...ott Architect or in plasma with Biosite Triage? The laboratory's point of view

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...alin (NGAL) has emerged as a potential interesting marker for the early detection of acute kidney injury (AKI) (1-3).

However, most of these studies were obtained with cumbersome techniques (Elisa), particularly difficult to implement in routine (3,4). Recently, two commercially available kits for NGAL determination appeared on the market. The first one, from Abbott Laboratories (Abbott Park, IL) is an automated immuno-assay that allows the determination of urinary NGAL on the Architect platform. The second one, the Triage NGAL Test (Biositeó Inverness Medical, Waltham, MA) is a point-of-care-immunoassay for the quantitative determination of NGAL in EDTA anticoagulated whole blood or plasma specimen. The aim of this study was to perform an analytical validation and an imprecision comparison of these new tests.

The Architect NGAL assay is a non-competitive two-site sandwich immunoassay that utilizes two mouse antibodies recognizing distinct NGAL epitopes. The Triage NGAL is a rapid fluorescence immunoassay to be used with the Triage Meters. All the tests have been performed by a well trained laboratory technician according to the manufacturersø instructions. Both tests have been correlated against an established and validated Elisa that uses mouse monoclonal antibody raised against human NGAL (#HYB211-05; AntibodyShop, Gentofte, Denmark) (5,6). We used the e-noval (Arlenda, Liège, Belgium) software for the statistical evaluation of the results.

Random fresh urine and EDTA samples were collected in healthy and chronic kidney disease volunteers who gave their informed consent. EDTA samples were immediately centrifuged, and aliquoted. All samples have been kept at -80°C until assayed in the two following weeks. Precision was evaluated in accordance to the recommended CLSI EP-5A2 guideline: six urine (for the Architect) and seven EDTA plasma (for the Triage) pools were assayed in triplicate once per day on five different days. The complete imprecision check thus implies 15 determinations for each pool, i.e. 90 determinations for urine and 105 for plasma. We also

(which characterizes the dispersion of the possible values around the true value, which is always unknown and which is method and matrix specific for this substance), the accuracy and the β-expectation limits. β-expectation tolerance limits with β=0.95 is the upper and lower values in-between which each future measurements of the same level has a probability of 95% to be found (7,8). The study was approved by the Institutional Review Board of the Centre Hospitalier Universitaire de Liège.

The results of the precision evaluation are shown in Table 1. The coefficient of variation (CV) did not exceed 6% for the Abbott Architect NGAL. For the Biosite Triage, the CV ranged from 5 to 16% for levels of 722 and 117µg/L, respectively.

Measurement uncertainty and β-expectation tolerance limits are also presented in Table 1. Measurement uncertainty ranged from 3.4 and 12.5% for the Abbott Architect vs. 10.4 to 33.3% for the Triage. The β-expectation tolerance intervals were computed at each concentration level with a probability β=95%. In other words, this means that, on average, 95% of the future results that will be generated by this method will be included in the computed tolerance intervals. Thus, the method will provide accurate results if the 95% β-expectation tolerance interval at each concentration level is fully included in the acceptance limits that we proposed at ±20%. This is illustrated in Figure 1a through an error profile for the Abbott Architect, showing that this method provided precise results from 22.5 to 1315 µg/L. These two concentrations define the dosing range of the method. It is thus guaranteed that each future result will be included in the ±20% with a probability of 95%.

On the other hand, Figure1b shows that the Biosite Triage NGAL will provide precise results between 619 and 722 µg/L. Only in this range, each future result obtained with this method will be comprised in the ±20% with a probability of 95%.

We presented here the results of the analytical validation of two newly commercially available kits for NGAL determination. The aim of our study was to compare the analytical

best of our knowledge, there is no publication on the analytical performances of the Biosite Triage NGAL. The analytical validation of the Abbott Architect NGAL was recently published (9). Even if the results seemed interesting, this work was performed by scientists employed by the Abbott Corporation. Our validation which confirms the good performance of the assay, is independent of any company, used a strong methodology that is very innovating in the field of Clinical Chemistry (10). The Abbott Architect NGAL is an automated method that has to be used in the laboratory whereas the Triage NGAL could be used by virtually any clinician at the bedside. For institutions that do not have the possibility to run the NGAL on the Architect, Biosite Triage NGAL could be an alternative as the Triage meters can be used or placed in a ward. Unfortunately, the analytical performances of this machine did not fulfil our expectations. Indeed, the method showed important analytical limitations, the main point being the high variation around the proposed cut-off for reference values (below 130 ng/ml). The clinical interpretation of a single NGAL measurement, with the help of a cut-offö value, is thus questionable with the Triage NGAL that presents a high degree of analytical variability around this cut-off value. Our results actually show that, for a patient presenting a value of 163 µg/L, the õtrue valueö could range, with a confidence of 95%, between 109 and 221 µg/L with the Triage (by comparison, a urine value of 141 µg/L could range in the same way between 125 and 158 µg/L). Moreover, it must be remembered that this high variation was observed when the analyses were performed in an optimal manner, with a very well trained and experienced technician. In the hands of many different inexperienced nurses in an overbooked ward, this variability could be worse.

On the other hand, the Abbott Architect NGAL gave much more precise results. Our results showed that between 22.5 and 1315 µg/L, 95% of the results will be in the ±20% maximum allowable total error, which is acceptable (although arbitrary) for an immuno-assay.

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rest of NGAL as a potential new marker for the early detection of AKI (1-5). These data, obtained with different Elisa methods, are somewhat difficult to translate directly into clinical practice. Our results show that when NGAL was determined in urine with Abbott Architect, imprecision was less than when it was determined in plasma with the Triage method. From our õanalyticalö point of view, Abbott Architect NGAL is, for the moment, the best method to detect AKI in patients.

Table 1. Precision and measurement uncertainty observed on six urine pools (Abbott Architect NGAL) and seven EDTA plasma pools (Biosite Triage NGAL). The standard deviation (SD) and coefficient of variation (CV) correspond to the total variability observed during the 5 days of the experiment. Uncertainty characterizes the dispersion of the values around the (unknown) true value. The β-expectation tolerance limits show, for each level tested, where 95% of the future results that will be generated by the methods could be situated.

Figure 1: Error profile of the Abbott Architect NGAL (1a) and Biosite Triage NGAL (1b). When the β-expectation limits (---) are comprised between the maximum total allowable error (í) (settled here at ±20%), the method is considered as valid. Each dot represents the result of one assay. We can see here that the Biosite Triage method is valid between 619 µg/L and 722 µg/L only, whereas the Abbott Architect method is valid throughout the whole measurement range studied.

Competing interests:

None of the Authors have any financial or non-financial interest to declare.

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ABBOTT ARCHITECT NGAL							
Pool	N	Mean (µg/L)	SD (µg/L)	CV (%)	Uncertainty (µg/L)	Uncertainty (%)	Beta-expectation tolerance limit (µg/L)	Beta-expectation tolerance limit (%)
1	15	22.47	0.72	3.2	0.73	6.5	[21.0 , 25.0]	[-6.7 , 7.3]
2	15	81.07	2.80	3.5	3.0	7.3	[74.4 , 87.8]	[-8.3 , 8.3]
3	15	141.4	5.80	4.1	6.3	8.2	[124.9 , 157.9]	[-11.7 , 11.7]
4	15	460.0	26.3	5.7	28.7	12.5	[385.3 , 534.7]	[-16.2 , 16.2]
5	15	927.5	15.3	1.7	16.0	3.5	[892.6 , 962.4]	[-3.8 , 3.8]
6	15	1315	24.0	1.9	25.5	3.9	[1257 , 1373]	[-4.4 , 4.4]
BIOSITE TRIAGE NGAL							
1	15	117	18.2	15.6	19.4	33.3	[72.22 , 161.1]	[-38.1 , 38.0]
2	15	163	25.3	15.5	26.1	32.0	[108.9 , 221.3]	[-33.1 , 35.7]
3	15	174	17.1	9.9	18.2	22.0	[132.6 , 214.5]	[-23.6 , 23.6]
4	15	199	29.0	14.6	30.0	30.2	[134.3 , 263.2]	[-32.4 , 32.4]
5	15	298	39.3	13.2	40.5	27.2	[210.6 , 385.0]	[-29.2 , 29.2]
6	15	427	67.6	15.8	69.8	32.7	[276.5 , 576.6]	[-35.1 , 35.1]
7	15	722	35.0	4.9	37.4	10.4	[636.0 , 807.3]	[-11.8 , 11.8]

Abbott Architect NGAL (1a) and Biosite Triage NGAL (1b). When the β-expectation limits (---) are comprised between the maximum total allowable error (í) (settled here at ±20%), the method is considered as valid. Each dot represents the result of one assay. We can see here that the Biosite Triage method is valid between 619 µg/L and 722 µg/L only, whereas the Abbott Architect method is valid throughout the whole measurement range studied.