Supplementary Tables and Figures for:

Title: Systematic Comparison of Published Host Gene Expression Signatures for Bacterial/Viral Discrimination

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List of Supplementary Materials:

Table S1. Conditions represented in validation datasets.

Condition	**Number of cases**
All Viral Infections	1679
Viral infection, NOS	451
Influenza	431
Respiratory Syncytial Virus	406
Rhinovirus	209
Enterovirus	58
Poly-viral	66
Adenovirus	31
Human Herpesvirus 6	10
Other	12
All Bacterial Infections	951
Bacterial Infection, NOS	469
Staphylococcus aureus	118
Escherichia coli	64
Burkholderia pseudomallei	45
Unspecified *Staphylococcus*	40
Streptococcus pneumoniae	39
Mycoplasma	30
Salmonella typhi	25
Coagulase-Negative *Staphylococcus*	16
Streptococcus pyogenes	14
Unspecified *Streptococcus*	12
Poly-bacterial	11
Other	68
All Non-Infectious Illnesses	537
SIRS, NOS	306
Systemic Lupus Erythematosus	110
Kawasaki Disease	90
Still’s Disease	31
Healthy	1427

Validation datasets include patients with a wide range of conditions. Species with less than 10 subjects are grouped into “Other”. NOS = Not Otherwise Specified. SIRS = Systemic Inflammatory Response Syndrome.

Table S2. Top 20 Genes in Composite Signature by Average Coefficient.

Rank	Bacterial vs. non-Bacterial			Viral vs. non-Viral		
	Gene Name	**Ensembl ID**	**Avg. Coefficient**	**Gene Name**	**Ensembl ID**	**Avg. Coefficient**
1	CETP	ENSG00000087237	0.0217	IFI27	ENSG00000165949	0.0702
2	RPGRIP1	ENSG00000092200	-0.0181	OTOF	ENSG00000115155	0.0190
3	FCER1A	ENSG00000179639	-0.0167	FCER1A	ENSG00000179639	-0.0188
4	IFI27	ENSG00000165949	-0.0165	LARP1	ENSG00000155506	0.0160
5	PDE9A	ENSG00000160191	-0.0151	OAS1	ENSG00000089127	0.0155
6	PLAC8	ENSG00000145287	0.0146	XAF1	ENSG00000132530	0.0140
7	SLPI	ENSG00000124107	0.0126	IRF9	ENSG00000213928	0.0140
8	JUP	ENSG00000173801	-0.0126	KREMEN1	ENSG00000183762	0.0138
9	ADGRE1	ENSG00000174837	0.0125	QARS	ENSG00000172053	-0.0134
10	ZNF823	ENSG00000197933	-0.0118	IFI44	ENSG00000137965	0.0132
11	LILRB1	ENSG00000104972	0.0115	AL136295.5	ENSG00000259529	0.0132
12	NRG1	ENSG00000157168	0.0113	KLRB1	ENSG00000111796	-0.0132
13	VPS13A	ENSG00000197969	0.0113	ADGRE3	ENSG00000131355	-0.0131
14	LTA4H	ENSG00000111144	0.0110	RSAD2	ENSG00000134321	0.0130
15	VAMP5	ENSG00000168899	0.0109	EEF1G	ENSG00000254772	-0.0125
16	YWHAE	ENSG00000108953	0.0109	LY6E	ENSG00000160932	0.0120
17	ACTR2	ENSG00000138071	0.0107	EEF1B2	ENSG00000114942	-0.0119
18	TSPO	ENSG00000100300	0.0107	EIF4B	ENSG00000063046	-0.0117
19	ANKRD20A11P	ENSG00000215559	-0.0106	AC000120.1	ENSG00000243107	-0.0116
20	ADK	ENSG00000156110	-0.0105	AP002990.1	ENSG00000255508	-0.0109

Relative gene importance was characterized by the average of each gene's coefficient in all models. For genes that mapped to multiple microarray probes, the coefficient with the largest magnitude was used for the average. This analysis was performed using coefficient data from the composite signature comprised of 864 genes.

Table S3. Heterogeneity in DOR of Bacterial and Viral Classification Signatures.

Signature	Bacterial vs. non-Bacterial			Viral vs. non-Viral		
	% Heterogeneity (95% CI)	Q-statistic	p-value	% Heterogeneity (95% CI)	Q-statistic	p-value
TS1	52 [27.1-68.4]	60.40	< 0.001	79.4 [72.3-84.7]	179.89	< 0.001
HL2	66.2 [51.3-76.5]	94.59	< 0.001	67.2 [54.3-76.5]	116.02	< 0.001
LC2	53.8 [30.1-69.5]	62.79	< 0.001	50.6 [26.6-66.7]	66.75	< 0.001
XW2	46.7 [19.8-64.5]	60.00	0.002	66.4 [53.2-75.9]	116.01	< 0.001
GS3	72.7 [59.6-81.5]	91.46	< 0.001	66.1 [51.2-76.5]	94.49	< 0.001
LS3	61.6 [44-73.7]	83.35	< 0.001	67 [54-76.4]	115.19	< 0.001
SB4	70.1 [55.1-80.1]	80.36	< 0.001	57.4 [36.9-71.2]	72.76	< 0.001
SK7	67.6 [51.3-78.4]	77.11	< 0.001	71.5 [59.6-79.9]	112.32	< 0.001
SB8	72.5 [58.7-81.7]	83.55	< 0.001	61.7 [43.8-73.9]	80.91	< 0.001
RC10	74.1 [62.3-82.2]	100.40	< 0.001	71.9 [60.7-80]	121.10	< 0.001
SN10	70 [54.6-80.2]	76.76	< 0.001	66 [50.7-76.6]	91.21	< 0.001
SR10	69.9 [56.8-79]	102.86	< 0.001	70.3 [58.9-78.5]	127.95	< 0.001
AK11	68.2 [53.7-78.2]	91.30	< 0.001	73.2 [62.6-80.8]	126.96	< 0.001
BF11	67.1 [52.7-77.1]	97.24	< 0.001	51.4 [30.2-66.2]	80.30	< 0.001
NC19	63.7 [47.3-74.9]	88.05	< 0.001	70.9 [60-78.8]	134.09	< 0.001
SL20	67.7 [52.8-77.9]	89.80	< 0.001	61 [43.9-72.8]	89.67	< 0.001
MW23	66.8 [51.9-77]	93.25	< 0.001	77.4 [69.6-83.2]	172.66	< 0.001
ZG25	71.9 [60.1-80.1]	113.70	< 0.001	71.4 [60.6-79.3]	133.06	< 0.001
MS29	71.8 [57.1-81.4]	77.89	< 0.001	75.9 [66.2-82.8]	128.58	< 0.001
PT29	69.8 [56.5-79.1]	99.36	< 0.001	65 [50.9-75.1]	108.67	< 0.001
RC31	73.9 [62.8-81.6]	114.74	< 0.001	62.9 [47.5-73.8]	99.79	< 0.001
HS33	74.2 [63.4-81.9]	116.50	< 0.001	70.5 [59.2-78.7]	128.82	< 0.001
HL34	72.5 [61.1-80.5]	116.32	< 0.001	77 [69-82.9]	169.47	< 0.001
ZG48	63.9 [47.6-75.1]	88.57	< 0.001	70.7 [59.5-78.8]	129.59	< 0.001
MR59	73.8 [63-81.5]	118.51	< 0.001	73.1 [63.2-80.4]	141.52	< 0.001
TW96	70.5 [57.8-79.4]	104.97	< 0.001	77.5 [69.6-83.3]	168.88	< 0.001
MW139	69.9 [57.1-78.9]	106.39	< 0.001	73.9 [64.4-80.8]	149.16	< 0.001
AK398	73.3 [61.9-81.3]	112.34	< 0.001	77.2 [68.9-83.3]	157.81	< 0.001
All	66 [50.9-76.4]	94.01	< 0.001	70.4 [59.3-78.5]	131.98	< 0.001

Heterogeneity in the Diagnostic Odds Ratio (DOR) was evaluated for each host gene expression signature in bacterial and viral classification. Percent heterogeneity with a 95% confidence interval is presented with the Q-statistic and p-value. Values were computed using the Mantel-Haenszel method.

Table S4. Predictive Values in Patient Subgroups.

	Bacterial vs. non-Bacterial				Viral vs. non-Viral			
Parameter	PPV (%)	NPV (%)	Prevalence (%)	N (subjects/studies)	PPV (%)	NPV (%)	Prevalence (%)	N (subjects/studies)
All subjects	65 (61-69)	89 (87-91)	32.7%	2887 / 31	84 (81-86)	84 (82-87)	46.9%	3584 / 37
Age	-	-	-	-	-	-	-	-
Adult	73 (68-79)	88 (85-92)	37.4%	1183 / 18	85 (80-89)	90 (87-93)	41.4%	1268 / 14
12 - 18 years	61 (42-81)	93 (84-100)	26.3%	132 / 6	80 (57-95)	94 (84-100)	35.6%	95 / 6
2 - 11 years	51 (41-61)	87 (80-94)	31.0%	373 / 7	73 (62-83)	82 (75-89)	38.0%	352 / 10
3 months - 1 year	51 (36-66)	89 (79-96)	28.9%	183 / 8	88 (83-93)	70 (61-77)	62.2%	576 / 17
<3 months	84 (75-92)	86 (79-93)	44.2%	320 / 8	90 (85-94)	67 (58-76)	68.7%	547 / 16
Race	-	-	-	-	-	-	-	-
All Subjects	60 (54-66)	90 (87-92)	31.5%	1389 / 12	81 (77-86)	79 (74-83)	49.3%	1157 / 12
Black	70 (59-80)	86 (78-93)	41.1%	311 / 11	78 (67-88)	76 (66-85)	50.2%	254 / 12
White	52 (43-60)	91 (88-95)	26.1%	684 / 11	82 (76-88)	78 (72-83)	50.6%	686 / 12
Asian	81 (60-95)	87 (70-100)	42.7%	87 / 9	69 (25-100)	93 (75-100)	29.5%	33 / 7
Other	37 (9-64)	91 (78-100)	19.1%	72 / 5	81 (58-100)	69 (48-88)	52.9%	79 / 6
Ethnicity	-	-	-	-	-	-	-	-
Hispanic or Latino	68 (56-78)	90 (83-96)	34.6%	302 / 9	85 (74-93)	85 (76-93)	46.0%	220 / 11
Not Hispanic or Latino	56 (49-63)	89 (85-92)	30.3%	407 / 4	81 (76-86)	77 (71-82)	50.7%	474 / 5

Positive predictive values (PPV) and negative predictive values (NPV) with 95% confidence intervals of bacterial and viral classification, stratified by different clinical parameters. N is represented by the number of subjects / the number of datasets used for validation. The “All Subjects” group under the “Race” category represents all subjects for which racial information was available.

Table S5. Overall Signature Performance in COVID-19 Classification.

Dataset Makeup	N	Viral vs. non-Viral		COVID vs. non-Viral		COVID vs. non-COVID	
		Median AUC	IQR	Median AUC	IQR	Median AUC	IQR
COVID + healthy	9	0.867	[0.823-0.893]	-	-	-	-
COVID + other infection + healthy	4	0.831	[0.789-0.855]	0.839	[0.801-0.865]	0.804	[0.735-0.831]

Viral vs. non-viral AUCs were calculated for each of the 29 signatures in thirteen COVID-19 datasets. The Viral vs. non-Viral metrics describe the ability of the signatures to classify COVID-19 as viral in nature when compared to healthy controls in nine datasets. These performance metrics can be compared to signature performance for viral classification more generally (Table 3). Four datasets included non-COVID infections such as other viral infections or bacterial infections. In this case, we report two additional performance measures. COVID vs. non-Viral describes the ability of the signatures to discriminate COVID-19 from other non-viral diseases (e.g., healthy or bacterial infection). The COVID vs. non-COVID comparison discriminated subjects with COVID-19 infection from all other phenotypes (other viral infections, bacterial infections, healthy). Mean AUCs were first generated for each signature across the datasets in the parameter group, weighted by the number of subjects in each validation dataset. The median of the weighted AUC values and IQR were then calculated and presented here. N represents the number of datasets for the specified cohort composition.

Fig. S1. Flow Diagram for the Inclusion of Validation Datasets. Transcriptome studies, consisting of microarray or RNA sequencing data, were systematically reviewed and selected from the Gene Expression Omnibus (GEO) and ArrayExpress with an approach similar to that outlined in the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement. After screening the initial 781 studies, 47 studies met our inclusion criteria.

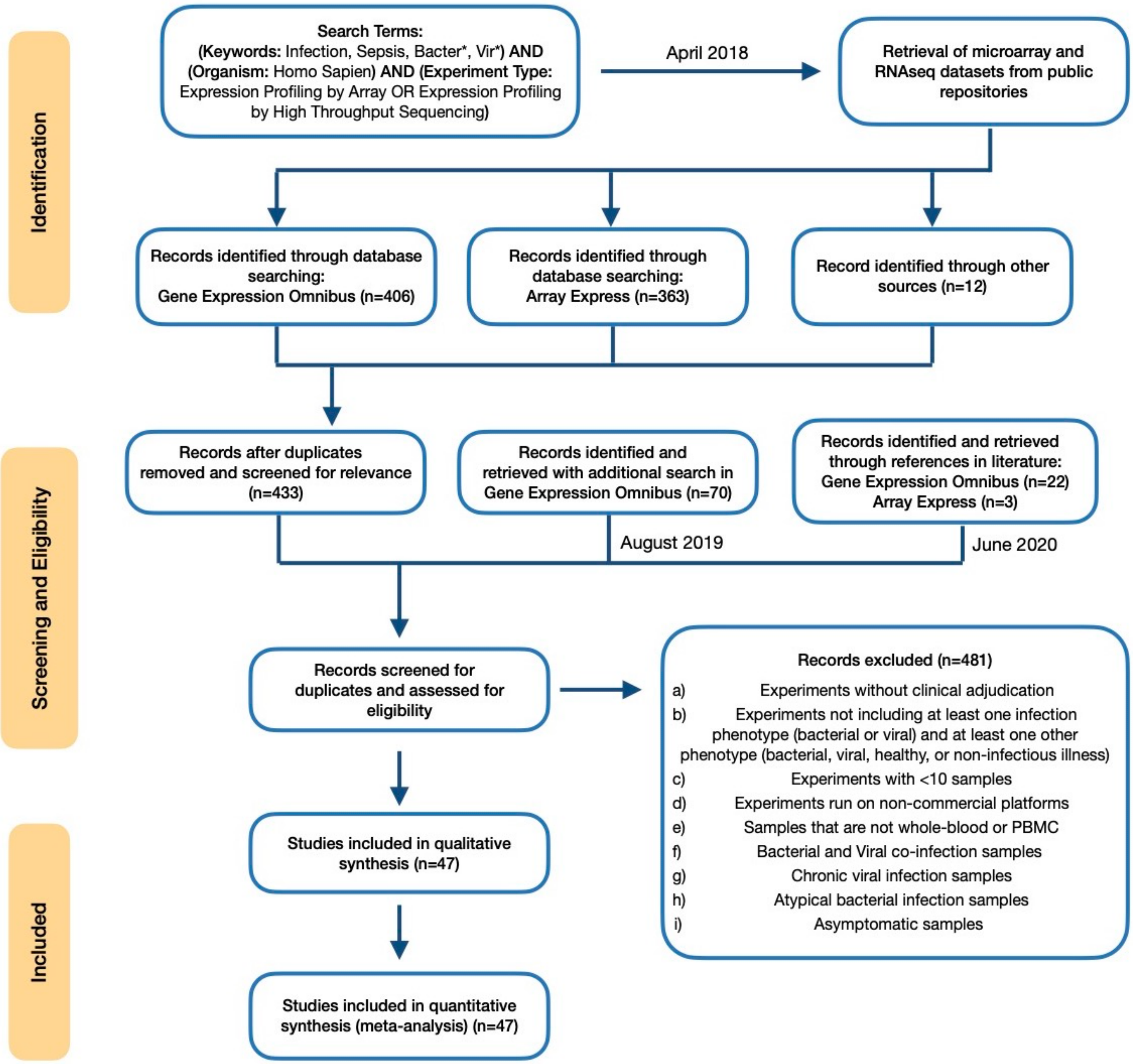
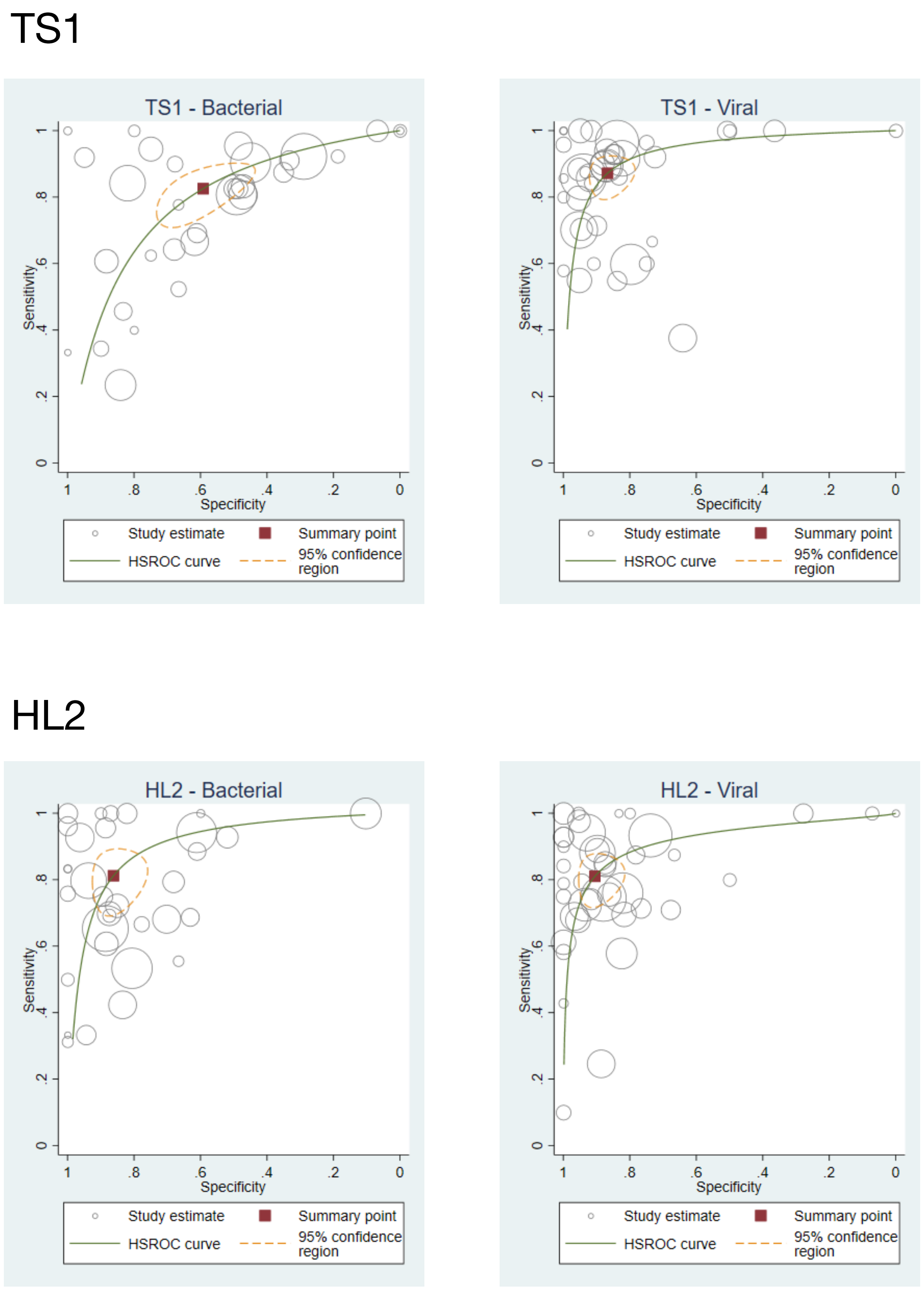

Identification
Screening and Eligibility
Included
Search Terms:
(Keywords: Infection, Sepsis, Bacter*, Vir*) AND (Organism: Homo Sapien) AND (Experiment Type: Expression Profiling by Array OR Expression Profiling by High Throughput Sequencing)
April 2018
Retrieval of microarray and RNAseq datasets from public repositories
Records identified through database searching: Gene Expression Omnibus (n=406)
Records identified through database searching: Array Express (n=363)
Record identified through other sources (n=12)
Records after duplicates removed and screened for relevance (n=433)
Records identified and retrieved with additional search in Gene Expression Omnibus (n=70)
Records identified and retrieved through references in literature: Gene Expression Omnibus (n=22) Array Express (n=3)
August 2019
June 2020
Records screened for duplicates and assessed for eligibility
Records excluded (n=481)
a) Experiments without clinical adjudication
b) Experiments not including at least one infection phenotype (bacterial or viral) and at least one other phenotype (bacterial, viral, healthy, or non-infectious illness)
c) Experiments with <10 samples
d) Experiments run on non-commercial platforms
e) Samples that are not whole-blood or PBMC
f) Bacterial and Viral co-infection samples
g) Chronic viral infection samples
h) Atypical bacterial infection samples
i) Asymptomatic samples
Studies included in qualitative synthesis (n=47)
Studies included in quantitative synthesis (meta-analysis) (n=47)

Fig. S2. HSROC Curves for Evaluated Signatures. Hierarchical summary ROC (HSROC) curves were generated for each signature, based on the signature's confusion matrices for all validation datasets. [1/14]

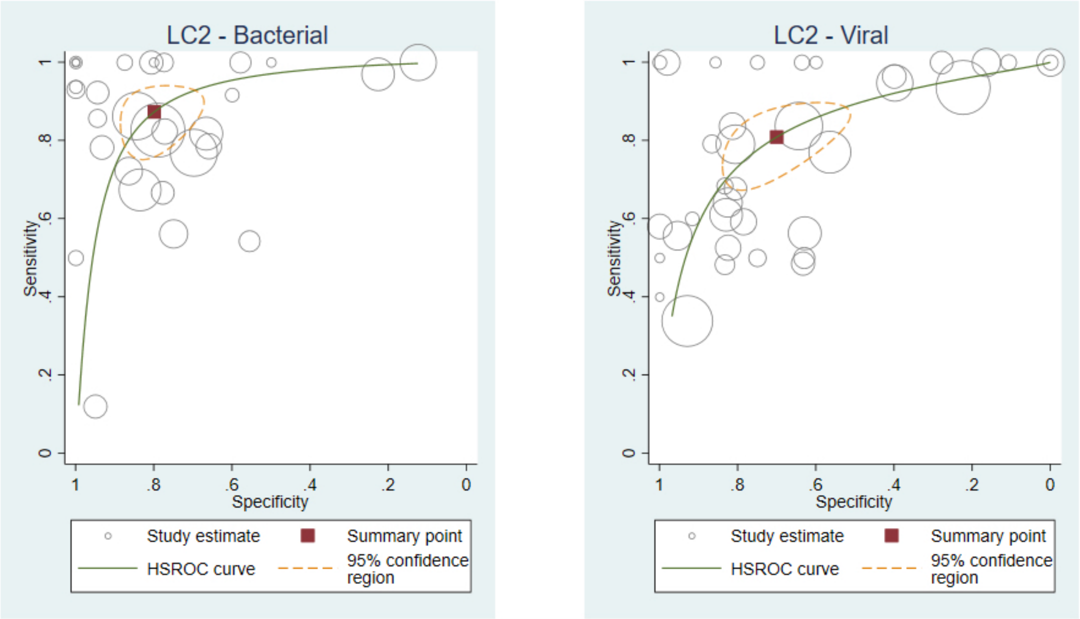


TS1

HL2

Fig. S2. HSROC Curves for Evaluated Signatures (continued) [2/14]

LC2



XW2

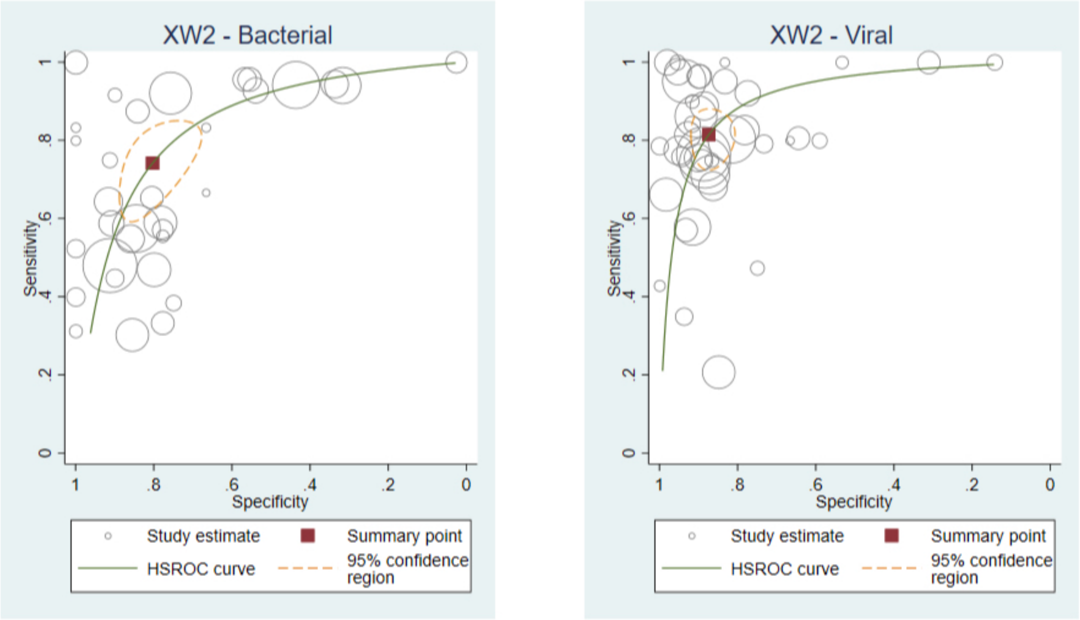
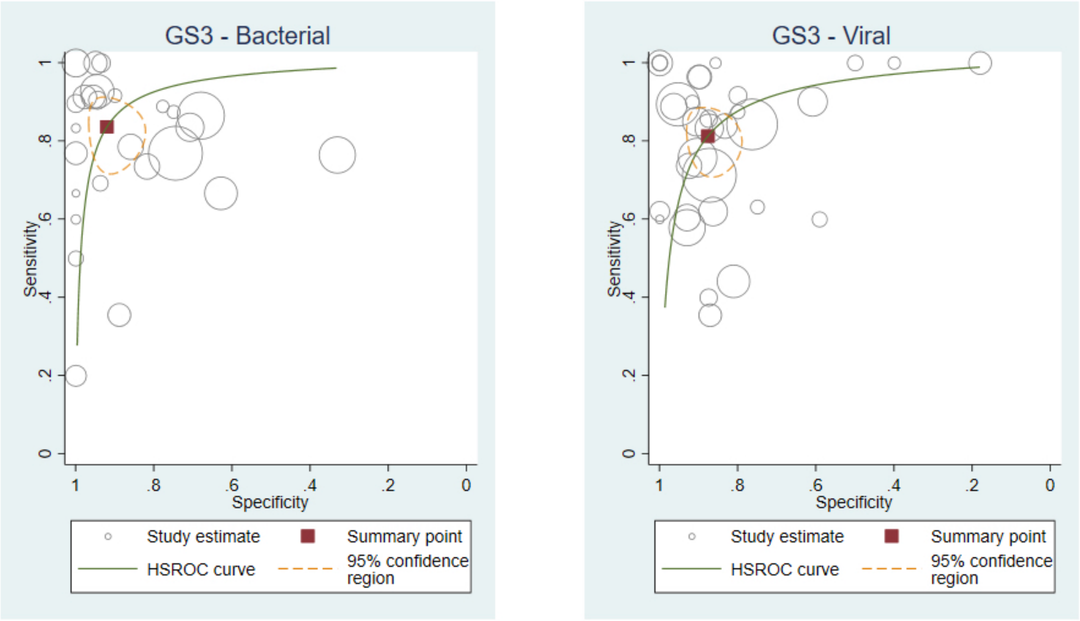


Fig. S2. HSROC Curves for Evaluated Signatures (continued) [3/14]

GS3



LS3

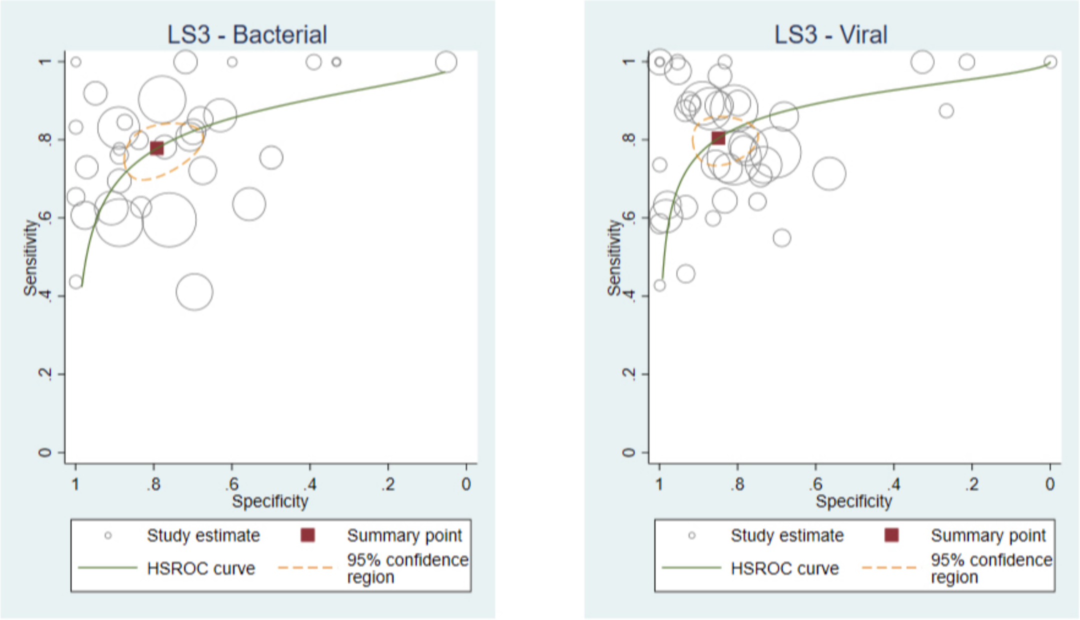
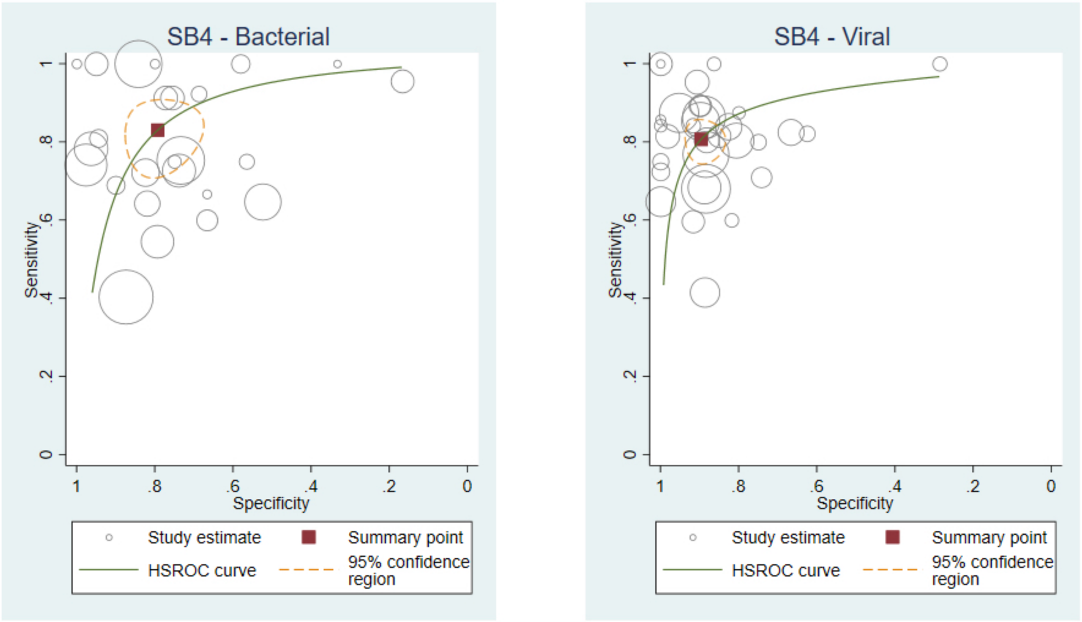


Fig. S2. HSROC Curves for Evaluated Signatures (continued) [4/14]

SB4



SK7

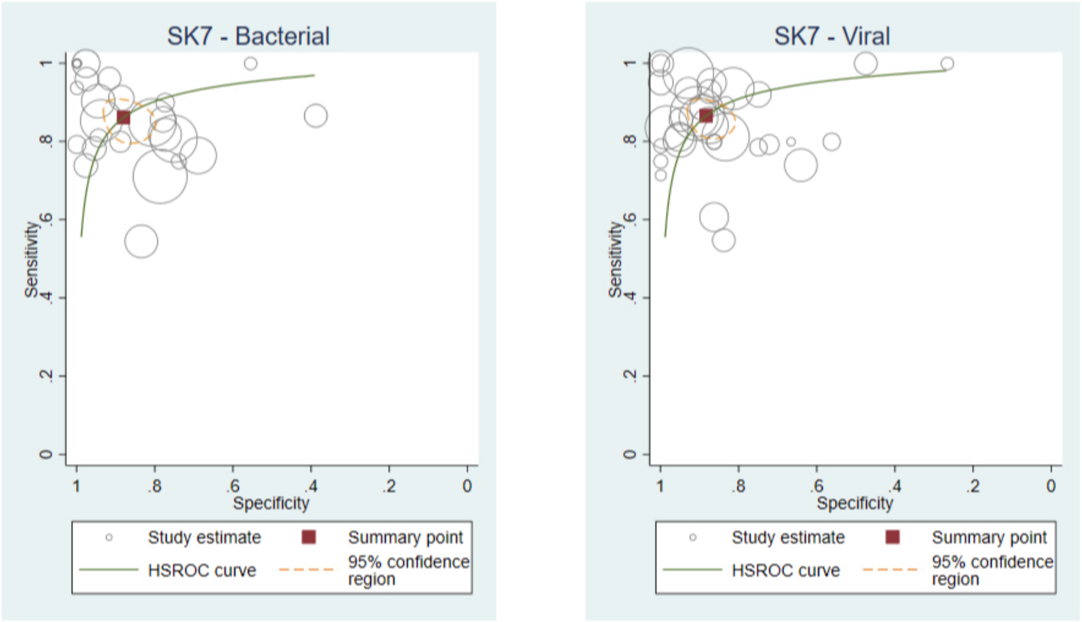
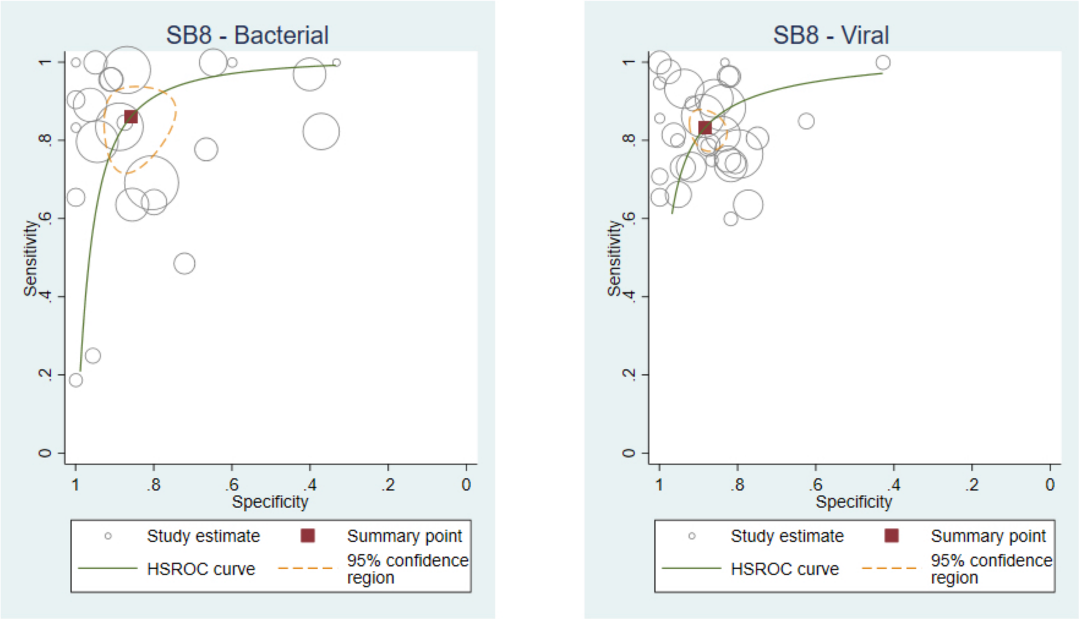


Fig. S2. HSROC Curves for Evaluated Signatures (continued) [5/14]

SB8



RC10

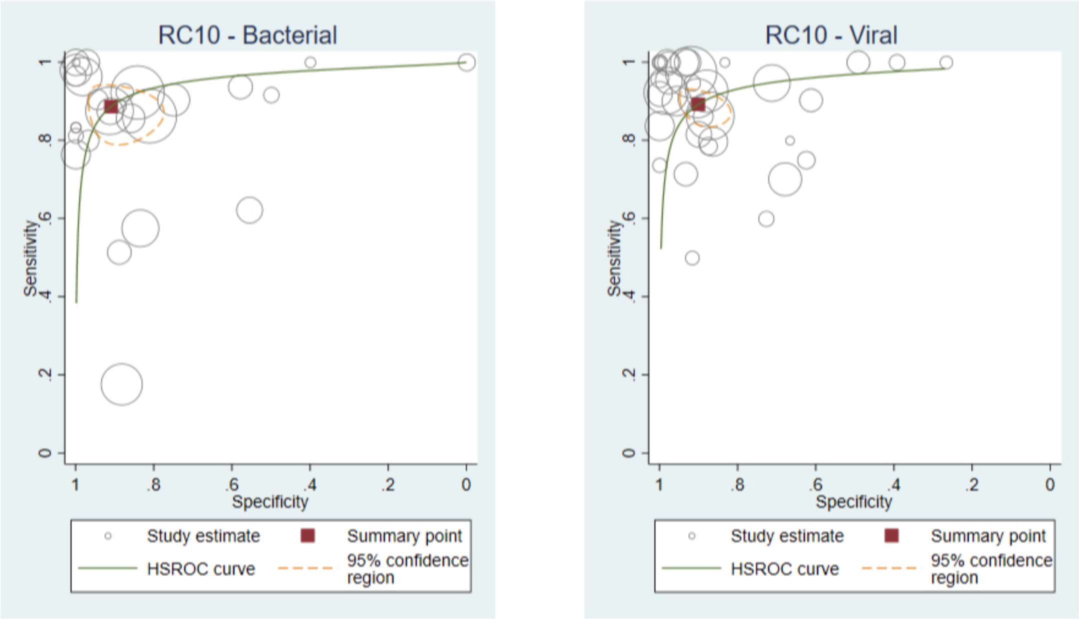
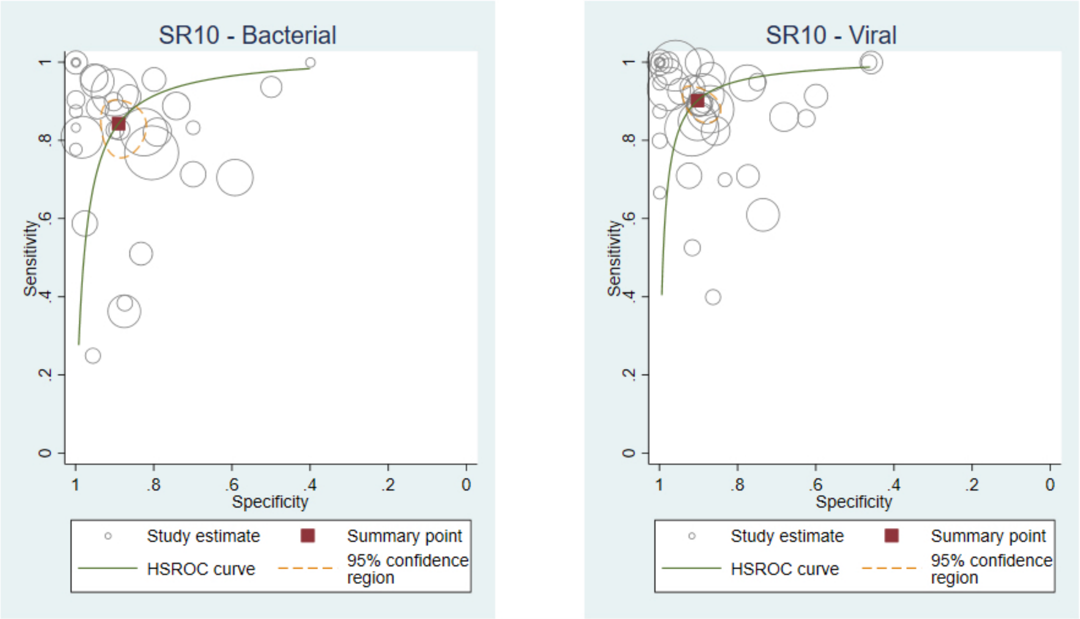


Fig. S2. HSROC Curves for Evaluated Signatures (continued) [6/14]

SR10



SN10

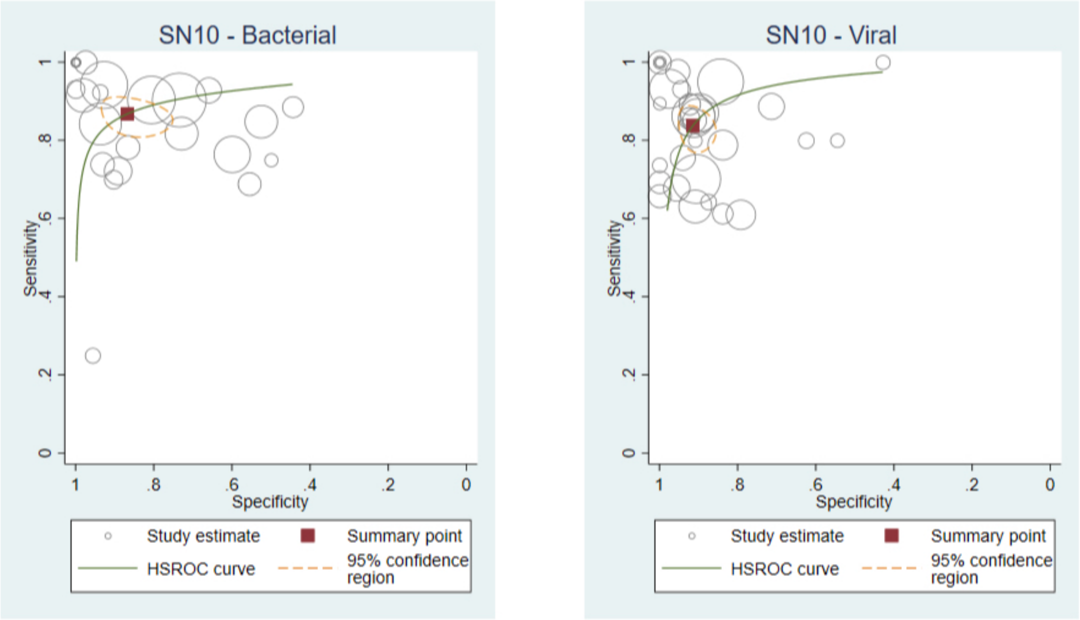
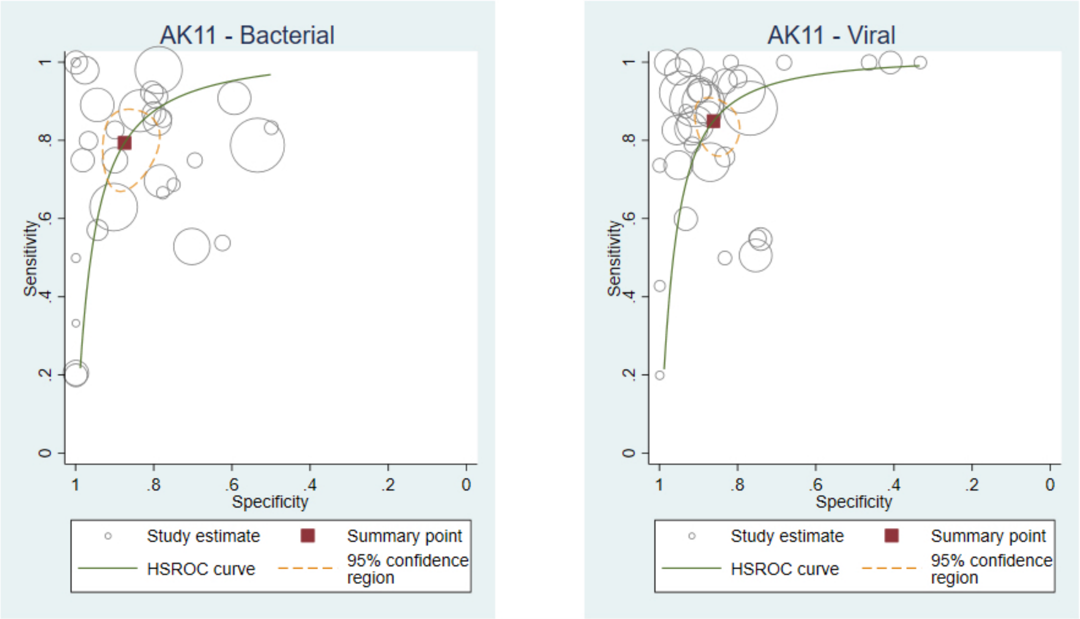


Fig. S2. HSROC Curves for Evaluated Signatures (continued) [7/14]

AK11



BF11

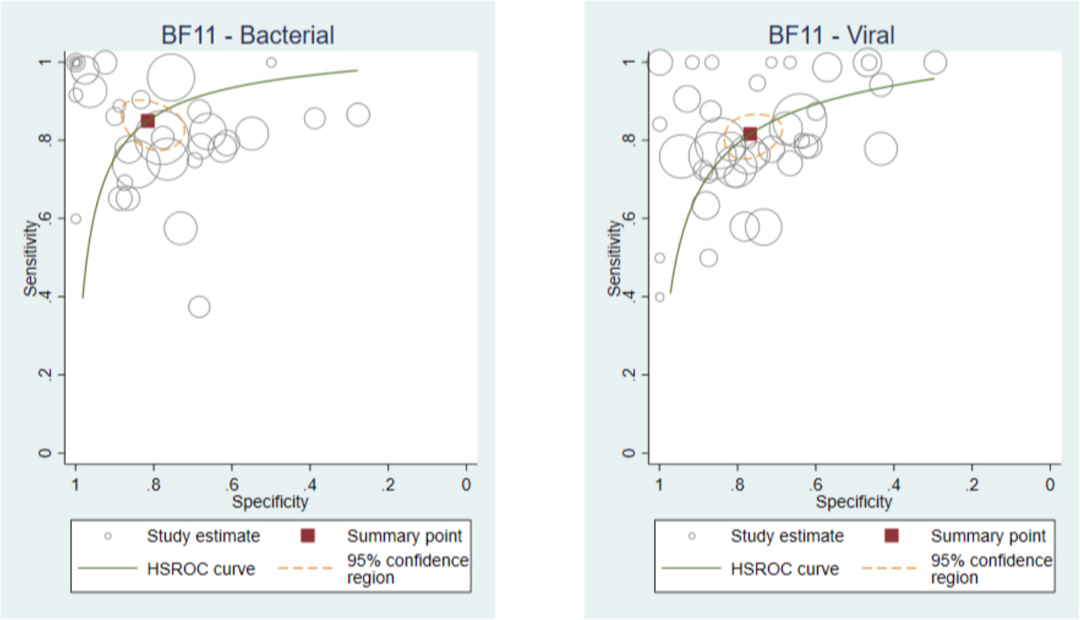
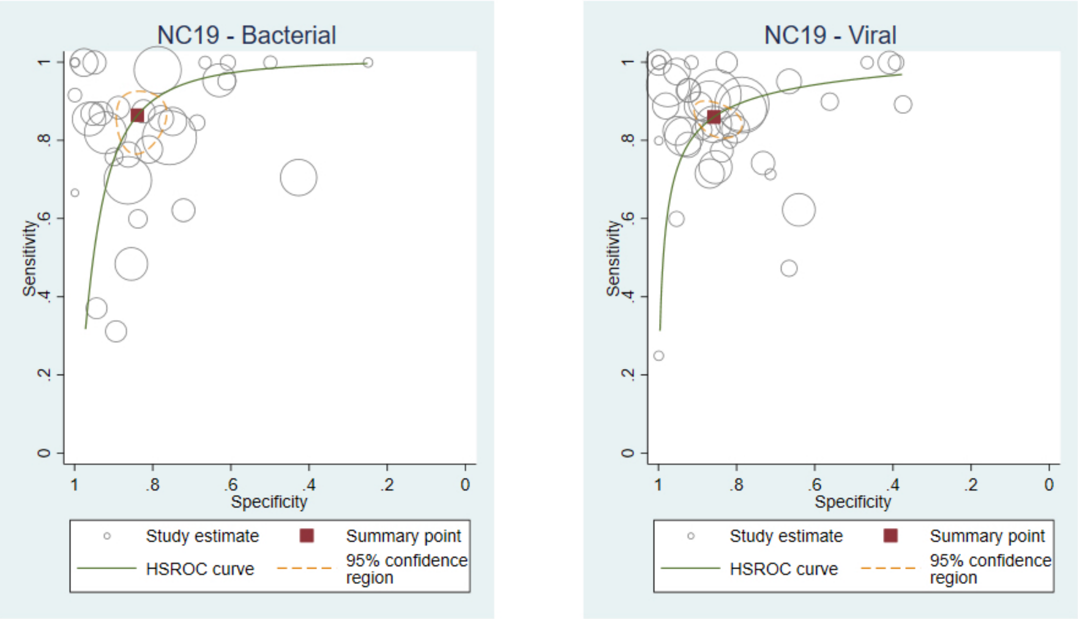


Fig. S2. HSROC Curves for Evaluated Signatures (continued) [8/14]

NC19



SL20

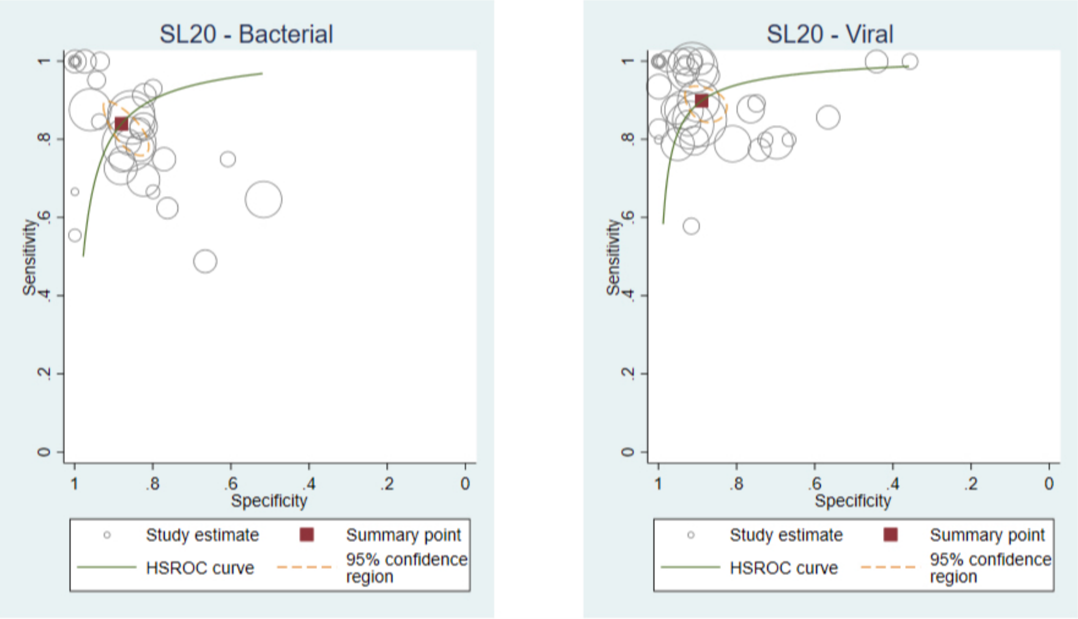
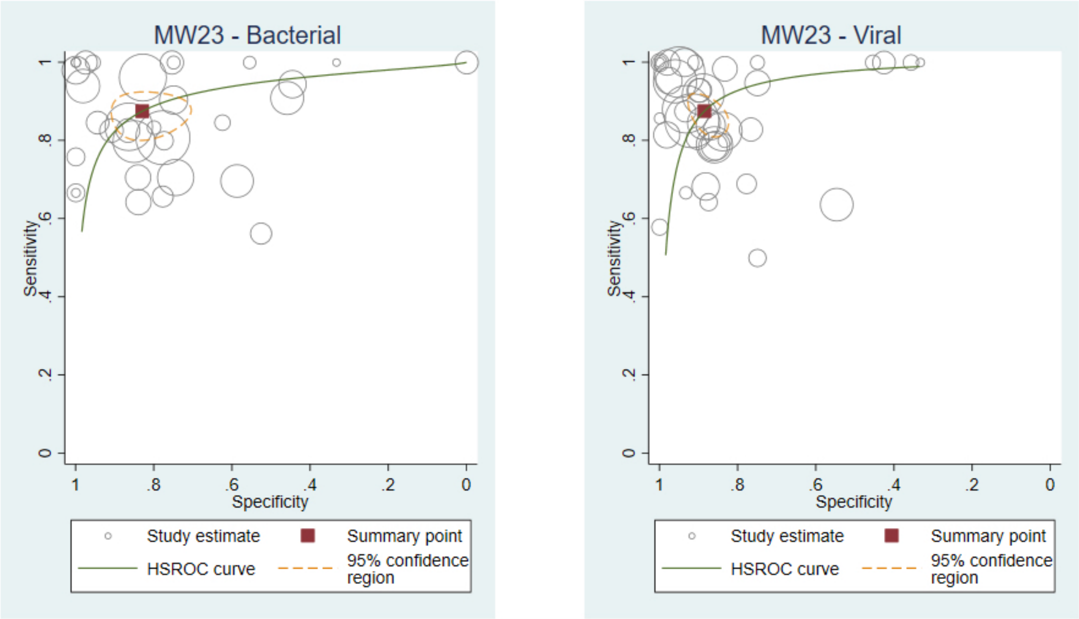


Fig. S2. HSROC Curves for Evaluated Signatures (continued) [9/14]

MW23



ZG25

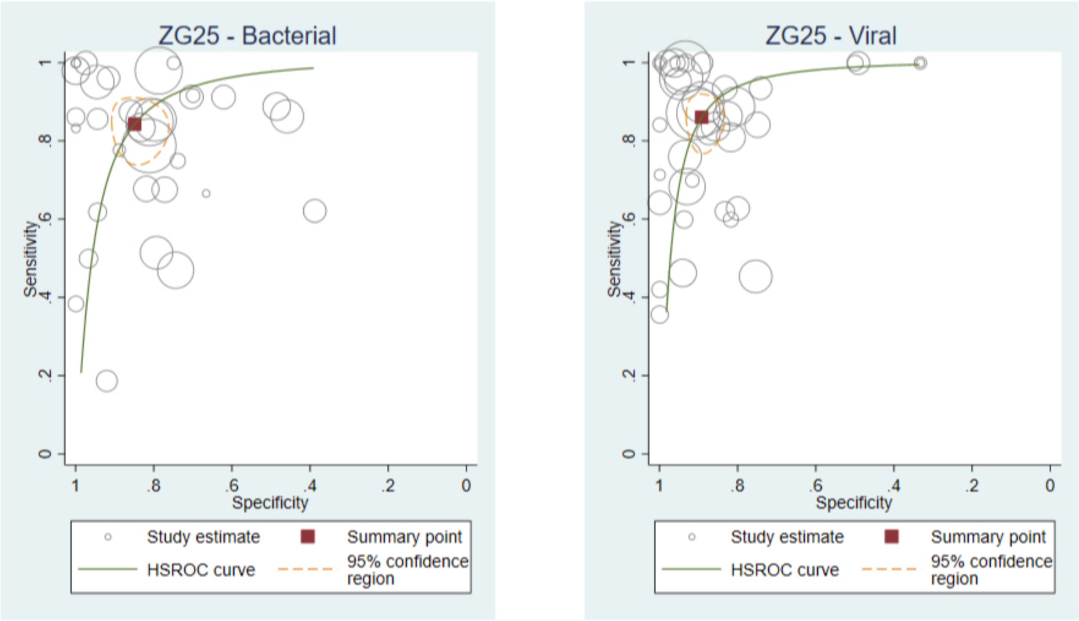
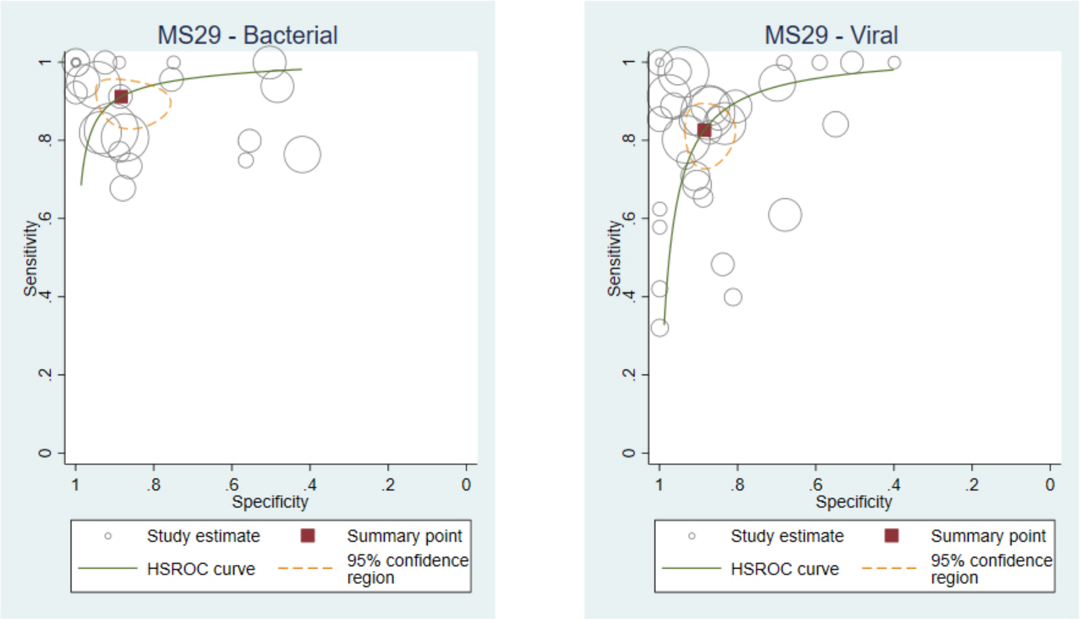


Fig. S2. HSROC Curves for Evaluated Signatures (continued) [10/14]

MS29



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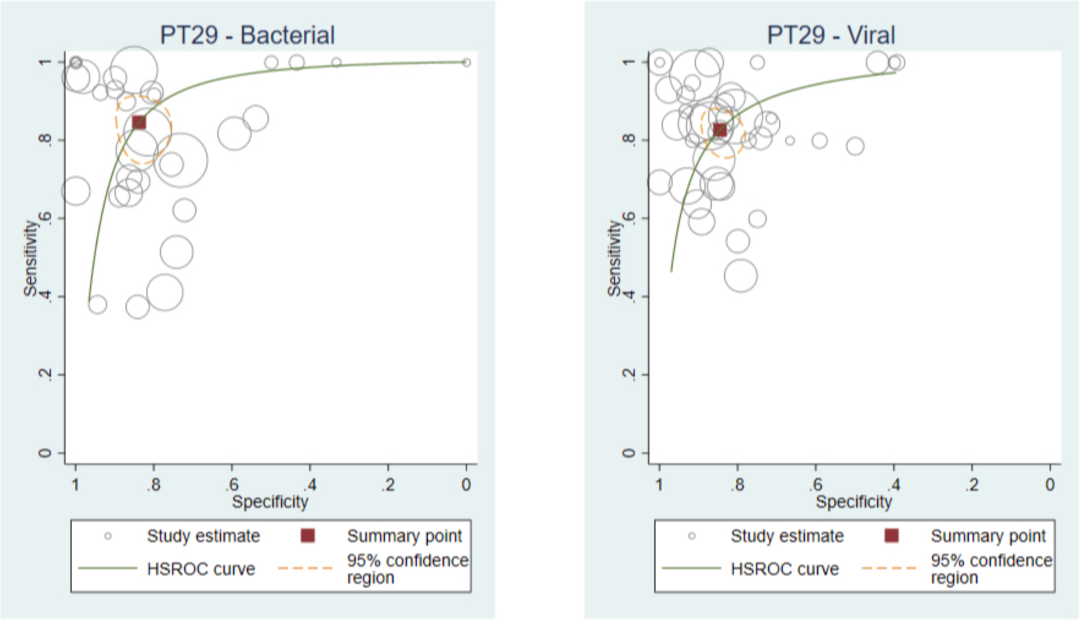
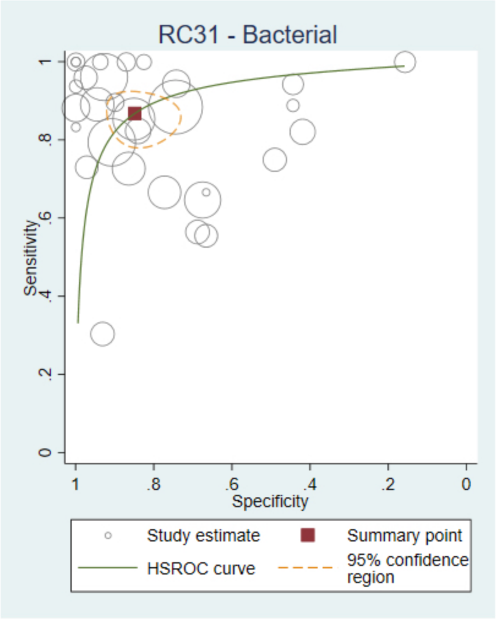
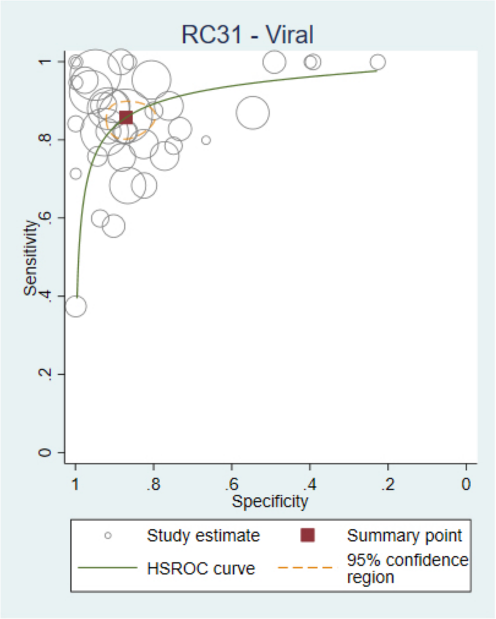


Fig. S2. HSROC Curves for Evaluated Signatures (continued) [11/14]

RC31





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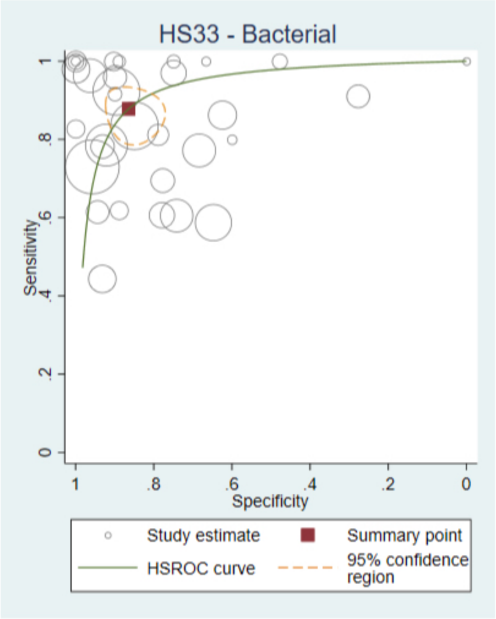


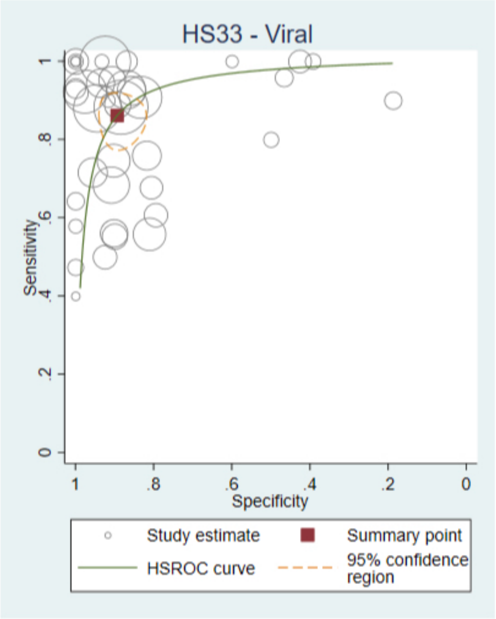
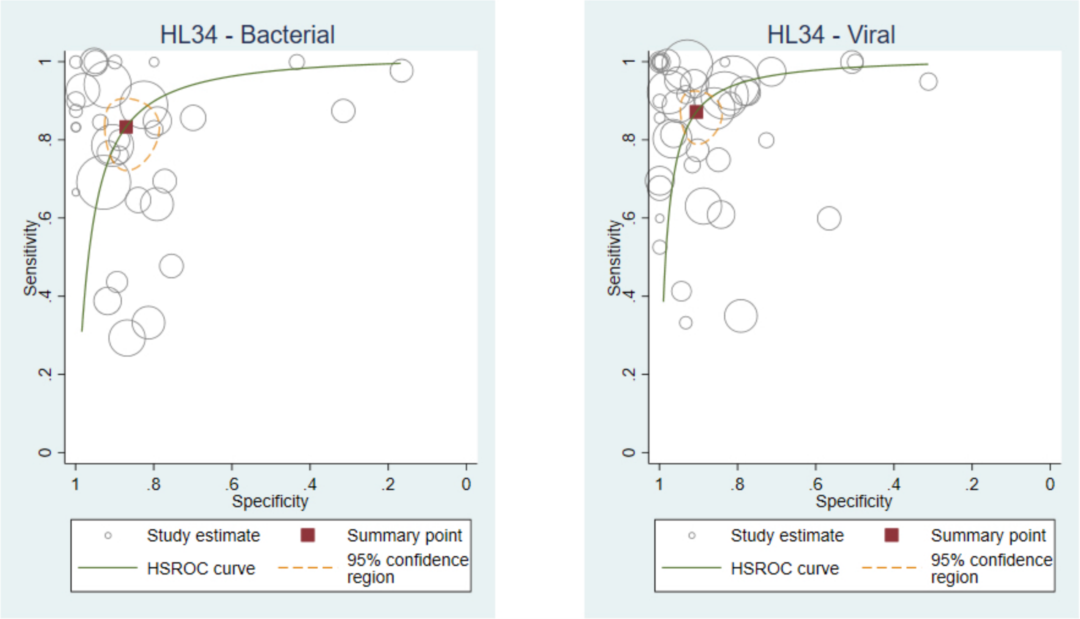


Fig. S2. HSROC Curves for Evaluated Signatures (continued) [12/14]

HL34



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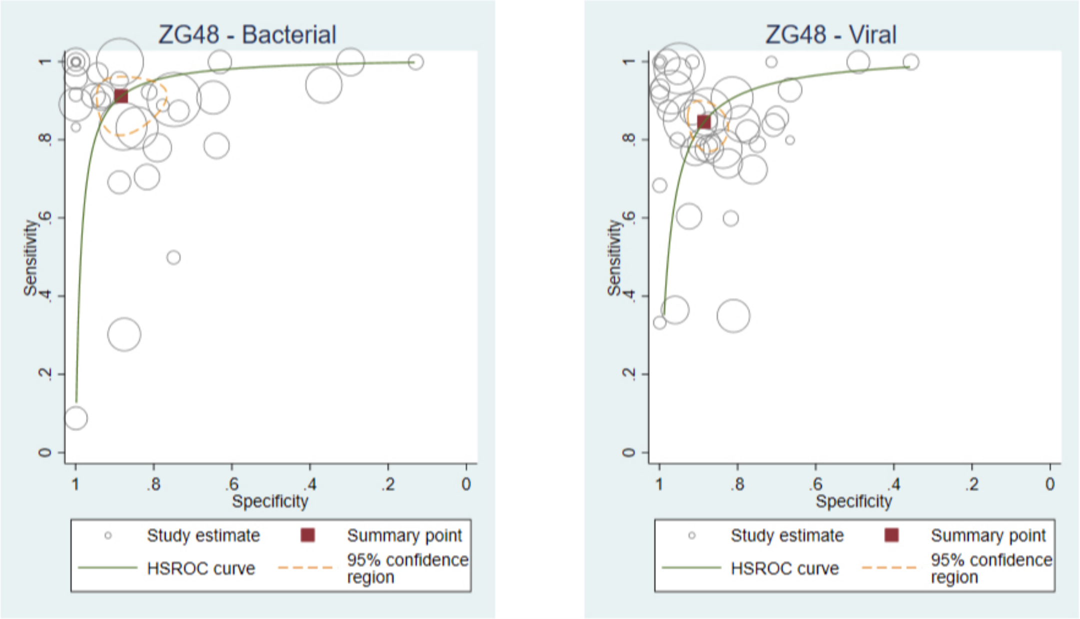
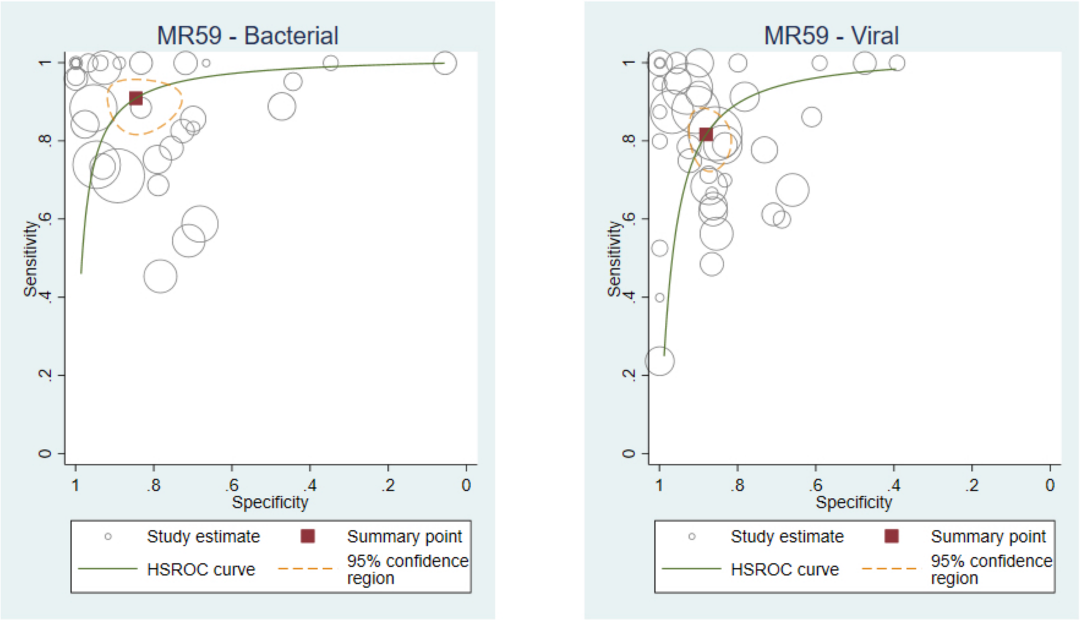


Fig. S2. HSROC Curves for Evaluated Signatures (continued) [13/14]

MR59



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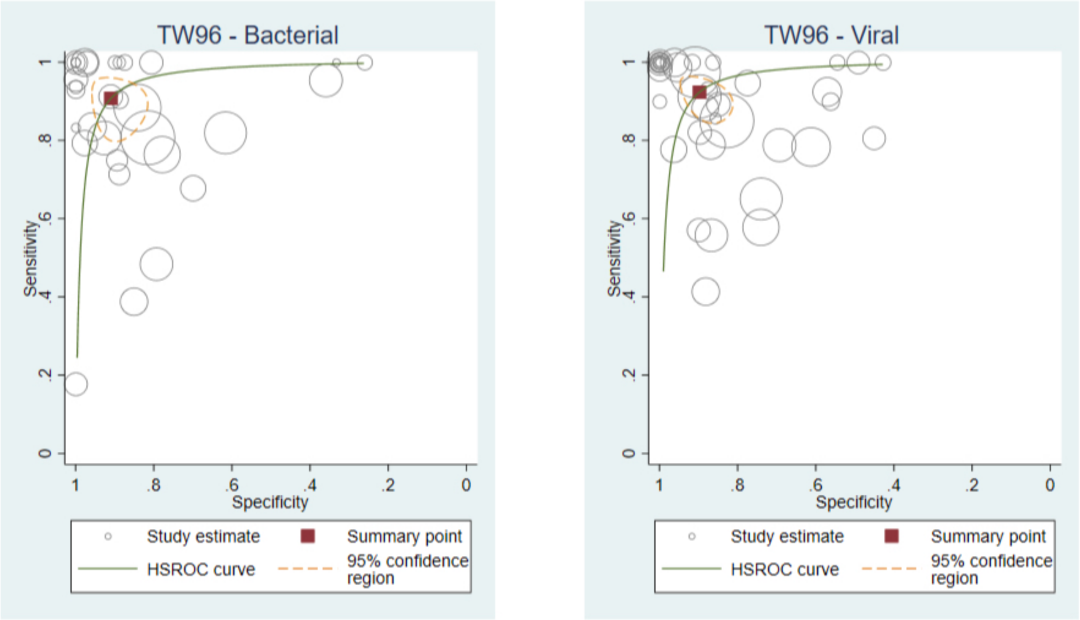
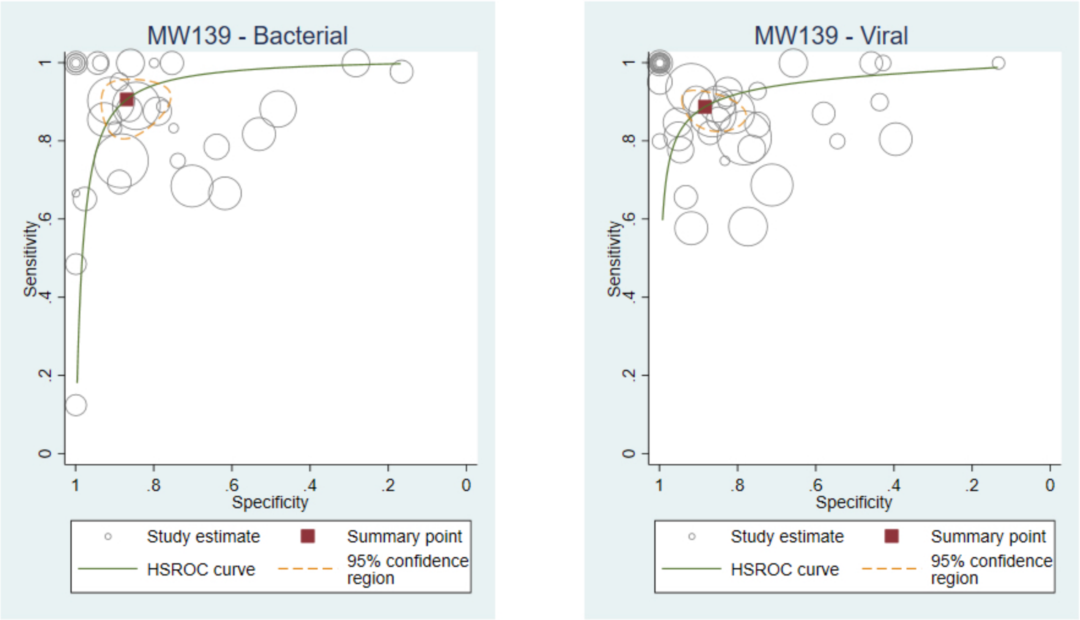


Fig. S2. HSROC Curves for Evaluated Signatures (continued) [14/14]

MW139



AK398

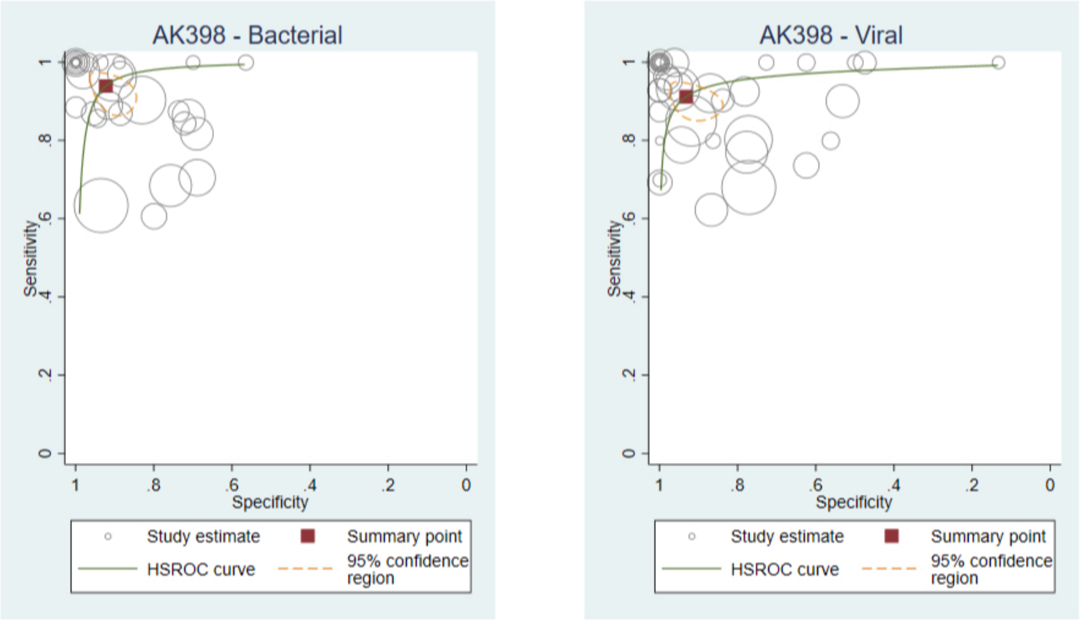
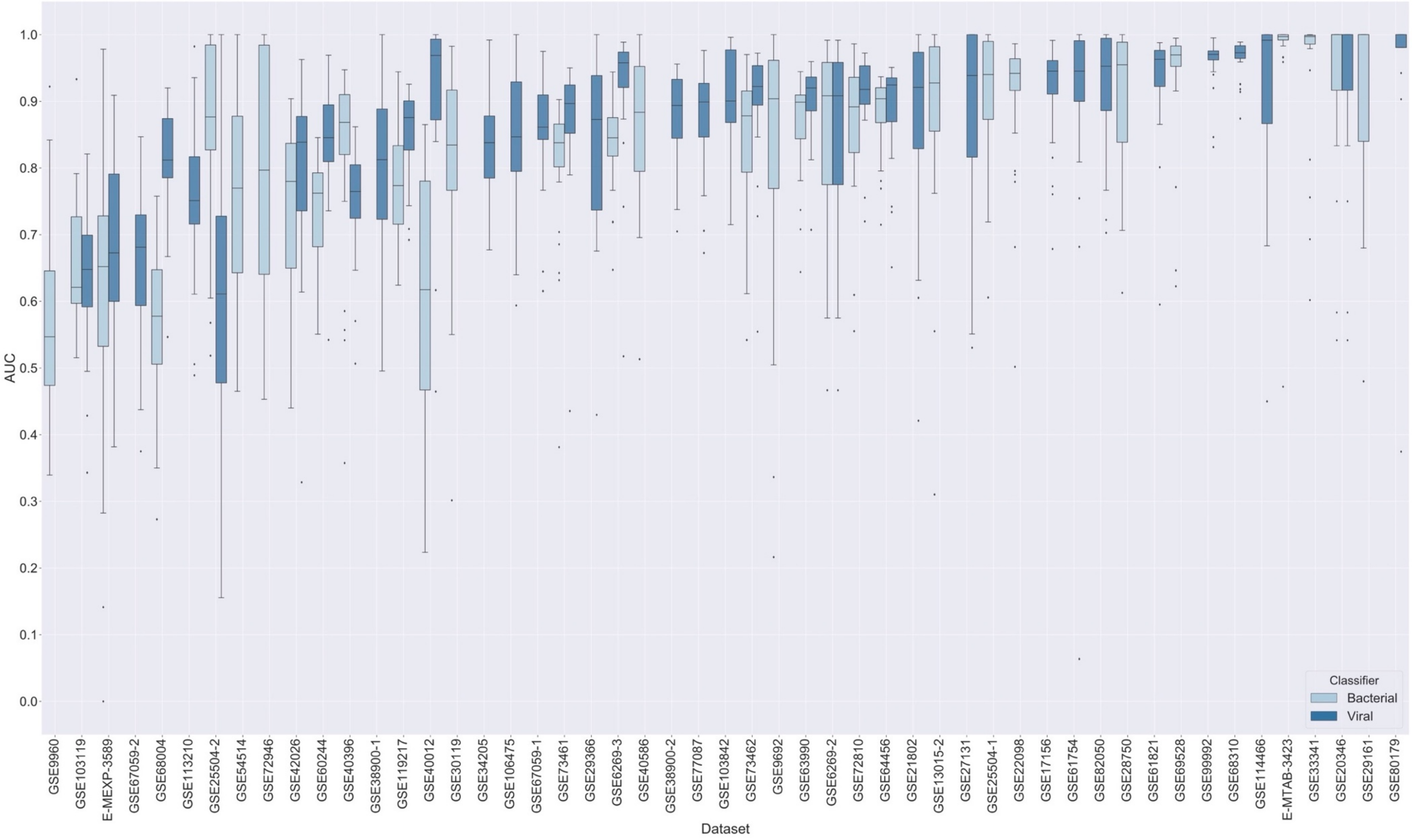


Fig. S3. Signature Performance by Validation Dataset. Box-plots were generated for each validation dataset's AUCs as measured across the 29 gene expression signatures for bacterial vs. non-bacterial and viral vs. non-viral classification.


AUC
Classifier
Bacterial
Viral
GSE9960
GSE103119
E-MEXP-3589
GSE67059-2
GSE68004
GSE113210
GSE25504-2
GSE54514
GSE72946
GSE42026
GSE60244
GSE40396
GSE38900-1
GSE119217
GSE40012
GSE30119
GSE34205
GSE106475
GSE67059-1
GSE73461
GSE29366
GSE6269-3
GSE40586
GSE38900-2
GSE77087
GSE103842
GSE73462
GSE9692
GSE63990
GSE6269-2
GSE72810
GSE64456
GSE21802
GSE13015-2
GSE27131
GSE25504-1
GSE22098
GSE17156
GSE61754
GSE82050
GSE28750
GSE61821
GSE69528
GSE99992
GSE68310
GSE114466
E-MTAB-3423
GSE33341
GSE20346
GSE29161
GSE80179
Dataset