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New Automated Chemiluminescent Anti-heparin/PF4 Immunoassay: Analytical Performance in HIT Suspected Patients.

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Abstract

Heparin-induced thrombocytopenia (HIT) is an iatrogenic immune thrombotic disorder in which platelet activating antibodies are mainly induced by heparin-platelet factor 4 (H-PF4) complexes. The detection of these antibodies aids in HIT diagnosis. The aim was to evaluate the performance of a new panel of two fully automated chemiluminescent assays (HemosIL® AcuStar HIT-IgG and HemosIL AcuStar HIT-Ab) for detecting circulating IgG, IgA, and IgM (GAM) antibodies against H-PF4 complex on a random access analyzer (ACL AcuStar™, IL). The new assays exhibited a good reproducibility in 2 quality control materials (CV% 4.0 – 8.0%). Plasmas from 79 HIT suspected patients screened with the "4T's" probability score and the serotonin release assay (SRA) were tested with the new assays. The results were compared to results from three commercially available kits [Zymutest IgG & GAM ELISAs (Hyphen Biomed) and Asserachrom® HPIA (Stago Diagnostica)], where applicable. The positivity of HIT-Ab in high score patients (12/14) and the co-positivity of HIT-IgG with SRA (8/10) were equal to that of the ELISAs. In low scoring and negative SRA patients, HIT-Ab negativity was less than the ELISAs (26/35 and 33/35, respectively). We also found a range of high positive values (HIT-IgG = 1.4 – 194.9 U/ml; HIT-Ab = 2.9 – 328.6 U/ml) in patients with positive SRA outcome and one of the commercial kits (Asserachrom HPIA) where the OD was greater than 2x the cut-off value, indicating high sensitivity. Overall, this new panel of automated assays is more useful than, and demonstrates a comparable technical performance with the ELISA kits at this stage of development. These very promising data must be confirmed in a future prospective large study.

Introduction

Heparin-induced thrombocytopenia (HIT) is an iatrogenic immune thrombotic disorder in which platelet activating antibodies are mainly induced by heparin-platelet factor 4 (H-PF4) complexes. The detection of a subset of these antibodies (IgG, IgA, and IgM) aids in HIT diagnosis. The American College of Chest Physicians recommends investigating for a diagnosis of HIT if a patient with current or previous 2 week exposure to heparin exhibits a fall in platelet count by > 50%, and/or a thrombotic event occurs, between days 5 and 14 (inclusive) following initiation of heparin, even in the case of heparin therapy cessation during the time of thrombosis or thrombocytopenia.

SCOPE

The aim was to evaluate the performance of a new panel of two fully automated chemiluminescent assays, HemosIL® AcuStar HIT-IgG(PF4-H) and HemosIL® AcuStar HIT-Ab(PF4-H) (Figure 1). The assays detect circulating IgG antibody and total antibodies (IgG, IgA, and IgM) to PF4-heparin complex in serum or citrated plasma.

The assays are completed on the ACL AcuStarTM, a fully automated, random access, chemiluminescent detection system.

The Assay Sequence

All steps, including sample predilution, are performed automatically

1. The sample is prediluted, mixed with the microparticles (MP) and assay buffer into the reaction cuvette, and incubated for ~8 minutes at 37°C, during which the aHIT antibodies, if present in the sample, are captured.
2. After a washing step the MP are resuspended in the tracer (isoluminol-labelled monoclonal antibody anti human IgG or IgM) and incubated for 10 additional minutes at 37°C during which the mAb attaches to the human IgG, IgA or IgM if captured by the particles
3. After a second washing step the cuvette is sent to the luminometer of the instrument, oxidizer and catalyst are injected and the emitted light measured during three seconds

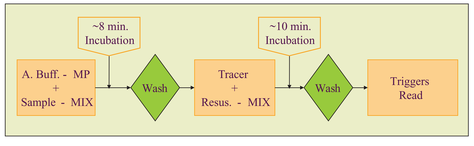
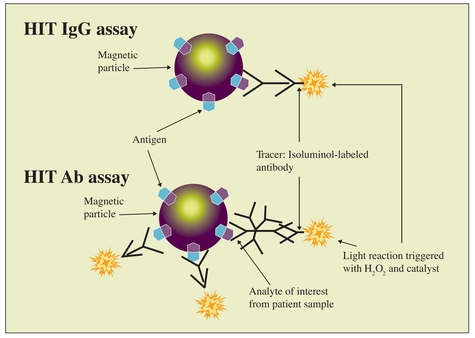


Figure 1
AcuStar HIT Assays Description

The Microparticles Reagent

Platelet factor 4 (PF4) complexed to polyvinyl sulfonate (PVS) is covalently coated onto paramagnetic particles.



Materials and Methods

The AcuStar HIT Multi-Controls are used for the assessment of the precision and the accuracy of both assays below and above the cut-off that is set at 1 Arbitrary Unit/mL.

Plasmas from 79 HIT suspected patients screened with the "4T's" probability score and the serotonin release assay (SRA) were tested with the new assays. For this study, samples were determined to be POSITIVE or NEGATIVE for antibody detection in accordance with SRA result (Table 1).

Table 1
Clinical Outcome (Serotonin Release Assay)

SRA Outcome		
POS	**NEG**	**Total**
10	69	79

The results of the new assays were compared to results from three commercially available kits: Zymutest HIA IgG (Hyphen Biomed), Zymutest HIA IgGAM (Hyphen Biomed) and Asserachrom® HPIA (Diagnostica Stago), where applicable.

Results

A total of 10 quality control data points were gathered during the study. Quality control materials were in control for the duration of the study, with series SD for the 2 levels of multi-controls ranging from 0.02 – 0.12. The new assays exhibited a good reproducibility in 2 quality control materials (CV% 4.0 – 8.0%) (Table 2).

Table 2
AcuStar reproducibility

	AcuStar HIT-$IgG_{(PF4-H)}$		AcuStar HIT-$Ab_{(PF4-H)}$	
n=10	**Low HIT Control**	**High HIT Control**	**Low HIT Control**	**High HIT Control**
Mean	0.5	2.7	0.5	2.7
SD	0.02	0.11	0.04	0.12
%CV	4.0	4.1	8.0	4.4

We compute the sensitivity and specificity vs. SRA for each assay (Table 3) and % agreement to each assay (Table 4) and summarize the analysis of positivity and negativity (Table 5).

Table 3
AcuStar sensitivity and specificity vs. SRA

n=79	AcuStar HIT Assay	SRA
Specificity (%)	HIT-$IgG_{(PF4-H)}$ HIT-$Ab_{(PF4-H)}$	90.9 73.9
Sensitivity	HIT-$IgG_{(PF4-H)}$ HIT-$Ab_{(PF4-H)}$	84.6 100.0

Table 4
AcuStar vs. Comparative Methods

	AcuStar HIT Assay	SRA n=79	Asswechrom HPIA n=79	Zymutest HIA IgGAM n=53	Zymutest HIA IgG n=25
Agreement (%)	HIT-$IgG_{(PF4-H)}$	89.9	87.2	82.7	83.3
	HIT-$Ab_{(PF4-H)}$	77.2	93.6	84.6	66.7

Table 5
Analysis of Positivity and Negativity

	SRA+ / ELISA+	SRA- / ELISA+	SRA- / ELISA-
n	10	16	53
AcuStar HIT-$IgG_{(PF4-H)}$	8/10 POS	9/16 POS	53/53 NEG
AcuStar HIT-$Ab_{(PF4-H)}$	10/10 POS	15/16 POS	49/53 NEG

In our analysis, we found that the positivity of HIT-Ab in high score patients (12/14) and the co-positivity of HIT-IgG with SRA (8/10) were equal to that of the ELISAs. In low scoring and negative SRA patients, HIT-Ab negativity was less than the ELISAs (26/35 and 33/35, respectively).

We also found a range of high positive values [HIT-IgG = 1.4 – 194.9 U/ml; HIT-Ab = 2.9 – 328.6 U/ml] in patients with positive SRA outcome and one of the commercial kits [Asserachrom HPIA] where the OD was greater than 2x the cut-off value, indicating high sensitivity.

Conclusions

Overall, this new panel of automated assays is more useful than, and demonstrates a comparable technical performance with the ELISA kits. These very promising data must be confirmed in a future prospective large study.

References

1. Warkentin TE, Greinacher A, Koster A, Lincoff AM. Andreas Greinacher, Andreas Koster and A. Treatment and Prevention of Heparin-Induced Thrombocytopenia: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines (8th Edition). Chest 2008;133;340-380.

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