

1 *Review article*

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3 **Clinical and Laboratory tests for the diagnosis of heparin-induced thrombocytopenia**

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1 **Summary**

2 | A rapid diagnostic work-up is required in patients with suspected heparin-induced  
3 thrombocytopenia (HIT). However, diagnosis of HIT is challenging due to a number of practical  
4 issues and methodological limitations. Many laboratory tests and a few clinical scoring systems  
5 are available but the individual characteristics and the diagnostic accuracy of these are hard to  
6 appraise. The 4Ts score is a well evaluated clinical assessment tool with the potential to rule-out  
7 HIT in many patients. Still, it requires Scoring tools such as the 4Ts are time consuming, require  
8 experience and ~~are-is~~ subject to a ~~relevant~~ inter-observer variability. Immunoassays such as  
9 enzyme-linked immunosorbent assays or recently developed rapid assays are able to exclude HIT  
10 in a number of patients. But, a Accuracy of immunoassays differs depending on type of assay,  
11 threshold, antibody specificity and even manufacturer. Due to a comparatively low positive  
12 predictive value, HIT cannot be confirmed with by immunoassays alone. In addition, only some  
13 of them are immediately accessible, particularly in small laboratories. While functional assays  
14 such as the serotonin release assay (SRA) and the heparin-induced platelet activation assay  
15 (HIPA) are considered a gold standard for diagnosis of HIT, they require a highly specialised  
16 laboratory. In addition, ~~and many~~ some of them are not adequately evaluated. In clinical practice,  
17 we recommend an integrated diagnostic approach combining not only clinical assessment (the  
18 4Ts score) but immunoassays and functional assays as well. We propose a clear diagnostic  
19 algorithm supporting clinical decision-making. Furthermore, ~~In this review,~~ we provide an  
20 overview of all current laboratory techniques for HIT and discuss diagnostic pathways and  
21 strategies to reduce diagnostic errors, and future perspectives.

22 **Keywords:** Heparin/adverse effects; Immunoassay/methods; Thrombocytopenia/chemically  
23 induced; Thrombocytopenia/diagnosis

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## 2 **Introduction**

3 Diagnostic work-up of patients with suspected heparin-induced thrombocytopenia (HIT) is  
4 hampered by major practical issues and a number of methodological limitations. ~~Often~~Not  
5 infrequently, suspicion is raised during ~~night shifts and~~ weekends when haematology consultants  
6 and elaborated laboratory services are not available. Thus, surgical registrars or intensive care  
7 unit consultants who are inexperienced with such patients may face major clinical decisions at  
8 times when there is little support. Most accurate diagnostic tests are functional assays, which are  
9 time-consuming, expensive and require a high level of laboratory expertise (1-3). Even in the  
10 best case scenario, results of these gold-standard tests will take at least two days and will only be  
11 available from Monday to Friday (4-6). However, the clinical decision regarding whether or not  
12 heparin should be stopped and treatment with an alternative anticoagulant started, must be made  
13 immediately within a few hours (7-10). Delaying this decision- may be life-threatening in  
14 patients with HIT (11), while treatment with alternative anticoagulants in non-HIT patients  
15 can be associated with major risks (12-14). Some clinical scoring systems and a number of  
16 immunoassays (Table 1) are currently available to help physicians select the most appropriate  
17 course of action. However, the diagnostic accuracy varies across these tests and all are associated  
18 with limitations (15). Given the large number of publications describing heterogeneous study  
19 designs and reporting imprecise and varying results, -it is hard to appraise the diagnostic  
20 characteristics of individual tests.

21 ~~With a focus on laboratory assays, w~~In the present article, we will review the currently available  
22 diagnostic clinical and laboratory tests, summarise their diagnostic accuracy data and discuss  
23 practical issues. We will also elaborate on test variations and discuss strategies to reduce over-  
24 diagnosis.

## 25 **Diagnostic pathways**

26 While estimating the value of diagnostic tests, it is helpful to appreciate the pathways in which  
27 they are used. Thus, we describe typical scenarios requiring a diagnosis of HIT that physicians  
28 may find themselves in, which will generally be informed by previous training and the technical  
29 infrastructure of the hospital. In virtually all situations, physicians must make an initial clinical

1 decision while ~~awaiting~~waiting for the results of the functional assay and the following scenarios  
2 may arise. First, ~~the associated laboratory does not provide access to a rapid assay, no immediate~~  
3 ~~access to a laboratory tests is available, neither a functional assay nor an immune~~immuno-assay,  
4 ~~because (e.g. enzyme-linked immunosorbent assay [ELISA] is conducted, which is usually~~  
5 ~~carried out once or~~ twice a week only). In this setting, ~~the~~an initial decision is solely made on the  
6 basis of the estimated clinical probability using one of the validated scoring tools. The accuracy  
7 of the decision critically depends on the characteristics and appropriate execution of the clinical  
8 test. The decision may be revised when the immunoassay test result arrives several days later.  
9 However, most authors and recent guidelines recommend against conducting an immunoassay in  
10 patients with a low risk score (9, 16, 17). In the second scenario, an immunoassay is available  
11 Monday to Friday and a functional assay once a week. As above, physicians must decide on the  
12 outcome of the clinical tool but decisions can be revised quickly. This strategy puts equal weight  
13 on the clinical scoring system as well as laboratory test results. In the third case, ~~an~~a  
14 immunoassay is conducted via a 24-hour service and the results of a functional assay will be  
15 reported at least once a week. In this preferable situation, physicians can consider clinical  
16 characteristics as well as results of immunoassays, and decisions will be modified accordingly  
17 ~~corrected~~ within a few days. However, ~~in this scenario~~ physicians may be tempted ~~(tend?)~~ to skip  
18 replace the fairly time-consuming task of gathering all information for clinical risk assessment  
19 with a laboratory test only (e.g. a rapid immunoassay), what places the patients at particular  
20 risks. filling a clinical assessment form and instead rely exclusively on the results from an  
21 immunoassay, which also have specific limitations. In all the above-mentioned scenarios, patient  
22 care can relevantly be improved with the help of the local haematology/~~coagulation~~ consultancy  
23 service. As experienced in clinical, haematology consultation which may reduces the number  
24 of false-classified 4Ts scorings and improves interpretation of laboratory results. In addition,  
25 prophylactic treatment with fondaparinux can often be implemented in unclear cases.  
26 Furthermore, it may save costs by reducing unnecessary testing and treatment with alternative  
27 anticoagulants.  
28 ~~it is also possible to request and assessment of clinical probability and interpretation of~~  
29 ~~laboratory results from the local haematology consultancy service. In clinical practice, many~~  
30 ~~inappropriate decisions can be corrected this way, but it is a time consuming and perhaps~~  
31 ~~expensive intervention.~~

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1 **Assessing the pretest probability: clinical scoring tools**

2 As illustrated above, standardised assessment of the clinical probability of HIT is an essential  
3 step in the work-up of patients with suspected HIT. If conducted correctly, the probability of HIT  
4 can be estimated *before* determination of a laboratory test. Several clinical assessment tools have  
5 been developed, the outputs of which not only affect the interpretation of any laboratory test  
6 result but may in some instances represent the only diagnostic test to guide therapeutic decisions  
7 (see Figure-~~2~~ 1).

8 *The 4Ts score*

9 The most extensively studied assessment tool, the 4Ts score, incorporates four typical clinical  
10 features of HIT: (i) thrombocytopenia, (ii) characteristic timing of thrombocytopenia, (iii)  
11 presence of thrombosis or other clinical sequelae, and (iv) the absence of other causes of  
12 thrombocytopenia, ~~(8), Table 1~~. The pretest probability is estimated to be low (0 to 3 points),  
13 intermediate (4 or 5 points), or high with 6 to 8 points (18, 19). A number of evaluation studies  
14 assessed the diagnostic accuracy of the 4Ts score (18, 20-30) and a meta-analysis suggested a  
15 high negative predictive value (99.8%; 95% CI: 97-100%) (19). This result was not influenced  
16 by the type of performer (laboratory or treating physician), the prevalence, or the clinical setting  
17 as studied in sensitivity analyses. According to ~~this meta-analysis study~~, the probability of  
18 suffering from HIT can be estimated to be 0.82% in low risk 4Ts scoring, ~~13.414%~~ 9 to  
19 22% in intermediate scoring and ~~50.636%~~ 40 to 82% in the high risk scoring.  
20 ~~However, these results are clearly unsatisfactory for the purpose of ruling-inconfirming HIT.~~  
21 ~~positive predictive value of an intermediate or even high 4Ts score was found to be~~  
22 ~~unsatisfactory (14%; 95% CI: 9-22% and 64%; 95% CI: 40-82%, respectively).~~ While the use of  
23 the 4Ts score as a screening test in the diagnostic pathways has been suggested, some  
24 methodological issues have been raised, in particular with regard to determination in clinical  
25 practice (31, 32). Most importantly, application of diagnostic accuracy measures to clinical  
26 practice was questioned because assessment of the 4Ts score was done by experts instead of  
27 referring physicians in most of the diagnostic accuracy studies (31). Indeed, a very recent, well-  
28 designed prospective study considering these issues reported a much more limited ~~diagnostic~~  
29 ~~accuracy~~ sensitivity of the 4Ts score than estimated in the above mentioned meta-analysis  
30 (sensitivity 81.3%; 95% CI: 67.7, 94.8; specificity 63.8%; 95% CI: 59.6-68.0%) and a limited

1 agreement between physicians and expert observers (Cohens kappa 0.43; 95% CI, 0.29-0.57)  
2 (33). In clinical practice, we experienced several misdiagnosed HIT cases due to low risk  
3 4Ts scorings and Figure 2 illustrates the dreadful course of a 30-year-old female patient who  
4 xxx died in the course of cerebral vein thrombosis. Thus, some authors conclude that a negative  
5 (≤3) 4T's score alone is insufficient to exclude HIT in clinical practice (24, 34).

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6 *The HEP score*

7 The HIT expert probability (HEP) score is another clinical assessment tool which incorporates  
8 more clinical features than the 4Ts score (magnitude of platelet count fall, timing of platelet  
9 count fall, nadir platelet count, thrombosis, skin necrosis, acute systemic reaction, bleeding and  
10 other causes of thrombocytopenia) (29). Each of these features is evaluated using a score ranging  
11 from -3 (inconsistent with a HIT diagnosis) to +3 (consistent with a HIT diagnosis). Application  
12 of the HEP score resulted in a higher inter-observer agreement than the 4Ts score in one  
13 evaluation study (29). A cut-off value of 5 was associated with a positive predictive value of  
14 55% and a negative predictive value of 97%, showing operating characteristics similar to those  
15 observed with the 4Ts score. Nevertheless, the HEP score is more complex and may be more  
16 time consuming than the 4Ts. In addition, the number of evaluation studies is much more  
17 limited.

18 *Other scoring systems*

19 Another, simple score to exclude HIT has been suggested by Messmore et al (35). The system is  
20 designed to arrive at low (0) or possible (1) probability scores depending on the presence or  
21 absence of typical HIT manifestations without knowledge of laboratory test results (except  
22 platelet counts). In one evaluation study, it was able to exclude patients without HIT efficiently  
23 and it might be more useful for physicians who are not HIT experts. Lillo-Le Louët and  
24 colleagues developed a score to assess the probability of HIT in patients following  
25 cardiopulmonary bypass surgery (20). This score incorporates 3 variables that were predictive  
26 for HIT in a retrospective study (a biphasic platelet count profile, an interval of >5 days from  
27 CPB to the first day of suspected HIT and a CPB duration of >118 minutes). In an independent  
28 study, this score demonstrated a negative predictive value of 78%, suggesting that it may have  
29 inadequate sensitivity to be used as a clinical screening test (36). However, both the Mesmore

1 and Lillo-Le Louët scores require more validation in larger prospective studies before firm  
2 conclusions regarding their diagnostic accuracy can be drawn.

### 3 **Immunoassays**

4 Acquired thrombocytopenia is a frequent finding in hospitalised patients treated with heparin.  
5 Often, HIT is difficult to exclude or to confirm based on clinical information alone and  
6 physicians rely heavily on laboratory tests. Two classes of assays are available: functional  
7 (platelet activation) assays and (PF4-dependent) immunoassays. Immunoassays are pivotal in the  
8 diagnostic work-up of patients with suspected HIT and rely on detection of antibody binding by  
9 ELISA or particle-based immunoassays. However, diagnostic accuracy of immunoassays is quite  
10 variable. As an example, Figure 4-3 illustrates the difference of the probability of having HIT  
11 after a positive (or negative test respectively) between two available assays.

#### 12 *Enzyme-linked immunosorbent assays (ELISAs)*

13 In ELISA, the target antigen (PF4/polyanion complexes) is bound to the solid phase, e.g.  
14 microtitre plate wells. Patient serum or plasma is added and an enzyme-labeled secondary  
15 antibody is used to detect the amount of anti-PF4/heparin antibodies bound in a semi-quantitative  
16 fashion. The intensity of the colour change, measured as optical density (OD), is proportional to  
17 the concentration of bound antibodies. The first polyspecific ELISA was developed by Amiral  
18 and Greinacher in 1992 (37, 38). Sensitivity was comparable to a heparin-induced platelet  
19 activation assay (HIPA) as evaluated in 209 patients with a clinical diagnosis of HIT (38). Since  
20 then, several in-house and commercially available assays have been developed and many studies  
21 have evaluated their performance characteristics (Table 1) (20, 21, 23, 24, 26-28, 39-63). A  
22 recent meta-analysis pooled this data and calculated the diagnostic accuracy according to  
23 different cut-off values: low threshold ( $OD \leq 0.7$ , according to or slightly above the  
24 manufacturer's instructions), intermediate threshold ( $OD 0.8$  to  $1.4$ ) and high threshold ( $OD >$   
25  $1.4$ ). Sensitivity of the polyspecific ELISA was excellent at low threshold (see Table 2) (64), ~~(63)~~  
26 but relevant differences were observed with regard to different thresholds and particular  
27 manufacturers. However, specificity was limited for all assays (Table 2), restricting their value as  
28 a confirmatory test. With regard to ELISA, a significant inter-laboratory variation was observed

1 in a North American proficiency testing programme, in particular with regard to weak positive  
2 results (65).

3 Following in vitro observations on the specificity of platelet-activating PF4/H-antibodies, IgG-  
4 specific ELISAs were developed and tested in a number of studies (23, 41, 43, 45, 46, 48, 56, 66-  
5 72). At low thresholds, sensitivity is again excellent (Table 2). However, even though several  
6 studies suggested a higher specificity than polyspecific assays (23), this observation was not  
7 generalizable in the above-mentioned meta-analysis (73). Data pooled from all available  
8 evaluation studies revealed a specificity of 85.4% for IgG-specific ELISAs (95% CI: 78.2-  
9 90.6%), and 86.8% for polyspecific ELISAs (95% CI: 82.0-90.5%). While these ELISA assays  
10 can be excellent screening tests, they do have the major drawbacks of being time consuming and  
11 requiring a specialised laboratory.

#### 12 *Particle-based immunoassays*

13 Several types of tests have been developed to overcome the drawbacks of ELISA assays: particle  
14 gel immunoassays (PaGIA), lateral flow immunoassays, chemiluminescent immunoassays  
15 ~~[CLIA]~~ and latex agglutination assays. PaGIA as well as lateral flow immunoassay can be  
16 implemented in routine laboratories, conducted 24-hours a day and technicians can perform these  
17 without specialised training. The polyspecific PaGIA is a particle agglutination assay uses the gel  
18 technique of ID-Micro typing with polymer particles coated with PF4/heparin complexes (52). It  
19 has been evaluated in a number of studies (21-24, 26, 33, 44, 45, 52, 54, 55, 71, 72, 74, 75). The  
20 sensitivity as well as the specificity of the PaGIA was excellent; the specificity was even higher  
21 than ELISA assays with low threshold (cutt-off according to manufacturer's instructions; Table  
22 2, (73)). The principle of the lateral-flow immunoassay, which is a different particle-based  
23 immunoassay, is well known from modern pregnancy tests: labeled antibody complexes are  
24 retained and become visible during capillary action (71). The diagnostic characteristics have  
25 been evaluated in several studies (59, 69-72, 75, 76) from which the data have been pooled and a  
26 high sensitivity and reasonable specificity have been confirmed (Table 2; (73)). Nevertheless,  
27 PaGIA and lateral flow immunoassays share two disadvantages. First, the results are assessed  
28 visually (even though automatic applications exist), which permits variation in interpretation.  
29 Second, the results are expressed positively or negatively and titration studies are necessary to  
30 determine the anti-PF4/H antibody concentration (24). ~~In addition, PaGIA is only available as a~~

1 ~~polyspecific test.~~ The particle immunofiltration assay is a different assay, but as yet has not been  
2 shown to demonstrate adequate diagnostic accuracy (73, 77).

3 A desirable characteristic of tests to be implemented in modern laboratories is that they can be  
4 automated allowing them to be run 24 hours a day. Two assays have been developed to meet  
5 this demand: the ~~chemiluminescent immunoassay-CLIA~~ (polyspecific HemosIL® AcuStar HIT-  
6 Ab and IgG-specific HemosIL® AcuStar HIT-IgG) and the latex agglutination assay  
7 (polyspecific HemosIL® HIT-Ab). Both assays can be used with the BIO-FLASH® analyzer  
8 (Inova Diagnostics, San Diego, CA, USA) or the ACL TOP coagulometers (Instrumentation  
9 ~~L~~aboratory, Bedford, MA, USA). Magnetic coated particles capture the PF4/heparin antibodies  
10 and in case of ~~chemiluminescent immunoassay-CLIA~~ emitted light is measured (78). The  
11 diagnostic accuracy of these assays has been investigated in several large cohorts with  
12 favourable results (56, 58, 78-81). At low threshold, sensitivity was very high for both the  
13 polyspecific and the IgG-specific tests (Table 2) (73). Furthermore, a combination of a high  
14 sensitivity with a high specificity was estimated for the polyspecific assay (intermediate  
15 threshold) as well as IgG-specific assay (low threshold). Coated latex beads are used instead of  
16 magnetic particles with the polyspecific latex agglutination assay. In one evaluation study,  
17 sensitivity was found to be excellent, specificity was moderate (80)(Table 2).

18 Diagnostic accuracy measures of rapid immunoassays have also been studied in another recent  
19 systematic review and meta-analysis comprising essentially the same primary studies cited above  
20 (82). A high sensitivity and specificity (corresponding to a high negative predictive value) was  
21 observed for some of the assays as well (PaGIA, lateral flow immunoassay and IgG-specific  
22 ~~chemiluminescent immunoassay-CLIA~~), suggesting their usefulness in diagnostic algorithms ~~as-~~  
23 ~~mentioned below.~~ In addition, implementation of rapid immunoassays is also supported by a  
24 study which modeled ~~evaluated?~~the cost impact (83).

## 25 **Functional assays**

26 A subset of PF4/heparin-antibodies is able to activate platelets and cause clinical HIT under  
27 certain conditions (8, 84). The presence of platelet-activating antibodies can only be established  
28 using functional assays. In all tests, patient plasma or serum is incubated with donor platelets  
29 which can be prepared in one of two different ways: either as (a) washed platelets, or as (b)

1 platelet rich plasma (PRP) or whole blood (1). Washed platelet assays are considered preferable  
2 over other PRP or whole blood tests, because remaining plasma/serum may influence the  
3 antigen-antibody interaction as well as platelet activation (2, 8, 9, 85, 86). Table 3 summarizes  
4 the characteristics of the assays most often used.

#### 5 *Washed platelet assays*

6 Both the serotonin release assay (SRA) and heparin induced platelet activation (HIPA) assay  
7 utilise washed platelets. Platelet activation is assessed by measurement of the release of <sup>14</sup>C-  
8 labeled serotonin from test platelets in SRA ~~and or~~ by visually determining the formation of  
9 platelet aggregates in HIPA (87, 88).

10 In the HIPA assay, washed platelets from four healthy unselected donors are incubated with  
11 patient serum in the presence of buffer or heparin (0.2 IU/mL and 100 IU/mL). Incubation takes  
12 place in a round-bottom microtitre plate, with spinning magnetic spheres as a source of shear  
13 force. Platelet aggregate formation is determined visually at 5-minute intervals; the test is  
14 positive if aggregation is observed within 30 minutes (at 0.2 IU/mL but not at 100 IU/mL  
15 heparin) using platelet suspensions from at least two of the four donors.

16 In the SRA, platelets obtained from a selected donor are pre-incubated with radioactive <sup>14</sup>C-  
17 serotonin. After washing, platelets are incubated with patient serum and heparin in flat-bottomed  
18 microtitre wells in duplicate on a plate shaker. After incubation for 60 minutes and  
19 centrifugation, supernatants of each reaction mixture are collected, and radioactivity is measured.  
20 Test results are expressed as percentage of serotonin release (compared to the 100% value  
21 obtained by detergent-induced platelet lysis). The test is considered positive if there is >20%  
22 release at low heparin concentrations (0.1 to 0.3 IU/mL) and <20% release at supratherapeutic  
23 heparin levels (100 IU/mL). However, a number of laboratories use a threshold of >50%  
24 serotonine release in order to increase specificity (89).

25 The SRA was initially validated using a set of samples from patients with different degrees of  
26 clinical probability of HIT and a very large set of controls obtained from patients with a broad  
27 spectrum of clinical characteristics (87, 90). Not only high sensitivity and specificity were  
28 observed, but also a clear trend between clinical probability of HIT and the SRA results. These  
29 findings were confirmed in a prospective study following up all patients with heparin treatment  
30 based on strict clinical criteria (86). Equivalent diagnostic characteristics have been observed in

1 the evaluation of the HIPA test. Initially, Greinacher and co-workers studied sensitivity in 34  
2 samples, followed by sera from 209 patients (38, 88). Both functional assays are considered the  
3 "gold standard" for diagnosing HIT. However, these assays are difficult to perform, require  
4 selected healthy platelet donors and are restricted to few reference laboratories. Moreover, the  
5 SRA requires the use of the radioisotope, <sup>14</sup>C-serotonin, which most laboratories try to avoid  
6 due to regulatory and safety issues.

7 Even though SRA and HIPA are considered as gold standard for the diagnosis of HIT, some  
8 cases with incongruous results were observed, eg. positive tests in combination with negative  
9 immunoassays and an atypical clinical presentation (91). These rare cases were generally  
10 considered to be "false-positive" (91). In clinical practice, it is important ~~not~~ to always use any  
11 laboratory assay ~~functional assays as the only test applied but to consider in combination with~~  
12 appropriate assessment of the clinical presentations and immunoassay test results as well.

13 Other washed platelet assays that either use ATP release detected by lumiaggregometry, platelet-  
14 derived microparticle generation measured by flow cytometry, or proteolysis of FcγRIIa (the  
15 receptor through which HIT immune complexes activate platelets) assessed by  
16 chemiluminescence have been described, but still require independent validation.

#### 17 *Whole blood assays*

18 Platelet-activating antibodies can be detected using the whole blood impedance analyser  
19 (Multiplate®, multiple electrode platelet aggregometry) in the presence of heparin. Blood from a  
20 selected donor is collected in hirudin-containing tubes. UFH is then added (0.5 or 100 IU/mL)  
21 and the suspension are incubated with patient citrated platelet-poor plasma (PPP) or heat-  
22 inactivated serum. Changes in impedance are then recorded over a 15 minute period (92). In a  
23 multicentre Australian study, this assay, which does not require platelet preparation,  
24 demonstrated a sensitivity and specificity of 90.3% and 89.0%, respectively (81, 92).

#### 25 *Other functional assays*

26 A number of other, less elaborate functional assays have been suggested; of these the heparin-  
27 induced platelet aggregation test (PAT) and flow cytometry are the most often used. In PAT,  
28 platelet aggregometry is performed in the presence of two heparin concentrations using PRP of  
29 one to four, selected or unselected donors (85, 93). However, evaluation studies have revealed

1 varying results, partly explained by the modifications and selection of donors (38, 90, 93, 94). In  
2 general, sensitivity was clearly inferior to SRA/HIPA.

3 Flow cytometry assays have been developed by a number of authors. Serum of patients and  
4 platelets from unselected donors are incubated with heparin and different measures of platelet  
5 activation are recorded (Annexin V (44, 95, 96), P-selectin (44, 95), and microparticles (97, 98)).  
6 Although these assays showed some agreement with the gold standard, standardisation and  
7 further evaluation studies are needed.

## 8 **Strategies to improve the specificity of immunoassays**

9 Several strategies have been developed and introduced to improve the specificity of  
10 immunoassays, increase their positive predictive value and limit the number of patients over  
11 treated.

### 12 *Determination of PF4/Heparin antibody titres*

13 A number of studies have observed that higher optical density values (in the case of ELISA type  
14 assays) are associated with an increased probability of having HIT (43, 99). Higher titres of  
15 antibodies have also been correlated with the likelihood of HIT in the case of PaGIA (24) and  
16 [chemiluminescent immunoassayCLIA](#) (58, 81). To confirm these observations, we pooled the  
17 data of all available evaluation studies in a recently conducted meta-analysis (64). The cutt-off  
18 values used in the primary studies were categorised into low, intermediate and high thresholds  
19 (corresponding to a low, intermediate, and high antibody titres). In line with previous  
20 observations, we found a remarkably increased specificity (or positive likelihood ratio) in all  
21 immunoassays (poly- and IgG-specific ELISA, PaGIA, poly- and IgG-specific [chemiluminescent](#)  
22 [immunoassayCLIA](#)) (73). However, the negative likelihood ratio increased as well,  
23 corresponding to a decline in sensitivity. In Table 2, we report a summary of the results that  
24 might help to define the best threshold.

### 25 *Application of IgG-specific assays*

26 In-vitro data suggest that IgG-specific antibodies account for the vast majority of HIT cases and  
27 several studies indeed observed an increased specificity of IgG-specific assays compared to  
28 polyspecific tests while sensitivity also remained high (23, 86, 100). We tried to confirm this

1 observation by pooling all available data in the above-mentioned meta-analysis. However, this  
2 could be replicated only in part (Table 2) (73). In addition, sensitivity was somewhat reduced, at  
3 least with intermediate and high cut-off values. In clinical practice, we recommend selecting an  
4 appropriate combination of antibody specificity and threshold according to the respective  
5 likelihood ratios (eg. polypecific ELISA/ [chemiluminescent immunoassay CLIA](#)/ PaGIA with  
6 intermediate threshold or IgG-specific ELISA/ [chemiluminescent immunoassay CLIA](#) with a low  
7 threshold).

#### 8 *Implementation of a high-dose heparin confirmation step*

9 It has been suggested that the specificity of HIT immunoassays could be improved by the  
10 implementation of a confirmatory step using supratherapeutic concentrations of heparin. This is  
11 because a persistently positive test despite high heparin concentrations can indicate an antibody  
12 that reacts against PF4, but not to the PF4/heparin complex. Such antibodies usually do not  
13 indicate HIT. While some studies support the use of this step, especially for weakly positive OD  
14 values <1.0 units, some of the clinically most relevant high-titre antibodies with strong platelet-  
15 activating capacity are not inhibited (101). A recent meta-analysis however did not find this  
16 strategy helpful (64). Sensitivity was found to be low, at least in a subgroup of samples with a  
17 high titre of antibodies (73, 85). Because of this limitation and the corresponding difficulties in  
18 interpretation, we recommend against implementing this in routine clinical practice.

#### 19 **Current challenges and future perspectives**

20 While the incidence of HIT in uncomplicated patients can be anticipated to decline due to the  
21 increasing use of [low molecular weight heparins and](#) alternative, non-heparin anticoagulants  
22 (102), HIT will remain a particular issue in specific patient populations, which have undergone  
23 cardiac surgery or are severely ill patients. Despite the progress in understanding the  
24 pathophysiology of HIT, there are still numerous diagnostic issues and treatment challenges.

#### 25 *The clinical dilemma*

26 The management of patients with suspected HIT is associated with two major risks: missing  
27 patients with HIT and overtreatment. Physicians rely heavily on immunoassay test results and  
28 immunoassays are an essential part of most diagnostic pathways as discussed above. However,  
29 as few as 10-15% of sera test positive for anti-PF4/heparin antibodies and only up to 50% of

1 these contain clinically relevant, platelet-activating antibodies characteristic of HIT. Therefore, a  
2 considerable risk of “overdiagnosis” and subsequent mistreatment of patients without HIT exists  
3 (14). These patients are exposed to relevant risks. Therapy with alternative anticoagulants is  
4 associated with a high rate of bleeding complications (12), severe anaphylactic reactions (13),  
5 higher costs, and requires more management generally than compared to heparin treatment (12,  
6 14). Thus, an important aim of clinical practice and scientific inquiry is to develop and  
7 implement diagnostic tests and algorithms that reduce the number of false-positive results.

8 On the other hand, increasing specificity should not be at the expense of test sensitivity, as  
9 missing a diagnosis of HIT is dangerous (64). The risk of severe thromboembolic complications,  
10 limb loss and even death is high in untreated HIT patients (11, 103). There is increasing  
11 awareness that a low risk 4Ts score does not exclude HIT in all cases (33, 34) and Figure 2  
12 illustrates a dreadful example. In addition, the sensitivity is below 95% in some immunoassays,  
13 suggesting that one in 20 HIT patients will be missed as well (64).-

#### 14 *Diagnostic algorithms*

15 In order to avoid the above-mentioned risks, the most important challenge in clinical practice is  
16 to estimate the probability of an individual patient having HIT. Our considerations above suggest  
17 that neither an immunoassay, nor a clinical assessment score alone is able to correctly diagnose  
18 HIT. However, combining different diagnostic approaches (clinical and laboratory) can improve  
19 diagnostic accuracy and may represent a strategy to solve this clinical dilemma (~~Figure 1~~).

20 Diagnostic algorithms are the most obvious way of combining clinical and laboratory tests for  
21 the diagnosis of HIT (24, 33). In Figure 21, we illustrate a recently adapted diagnostic algorithm,  
22 (8). Assessing the clinical probability is suggested for all patients with suspected HIT. Given an  
23 appropriate application of the 4Ts score, HIT can be excluded in ~~all~~ most patients with a low risk  
24 scoring. However, conducting the 4Ts score correctly is difficult (31) and determination of an  
25 immunoassay is suggested in all cases where there are uncertainties (eg. unclear heparin  
26 exposure, missing platelet numbers).-

27 In contrast, HIT should be considered if an applied 4Ts score is high. In all other cases,  
28 determination of a quantitative immunoassay is recommended. However, the diagnostic accuracy  
29 varies between different assays and we recommend selecting a test with a high sensitivity as well  
30 as a high specificity (64). For example, we recommend choosing an intermediate threshold (cut-

1 off value) in the case of polyspecific ELISA, PaGIA, as well as polyspecific chemiluminescent  
2 immunoassay~~CLIA~~. HIT can be essentially ruled-out if the immunoassay is negative or highly  
3 suspected if high titres of antibodies are demonstrated (eg.  $OD \geq 3.0$ ). Even though HIT must be  
4 assumed in all other cases with a positive immunoassay, determination of a functional assay is  
5 recommended if possible. Depending on the individual setting, a functional assay will be  
6 conducted in more cases as well.

7 There are nevertheless other ways of combining ~~different~~ diagnostic tests as well and all have the  
8 potential of reducing the number of false-positive and false-negative classifications. For  
9 example, a clinical scoring system and an immunoassay can be determined in parallel as  
10 suggested by several authors (2, 34, 104), and probabilities ~~of clinical scoring systems and~~  
11 ~~immunoassays~~ can be combined with the use of likelihood ratios and Bayes' theorem (24, 104,  
12 105). However, prospective studies evaluating these tools are still needed.

### 13 *Conclusion*

14 HIT is a life-threatening situation that requires an immediate diagnostic work-up. Not only  
15 missing a patient with HIT can result in catastrophic consequences, but overtreatment also  
16 carries a significant risk. The diagnostic work-up is, however, difficult due to a number of  
17 practical issues and limitations in the diagnostic accuracy of available assays. The diagnostic  
18 pathway should be adjusted to the individual setting using well-defined diagnostic algorithms.  
19 The first step should include the assessment of the clinical probability according to a validated  
20 scoring system and laboratory investigations should additionally be performed if the probability  
21 is intermediate or high. An immunoassay with adequate sensitivity and specificity should be used  
22 to avoid over-treatment or failure to recognise HIT. Future efforts to address these challenges  
23 should focus on the improvement and clinical evaluation of diagnostic algorithms.

1 **Conflict of Interest**

2 MN has received research grants or lecture fees from Bayer and CSL Behring.

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## Tables

**Table 1:** Available immunoassays for the diagnosis of HIT (adapted from (73))

Type of assay	Available antibody specificities	Measurement scale	Practical issues	Manufacturers
Enzyme-linked immunosorbent assay (ELISA)	Polyspecific IgG specific	Optical density <i>Low, intermediate and high threshold*</i>	Requires specialised laboratory, determination in batches, daily determination rarely possible	Genetic testing institute [GTI] Diagnostics, Waukesha, WI, USA ( <i>GTI-PF4; HAT; PF4-Enhanced; GTI-IgG</i> ) Hyphen-BioMed, Neuville-Sur-Oise, France ( <i>Zymutest HIA IgGAM; Zymutest HIA IgG</i> ) Diagnostica Stago, Asnières-sur-Seine, France ( <i>Asserachrom HPIA</i> ) Gen-Probe-Waukesha, Waukesha, WI, USA ( <i>Gen-Probe PF4</i> ) <sup>#</sup> Technoclone GmbH, Vienna, Austria ( <i>Technozym</i> )
Particle gel immunoassay (PaGIA)	Polyspecific	Visual assessment of agglutination <i>Quantification using titration studies</i> <sup>°</sup>	Determination in standard laboratories possible, 24-hour service, observer-dependent	Diamed, Cressier sur Morat, Switzerland ( <i>ID-H/PF4 PaGIA</i> )
Particle immunofiltration assay	Polyspecific	Visual assessment	Observer-dependent	Akers Biosciences Inc, Thorofare, NJ, USA ( <i>HealthTEST</i> )
Lateral flow immunoassay	IgG specific	Visual or automated assessment <sup>°</sup>	Determination in standard laboratories possible, 24-hour service	Diagnostica Stago, Asnières-sur-Seine, France ( <i>STic EXPERT HIT</i> ) Milenia Biotec, Giessen, Germany ( <i>Milenia QuickLine HIT</i> )
Chemiluminescent immunoassay-(CLIA)	Polyspecific IgG specific	Detection of emitted light <i>Low, intermediate and high threshold†</i>	Automated determination possible, 24-hour service, expensive	Instrumentation Laboratory, Bedford, MA, USA ( <i>HemosIL AcuStar HIT-Ab; HemosIL AcuStar HIT-IgG</i> )
Latex agglutination assay	Polyspecific	Inhibition of agglutination	Automatized determination possible, 24-hour service, expensive	Instrumentation Laboratory, Bedford, MA, USA ( <i>HemosIL HIT-Ab</i> )

\* low threshold: below or equal to OD 0.7, intermediate threshold: between OD 0.8 and 1.4, high threshold: above OD 1.4; ° positive/negative; † low threshold: below 1.0 U/ml, intermediate threshold: between 1.0 and 2.8 U/ml, high threshold: above 2.8 U/ml; # technically identical with GTI assay

**Table 2:** Diagnostic accuracy of immunoassays for diagnosis of HIT<sup>+</sup>

Type of test	Sensitivity	Specificity	Likelihood ratio	
			Positive (95% CI)	Negative (95% CI)
<b>Polyspecific ELISA</b>				
<i>Low threshold*</i>	96.7 (89.7, 99.0)	86.8 (82.0, 90.5)	7.3 (5.4, 10.0)	0.04 (0.01, 0.12)
<i>Intermediate threshold*</i>	98.4 (90.8, 99.7)	94.9 (90.5, 97.3)	19.3 (10.4, 36.0)	0.02 (0.00, 0.1)
<i>High threshold*</i>	15.0 (14.5, 15.5)	100 (99.3, 100)	73.4 (28.2, 190.9)	0.3 (0.2, 0.5)
<b>IgG-specific ELISA</b>				
<i>Low threshold*</i>	98.3 (95.1, 99.4)	85.4 (78.2, 90.6)	6.7 (4.5, 10.2)	0.02 (0.01, 0.05)
<i>Intermediate threshold*</i>	91.2 (86.2, 94.5)	93.5 (89.1, 96.2)	14.1 (8.1, 24.5)	0.09 (0.05, 0.15)
<i>High threshold*</i>	60.9 (59.7, 62.1)	99.4 (97.6, 100)	97.0 (53.0, 177.6)	0.4 (0.3, 0.5)
<b>PaGIA</b>				
<i>Low threshold°</i>	96.5 (89.8, 98.9)	93.7 (83.1, 97.8)	15.3 (5.5, 42.3)	0.04 (0.01, 0.11)
<i>Intermediate threshold°</i>	98.9	95.9	24.1	0.01
<b>Lateral flow immunoassay</b>	98.4 (85.3, 99.9)	90.3 (84.4, 94.1)	10.1 (6.2, 16.5)	0.02 (0.00, 0.18)
<b>Particle immunofiltration assay</b>	0.0	70.1	2.3	0.5
<b>Latex agglutination assay</b>	100.0	75.6	3.7	0.0
<b>Polyspecific <del>CL</del>chemiluminescent immunoassay<sup>A</sup></b>				
<i>Low threshold†</i>	98.9 (92.7, 99.8)	85.6 (79.3, 90.3)	6.9 (4.7, 10.0)	0.01 (0.00, 0.09)
<i>Intermediate threshold†</i>	97.9 (94.6, 100.0)	93.1 (90.4, 95.8)	13.5 (9.5, 18.9)	0.0 (0.0, 0.1)
<i>High threshold†</i>	98.3 (69.5, 99.9)	97.5 (94.4, 98.9)	39.5 (17.5, 89.2)	0.0 (0.0, 0.40)
<b>IgG-specific <del>chemiluminescent immunoassay</del>CLIA</b>				
<i>Low threshold†</i>	98.8 (69.2, 100.0)	94.6 (90.7, 96.9)	18.3 (10.6, 31.5)	0.01 (0.00, 0.40)
<i>Intermediate threshold†</i>	78.6 (75.9, 81.2)	98.7 (94.6, 100)	42.3 (20.1, 88.7)	0.2 (0.1, 0.3)
<i>High threshold†</i>	74.2 (71.9, 76.5)	99.1 (95.4, 100)	47.8 (23.2, 98.7)	0.2 (0.1, 0.4)

\* According to results of a recent meta-analysis (73), please note differences between individual manufacturers; \* low threshold: below or equal to OD 0.7, intermediate threshold: between OD 0.8 and 1.4, high threshold: above OD 1.4; ° low threshold: positive/negative, intermediate threshold: titer 2 to 3; † low threshold: below 1.0 U/ml, intermediate threshold: between 1.0 and 2.8 U/ml, high threshold: above 2.8 U/ml

**Table 3:** Commonly used functional assays for diagnosis of HIT

Type of test	Analytic principle	Endpoint	Platelets used	Confirmation step	Validation
Serotonin release assay (SRA)	Stimulation of platelet serotonin release by patient serum in the presence of heparin	Detection of change in <sup>14</sup> C	Washed, <sup>14</sup> C-radiolabeled platelets from one selected donor	Suppression with high-dose heparin and inhibition using an FcγRIIA blocking antibody	High agreement with clinical HIT (86, 87)
Heparin-induced platelet activation assay (HIPA)	Detection of platelet aggregation induced by patient serum in the presence of heparin	Visual assessment of aggregation in microtitre plates	Washed platelets from four unselected donors	Suppression with high-dose heparin and inhibition using an FcγRIIA blocking antibody	High agreement with clinical HIT (38, 88)
Heparin-induced platelet aggregation test (PAT)	Activation of platelets (citrated PRP) in the presence of patient plasma and heparin	Detection of aggregation by aggregometry	PRP of one to four, selected or unselected donors	Suppression with high-dose heparin	Varying agreement with SRA, depending on platelet donor (94), lower sensitivity than SRA/HIPA with clinical criteria (38, 90, 93)
Flow cytometry	Detection of markers for platelet activation (eg. CD45/GPIIb; platelet microparticles; CD62; annexin V)	Increase of platelet activation markers of donor platelets in presence of heparin	PRP of unselected donors	None	Some agreement with SRA (44, 95-98), requires standardisation and further evaluation
Whole blood impedance aggregometry (Multiplate®)	Activation of whole blood platelets in the presence of patient plasma and heparin	Changes in impedance	Whole blood from one selected donor	Suppression with high-dose heparin	Adequate agreement with SRA in two studies (81, 106), requires confirmation

## Figure legends

**Figure 1: Suggested diagnostic algorithm for diagnosis of HIT** (adapted from (8)). The algorithm must be adapted according to the individual setting, taking the availability of laboratory tests such as functional assays into account. Of note, using this algorithm some HIT patients with a low risk 4Ts scoring will be missed, particularly in cases with inadequately determination of the 4Ts score. Thus, several authors suggested conducting an immunoassays in all patients with suspected HIT (24, 33, 34). However, this approach needs careful interpretation of immunoassay test results to avoid over-treatment.

**Figure 2: Diagnostic challenges in clinical practice.** The 35-year-old female patient was admitted to hospital with fever and abdominal pain; the platelet count was  $70 \times 10^9/L$ . She underwent uterine embolization and curettage 10 days earlier because of vaginal bleeding due to ectopic cervical pregnancy. HIT was rejected because of a low risk 4Ts scoring (3/8 points) and no immunoassay test was conducted (in accordance with recent guidelines). Patient suffered extensive intracranial haemorrhage three days later and cerebral venous thrombosis as well as HIT was diagnosed. Despite immediate start with lepirudin and intensive medical support, patient died on day 33.

**Figure 3: Probability of having HIT with a particular immunoassay test result according to pre-test probability.** The probability of having HIT is represented by the post-test probability on the Y-axis, the clinical probability (as measured by a clinical assessment tool) is illustrated by the pre-test probability on the X-axis. Two different immunoassays are shown with curves illustrative of the probability of HIT with a positive and negative immunoassay results as indicated. It is obvious that the probability of having HIT remains low in patients with a low clinical probability despite a positive immunoassay test result. In contrast, the probability of HIT is increasing in patients with a high clinical probability, even with a negative immunoassay test result (applies mainly to assays with a limited sensitivity).

**Figure 2: Suggested diagnostic algorithm for diagnosis of HIT** (adapted from (8)). The algorithm must be adapted according to the individual setting, taking the availability of laboratory tests such as functional assays into account. (24, 33, 34)

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