and life threatening and can provoke limb amputation in some patients. The first action in presence of HIT suspicion is to withdraw heparin and to switch to an alternative anticoagulant. However, if HIT is excluded, patients can benefit again from the high therapeutic and antithrombotic efficacy of this drug, which remains superior to all the substitutive anticoagulant treatments. HIT is suspected in presence of a platelet count drop > 50% (eventually 30%) on 2 successive counts, or a platelet count < 100 G/L, and in presence of a significant clinical probability (4Ts score) (2, 3, 16, 24-28). This clinical evaluation is addressed later in this report. In addition to the clinical probability, laboratory testing of patients’ plasma is required for establishing the HIT diagnosis (11, 16, 29). Laboratory investigation involves the immunological measurement of heparin dependent antibodies, and, when positive, confirmation of diagnosis with a functional assay is required to demonstrate platelet activation (2, 3, 16, 30, 31). The confirmation test is performed at a low and high heparin concentration, and platelet activation occurs only at low heparin concentration, but not at high. In any case, if the immuno-assay is negative, HIT can be excluded with a high probability, and heparin can be continued (if clinical examination favors this decision). Conversely, higher is the IgG antibody concentration measured with the immunoassay, and higher is the probability of HIT (32).

Nevertheless, immunoassays lack of “clinical specificity”, as many antibodies, although IgG, remain asymptomatic (13, 29, 33).
Generation of heparin dependent antibodies and clinical development of HIT are based on 2 different timings. Whilst presence of antibodies reflects the immune response, pathogenicity requires the binding of these antibodies, mainly IgGs, with enough affinity, onto the target antigen (HPF4 complexes) bound onto platelets or other blood cells, including monocytes and endothelium (19, 34, 35). Heparin dependent antibodies then target their deleterious and activating effect to these cells. Different conditions are requested for generation of antibodies, and for development of the HIT clinical complications (20). Immuno-stimulation needs the presence of the antigen, which is formed when heparin and PF4 concentrations are in a stoichiometric ratio forming ultra-large complexes. This occurs in presence of about 27 IU (about 150 μg) of heparin (specific activity of about 180 IU/mg for UFH) per mg of PF4-tetramer (figure 1).

**Figure 1:**
Formation of ultra-large, stoichiometric HPF4 complexes in presence of heparin: in presence of a heparin concentration with an equivalent electro-negative content to the electro-positive charges on PF4 tetramers, ultra-large complexes are form, as shown on the central picture; if PF4 or heparin are in excess, no complexes can be formed.
There is a tight interaction between the electronegative heparin charge, and the ring of positive charges exposed on PF4 tetramers (11, 12, 20). This binding alters the PF4 structure and generates neo-epitopes exposed on this protein and can render it immunogenic. Generated HPF4 complexes can then bind heparin-dependent antibodies when present (Figure 2).

Figure 2:
At the onset of heparin therapy, the PF4 mobilizable pool on endothelial cells is displaced by heparin, and high concentrations of PF4 can be released into blood circulation and can form multi-molecular ultra-large HPF4 complexes in presence of the appropriate heparin concentration. Endothelial cell PF4 amount is higher in vascular diseases, or all clinical contexts with blood activation and PF4 release from platelets, as a complex with a proteoglycan dimer; it binds to endothelial cell glyco-amino-glycans and is displaced by heparin.

Total amount of PF4 releasable from blood is in the range of 3-10 μg/ml, which means that stoichiometric HP4 complexes are formed in presence of heparin concentrations from 0.08 to 0.27 IU/ml, should all PF4 be released (which can only occur at “blood coagulation” sites). In addition, when patients are treated with heparin (UFH or LMWH), at the onset of treatment, there is a boost of PF4 into blood circulation (PF4 is released from its “endothelial storage pool”), which intensity depends on the patient’s clinical state (hypercoagulability/blood activation grade), as shown on figure 2. In blood circulation, PF4 concentrations can then range from < 100 ng/ml up to 1,000 ng/ml, corresponding to required heparin concentrations in blood circulation from 0.0027 to 0.027 IU/ml. This means that even at the onset of heparin treatment, when the highest PF4 concentration is present in
blood circulation, immunogenic complexes are formed in presence of very low heparin concentrations. However, at blood activation or inflammation sites, much higher PF4 concentrations can be present in the microenvironment and can form HPF4 immunogenic complexes in presence of higher heparin concentrations. With the continuation of heparin therapy, once the PF4 releasable pool has been mobilized from endothelial cells, PF4 concentrations return to much lower values in blood circulation (< 10 ng/ml), excepted at inflammatory and blood activation sites. When heparin is given through injection, its blood concentration presents important variations between 2 injections, and conditions for stoichiometric HPF4 complexes formation can be met in some cases, and this stimulates the immune system. When immuno-stimulation occurs, IgG antibodies are present few days later (13). However, pathogenicity can only develop if antibodies bind to their target antigen with enough affinity, i.e. if they can react with HPF4 complexes exposed onto platelets, or some blood white cells (monocytes, ...) and endothelial cells (12, 21, 34-36). This condition requires again the presence of stoichiometric ultra-large PF4 complexes in blood circulation, or at pathological sites. If no complexes are available, antibodies cannot bind to platelets and blood cells, and cannot induce HIT. This condition of a lack of target antigen for binding antibodies is rapidly met following heparin withdrawal, when HIT is suspected. If complexes are present in blood circulation, or more probably in the microenvironment at pathological sites associated with inflammation, blood activation and chemoattraction of platelets and white blood cells, then antibodies can bind to these cells, which expose HPF4 complexes. They induce then the pathogenic multifactorial mechanisms: platelet activation and aggregation, interaction with leukocytes forming aggregates, activation of monocytes and endothelial cells, release of Tissue Factor (TF), all contributing to development of thrombosis (2, 3, 16, 23, 34-36).
PATHOGENICITY OF HEPARIN DEPENDENT ANTIBODIES

Many of the heparin dependent antibodies, including those with the IgG isotype, are asymptomatic, and only a subgroup of antibodies is positive in platelet activation assays. What differentiates pathogenic from asymptomatic antibodies is not fully understood, with the exception that there is some relationship between antibody concentration and occurrence of HIT, and that antibodies with the highest affinity to HPF4 are those with the strongest capacity to activate platelets (19, 20, 32). Probably, the immune response is first targeted to HPF4 complexes, but in some patients, antibodies can also react with PF4 alone as the consequence of epitope spreading, and then the deleterious antibodies’ effect is higher. In most of cases, pathogenic antibodies are of the IgG isotype. Interestingly, patients who develop HIT generate directly and rapidly IgG isotypes, although IgM and IgA isotypes can be present (13). Conversely, in unselected UFH or LMWH treated patients, development of IgM, and IgA anti-HP4 antibodies, and sometimes IgG isotype, is a frequent feature, with a much higher incidence in patients with Extra Corporeal Circulation (ECC), without thrombocytopenia (37). If HIT does not develop, which is the usual context, antibody concentration tends then to decrease with continuation of heparin treatment (13, 37). Follow-up of the development kinetics of IgG isotypes in non-thrombocytopenic, heparin-treated, patients shows that the first generated antibodies are mainly IgM and IgA isotypes, few IgG isotypes being formed slightly later (37). The rapid generation of IgG isotypes in patients developing HIT is in line with the studies suggesting that the HIT immune response could be secondary and anamnestic (after an earlier PF4-dependent immune stimulation) (3, 22, 24, 33). A possible example is the association between periodontitis and the early development of antibodies to HPF4. For distinguishing asymptomatic from symptomatic antibodies, their presence and concentration must be compared with the clinical probability of HIT, and they must be tested for platelet activation with platelet functional assays. The unresolved question is to know if asymptomatic antibodies can become symptomatic if exposure to heparin is prolonged (although the risk of developing HIT decreases with time of heparin therapy, and becomes rare after 15 days of treatment), or if they can be pathogenic if the target antigen (ultra-large HPF4 complexes) is formed and exposed onto blood
and endothelial cells, focusing the deleterious immune attack. Two possibilities can be considered for the understanding of pathogenic antibodies development: the intensity of the immune response following heparin treatment; the presence of the target antigen in an amount enough for binding antibodies, when present, and targeting them to platelets and blood cells. Interestingly, in functional assays, antibodies activate directly platelets in presence of a low heparin concentration, whether a washed platelet suspension or platelet rich plasma is used, without any need of an exogeneous addition of PF4. This means that HPF4 complexes are nevertheless present for binding antibodies. This suggest that in presence of low heparin concentrations (0.1 to 1.0 IU/ml) pathogenic anti-HPF4 antibodies can bind to platelets and activate them. Probably, some PF4 is already available on the platelet surface (21, 38), for initiating the antibody binding to platelets, followed by their activation, which amplifies the system: more PF4 is released, and more complexes are formed contributing to the complete platelet activation/aggregation. The required heparin concentration (0.1 to 1.0 IU/ml) is then compliant with the total releasable amount of PF4 from platelet rich plasma (3 to 10 μg/ml). This observation can also explain the variability from plasma to plasma (the total amount of platelet bound PF4 being variable, in addition to FcγRIIA platelet surface heterogeneity). It also allows understanding the propensity for a better platelet reactivity and homogeneity in assays using washed platelets, as this process can release some PF4, which binds onto the platelet surface.
CLINICAL SUSPICION

In heparin (UFH, LMWH) treated patients, occurrence of HIT can have a high incidence in cardiology patients (3 to 8 %) and range from > 3% (patients treated UFH in orthopedic surgery) to < 0.2 % (clinical patients treated with LMWH) (1, 33, 39). Variability of HIT incidence can result from patient recruitment heterogeneity and heparin anticoagulant protocols used, but also from the criteria for reporting occurrence of HIT (4, 5). During heparin therapy platelet counts are monitored, at least every 2 days or more frequently for patients at high risk. The very first HIT suspicion warning concerns the platelet count drop by > 50% (but a decrease >30% is already a warning), or < 100 G/L. The platelet count decrease’s magnitude is a criterion for severity of HIT. Thrombocytopenia is always moderate in HIT, and platelet counts remain usually > 20 G/L, rarely in the range 10 to 20 G/L. A very low platelet count, < 10 G/L is not in favor of HIT. If no direct cause of thrombocytopenia is present, HIT must be suspected in presence of a platelet count drop, and the first urgent action is to withdraw heparin treatment, and to replace it with another anticoagulant. However, the alternative treatments are not as efficient as heparin in many clinical situations, and especially in cardiac patients or those with vascular diseases. Therefore, HIT must be confirmed as soon as possible, to avoid depriving patients from the efficient therapy they need. Confirmation of HIT involves a 3-step approach: establishing the clinical probability; measuring IgG heparin dependent antibodies; testing for the platelet activation capacity of antibodies. Clinical probability is assessed using the 4T’s approach, as proposed by Warkentin, and it involves the Timing for thrombocytopenia, Thrombocytopenia with the magnitude of platelet fall, the presence of Thrombosis, and the possible presence of other causes for Thrombocytopenia (table 1) (24, 27, 28).

Table 1: Clinical Probability of HIT:

In clinical practice, the diagnosis of HIT is often difficult. Patients at highest risk of HIT often have multisystem disease and may have several causes of thrombocytopenia. The probability that a patient has HIT can be estimated using the “4 T’s”.

<table>
<thead>
<tr>
<th>Estimated the Pretest Probability of HIT: The ‘4 T’s’.</th>
<th>Points (0, 1, or 2 for each of 4 categories: maximum possible score = 8)</th>
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<tbody>
<tr>
<td>Thrombocytopenia</td>
<td>2 1 0</td>
</tr>
<tr>
<td>&gt;50% fall from baseline or platelet nadir 20 to 100 x 10^9/L</td>
<td>30 to 50% fall from baseline or platelet nadir 10 to 19 x 10^9/L</td>
</tr>
<tr>
<td>Timing of platelet count fall or other sequelae</td>
<td>Clear onset between days 5 and 10, or less than 1 day (if heparin exposure within past 100 days).</td>
</tr>
<tr>
<td>Thrombosis or other sequelae (e.g., skin lesions)</td>
<td>New thrombosis; skin necrosis; post heparin bolus acute systemic reaction</td>
</tr>
<tr>
<td>other cause for thrombocytopenia</td>
<td>No other cause for platelet count fall is evident</td>
</tr>
</tbody>
</table>

Pretest probability score: 6 to 8 = High; 4 to 5 = Intermediate; 0-3 = Low

Adapted from Warkentin TE. Br J Haematol. 2003;121:535-55.
Other evaluation methods exist for the clinical probability of HIT, as the expert panel probability (HIT Expert Probability score/ HEP) (26, 40).

Measurement of IgG heparin dependent antibodies, usually targeted to HPF4, are tested by Elisa, and new methodologies are now available: lateral immuno-filtration, chemiluminescence (41, 42). Functional assays are all based on the capacity of heparin dependent antibodies to activate platelets at a low heparin concentration, but not at a high one (30, 31). A positive immuno-assay, combined with a positive platelet activation assay, at low heparin concentration, allows confirming HIT, whilst a negative immuno-assay can rule out this complication. However, in rare cases variant HIT forms can be present and are provoked by heparin dependent antibodies targeted to non-PF4 antigens, or eventually by other isotypes than IgG (12, 14).

Heparin dependent IgG antibodies, targeted to HPF4 complexes, are transient, and disappear rapidly, after about 2 months when tested for platelet activation, and after 3 to 4 months when tested for Elisa reactivity. Some patients with HIT have antibodies also reactive with PF4 alone, as the result of the epitope spreading during the immune response.

Anti-HPF4 antibodies are allo-antibodies, as they are induced by heparin binding to PF4. In rare cases true autoantibodies to PF4, can pre-exist the heparin treatment (43). They react with HPF4 complexes and can then become pathogenic in presence of heparin. This mechanism is like that described in presence of pre-existing antibodies to IL-8.
MEASUREMENT OF HEPARIN DEPENDENT ANTIBODIES WITH IMMUNOASSAYS

Immunoassays for heparin dependent antibodies have been introduced since the discovery of PF4 as the major target antigen in presence of heparin. Different immunoassay presentations are available, and interestingly heparin can be replaced by polyanions such as Polyvinyl-Sulfonate (PVS), providing evidence that the location of the reactive epitopes of heparin dependent antibodies are on the PF4 molecule (2, 3, 10, 11, 16). PF4, in presence of heparin (UFH or LMWH) at stoichiometric concentrations, forms ultra-large, multimolecular complexes and its native structure is then altered as shown on figure 3.

This alteration exposes PF4 epitopes, characteristic of the molecule involvement in heparin complexes, which can bind heparin dependent antibodies. Enzyme Linked Immuno-Sorbent Assays (Elisa) for heparin dependent antibodies, use stoichiometric HPF4 complexes, or complexes of PF4 with PVS for coating the Elisa microwell, which then captures antibodies, when present. An Anti- IgG linked with peroxidase, or another label, is then introduced for characterizing immobilized IgG antibodies. A more dynamic assay has been introduced in the form of plates coated with a limited amount of protamine sulfate complexed with an excess of heparin, which is then biologically available (Zymutest HIA). The tested sample is introduced in the heparin containing well, along
with a platelet lysate (source of PF4, and other platelet releasable proteins), mimicking what occurs at pathogenic sites where platelets concentrate and are activated, releasing their content. When antibodies are present, platelet PF4 forms complexes with heparin and binds antibodies: the reactivity kinetics for antibodies’ binding are directly dependent on their affinity. There is now evidence that heparin dependent / anti-HPF4 antibodies with the IgG isotype are those with the highest significance and association with symptomatic HIT, although in many cases they can remain asymptomatic (13).

Retrospective analysis in patients diagnosed with HIT, and positive IgG anti-HPF4 antibodies, could help in understanding the risk incidence of asymptomatic IgGs to switch to symptomatic if heparin is continued. If patient plasma or serum samples from the days preceding the diagnosis of HIT are available, testing and isotyping antibodies could help to document this concern. Prospective studies, in patients with prolonged heparin therapy and without HIT, have shown that the various IgG, IgA and IgM isotypes have close kinetics profiles, although IgG reactivity uses to remain low, and rarely increases later (37). Using an excess of heparin in the Elisa (by diluting the sample in presence of a high heparin concentration) can significantly reduce the antibody reactivity, providing some evidence on the heparin dependence (42). This is explained by the disruption of HPF4 complexes by a high heparin concentration. However, in some HIT cases, as for example those with a strong reactivity to PF4 alone, there is not, or only a low, inhibition by an excess of heparin (43). Complementary investigations are then needed for establishing the diagnosis of HIT, especially with the evaluation of the clinical probability (28). There is nevertheless a clear association between the immunoassay reactivity and the disease risk. For most of the Elisas for heparin dependent antibodies, the clinical cut-off value is defined for an OD of 0.50, but almost all the patients with HIT have an OD > 1.00, or much higher (32).
FUNCTIONAL ASSAYS FOR HIT CONFIRMATION

Diagnosis of HIT, in patients with a significant HIT clinical probability (4Ts’ score or HIT Expert Probability score), and positive for heparin dependent antibodies, must be confirmed with functional assays. These assays are performed at a low (0.10 to 1.00 IU/ml) and a high (10 to 100 IU/ml) heparin concentration, and possibly without heparin, as this helps identification of aspecific reactions. Positive criteria for assayed samples are a positive platelet activation at a low, but not at a high, heparin concentration. Many different assays are available and have been recently reviewed elegantly by F. Mullier’s group. Assays are designed with the use of platelet rich citrated plasma, or with washed platelets. Platelet donors need to be characterized and selected, as there is an important donor’s variability for platelets’ activation by heparin dependent antibodies. FcγRIIA (CD32) platelet surface density and polymorphism (His or Arg at position 131) could be one of the parameters explaining the platelet donor variability. However, sensitivity is higher when washed platelets are used, and platelet donors’ variability is lower, probably as the result of a slight platelet activation and PF4 release and binding onto the platelet surface during washing process.

Among functional assays, the C14-Serotonin Release Assay (SRA) is considered as the gold standard for the diagnosis of HIT (30). Usually, four donors are used, and the assay is positive if SRA is > 20% at low heparin concentration and < 20% at high concentration, or without heparin. Some inconclusive results can be faced, especially when SRA is borderline or slightly positive, and differences without heparin or at a high concentration are weak. These inconclusive cases account for most of discrepancies when comparing assays’ performances. SRA is considered to offer up to date the highest specificity and sensitivity for diagnosis of HIT. Recently, an assay variant performed with PF4 was proposed (PF4-SRA) and reported as offering a higher sensitivity but probably a lower specificity.

The Platelet Aggregation Test (PAT) is frequently used in laboratories where SRA is not available and it is performed with platelet rich plasmas (PRP) from 4 selected donors tested separately, each PRP being mixed with the tested patient plasma at a low and a high heparin concentration. As for SRA, the test is positive if at least platelets from 2 out of the 4 donors are activated at a low heparin concentration, but not at a high one.
Another functional assay, Heparin Induced Platelet Aggregation (HIPA), is available and is performed with washed platelets (from 4 selected donors), mixed with the tested plasma, introduced in a micro-Elisa well (31). Platelet aggregation is evaluated by the naked eye and can highly be operator specific. The assay is positive when 2 out of the 4 donors produce a positive aggregation. This HIPA method is mainly used in Germany, and in few other laboratories outside this country. Other assays are available, including the generation of microparticles, release of ATP, measurement of expressed platelet membrane activation proteins (flow cytometry), procoagulant activity tested as thrombin generation assay (TGA), and any methodology able to detect platelet activation. However, these specialized assays remain used in limited laboratories, and mainly for research applications (44).

Recently, a new standardized and easy to use calibrated Flow Cytometry Assay, is being introduced (Emosis HIT Confirm®*): platelet rich plasma (from a platelet donor) is mixed with the tested plasma in presence of a low and a high heparin concentration and platelet activation is measured through the expression of P-Selectin (CD62) and GP IIb (CD41) on the platelet membrane. The simultaneous measurement of CD41 and CD62 expression, allows auto-calibrating the assay, and reducing the platelet donor variability. The level of platelet activation is defined comparatively to another platelet agonist (TRAP), and the measurement of platelet surface expressed CD41/CD62, which allows to render a quantitative result. This assay is easy to set up and to practice, and all reagents are ready to use. The assay principle is depicted on figure 4.

*CE-marked in Europe, RUO in the USA

Figure 4:
This figure shows the assay principle for the Emosis HIT Confirm® method. This functional one-step assay is designed with a Flow Cytometry technique: tested patient plasma is incubated with a normal donor platelet rich plasma at a low or high heparin concentration; platelet rich plasma is also incubated with TRAP; CD41 and CD62 exposed on platelet surface through activation are identified with anti-CD 41-PE or anti-CD 62 FITC, by Flow Cytometry. The total assay time is of 30 min.
Fresh platelet rich plasma from only a healthy donor is needed, without any selection if this PRP is reactive with a positive control. The present experience shows that this assay can offer at least the same specificity and sensitivity than SRA (Figure 5), as recently reported at the XXVI ISTH congress in 2017 (Berlin, Germany), by Tardy B et al. It can be used in any laboratory equipped with a Flow Cytometer (40).

No doubt that this Emosis HIT Confirm® assay can rapidly become the method of choice for testing platelet activation by heparin dependent antibodies.

### Examples and data set:

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<th>Test vs Ref</th>
<th>FCA vs SRA</th>
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** Cut-off at Youden point: 13

### Examples and results:

<table>
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<table>
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*8 Ref ; + 5 Ref -
CONCLUSIONS

Today, HIT remains a critical clinical context in many hospital settings, when heparin, mainly unfractionated, is used in cardiology, intensive care units, or ECC, including CPB and increasingly ECMO (4, 6-8, 33). This is a paradoxal disease, as this anticoagulant drug can provoke thrombocytopenia associated with thrombosis in affected patients. Detecting the risk of HIT is mandatory, as it requires an immediate heparin withdrawal, and to switch to another anticoagulant therapy. However, if ruled out, heparin can be continued as it is the anticoagulant of choice for some disorders, such as in cardiology or vascular disease patients. Until now, diagnosis of HIT mainly involves the clinical probability evaluation, testing for presence of heparin-dependent antibodies (IgG isotype) with an immuno-assay, and confirmation with a functional assay (rarely available on site). This diagnosis remains inaccurate due to the low specificity of the immunological testing, many of the antibodies being asymptomatic. Use of a functional assay is mandatory for confirming HIT. Those available until now are complicate, and restricted to few laboratories, or only used for academic applications (30, 31, 44). Results are then reported with a high delay, and not useful for the immediate management of affected patients.

The new Flow Cytometry functional assay introduced, Emosis HIT Confirm®, overwhelms these limitations, and allows using this functional assay, on demand, in any site equipped with a flow cytometer (40). Only a platelet rich plasma from a single healthy donor is required, without any need for a cumbersome donor selection, and multiple testing. Only reactivity verification with a positive control is needed. This assay is a one-step method, requiring only 10 μl of tested patient’s plasma; it is performed within 30 minutes with ready to use, liquid reagents. It is always available on-site and on-demand, gives clear and easy-repeatable results, which are not operator-dependent. This introduces new possibilities, and a unique tool for a rapid confirmation of HIT, thus allowing to implement the most appropriate patient management in each clinical context.
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