

Evaluation of a Rapid Particle Gel Immuno Assay (PaGIA) for Detection of Heparin/PF4 Antibodies (HPA)

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PURPOSE OF THE STUDY

- An estimated 2-4% of patients receiving heparin will develop immune mediated thrombocytopenia
- Of these, 25% will go on to develop thrombotic complications.
- Diagnosis of Heparin Induced Thrombocytopenia (HIT) involves both clinical and appropriate laboratory assessment.
- The purpose of this study** was to compare the performance of a rapid test for detecting HPA to a standard ELISA screening test.

BACKGROUND

- The diagnosis of HIT involves the demonstration of HPA in plasma or serum.
- HPA activate platelets and endothelial cells leading to thrombosis.
- HPA can be detected by functional assays (serotonin release, heparin induced platelet aggregation, flow cytometry) or immunologic tests (ELISA).
- Functional assays are complex and labor- as well as time-intensive.



GENERAL METHODS

HIT ASSAYS

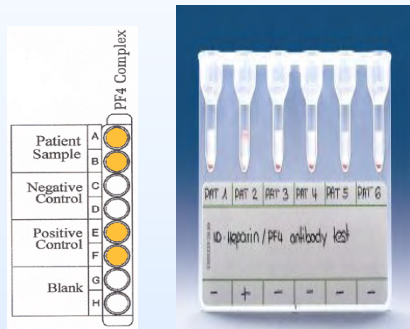
- A rapid test for detecting HPA, **PaGIA (Diamed)**, was evaluated. PaGIA uses particles coated with heparin/PF4. Particle agglutination occurs in the presence of HPA, and is noted visually.
- Results were compared to a standard ELISA (GTI).
- The GTI ELISA detects antibodies to PF4-polyvinyl sulfate constructs. A positive reaction is defined by an OD (405 nm) >0.400 and a reduction in reactivity (>50%) by excess heparin.

STUDY

- Tests were performed by both methods on serum samples (n=219) prepared in the clinical laboratory within 3 hours of sample receipt.
- Results were compared without information regarding clinical history and outcome.
- If sufficient serum was available, samples yielding discordant results were evaluated for HPA isotyping (IgM, IgG, IgA) by a second ELISA (Aniara/Hyphen Biomed), and a serotonin release assay was performed courtesy of Dr. John Francis, Florida Hospital Institute of Translational Research, Orlando, FL.

RESULTS

Representative Assay Endpoints:



GTI ELISA

PaGIA Diamed

Method Comparison

PaGIA Diamed	GTI - ELISA	
	POSITIVE	NEGATIVE
POSITIVE	17 (7.8%)	2 (1%)
NEGATIVE	19 (8.6%)	181 (82.6%)

- There was general agreement between HIT test results obtained with GTI ELISA and Diamed PaGIA kits.
- Samples (n=19) that were negative by PaGIA but positive by ELISA demonstrated ELISA reactivity ranging from OD 0.472-2.624.
- Samples (n=2) that were negative by ELISA and positive by PaGIA were hemolyzed. Hemolysis is a recognized source of interference.

Characterization of Available Samples with Discordant Assay Results (ELISA vs PaGIA)

I.D.	ELISA (O.D.) (Interp)	PaGIA Result	Heparin PF4 Antibody Isotype (O.D.)			Serotonin Release
			IgG	IgM	IgA	
178-3651	0.233 (neg)	Pos (hemolyzed)	0.105	0.050	0.060	Negative
241-2009	0.488 (pos)	Neg	0.076	0.004	0.048	Negative
168-2580	0.555 (pos)	Neg	0.198	0.048	0.595	Negative
261-2289	0.588 (pos)	Neg	0.249	0.014	0.068	Negative
207-2101	0.595 (pos)	Neg	0.516	0.027	0.079	Negative
289-2192	0.646 (pos)	Neg	0.300	0.124	0.207	Negative
211-1856	0.669 (pos)	Neg	0.032	0.002	0.059	Negative
235-1118	0.691 (pos)	Neg	0.091	0.066	0.078	Negative
282-2305	0.763 (pos)	Neg	0.148	0.039	0.109	Negative
297-1647	0.767 (pos)	Neg	0.165	0.066	0.09	Negative
282-2252	1.159 (pos)	Neg	0.052	0.124	0.069	Negative
173-2482	2.624 (pos)	Neg	1.744	0.052	0.986	Negative

- PaGIA results agreed with results of serotonin release assay, except in the case of hemolysis.

CONCLUSIONS

- PaGIA is a rapid screening test with potentially better specificity for HPA than the GTI ELISA.
- Since IgG HPA are more thrombogenic than IgM or IgA HPA, combining information from screening and isotype testing may enhance overall HPA test sensitivity.