Functional Heparin-Induced Thrombocytopenia Pilot Study

In Autumn 2024 we performed a pilot study on testing for functional Heparin-Induced-Thrombocytopenia (HIT). The purpose of this pilot study was to investigate the feasibility of external quality assessment surveys for functional HIT. Forty-four laboratories participated in this pilot; 33 laboratories actually reported results. Here is a summary of the results obtained in this pilot study.

Background

Since 2009 ECAT has been organising regular external quality assessment surveys on the subject of immunological HIT testing. Several attempts to set up surveys concerning functional HIT testing were unsuccessful.
Recently the development of a monoclonal antibody which demonstrates functional HIT properties has been reported. This K070 monoclonal antibody, a human-mouse recombinant chemeric monoclonal antibody, IgG1 isotype, is targeted at heparin and PF4 complexes [1].

Set-up

Two lyophilised citrated plasma samples were distributed. One sample was a negative control sample. The second sample was citrated plasma spiked with 200 µg/mL monoclonal antibody resulting in a positive functional HIT sample. The participants had measured the samples with their regular functional HIT assay. Both results and interpretation were reported.

Results and Discussion

Table 1 shows the methods used by the participants.

Table1: The methods for functional HIT testing used by the participants.

Type of Method	Frequency
Aggregation / ATP-release	1
Aggregometry	1
Flow cytometry / FACS	4
Heparin-induced platelet activation Assay (HIPA)	13
HIMEA	3
Serotonin Release Assay (SRA)	5

Four participants reported results for a test which was not a functional HIT assay: Chemiluminescent Immunoassay (n=2) and Immunoturbidimetric assay (n=2). These results were not included in the evaluation.

The participants were asked to classify the results obtained into HIT-positive, equivocal or negative. Table 2 shows an overview of the classification given divided per method used, for both samples.

Table 2: The classification given by the participants.

	Negative Control				Positive Control			
Type of Method	Negative	Equivocal	Positive	None	Negative	Equivocal	Positive	None
Aggregation / ATP-release	1	0	0	0	0	1	0	0
Aggregometry	1	0	0	0	0	0	1	0
Flow cytometry / FACS	4	0	0	0	0	0	4	0
HIPA	12	0	1	0	0	0	13	0
HIMEA	2	0	0	1	0	0	2	1
SRA	3	0	2	0	0	0	5	0
Total	**23**	**0**	**3**	**1**	**0**	**1**	**25**	**1**
Perc. Correct classification	**88%**				**96%**			

A minority of the participants classified the negative control sample as positive. It seems that the Serotonin-Release Assay is more susceptible to producing false positive results with this negative citrated plasma control sample. Almost all participants found a positive result for the positive control sample.

Eight participants only reported qualitative results, while 18 participants reported quantitative results.

A heterogeneous pattern in the way quantitative results were reported was observed. A summary of the results is given in table 3.

Table 3:

		Negative Control		Positive Control	
Unit	**N**	**Median**	**Range**	**Median**	**Range**
% activation	2	3.2	1.4 – 5.0	70.9	0.7 – 80.7
% max aggregation	5	6.0	2.0 – 8.0	50.0	36.0 – 83.0
% release	5	0.0	0.0 – 98.0	92.8	83.0 – 100
AUC	3	47.0	0.0 – 50.0	230.0	190 - 1036
minutes	1	15.0	-	3.0	-
U	1	0.0	-	117.0	-

Some participants also reported results of testing with several concentrations of heparin to support their final reported results.

Conclusion

This pilot study on functional HIT testing has been successful. The vast majority of participants correctly identified the negative and positive samples. Because of the heterogeneity in how results were reported it remains difficult to perform extended statistical analyses, including the performance assessment. We hope this will become possible when more laboratories participate in this survey. In 2025 ECAT will start with regular surveys for functional HIT testing.

References:

1. Amiral, J., N. Bouveyron, E. Legros and K. Kobbayashi, Standardization of HIT Diagnostic Assays, with K070, a chemeric human-mouse antibody, mimicking heparin dependent pathogenic antibodies. Medical Reserach Archives, 2023; [Online], November 30, 2023: https://doi.org/10.18103/mra/v11i11.4760.