Reliable Detection of Duffy x (Fy^x) – A Weak Variant of Duffy b (Fy^b) by a New Reagent Using Lateral Flow Technique.

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BACKGROUND

With the advent of molecular techniques occasional blood group phenotyping errors became apparent. In particular, a number of weak phenotypes, previously typed false-negatively, were discovered in several blood group systems. For the Duffy (DARC) system and Fy^x, this was reported as early as 1997 [1]. Fy^x is a weak Fy^b phenotype with a pronounced quantitative reduction of the number of Fy^b antigens on the erythrocyte surface, thus provoking the risk of false-negative results in blood group phenotyping. Today, to our knowledge, there is no serological reagent for the reliable detection of Fy^x.

AIMS

Sensitivity of a novel Anti-Fy^b reagent should be evaluated, especially focusing on the ability to detect Fy^x in a selected cohort with Fy^x positive individuals.

METHODS

Freshly drawn and EDTA anticoagulated samples were from 42 random individuals previously determined serologically for Fy^a and Fy^b and 21 samples with standard Fy serotypes and FY* genotypes previously derived by MALDI-TOF MS [2]. The 9 previously known FY*A/ FY*02W.01/02 heterozygous individuals were of specific interest for evaluation purpose and included 3 samples with previously identified phenotypic Fy^x positivity and 6 previously serologically "overseen" Fy^x cases (Table 1). All samples were tested by one person without prior knowledge of the existent phenotypes.

Fy^b serology using a MDmulticard lateral flow blood grouping device (Medion Grifols Diagnostics, Duedingen, Switzerland) was performed as follows: 100 µl of diluted whole blood were transferred to the application zone of the MDmulticard cassette, followed by 300 µl of a rinsing solution. Results were interpreted after 5 minutes. Positive results were interpretable as distinct red bands, whereas negative results lack the respective bands.

RESULTS

All samples with genotypic Fy^x positivity were correctly recognized as Fy^b positive by the MDmulticard device (Figure 1A), but not always with Gel Technique and Tube method (Figure 1B; e.g. sample 158 is negative with all 4 Anti-Fy^b reagents, sample 150 shows a doubtful reaction with Anti-Fy^b monoclonal and a weak with ID-Card system).

All 42 random phenotype-only samples except one were concordant with the serological pre-values (Table 1). The discrepant sample had a recorded Fy(a+b-) phenotype (routine serology), but showed a Fy(a+Fybweak+) phenotype with lateral flow technique. Presumably and in line with unexpected, but plausible statistics, this sample was supposedly of Fy(a+bweak+) phenotype, previously undetected for Fy^x by the routine method. Retesting of the respective sample was prohibited by the mandatory ethical provisions, i.e. anonymization.

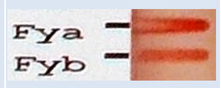
MDmulticard lateral flow technique				
allel-1 | allel-2	Sample n°		Sample n°	
FY*A | FY*02W.01/02	158		157	
	144		149	
FY*B | FY*02W.01/02	122		145	
FY*02W.01/02 | FY*02W.01/02	150		165	
ND	114		103	

Figure 1A Examples of results obtained with lateral flow technique

MALDI TOF MS			Serological prevalues			MDmulticard		
allel-1 | allel-2	Phenotype deduced from genotype	n=	Fy (a+b-)	Fy (a+b+)	Fy (a-b+)	Fy (a+b-)	Fy (a+b+)	Fy (a-b+)
FY*A | FY*02W.01/02	Fy(a+b+)	9	6	3	0	0	9	0
FY*B | FY*02W.01/02	Fy(a-b+)	10	0	0	10	0	0	10
FY*02W.01/02 | FY*02W.01/02	Fy(a-b+)	2	0	0	2	0	0	2
ND	n.a.	42	15	15	12	14	16	12
TABLE 1 Summary results		63	21	18	24	14	25	24

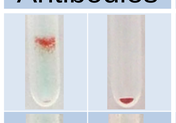
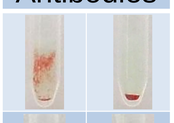
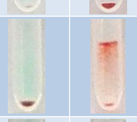
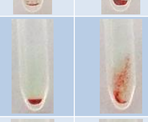
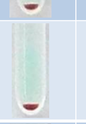
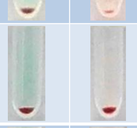
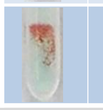
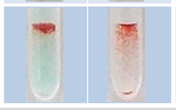
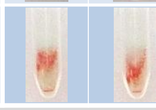
	DG Gel System				ID-Card System		Tube method	
	Anti-Fya	Anti-Fyb	Anti-Fya	Anti-Fyb	Anti-Fya	Anti-Fyb	Anti-Fya	Anti-Fyb
	Polyclonal Antibodies		Monoclonal Antibodies		Polyclonal Antibodies		Polyclonal Antibodies	
Sample 158							2+	-
Sample 122							-	2+
Sample 150							-	1+
Sample 114							3+	2+

Figure 1B Examples of blood group phenotyping results obtained with other techniques

SUMMARY/CONCLUSIONS

The MDmulticard Anti-Fy^b reagent seems to reliably detect all Fy^b and Fy^x positive phenotypes. This study is ongoing as more data are needed in order to have statistically significant results. Technically, the combination of well selected clones with appropriate diagnostic techniques may lead to novel methods with increased diagnostic sensitivity. As shown previously, genotyping may serve as a valuable tool to create more specific and better characterized testing panels [3].

BIBLIOGRAPHY

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GRIFOLS

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