

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

24

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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%~~ (95% CI: 9 to
19 22%) in intermediate scoring and ~~50.636%~~ (95% CI: 40 to 82%) in the high risk scoring.
20 ~~However, the~~ 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 ¹⁴C-
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 ¹⁴C-
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, ¹⁴C-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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7 **References**

- 8 1. Bakchoul T. Current insight in the laboratory diagnosis of hit. International journal of
9 laboratory hematology 2014; 36: 16.
- 10 2. Cuker A. Clinical and laboratory diagnosis of heparin-induced thrombocytopenia: an
11 integrated approach. Seminars in thrombosis and hemostasis 2014; 40(1): 106-14.
- 12 3. Warkentin TE, Greinacher A, Gruel Y, et al. Laboratory testing for heparin-induced
13 thrombocytopenia: a conceptual framework and implications for diagnosis. Journal of
14 thrombosis and haemostasis : JTH 2011; 9(12): 2498-500.
- 15 4. Otis SA, Zehnder JL. Heparin-induced thrombocytopenia: current status and diagnostic
16 challenges. American journal of hematology 2010; 85(9): 700-6.
- 17 5. Cuker A, Cines DB. How I treat heparin-induced thrombocytopenia. Blood 2012;
18 119(10): 2209-18.
- 19 6. Price EA, Hayward CP, Moffat KA, et al. Laboratory testing for heparin-induced
20 thrombocytopenia is inconsistent in North America: a survey of North American specialized
21 coagulation laboratories. Thrombosis and haemostasis 2007; 98(6): 1357-61.
- 22 7. Greinacher A. Heparin-induced thrombocytopenia. Journal of thrombosis and
23 haemostasis : JTH 2009; 7 Suppl 1: 9-12.
- 24 8. Greinacher A. CLINICAL PRACTICE. Heparin-Induced Thrombocytopenia. The New
25 England journal of medicine 2015; 373(3): 252-61.
- 26 9. Linkins LA, Dans AL, Moores LK, et al. Treatment and prevention of heparin-induced
27 thrombocytopenia: Antithrombotic Therapy and Prevention of Thrombosis, 9th ed: American
28 College of Chest Physicians Evidence-Based Clinical Practice Guidelines. Chest 2012; 141(2
29 Suppl): e495S-530S.
- 30 10. Warkentin TE. How I diagnose and manage HIT. Hematology / the Education Program
31 of the American Society of Hematology American Society of Hematology Education Program
32 2011; 2011: 143-9.
- 33 11. Warkentin TE, Kelton JG. A 14-year study of heparin-induced thrombocytopenia. The
34 American journal of medicine 1996; 101(5): 502-7.

- 1 12. Tardy B, Lecompte T, Boelhen F, et al. Predictive factors for thrombosis and major
2 bleeding in an observational study in 181 patients with heparin-induced thrombocytopenia
3 treated with lepirudin. *Blood* 2006; 108(5): 1492-6.
- 4 13. Greinacher A, Lubenow N, Eichler P. Anaphylactic and anaphylactoid reactions
5 associated with lepirudin in patients with heparin-induced thrombocytopenia. *Circulation* 2003;
6 108(17): 2062-5.
- 7 14. Cuker A. Heparin-induced thrombocytopenia (HIT) in 2011: an epidemic of
8 overdiagnosis. *Thrombosis and haemostasis* 2011; 106(6): 993-4.
- 9 15. Warkentin TE, Linkins LA. Immunoassays are not created equal. *Journal of thrombosis*
10 *and haemostasis : JTH* 2009; 7(8): 1256-9.
- 11 16. Alatri A, Armstrong AE, Greinacher A, et al. Results of a consensus meeting on the use
12 of argatroban in patients with heparin-induced thrombocytopenia requiring antithrombotic
13 therapy - a European Perspective. *Thrombosis research* 2012; 129(4): 426-33.
- 14 17. Watson H, Davidson S, Keeling D, et al. Guidelines on the diagnosis and management of
15 heparin-induced thrombocytopenia: second edition. *British journal of haematology* 2012; 159(5):
16 528-40.
- 17 18. Lo GK, Juhl D, Warkentin TE, et al. Evaluation of pretest clinical score (4 T's) for the
18 diagnosis of heparin-induced thrombocytopenia in two clinical settings. *Journal of Thrombosis*
19 *and Haemostasis* 2006; 4(4): 759-65.
- 20 19. Cuker A, Gimotty PA, Crowther MA, et al. Predictive value of the 4Ts scoring system
21 for heparin-induced thrombocytopenia: a systematic review and meta-analysis. *Blood* 2012;
22 120(20): 4160-7.
- 23 20. Lillo-Le Louet A, Boutouyrie P, Alhenc-Gelas M, et al. Diagnostic score for heparin-
24 induced thrombocytopenia after cardiopulmonary bypass. *Journal of thrombosis and haemostasis*
25 *: JTH* 2004; 2(11): 1882-8.
- 26 21. Pouplard C, Gueret P, Fouassier M, et al. Prospective evaluation of the '4Ts' score and
27 particle gel immunoassay specific to heparin/PF4 for the diagnosis of heparin-induced
28 thrombocytopenia. *Journal of thrombosis and haemostasis : JTH* 2007; 5(7): 1373-9.
- 29 22. Bryant A, Low J, Austin S, et al. Timely diagnosis and management of heparin-induced
30 thrombocytopenia in a frequent request, low incidence single centre using clinical 4T's score and
31 particle gel immunoassay. *British journal of haematology* 2008; 143(5): 721-6.
- 32 23. Bakchoul T, Giptner A, Najaoui A, et al. Prospective evaluation of PF4/heparin
33 immunoassays for the diagnosis of heparin-induced thrombocytopenia. *Journal of thrombosis*
34 *and haemostasis : JTH* 2009; 7(8): 1260-5.
- 35 24. Nellen V, Sulzer I, Barizzi G, et al. Rapid exclusion or confirmation of heparin-induced
36 thrombocytopenia: A single-center experience with 1,291 patients. *Haematologica* 2012; 97(1):
37 89-97.
- 38 25. Tawfik NM, Hegazy MA, Hassan EA, et al. Egyptian experience of reliability of 4T's
39 score in diagnosis of heparin induced thrombocytopenia syndrome. *Blood Coagulation and*
40 *Fibrinolysis* 2011; 22(8): 701-5.
- 41 26. Denys B, Stove V, Philippe J, et al. A clinical-laboratory approach contributing to a rapid
42 and reliable diagnosis of heparin-induced thrombocytopenia. *Thrombosis research* 2008; 123(1):
43 137-45.

- 1 27. Crowther MA, Cook DJ, Albert M, et al. The 4Ts scoring system for heparin-induced
2 thrombocytopenia in medical-surgical intensive care unit patients. *Journal of critical care* 2010;
3 25(2): 287-93.
- 4 28. Berry C, Tcherniantchouk O, Ley EJ, et al. Overdiagnosis of heparin-induced
5 thrombocytopenia in surgical ICU patients. *Journal of the American College of Surgeons* 2011;
6 213(1): 10-7.
- 7 29. Cuker A, Arepally G, Crowther MA, et al. The HIT Expert Probability (HEP) Score: A
8 novel pre-test probability model for heparin-induced thrombocytopenia based on broad expert
9 opinion. *Journal of Thrombosis and Haemostasis* 2010; 8(12): 2642-50.
- 10 30. Demma LJ, Winkler AM, Levy JH. A diagnosis of heparin-induced thrombocytopenia
11 with combined clinical and laboratory methods in cardiothoracic surgical intensive care unit
12 patients. *Anesthesia and analgesia* 2011; 113(4): 697-702.
- 13 31. Nagler M, Fabbro T, Wuillemin WA. Prospective evaluation of the interobserver
14 reliability of the 4Ts score in patients with suspected heparin-induced thrombocytopenia. *Journal*
15 *of thrombosis and haemostasis : JTH* 2012; 10(1): 151-2.
- 16 32. Nagler M, Angelillo-Scherrer A. Diagnostic value of the 4Ts score for heparin-induced
17 thrombocytopenia in the critically ill. *Journal of critical care* 2014; 29(6): 1126-7.
- 18 33. Linkins LA, Bates SM, Lee AY, et al. Combination of 4Ts score and PF4/H-PaGIA for
19 diagnosis and management of heparin-induced thrombocytopenia: prospective cohort study.
20 *Blood* 2015; 126(5): 597-603.
- 21 34. Favaloro EJ. Toward improved diagnosis of HIT. *Blood* 2015; 126(5): 563-4.
- 22 35. Messmore HL, Fabbri N, Bird ML, et al. Simple scoring system for early management
23 of heparin-induced thrombocytopenia. *Clinical and Applied Thrombosis/Hemostasis* 2011;
24 17(2): 197-201.
- 25 36. Piednoir P, Allou N, Provenchere S, et al. Heparin-induced thrombocytopenia after
26 cardiac surgery: an observational study of 1,722 patients. *Journal of cardiothoracic and vascular*
27 *anesthesia* 2012; 26(4): 585-90.
- 28 37. Amiral J, Bridey F, Dreyfus M, et al. Platelet factor 4 complexed to heparin is the target
29 for antibodies generated in heparin-induced thrombocytopenia. *Thrombosis and haemostasis*
30 1992; 68(1): 95-6.
- 31 38. Greinacher A, Amiral J, Dummel V, et al. Laboratory diagnosis of heparin-associated
32 thrombocytopenia and comparison of platelet aggregation test, heparin-induced platelet
33 activation test, and platelet factor 4/heparin enzyme-linked immunosorbent assay. *Transfusion*
34 1994; 34(5): 381-5.
- 35 39. Arepally G, Reynolds C, Tomaski A, et al. Comparison of PF4/heparin ELISA assay with
36 the 14C-serotonin release assay in the diagnosis of heparin-induced thrombocytopenia. *American*
37 *journal of clinical pathology* 1995; 104(6): 648-54.
- 38 40. Shelat SG, Tomaski A, Pollak ES. Serologic results in >1000 patients with suspected
39 heparin-induced thrombocytopenia. *Clinical and applied thrombosis/hemostasis : official journal*
40 *of the International Academy of Clinical and Applied Thrombosis/Hemostasis* 2008; 14(4): 410-
41 4.

- 1 41. Morel-Kopp MC, Aboud M, Tan CW, et al. Heparin-induced thrombocytopenia:
2 Evaluation of IgG and IgGAM ELISA assays. *International journal of laboratory hematology*
3 2011; 33(3): 245-50.
- 4 42. Greinacher A, Juhl D, Strobel U, et al. Heparin-induced thrombocytopenia: A prospective
5 study on the incidence, platelet-activating capacity and clinical significance of antiplatelet factor
6 4/heparin antibodies of the IgG, IgM, and IgA classes. *Journal of thrombosis and haemostasis* :
7 *JTH* 2007; 5(8): 1666-73.
- 8 43. Warkentin TE, Sheppard JI, Moore JC, et al. Quantitative interpretation of optical density
9 measurements using PF4-dependent enzyme-immunoassays. *Journal of thrombosis and*
10 *haemostasis* : *JTH* 2008; 6(8): 1304-12.
- 11 44. Vitale M, Tazzari P, Ricci F, et al. Comparison between different laboratory tests for the
12 detection and prevention of heparin-induced thrombocytopenia. *Cytometry* 2001; 46(5): 290-5.
- 13 45. Nasr R, Owaidah T. Validation of monospecific elisa test as a screening test for heparin
14 induced thrombocytopenia compare to other immunological and functional assays. *Journal of*
15 *thrombosis and haemostasis* : *JTH* 2011; 9: 333.
- 16 46. Schwarz N, Luxembourg B, Kroll H, et al. Performance of anti-heparin/platelet factor 4-
17 assays in the diagnosis of heparin-induced thrombocytopenia (HIT) defined by clinical criteria.
18 *Journal of Thrombosis and Haemostasis* 2009; 7 (S2): 869.
- 19 47. Andrews DM, Cubillos GF, Paulino SK, et al. Prospective observational evaluation of the
20 particle immunofiltration anti-platelet factor 4 rapid assay in MICU patients with
21 thrombocytopenia. *Critical Care* 2013; 17(4).
- 22 48. Pouplard C, Leroux D, Regina S, et al. Effectiveness of a new immuno-assay for the
23 diagnosis of heparin-induced thrombocytopenia and improved specificity when detecting IgG
24 antibodies. *Thrombosis and haemostasis* 2010; 103(1): 145-50.
- 25 49. Juhl D, Eichler P, Lubenow N, et al. Incidence and clinical significance of anti-
26 PF4/heparin antibodies of the IgG, IgM, and IgA class in 755 consecutive patient samples
27 referred for diagnostic testing for heparin-induced thrombocytopenia. *European journal of*
28 *haematology* 2006; 76(5): 420-6.
- 29 50. Farahani N, Tcherniantchouk O, Kitahara S. Cost-effective HIT diagnosis: Utilizing IgG-
30 specific PF4 assays reduces the number of confirmatory serotonin release assays without missing
31 true HIT. *Journal of thrombosis and haemostasis* : *JTH* 2013; 11: 937.
- 32 51. Haouach K, Admou B, Lauriant P, et al. Contributions of biological tests and the 4 Ts
33 score in the diagnosis of heparin induced thrombocytopenia. *The Pan African medical journal*
34 2012; 13.
- 35 52. Eichler P, Raschke R, Lubenow N, et al. The new ID-heparin/PF4 antibody test for rapid
36 detection of heparin-induced antibodies in comparison with functional and antigenic assays.
37 *British journal of haematology* 2002; 116(4): 887-91.
- 38 53. Izban KF, Lietz HW, Hoppensteadt DA, et al. Comparison of two PF4/heparin ELISA
39 assays for the laboratory diagnosis of heparin-induced thrombocytopenia. *Seminars in*
40 *thrombosis and hemostasis* 1999; 25(2 SUPPL. 1): 51-6.
- 41 54. Kapadia FN, Ketkar AS, Deshpande AS, et al. Evaluation of various laboratory assays in
42 detection of heparin-induced thrombocytopenia in an adult general ICU. *International journal of*
43 *laboratory hematology* 2013; 35(2): 137-43.

- 1 55. Tazzari PL, Ricci F, Vitale M, et al. Heparin-induced thrombocytopenia: Detection of
2 antiheparin/PF4 antibodies by means of heparin/PF4-coated beads and flow cytometry.
3 *Transfusion medicine (Oxford, England)* 2002; 12(3): 193-8.
- 4 56. Van Hoecke F, Devreese K. Evaluation of two new automated chemiluminescent assays
5 (HemosILAcuStar HIT-IgG and HemosILAcuStar HIT-Ab) for the detection of heparin-induced
6 antibodies in the diagnosis of heparin-induced thrombocytopenia. *International journal of*
7 *laboratory hematology* 2012; 34(4): 410-6.
- 8 57. Javela K, Koskinen S. Comparison of a heparin-platelet factor 4 (PF4)-ELISA IgG/M/A
9 and the heparin-induced platelet activation assay (HIPA) with a PF4-ELISA IgG. *Vox sanguinis*
10 2010; 99: 27-8.
- 11 58. Minet V, Bailly N, Douxfils J, et al. Assessment of the performances of AcuStar HIT and
12 the combination with heparin-induced multiple electrode aggregometry: A retrospective study.
13 *Thrombosis research* 2013; 132(3): 352-9.
- 14 59. Kiefel V, Schonberner-Richter I, Lange B. Lateral flow immunoassay-a useful tool for
15 detection of PF4/heparin antibodies in HIT? *Hamostaseologie* 2012; 32 (1): A83-A4.
- 16 60. Look KA, Sahud M, Flaherty S, et al. Heparin-induced platelet aggregation vs platelet
17 factor 4 enzyme-linked immunosorbent assay in the diagnosis of heparin-induced
18 thrombocytopenia- thrombosis. *American journal of clinical pathology* 1997; 108(1): 78-82.
- 19 61. Pierce W, Mazur J, Greenberg C, et al. Evaluation of heparin-induced thrombocytopenia
20 (HIT) laboratory testing and the 4Ts scoring system in the intensive care unit. *Annals of clinical*
21 *and laboratory science* 2013; 43(4): 429-35.
- 22 62. Verma AK, Levine M, Shalansky SJ, et al. Frequency of heparin-induced
23 thrombocytopenia in critical care patients. *Pharmacotherapy* 2003; 23(6): 745-53.
- 24 63. Shaheed G, Malkovska V, Mendoza J, et al. PF4 ENHANCED assay for the diagnosis of
25 heparin-induced thrombocytopenia in complex medical and surgical patients. *Critical care*
26 *medicine* 2007; 35(7): 1691-5.
- 27 64. Nagler M, Bachmann LM, Ten Cate H, et al. Diagnostic value of immunoassays for
28 heparin-induced thrombocytopenia: a systematic review and meta-analysis. *Blood* 2016; 127(5):
29 546-57.
- 30 65. Smock KJ, Ledford-Kraemer MR, Meijer P, et al. Proficiency testing results for heparin-
31 induced thrombocytopenia in north america. *Seminars in thrombosis and hemostasis* 2014; 40(2):
32 254-60.
- 33 66. Althaus K, Strobel U, Warkentin TE, et al. Combined use of the high heparin step and
34 optical density to optimize diagnostic sensitivity and specificity of an anti-PF4/heparin enzyme-
35 immunoassay. *Thrombosis research* 2011; 128(3): 256-60.
- 36 67. Bakchoul T, Giptner A, Bein G, et al. Performance characteristics of two commercially
37 available IgG-specific immunoassays in the assessment of heparin-induced thrombocytopenia
38 (HIT). *Thrombosis research* 2011; 127(4): 345-8.
- 39 68. Denys B, Devreese K. A clinical-laboratory approach contributing to a rapid and reliable
40 diagnosis of heparin-induced thrombocytopenia: An update. *Thrombosis research* 2009; 124(5):
41 642-3.

- 1 69. Kolde HJ, Dostatni R, Mauracher S. Rapid and simple IgG specific test for the exclusion
2 of heparin induced thrombocytopenia (HIT). *Clinical chemistry and laboratory medicine* :
3 CCLM / FESCC 2011; 49(12): 2065-8.
- 4 70. Novelli C, Erba N, Gatti A, et al. Evaluation of a new rapid test for detection of anti PF4-
5 heparin antibodies in hit diagnosis. *Blood Transfus* 2012; 10: s69.
- 6 71. Sachs UJ, von Hesberg J, Santoso S, et al. Evaluation of a new nanoparticle-based lateral-
7 flow immunoassay for the exclusion of heparin-induced thrombocytopenia (HIT). *Thrombosis*
8 and haemostasis 2011; 106(6): 1197-202.
- 9 72. Seidel H, Hoffmann T, Wulfig AH, et al. Laboratory and clinical performance of rapid
10 immunoassays in diagnosis of heparin induced thrombocytopenia: Comparison of lateral flow
11 and gel centrifugation with ELISA technique. *Hamostaseologie* 2013; 33 (1): A71.
- 12 73. Nagler M, Bachmann LM, Ten Cate H, et al. Diagnostic value of immunoassays for
13 heparin-induced thrombocytopenia: a systematic review and meta-analysis. *Blood* 2015.
- 14 74. Alhaliq AR, Joshua D, Kershaw G, et al. The Diamed assay has a poor positive predictive
15 value for HIT in a tertiary hospital referral setting [1]. *International journal of laboratory*
16 *hematology* 2007; 29(1): 69-70.
- 17 75. Leroux D, Hezard N, Lebreton A, et al. Prospective evaluation of a rapid nanoparticle-
18 based lateral flow immunoassay (STic Expert(R)) HIT) for the diagnosis of heparin-induced
19 thrombocytopenia. *British journal of haematology* 2014; 166(5): 774-82.
- 20 76. Kolde HJ, Habrecht U, Von Hesberg J, et al. Multicentric validation of a rapid assay for
21 heparin-induced thrombocytopenia with different specimen types. *Blood coagulation &*
22 *fibrinolysis : an international journal in haemostasis and thrombosis* 2014; 25(1): 6-9.
- 23 77. Warkentin TE, Sheppard JI, Raschke R, et al. Performance characteristics of a rapid
24 assay for anti-PF4/heparin antibodies: The particle immunofiltration assay [5]. *Journal of*
25 *Thrombosis and Haemostasis* 2007; 5(11): 2308-10.
- 26 78. Legnani C, Cini M, Pili C, et al. Evaluation of a new automated panel of assays for the
27 detection of anti-PF4/heparin antibodies in patients suspected of having heparin-induced
28 thrombocytopenia. *Thrombosis and haemostasis* 2010; 104(2): 402-9.
- 29 79. Archambault AJ, Dolan EJ, Arkin CF. Comparison of the Hemosil HIT-AB1 to the
30 standard ELISA assay for heparin-induced thrombocytopenia (HIT) antibody. *American journal*
31 *of hematology* 2012; 87: S184.
- 32 80. Althaus K, Hron G, Strobel U, et al. Evaluation of automated immunoassays in the
33 diagnosis of heparin induced thrombocytopenia. *Thrombosis research* 2013; 131(3): e85-e90.
- 34 81. Minet V, Baudar J, Bailly N, et al. Rapid exclusion of the diagnosis of immune HIT by
35 AcuStar HIT and heparin-induced multiple electrode aggregometry. *Thrombosis research* 2014;
36 133(6): 1074-8.
- 37 82. Sun L, Gimotty PA, Lakshmanan S, et al. Diagnostic accuracy of rapid immunoassays for
38 heparin-induced thrombocytopenia. A systematic review and meta-analysis. *Thrombosis and*
39 *haemostasis* 2016; 115(5).
- 40 83. Caton S, O'Brien E, Pannelay AJ, et al. Assessing the clinical and cost impact of on-
41 demand immunoassay testing for the diagnosis of heparin induced thrombocytopenia.
42 *Thrombosis research* 2016; 140: 155-62.

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Formatiert: Deutsch
(Deutschland)

- 1 84. Warkentin TE. PF4-dependent immunoassays and inferential detection of HIT antibodies.
2 Journal of Thrombosis and Haemostasis 2007; 5(2): 232-4.
- 3 85. Bakchoul T, Zollner H, Greinacher A. Current insights into the laboratory diagnosis of
4 HIT. International journal of laboratory hematology 2014; 36(3): 296-305.
- 5 86. Warkentin TE, Sheppard JAI, Moore JC, et al. Laboratory testing for the antibodies that
6 cause heparin-induced thrombocytopenia: How much class do we need? Journal of Laboratory
7 and Clinical Medicine 2005; 146(6): 341-6.
- 8 87. Sheridan D, Carter C, Kelton JG. A diagnostic test for heparin-induced
9 thrombocytopenia. Blood 1986; 67(1): 27-30.
- 10 88. Greinacher A, Michels I, Kiefel V, et al. A rapid and sensitive test for diagnosing
11 heparin-associated thrombocytopenia. Thrombosis and haemostasis 1991; 66(6): 734-6.
- 12 89. Warkentin TE. HIT paradigms and paradoxes. Journal of thrombosis and haemostasis :
13 JTH 2011; 9 Suppl 1: 105-17.
- 14 90. Favaloro EJ, Bernal-Hoyos E, Exner T, et al. Heparin-induced thrombocytopenia:
15 laboratory investigation and confirmation of diagnosis. Pathology 1992; 24(3): 177-83.
- 16 91. Warkentin TE, Arnold DM, Nazi I, et al. The platelet serotonin-release assay. American
17 journal of hematology 2015; 90(6): 564-72.
- 18 92. Morel-Kopp MC, Aboud M, Tan CW, et al. Whole blood impedance aggregometry
19 detects heparin-induced thrombocytopenia antibodies. Thrombosis research 2010; 125(5): e234-
20 e9.
- 21 93. Stricker H, Lammle B, Furlan M, et al. Heparin-dependent in vitro aggregation of normal
22 platelets by plasma of a patient with heparin-induced skin necrosis: specific diagnostic test for a
23 rare side effect. The American journal of medicine 1988; 85(5): 721-4.
- 24 94. Chong BH, Burgess J, Ismail F. The clinical usefulness of the platelet aggregation test for
25 the diagnosis of heparin-induced thrombocytopenia. Thrombosis and haemostasis 1993; 69(4):
26 344-50.
- 27 95. Tomer A, Masalunga C, Abshire TC. Determination of heparin-induced
28 thrombocytopenia: A rapid flow cytometric assay for direct demonstration of antibody-mediated
29 platelet activation. American journal of hematology 1999; 61(1): 53-61.
- 30 96. Gobbi G, Mirandola P, Tazzari PL, et al. New Laboratory Test in Flow Cytometry for the
31 Combined Analysis of Serologic and Cellular Parameters in the Diagnosis of Heparin-Induced
32 Thrombocytopenia. Cytometry Part B - Clinical Cytometry 2004; 58(1): 32-8.
- 33 97. Lee DH, Warkentin TE, Denomme GA, et al. A diagnostic test for heparin-induced
34 thrombocytopenia: Detection of platelet microparticles using flow cytometry. British journal of
35 haematology 1996; 95(4): 724-31.
- 36 98. Mullier F, Bailly N, Cornet Y, et al. Contribution of platelet microparticles generation
37 assay to the diagnosis of type II heparin-induced thrombocytopenia. Thrombosis and haemostasis
38 2010; 103(6): 1277-81.
- 39 99. Greinacher A, Ittermann T, Bagemuhl J, et al. Heparin-induced thrombocytopenia:
40 towards standardization of platelet factor 4/heparin antigen tests. Journal of thrombosis and
41 haemostasis : JTH 2010; 8(9): 2025-31.

Formatiert: Deutsch
(Deutschland)

Formatiert: Deutsch
(Deutschland)

- 1 100. Lindhoff-Last E, Gerdson F, Ackermann H, et al. Determination of heparin-platelet factor
2 4-IgG antibodies improves diagnosis of heparin-induced thrombocytopenia. *British journal of*
3 *haematology* 2001; 113(4): 886-90.
- 4 101. Warkentin TE, Sheppard JI. No significant improvement in diagnostic specificity of an
5 anti-PF4/ polyanion immunoassay with use of high heparin confirmatory procedure [10]. *Journal*
6 *of Thrombosis and Haemostasis* 2006; 4(1): 281-2.
- 7 102. McGowan KE, Makari J, Diamantouros A, et al. Reducing the hospital burden of
8 heparin-induced thrombocytopenia: impact of an avoid-heparin program. *Blood* 2016; 127(16):
9 1954-9.
- 10 103. Wallis DE, Workman DL, Lewis BE, et al. Failure of early heparin cessation as treatment
11 for heparin-induced thrombocytopenia. *The American journal of medicine* 1999; 106(6): 629-35.
- 12 104. Cuker A. Does my patient have HIT? There should be an app for that. *Blood* 2016;
13 127(5): 522-4.
- 14 105. Raschke RA, Curry SC, Warkentin TE, et al. Improving clinical interpretation of the anti-
15 platelet factor 4/heparin enzyme-linked immunosorbent assay for the diagnosis of heparin-
16 induced thrombocytopenia through the use of receiver operating characteristic analysis, stratum-
17 specific likelihood ratios, and Bayes theorem. *Chest* 2013; 144(4): 1269-75.
- 18 106. Morel-Kopp MC, Tan CW, Brighton TA, et al. Validation of whole blood impedance
19 aggregometry as a new diagnostic tool for HIT: results of a large Australian study. *Thrombosis*
20 *and haemostasis* 2012; 107(3): 575-83.

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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>) [#] Technoclone GmbH, Vienna, Austria (<i>Technozym</i>)
Particle gel immunoassay (PaGIA)	Polyspecific	Visual assessment of agglutination <i>Quantification using titration studies</i> [°]	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 [°]	Determination in standard laboratories possible, 24-hour service	Diagnostica Stago, Asnières-sur-Seine, France (<i>STic EXPERT HIT</i>) Milenia Biotec, Giessen, Germany (<i>Milena 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⁺

Type of test	Sensitivity	Specificity	Likelihood ratio	
			(percentages)	Positive (95% CI)
Polyspecific ELISA				
<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)
IgG-specific ELISA				
<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)
PaGIA				
<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
Lateral flow immunoassay	98.4 (85.3, 99.9)	90.3 (84.4, 94.1)	10.1 (6.2, 16.5)	0.02 (0.00, 0.18)
Particle immunofiltration assay	0.0	70.1	2.3	0.5
Latex agglutination assay	100.0	75.6	3.7	0.0
Polyspecific CLchemiluminescent immunoassay^A				
<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)
IgG-specific chemiluminescent immunoassayCLIA				
<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 ¹⁴ C	Washed, ¹⁴ 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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