Validation of a commercial kit for *Pseudomonas aeruginosa* antibodies

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Conclusion: The E15 anti-*Pseudomonas aeruginosa*-IgG EIA test (Mediagnost GmbH), which is based on three antigens, Alkaline protease, Elastase and Exotoxin A, has been integrated into the routine diagnostic arsenal at Karolinska University Hospital.

Lung damage secondary to chronic bacterial infection (predominantly *Pseudomonas aeruginosa* (PA)) is the main determinant of morbidity and mortality in patients with cystic fibrosis (CF). Eradication of early infection and prevention of chronic infection is associated with preserved lung function. Antibody testing against PA enzymes can be a marker for successful eradication since elevated levels of antibodies are a risk factor for developing chronic infection.

At Karolinska University Hospital, an anti-PA-IgG in-house EIA has been used. A drop in antibody titer was observed during 2014 for a new batch of the coating antigen, which never reestablished its previous quality. Therefore, the commercial E15 anti-PA-IgG EIA (Mediagnost GmbH) was evaluated.

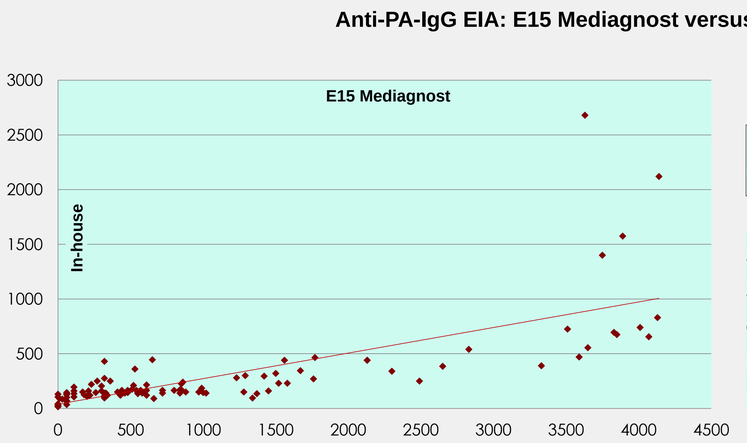

Figure 1. The OD-values for anti-PA(EXO)-IgG multiplied with the dilution factor for the Mediagnost and the in-house tests are plotted against each other. Some outliers are observed, which can be expected. This effects the correlation factor. The results from E15 Mediagnost align with a wider spread than with the in-house test.

		In-house		
		Pos	Neg	
E15 Mediagnost	Pos	68 (31)	2 (3)	70 (36)
	Neg	15 (3)	25 (6)	40 (7)
		83 (34)	27 (9)	110 (43)

Sensitivity: 82% (91%)

Specificity: 93% (67%)

Concordance: 85% (91%)

Positive predictive value: 97% (92%)

Negative predikctive value: 63% (86%)

Figure 2. A summary of the 110 clinical samples that were analyzed with anti-PA(EXO)-IgG, non-prenthesis numbers, and of the additional analysis of 43 clinical samples using the complete E15 anti-PA-IgG EIA kit, prenthesis numbers.

Methods

The E15 anti-PA-IgG EIA kit (Mediagnost GmbH) was compared to the anti-PA-IgG in-house EIA. The kit cover three antigens for PA; Exotoxin A (EXO), Alkaline protease (AP) and Elastase (ELA), only EXO is used as coating antigen in the in-house EIA. First, anti-PA(EXO)-IgG was analyzed and compared to previous in-house EIA results. In total, 110 samples from CF-patients from 2013 were included; 45 borderline, 25 positive, 11 negative, 10 with significant titer elevations and 3 from patients sampled over time. Second, 43 out of the 110 samples were reanalyzed for all three antigens using the complete setup of the E15 anti-PG-IgG kit.

Results

The OD-values multiplied with the dilution factor for the Mediagnost and the in-house tests are plotted in Figure 1. Borderline EXO results were interpreted as positive. First, for the EXO analyzes only, a sensitivity of 82% (68/83) a specificity of 93% (25/27), a concordance of 85% (93/110), a positive predictive value of 97% (93/110) and a negative predictive value of 63% (25/40) were generated. Six previously positive patients were repeatedly negative for EXO in the anti-PA(EXO)-IgG EIA kit analyses. When introducing the two additional PA-antigens, 3/6 results were interpreted as positive, thereby increasing the E15 anti-PA-IgG EIA kit sensitivity to 91% (31/34), for the selection of clinical samples included in this evaluation. A summary of the results is presented in Figure 2.

Discussion

The rational for interpreting borderline EXO results as positive, is that they were positive in our in-house EIA, or vise versa. A few samples generated discordant results close to the cutoff limits, negative/borderline or borderline/positive, such small variations are acceptable.

By introducing the two additional PA-antigens in the assay the E15 kit performance is improved. The concordance (91%) and specificity (91%) of the E15 kit is acceptable when results of all three antigens are included. Unfortunately, lack of recent experience regarding AP and ELA at Karolinska University Laboratory require trust in publications of their diagnostic value. The three patients that still remained negative using the E15 kit are young children, where two had borderline results for anti-PA(AP)-IgG.

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