COMPARISON OF THE DETECTION OF P2Y12-RECEPTOR BLOCKADE IN PRE-ANGIOCATH SUBJECTS WITH CARDIOVASCULAR DISEASE BY LIGHT-TRANSMITTANCE AND WHOLE-BLOOD AGGREGOMETRY, VERIFY NOW® P2Y12 AND INNOVANCE® PFA P2Y

David L. McGlasson, MS, MLS (ASCP) CM
Anand D. Shah, MD.
Wilford Hall Medical Center, Lackland AFB, TX

ABSTRACT

- The purpose of this study was to compare the results of the INNOVANCE® PFA P2Y* (P2Y), a new test cartridge for the PFA-100 ® system to those obtained by light transmittance (LTA) with 20 µM ADP and whole blood aggregometry (WBA) using 5 and 10 µM of ADP on the Chrono-Log 700, and the Verify Now ® P2Y12 cartridge by Accumetrics. Blood was collected with 3.2% and 3.8% sodium citrate from 102 subjects with cardiovascular disease after receiving clopidogrel (6-24 hours post loading with 300 or 600 mg) or after 7 days of 75 mg daily P2Y12 receptor blockade was detected with P2Y using a cut-off of >106 sec. Only the P2Y system was tested with 3.2 and 3.8% sodium citrate. All others were tested with 3.2% sodium citrate only. Cut offs for other systems were VerifyNow >20%; WBA 5 <5 ohms; WBA 10 <8 ohms; LTA <50% amplitude.
- The following results indicate the comparison of methods for detection of the influence of clopidogrel: Sensitivity (%): P2Y 3.2%=59%; P2Y 3.8%=95%; VerifyNow®=60%; WBA 5µM=88%; WBA 10 µM=89%; LTA 20 µM=72%.
- The total concordance (%) for this set of post drug patients was computed and the results are as follows: P2Y 3.2% to VerifyNow®=71%; to WBA 5 and 10µM=64 and 65% respectively; to LTA 20µM=69%. P2Y 3.8% to VerifyNow®=71%; to WBA 5 and 10µM=90% for both; LTA 20µM=76%. VerifyNow® to WBA 5 and 10µM=68 and 67% respectively; LTA 20µM=72%.
- The P2Y 3.8% result of 95% compares favorably with the results obtained in both WBA ADP concentrations. The P2Y 3.2% data compares closely with the VerifyNow® cartridge system. Concordance with the Verify Now® cartridge system was favorable at 71% for both P2Y sodium citrate concentrations. However, when comparing with WBA the 3.8% citrate results with the P2Y cartridge was 90%. The INNOVANCE® PFA-P2Y agrees favorably with other methods for detection for P2Y12-receptor blockade agents.

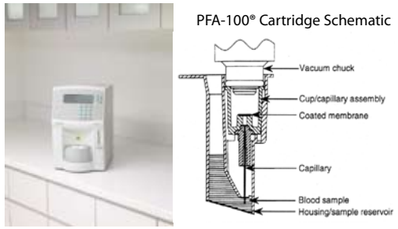
INTRODUCTION

- Our purpose is to determine the accuracy with which a new technology from Siemens Healthcare Diagnostics, Inc. can quantify the effects of the anti-platelet medication clopidogrel on platelet function.
- In this study we compared the results of the INNOVANCE® PFA P2Y* (P2Y), a new test cartridge for the PFA-100® system to the following test systems:
- Light transmittance aggregometry (LTA) with 20 µM ADP and whole blood aggregometry (WBA) using 5 and 10 µM ADP performed on a Chrono-Log 700 platelet aggregometer.
- Verify Now ® P2Y12 cartridge by Accumetrics.
- We anticipate that patients receiving clopidogrel as anti-platelet therapy for coronary artery disease will demonstrate platelet inhibition (when tested with P2Y) using the PFA-100® system.
- The performance characteristics of the P2Y cartridge used in this protocol have not been established for the US.

MATERIALS AND METHODS

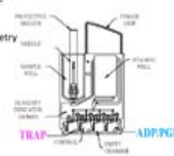
- Blood was collected with 3.2% and 3.8% sodium citrate from 102 subjects with cardiovascular disease after receiving clopidogrel (6-24 hours post loading) with 300 mg [n=35] or 600 mg [n=7]) or after 7 days of 75 mg daily [n=60].
- P2Y12 receptor blockade was detected with P2Y using a cut-off of >106 seconds (provided by manufacturer for this study).
- Only the P2Y was tested with 3.2 and 3.8% sodium citrated blood.
- The VerifyNow (VNP) system was only tested with 3.2% sodium citrate.
- Cut off for the VNP was >20% inhibition (provided by manufacturer in personal communication).
- Cut-off using LTA with 20 µM ADP on the Chrono-Log 700 platelet aggregometer was <50% amplitude (in-house method validation).
- WBA with 5 µM ADP <5 ohms and 10 µM <8 ohms (in-house method validation).

PFA-100® Cartridge Schematic



VerifyNow® P2Y12

- RAPID
  - Result available in <3 minutes
- EASY
  - Whole blood - no sample preparation
  - Automatic sampling from closed tube
  - Factory calibrated reagents
- ACCURATE
  - More specific than optical aggregometry
  - Can measure % platelet inhibition without weaning patient off drug
- COST-EFFECTIVE
  - Reimbursement
  - CPT code 85576 (2 times)
  - FDA cleared



Chrono-Log Model 700

RESULTS

- Sensitivity is determined by dividing the number of true positives (TP) by the TP plus the false negatives (FN) X 100% (TP/TP+FN).
- Detection rates of P2Y12-receptor blockade for each method are show in the table below:

Method	PFA P2Y 3.2%	PFA P2Y 3.8%	VN P2Y12	LTA 20 µM	WBA 5 µM	WBA 10 µM
Sensitivity	59%	95%	60%	88%	89%	72%

- Concordance is the agreement between two methods cut-offs usually expressed in percent (%)
- The total concordance for this set of post drug patients was computed and the results are as follows:

	VN P2Y12	WBA 5 µM	WBA 10 µM	LTA 20 µM
P2Y 3.2%	71%	64%	65%	69%

	VN P2Y12	WBA 5 µM	WBA 10 µM	LTA 20 µM
P2Y 3.8%	71%	90%	90%	76%

	WBA 5 µM	WBA 10 µM	LTA 20 µM
VN P2Y12	68%	67%	72%

DISCUSSION

- The P2Y 3.8% results of 95% compares favorably with the results obtained in both WBA ADP concentrations. The P2Y 3.2% data compares closely with the VerifyNow® cartridge system.
- Concordance with the VerifyNow® cartridge system was favorable at 71% for both P2Y sodium citrate concentrations.
- However, when comparing with WBA the 3.8% citrate results with the P2Y cartridge was 90%.
- The INNOVANCE® PFA-P2Y agrees favorably with other methods for detection of P2Y12 receptor blockade induced by clopidogrel.

REFERENCES

- Rechner AR, Merz M, Christie D, Giannnitsis E, Rade J, Kickler TS, Parades FA, Ahnadi C, de Fillippi CR, Christenson RH, Shah AD, McGlasson DL. **Comparison of the detection of P2Y12-receptor blockade in cardiovascular disease patients by whole blood aggregometry, classic light transmittance aggregometry and INNOVANCE PFA P2Y®**. Symposium of the Nederlandse Vereniging voor thrombose en Hemostase (NVTH). Nuremberg, Germany, February 24-28, 2010
- Rechner AR, Merz M, Christie D, Giannnitsis E, Rade J, Kickler TS, Parades FA, Ahnadi C, de Fillippi CR, Christenson RH, Shah AD, McGlasson DL. **Detection of P2Y12-receptor blockade in cardiovascular disease patients using INNOVANCE PFA-P2Y® for the PFA-100 system**. Symposium of the Nederlandse Vereniging voor thrombose en Hemostase (NVTH). Nuremberg, Germany, February 24-28, 2010
- Ivandic BT, Schlick P, Staritz P, Kurz K, Katus HA, Giannitsis E. Determination of clopidogrel resistance by whole blood platelet aggregometry and inhibitors of the P2Y12 receptor. Clin Chem 2006;52:383-388.
- Sibbing D, Braun S, Jawansky S et al. Assessment of ADP induced platelet aggregation with light transmission aggregometry and multiple electrode platelet aggregometry before and after clopidigrel treatment. Thromb Haemost 2008;99:121-126.
- Ferreiro JL, Sibbing D, Angiolillo DJ. Platelet function testing and risk of bleeding complications. Thromb
- Haemost 2010;103:1128-1135.
- Von Beckerath N, Sibbing D, Jawansky S et al: Assessment of platelet response to clopidogrel with multiple electrode aggregometry, the VerifyNow P2Y12 analyzer and platelet vasodilator-stimulated phosphoprotein flow cytometry. Blood Coag and Fibrinolysis 2010;21:46-52
- VerifyNow Accumetrics is based in San Diego, CA, United States
- Chrono-Log is based in Havertown, PA, United States

Funding for meeting registration to present this poster was received from Siemens Healthcare Diagnostics, Inc.