Philippine COVID-19 Living Clinical Practice Guidelines
Institute of Clinical Epidemiology, National Institutes of Health, UP Manila
In cooperation with the Philippine Society for Microbiology and Infectious Diseases
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EVIDENCE SUMMARY

Should heparin induced thrombocytopenia (HIT) test kits be used for COVID-19 vaccine induced thrombosis with thrombocytopenia (VITT)?

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RECOMMENDATION

We suggest against the use of PF4 antibody ELISA Heparin Induced Thrombocytopenia (HIT) test kits and non-ELISA rapid HIT test kits for COVID-19 Vaccine Induced Thrombosis and Thrombocytopenia (VITT). *(Very low certainty of evidence; Weak recommendation)*

Consensus Issues
The panel unanimously decided against the use of HIT test kits for COVID-19 VITT, citing that the test has a slow turnaround time and immediate clinical management remains the same regardless of the test result. Concerns were also raised on its cost-effectiveness as well as availability in the local setting. Test kits employing ELISA demonstrate relatively high sensitivity and specificity but have limited availability locally.

Key Findings

- One cross-sectional observational study [1] was included on the use of anti-platelet factor 4 (anti-PF4) assay test kits for the diagnosis of COVID-19 vaccine-induced thrombosis and thrombocytopenia (VITT).
- The overall certainty of evidence was very low because of serious concerns of risk of bias, inconsistency, and imprecision.
- The study analyzed samples from 43 patients with suspected VITT using six brands of anti-PF4 ELISA test kits and four brands of anti-PF4 non-ELISA test kits. Sensitivity ranged from 0.71-0.97 and specificity ranged from 0.56-1.00 for the ELISA test kits, while sensitivity ranged from 0.00-0.45 and specificity ranged from 0.67-1.00 for the non-ELISA test kits.

Introduction

Rare cases presenting with signs and symptoms of thrombosis and thrombocytopenia have been reported following vaccination with viral vector-based COVID-19 vaccines, defined as COVID-19 vaccine-induced thrombosis and thrombocytopenia (VITT). Clinical presentation of COVID-19 VITT closely resembles heparin-induced thrombocytopenia (HIT), which involves platelet factor 4 (PF4) antibodies complexed with heparin.[2] Interactions between COVID-19 vaccines and platelets or PF4 may play a role in the pathophysiology of VITT, hence prompting the use of PF4 antibody test kits typically applied in the diagnostic workup of HIT to be investigated for COVID-19 VITT.

Review Methods

The review searched for studies that enrolled patients with suspected VITT after administration of COVID-19 vaccines who were tested with anti-PF4 assay HIT test kits, using sensitivity and specificity as the outcomes of interest.

A search was done through PubMed and Google Scholar from inception to 03 Oct 2021 using the following search terms: "(SARS-CoV 2 vaccine OR COVID-19 vaccine OR COVID vaccine) AND (vaccine induced immune thrombotic thrombocytopenic OR Vaccine-induced immune thrombosis with thrombocytopenia OR vaccine associated immune thrombotic thrombocytopenic OR VITT OR VATT) AND (PF4 OR ELISA OR anti-PF4 assay OR heparin induced thrombocytopenia assay)." References and citations were cross-referenced for relevant articles. Initial intended inclusion criteria were randomized controlled trials, observational studies, or systematic reviews that enrolled patients with suspected VITT after administration of COVID-19 vaccines tested with anti-PF4 assay HIT test kits. Exclusion criteria were studies that tested anti-PF4 test kits in non-COVID vaccine related settings, and study designs that were limited to only case studies, case series, or consensus reports.

Results

Characteristics of Included Studies

Initial search yielded 55 studies, majority of which were case reports, case series, consensus reports, or proposed guidelines. No randomized controlled trials (RCT) or case-control studies were found. The search yielded three systematic reviews [2-4] that summarized findings from only case reports and case series regarding COVID-19 VITT. The search also yielded two cross-sectional studies [1,5] that compared sensitivities of different brands and methods of anti-PF4 antibody assays among patients with clinical signs and symptoms of suspected VITT. One [5] of the two cross-sectional studies was excluded because it only analyzed samples from one patient across different brands of the assay.

One cross-sectional observational study was ultimately included in the analysis. Platton et al. [1] evaluated 50 samples from 43 patients that were referred to the UK Expert Haematology Panel multidisciplinary team for suspected VITT five to 28 days after the administration of ChAdOx1 nCoV-19 vaccines.

Samples were tested using the index test of PF4 antibody test kits traditionally used for HIT. Six brands of anti-PF4 ELISA test kits (four IgG-specific and two polyspecific) and four brands of anti-PF4 non-ELISA test kits were included. The reference standard was clinical presentation suggestive of VITT as evaluated by the UK Expert Haematology Panel. Based on signs and symptoms of thrombosis, thrombocytopenia, and elevated D-dimer, patients with suspected VITT were classified into possible VITT, probable VITT, or unlikely VITT. Samples were taken before treatment initiation and were measured for optical density according to manufacturer specifications to determine positivity or negativity of test result. The computed sensitivity and specificity are summarized in Table 1.

Diagnostic Accuracy

The accuracy of HIT tests varied across test kit types and brands as shown in Table 1 and in the paired forest plots and SROC curves in Appendix 6.

A. ELISA test kits

ELISA test kits showed moderate to high sensitivity across test brands ranging from 0.71 to 0.97. Specificity ranged from 0.56 to 1.00, with IgG-specific test kits appearing to have higher

specificities (Range 0.78-1.00) compared to polyspecific test kits (Range 0.56-0.67). Among the six different ELISA test kit brands, **Asserachrom HPIA IgG ELISA** showed the highest accuracy with a sensitivity of 0.91 (95% CI 0.76-0.98) and specificity of 1.00 (95% CI 0.660-1.00).

B. Non-ELISA test kits

Compared to ELISA test kits, non-ELISA test kits demonstrated poorer sensitivity ranging from 0 to 0.45, but with high specificity from 0.67 to 1.00. Of the four non-ELISA test kits evaluated in the study, Diamed PaGIA gel rapid test showed the highest sensitivity at 0.45 (95% CI 0.28-0.64) but also the poorest specificity at 0.67 (95% CI 0.30-0.93).

Table 1. Sensitivity and specificity of brands of anti-PF4 assays

Test	Sensitivity (95% CI)	Specificity (95% CI)
ELISA test kits, IgG-specific		
AESKULISA HiT II IgG ELISA	0.71 (0.53-0.85)	0.89 (0.52-1.00)
Asserachrom HPIA IgG ELISA	0.91 (0.76-0.98)	1.00 (0.66-1.00)
Lifecodes PF4 IgG ELISA	0.94 (0.80-0.99)	0.78 (0.40-0.97)
Zymutest HIA IgG ELISA	0.94 (0.80-0.99)	0.78 (0.40-0.97)
ELISA test kits, polyspecific		
Asserachrom HPIA polyspecific ELISA	0.97 (0.85-1.00)	0.67 (0.30-0.93)
Lifecodes PF4 Enhanced polyspecific ELISA	0.97 (0.85-1.00)	0.56 (0.21-0.86)
Non-ELISA test kits		
Diamed PaGIA gel rapid test	0.45 (0.28-0.64)	0.67 (0.30-0.93)
HemosIL AcuStar HIT-IgG rapid test	0.06 (0.01-0.20)	1.00 (0.66-1.00)
HemosIL HIT-Ab rapid test	0.00 (0.00-0.19)	1.00 (0.63-1.00)
STic Expert rapid test	0.04 (0.00-0.21)	1.00 (0.16-1.00)

Certainty of evidence

The overall certainty of evidence was rated very low because of serious risk of bias and imprecision from a small sample size. Using the QUADAS-2 tool [6], the study was assessed to be at risk of bias due to unclear concerns with patient selection (consecutive enrolment and unnecessary exclusions), blinded interpretation of index tests, flow, and timing of conduct of index tests and reference standard, and applicability concerns due to unreported baseline patient characteristics. A summarized tabular representation of QUADAS-2 results is in Appendix 4. Concerns with imprecision were attributed to evidence coming from a single observational study, of which the sample size was small.

Other Factors in Evidence to Decision

Immunoassay tests like anti-PF4 ELISA are observed to have relatively high sensitivity and efficiency due to compatibility with testing in large batches. However, these are also time-consuming with slow turnaround time especially compared to rapid tests and can be expensive in commercial use. Meanwhile, non-ELISA tests for anti-PF4 such as particle gel immunoassay, lateral flow assay, and automated latex-based assays have much faster turnaround time suited to urgent testing, but have more variations in sensitivity, specificity, and expense, and have interobserver variability.[7]

Despite its high sensitivity, the literature notes that there is still likelihood of false negative results with ELISA anti-PF4 test kits. The likelihood of false negatives is even greater in non-ELISA test

kits.[10] Thus, the interpretation of anti-PF4 test kits must be done alongside the complete clinical picture of patients with suspected VITT, including platelet count, D-dimer, and imaging as pertinent.

Estimated costs for various anti-PF4 HIT test assays were $52 (P2,623) for ELISA, $84 (P4,274) for AcuStar IgG rapid test, and $120 (P6,042) for Diamed PaGIA.[8] Anti-PF4 HIT test kits have very limited availability in the Philippines, found only as non-ELISA tests in two hospitals in Metro Manila with estimated costs ranging from P9,363 to PHP13,800.

For applicability and appropriateness in the local context, as of May 2021 the Department of Health (DOH) acknowledged reports of COVID-19 VITT following administration of viral vector-based vaccines such as the ChAdOx1 nCoV-19 vaccine, which is among the COVID-19 vaccines distributed in the country. After a temporary suspension in administration, the DOH resumed vaccination with ChAdOx1 nCoV-19 and reported no local cases of VITT in the Philippines.[9]

Additionally, anti-PF4 testing may have prolonged turnaround times and VITT is an urgent medical condition. Requesting physicians should not wait for anti-PF4 results and are advised to immediately treat patients with a high index of suspicion of VITT.[10,14] It is also important to note that patients with COVID-19 disease itself can also present with positive results in anti-PF4 antibody testing, likely in association with prothrombotic state in COVID-19 disease.[15] It is recommended that monitoring and surveillance of COVID-19 vaccine-related adverse events continue to be strengthened, including the expansion of novel avenues for active reporting of adverse events.[11]

Recommendations from Other Groups

Several international organizations have made recommendations in support for anti-PF4 testing in COVID-19 VITT.[10-14] These groups have also specified that anti-PF4 ELISA test kits be used. These recommendations are summarized in Table 2. No local guidelines on testing for suspected VITT were found.

Table 2. Recommendations from other groups for testing in COVID-19 VITT

Organization	Date	Recommendation
National Institute for Health and Care Excellence (NICE)	July 29, 2021	• For probable VITT, use ELISA for anti-PF4 antibodies to confirm the diagnosis.
US CDC Advisory Committee on Immunization Practices (ACIP)	May 12, 2021	• For Tier 1 TTS (thrombosis in unusual location), positive hepatin-PF4 ELISA is supportive but not required • For Tier 2 TTS (thrombosis in common location only), positive PF4 ELISA is required
American Society of Hematology (ASH)	August 12, 2021	• PF4-ELISA as part of initial work-up of suspected TTS; draw blood prior to any therapy
Expert Haematology Panel / British Society of Haematology	August 30, 2021	• PF4 antibodies are detected by ELISA • Not by other HIT assay methods – negative results and cannot be relied upon
International Society of Thrombosis and Hematology (ISTH)	April 20, 2021	• Not all HIT assays can detect PF4 antibodies – ELISA is most reliable • If reliable PF4 assay is negative: VITT is excluded • If reliable PF4 assay is positive: VITT is likely • If PF4 assay unavailable: >4x elevated D-dimer is highly suggestive of VITT

Research Gaps

More studies with higher certainty of evidence are needed to improve guidance on testing for VITT. One observational study was included for this review. To date, majority of published studies on anti-PF4 HIT testing for VITT are case series, case reports, and consensus statements.

A search for ongoing studies in *ClinicalTrials.gov* found an observational study based in the US titled the National Vaccine Adverse Event Reporting Survey and Etiology (NVAERS) which aims to enroll 100,000 participants for reporting of any adverse events, including thrombosis, within 60 days after receipt of any vaccine dose, including but not limited to vaccines for COVID, hepatitis, measles, seasonal influenza, and other diseases. No other ongoing COVID-19 studies related to HIT test kits or VITT were found.

References

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Appendix 1. Evidence to Decision

Table 1. Summary of initial judgements prior to the panel discussion (N = 9)

FACTORS	JUDGEMENT						RESEARCH EVIDENCE/ADDITIONAL CONSIDERATIONS
Problem	No (4)	Yes (5)					No local VITT events reported.
Benefits	Large (1)	Moderate (5)	Small (2)	Uncertain (1)			The study by Platton et all suggests moderate to high sensitivity and moderate to high specificity for ELISA test kits. In combination with clinical diagnosis, it may contribute to the diagnosis of VITT.
Harms	Large (1)	Moderate (6)	Small (1)	Uncertain (1)			The results from Platton's study show there is some risk of misdiagnosis, particularly with false negative test results with non-ELISA tests. This may lead to harmful consequences if VITT is missed or undiagnosed.
Balance of Benefits and Harms	Favors the use of HIT test	Probably favors the use of HIT test (7)	Does not favor the use of HIT test (2)				The moderate to high sensitivity and specificity shown in Platton 2021 suggests its contribution to the workup of COVID-19 VITT, but more studies with improved certainty are needed.
Certainty of Evidence	High	Moderate	Low (5)	Very low (4)			Only one observational study [1] was included in the analysis. It had uncertain risk of bias and uncertain concerns of applicability, hence low certainty of evidence.
Accuracy	Very Accurate	Accurate (2)	Inaccurate (4)	Very Inaccurate	Uncertain (3)		The sensitivity across brands of ELISA anti-PF4 test kits ranged from 0.71-0.97, while specificity ranged from 0.56-1.00, suggesting its accuracy. However, sensitivity ranged from 0.00-0.45 and specificity ranged from 0.56-1.00 in non-ELISA kits.

Values	Important uncertainty or variability (3)	Possibly important uncertainty or variability (6)	Possibly NO important uncertainty or variability	No important uncertainty or variability			The risk of underdiagnosis of VITT due to false negatives may be an important point of uncertainty, as the prothrombotic condition necessitates hospitalization and anticoagulant therapy. However, other means of diagnosis of VITT are also important, such as clinical presentation, platelet count, D-dimer, and imaging.
Resources Required	Uncertain (2)	Large cost (3)	Moderate Cost (4)	Negligible cost or savings	Moderate savings	Large savings	Immunoassays are expensive, including the ELISA test kits included in the study. However, missing the diagnosis of VITT is potentially more expensive given hospitalization and anticoagulation treatment are part of the management of VITT.
Certainty of evidence of required resources	No included studies (5)	Very low (2)	Low	Moderate (2)	High		No evidence found.
Cost effectiveness	No included studies (2)	Favors HIT tests	Does not favor either HIT tests or the comparator (1)	Favors comparison (6)			The reference standard of clinical diagnosis is more cost-effective than immunoassay testing.
Equity	Uncertain (2)	Reduced (4)	Probably no impact (3)	Increased			Anti-PF4 immunoassay testing is expensive and not readily accessible.
Acceptability	Uncertain (8)	No (1)	Yes				While COVID-19 VITT is of clinical concern, applicability to the local setting is uncertain because despite availability of viral vector COVID vaccines, the DOH has not reported any cases of VITT in the Philippines to date. Information about accessibility and cost of anti-PF4 testing in the local setting is not readily available.
Feasibility	Uncertain (5)	No (3)	Yes (1)				

Appendix 2. Search Yield and Results

Database	Search Terms	Yield
PubMed and Google Scholar	"*(SARS-CoV 2 vaccine OR COVID-19 vaccine OR COVID vaccine) AND (vaccine induced immune thrombotic thrombocytopenic OR Vaccine-induced immune thrombosis with thrombocytopenia OR vaccine associated immune thrombotic thrombocytopenic OR VITT OR VATT) AND (PF4 OR ELISA OR anti-PF4 assay OR heparin induced thrombocytopenia assay)*	55 studies

Appendix 3. Characteristics of Included Studies

Title/ Author	Study design	Country	Number of patients	Population	Index Test / Exposure	Reference Standard / Control	Outcomes
Platton 2021	Cross-sectional, observational study	UK	50 samples from 43 patients	Patients with suspected VITT (based on clinical presentation, platelet count, D-dimer level)	IgG specific anti-PF4 ELISA (Asserachrom, Lifecodes, Hyphen Biomed, Zymutest, AESKULISA) Polyspecific anti-PF4 ELISA (IgG, IgA, IgM) (Asserachrom, Lifecodes) Rapid anti-PF4 non-ELISA assays (Diamed, STic Expert, HemosIL Acustar HIT-IgG, HemosIL HIT-Ab)	Clinical diagnosis of VITT (VITT unlikely, VITT possible, VITT probable)	Optical density; Anti-PF4 assay result (positive or negative); Sensitivity; Specificity

Appendix 4. Detailed Study Appraisal

Table 1. QUADAS-2 summarized results for Platton 2021

Study	Risk of Bias				Applicability Concerns		
	Patient Selection	Index Test	Reference Standard	Flow and Timing	Patient Selection	Index Test	Reference Standard
Platton 2021	?	?	😊	?	?	😊	😊

😊 = low risk, ☹ = high risk, ? = unclear risk

Table 2. QUADAS-2 signaling questions for Platton 2021

PATIENT SELECTION	
A. Risk of Bias	
Patient sampling	50 samples from 43 patients with suspected VITT that were referred to the UK Expert Haematology Panel multi-disciplinary team. All samples were analyzed before therapeutic treatment of VITT.
Was a consecutive or random sample of patients enrolled?	Unclear
Was a case-control design avoided?	Yes
Did the study avoid inappropriate exclusions?	Unclear
Could the selection of patients have introduced bias?	Unclear risk
B. Concerns Regarding Applicability	
Patient characteristics and setting	Patient baseline characteristics were not reported. Study was conducted in the UK.
Are there concerns that the included patients and setting do not match the review question?	Unclear concern
INDEX TEST	
Index tests	Anti-platelet 4 (anti-PF4) serologic assays measure the presence of anti-platelet 4-heparin antibodies. They can be ELISA-based or non-ELISA based, such as particle gel, lateral flow, chemoluminescence, and latex-agglutination. Anti-PF4 assays are traditionally used for screening for HIT. The results are positive or negative, based on optical density (OD) predetermined cutoffs set by laboratories. Both ELISA-based and non-ELISA methods, as well as different brands among these, were assessed.
A. Risk of Bias	
Were the index test results interpreted without knowledge of the results of the reference standard?	Unclear
If a threshold was used, was it pre-specified?	Yes

Could the conduct or interpretation of the index test have introduced bias?	Unclear risk
B. Concerns Regarding Applicability	
Are there concerns that the index test, its conduct, or interpretation differ from the review question?	Low concern
REFERENCE STANDARD	
A. Risk of Bias	
Target condition and reference standard	VITT is described as signs and symptoms of thrombosis and thrombocytopenia following immunization with COVID-19 vaccines. Aside from clinical signs of thrombosis at unusual sites and thrombocytopenia, it is also associated with elevated D-dimer and anti-PF4 antibodies. It is similar in presentation to heparin-induced thrombocytopenia (HIT) but unlike HIT, patients with VITT have no known exposure to heparin and instead occurs following inoculation with COVID-19 vaccines.For the study, patients were assessed as suspected VITT and then divided into probable VITT, possible VITT, and unlikely VITT.
Is the reference standard likely to correctly classify the target condition?	Yes
Were the reference standard results interpreted without knowledge of the index tests?	Yes
Could the reference standard, its conduct, or its interpretation have introduced bias?	Low risk
B. Concerns Regarding Applicability	
Are there concerns that the target condition as defined by the reference standard does not match the question?	Low concern
FLOW AND TIMING	
A. Risk of Bias	
Flow and timing	All patients in whom the anti-PF4 assays were tested were assessed for suspected VITT. The time interval between clinical assessment of VITT and testing with the index test was not explicitly reported.
Was there an appropriate interval between index test and reference standard?	Unclear
Did all patients receive the same reference standard?	Yes
Were all patients included in the analysis?	Yes
Could the patient flow have introduced bias?	Unclear risk

Appendix 5. GRADE Evidence Profile

Outcomes	No of studies (patient)	Study design	Factors that may decrease certainty of evidence					Effect per 1,000 patients tested	Test Accuracy CoE
			Risk of bias	Indirectness	Inconsistency	Imprecision	Publication Bias	Pre-test probability of 0.00142%	
True positives (patients with VITT)	1 study (43 patients)	Cross-sectional (cohort type accuracy study)	seriousa	not serious	not serious	seriousb	none	0 to 0	⊕⊕◯◯ Low
False negatives (patients incorrectly classified as not having VITT)								0 to 0	
True negatives (patients without VITT)	1 study (43 patients)	Cross-sectional (cohort type accuracy study)	seriousa	not serious	not serious	seriousb	none	560 to 1,000	⊕⊕◯◯ Low
False positives (patients incorrectly classified as having VITT)								0 to 440	

*Pretest probability is based on incidence estimates by NICE of 14.2 cases of VITT per million doses [9].

Appendix 6. Forest Plots

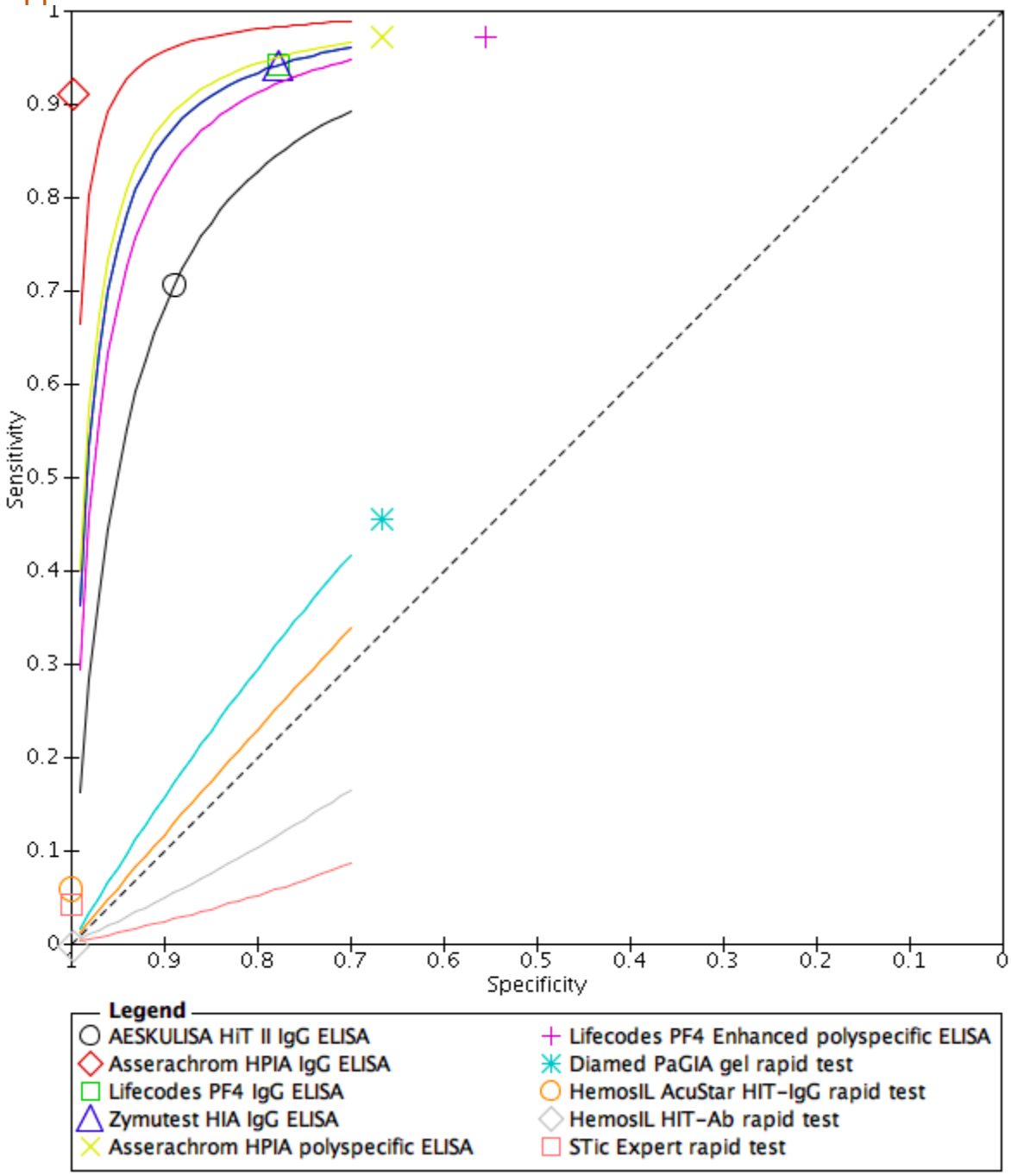

Figure 1. Summary receiver operating characteristic curves (SROC) for different HIT test kits.

AESKULISA HiT II IgG ELISA

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)
Platton 2021	24	1	10	8	0.71 [0.53, 0.85]	0.89 [0.52, 1.00]		

Asserachrom HPIA IgG ELISA

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)
Platton 2021	31	0	3	9	0.91 [0.76, 0.98]	1.00 [0.66, 1.00]		

Lifecodes PF4 IgG ELISA

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)
Platton 2021	32	2	2	7	0.94 [0.80, 0.99]	0.78 [0.40, 0.97]		

Zymutest HIA IgG ELISA

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)
Platton 2021	32	2	2	7	0.94 [0.80, 0.99]	0.78 [0.40, 0.97]		

Figure 2. ELISA test kits, IgG-specific

Asserachrom HPIA polyspecific ELISA

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)
Platton 2021	33	3	1	6	0.97 [0.85, 1.00]	0.67 [0.30, 0.93]		

Lifecodes PF4 Enhanced polyspecific ELISA

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)
Platton 2021	33	4	1	5	0.97 [0.85, 1.00]	0.56 [0.21, 0.86]		

Figure 3. ELISA test kits, polyspecific

Diamed PaGIA gel rapid test

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)
Platton 2021	15	3	18	6	0.45 [0.28, 0.64]	0.67 [0.30, 0.93]		

HemosIL AcuStar HIT-IgG rapid test

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)
Platton 2021	2	0	32	9	0.06 [0.01, 0.20]	1.00 [0.66, 1.00]		

HemosIL HIT-Ab rapid test

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)
Platton 2021	0	0	18	8	0.00 [0.00, 0.19]	1.00 [0.63, 1.00]		

STic Expert rapid test

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)
Platton 2021	1	0	23	2	0.04 [0.00, 0.21]	1.00 [0.16, 1.00]		

Figure 4. Non-ELISA test kits