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Analytical validation of the new Hemoclot™ LA test in comparison with HemosIL® Silica Clotting Time and HemosIL® dRVVT assay on the ACL-TOP analyzer

M. SCHOORL, C. DAS, M. CHEVALLIER, J. van PELT, R.K. SCHINDHELM and M. SCHOORL

The antiphospolipid syndrome (APS) is a systemic autoimmune syndrome, characterized by thrombotic events and/or by recurrent miscarriages, and by the persistent presence of circulating antiphospholipid antibodies (aPL). The diagnostic criteria are defined by strict guidelines and require clinical characteristics (vascular thrombosis or pregnancy complications) and the persistent presence of aPL: lupus anticoagulant (LA), and/or anticardiolipin antibodies (aCL >99th percentile), and/or anti-β2-glycoprotein I antibodies (a-β2GpI >99th percentile) (1-4).

LA are antibodies directed against negatively charged phospholipids/protein complexes, resulting in prolonged clotting times in phospholipid dependent tests (e.g. APTT). Diagnosis of antiphospholipid syndrome (APS) relies predominantly on laboratory results. Therefore, adequate laboratory detection of antiphospholid antibodies, such as LA is clinical relevant. Assays must be sufficiently sensitive and highly specific to classify APS-positive patients correctly, as they have impact on clinical decisions for oral anticoagulant treatment (3, 5-7). Patients with thrombosis and aPL antibodies may be given indefinite oral anticoagulant treatment. Falsely diagnosed patients may be exposed to a high risk of bleeding, without having any benefit of such treatment. False-negative results have serious consequences for patients suspected for APS because they need long-term anticoagulation to prevent recurrences (7).

In this study, the analytical performances of the Hemoclot™ LA test (Hyphen BioMed), HemosIL® Silica Clotting Time (SCT) and HemosIL® dRVVT assays (Instrumentation Laboratory) were evaluated on the ACL-TOP analyzer. The analytical LA results of all tests were compared with the LA results obtained from the Sanquin reference laboratory.

Subjects and Methods

A group of 29 subjects suspect for LA participated in the study. A group of 70 blood donors (35 men and 35 women, Sanquin, Amsterdam, The Netherlands) was selected in order to determine the upper limit reference ranges (99th percentile cut-off).

Blood samples for LA were collected into sodiumcitrate tubes (0,109 Mol, Vacutainer®, Becton Dickinson, Plymouth, UK). Samples were two times centrifuged at 2500g in order to separate plasma from the cellular fraction and to obtain platelet poor plasma. Within 4 h after collection, plasma aliquots were stored at -70°C until analysis on the ACL-TOP analyser (Instrumentation Laboratory, Milan, Italy).

Hemoclot™ LA test

The Hemoclot™ LA testkit (Hyphen BioMed, Neuville-sur-Oise, France) consists of Hemoclot™ La-S and Hemoclot™ LA-C reagents. Hemoclot™ La-S is a simplified diluted Russell's Viper Venom (dRVV) reagent with a low concentration of phospholipids to screen for the presence of LA. Hemoclot™ LA-C is a dRVV reagent with a high phospholipid content to confirm the presence of LA. The higher phospolipid concentration neutralizes the LA present in the test plasma and shortens the clotting time. The reagents contain a heparin neutralizing substance which neutralizes up to 1 U/ml heparin.

For quality control purposes Biophen Normal control plasma and LA Control plasma (Hyphen Biomed, Neuville-sur-Oise, France) were used.

HemosIL® SCT

Reagents of the HemosIL® SCT assay (Instrumentation Laboratory, Milan, Italy) include low and high concentrations of phospholipids in order to screen and confirm the presence of LA. Heparin interference up to 0.4 U/mL is neutralized by polybrene. For quality control purposes LA Positive and LA Negative control plasmas (Instrumentation Laboratory, Milan, Italy) were used.

HemosIL® dRVVT test

The dRVVT Screen and dRVVT Confirm assays (Instrumentation Laboratory, Milan, Italy) are improved dRVVT reagents. The dRVVT Screen assay is poor in phospholipid, making it sensitive to LA. The additional amount of phospholipid in dRVVT Confirm neutralizes LA to give shorter clotting times. Heparin interference up to 1 U/mL is neutralized by polybrene. LA Positive and LA Negative control plasmas (Instrumentation Laboratory, Milan, Italy) were used for quality control purposes.

Analytical performance

All LA tests were performed according to the instructions of the manufacturer. To determine intra-

Laboratorium voor Klinische Chemie, Hematologie & Immunologie, Noordwest Ziekenhuisgroep, Wilhelminalaan 12, 1815 JD Alkmaar

E-mail: m.i.schoorl@nwz.nl

Table 1. Intra-assay and day-to-day reproducibility (CV%) of the Hemoclot™ LA, HemosIL® SCT and HemosIL®dRVVT screen and confirm assays. For intra-assay reproducibility (n=10) two patient samples were used. For day-to-day reproducibility (n=8), normal and LA positive control plasmas from the manufacturers' were used.

		Intra assay %CV	Day-to-Day %CV		Manufacturer %CV
			normal	positive	
Hemoclot LA	screen	0.7	2.1	2.6	<3
	confirm	1.4	1.6	1.6	<3
HemosIL SCT	screen	1.1	2.2	2.5	<4
	confirm	3.0	2.8	1.6	<4
HemosIL dRVVT	screen	0.8	3.3	4.0	<4
	confirm	1.0	2.6	1.2	<4

assay precision, two patient samples were determined in tenfold. Day-to-day variation was determined in eightfold with the positive and negative control plasmas of the respective LA tests.
Results of the LA tests in the group suspect for LA were compared with the LA results obtained from the Sanquin reference laboratory (LA dRVVT test).
Statistical evaluation of results was performed with IBM SPSS statistics 20 for Windows. The 99th percentile cut-off was determined using a nonparametric method of percentile calculation. Values ≥ cut-off were defined as LA positive.

Results

Intra-assay and day-to-day reproducibility is demonstrated in Table 1. Results were within the specifications of the manufacturers.
The 99th percentile cut-off values of the normalized LA ratio were all higher in comparison with the cut-off values of the manufacturer: Hemoclot™ LA test 1.27 (manufacturer 1.20), HemosIL® SCT assay 1.31 (manufacturer 1.16) and HemosIL® dRVVT assay 1.24 (manufacturer 1.20).
Using the manufacturer's cut-off or the 99th percentile cut-off, the Hemoclot™LA assay resulted in 100%, respectively 97% agreement for LA interpretation (Table 2). The HemosIL®dRVVT assays resulted in extra LA positive cases compared to Hemoclot™LA and the results from the reference laboratory. Using the manufacturers' cut-off, the HemosIL® SCT and HemosIL®dRVVT assays resulted in four, respectively three extra LA positive cases compared to Hemoclot™LA and the results from the reference laboratory. Using the 99th percentile cut-off, the HemosIL® SCT assay resulted in four false negative cases compared to Hemoclot™LA and the results from the reference laboratory.
With use of the manufacturer's or 99th percentile cut-offs the Hemoclot™LA and HemosIL®dRVVT assays demonstrated both >90% correct results compared to the dRVVT test from the reference laboratory. The HemosIL® SCT assay demonstrated 76% correct results using the cut-off from the manufacturer. The % correct results increased to 83% using the 99th percentile cut-off.
Five cases were LA positive with all three assays.

Table 2. Positive and negative predictive values (%) and the percentage correct LA results compared to the dRVVT LA result from the Sanquin reference laboratory, using the cut-off from the manufacturer or the 99th percentile cut-off for LA positive interpretation. PPV = positive predictive value ; NPV = negative predictive value

	cut-off manufacturer			99th percentile cut-off		
	PPV	NPV	% correct results	PPV	NPV	% correct results
	%	%	%	%	%	%
Hemoclot LA	100	100	100	100	95	97
HemosIL SCT	60	84	76	83	83	83
HemosIL dRVVT	75	100	90	82	100	93

Conclusion

Hemoclot™LA, HemosIL® SCT and HemosIL® dRVVT assays are easy to perform on the ACL TOP analyzer. Intra- and day-to-day reproducibility demonstrated acceptable performance characteristics for all assays.
A moderate agreement was obtained between the HemosIL® SCT and the dRVVT assays using the cut-off from the manufacturer. Using a 99th percentile cut-off leads to a better performance for this assay.
The HemosIL®dRVVT assay demonstrated 10% more positive results compared to the Hemoclot™LA dRVVT assay. The Hemoclot™LA assay demonstrated excellent agreement with the LA results from the Sanquin reference laboratory.

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