



Phleum pratense may be taken as a marker allergen for gx3 grass mix

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Background: Allergen extract mixes are often used in routine diagnostics. Reflex testing after a positive mix result is poorly performed to identify the responsible allergen. The grass mix gx3 contains grasses that belong to the Pooideae family and are known to have IgE cross reactivity. The aim of the study was to investigate if one grass pollen allergen extract can be used instead of the mix.

Method: 30 serum samples from Northern European routine patients (biobank) were tested simultaneously on the ImmunoCAP gx3 mix (ThermoFisher) and the MUXF allergen (cross reactive carbohydrate determinants, CCD). The grass pollen extracts included in gx3 were tested with the IDS Specific IgE assays (formerly Allersys®, Omega Diagnostics) and the ImmunoCAP allergens. Samples were also tested on the component mix rPhl p 1, rPhl p 5b on both platforms. Anti-CCD IgE antibodies were blocked with the inhibitor from HÄMOSAN Life Science Services® GmbH according to the instructions for use. 0.35 kUa/L was used as a cut-off for positive and negative results.

Higher number of samples confirms Timothy grass as a potential marker for gx3

	Allergen mix gx3	
	positive agreement	negative agreement
Timothy grass	96.6 %	100 %
Timothy grass	98.3 %	100 %

N= 80 samples in total, 58 positive for gx3, 22 negative for gx3; Grey shaded, black font: IDS allergy assays; Dark grey shaded, white font: ImmunoCAP allergy assays

Agreement between allergen mix gx3 and single allergens

	Allergen mix gx3		Sample nr	Sweet vernal G001	Rye grass G005	Timothy G006	Cultiv. Rye G012	Velvet G013	Phl p1, 5b G213	Mix gx3	Sweet vernal g1	Rye grass g5	Timothy g6	Cultiv. Rye g12	Velvet g13	Phl p1, 5b g213	MUXF o214
	positive agreement	negative agreement															
Sweet vernal g.	84.2 %	100 %	3														
Rye grass	84.2 %	100 %	4														
Timothy grass	89.5 %	100 %	6														
Cultivated rye	78.9 %	100 %	7														
Velvet grass	89.5 %	100 %	2														
rPhl p 1, rPhl p 5b	84.2 %	100 %	8														
Sweet vernal g.	89.5 %	100 %	5														
Rye grass	94.7 %	100 %	1														
Timothy grass	94.7 %	100 %	10														
Cultivated rye	89.5 %	100 %	9														
Velvet grass	94.7 %	100 %	17														
rPhl p 1, rPhl p 5b	84.2 %	100 %	14														
Sweet vernal g.	89.5 %	100 %	15														
Rye grass	94.7 %	100 %	20														
Timothy grass	94.7 %	100 %	21														
Cultivated rye	89.5 %	100 %	22														
Velvet grass	94.7 %	100 %	16														
rPhl p 1, rPhl p 5b	84.2 %	100 %	23														
			24														
			25														
			26														
			13														
			11														
			28														
			29														
			19														
			30														
			18														
			12														

Grey shaded, black font: IDS allergy assays; Dark grey shaded, white font: ImmunoCAP allergy assays

11 samples were negative (red) and 19 samples positive (green) for gx3. All samples were negative for MUXF but 4 had detectable levels (> 0.1 < 0.35 kUa/L).

Sample 14:
The mix result is positive and the single allergens included in the mix are negative. G213 was negative on both platforms as well as the SPT result for grass allergens too which suggest no grass pollen allergy for this patient.

Sample 15:
The mix result is positive as well as some grass extracts but the molecules are negative which suggests no grass pollen allergy.

Sample 20:
Disagreement between both platforms except for Phl p 1, 5b which suggest no grass pollen allergy; MUXF level: 0.28 kUa/L

Blocking of anti-CCD antibodies converts positive result into negative one

Sample nr	Sweet vernal G001	Rye grass G005	Timothy G006	Cultiv. Rye G012	Velvet G013	Phl p1, 5b G213	Mix gx3	Sweet vernal g1	Rye grass g5	Timothy g6	Cultiv. Rye g12	Velvet g13	Phl p1, 5b g213	MUXF o214
20: 1 st run	0.34	<0.1	0.28	0.14	0.13	<0.1	0.61	0.59	0.58	0.59	0.66	0.59	<0.1	0.28
20: 2 nd run	not tested	0.17						0.59						0.27
20: 2 nd - BLOCK		<0.1							0.11					<0.1

Grey shaded, black font: IDS allergy assays; Dark grey shaded, white font: ImmunoCAP allergy assays, unit = kUa/L.

Conclusion: The IDS allergy assays Timothy and Velvet grass showed the best concordance to the grass mix gx3. For the ImmunoCAP system Rye grass, Timothy and Velvet grass revealed the best agreement. Inclusion of further samples and testing on timothy grass revealed that timothy grass can be used instead of gx3 in this patient population. The disagreement between both platform may be explained by the interference of anti-CCD antibodies as blocking of those resulted in a negative ImmunoCAP result for the tested allergen. There was potentially a false positive result in the gx3 assay confirmed by negative results in the individual grass allergens, the component mix and clinical information. Not surprising, molecular allergen testing is useful to confirm genuine sensitisation and to prove positive mix and extract results.