Received: 30 April 2021 | Accepted: 3 May 2021

DOI: 10.1111/jth.15362

BRIEF REPORT

Evaluation of laboratory assays for anti-platelet factor 4 antibodies after ChAdOx1 nCOV-19 vaccination

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Abstract

Introduction: Vaccine-induced immune thrombocytopenia and thrombosis (VITT) following ChAdOx1 nCOV-19 vaccine has been described, associated with unusual site thrombosis, thrombocytopenia, raised D-dimer, and high-titer immunoglobulin-G (IgG) class anti-platelet factor 4 (PF4) antibodies. Enzyme-linked immunosorbent assays (ELISA) have been shown to detect anti-PF4 in patients with VITT, but chemiluminescence assays do not reliably detect them. ELISA assays are not widely available in diagnostic laboratories, and, globally, very few laboratories perform platelet activation assays.

Methods: Assays that are commercially available in the United Kingdom were evaluated for their ability to identify anti-PF4 antibodies in samples from patients with suspected VITT. Four IgG-specific ELISAs, two polyspecific ELISAs, and four rapid assays were performed on samples from 43 patients with suspected VITT from across the United Kingdom. Cases were identified after referral to the UK Expert Haematology Panel multidisciplinary team and categorized into unlikely, possible, or probable VITT.

Results and Discussion: We demonstrated that the HemosIL AcuStar HIT-IgG, HemosIL HIT-Ab, Diamed PaGIA gel, and STic Expert assays have poor sensitivity for VITT in comparison to ELISA. Where these assays are used for heparin-induced thrombocytopenia (HIT) diagnosis, laboratories should ensure that requests for suspected VITT are clearly identified so that an ELISA is performed. No superiority of IgG-ELISAs over polyspecific ELISAs in sensitivity to VITT could be demonstrated. No single ELISA method detected all possible/probable VITT cases; if a single ELISA test is negative, a second ELISA or a platelet activation assay should be considered where there is strong clinical suspicion.

KEYWORDS
enzyme-linked immunosorbent assay, immunoassay, platelet activation, thrombocytopenia, thrombosis

Manuscript Handled by: David Lillicrap

Final decision: David Lillicrap, 03 May 2021

1 | INTRODUCTION

Vaccine-induced immune thrombocytopenia and thrombosis (VITT) following administration of the ChAdOx1 nCOV-19 vaccine has recently been described,[1–3] associated with thrombosis at unusual sites, thrombocytopenia, raised D-dimer, and high titers of immunoglobulin G (IgG) class anti-platelet factor 4 (PF4) antibodies.

Authors have described using the Zymutest HIA IgG enzyme-linked immunosorbent assay (ELISA),[4] the Lifecodes PF4 IgG ELISA,[2,3] and the Asserachrom HPIA IgG ELISA[3] to successfully detect anti-PF4 in patients with VITT, but have also reported that the HemosIL AcuStar HIT-$IgG_{(PF4\text{-}H)}$ chemiluminescence method does not reliably detect them.[3] At the time of writing, there is a single case report of VITT with a negative anti-PF4 assay using an unidentified lateral flow device,[5] and another where the results of anti-PF4 assays have not been reported.[6]

Essentials

- The performance of immunoassays for anti-PF4 antibodies in vaccine-induced immune thrombocytopenia and thrombosis (VITT) was assessed.
- Patients with possible and probable VITT were tested using eleven commercially available immunoassays.
- Immunoglobulin-G-specific and polyspecific enzyme-linked immunosorbent assays (ELISAs) showed acceptable sensitivity to anti-PF4 antibodies in VITT.
- Rapid methods showed poor sensitivity for anti-PF4 antibodies in VITT.
- No single ELISA method detected all cases of VITT.

TABLE 1 Results of anti-PF4 assays

	AESKULISA HiT II			Asserachrom HPIA IgG		Lifecodes PF4 IgG		Zymutest HIA IgG	
		OD	U/ml		OD		OD		OD
Study no.	Positive or Negative	Cutoff 1.88	Cutoff 18.0	Positive or Negative	Cutoff 0.21	Positive or Negative	Cutoff 0.40	Positive or Negative	Cutoff 0.47
Probable cases									
VITT01	Positive	2.25	22.5	Positive	0.37	Positive	1.18	Positive	0.87
VITT02	Positive	3.96	>300.0	Positive	2.37	Positive	1.90	Positive	3.36
VITT04	Positive	3.95	>300.0	Positive	2.70	Positive	1.95	Positive	3.37
VITT05	Positive	3.42	>300.0	Positive	2.29	Positive	1.85	Positive	3.03
VITT07	Positive	3.23	229.3	Positive	2.22	Positive	1.96	Positive	3.30
VITT08	Positive	3.01	137.6	Positive	1.91	Positive	1.42	Positive	2.74
VITT10	Negative	1.43	3.3	Negative	0.18	Positive	1.33	Negative	0.42
VITT12	Negative	1.79	7.7	Positive	0.38	Positive	1.47	Positive	1.47
VITT13	Positive	2.99	131.5	Positive	0.31	Positive	1.70	Positive	2.57
VITT14	Negative	0.67	0.5	Positive	1.06	Positive	1.15	Positive	1.43
VITT15	Positive	3.75	>300.0	Positive	2.74	Positive	1.87	Positive	3.51
VITT17	Positive	3.27	253.2	Positive	1.56	Positive	1.46	Positive	3.08
VITT18	Positive	3.19	210.1	Negative	0.19	Positive	1.55	Positive	2.48
VITT19	Positive	3.17	199.9	Positive	0.79	Positive	1.72	Positive	2.59
VITT23	Negative	1.42	3.2	Positive	2.23	Negative	0.27	Positive	2.43
VITT24	Positive	3.55	>300.0	Positive	1.70	Positive	1.76	Positive	2.98
VITT25	Negative	0.84	0.8	Negative	0.10	Negative	0.10	Negative	0.11
VITT26	Positive	2.92	110.9	Positive	0.35	Positive	1.26	Positive	1.73
VITT27	Negative	1.70	6.2	Positive	2.69	Positive	1.93	Positive	3.11
VITT28	Positive	2.88	101.6	Positive	0.46	Positive	1.28	Positive	2.39
VITT29	Positive	2.60	52.1	Positive	0.36	Positive	1.47	Positive	1.18
VITT31	Positive	3.88	>300.0	Positive	1.83	Positive	1.24	Positive	3.11
VITT33	Negative	1.85	8.8	Positive	1.03	Positive	1.08	Positive	1.14

Therefore, we have evaluated assays currently available commercially in the United Kingdom for their ability to identify anti-PF4 antibodies in samples from patients with suspected or confirmed VITT.

2 | METHODS

Fifty samples from 43 patients with suspected VITT from across the United Kingdom were received for analysis. Sample analysis took place within a central laboratory group for consistency. Cases had been identified after referral to the UK Expert Haematology Panel multidisciplinary team, established on March 22, 2021, to review and consider all cases of suspected VITT on the grounds of clinical presentation, radiological evidence of thrombosis, and local laboratory results for platelet count, coagulation parameters, and anti-PF4 testing. Case definition is: presentation between 5 and 28 days post-ChAdOx1 nCOV-19 vaccine; thrombosis and thrombocytopenia (platelets <150 × 10^9/L), or isolated thrombocytopenia; evidence of extreme activation of the coagulation system (D-dimers >4000 μg/L, or >2000 μg/L with a strong clinical index of suspicion). These cases are categorized into those with unlikely, possible, or probable VITT. All samples analyzed in this study were collected before treatment for VITT.

The available assays for anti-PF4 testing that were assessed in this study can be split into three groups: IgG-specific ELISAs; poly-specific (IgG, IgA, and IgM) ELISAs; and rapid assays.

The IgG-specific ELISAs were performed on all 43 samples. These assays were Asserachrom HPIA IgG (Stago UK Ltd, Theale, UK), Lifecodes PF4 IgG (Immucor, Solihull, UK), Hyphen Biomed Zymutest HIA IgG (Quadratech Diagnostics, UK), and AESKULISA HiT II (AEKSU UK, London, UK).

Asserachrom HPIA		Lifecodes PF4 Enhanced		HemosIL AcuStar HIT-IgG$_{(PF4-H)}$		HemosIL HIT-Ab$_{(PF4-H)}$		Diamed gel	STic Expert
	OD		OD		U/ml		U/ml		
Positive or Negative	Cutoff 0.54	Positive or Negative	Cutoff 0.40	Positive or Negative	Cutoff 1.00	Positive or Negative	Cutoff 1.0	Positive or Negative	Positive or Negative
Positive	1.14	Positive	1.89	Negative	0.04	Negative	0.3	Negative	Negative
Positive	2.92	Positive	1.91	Negative	0.12	Negative	0.3	Negative	Negative
Positive	3.11	Positive	1.91	Negative	0.19	Negative	0.4	Negative	Negative
Positive	2.49	Positive	1.91	Negative	0.85	Negative	0.2	1+	Negative
Positive	2.62	Positive	1.90	Positive	1.72	Negative	0.0	2+	Negative
Positive	2.20	Positive	1.90	Negative	0.14	Negative	0.0	1+	Negative
Negative	0.27	Positive	1.89	Negative	0.07	Negative	0.4	Negative	Negative
Positive	0.63	Positive	1.88	Negative	0.06			Negative	Negative
Positive	0.93	Positive	1.91	Negative	0.18			Negative	Negative
Positive	1.25	Positive	1.86	Negative	0.54			1+	Negative
Positive	2.77	Positive	1.90	Negative	0.18	Negative	0.3	3+	Positive
Positive	2.51	Positive	1.91	Negative	0.13			Negative	Negative
Positive	0.57	Positive	1.89	Negative	0.05			1+	
Positive	1.87	Positive	1.90	Negative	0.07			Negative	Negative
Positive	1.69	Positive	0.98	Negative	0.80			2+	
Positive	2.53	Positive	1.91	Negative	0.24			Negative	Negative
Negative	0.21	Negative	0.37	Negative	0.12			Negative	
Positive	0.94	Positive	1.86	Negative	0.10			1+	Negative
Positive	3.28	Positive	1.91	Negative	0.27			Negative	
Positive	1.69	Positive	1.90	Negative	0.17			Negative	Negative
Positive	1.29	Positive	1.90	Positive	1.04			Negative	Negative
Positive	3.12	Positive	1.91	Negative	0.13	Negative	0.1	Negative	Negative
Positive	1.34	Positive	1.89	Negative	0.51	Negative	0.3	3+	

(Continues)

TABLE 1 (Continued)

Study no.	AESKULISA HiT II			Asserachrom HPIA IgG		Lifecodes PF4 IgG		Zymutest HIA IgG	
		OD	U/ml		OD		OD		OD
	Positive or Negative	Cutoff 1.88	Cutoff 18.0	Positive or Negative	Cutoff 0.21	Positive or Negative	Cutoff 0.40	Positive or Negative	Cutoff 0.47
VITT36	Negative	1.40	3.1	Positive	0.89	Positive	0.76	Positive	1.58
VITT38	Positive	3.12	179.7	Positive	1.43	Positive	1.29	Positive	2.22
VITT40	Positive	2.52	42.7	Positive	1.32	Positive	1.56	Positive	1.87
VITT44	Positive	3.89	>300.0	Positive	2.63	Positive	1.80	Positive	3.19
Possible cases									
VITT06	Negative	1.83	8.4	Positive	0.57	Positive	1.79	Positive	1.46
VITT09	Positive	3.96	>300.0	Positive	2.62	Positive	1.81	Positive	3.43
VITT11	Positive	3.77	>300.0	Positive	1.60	Positive	1.75	Positive	3.09
VITT30	Positive	3.20	214.6	Positive	1.49	Positive	1.58	Positive	2.62
VITT37	Positive	3.30	271.8	Positive	0.68	Positive	1.45	Positive	2.33
VITT39	Negative	1.41	3.1	Positive	2.35	Positive	1.90	Positive	2.94
VITT45	Positive	3.83	>300.0	Positive	2.09	Positive	1.76	Positive	3.03
Unlikely cases									
VITT03	Negative	1.84	8.6	Negative	0.03	Negative	0.05	Negative	0.05
VITT20	Negative	0.91	1.0	Negative	0.12	Negative	0.08	Negative	0.14
VITT21	Negative	0.43	0.3	Negative	0.08	Negative	0.07	Positive	1.74
VITT22	Negative	0.42	0.3	Negative	0.19	Negative	0.07	Negative	0.07
VITT32	Negative	0.43	0.3	Negative	0.10	Negative	0.07	Negative	0.08
VITT35	Negative	0.28	0.2	Negative	0.14	Negative	0.09	Negative	0.19
VITT41	Positive	2.63	56.0	Negative	0.18	Positive	1.49	Positive	0.82
VITT42	Negative	0.49	0.4	Negative	0.08	Positive	0.50	Negative	0.16
VITT43	Negative	0.57	0.4	Negative	0.06	Negative	0.11	Negative	0.13

Abbreviations: Ig, immunoglobulin; OD, optical density; PF4, platelet factor 4; VITT, vaccine-induced immune thrombocytopenia and thrombosis.

The polyspecific ELISAs were also performed on all 43 samples. These assays were Asserachrom HPIA (Stago UK Ltd, Theale, UK) and Lifecodes PF4 Enhanced (Immucor, Solihull, UK).

The rapid assays performed were polyspecific Diamed PaGIA gel (BioRad Laboratories Ltd, Watford, UK), IgG-specific STic Expert lateral flow device (Stago UK Ltd, Theale, UK), IgG-specific HemosIL AcuStar HIT-$IgG_{(PF4\text{-}H)}$ (Werfen Ltd, Warrington, UK), and IgG-specific HemosIL HIT-$Ab_{(PF4\text{-}H)}$ (Werfen Ltd). The HemosIL AcuStar HIT-IgG assay was performed on all 43 samples; the Diamed PaGIA gel was performed on 42 samples, one being unsuitable because of limited sample volume; and the HemosIL HIT-Ab and STic Expert assays were performed on 26 samples in the order they were received because of limited reagent availability.

All assays were performed according to the manufacturer's instructions for use. Results were interpreted as positive or negative for anti-PF4 antibodies using the manufacturer's cutoffs that have been derived for the diagnosis of HIT. For the Lifecodes assays, the cutoff was defined by the manufacturer as optical density (OD) 0.40; for the HemosIL assays, the cutoff was defined by the manufacturer as 1.0 U/ml. For the remaining ELISAs, a kit-specific cutoff in relation to a kit reference plasma was used (Table 1).

GraphPad Prism 9.1 (GraphPad Software, CA, USA) was used for statistical analysis of assay sensitivity and specificity.

3 | RESULTS AND DISCUSSION

Table 1 shows the results for all assays; the results for patients who were categorized as possible or probable VITT are shown in Figure 1.

Of the 43 samples tested, 23 had OD for all six ELISAs that were above the assay-specific cutoff (positive); all of these 23 samples were from patients with possible or probable VITT.

Eight samples were positive by five of the six ELISAs. Seven had OD below the assay-specific cutoff (negative) by AEKSULISA HiT II from six patients with probable VITT and one with possible VITT. One was negative by Asserachrom HPIA IgG from a patient with probable VITT.

Using HemosIL AcuStar HIT-IgG for the 31 samples that were positive by five or six ELISAs, two had a positive result (of 1.04 U/ml

Asserachrom HPIA		Lifecodes PF4 Enhanced		HemosIL AcuStar HIT-IgG$_{(PF4-H)}$		HemosIL HIT-Ab$_{(PF4-H)}$		Diamed gel	STic Expert
	OD		OD		U/ml		U/ml		
Positive or Negative	Cutoff 0.54	Positive or Negative	Cutoff 0.40	Positive or Negative	Cutoff 1.00	Positive or Negative	Cutoff 1.0	Positive or Negative	Positive or Negative
Positive	1.51	Positive	1.82	Negative	0.25				Negative
Positive	2.78	Positive	1.88	Negative	0.33	Negative	0.1	Negative	
Positive	2.20	Positive	1.90	Negative	0.77	Negative	0.0	Negative	
Positive	2.88	Positive	1.91	Negative	0.20	Negative	0.0	1+	Negative
Positive	0.63	Positive	1.90	Negative	0.47	Negative	0.0	1+	Negative
Positive	3.24	Positive	1.91	Negative	0.95	Negative	0.4	1+	Negative
Positive	2.45	Positive	1.90	Negative	0.07			Negative	Negative
Positive	2.09	Positive	1.90	Negative	0.71			2+	Negative
Positive	1.42	Positive	1.90	Negative	0.19	Negative	0.0	Negative	
Positive	2.70	Positive	1.91	Negative	0.31	Negative	0.0	1+	
Positive	2.66	Positive	1.90	Negative	0.16	Negative	0.3	1+	
Negative	0.11	Negative	0.29	Negative	0.00	Negative	0.9	Negative	Negative
Negative	0.13	Negative	0.33	Negative	0.04			Negative	
Negative	0.15	Negative	0.26	Negative	0.10	Negative	0.0	Negative	
Negative	0.26	Negative	0.15	Negative	0.03	Negative	0.0	Negative	
Negative	0.20	Positive	0.42	Negative	0.18	Negative	0.0	Negative	Negative
Negative	0.16	Negative	0.33	Negative	0.06	Negative	0.0	2+	
Negative	0.42	Positive	1.90	Negative	0.06	Negative	0.0	Negative	
Negative	0.35	Positive	1.55	Negative	0.04	Negative	0.0	1+	
Negative	0.20	Positive	0.56	Negative	0.81	Negative	0.0	3+	

and 1.72 U/ml) and 29 had a negative result (<1.00 U/ml). Using Diamed PaGIA gel in 30 of these samples, 14 had a positive result and 16 had a negative result. Using STic Expert in 23 of these samples, one had a positive result, 20 had a negative result, and two had a test line that was less intense than the kit reference (negative). Using HemosIL HIT-Ab in 17 of these samples, all had a negative result (<1.0 U/ml).

Two samples were positive by four ELISA assays: one from a probable VITT patient was positive by Lifecodes PF4 Enhanced, Asserachrom HPIA IgG, Asserachrom HPIA, Zymutest HIA IgG, and Diamed PaGIA gel (2+), but HemosIL Acustar HIT-IgG was negative; one from an unlikely VITT patient was positive by Lifecodes PF4 IgG, Lifecode PF4 Enhanced, Zymutest HIA IgG, and AUSKULISA HiT II, but Diamed PaGIA gel, HemosIL AcuStar HIT-IgG, and HemosIL HIT-Ab were negative.

Two samples were positive by two ELISA assays (Lifecodes PF4 IgG and Lifecodes PF4 Enhanced). One was from a probable VITT patient with Diamed PaGIA gel negative and the other from an unlikely VITT patient with Diamed PaGIA gel positive (1+); HemosIL AcuStar HIT-IgG, HemosIL HIT-Ab, and STic Expert were negative for both samples.

Four samples had positive results by one ELISA assay. Three samples had positive results with the Lifecodes PF4 Enhanced assay: one sample from an unlikely VITT patient was negative by all rapid assays; one sample from an unlikely VITT patient was positive (3+) by Diamed PaGIA gel and negative by all other rapid assays; one sample from a probable VITT patient was negative by all rapid assays. The fourth sample was from an unlikely VITT patient and was positive with the Zymutest HIA IgG assay and negative by all other rapid assays (STic Expert not tested).

Four samples had results for all six ELISAs that were negative, all from patients in whom VITT was unlikely; one was positive using the Diamed PaGIA gel (2+) and all the other rapid assays were negative.

Comparing test results with the clinical phenotype as evaluated by the clinical expert group enabled calculation of assay sensitivity and specificity for VITT. These data are presented in Table 2.

We have demonstrated that, although the HemosIL AcuStar HIT-IgG, HemosIL HIT-Ab, Diamed PaGIA gel, and STic Expert

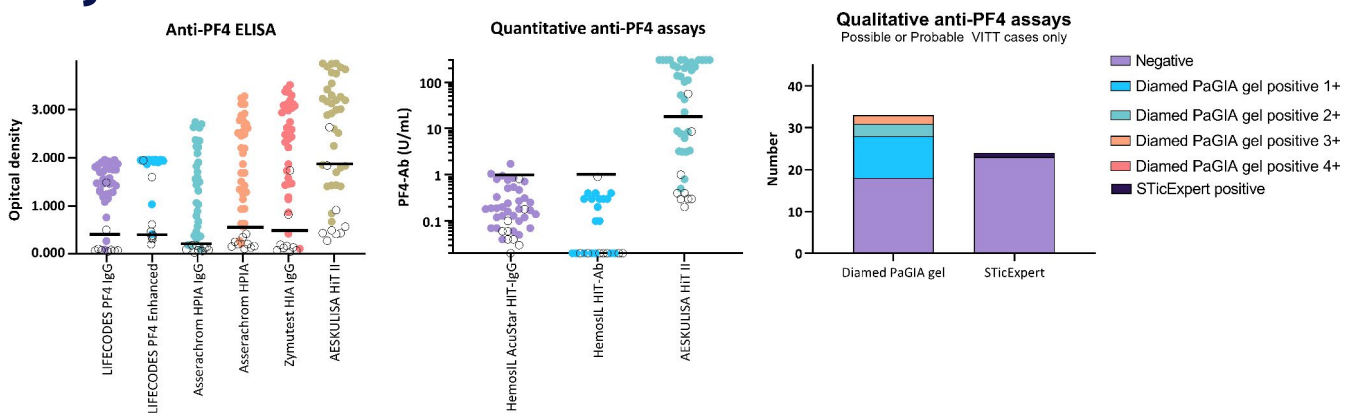

FIGURE 1 Results for anti-PF4 assays for samples from patients with suspected vaccine-induced immune thrombocytopenia and thrombosis (VITT). Solid circles, VITT possible or probable; empty circles, VITT unlikely; solid black line, assay-specific cutoff for assay. ELISA, enzyme-linked immunosorbent assay; PF4, platelet factor 4

TABLE 2 Sensitivity and specificity of assays for possible and probable VITT and for HIT

Assay	Sensitivity for VITT % (95% CI)	Specificity for VITT % (95% CI)	Sensitivity for HIT % (95% CI)	Specificity for HIT % (95% CI)
IgG-specific ELISAs				
AEKSULISA HiT II	70.6 (53.8–83.2)	88.9 (56.5–99.4)	91[a]	97[a]
Asserachrom HPIA IgG	91.1 (77.0–97.0)	100.0 (70.1–100.0)	72.0 (68.4–75.5)[7]	93.8 (90.3–97.4)[7]
Lifecodes PF4 IgG	94.1 (80.9–99.0)	77.8 (45.3–96.1)	99.6 (22.7–100.0)[7]	89.9 (86.2–92.6)[7]
Zymutest HIA IgG	94.1 (80.9–99.0)	77.8 (45.3–96.1)	99.2 (86.4–100.0)[7]	85.8 (77.1–91.5)[7]
Polyspecific ELISAs				
Asserachrom HPIA	94.1 (80.9–99.0)	100.0 (70.1–100.0)	92.7 (73.6–98.3)[7]	87.3 (79.9–92.3)[7]
Lifecodes PF4 Enhanced	100.0 (89.9–100.0)	55.6 (26.7–81.1)	99.9 (90.9–100.0)[7]	87.4 (79.2–92.7)[7]
Rapid tests				
Diamed PaGIA gel	45.5 (29.8–62.0)	66.7 (35.4–87.9)	96.5 (89.8–98.9)[7]	93.7 (83.1–97.8)[7]
HemosIL AcuStar HIT-$IgG_{(PF4-H)}$	5.9 (1.0–19.1)	100.0 (70.1–100.0)	98.8 (69.2–100.0)[7]	94.6 (90.7–96.9)[7]
HemosIL HIT-$Ab_{(PF4-H)}$	0.0 (0.0–17.6)	100.0 (67.6–100.0)	100.0[7]	84.3[7]
STic expert	4.2 (0.2–20.2)	100.0 (17.8–100.0)	98.4 (85.3–99.9)[7]	90.3 (84.4–94.1)[7]

Abbreviations: 95% CI, 95% confidence interval; ELISA, enzyme-linked immunosorbent assay; HIT, heparin-induced thrombocytopenia; IG, immunoglobulin; VITT, vaccine-induced immune thrombocytopenia and thrombosis.

aManufacturer's data.

assays have a high sensitivity for HIT,[7] they have poor sensitivity for VITT in comparison to ELISA (Table 2). We have also shown that, although IgG-specific ELISAs are considered better than polyspecific assays for the diagnosis of HIT,[8] there is little difference in the assays for the detection of VITT. However, our study looked at only a small number of samples and had a strong bias toward patients with possible or probable VITT, making it difficult to recommend whether an IgG-specific ELISA or polyspecific ELISA is of more clinical use.

It is unclear why certain assays are insensitive to VITT and whether the concentrations and compositions of the PF4 complexes account for the differences. The Diamed PaGIA gel uses PF4 bound to heparin, similar to the assay principle used in the sensitive Zymutest HIA IgG, Asserachrom HPIA IgG, and Asserachrom HPIA assays. The two insensitive HemosIL assays use PF4 bound to polyvinyl sulphate, similar to the assay principle used in the two sensitive Lifecodes assays. The STic Expert assay uses PF4 bound to an unspecified polyanion, and the AEKSULISA assay does not specify the composition of the kit.

There were two specific problems observed during this study. First, many positive results for Diamed PaGIA gel were only weakly positive (1+ or 2+) (Table 1). In local experience, such reactions are rarely positive by ELISA in HIT. Second, the OD for samples tested by both Lifecodes assays were never higher than 1.90, suggesting that the antibody in the assay was exhausted in the reaction, and that higher dilutions of patient sample are required for accurate OD readings using these two assays.

The next stage would be to determine whether the anti-PF4 antibodies detected cause platelet activation. Further studies should also investigate the presence or absence of anti-PF4 antibodies in different patient

groups that may include healthy nonvaccinated patients, healthy patients postvaccination with ChAdOx1, and thrombocytopenic patients without raised D-dimers or thrombosis postvaccination with ChAdOx1.

We conclude that none of the rapid assays tested, which may be suitable for the exclusion of HIT, is suitable for the exclusion of VITT. Centers where such rapid assays are in use for the diagnosis of HIT should ensure that requests for diagnosis of VITT should be clearly distinguished from those for diagnosis of HIT so that the correct tests are performed.

Clinicians should be aware that ELISA assays are not widely available in diagnostic laboratories, and a very small number of laboratories globally are able to perform platelet activation assays. Our study showed no single ELISA method appears to detect all cases of VITT, and therefore if a single ELISA test is negative, a second ELISA or platelet activation assay should be considered where there is strong clinical suspicion.

AUTHOR CONTRIBUTIONS

Mr. Platton and Dr. Pavord, Prof. Makris, and Prof. Scully devised the study; Mr. Platton, Mr. Bartlett, and Mr. Singh performed the sample analysis. Mr. Platton wrote the first draft of the manuscript; all authors contributed to the review and revision of the manuscript.

CONFLICT OF INTEREST

All authors declare no relevant conflicts of interest.

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How to cite this article: Platton S, Bartlett A, MacCallum P, et al. Evaluation of laboratory assays for anti-platelet factor 4 antibodies after ChAdOx1 nCOV-19 vaccination. *J Thromb Haemost*. 2021;19:2007–2013. https://doi.org/10.1111/jth.15362