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2.0 Aim

This report summarizes studies for the verification of ALFA.

3.0 Relevant Documents

- DIN EN ISO 13612:2002 (Performance evaluation of in vitro diagnostic medical devices)
- Instructions for use ALFA (current version)
- Instructions for use ALLERG-O-LIQ (current version)
- Verification Plan ALFA

4.0 Methods

Single determinations were carried out according to the instructions for use for both ALLERG-O-LIQ and ALFA.

5.0 Results

5.1 Analytical Sensitivity and Specificity of ALFA Seasonal *Screen*

50 sera were tested for the presents of specific IgE antibodies against Birch (t3), Bermuda Grass (g2), Perennial Rye Grass (g5), Timothy Grass (g6), June Grass (g8), Cultivated Rye (g12), Mugwort (w6), *Alternaria alternata* (m6) and *Aspergillus fumigatus* (m3) on ALLERG-O-LIQ system. The maximum IU/mL value (ALLERG-O-LIQ) of each serum was used as a reference value for the comparison of ALLERG-O-LIQ results to results of ALFA (Seasonal Screen).
ALFA Basis Set 07130112 and ALFA Seasonal Screen 071610S was used.

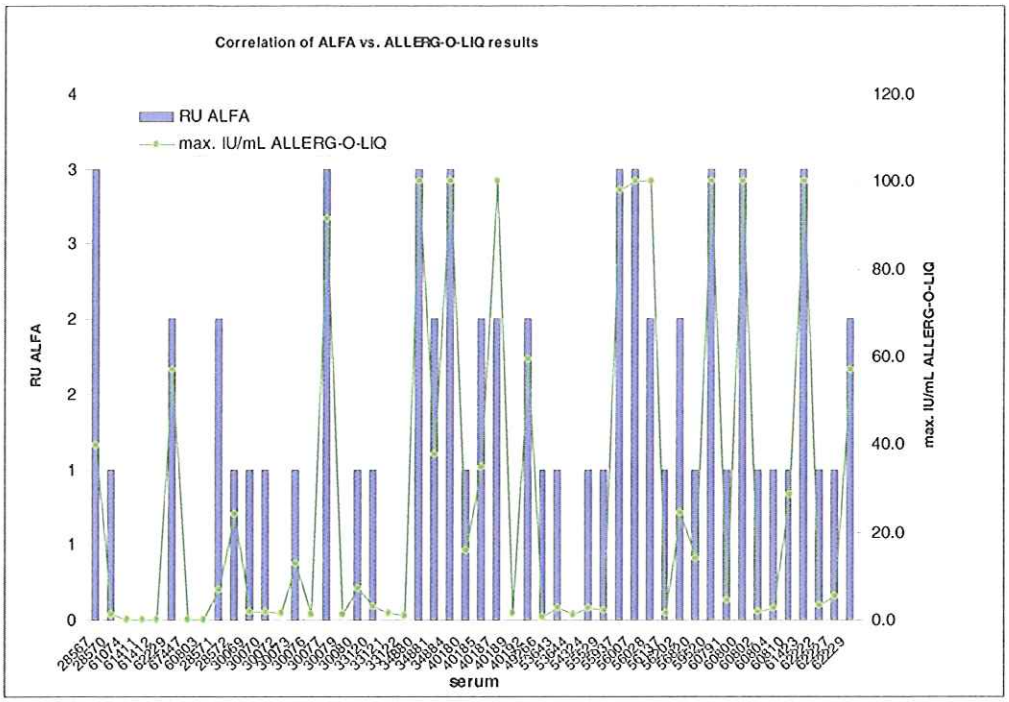


Figure 1 Comparison of ALFA vs. ALLERG-O-LIQ results (REF Y:\ALFA\Journale\ALFA061a.xls)

Table 1 Sensitivity and Specificity of ALFA Seasonal Screen (REF Y:\ALFA\Journale\ALFA061a.xls)

IU/mL ranges	Number of pos sera tested	Number of pos sera tested	Sensitivity (ALFA) in % compared to ALLER-O-LIQ	Specificity ALFA*
0.35 IU/mL – 100 IU/mL	42	8	80	100%
0.7 IU/mL – 100 IU/mL	41	9	82	100%
1.5 IU/mL – 100 IU/mL	34	16	100	100%
2.0 IU/mL – 100 IU/mL	30	20	100	100%

*No false positive results were obtained.

If low titer sera (0.35-0.70 IU/mL) are tested, sensitivity is ≈ 80%. Sera in the range of 1.5-2 IU/mL are found positive with a sensitivity of ≈ 100%. Specificity was found at 100%, no false positive results were obtained.

5.2 Inter Assay Variation

2 negative and 3 positive sera were tested on ALFA Basis Set 06122010 using ALFA Seasonal Screen 071610S. Minor variations in signal intensities were observed. However, the agreement of the qualitative results was found at 100%.

Table 2 Inter Assay Variation of ALFA (REF Y:\ALFA\Journale\ALFA061a.xls)

	071610S VB1 06122010 *			
	Assay 1	*Assay 2*	*Assay 3*	*Assay 4*
Sera	Test Line	Test Line	Test Line	Test Line
67447	-	-	-	-
67449	-	-	-	-
56007	++++	+++	++++	++++
55529	+	+	+	+
56137	++	++	+++	+++
67447	-	-	-	-
67449	-	-	-	-
56007	++++	++++	+++	++++
55529	+	+	+	+
56137	++	++	+++	+++
67447	-	-	-	-
67449	-	-	-	-
56007	+++	+++	++++	+++
55529	+	+	+	+
56137	++	++	+++	+++

* read-out was done after 25 min, Control Line gave a strong signal (++++) in any case

The correlation of the qualitative interpretation of the results was found at 100%.

5.3 Inter Observer Variation (agreement of qualitative results)

Samples were tested on ALFA Basis Set 06122010 with Seasonal Screen 071610S.

Table 3 Inter Observer Variation of ALFA

% agreement between observers (qualitative)			
observer	1	2	3
1		100	100
2			100

REF: Y:\ALFA\Journale\ALFA061a.xls

No false positive results were obtained. The agreement of the qualitative result between Observer 1, 2 and 3 was found at 100%.

5.4 Comparison whole blood samples vs. sera

Whole Blood samples as well as serum samples of 5 subjects were tested on ALFA *Basis Set* 06122010 using ALFA Seasonal and ALFA Perennial Screens. For one patient a dilution of biotinylated W1 extract was tested.

Table 4 Comparsion of whole blood samples vs. corresponding serum samples

ALFA Basis Set 06122010							
				whole blood		serum	
whole blood or serum	spez.	~max. IU/mL	screen or single allergen	result after 20min test line	result after 20min control line	result after 20min test line	result after 20min control line
RL	neg.	0	071610S	-	++++	-	++++
MH	neg.	0	121610P	-	++++	-	++++
MG	neg.	3	071610S	+	++++	+	++++
PE	pos.	8	012310W1	++	++++	++	++++
MA	pos.	5	121610P	++	++++	+	++++
MA	pos.	100	071610S	++++	++++	++++	++++

ALFA results obtained using whole blood samples, are in good agreement with results obtained using serum samples of the same subject; however signal intensities were slightly weaker for the whole blood samples. Nevertheless the agreement of the qualitative results remained at 100%. A broader study is under way to support these findings.

5.5 Stability Studies

5.5.1 Stability of ALFA Test Kits (POCT)

ALFA "Point of Care" Test Kits were stored at 4°C and 37°C for six weeks and tested using two negative and three positive sera. Determinations were done in duplicate.

Table 5 Stability of ALFA Test Kits (POCT)

ALFA POC Test	stored at		
	4°C	37°C	
Sera	**071610S VB1 06122010**		
67447	-	-	6 weeks at 37°C or 4°C
67449	-	-	
56007	++++	+++	
55529	+	+	
56137	++	++	
67447	-	-	6 weeks at 37°C or 4°C
67449	-	-	
56007	++++	++++	
55529	+	+	
56137	++	++	
67447	-	-	6 weeks at 37°C or 4°C
67449	-	-	
56007	+++	+++	
55529	+	+	
56137	++	++	

No significant reduction in signal could be observed. The correlation of the qualitative interpretation of the results was found at 100%.

5.5.2 Stability of ALFA Test Kit Components

ALFA "Point of Care" Test Kit components were stored at 4°C and 37°C for six weeks and tested using two negative and three positive sera. Determinations were done in duplicate.

Table 6 Stability of ALFA Test Kit Components

	stored at		
	4°C	37°C	
Sera	ALFA *Basis Set* VB1 06122010		
67447	-	-	ALFA Perennial Screen 111610P at 37°C for 6 weeks
67449	-	-	
65346/1	++++	++++	
55529	+	+	
56137	+++	+++	
67447	-	-	ALFA Perennial Screen 111610P at 37°C for 6 weeks
67449	-	-	
65346/1	+++	++++	
55529	+	+	
56137	+++	+++	
67447	-	-	ALFA Perennial Screen 111610P at 37°C for 6 weeks
67449	-	-	
65346/1	++++	+++	
55529	+	+	
56137	+++	+++	

No significant reduction in signal could be observed. The correlation of the qualitative interpretation of the results was found at 100%.

6.0 Conclusions

6.1 Analytical Sensitivity and Specificity (ALFA Seasonal Screen vs. ALLERG-O-LIQ)

If low titer sera (0.35-0.70 IU/mL) are tested, sensitivity is ≈ 80%. Sera in the range of 1,5-2 IU/mL are found positive with a sensitivity of ≈ 100%.

6.2 Inter Assay Variation

Results of the same sera tested on 4 assays varied slightly. However, the correlation of the qualitative interpretation of the results was found at 100%.

6.3 Inter Observer (agreement of qualitative results)

The agreement between Observer 1, 2 and 3 was found at 100%.

6.4 Comparison whole blood samples vs. sera

ALFA results obtained using whole blood samples, are in good agreement with results obtained using serum samples of the same subject; however signal intensities were slightly weaker for the whole blood samples. Nevertheless the agreement of the

qualitative results remained at 100%. A broader study is under way to support these findings.

6.5 Stability Studies

After six weeks of storage at 4°C and 37°C respectively, no significant reduction in signal was observed. The correlation of the qualitative interpretation of the results was found at 100% for both ALFA "Point of Care" Test Kits as well as the single kit components.

7.0 History of the Document

Revision	Änderung	Erstellt von	Gültig ab
02	Berichtigung der Tabelle für Sensitivität und Spezifität (Tab.1)	RL	
01	Neuerstellung	RL	19.042007
-	Dokument entfällt oder Dokument geht in Dokument XY auf	eingezogen von:	gültig bis: