ReSeqTB

Relational Sequencing TB Data Platform

A Standardized System for Grading Mutations in *Mycobacterium tuberculosis* for Association with Drug Resistance

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The Need

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"A lack of user-friendly **data analysis and interpretation tools** has been frequently cited as a major barrier to routine use of WGS techniques."

"Our experience with WGS in the clinical context of drug-resistant TB highlights **the urgent need for an internationally recognized database for standardized genotype-phenotype correlation interpretation** that can be added to and interrogated by clinicians and microbiologists who will be increasingly accessing WGS platforms at the local hospital level."

Wyres KL, et al. Pathogens 2014; 3(2):437-58.
Witney AA, et al. J Clin Microbiol 2015; 53(5):1473-83.

The ReSeqTB Solution: A Standardized System for Grading Mutations

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- Driven by Experts and the community
- Grading criteria for confidence binning of mutations
  - Frequency of a mutation in phenotypically resistant or sensitive strain
  - Homoplasy – similar trait acquired by isolates from different lineages
  - Associated MIC values and the degree of change versus a sensitive strain
  - Functional genetics associated with the mutation
- Every SNP will be associated with a "score" or a "grading index" according to the information available
- Grading drug resistance associated mutations
  - 5 bins based upon the strength of associated data for individual mutations
  - Bins are defined using a statistical approach and then revised according to the expert panels confidence of association with resistance of a mutation

Statistical Approach and Thresholds

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Observed frequency of a mutation found in phenotypically resistant of sensitive strain

- Literature data were used to calculate the frequency of each mutation in resistant (DR) and susceptible (DS) MTBC isolates
- Likelihood ratio (LR) and odd ratio (OR) were used; p-values and 95% confidence intervals associated with LR and OR have been also considered
- Thresholds used most commonly in evidence-based medicine have been adapted to grade *M. tuberculosis* mutations:

LR+ ∨ OR *p*- value	value	Interpretation	Symbol
<0.05	**≥ 10**	**High Confidence for association with resistance** – strong association of the mutation with phenotypic drug resistanc; sufficient evidence that the mutation confers or is strongly associated with drug resistance	
<0.05	**5 ≤ ... < 10**	**Moderate Confidence for association with resistance** – moderate association of the mutation with phenotypic drug resistanc; additional data desirable for improved evidence that the mutation confers or is strongly associated with drug resistance	
<0.05	**1 ≤ ... < 5**	**Minimal Confidence for association with resistance** – weak association of the mutation with phenotypic drug resistanc; inconclusive evidence that the mutation confers or is strongly associated with drug resistance. Substantial additional data required	
<0.05	**< 1**	**No association with resistance** – No evidence of association between the mutation and drug resistance	
≥0.05	-	**Indeterminate** – no statistically significant threshold reached; additional data required	Indeter

Implementation – Systematic review of literature data

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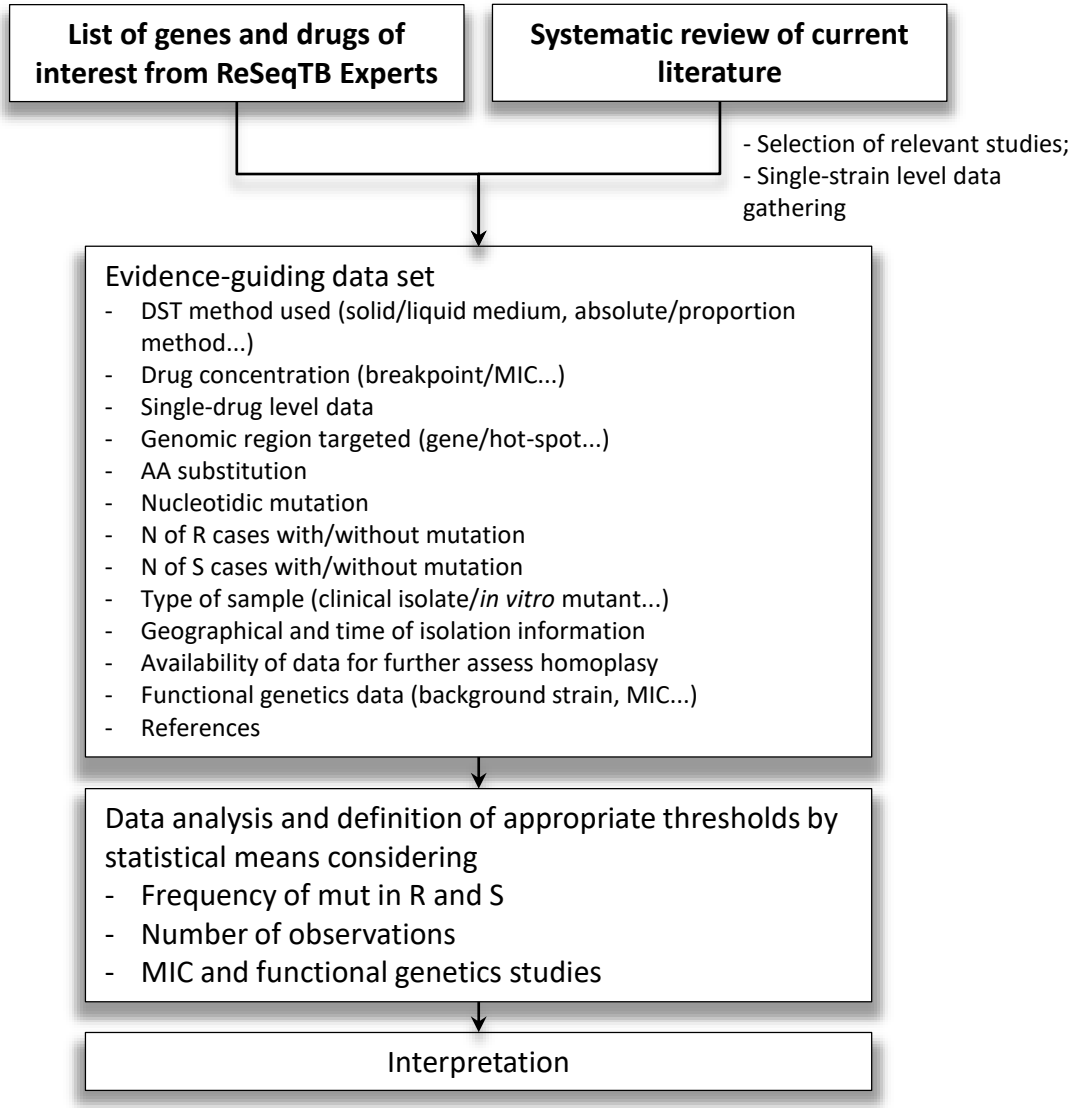

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Implementation – Systematic review of literature data

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- 11 drugs, 16 genomic loci
- 1748 unique papers screened
- Data: sequencing AND phenotypic DST
- Data from up to 43 countries and up to 13,424 isolates

Drug	Collected data				Number of studies	
	loci of interest	Total # of isolates	Isolation time frame	Represented countries	Screened	Included
Rifampicin (R)	*rpoB*	13424	1999-2014	37	459	95
Isoniazid (H)	*katG*	11847	1992-2014	42	650	127
	inhA-mabA	9407				
	furA	361				
	mshA	288				
Ethionamide & prothionamide (ETO/PTO)	*inhA-mabA*	346				
	ethA	181				
	mshA	117				
Ofloxacin (OFX)	*gyrA*	5911	1991-2013	36	243	75
	gyrB	3078				
Moxifloxacin (MFX)	*gyrA*	1019				
	gyrB	735				
Levofloxacin (LFX)	*gyrA*	449				
	gyrB	218				
Pyrazinamide (Z)	*pncA*	4949	1990-2014	36	378	81
Streptomycin (S)	*rpsL*	3263	1985-2013	43	423	104
	tap	0				
	rrs	2598				
	whiB7	0				
	gidB	812				
Amikacin (AM)	*rrs*	2105				
Capreomycin (CM)	*rrs*	2533				
	tlyA	1854				
Kanamycin (KM)	*rrs*	1727				
	tap	56				
	eis	2029				
	whiB7	56				

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Implementation – Results

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Drug (phenotypic testing)		Gene	High confidence mutations	Moderate confidence mutations	Minimal confidence mutations	No association with resistance
First-line	R	*rpoB*	D516A, D516F, D516G, D516G+L533P, D516ins, D516N, D516V, D626E, Del N518, F505V+D516Y, F514dupl, H526C, H526D, H526F, H526G, H526L, H526R, H526Y, M515I+D516Y, Q513-F514ins, Q513H+L533P, S512T, Q513K, Q513L, Q513P, S522Q, S531F, S531L, S531Q, S531W	D516Y, H526P, L533P, S522L	H526N, I572F, L511P	
	H	*inhA-mabA*		c-15t		g-47c, t-80g, T4I, g-102a
		katG	S315I, S315N, S315T, Pooled frameshifts and premature Stop codons			A110V, L499M, R463L
		furA				L68F
		mshA				N111S, A187V
Second-line (group A)	MFX	*gyrA*	A90V, D94A, D94G, D94N, D94Y, G88C, S91P			E21Q, G247S, G668D, S95T, V712L
	OFX/LFX	*gyrA*	A90V, D94A, D94G, D94H, D94N, D94Y, G88A, G88C, S91P	D89N		E21Q, G247S, G668D, S95T, T80A, V712L
		gyrB	A504V, E459K			
Second-line (group B)	AM	*rrs*	a1401g, g1484t			
	KM	*eis*	c-14t, g-10a		c-12t, g-37t	a1338c
		rrs	a1401g, c1402t, g1484t			
	CM	*rrs*	a1401g, c1402t, g1484t			c517t
		tlyA	N236K, Pooled frameshifts and premature Stop codons			D149H
	S	*rpsL*	K43G, K43R, K43T, K88Q, K88R, T40I			
		rrs	a514c, a514t, c462t, c513t, c517t			
		gidB				E92D, L16R, V110G, Pooled frameshifts and premature Stop codons

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Drug (phenotypic testing)		Gene	High confidence mutations	Moderate confidence mutations	Minimal confidence mutations	No association with resistance
Second-line (group C)	ETO/PTO	*inhA*	c-15t+I194T, c-15t+S49A	c-15t		
		ethA				Q347Stop
Second-line (group D)	Z	*pncA*	a-11g, A134V, A3E, A46V, C138Y, C14R, C72R, D12A, D12N, D49G, D49N, D63G, D8E, D8G, D8N, F94L, F94S, G108R, G132A, G132D, G132S, G162D, G17D, G24D, G97C, G97D, G97S, H137P, H51Q,H51R, H57D, H57P, H57R, H57Y, H71D, H71Q, H71Y, H82R, I6T, indel - R148ins (inframe), K96N, K96R, L116P, L116R, L120P, L151S, L159P, L172P, L19P, L4S, L85P, L85R, M175T, M175V, P54S, P62L, P62Q, Q10P, Q141P, R123P, S104R, S59P, S66P, S67P, t-12c, T135N, T135P, T142A, T142K, T142M, T160P, T168P, T76P, t-7c, V125F, V125G, V128G, V139G, V139L, V155G, V180F, V180G, V7G, W68C,W68R, Y103H, Y34D, Pooled frameshifts and premature Stop codons	A171E, K96E, K96T, M175I, P54L, Q10R, W68G	D12G, F58L, H71R, I133T, V139A	I31T, I6L, indel - c-125del, K48T, L35R, T114M, T47A

- 389 mutations (including frameshifts and premature stop codons) had minimal to high confidence values with regards to predicting drug resistance in MTBC

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Advantages of the Grading System

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Mutations not associated with DR

Drug	Gene	Mutation
H	*katG*	A110V, L499M, R463L
	inhA-mabA	g-102a, g-47c, t-80g, T4I
	mshA	A187V, N111S
MFX	*gyrA*	E21Q, G247S, G668D, S95T, V712L
OFX, LFX	*gyrA*	E21Q, G247S, G668D, S95T, T80A, V712L
KM	*rrs*	a1338c
CM	*rrs*	c517t
	tlyA	D149H
S	*rrs*	a1401g
	gidB	E92D, L16R, V110G, frameshifts and premature Stop codons
Z	*pncA*	I31T, I6L, c-125del, K48T, L35R, T114M, T47A
ETO/PTO	*ethA*	Q347Stop

Identification of potential phenotypic testing reproducibility issues

Drug	Gene	Mutation	Found in DR (TP)	Found in DS (FP)	Sensitivity		Specificity		Accuracy		Confidence value
					%	95% CIs	%	95% CIs	%	95% CIs	
R	*rpoB*	S531L	4416	20	53.2	52.2-54.3	99.6	99.4-99.8	71.0	70.2-71.7	
H	*katG*	S315T	4130	30	53.6	52.5-54.7	96.2	94.6-97.4	57.6	56.5-58.6	
OFX/OFX	*gyrA*	A90V	397	4	10.9	9.9-11.9	99.9	99.7-100.0	55.6	54.4-56.7	
AM	*rrs*	a1401g	600	5	74.2	71.0-77.2	99.6	99.1-99.9	89.8	88.5-91.1	
S	*rpsL*	K43R	358	6	62.9	58.8-66.9	98.1	95.8-99.3	75.3	72.3-78.1	
...	...	...	...	...	...	...	...	...	...	...	...

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Advantages of the Grading System

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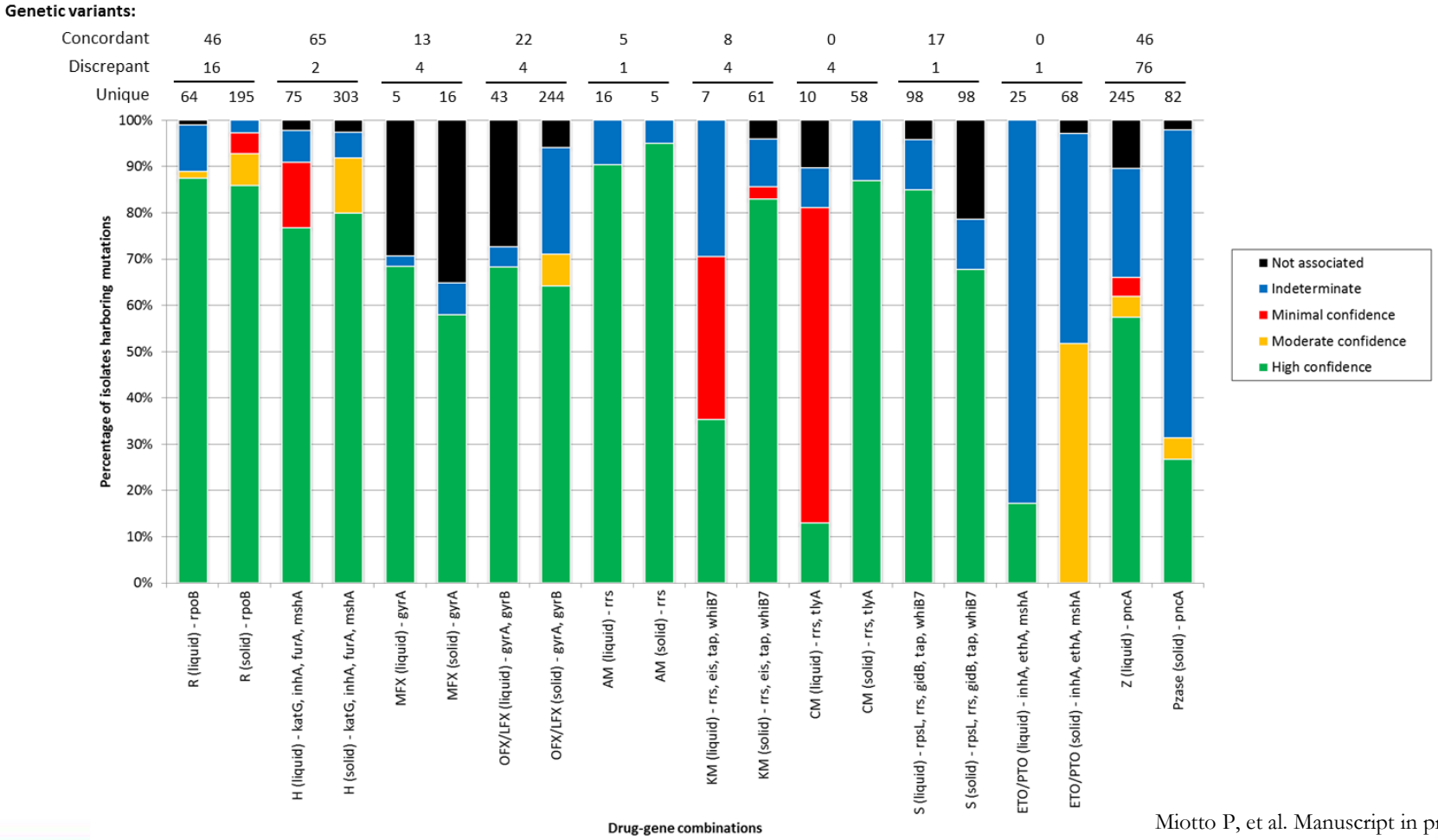
- Specific mutations showed different confidence levels depending upon the medium considered for phenotypic DST
- The number of these discrepancies varied from just one variant for drugs such as AM, S, and ETO/PTO to 76 for Z



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Advantages of the Grading System

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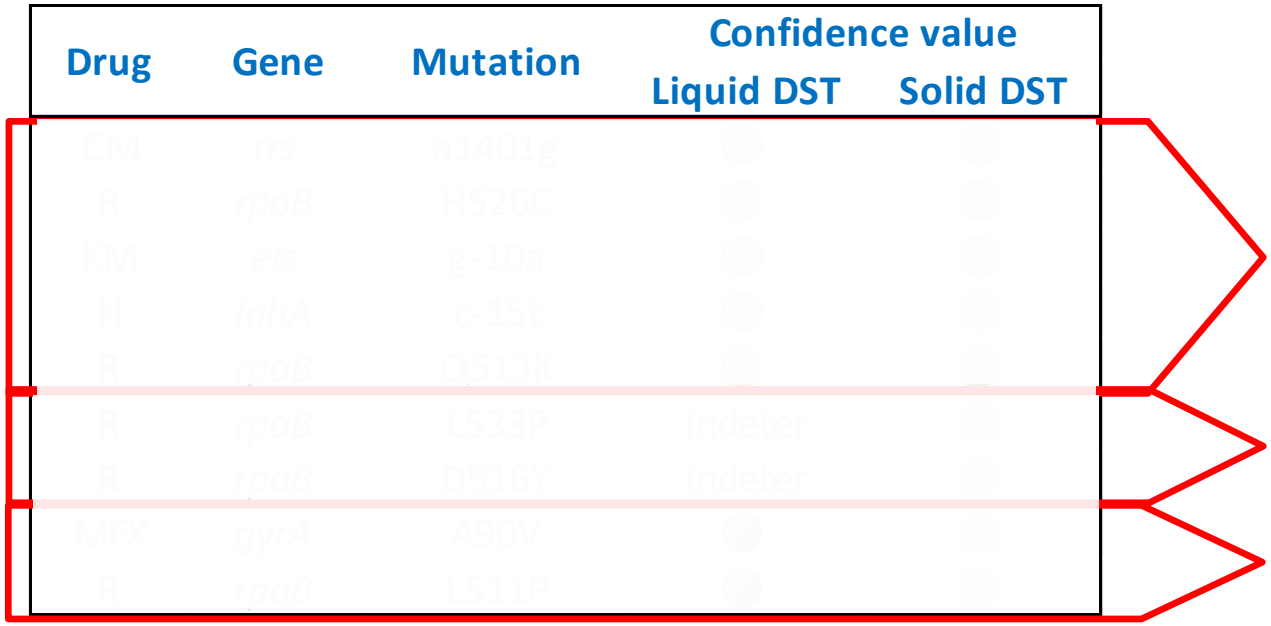

Cases testing differently on different media

MIC close to liquid testing critical concetration

Cases missed by liquid phenotypic testing

MOX liquid CC: 2 mg/L

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Implications of the Grading System

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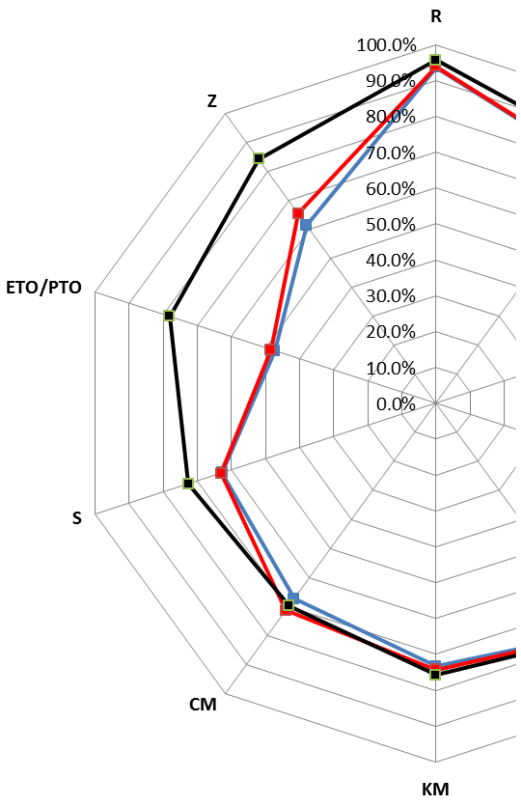
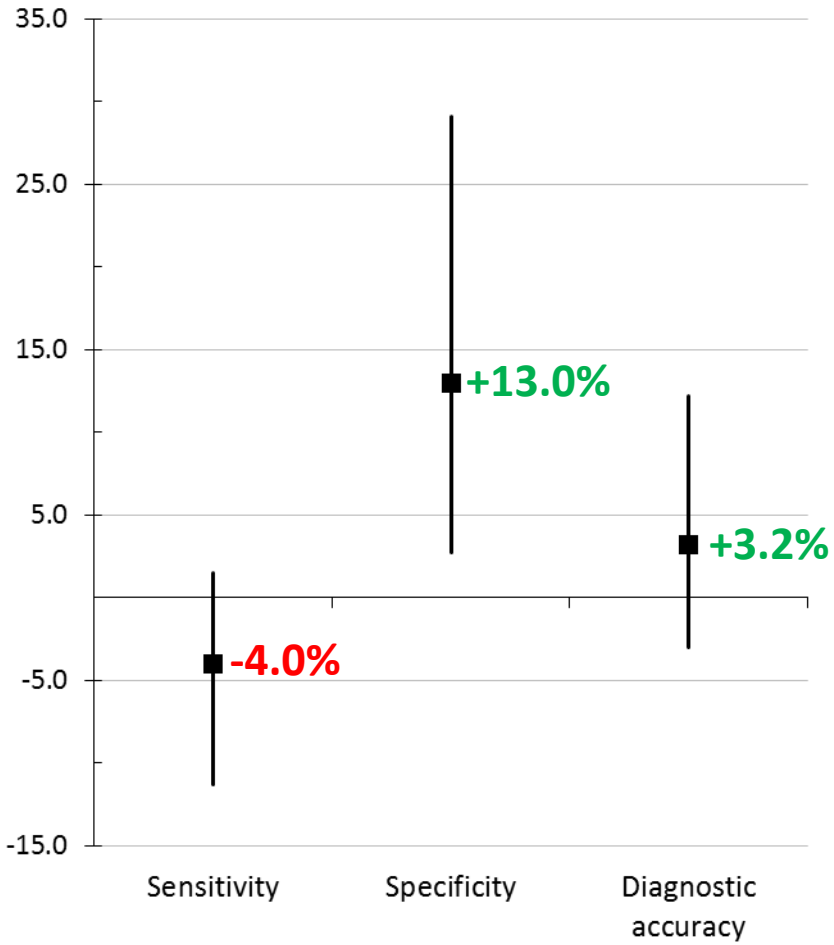
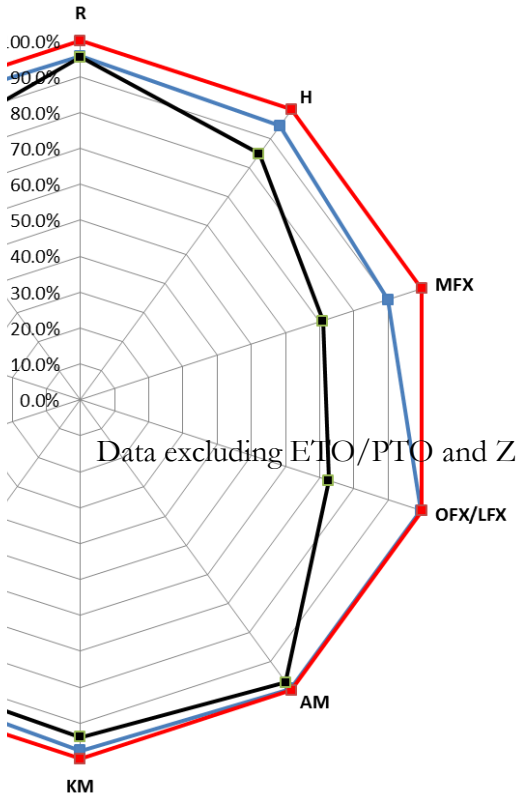
Sensitivity







Data excluding ETO/PTO and Z

- The use of graded mutations allows for improvement in specificity with a relatively small decrease in sensitivity
- Reduction of Very Major Errors (DR reported as DS)

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Summary

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- A total of 389 high, moderate, and minimal confidence mutations have been determined

- Advantages of the grading system:
  - suitable for automated classification of mutations
  - LR is a universal measure of association which is not affected by local prevalence
  - unlike sensitivities and specificities, LRs do not lead to an exaggeration of the benefits of a test or strength of an association

- The validation process is driven by statistics based on frequency of mutations, this will be improved by integrating
  - Homoplasy data
  - MIC data
  - functional genetic studies

- The validation process will need to be evaluated periodically and refined as tools and our understanding increase

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