Supplementary Materials

Identification of clinical and urine biomarkers for uncomplicated urinary tract infection using machine learning algorithms

Amal A. H. Gadalla[1*], Ida M. Friberg[2], Ann Kift-Morgan[2] Jingjing Zhang[2], Matthias Eberl[2,3], Nicholas Topley[2,3], Ian Weeks[3,4], Simone Cuff[2,3,4], Mandy Wootton[5], Micaela Gal[1], Gita Parekh[6], Paul Davis[6], Clive Gregory[1], Kerenza Hood[7], Kathryn Hughes[1], Christopher Butler[1,8†] and Nick A Francis[1,3†]

[1]Division of Population Medicine, School of Medicine, College of Biomedical and Life Sciences, Cardiff University, Cardiff, United Kingdom

[2]Division of Infection & Immunity, School of Medicine, College of Biomedical and Life Sciences, Cardiff University, Cardiff, United Kingdom

[3]Systems Immunity Research Institute, Cardiff University, Cardiff, United Kingdom

[4]Clinical Innovation Hub, School of Medicine, College of Biomedical and Life Sciences, Cardiff University, Cardiff, United Kingdom

[5]Specialist Antimicrobial Chemotherapy Unit, Public Health Wales Microbiology Cardiff, University Hospital of Wales, Cardiff, United Kingdom

[6]Mologic Ltd., Bedford Technology Park, Thurleigh, Bedford, United Kingdom

[7]Centre for Trials Research, School of Medicine, College of Biomedical and Life Sciences, Cardiff University, Cardiff, United Kingdom

[8]Nuffield Department of Primary Care Health Sciences, University of Oxford, Oxford, United Kingdom

[*]**Corresponding author:** GadallaA1@cardiff.ac.uk

[†] joint last authors

Supplementary Materials

Table S1: Performance of selection and merged models on test data subset

Data set	Algorithm	AUC	PPV	NPV	LR+	LR-	F1 score[1]	Selected predictors
PHE UTI classification, UTI prevalence 53.7%								
Clinical markers with cloudiness	RF+RFE	0.66 (0.53-0.79)[2]	0.68 (0.49-0.83)	0.65 (0.43-0.83)	1.80 (1.10-3.08)	0.47 (0.24-0.87)	0.70 (0.58-0.82)	Cloudiness
Clinical markers with turbidity	RF+RFE	0.73 (0.61-0.85)	0.77 (0.54-0.91)	0.63 (0.44-0.78)	2.95 (1.26-6.80)	0.51 (0.33-0.81)	0.67 (0.54-0.80)	Turbidity
Immunological markers	RF+RFE	0.83 (0.72-0.94)	0.77 (0.57-0.89)	0.75 (0.53-0.89)	2.82 (1.47-5.46)	0.29 (0.14-0.60)	0.77 (0.66-0.88)	14 biomarkers selected[3]
Selected clinical + selected immunological markers	RF	#						
Clinical markers with cloudiness	SVM+RFE	0.66 (0.53-0.79)	0.68 (0.49-0.83)	0.65 (0.43-0.83)	1.80 (1.10-3.08)	0.47 (0.24-0.87)	0.70 (0.54-0.80)	Cloudiness
Clinical markers with turbidity	SVM+RFE	0.73 (0.61-0.85)	0.77 (0.54-0.91)	0.63 (0.44-0.78)	2.95 (1.26-6.80)	0.51 (0.33-0.81)	0.67 (0.54-0.80)	Turbidity
Immunological markers	SVM+RFE	0.81 (0.69-0.93)	0.79 (0.59-0.91)	0.73 (0.52-0.88)	3.17 (1.53-6.54)	0.32 (0.16-0.62)	0.77 (0.66-0.88)	NGAL
Selected clinical with cloudy+ selected immunological markers	SVM	0.81 (0.68-0.93)	0.85 (0.65-0.95)	0.78 (0.57-0.90)	4.94 (1.98-12.40)	0.25 (0.12-0.51)	0.82 (0.72-0.92)	Cloudiness and NGAL
Selected clinical with turbidity+ selected immunological markers	SVM	0.79 (0.66-0.92)	0.76 (0.56-0.89)	0.72 (0.54-0.87)	2.71 (1.40-5.25)	0.33 (0.17-0.66)	0.76 (0.65-0.87)	Turbidity and NGAL
EAU UTI classification, UTI prevalence 64.8%								
Clinical markers with cloudiness	RF+RFE	0.69 (0.55-0.82)	0.79 (0.61-0.91)	0.57 (0.34-0.77)	2.00 (1.08-3.75)	0.41 (0.21-0.76)	0.76 (0.65-0.87)	Cloudiness
Clinical markers with turbidity	RF+RFE	0.68 (0.55-0.81)	0.82 (0.60-0.94)	0.48 (0.31-0.67)	2.57 (1.03-6.49)	0.58 (0.39-0.85)	0.66 (0.53-0.79)	Turbidity

Supplementary Materials

Immunological markers	RF+RFE	0.80 (0.68-0.93)	0.75 (0.58-0.87)	0.64 (0.36-0.86)	1.62 (1.04-2.55)	0.30 (0.12-0.76)	0.80 (0.69-0.91)	NGAL, MMP9, IL-17A, IFN-γ, Fibrinogen, IL-1β, IL-16 and CCL2
Selected clinical + selected immunological markers	RF	#						
Clinical markers with cloudiness	SVM+RFE	0.72 (0.58-0.86)	0.71 (0.55-0.84)	0.58 (0.29-0.83)	1.37 (0.94-1.96)	0.38 (0.14-1.04)	0.78 (0.67-0.89)	Cloudiness and severity of being unwell
Clinical markers with turbidity	SVM+RFE	0.82 (0.70-0.93)	0.77 (0.60-0.88)	0.67 (0.39-0.87)	1.83 (1.11-2.96)	0.26 (0.11-0.67)	0.81 (0.71-0.91)	Turbidity, severity of being unwell and severity of foul smell in urine
Immunological markers	SVM+RFE	0.85 (0.73-0.96)	0.74 (0.59-0.86)	0.73 (0.39-0.93)	1.57 (1.06-2.35)	0.21 (0.06-0.69)	0.82 (0.72-0.92)	NGAL and MMP9
Selected clinical with cloudiness+ selected immunological markers	SVM	0.71 (0.55-0.87)	0.73 (0.57-0.85)	0.70 (0.35-0.92)	1.46 (1.01-2.07)	0.22 (0.07-0.82)	0.81 (0.71-0.91)	Cloudiness, severity of being unwell, NGAL and MMP9
Selected clinical with Turbidity+ selected immunological markers	SVM	0.85 (0.73-0.96)	0.77 (0.61-0.88)	0.82 (0.48-0.97)	1.77 (1.16-2.77)	0.13 (0.03-0.52)	0.85 (0.75-0.95)	Turbidity, severity of being unwell, severity of foul smell in urine, NGAL and MMP9

[1] F1-score: harmonic mean of precision and recall

[2] 95% confidence interval of the performance metric

[3] NGAL, MMP9, IL-1β, HSA, Desmosine, IL-16, CCL3, Fibrinogen, CCL17, IL-17A, MMP8, IL-12p70, IL-8 and CCL13

[#] merging was not considered due to the large number of selected predictors

AUC: Area under the curve

PPV: Positive predictive value

NPP: Negative predictive value

LR+ and LR-: positive and negative likelihood ratio

Supplementary Materials

SVM: Support vector machine

RF: Random Forest

RFE: Recursive Feature Elimination

NGAL: Neutrophil gelatinase-associated lipocalin

MMP: Matrix metalloproteinase

IL: Interleukin

HSA: Human serum albumin

CCL: CC chemokine ligands

IFN-γ: Interferon-γ

Supplementary Materials

Table S2: Hyperparameters for models following predictors' selection. For the selected predictors please refer to Table 2 and Table S1.

Data set	RF: mtry	SVM: sigma	SVM: C	Selected predictors
POETIC UTI classification, UTI prevalence 42.6%				
Clinical markers with cloudiness	One predictor			Cloudiness
Clinical markers with turbidity	One predictor			Turbidity
Immunological markers	2			IL-1β and MMP9
Selected clinical with cloudiness + selected immunological markers	2			Cloudiness, IL-1β and MMP9
Selected clinical with turbidity + selected immunological markers	3			Turbidity, IL-1β and MMP9
Clinical markers with cloudiness		0.25	0.25	Cloudiness
Clinical markers with turbidity		0.38	1	Turbidity and age category
Immunological markers		0.66	0.25	MMP9, NGAL, IL-8/CXCL8 and IL-1β
Selected clinical with cloudy+ selected immunological markers		0.51	0.25	Cloudiness, MMP9, NGAL, IL-8/CXCL8 and IL-1β
Selected clinical with turbidity+ selected immunological markers		0.24	0.25	Turbidity, age category, MMP9, NGAL, IL-8/CXCL8 and IL-1β
PHE UTI classification, UTI prevalence 53.7%				
Clinical markers with cloudiness	One predictor			Cloudiness

Supplementary Materials

Clinical markers with turbidity	One predictor			Turbidity
Immunological markers	Not taken further			14 biomarkers selected[3]
Selected clinical + selected immunological markers	Not trained			
Clinical markers with cloudiness		0.25	0.25	Cloudiness
Clinical markers with turbidity		0.30	1	Turbidity
Immunological markers		97.6	0.5	NGAL
Selected clinical with cloudiness+ selected immunological markers		21.5	1	Cloudiness and NGAL
Selected clinical with Turbidity+ selected immunological markers		55.4	0.5	Turbidity and NGAL
EAU UTI classification, UTI prevalence 64.8%				
Clinical markers with cloudiness	One predictor			Cloudiness
Clinical markers with turbidity	One predictor			Turbidity
Immunological markers	Not taken further			NGAL, MMP9, IL-17A, IFN-γ, Fibrinogen, IL-1β, IL-16 and CCL2
Selected clinical + selected immunological markers	Not trained			
Clinical markers with cloudiness		1.59	1	Cloudiness and severity of being unwell

Supplementary Materials

Clinical markers with turbidity	0.44	1	Turbidity, severity of being unwell and severity of foul smell in urine
Immunological markers	2.27	0.25	NGAL and MMP9
Selected clinical with cloudiness+ selected immunological markers	0.32	0.25	Cloudiness, severity of being unwell, NGAL and MMP9
Selected clinical with Turbidity+ selected immunological markers	0.15	0.25	Turbidity, severity of being unwell, severity of foul smell in urine, NGAL and MMP9

Supplementary Materials

Table S3: Uro-pathogen or potential significant isolate used to define UTI positivity

Potentially significant isolates	**Uropathogens**
Enterobacteriaceae (*E. coli, Klebsiella species*)	*E. coli*
Proteus	*Klebsiella* species
Enterococcus species	*Enterobacter* species
Staphylococcus aureus	*Citrobacter* species
Staphylococcus saprophyticus	*Serratia* species
Coagulase negative *Staphylococcus* species	*Morganella* species
Pseudomonas	*Proteus* species
Yeasts	*Pantoea* species
	Group B *Streptococcus* species
	Staphylococcus saprophyticus
	Salmonella species

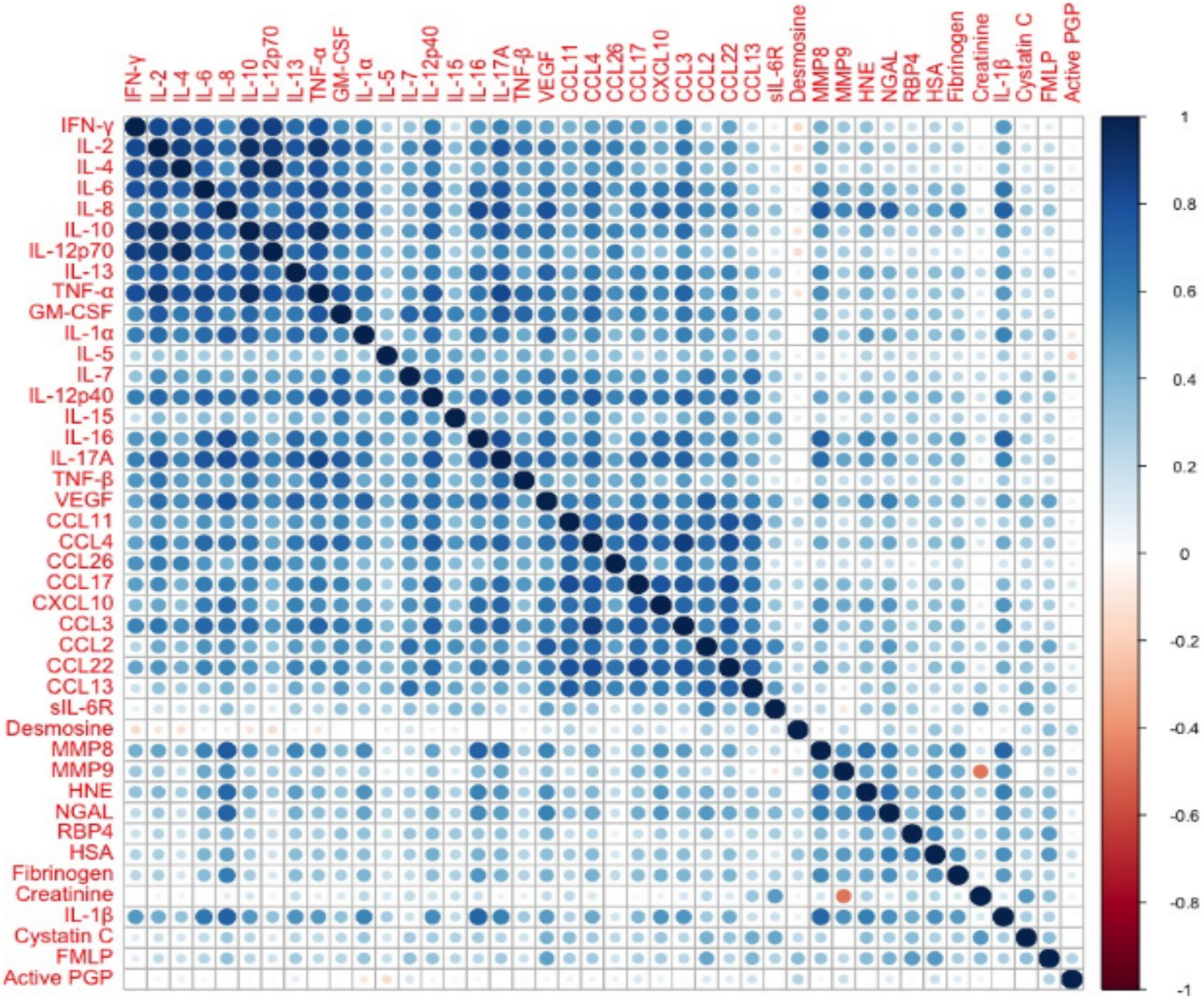


Figure S1: Correlation between immunological markers. Circle size and colour scale represent correlation strength and direction, respectively, between the 42 examined immunological markers. Spearman correlation test was used. R package "corrplot" was used for this visual presentation.

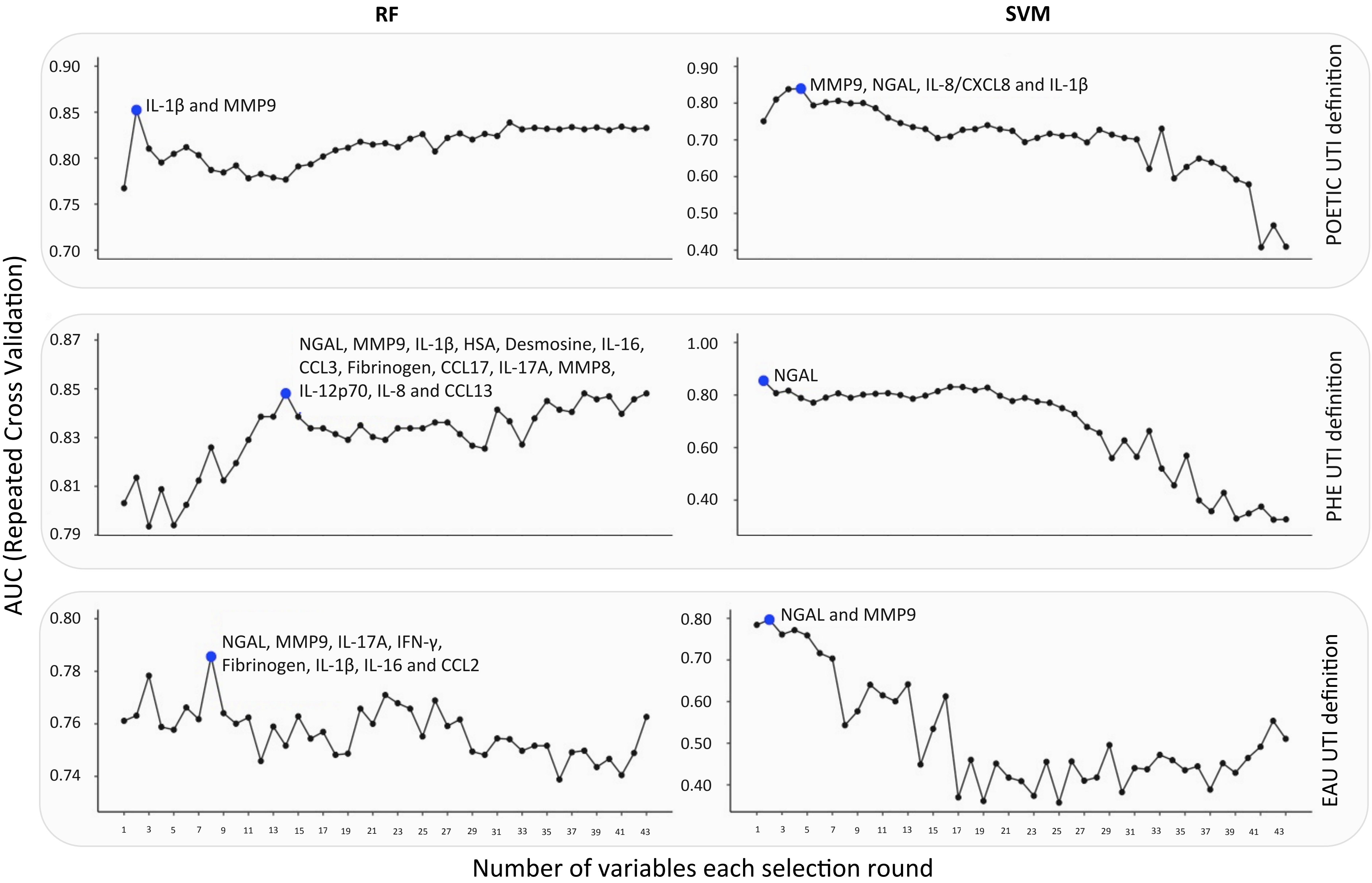


Figure S2: Feature selection among immunological markers using different UTI classification guidelines. POETIC: Point of care testing for urinary tract infection in primary care, PHE: Public Health England, EAU: European Association of Urology, AUC: Area under the ROC curve, RF: Random forest and SVM: Support vector machine

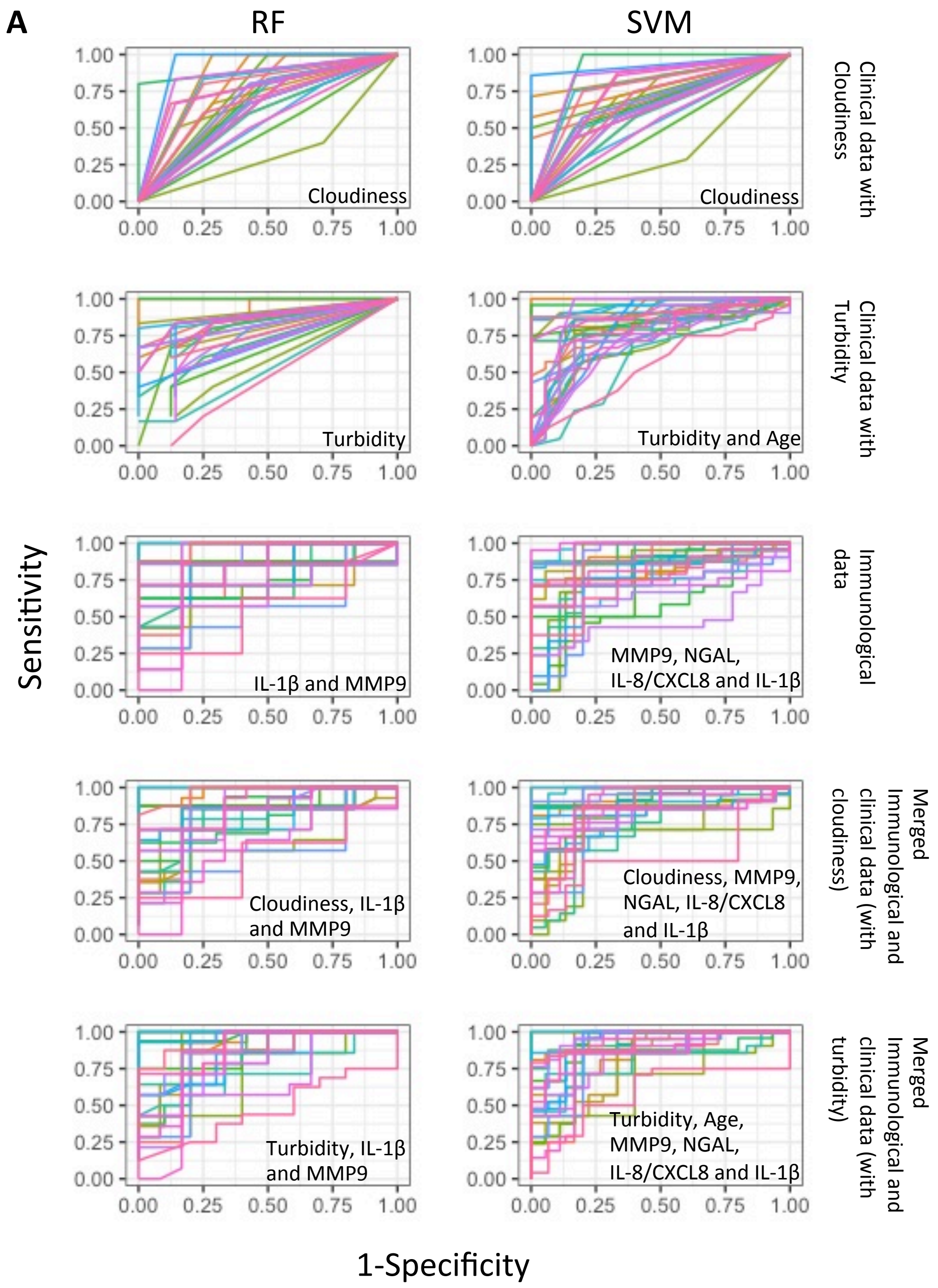
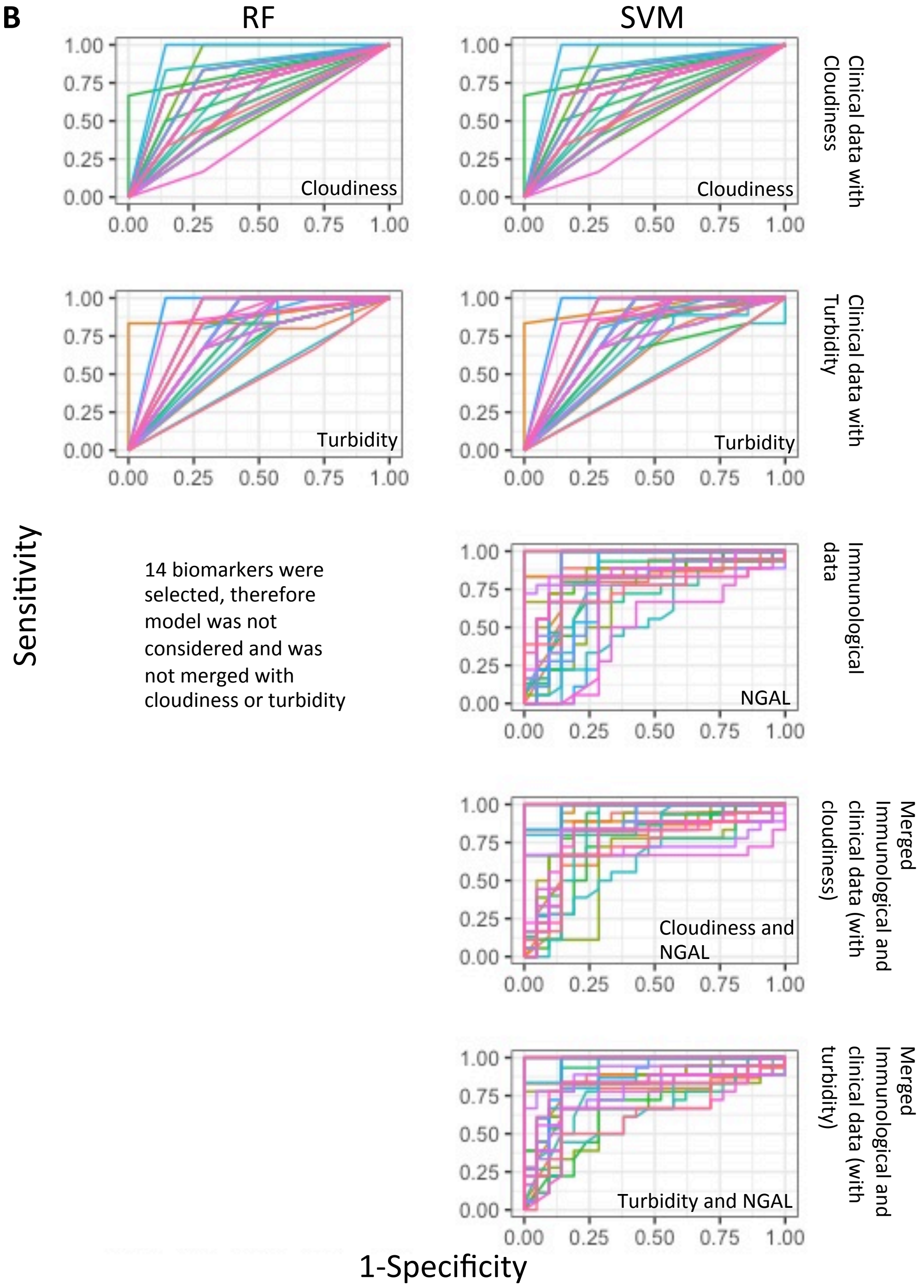
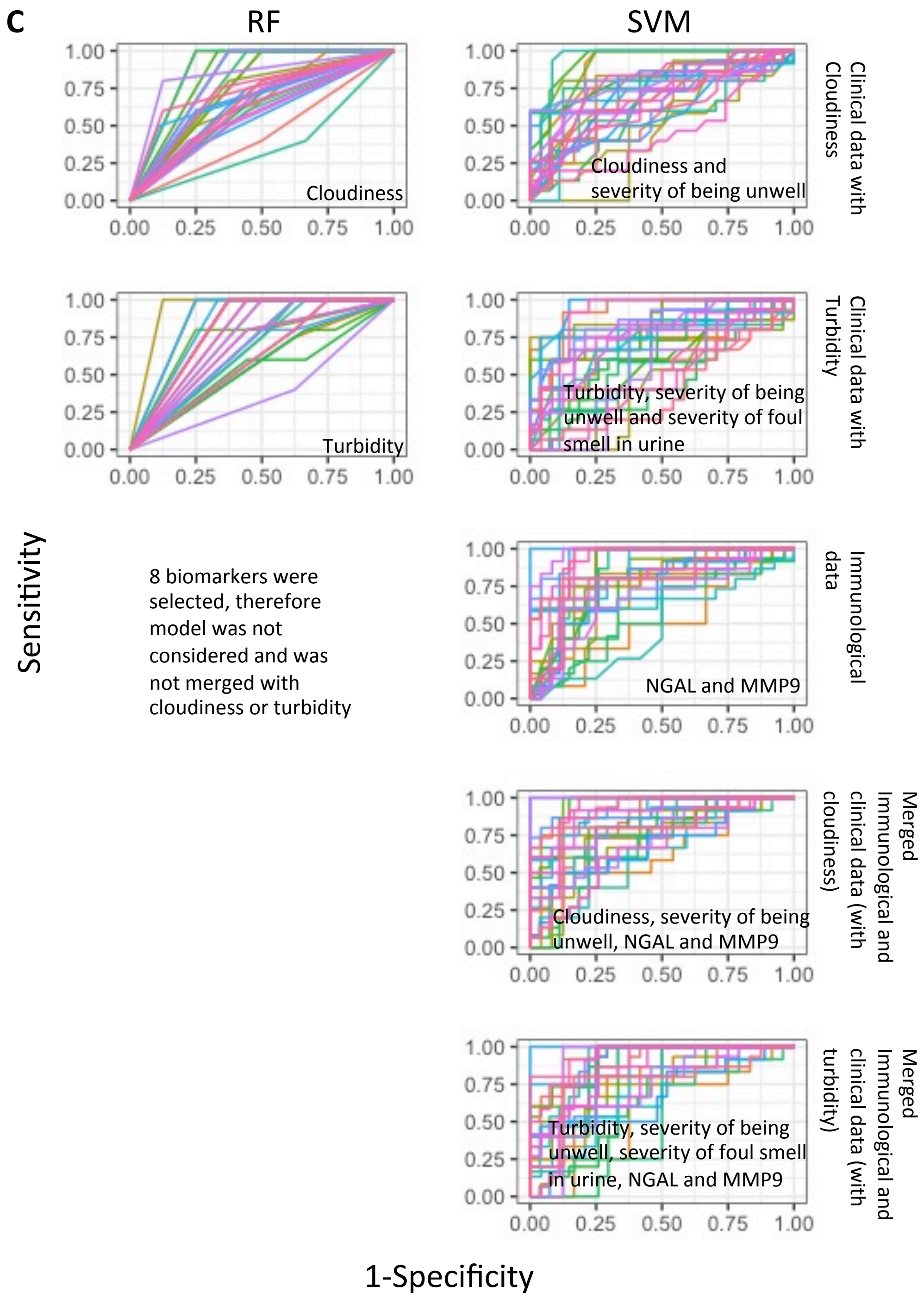


Figure S3: Area under the ROC curve for three repeats of 10-fold cross-validation from models following selection using RF- or SVM- recursive feature elimination. **A.** UTI classified according to the POETIC protocol (Point of care testing for urinary tract infection in primary care trial). **B.** UTI classified according to the Public Health England (PHE) guidelines. **C.** UTI classified according to the European Association of Urology (EAU) guidelines. Predictors included in the model following selection are given in text boxes within each plot. RF: random forest, SVM: support vector machine



14 biomarkers were selected, therefore model was not considered and was not merged with cloudiness or turbidity



8 biomarkers were selected, therefore model was not considered and was not merged with cloudiness or turbidity