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# Supplementary appendix

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*Supplementary Materials to*

# Host gene expression signatures to identify infection type and organ dysfunction in children evaluated for sepsis: a multicenter cohort study

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**Supplement.**

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**eMethods 2. List of ethics approval numbers and approved protocol modifications.**

*Children's Health Queensland Human Research Ethics Committee Approval Number:* ***HREC/17/QRCH/85***

| **Version Number** | **Version Date and Number** | **List of Modifications** |
|---|---|---|
| 1 (Original) | 09/06/2017 | - |
| 2.0 | 25/11/2017 | • Updates to investigator team<br>• Addition of Gold Coast University Hospital site<br>• Removal of SIRS criteria<br>• Increased upper age limit to <18 years<br>• Specify fluc swab/NPA use for viral diagnostics<br>• Inclusion of data capture for parental concern and clinical assessment<br>• Phone consent option for patients discharged rapidly prior to contact with study team |
| 3.0 | 26/10/2018 | • Clarification of data and blood storage<br>• Updates to investigator team<br>• Addition of Mt Isa and Thursday Island sites details<br>• Clarification of staff and parental questionnaire to assess level of concerns upon presentation<br>• Further detail on gene expression analyses<br>• Further detail on pathogen specific analyses |
| 3.1 | 05/06/2019 | • Included use of RESPOND study samples<br>• Investigations on bacterial and viral pathogen genomics specified<br>• Updates to investigator team |
| 3.2 | 04/10/2019 | • Updates to investigator team<br>• Exclusion criteria: immunocompromised patient added<br>• Blood volume for citrate tube increased to 1.8mL due to tubes shortage and difficulty to get all aliquots with 1mL<br>• Study completion timeframe extended |
| 3.3 | 13/12/2019 | • Updates to investigator team<br>• Use of biobank specified, including measurement of procalcitonin and Vitamin C levels in stored sera from this study<br>• Updated staff and parental questionnaire to assess level of concerns upon presentation |
| 3.4 | 31/12/2019 | • Updated to include collection of parental and clinician (medical and nursing) surveys independent of blood collection |

| Version Number | Version Date and Number | List of Modifications |
|---|---|---|
| 3.5 | 08/07/2020 | • Extended recruitment to 1600 patients and study completion timeframe extended<br>• Updates to investigator team<br>• Consent considerations due to COVID included<br>• Volume of blood reduced per sampling point (no citrate tube)<br>• Second blood sampling time point added if feasible<br>• Additional detail provided regarding use of biobanked samples (PCT/NTproANP, Septicyte, metabolomics, endothelial markers)<br>• Removal of immunocompromised patients as an exclusion criteria |
| 3.6 | 18/10/2020 | • Updates to investigator team<br>• Updated sample analysis process |
| 3.7 | 23/02/2021 | • Updates to investigator team |
| 3.8 | 07/10/2021 | • Updates to investigator team |
| 3.9 | 09/01/2022 | • Updates to investigator team<br>• Addition of a comparison cohort of N=50 children after cardiopulmonary bypass surgery as a non-infectious inflammatory control of patients with high disease severity<br>• Addition of single-cell sequencing<br>• Further detail added about the relationship with the RESPOND trial<br>• Study completion timeframe extended study completion timeframe extended<br>• Specification of the volume of EDTA blood to be obtained (3ml rather than 1-2ml) to ensure sufficient sample volume for the analyses |

**eMethods 3. Inclusion and exclusion criteria.**

**Inclusion criteria**

- All children aged 1-month to 17 years undergoing evaluation for suspected sepsis.
- Evaluation for suspected sepsis which must include blood culture sampling
- Consent of parents/guardian.

The state of Queensland, Australia, where this study was conducted, started at the end of 2017 a campaign. One of the key messages to clinicians was "Could this be sepsis"[1,2]. The study screening procedures educated ED and ICU staff at the participating four sites to enrol patients if blood cultures were obtained and as part of an evaluation for suspected sepsis.

**Exclusion criteria**

- Neonatal patients ≤1-month of age
- Patients with immune suppression (defined as active chemotherapy for cancer, or active treatment with systemic immunosuppressive drugs as this may alter whole blood gene expression)
- Inability to obtain consent from parents/guardians

**Consent of participants**

Delayed consent ("Consent-to-continue" or "deferred consent") was used in this study for the following reasons. Firstly, it is an observational and not an interventional study and the laboratory measurements will not affect treatment. Secondly, prospective recruitment with obtaining parental consent is likely to lead to delays in blood sampling which would preclude direct comparison with blood cultures. Thirdly, the Australian National Statement Section 4.4.6 recognizes that in emergency care research recruitment into a research project often has to be achieved quickly, and that a waiver of consent may be granted provided the conditions of the N.S paragraph 2.3.6 are satisfied. Acute care studies have demonstrated the difficulties for parents to make decisions at time of high stress, supporting delayed consent approaches. We therefore utilised a delayed consent process for this study. Consent was sought from the parent/guardian by the Study Coordinator, Registrar, Consultant or local Nurse Champion as soon as possible once the child has been stabilized and the parent/guardian has had time to adjust to the emergency environment.

**eMethods 4. Clinical phenotyping algorithm.**

The clinical phenotyping followed a two-step procedure (**eFigure 1**). In a first step, the microbiologic etiology (bacterial versus viral infection) was classified. In a second step, the presence of organ dysfunction within 24 hours of sampling was assessed (organ dysfunction versus no organ dysfunction).

**Presence of bacterial versus viral infection.** Bacterial versus viral infections were classified on a 6-item order ranging from confirmed bacterial, probable bacterial, undefined infectious illness, probable viral, to confirmed viral infection, and non-infectious illness (including controls). Bacterial infections were confirmed by cultures of sterile sites by standard pathology services which must be compatible with the clinical presentation. Probable bacterial infections were microbiologic unconfirmed infections where the clinical presentation (bacterial syndrome, increased C-reactive protein, decision by the treating physician to treat for at least 5 days with antibiotics) was indicative of bacterial infection. Confirmed viral infection were based on routine diagnostics (influenza A and B, respiratory syncytial Virus (RSV), parainfluenza 1-3, human metapneumovirus (hMPV), adenovirus, enterovirus) and add-on viral diagnostics of specimens as clinically indicated (such as Enterovirus-PCR in infants with suspected sepsis or central nervous system infection). During the study it was routine practice to perform a standardized PCR panel for viral respiratory pathogens (influenzavirus A and B, parainfluenzavirus 1-3, RSV, hMPV, Rhino/Enterovirus, Adenovirus) using nasopharyngeal aspirates. respiratory PCR results were considered if compatible with the clinical phenotype. Probable viral infections were microbiologic unconfirmed infections where the clinical presentation (viral syndrome such as for example bronchiolitis, low C-reactive protein) was indicative of a viral infection. Non-infectious illness was defined as patients with signs and symptoms of illness in the absence of signs and symptoms of infection, including surgical controls. Combined Bacterial/Viral infections were classified if a secondary infection with a different pathogen class (i.e., bacterial infection leading to presentation, with viral co-infection) was confirmed.

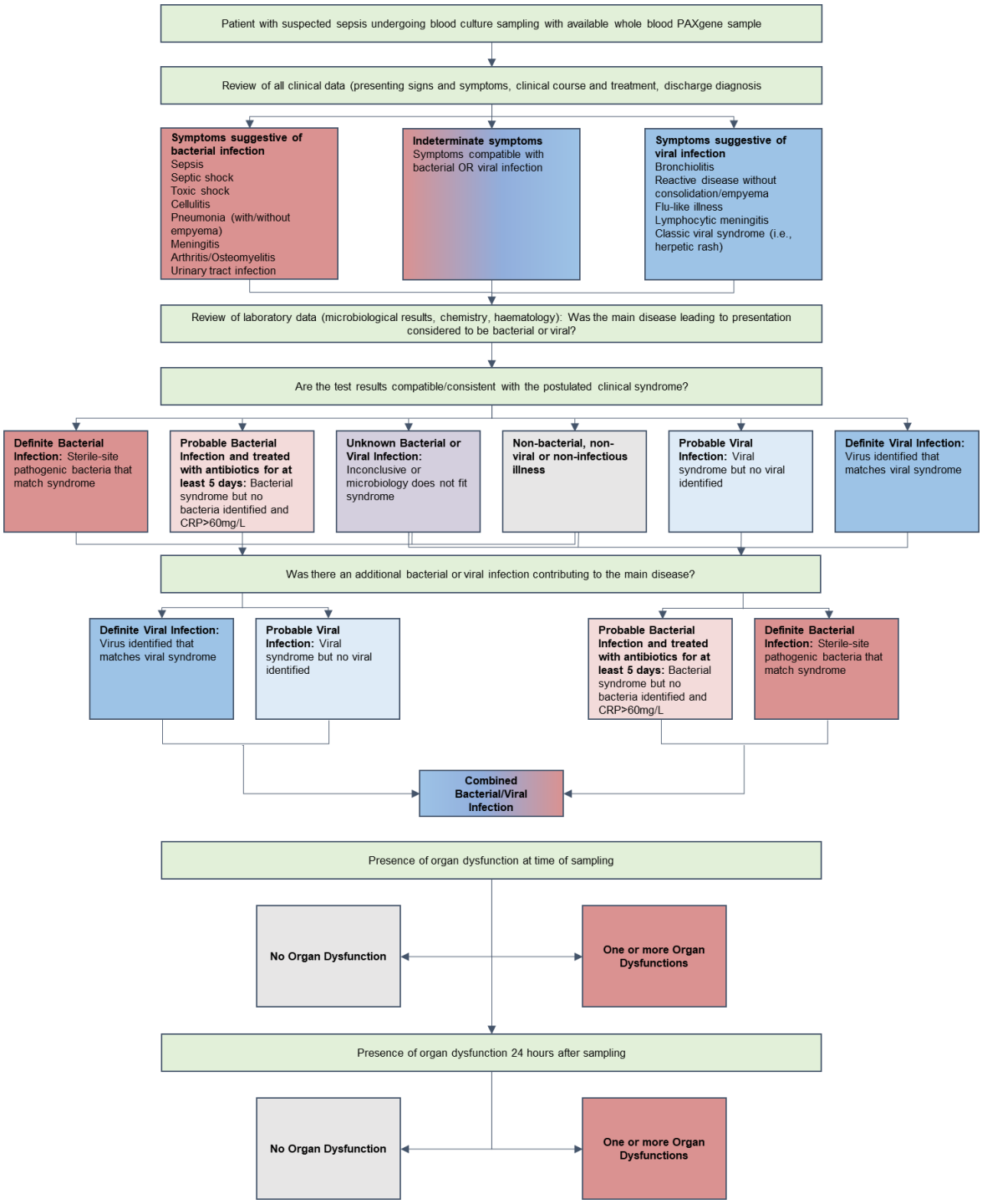
**Presence of organ dysfunction.** Severity was assessed at baseline (at time of blood sampling) and at 24 hours after blood sampling using clinical, laboratory, and organ support criteria for organ dysfunction as defined by the 2005 International Pediatric Sepsis Definition Consensus Conference[3,4]. Accordingly, presence of between one and six of the following organ dysfunctions (cardiovascular, respiratory, neurologic, renal, haematologic and hepatic) was adjudicated:

- **Cardiovascular:** systemic hypotension (as per age-specific cut-offs for systolic blood pressure), OR need for vasoactive drugs, OR increased base excess/capillary refill/lactate
- **Respiratory:** need for invasive or non-invasive mechanical ventilation, OR $PaO_2$ / $FiO_2$ <300, PR $PaCo_2$ >65 mmHg, of need of $FiO_2$ >50% to maintain oxygen saturations ≥92%
- **Neurologic:** Glasgow Coma Score <11
- **Renal:** serum creatinine increase ≥ 2 times upper limit of normal for age
- **Haematologic:** Platelet count <80,000 /$mm^3$
- **Hepatic:** total bilirubin ≥69 umol/l OR ALT 2 times upper limit

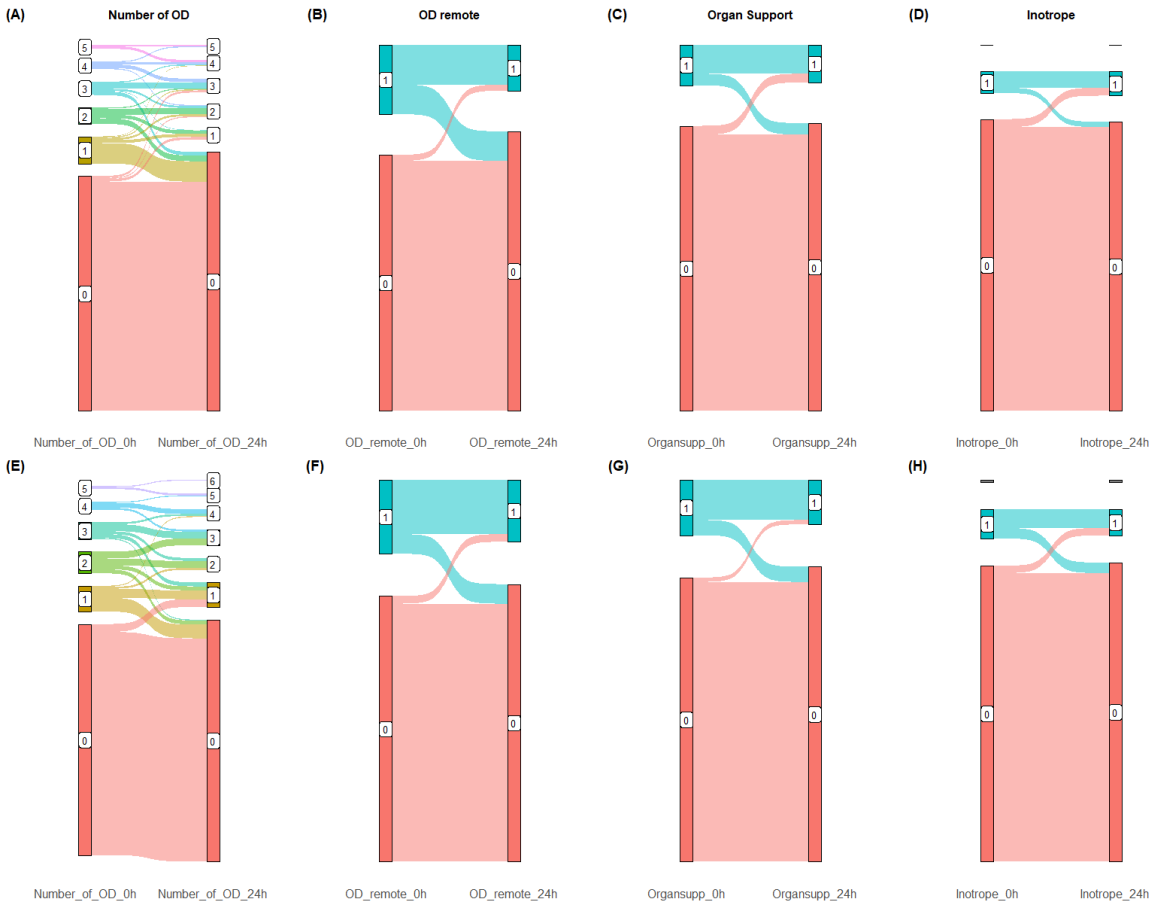
As there is no gold standard for organ dysfunction, and given that the clinical relevance of some organs may vary, we investigated in addition the following **secondary severity outcomes:**

- **Organ dysfunction remote to the site of infection:** this outcome was chosen because organ dysfunction remote from the site of infection (i.e. renal failure or shock in a patient with pneumonia) may indicate more severe systemic processes; compared to organ dysfunction at the site of infection (i.e. respiratory failure in a patient with pneumonia; neurological failure in a child with meningitis). This is based on a recent global survey of the Pediatric Sepsis Definition Taskforce.[5] Shock was always considered as organ dysfunction remote of the site of infection)
- **Need for organ support:** defined as inotropes, invasive or noninvasive ventilation, renal replacement therapy, or ECMO. This outcome was chosen as it indicates a higher severity, given the need for organ support.
- **Administration of inotropes:** defined as vasoactives or inotropes (adrenaline, noradrenaline, milrinone, dobutamine, dopamine, vasopressin)
- **Cardiovascular, respiratory, or neurological organ dysfunction:** presence of at least of these three organ dysfunctions, given that they were shown to be more relevant to outcomes compared to other organ failures.[6]
- **Multi-organ dysfunction (MOD):** Presence of at least two organ dysfunctions[7], which is associated with substantially higher mortality and worse short- and long-term outcomes compared to single organ dysfunction.
- **Improving/worsening organ dysfunction 24 hours after sampling (OD better; OD worse):** using the count of organ dysfunctions at 24 hours after study blood sampling compared to the count of organ dysfunction at time of sampling to reflect disease dynamics.
- **Improving/worsening multi-organ dysfunction 24 hours after sampling (MOD better; MOD worse):** using the count of organ dysfunctions at 24 hours after study blood sampling compared to the count of organ dysfunction at time of sampling to reflect disease dynamics.
- **Presence of individual organ dysfunction:** specific for each of the six organs listed above.

Changes in organ dysfunction from baseline to 24-hours after sampling is shown in **eFigure 2**.




**eFigure 1. Classification of patients into diagnostic groups by type of infection and by organ dysfunction.**




**eFigure 2.** Sankey diagram showing the course of organ dysfunction from presentation to 24-hours after presentation in both discovery (A, B, C, D) and RAPIDS validation (E, F, G, H) cohorts; **A**, **E** - number of organs affected (number of OD); **B**, **F** - OD remote to the site of infection (0 – No, 1 – Yes); **C**, **G** - need of organ support (0 – No, 1 – Yes); **D**, **H** - administration of inotropes (0 – No, 1 – Yes).

**eMethods 5. Data monitoring plan.**

| Version No. | Version Date | Major Changes |
|---|---|---|
| Version 1.0 | 01/07/2021 | Initial version |

**Purpose**
This data monitoring plan describes the nature and extent of data monitoring activities to be performed for the Rapid Pediatric Infection Diagnosis in Sepsis (RAPIDS) study. It was developed by the Coordinating Principal Investigator in collaboration with the Study Statistician and the Study Management Team, after the conduct of a risk assessment.

Monitoring activities will be performed in accordance with:
- The Integrated Addendum to ICH E6 (R1): Guideline for Good Clinical Practice E6 (R2) *(ICH-GCP)*;
- Risk-based management and Monitoring of Clinical Trials Involving Therapeutic Goods, NHMRC, 2018;
- Protocol-specific requirements; and
- Applicable policies and procedures at each participating site.

**Roles and Responsibilities**
The data monitoring activities for this study will be developed and supported by the staff from the coordinating site, Child Health Research Centre, The University of Queensland, and Queensland Children's Hospital (QCH). Each participating site will be monitored by the QCH Research Coordinators.

All Monitors are qualified by education and experience to monitor the conduct of the study according to applicable standard operating procedures (SOPs), ICH-GCP, and local requirements.

**Risk Assessment**
Prior to the development of the data monitoring plan, critical data and processes were identified by the Study Management Team. A risk assessment was conducted to determine the extent and nature of data monitoring activities proportionate to the risk associated with the critical data and processes. The risk assessment was conducted in accordance with the research group's Data Monitoring Work Instruction and Risk Assessment Template.

**Overview of Monitoring Activities**
There will be three types of data monitoring used for this study:
1. Onsite or remote monitoring will be conducted routinely to verify that data recorded on the case report forms are accurate, complete, and verifiable from source documentation. Source data verification of all data items relating to the calculation of organ dysfunction within 24 hours of presentation, and all infection phenotype data items will be completed for every patient admitted to the ICU and every patient with organ dysfunction within 24 hours of presentation. The original REDCap study database will be enhanced to facilitate the source data verification of the relevant data items.
2. Remote monitoring by the coordinating site will be conducted routinely to review automated range and logic discrepancies generated from data quality rules in the REDCap study database.
3. Centralised monitoring will be conducted routinely to evaluate rates of recruitment across study sites.

**Monitoring Reports**
Source data verification results will be documented in a data monitoring report. This report will be generated from the data verified by the monitor in the verification forms in the REDCap study database.

The coordinating site will complete any required updates to the case report form data in the REDCap study database in a timely manner.

**Review**
The Study Management Team will review the data monitoring plan in response to outcomes of monitoring activities that identify deficiencies and new risks that were not previously considered. Where applicable, an updated version of the monitoring plan will be issued.

**References**
Department of Health, Education, and Welfare, Office of the Secretary, Protection of Human Subjects. Belmont Report: Ethical Principles and Guidelines for the Protection of Human Subjects of Research, Report of the National Committee for the Protection of Human Subjects of Biomedical and Behavioral Research. DHEW Publication No. (OS) 78-0013 and No. (OS) 78-0014. 18 April 1979.

The Greenberg Report (1988). Organization, review, and administration of cooperative studies: a report from the heart special project committee to the National Advisory Heart Council. Controlled Clinical Trials 9 (2): 137-148.

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UNICEF/UNDP/World Bank/WHO (31 March 2004). Operational Guidelines for the Establishment and Functioning of Data and Safety Monitoring Boards. Geneva, Switzerland.

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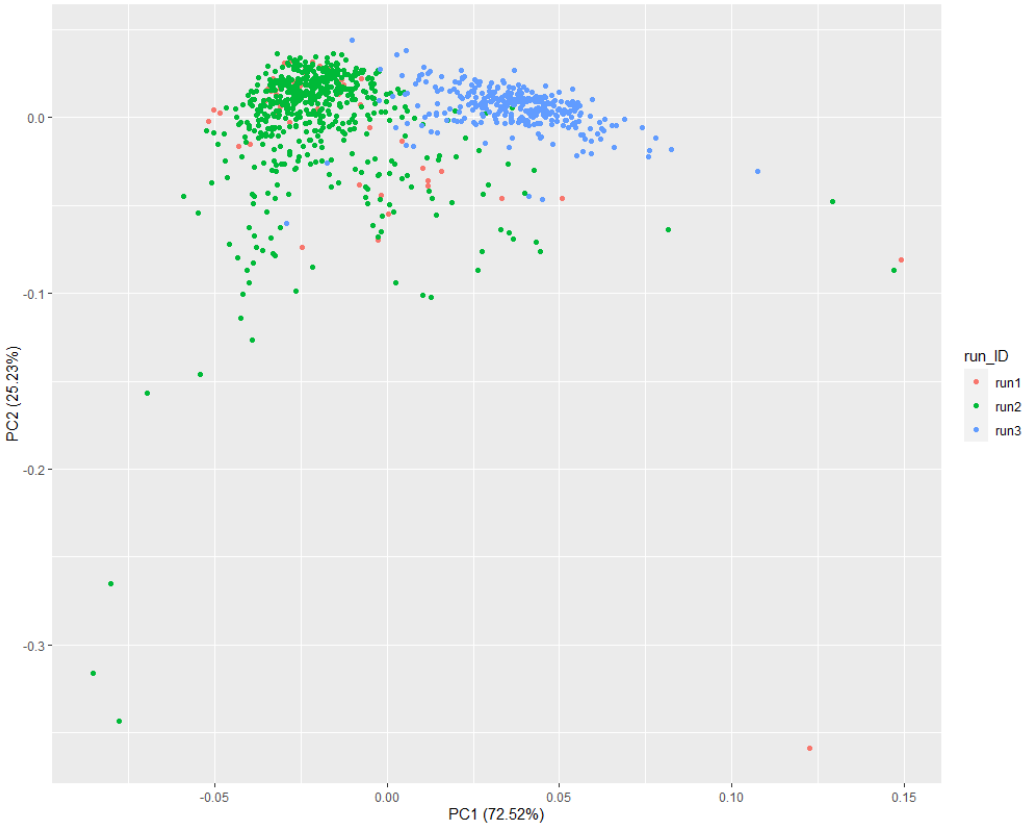
**eMethods 6. RNA Sequencing analysis and FSPLS methods for disease class procedures.**

*RNA Sequencing data*

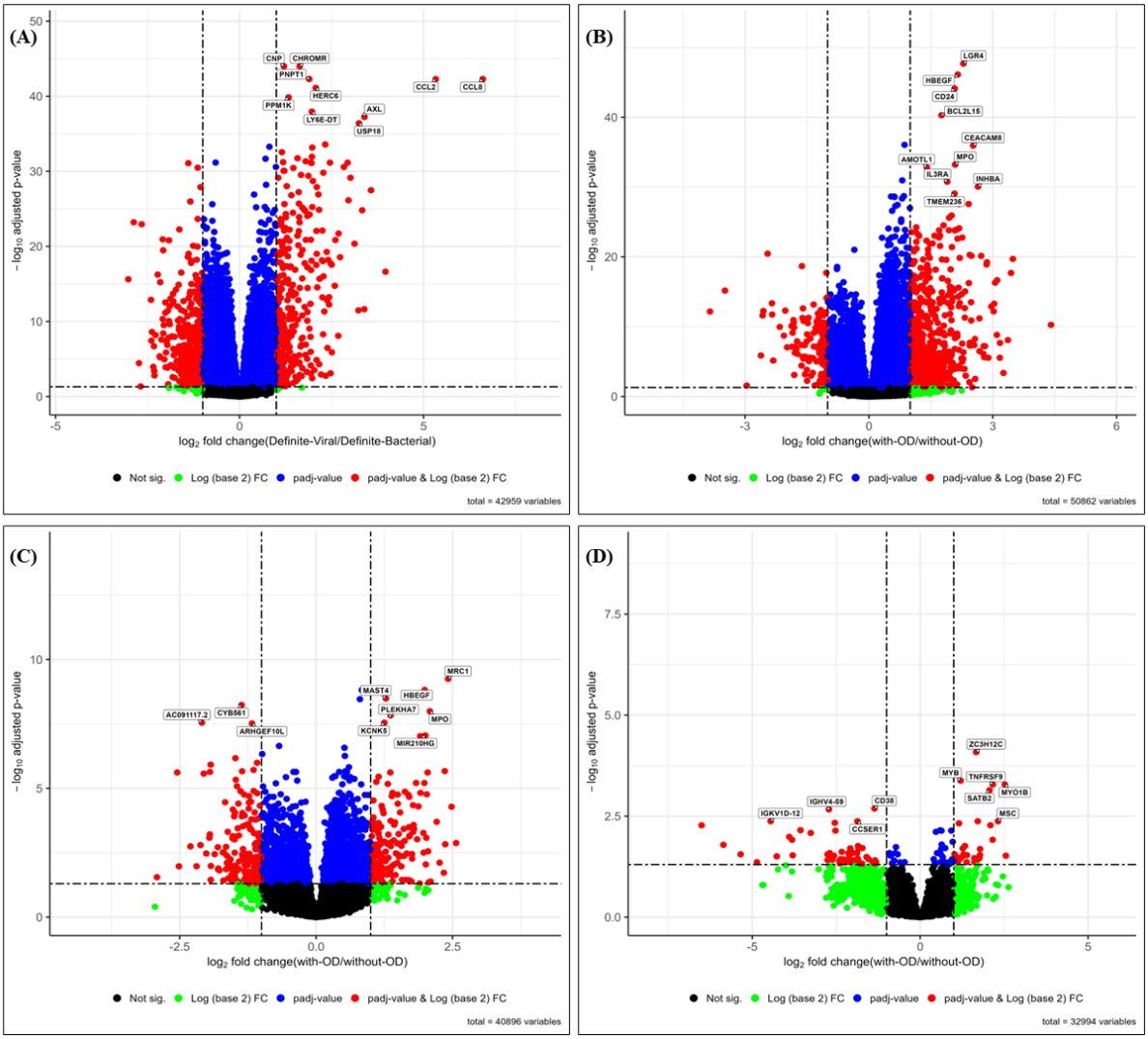
Sequencing was performed in three batches and the sequencing configuration was 75bp single-end (50 samples), 100bp single-end (545 samples) and 100bp paired-end (316 samples), respectively. The first two batches of samples were used for discovery (n=595) and the third batch was used for validation (n=316). Principal component analysis (PCA) was performed to identify any outliers (**eFigure 3**). Principal components (PCs) which explain greater than 5% of variance were retained. There were only 2 PCs with >5% variance (PC1 – 72.52%, PC2 – 25.23%) and these are plotted in eFigure3. Run 3 clustered together, however this group was only used for the validation of the signatures. Hence, we did not perform any corrections to the data.




**eFigure 3:** Principal Component Analysis (PCA) performed on the discovery and validation cohort with the gene expression counts. Points represent samples and are coloured by RNA-seq run.

*Differential Expression Analysis*

Genes which had absolute log2 fold-change (LFC) of >1 and adjusted p-value of <0.05 were considered as differentially expressed. Log2 fold change of >1 would identify genes which are 2 times different in expression levels between the comparison groups and this will assist to identify genes which are significantly differentially expressed between groups. DESeq2 adjusted P-values are from the Wald test using Benjamini and Hochberg method (BH-adjusted p values) and adjusted p-value of <0.05 will identify genes which are significantly differentially expressed between groups. Differential expression analysis was performed on infection type (definite bacterial versus definite viral) and organ dysfunction (with OD versus without OD) phenotypes. Outcomes are listed in eFigure 4 and eTable 3.



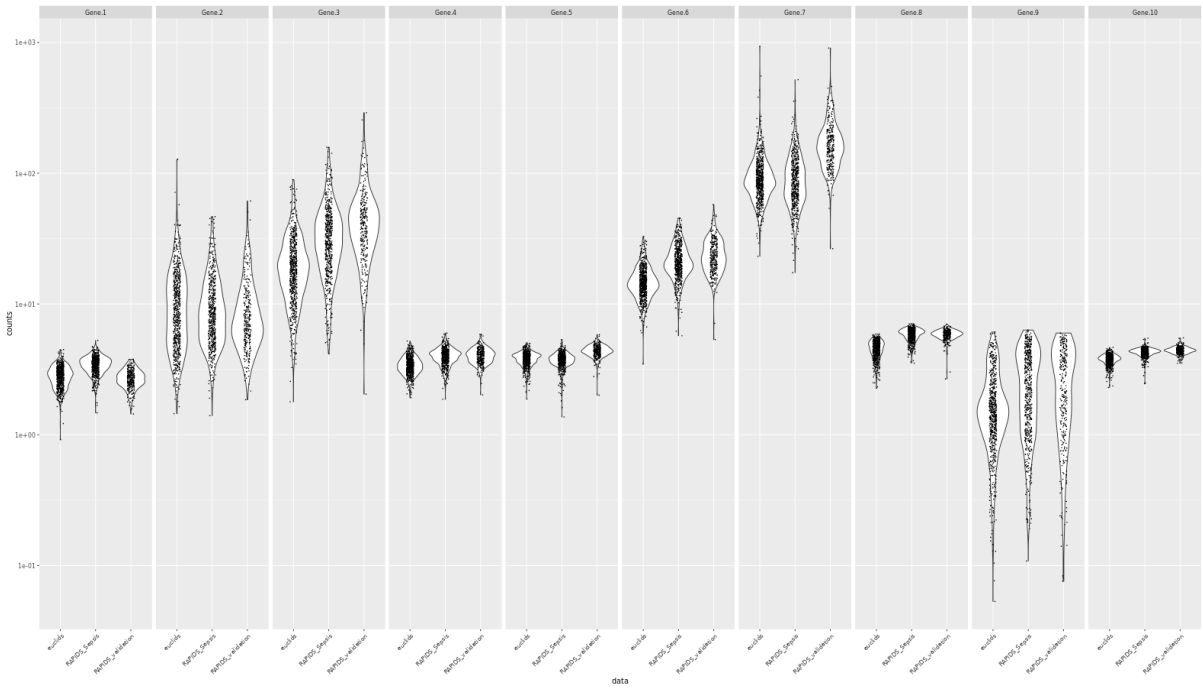

**eFigure 4.** Volcano plots showing log2 fold change (LFC) values and adjusted -log10 p-values from differential expression analysis comparing (A) patients with definite bacterial (DB) (n=172 patients) versus definite viral (DV) (n=110 patients) infections; (B) patients with organ dysfunction (OD) at 24-hours after sampling (n = 87) versus patients without OD (n=508); (C) patients with definite bacterial infections with OD at 24-hours after sampling (n = 44) versus without OD (n=192); (D) patients with definite viral infections with OD at 24-hours after sampling (n = 11) versus patients without OD (n=186). Red points represents genes with adjusted p-values < 0.05 and absolute LFC > 1 or < -1; Blue points represents genes with adjusted p-values < 0.05 and absolute LFC < 1 or >-1; Green points represents genes with adjusted p-values > 0.05 and absolute LFC > 1 or < -1; Grey points represents genes which are not significant. Top 10 differentially expressed genes based on -log10 adjusted p-values are labelled for each comparison.

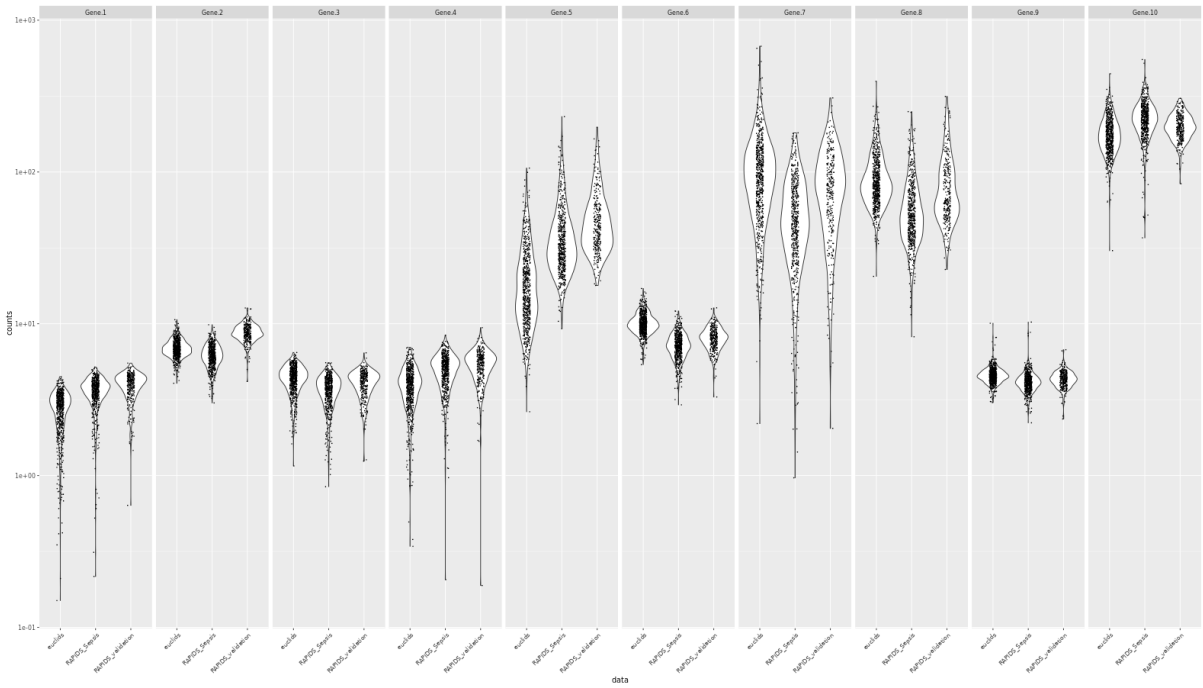
*FSPLS methods*

To identify gene signatures, we applied an in-house forward selection algorithm Forward Selection – Partial Least Squares (FS-PLS) to discover novel transcript signature. A previous iteration of our algorithm was reported in Herberg et al[8] and Gliddon et al[9]. The gene expression data are first converted from count data to transcripts per million mapped reads by dividing by the total library size for each sample. Next, we applied a series of variance stabilising transformations to the data, including log(x+1), Anscombe transformation and inverse binomial[10]. We next divided the data into 10 random 10% chunks (by sample) for fitting the model using 10-fold cross validation. For each of these, we used the remaining 90% of the data to fit a forward selection model, using a logistic link function. At each round of forward selection, we predicted the held-out samples, and terminated the forward selection when a goodness of fit test on these held-out samples no longer increased. We used the area-under-the curve (AUC) as the goodness of fit function.

We modified the FSPLS approach to enable simultaneous comparison of multiple disease groups. This allowed us to discover a single set of gene signature to distinguish various phenotypes. For disease-class signature discovery, FSPLS was run with 5 different comparisons, including DB versus DV; DB versus PV; DV versus PB; DB versus NI and DV versus NI comparisons. For OD signature analysis, FSPLS was run with two comparisons, those with versus those without OD at time of sampling (0 hours) and those with versus those without OD at 24-hours post sampling.

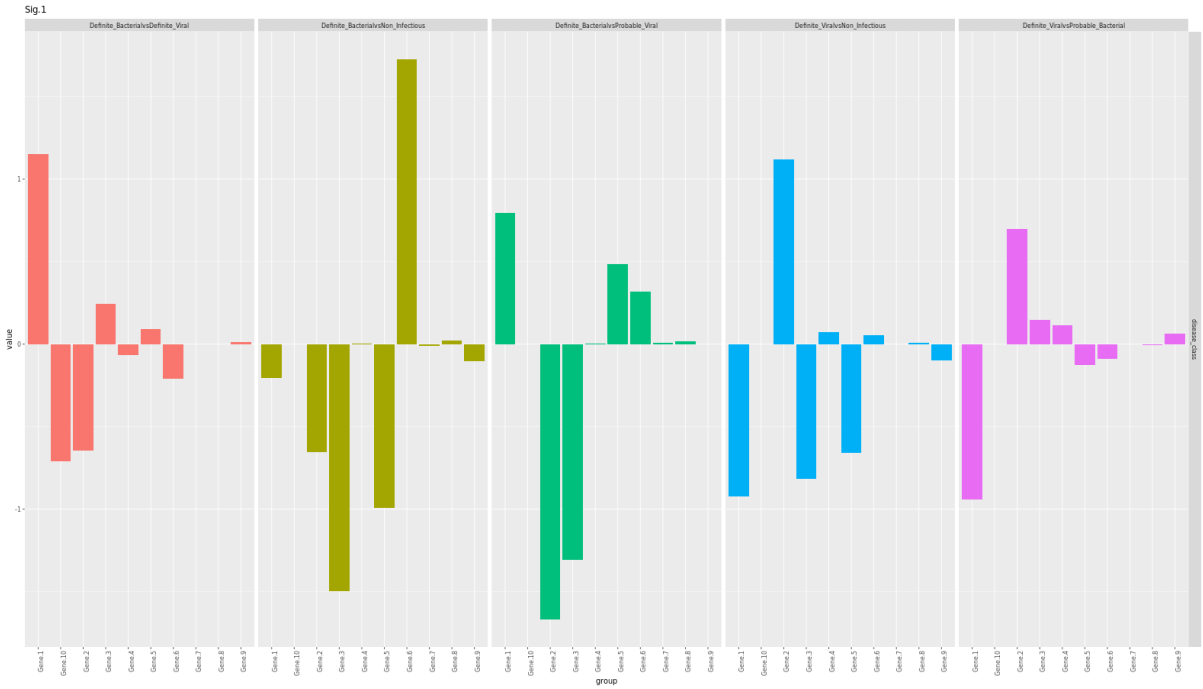
To minimize the signature size, maximum gene numbers in the signature was set at 10. The expression levels of the individual genes in the signatures were comparable across the discovery and validation cohorts (**eFigure 5**).






**eFigure 5**: Gene counts distribution of the 10 genes in disease-class signature (top panel) and disease-severity signature (bottom panel) across the discovery and validation cohorts

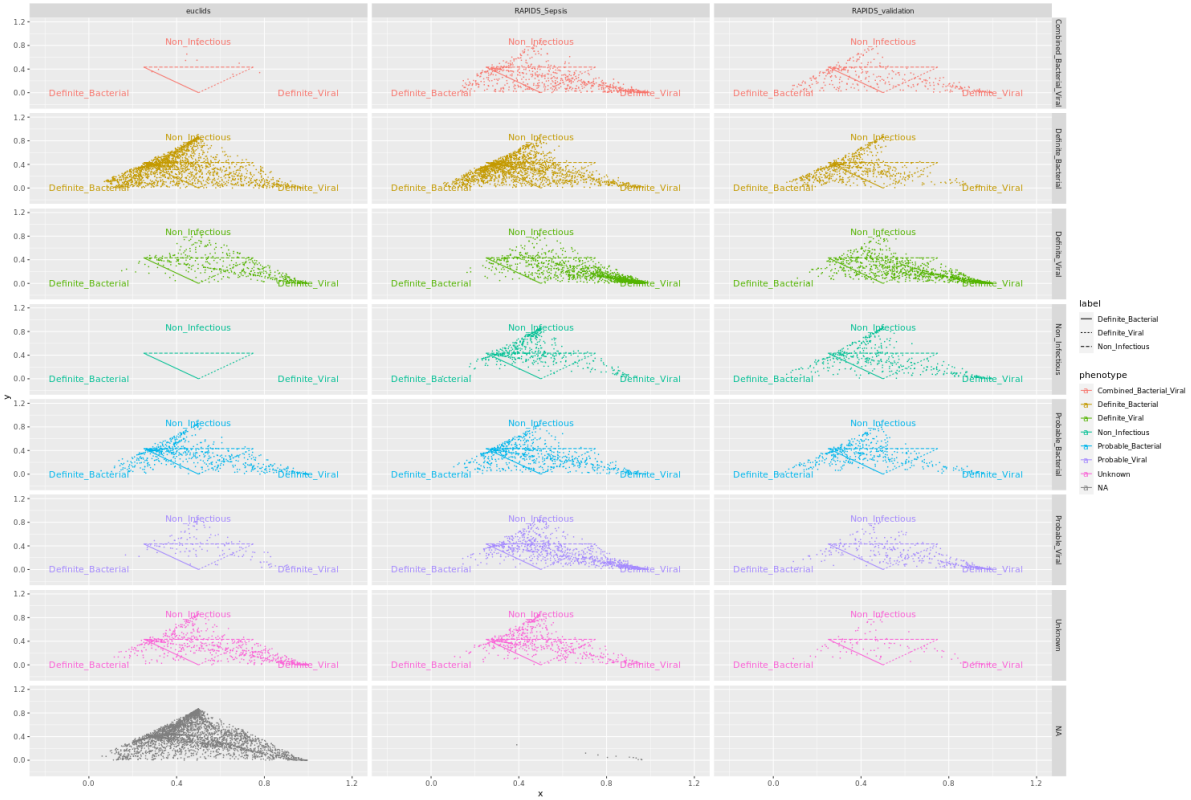
Weights for each gene in the signature for each phenotype differed (**eFigure 6**) and these gene weights were used for the validation of the signature. To test the performance of previous reported signatures, we trained these signatures on our data and determined the weights for each gene in the signature and used these to determine the performance for each phenotype.




**eFigure 6.** Weights of the genes in the disease-class signature for different phenotypes.

To integrate the disease-class and disease-severity signatures in a combined prediction model, we first applied a stratification using the disease-class signature to predict the probability of being definite bacterial or definite viral or non-infectious, as these phenotypes were well-

defined (**eFigure 7** shows the distribution of samples in the ternary plot for each phenotype in the corresponding cohorts). Then, for each strata, i.e. for each predicted disease-class group, the disease-severity signature was applied to predict the likelihood of developing organ dysfunction.




**eFigure 7.** Distribution of the sample predictions into definite bacterial (DB), definite viral (DV) and non-infectious (NI) for each phenotype in the discovery and both validation cohorts. The three axes of the triangle are probability of DB, probability of DV and probability of NI. The upside-down triangle shows the lines which define greater than 0.5 probability for each infection type.

Model calibration:
FS-PLS has been carefully designed so that model normalisation is not required prior to running (e.g. centralisation or standardisation), other than library size normalisation. Moreover, all the variance stabilising transformations are written in a functional form and do not rely on any calculation on the data (e.g. standard deviation). The model weights obtained from model fitting are derived in this space of library size normalised count data, and hence can be applied directly to validation datasets normalised by library size in the same way.

Computational requirements:
The model fitting was carried out on a single 8 CPU node of our HPC cluster utilising 16GB of memory. The model fitting for the disease-class signature required 492 seconds and the disease-severity signature required 462 seconds.

Statistical significance:
Previous version of FS-PLS would terminate model fitting if the orthogonal component of the next variable selected was not significantly associated with the outcome (at alpha of 0.05). However, in the current version a cross-validation scheme was used instead. In more detail, the training set was randomly split into 10. At each stage of the forward iteration, the entire process

of selection of the next best variable, and model fitting was carried out on the 10 different 90% subsets, with a prediction made on each remaining 10%. In this way each sample had an out-of-sample prediction made (for each of the different multi-way comparisons). Based on these out-of-sample predictions, a receiver operating curve was generated, and the area-under-the curve (AUC) was calculated and summed across the comparisons. The iterations terminate if the summed is less or equal to the previous iteration.

**eMethods 7. Previously reported gene expression signatures used in the analysis to identify infection type and severity in children with infection.**

We searched publications in English language in PubMed since January 2011 with the terms “sepsis OR septic shock”, “infection”, “bacterial”, “viral” AND “transcriptomics OR multiarray OR RNAseq”.

| Reference | Genes included in signature | Year of Publication | Number of samples | Cohort | Outcome | Signature assessed for |
|---|---|---|---|---|---|---|
| ***Herberg***[8] | *"IFI44L","FAM89A"* | 2016 | Discovery: 240<br>Validation: 130 | Paediatric | Bacterial vs Viral (AUC 0.96 (95% CI 0.87 - 1.00)) | Disease-class |
| ***McHugh***[11] | *"LAMP1","PLA2G7","PLAC8","CEACAM4"* | 2015 | Discovery: 105<br>Validation: 345 | Adult | Sepsis vs Infection negative systemic inflammation (AUC 0.95 (95% CI 0.91 - 1.00)) | Disease-class |
| ***Sweeney***[12] | *"IFI27","JUP","LAX1","HK3","TNIP1","GPAA1","CTSB"* | 2016 | Discovery: 426<br>Validation: 341 | Adult and Paediatric | Bacterial vs Viral (AUC 0.91 (95% CI 0.82 – 0.96)) | Disease-class |
| ***Sampson***[13] | *"ISG15","IL16","OASL","ADGRE5"* | 2017 | Discovery: 417<br>Validation: 332 | Adult and Paediatric | Viral vs Non-viral (AUC 0.90 – 1.00) | Disease-class |
| ***Tang***[14] | *"IFI27"* | 2017 | Discovery: 111<br>Validation: 521 | Adult and Paediatric | Influenza vs Bacterial (AUC 0.91 (95% CI 0.83 – 0.95)) | Disease-class |
| ***Wong***[15] | *"CCL3","CXCL8","GZMB","HSPA1B","MMP8"* | 2012 | Discovery: 220<br>Validation: 135 | Paediatric | Septic shock outcome (AUC 0.96 (95% CI 0.87 - 1.00)) | Disease-class |
| ***Li***[16] | *"MYBL1","KLRG1","STOM","MS4A4A"* | 2017 | Discovery: 99<br>Validation: 45 | Paediatric | Sepsis vs Controls | Disease-class |
| ***Li***[17] | *"HERC6","IGF1R","NAGK"* | 2021 | Discovery: 83<br>Validation: 781 | Adult | Bacterial vs Viral (AUC 0.91 (95% CI 0.89 – 0.93)) | Disease-class |

| | | | | | | |
|---|---|---|---|---|---|---|
| | | | Discovery: 532<br>Validation: 569 | Paediatric | Serious bacterial infections (concordance statistic 0.84 (95% CI 0.78 – 0.90)) | Disease-severity |
| ***Irwin[18]*** | *"RETN","LCN2","GNLY" "B4GALT5","AFF1","LDLR","ATXN7L3","LARP4B","SLC36A1","TRPM2","ATXN1","SLC41A3","MED13L","STOM","B4GALT5","MIDN","HVCN1","LDLR","CFLAR","SPATA13","EIF4G3","METTL7B","DOK3","ICAM2","IL1R1","LGALS2","LSG1","RPL13A","RPS13","SGSH"* | 2017 | Discovery: 63<br>Validation: 91 | Adult | Sepsis vs Post-operative inflammation (AUC 0.84) | Disease-severity |
| ***Lukaszewski[19]*** | *"C19orf59","CCL22","CD14","CD300LF","CYP1B1","DHRS9","FCER1G","FPR1","FPR2","GK","HIST2H2AA3","HK2","HK3","HPSE","LILRA5","MGST1","PDLIM7","PLAUR","PSTPIP2","RAB13","RETN","RHBDD2","S100A4","S100A9","S100A12","SERPINA1","UPP1","CPVL","CST3","LY86","PROCR"* | 2022 | Multiple datasets were used and split into training and test data set with random sampling | Adult | Sepsis vs Controls (AUC 0.96)<br>Sepsis vs No sepsis (AUC 0.64) | Disease-severity |
| ***Pena[20]*** | *"ATP9A","IL1R1","GADD45A","ARG1","PFKFB2","MLLT1","ANXA3","AGFG1","NSUN7","KREMEN1","MIR646HG","RIOK3","BNIP3L","TLCD4","SPTA1","TSPAN5","GLRX5","IFIT1B","ADAM23","MAP7","CACNA2D3","GPR34","GRAMD1C","PLCB1","DYNC2H1","TPRG1","ZNF60* | 2014 | Discovery: 182<br>Validation: 84 | Adult | High vs low severity (AUC 0.80) | Disease-severity |
| ***Baghela[21]*** | | 2022 | | | | |

| | | | |
|---|---|---|---|
| | *0","PLEKHO1","APOL1","EPSTI1","RSAD2","IFITM3","SERPING1","TPPP3","GTSE1","CDC45","CENPF","KIF14","PDIA4","KIF15"* | | |
| ***Baghela[21] – Reduced Severity*** | *"TNIP3","DSP","RHAG","G0S2","ITGB4","GPR84","FAM83A","PCOLCE2","CXCL8","SDC2","PRTN3","ELANE"* | High vs low severity (AUC 0.80) | Disease-severity |
| ***Baghela[21] – Reduced CR*** | *"CD300LF","CPVL","CST3","HK3","MGST1","RAB13","RETN","S100A12"* | High vs low severity (AUC 0.77) | Disease-severity |
| ***Baghela[21]– Reduced Mortality*** | *"HGF","DHRS9","SIGLEC1","MS4A4A","OAS2","MMP8","RGL1","SLC51A","OSBP2","IFIT1"* | Survived vs died (AUC 0.67) | Disease-severity |

**eMethods 8:** Novel disease-class and disease-severity signature genes

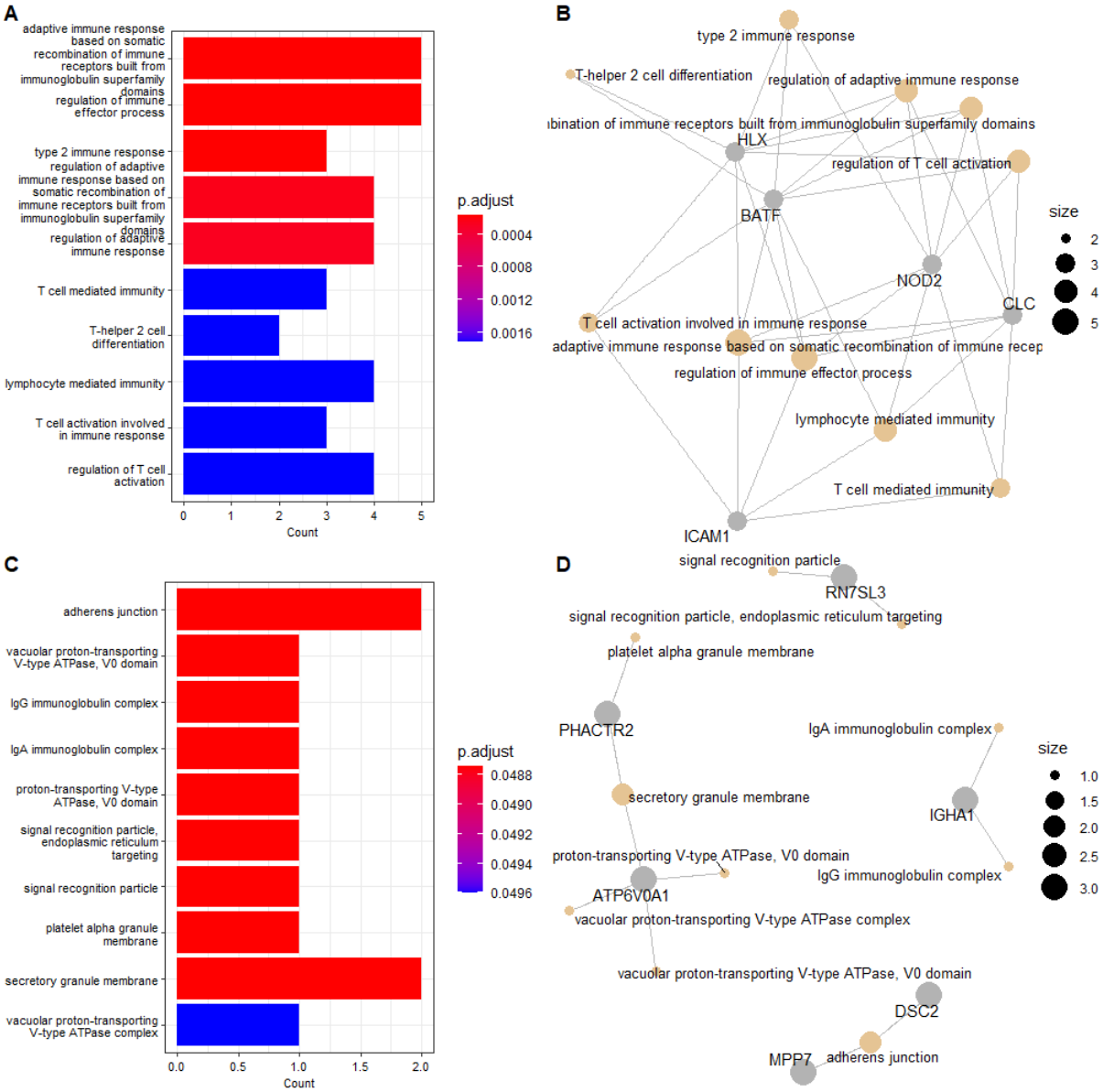
| Signature | Gene name | Gene description* |
|---|---|---|
| **Disease-class** | *USP18* | The *USP18* gene encodes a type 1 interferon (IFN)-stimulated gene that had dual functions: it is a negative regulator of type 1 IFN signalling and is also an isopeptidase that is a member of the deubiquitinating protease family of enzymes. |
| | *NCF1B* | The *NCF1B* gene is predicted to enable superoxide-generating NADPH oxidase activator activity and to be involved in respiratory burst and superoxide anion generation. Also, predicted to be part of NADPH oxidase complex and to be active in cytoplasm. |
| | *BATF* | The *BATF* gene is a transcription factor expressed in hematopoietic cells. |
| | *CLC* | The *CLC* gene referred as Charcot-Leyden crystals, are naturally occurring hexagonal bipyramidal crystals found in human tissues and secretions in association with increased numbers of peripheral blood or tissue eosinophils in parasitic and allergic processes. |
| | *S100A11* | THE *S100A11* gene is part of the S100 proteins, which are 10- to 12-kD molecules that have a canonical EF hand at their C termini and a modified, S100-specific EF hand at their N termini. Binding of Ca(2+) to EF-hand motifs changes the conformation and hence the function of S100 proteins. |
| | *ZBED1* | The *ZBED1* gene is part of the ZBED proteins, originated from domesticated hAT DNA transposons and encode regulatory proteins with diverse, fundamental functions in vertebrates. |
| | *PTGES3* | The *PTGES3* gene encodes an enzyme that converts prostaglandin endoperoxide H2 (PGH2) to prostaglandin E2 (PGE2). This protein functions as a co-chaperone with heat shock protein 90 (HSP90), localizing to response elements in DNA and disrupting transcriptional activation complexes. |
| | *HLX* | The *HLX* gene enables sequence-specific DNA binding activity. It is predicted to be involved in cell differentiation and regulation of transcription by RNA polymerase II. Also, predicted to act upstream of or within several processes, including animal organ development; enteric nervous system development; and regulation of T-helper cell differentiation. |
| | *NOD2* | The *NOD2* gene belongs to the nucleotide-binding oligomerization domain (NOD)-like receptor family of pattern-recognition receptors (PRRs). Inflammatory responses are triggered when PRRs detect tissue damage or microbial infection. |

| | | |
|---|---|---|
| | *ICAM1* | The *ICAM1* gene is an inducible glycoprotein of the immunoglobulin (Ig) superfamily that contains 5 distinct Ig-like domains, a transmembrane domain, and a short cytoplasmic tail. It was first discovered as a ligand for LFA1 and then as a counter receptor for MAC1. |
| **Disease-severity** | *AATBC* | The *AATBC* (Apoptosis Associated Transcript In Bladder Cancer) is an RNA Gene, and is affiliated with the lncRNA class. Diseases associated with *AATBC* include Bladder Cancer. |
| | *MAFG* | The *MAFG* gene can chimerize with p45 nuclear factor erythroid-2 (NFE2) and supports the expression of globin genes and promotes erythroid differentiation. MAFG is expressed in CNS neurons and involved in encephalomyelitis. |
| | *VAV1* | The *VAV1* gene is a member of the VAV gene family. The VAV proteins are guanine nucleotide exchange factors (GEFs) for Rho family GTPases that activate pathways leading to actin cytoskeletal rearrangements and transcriptional alterations. The encoded protein is important in hematopoiesis, playing a role in T-cell and B-cell development and activation. The encoded protein has been identified as the specific binding partner of Nef proteins from HIV-1. Coexpression and binding of these partners initiates profound morphological changes, cytoskeletal rearrangements and the JNK/SAPK signaling cascade, leading to increased levels of viral transcription and replication. |
| | *MS4A7* | The *MS4A7* gene encodes a member of the membrane-spanning 4A gene family, members of which are characterized by common structural features and similar intron/exon splice boundaries and display unique expression patterns in hematopoietic cells and nonlymphoid tissues. This family member is associated with mature cellular function in the monocytic lineage, and it may be a component of a receptor complex involved in signal transduction. |
| | *IGHA1* | The *IGHA1* gene contributes to immunoglobulin receptor binding activity. It is involved in antibacterial humoral response; glomerular filtration; and positive regulation of respiratory burst. It is located in extracellular space and it is part of monomeric IgA immunoglobulin complex and secretory dimeric IgA immunoglobulin complex. |
| | *ATP6V0A1* | The *ATP6V0A1* gene encodes a component of vacuolar ATPase (V-ATPase), a multisubunit enzyme that mediates acidification of eukaryotic intracellular organelles. V-ATPase dependent organelle acidification is necessary for such intracellular processes as protein sorting, zymogen activation, receptor-mediated endocytosis, and synaptic vesicle proton gradient generation. |

| | | |
|---|---|---|
| | *RN7SL3* | The *RN7SL3* gene is part of the signal recognition particle (SRP), which is a cytoplasmic ribonucleoprotein complex that mediates co-translational insertion of secretory proteins into the lumen of the endoplasmic reticulum. |
| | *MPP7* | The *MPP7* gene encodes the protein which is a member of the p55 Stardust family of membrane-associated guanylate kinase (MAGUK) proteins. It is involved in the establishment of epithelial cell polarity. This family member forms a complex with the polarity protein DLG1 (discs, large homolog 1) and facilitates epithelial cell polarity and tight junction formation. |
| | *DSC2* | The *DSC2* gene encodes a member of the desmocollin protein subfamily. Desmocollins, along with desmogleins, are cadherin-like transmembrane glycoproteins that are major components of the desmosome. Desmosomes are cell-cell junctions that help resist shearing forces and are found in high concentrations in cells subject to mechanical stress. Mutations in this gene are associated with arrhythmogenic right ventricular dysplasia-11, and reduced protein expression has been described in several types of cancer. |
| | *PHACTR2* | The *PHACTR2* gene is predicted to enable actin binding activity and to be involved in actin cytoskeleton organization. It is located in plasma membrane and platelet alpha granule membrane. It is implicated in Parkinson's disease and multiple sclerosis and reported as a biomarker of Alzheimer's disease. |

* - descriptions were derived from Genecards – the human gene database (www.genecards.org)

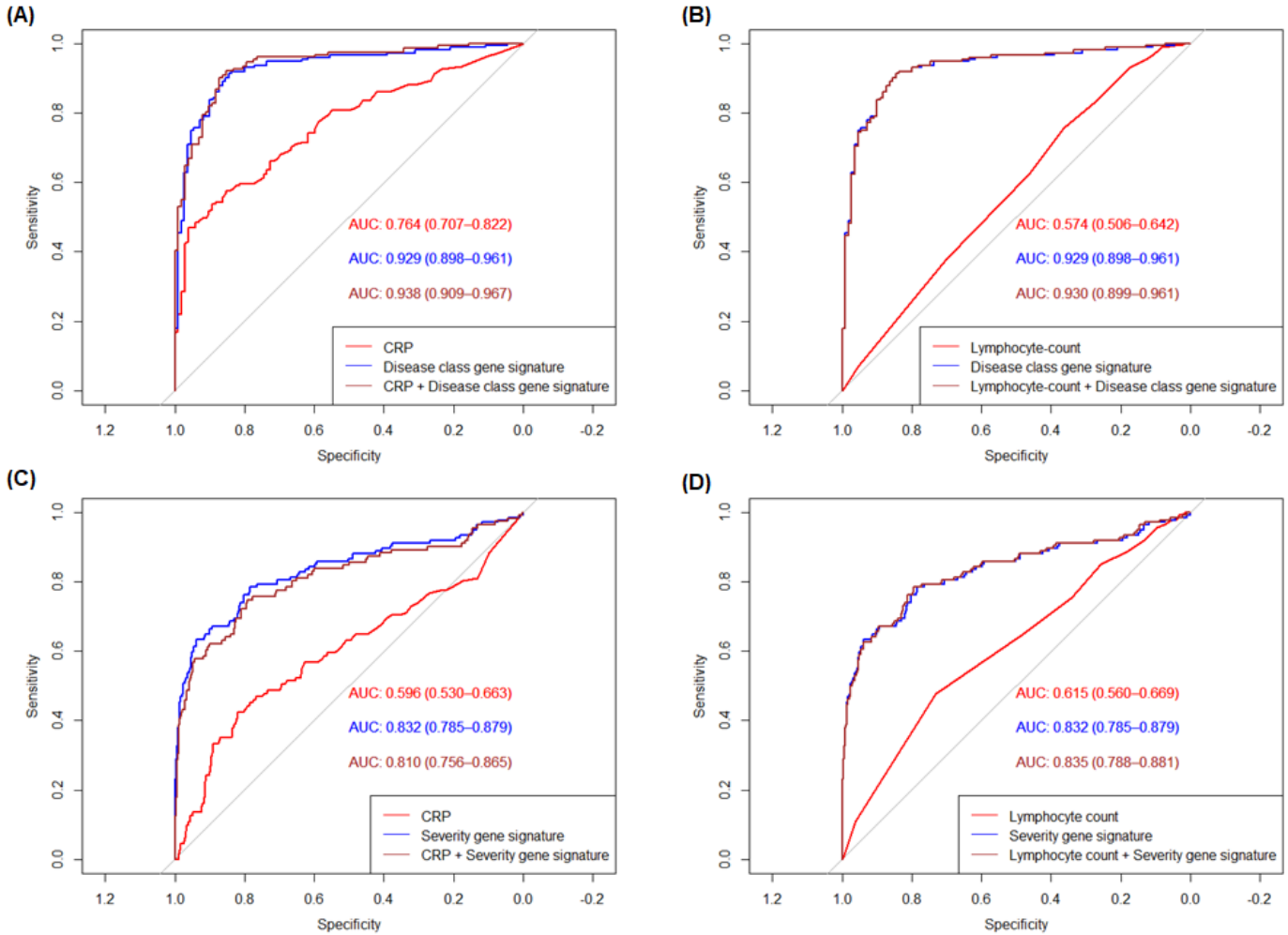
We assessed the enriched Gene Ontology (GO) terms in disease-class and disease-severity signature genes using ClusterProfiler (**eFigure 8**). Disease-class signature genes have immune response GO terms enriched indicating the immune response involved in different infection types. Disease-severity signature genes have immunoglobulin complex, signal recognition and proton transporting GO terms enriched, explaining the involvement of various organ dysfunctions and the subsequent biological response pathways.




**eFigure 8:** Enriched Gene Ontology terms in novel disease-class signature genes and novel disease-severity signature genes. Top 10 GO enriched terms are shown in the bar plots for (A) disease-class signature and (C) disease-severity signature. Count indicates the number of genes in the signature associated with the GO term and the p-adjusted value shows the enrichment score of the GO term. Network of the signature genes for the top 10 GO enriched terms for (B) disease-class signature and (D) disease-severity signature are shown in the network pots. Grey circles are the genes, and the brown circles are the GO enriched terms. Size of the brown circle is based on the enrichment scores associated to the GO term.

**eMethods 9:** Severity and disease class weights

The severity and disease class signature weights are available from https://github.com/lachlancoin/RAPIDS




**eFigure 9:** Performance of gene signatures combined with clinical measurements. Disease-class signature predictions for definite bacterial vs definite viral combined with **(A)** CRP measurements and **(B)** Lymphocyte cell count measurements at baseline. Disease-severity signature predictions for presence of organ dysfunction at baseline combined with **(C)** CRP measurements and **(D)** Lymphocyte cell count measurements at baseline.

**eTable 1: Clinical and severity characteristics of the discovery and validation cohorts of children evaluated for sepsis.**

| **Characteristic** | **Category** | **Discovery N=595** | **RAPIDS Validation N=312** | **EUCLIDS Validation N=362** |
|---|---|---|---|---|
| Sampling location *n (%)* | ED | 482 (81.0) | 231 (74.0) | |
| | PICU | 113 (19.0) | 81 (26.0) | |
| Time from PICU admission to sampling (hours) *median (IQR)* | | 1.9 (-1.5, 8.2) | 9.2 (2.7, 28.9) | |
| Organ dysfunction *n (%)* | Respiratory Dysfunction | | | |
| | Baseline | 67 (11.3) | 49 (15.7) | 157 (43.4) |
| | 24hrs | 63 (10.6) | 43 (13.8) | |
| | Cardiovascular Dysfunction | | | |
| | Baseline | 85 (14.3) | 41 (13.1) | 137 (37.9) |
| | 24hrs | 56 (9.4) | 37 (11.9) | |
| | Neurologic Dysfunction | | | |
| | Baseline | 79 (13.3) | 48 (15.4) | 69 (19.1) |
| | 24hrs | 67 (11.3) | 43 (13.8) | |
| | Renal Dysfunction | | | |
| | Baseline | 22 (3.7) | 9 (2.9) | 24 (6.6) |

| **Characteristic** | **Category** | **Discovery N=595** | **RAPIDS Validation N=312** | **EUCLIDS Validation N=362** |
|---|---|---|---|---|
| | 24hrs | 9 (1.5) | 6 (1.9) | |
| | Hematologic Dysfunction | | | |
| | Baseline | 37 (6.2) | 16 (5.1) | 31 (8.6) |
| | 24hrs | 22 (3.7) | 18 (5.8) | |
| | Hepatic Dysfunction | | | |
| | Baseline | 5 (0.8) | 5 (1.6) | 2 (0.6) |
| | 24hrs | 4 (0.7) | 3 (1.0) | |
| Mechanically ventilated *n (%)* | No | 505 (85.9) | 261 (83.7) | 203 (56.1) |
| | Yes | 83 (14.1) | 51 (16.4) | 159 (43.9) |
| Renal Replacement Therapy *n (%)* | No | 575 (98.0) | 307 (98.4) | 354 (97.8) |
| | Yes | 12 (2.0) | 5 (1.6) | 8 (2.2) |
| Extracorporeal Life Support *n (%)* | No | 577 (98.3) | 310 (99.4) | 360 (99.5) |
| | Yes | 10 (1.7) | 2 (0.6) | 2 (0.6) |
| Corticosteroids within 48hrs prior to study bloods *n (%)* | No | 559 (94.1) | 282 (91.3) | |
| | Yes | 35 (5.9) | 27 (8.7) | |

ED – Emergency Department, PICU – Paediatric Intensive Care Unit, IQR – Interquartile Range

**eTable 2: Microbiologic characteristics of the discovery and validation cohorts of children evaluated for sepsis.** Only cases where microbiologic results were positive are shown.

| **Pathogen Type** | **Discovery N=595** | **RAPIDS Validation N=312** | **EUCLIDS Validation N= 362** |
|---|---|---|---|
| Bacterial *n (%)* | 209 (35.1) | 77 (24.7) | 189 (52.2) |
| *Streptococcus pneumoniae* | 22 (3.7) | 8 (2.6) | 29 (8.0) |
| *Staphylococcus aureus* | 53 (8.9) | 19 (6.1) | 33 (9.1) |
| Group A *streptococcus* | 26 (4.4) | 2 (0.6) | 16 (4.4) |
| Group B *streptococcus* | 1 (0.2) | 0 (0) | 3 (0.8) |
| Coagulase-negative *staphylococci* | 2 (0.3) | 2 (0.6) | 1 (0.3) |
| *Escherichia coli* | 40 (6.7) | 16 (5.1) | 6 (1.7) |
| *Pseudomonas aeruginosa* | 4 (0.7) | 12 (3.8) | 4 (1.1) |
| *Enterococcus species* | 4 (0.7) | 3 (1.0) | 3 (0.8) |
| *Klebsiella species* | 8 (1.3) | 7 (2.2) | 1 (0.3) |
| *Neisseria meningitidis* | 2 (0.3) | 0 (0) | 75 (20.7) |
| *Haemophilus influenza* | 8 (1.3) | 2 (0.6) | 2 (0.6) |
| Other Gram-positive pathogens | 13 (2.2) | 9 (2.9) | 5 (1.4) |
| Other Gram-negative pathogens | 10 (1.7) | 10 (3.2) | 6 (1.7) |
| *Mycoplasma* | 22 (3.7) | 1 (0.3) | 2 (0.6) |
| *Bordetella pertussis* | 0 (0) | 0 (0) | 1 (0.3) |
| *Mycobacteria* | 1 (0.2) | 0 (0) | 0 (0) |
| Other | 19 (3.2) | 22 (7.1) | 2 (0.6) |
| Viral *n (%)* | 156 (26.2) | 123 (39.4) | 39 (10.8) |
| respiratory syncytial virus | 49 (8.2) | 24 (7.7) | 4 (1.1) |
| influenza A | 37 (6.2) | 22 (7.1) | 2 (0.6) |
| influenza B | 8 (1.3) | 8 (2.6) | 0 (0) |
| parainfluenza 1 | 5 (0.8) | 1 (0.3) | 0 (0) |
| parainfluenza 2 | 4 (0.7) | 0 (0) | 0 (0) |

| | | | |
|---|---|---|---|
| parainfluenza 3 | 10 (1.7) | 13 (4.2) | 1 (0.3) |
| parainfluenza 4 | 0 (0) | 0 (0) | 0(0) |
| human metapneumovirus (HMPV) | 14 (2.4) | 16 (5.1) | 3 (0.8) |
| adenovirus | 28 (4.7) | 18 (5.8) | 6 (1.7) |
| herpes simplex virus (HSV) | 2 (0.3) | 0 (0) | 0 (0) |
| enterovirus | 3 (0.5) | 1 (0.3) | 9 (2.5) |
| parechovirus | 1 (0.2) | 1 (0.3) | 2 (0.6) |
| Severe acute respiratory coronavirus-2 | 0 (0) | 0 (0) | 0 (0) |
| Other | 20 (3.4) | 28 (9.0) | 12 (3.3) |
| Fungal *n (%)* | 3 (0.5) | 2 (0.6) | |
| *Candida* | 3 (0.5) | 0 (0) | 0 |
| *Aspergillus* | 0 (0) | 1 (0.3) | 0 |
| Other | 0 (0) | 1 (0.3) | 0 |
| Parasitic *n (%)* | 0 (0) | 1 (0.3) | 0 |
| *Blastocystis hominis* | 0 (0) | 1 (0.3) | 0 |

**eTable 3: Number of differentially expressed (DE) genes and top 10 DE genes for infection types and disease severity in the discovery cohort.**

| Comparison | Number of DE genes[#] | Top 10 DE genes* |
|---|---|---|
| DV versus DB | 886 | *CNP, CHROMR, PNPT1, CCL2, CCL8, HERC6, PPM1K, LY6E-DT, AXL, USP18* |
| With versus without OD at 24-hours | 1028 | *LGR4, HBEGF, CD24, BCL2L15, CEACAM8, MPO, AMOTL1, IL3RA, INHBA, TMEM236* |
| With versus without OD at 24-hours with DV infections | 86 | *ZC3H12C, MYB, MYO1B, TNFRSF9, SATB2, CD38, IGHV4-59, CCSER1, IGKV1D-12, MSC* |
| With versus without OD at 24-hours with DB infections | 366 | *MRC1, HBEGF, MAST4, CYB561, MPO, PLEKHA7, AC091117.2, KCNK5, ARHGEF10L, MIR210HG* |

[#] - Differentially expressed genes with adjusted p-values < 0.05 and absolute LFC > 1 or < -1
*- Top 10 differentially expressed genes with low adjusted p-values
DB – Definite Bacterial, DV – Definite Viral, OD – Organ Dysfunction

**eTable 4: Performance of the novel disease-class signature and previously published host transcriptomic signatures in distinguishing infection types.**

| Phenotype | Cohort | Statistics | Novel Signature | Herberg et al | McHugh et al | Tang et al | Wong et al | Sweeney et al | Sampson et al | Li et al | Li et al (2021) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Definite Bacterial vs Definite Viral | Discovery (n=595) | Prevalence (95% CI) | 0.390 (0.333-0.450) | | | | | | | | |
| | | Sensitivity (95% CI) | 0.891 (0.814-0.940) | 0.836 (0.751-0.898) | 0.709 (0.614-0.790) | 0.864 (0.782-0.919) | 0.791 (0.701-0.860) | 0.864 (0.782-0.919) | 0.882 (0.803-0.933) | 0.664 (0.566-0.749) | 0.855 (0.772-0.912) |
| | | Specificity (95% CI) | 0.855 (0.791-0.902) | 0.785 (0.714-0.842) | 0.692 (0.616-0.759) | 0.744 (0.671-0.806) | 0.692 (0.616-0.759) | 0.785 (0.714-0.842) | 0.762 (0.690-0.822) | 0.587 (0.510-0.661) | 0.802 (0.733-0.857) |
| | | NPV (95% CI) | 0.925 (0.869-0.959) | 0.882 (0.818-0.927) | 0.788 (0.713-0.849) | 0.895 (0.830-0.938) | 0.838 (0.765-0.892) | 0.900 (0.838-0.941) | 0.910 (0.848-0.949) | 0.732 (0.649-0.802) | 0.896 (0.834-0.938) |
| | | PPV (95% CI) | 0.797 (0.713-0.862) | 0.713 (0.626-0.788) | 0.595 (0.506-0.679) | 0.683 (0.598-0.758) | 0.621 (0.535-0.701) | 0.720 (0.634-0.793) | 0.703 (0.618-0.776) | 0.507 (0.423-0.591) | 0.734 (0.648-0.807) |
| | RAPIDS Validation (n=312) | Prevalence (95% CI) | 0.613 (0.534-0.688) | | | | | | | | |
| | | Sensitivity (95% CI) | 0.750 (0.652-0.829) | 0.820 (0.728-0.887) | 0.520 (0.418-0.620) | 0.780 (0.684-0.854) | 0.620 (0.517-0.714) | 0.790 (0.695-0.862) | 0.660 (0.558-0.750) | 0.600 (0.497-0.695) | 0.870 (0.784-0.926) |
| | | Specificity(95% CI) | 0.937 (0.837-0.979) | 0.825 (0.705-0.906) | 0.762 (0.635-0.856) | 0.857 (0.741-0.929) | 0.778 (0.652-0.869) | 0.905 (0.798-0.961) | 0.937 (0.837-0.979) | 0.698 (0.568-0.804) | 0.762 (0.635-0.856) |
| | | NPV (95% CI) | 0.702 (0.591-0.795) | 0.743 (0.622-0.837) | 0.500 (0.402-0.598) | 0.711 (0.594-0.806) | 0.563 (0.453-0.668) | 0.731 (0.616-0.822) | 0.634 (0.528-0.730) | 0.524 (0.413-0.633) | 0.787 (0.660-0.877) |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | **PPV (95% CI)** | 0.949 (0.869-0.984) | 0.882 (0.794-0.937) | 0.776 (0.655-0.865) | 0.897 (0.808-0.949) | 0.816 (0.707-0.892) | 0.929 (0.847-0.971) | 0.943 (0.853-0.982) | 0.759 (0.648-0.846) | 0.853 (0.766-0.913) |
| | **EUCLIDS Validation (n=362)** | **Prevalence (95% CI)** | 0.172 (0.126-0.229) | | | | | | | | |
| | | **Sensitivity (95% CI)** | 0.846 (0.688-0.936) | 0.846 (0.688-0.936) | 0.949 (0.814-0.991) | 0.821 (0.659-0.919) | 0.308 (0.175-0.477) | 0.846 (0.688-0.936) | 0.692 (0.523-0.825) | 0.641 (0.472-0.783) | 0.872 (0.718-0.952) |
| | | **Specificity(95% CI)** | 0.872 (0.814-0.915) | 0.840 (0.778-0.888) | 0.144 (0.098-0.204) | 0.803 (0.738-0.856) | 0.920 (0.869-0.953) | 0.750 (0.681-0.809) | 0.899 (0.844-0.936) | 0.782 (0.715-0.837) | 0.750 (0.681-0.809) |
| | | **NPV (95% CI)** | 0.965 (0.921-0.986) | 0.963 (0.918-0.985) | 0.931 (0.758-0.988) | 0.956 (0.907-0.980) | 0.865 (0.808-0.908) | 0.959 (0.909-0.983) | 0.934 (0.884-0.964) | 0.913 (0.856-0.950) | 0.966 (0.918-0.987) |
| | | **PPV (95% CI)** | 0.579 (0.441-0.706) | 0.524 (0.395-0.650) | 0.187 (0.137-0.250) | 0.464 (0.344-0.587) | 0.444 (0.260-0.644) | 0.412 (0.305-0.528) | 0.587 (0.433-0.727) | 0.379 (0.265-0.507) | 0.420 (0.313-0.535) |
| **Definite Bacterial vs Probable Viral** | **Discovery (n=595)** | **Prevalence (95% CI)** | 0.336 (0.279-0.397) | | | | | | | | |
| | | **Sensitivity (95% CI)** | 0.885 (0.794-0.941) | 0.724 (0.616-0.812) | 0.759 (0.653-0.841) | 0.678 (0.568-0.772) | 0.828 (0.728-0.897) | 0.851 (0.754-0.915) | 0.690 (0.580-0.782) | 0.724 (0.616-0.812) | 0.724 (0.616-0.812) |
| | | **Specificity(95% CI)** | 0.762 (0.690-0.822) | 0.692 (0.616-0.759) | 0.692 (0.616-0.759) | 0.674 (0.598-0.743) | 0.599 (0.521-0.672) | 0.721 (0.647-0.785) | 0.686 (0.610-0.753) | 0.576 (0.498-0.650) | 0.750 (0.677-0.811) |
| | | **NPV (95% CI)** | 0.929 (0.87-0.964) | 0.832 (0.759-0.887) | 0.850 (0.778-0.903) | 0.806 (0.730-0.865) | 0.873 (0.796-0.925) | 0.905 (0.840-0.946) | 0.814 (0.739-0.872) | 0.805 (0.722-0.869) | 0.843 (0.774-0.895) |
| | | **PPV (95% CI)** | 0.653 (0.559-0.736) | 0.543 (0.448-0.635) | 0.555 (0.461-0.645) | 0.513 (0.419-0.607) | 0.511 (0.425-0.595) | 0.607 (0.514-0.693) | 0.526 (0.431-0.620) | 0.463 (0.378-0.551) | 0.594 (0.494-0.687) |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | **RAPIDS Validation (n=312)** | **Prevalence (95% CI)** | 0.337 (0.245-0.442) | | | | | | | |
| | | **Sensitivity (95% CI)** | 0.906 (0.738-0.975) | 0.812 (0.630-0.921) | 0.562 (0.379-0.732) | 0.688 (0.499-0.833) | 0.812 (0.630-0.921) | 0.844 (0.665-0.941) | 0.750 (0.562-0.879) | 0.750 (0.562-0.879) | 0.812 (0.630-0.921) |
| | | **Specificity(95% CI)** | 0.571 (0.441-0.693) | 0.730 (0.601-0.831) | 0.762 (0.635-0.856) | 0.778 (0.652-0.869) | 0.698 (0.568-0.804) | 0.619 (0.488-0.736) | 0.889 (0.778-0.950) | 0.619 (0.488-0.736) | 0.698 (0.568-0.804) |
| | | **NPV (95% CI)** | 0.923 (0.780-0.980) | 0.885 (0.759-0.952) | 0.774 (0.647-0.867) | 0.831 (0.706-0.911) | 0.880 (0.750-0.950) | 0.886 (0.746-0.957) | 0.875 (0.763-0.941) | 0.830 (0.687-0.919) | 0.880 (0.750-0.950) |
| | | **PPV (95% CI)** | 0.518 (0.382-0.652) | 0.605 (0.445-0.746) | 0.545 (0.366-0.715) | 0.611 (0.435-0.764) | 0.578 (0.422-0.720) | 0.529 (0.386-0.668) | 0.774 (0.585-0.897) | 0.500 (0.364-0.636) | 0.578 (0.422-0.720) |
| | **EUCLIDS Validation (n=362)** | **Prevalence (95% CI)** | 0.060 (0.033-0.105) | | | | | | | |
| | | **Sensitivity (95% CI)** | 0.917 (0.598-0.996) | 0.583 (0.286-0.835) | 0.917 (0.598-0.996) | 0.583 (0.286-0.835) | 0.333 (0.113-0.646) | 1.000 (0.699-1.000) | 0.583 (0.286-0.835) | 0.667 (0.354-0.887) | 0.917 (0.598-0.996) |
| | | **Specificity(95% CI)** | 0.904 (0.851-0.941) | 0.894 (0.838-0.932) | 0.277 (0.215-0.347) | 0.718 (0.647-0.780) | 0.941 (0.895-0.969) | 0.074 (0.043-0.124) | 0.920 (0.869-0.953) | 0.734 (0.664-0.794) | 0.612 (0.538-0.681) |
| | | **NPV (95% CI)** | 0.994 (0.963-1.000) | 0.971 (0.930-0.989) | 0.981 (0.886-0.999) | 0.964 (0.914-0.987) | 0.957 (0.914-0.980) | 1.000 (0.732-1.000) | 0.972 (0.932-0.990) | 0.972 (0.925-0.991) | 0.991 (0.946-1.000) |
| | | **PPV (95% CI)** | 0.379 (0.213-0.576) | 0.259 (0.119-0.466) | 0.075 (0.040-0.133) | 0.117 (0.052-0.232) | 0.267 (0.089-0.552) | 0.065 (0.035-0.113) | 0.318 (0.147-0.549) | 0.138 (0.066-0.259) | 0.131 (0.070-0.226) |
| **Definite Viral vs** | **Discovery (n=595)** | **Prevalence (95% CI)** | 0.368 (0.297-0.445) | | | | | | | |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Probable Bacterial** | | **Sensitivity (95% CI)** | 0.844 (0.727-0.919) | 0.734 (0.607-0.833) | 0.719 (0.590-0.821) | 0.719 (0.590-0.821) | 0.734 (0.607-0.833) | 0.828 (0.709-0.907) | 0.750 (0.623-0.846) | 0.594 (0.464-0.712) | 0.734 (0.607-0.833) |
| | | **Specificity(95% CI)** | 0.809 (0.721-0.875) | 0.773 (0.681-0.845) | 0.636 (0.539-0.724) | 0.800 (0.711-0.868) | 0.664 (0.566-0.749) | 0.773 (0.681-0.845) | 0.764 (0.671-0.837) | 0.527 (0.430-0.622) | 0.764 (0.671-0.837) |
| | | **NPV (95% CI)** | 0.899 (0.818-0.948) | 0.833 (0.744-0.897) | 0.795 (0.693-0.871) | 0.830 (0.742-0.894) | 0.811 (0.712-0.883) | 0.885 (0.800-0.939) | 0.840 (0.750-0.903) | 0.690 (0.579-0.784) | 0.832 (0.741-0.896) |
| | | **PPV (95% CI)** | 0.720 (0.603-0.815) | 0.653 (0.531-0.759) | 0.535 (0.425-0.642) | 0.676 (0.551-0.782) | 0.560 (0.447-0.666) | 0.679 (0.563-0.778) | 0.649 (0.528-0.754) | 0.422 (0.320-0.531) | 0.644 (0.522-0.750) |
| | **RAPIDS Validation (n=312)** | **Prevalence (95% CI)** | 0.281 (0.209-0.364) | | | | | | | | |
| | | **Sensitivity (95% CI)** | 0.923 (0.780-0.980) | 0.769 (0.603-0.883) | 0.821 (0.659-0.919) | 0.821 (0.659-0.919) | 0.795 (0.631-0.901) | 0.846 (0.688-0.936) | 0.923 (0.780-0.980) | 0.513 (0.350-0.673) | 0.769 (0.603-0.883) |
| | | **Specificity(95% CI)** | 0.510 (0.409-0.611) | 0.780 (0.684-0.854) | 0.420 (0.323-0.523) | 0.600 (0.497-0.695) | 0.490 (0.389-0.591) | 0.660 (0.558-0.750) | 0.400 (0.305-0.503) | 0.600 (0.497-0.695) | 0.750 (0.652-0.829) |
| | | **NPV (95% CI)** | 0.944 (0.837-0.986) | 0.897 (0.808-0.949) | 0.857 (0.721-0.936) | 0.896 (0.791-0.953) | 0.860 (0.737-0.933) | 0.917 (0.821-0.966) | 0.930 (0.799-0.982) | 0.759 (0.648-0.846) | 0.893 (0.802-0.947) |
| | | **PPV (95% CI)** | 0.424 (0.319-0.535) | 0.577 (0.433-0.710) | 0.356 (0.259-0.464) | 0.444 (0.329-0.566) | 0.378 (0.275-0.492) | 0.493 (0.370-0.616) | 0.375 (0.280-0.480) | 0.333 (0.220-0.468) | 0.545 (0.407-0.678) |
| | | **Prevalence (95% CI)** | 0.606 (0.503-0.701) | | | | | | | | |

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|---|---|---|---|---|---|---|---|---|---|---|---|
| | **EUCLIDS Validation (n=362)** | **Sensitivity (95% CI)** | 0.833 (0.710-0.913) | 0.667 (0.532-0.780) | 0.117 (0.052-0.232) | 0.817 (0.691-0.901) | 0.867 (0.749-0.937) | 0.767 (0.637-0.862) | 0.817 (0.691-0.901) | 0.433 (0.308-0.567) | 0.717 (0.584-0.822) |
| | | **Specificity(95% CI)** | 0.667 (0.497-0.804) | 0.692 (0.523-0.825) | 0.923 (0.780-0.980) | 0.769 (0.603-0.883) | 0.256 (0.136-0.424) | 0.821 (0.659-0.919) | 0.590 (0.422-0.740) | 0.718 (0.549-0.845) | 0.744 (0.576-0.864) |
| | | **NPV (95% CI)** | 0.722 (0.546-0.852) | 0.574 (0.423-0.714) | 0.404 (0.303-0.514) | 0.732 (0.568-0.852) | 0.556 (0.313-0.776) | 0.696 (0.541-0.818) | 0.676 (0.494-0.820) | 0.452 (0.327-0.582) | 0.630 (0.475-0.764) |
| | | **PPV (95% CI)** | 0.794 (0.670-0.881) | 0.769 (0.628-0.870) | 0.700 (0.354-0.919) | 0.845 (0.721-0.922) | 0.642 (0.527-0.743) | 0.868 (0.740-0.941) | 0.754 (0.629-0.849) | 0.703 (0.528-0.836) | 0.811 (0.676-0.901) |
| **Definite Bacterial vs Non-Infectious** | **Discovery (n=595)** | **Prevalence (95% CI)** | 0.207 (0.157-0.269) | | | | | | | | |
| | | **Sensitivity (95% CI)** | 0.978 (0.868-0.999) | 0.867 (0.725-0.945) | 0.889 (0.752-0.958) | 0.689 (0.532-0.814) | 0.800 (0.649-0.899) | 0.867 (0.725-0.945) | 0.733 (0.578-0.849) | 0.822 (0.674-0.915) | 0.556 (0.401-0.700) |
| | | **Specificity(95% CI)** | 0.686 (0.610-0.753) | 0.523 (0.446-0.599) | 0.581 (0.504-0.655) | 0.488 (0.412-0.565) | 0.477 (0.401-0.554) | 0.622 (0.545-0.694) | 0.587 (0.510-0.661) | 0.547 (0.469-0.622) | 0.587 (0.510-0.661) |
| | | **NPV (95% CI)** | 0.992 (0.947-1.000) | 0.938 (0.864-0.974) | 0.952 (0.887-0.982) | 0.857 (0.769-0.917) | 0.901 (0.816-0.951) | 0.947 (0.883-0.978) | 0.894 (0.818-0.942) | 0.922 (0.847-0.963) | 0.835 (0.754-0.894) |
| | | **PPV (95% CI)** | 0.449 (0.349-0.553) | 0.322 (0.242-0.414) | 0.357 (0.270-0.454) | 0.261 (0.186-0.350) | 0.286 (0.211-0.374) | 0.375 (0.284-0.476) | 0.317 (0.231-0.417) | 0.322 (0.239-0.416) | 0.260 (0.179-0.362) |
| | | **Prevalence (95% CI)** | 0.382 (0.289-0.484) | | | | | | | | |

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|---|---|---|---|---|---|---|---|---|---|---|---|
| | **RAPIDS Validation (n=312)** | **Sensitivity (95% CI)** | 0.667 (0.497-0.804) | 0.487 (0.327-0.650) | 0.308 (0.175-0.477) | 0.538 (0.374-0.696) | 0.692 (0.523-0.825) | 0.821 (0.659-0.919) | 0.103 (0.033-0.252) | 0.744 (0.576-0.864) | 0.308 (0.175-0.477) |
| | | **Specificity(95% CI)** | 0.492 (0.365-0.620) | 0.571 (0.441-0.693) | 0.857 (0.741-0.929) | 0.492 (0.365-0.620) | 0.619 (0.488-0.736) | 0.460 (0.336-0.590) | 0.952 (0.858-0.988) | 0.556 (0.426-0.679) | 0.746 (0.618-0.844) |
| | | **NPV (95% CI)** | 0.705 (0.546-0.828) | 0.643 (0.503-0.763) | 0.667 (0.552-0.765) | 0.633 (0.483-0.762) | 0.765 (0.622-0.868) | 0.806 (0.634-0.912) | 0.632 (0.526-0.726) | 0.778 (0.625-0.883) | 0.635 (0.515-0.742) |
| | | **PPV (95% CI)** | 0.448 (0.320-0.584) | 0.413 (0.273-0.567) | 0.571 (0.344-0.774) | 0.396 (0.268-0.540) | 0.529 (0.386-0.668) | 0.485 (0.361-0.610) | 0.571 (0.202-0.882) | 0.509 (0.374-0.642) | 0.429 (0.250-0.626) |
| **Definite Viral vs Non-Infectious** | **Discovery (n=595)** | **Prevalence (95% CI)** | 0.29 (0.222-0.37) | | | | | | | | |
| | | **Sensitivity (95% CI)** | 0.911 (0.779-0.971) | 0.889 (0.752-0.958) | 0.867 (0.725-0.945) | 0.889 (0.752-0.958) | 0.822 (0.674-0.915) | 0.933 (0.807-0.983) | 0.867 (0.725-0.945) | 0.844 (0.699-0.930) | 0.867 (0.725-0.945) |
| | | **Specificity(95% CI)** | 0.818 (0.731-0.883) | 0.782 (0.691-0.853) | 0.709 (0.614-0.790) | 0.800 (0.711-0.868) | 0.718 (0.623-0.798) | 0.809 (0.721-0.875) | 0.773 (0.681-0.845) | 0.709 (0.614-0.790) | 0.782 (0.691-0.853) |
| | | **NPV (95% CI)** | 0.957 (0.888-0.986) | 0.945 (0.871-0.980) | 0.929 (0.845-0.971) | 0.946 (0.873-0.980) | 0.908 (0.822-0.957) | 0.967 (0.901-0.992) | 0.934 (0.857-0.973) | 0.918 (0.832-0.963) | 0.935 (0.858-0.973) |
| | | **PPV (95% CI)** | 0.672 (0.539-0.784) | 0.625 (0.495-0.740) | 0.549 (0.427-0.666) | 0.645 (0.513-0.760) | 0.544 (0.419-0.664) | 0.667 (0.536-0.777) | 0.609 (0.479-0.726) | 0.543 (0.420-0.661) | 0.619 (0.488-0.736) |
| | | **Prevalence (95% CI)** | 0.281 (0.209-0.364) | | | | | | | | |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | **RAPIDS Validation (n=312)** | **Sensitivity (95% CI)** | 0.872 (0.718-0.952) | 0.615 (0.447-0.762) | 0.410 (0.260-0.578) | 0.744 (0.576-0.864) | 0.769 (0.603-0.883) | 0.769 (0.603-0.883) | 0.744 (0.576-0.864) | 0.718 (0.549-0.845) | 0.667 (0.497-0.804) |
| | | **Specificity(95% CI)** | 0.380 (0.286-0.483) | 0.800 (0.706-0.871) | 0.900 (0.820-0.948) | 0.590 (0.487-0.686) | 0.520 (0.418-0.620) | 0.600 (0.497-0.695) | 0.590 (0.487-0.686) | 0.580 (0.477-0.677) | 0.770 (0.673-0.846) |
| | | **NPV (95% CI)** | 0.884 (0.741-0.956) | 0.842 (0.750-0.906) | 0.796 (0.708-0.864) | 0.855 (0.745-0.925) | 0.852 (0.733-0.926) | 0.870 (0.762-0.935) | 0.855 (0.745-0.925) | 0.841 (0.728-0.914) | 0.856 (0.762-0.918) |
| | | **PPV (95% CI)** | 0.354 (0.261-0.459) | 0.545 (0.390-0.693) | 0.615 (0.407-0.791) | 0.414 (0.300-0.538) | 0.385 (0.279-0.502) | 0.429 (0.313-0.552) | 0.414 (0.300-0.538) | 0.400 (0.287-0.524) | 0.531 (0.384-0.672) |

**eTable5: Performance of novel disease-class signature in distinguishing other disease-class phenotypes such as Combined Bacterial Viral infection and Unknown infection status.**

| ***Phenotype**** | ***Discovery (n=595)*** | | | ***RAPIDS Validation (n=312)*** | | |
|---|---|---|---|---|---|---|
| | **AUC** | **95% CI low** | **95% CI high** | **AUC** | **95% CI low** | **95% CI high** |
| *DBvsCBV* | 0.739 | 0.663 | 0.815 | 0.587 | 0.449 | 0.724 |
| *DBvsUnknown* | 0.748 | 0.680 | 0.815 | 0.693 | 0.465 | 0.921 |
| *DVvsCBV* | 0.723 | 0.643 | 0.804 | 0.808 | 0.710 | 0.906 |
| *DVvsUnknown* | 0.790 | 0.713 | 0.868 | 0.750 | 0.567 | 0.933 |
| *PBvsPV* | 0.780 | 0.704 | 0.855 | 0.758 | 0.639 | 0.877 |
| *PBvsNI* | 0.526 | 0.416 | 0.637 | 0.616 | 0.490 | 0.742 |
| *PBvsCBV* | 0.572 | 0.472 | 0.672 | 0.562 | 0.417 | 0.706 |
| *PBvsUnknown* | 0.536 | 0.431 | 0.641 | 0.521 | 0.278 | 0.765 |
| *PVvsNI* | 0.786 | 0.703 | 0.870 | 0.675 | 0.544 | 0.806 |
| *PVvsCBV* | 0.710 | 0.625 | 0.794 | 0.758 | 0.635 | 0.881 |
| *PVvsUnknown* | 0.779 | 0.697 | 0.860 | 0.694 | 0.485 | 0.904 |
| *CBVvsNI* | 0.559 | 0.450 | 0.668 | 0.660 | 0.522 | 0.797 |
| *CBVvsUnknown* | 0.545 | 0.439 | 0.651 | 0.593 | 0.376 | 0.809 |
| *NIvsUnknown* | 0.492 | 0.376 | 0.609 | 0.430 | 0.184 | 0.676 |
| *BacterialvsViral* | 0.880 | 0.848 | 0.912 | 0.859 | 0.810 | 0.908 |

* DB – Definite Bacterial; DV – Definite Viral; PB – Probable Bacterial; PV – Probable Viral; NI – Non-infectious; CBV – Combined Bacterial Viral; Unknown – Unknown infection type; Bacterial – Definite Bacterial and Probable Bacterial combined and Viral - Definite Viral and Probable Viral combined

AUC – Area Under the Curve; CI – Confidence Interval

**eTable 6: Performance of the novel disease-severity signature and previously published host transcriptomic signatures in identifying organ dysfunction.**

| Phenotype | Cohort | Statistics | Novel Signature | Irwin et al | Lukaszewski et al | Pena et al | Baghela et al | Baghela et al - Severity | Baghela et al - CR | Baghela et al - Mortality |
|---|---|---|---|---|---|---|---|---|---|---|
| OD at 24hrs | Discovery (n=595) | Prevalence (95% CI) | 0.225 (0.193-0.261) | | | | | | | |
| | | Sensitivity (95% CI) | 0.791 (0.711-0.854) | 0.575 (0.486-0.659) | 0.687 (0.600-0.762) | 0.694 (0.608-0.769) | 0.731 (0.647-0.802) | 0.724 (0.639-0.796) | 0.679 (0.592-0.756) | 0.664 (0.577-0.742) |
| | | Specificity (95% CI) | 0.742 (0.699-0.781) | 0.733 (0.69-0.773) | 0.683 (0.638-0.725) | 0.729 (0.685-0.768) | 0.696 (0.652-0.738) | 0.718 (0.674-0.758) | 0.727 (0.683-0.766) | 0.679 (0.634-0.721) |
| | | NPV (95% CI) | 0.924 (0.891-0.948) | 0.856 (0.816-0.888) | 0.882 (0.843-0.913) | 0.891 (0.854-0.92) | 0.899 (0.862-0.927) | 0.899 (0.863-0.927) | 0.886 (0.849-0.916) | 0.874 (0.834-0.906) |
| | | PPV (95% CI) | 0.471 (0.405-0.538) | 0.385 (0.318-0.457) | 0.387 (0.325-0.452) | 0.427 (0.361-0.495) | 0.412 (0.349-0.477) | 0.427 (0.363-0.495) | 0.419 (0.353-0.488) | 0.376 (0.314-0.441) |
| | RAPIDS Validation (n=312) | Prevalence (95% CI) | 0.244 (0.198-0.296) | | | | | | | |
| | | Sensitivity (95% CI) | 0.987 (0.919-0.999) | 0.395 (0.287-0.514) | 0.816 (0.707-0.892) | 0.750 (0.635-0.839) | 0.579 (0.460-0.690) | 0.711 (0.594-0.806) | 0.776 (0.664-0.861) | 0.776 (0.664-0.861) |
| | | Specificity (95% CI) | 0.089 (0.057-0.135) | 0.869 (0.817-0.908) | 0.521 (0.456-0.586) | 0.542 (0.477-0.607) | 0.775 (0.716-0.826) | 0.716 (0.653-0.772) | 0.483 (0.418-0.549) | 0.648 (0.583-0.708) |
| | | NPV (95% CI) | 0.955 (0.751-0.998) | 0.817 (0.762-0.861) | 0.898 (0.831-0.941) | 0.871 (0.803-0.918) | 0.851 (0.795-0.895) | 0.885 (0.829-0.925) | 0.870 (0.798-0.920) | 0.900 (0.842-0.939) |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | **PPV (95% CI)** | 0.259 (0.210-0.314) | 0.492 (0.363-0.622) | 0.354 (0.285-0.431) | 0.345 (0.274-0.424) | 0.454 (0.353-0.558) | 0.446 (0.357-0.539) | 0.326 (0.259-0.400) | 0.415 (0.334-0.501) |
| **OD at 24hrs in Definite Bacterial Infection** | **Discovery (n=595)** | **Prevalence (95% CI)** | 0.318 (0.223-0.429) | | | | | | | |
| | | **Sensitivity (95% CI)** | 0.963 (0.791-0.998) | 0.815 (0.613-0.930) | 0.889 (0.697-0.971) | 0.815 (0.613-0.930) | 0.963 (0.791-0.998) | 0.926 (0.742-0.987) | 0.889 (0.697-0.971) | 0.926 (0.742-0.987) |
| | | **Specificity (95% CI)** | 0.776 (0.644-0.871) | 0.569 (0.433-0.696) | 0.724 (0.589-0.830) | 0.724 (0.589-0.830) | 0.828 (0.701-0.910) | 0.672 (0.535-0.786) | 0.724 (0.589-0.830) | 0.724 (0.589-0.83) |
| | | **NPV (95% CI)** | 0.978 (0.870-0.999) | 0.868 (0.711-0.951) | 0.933 (0.807-0.983) | 0.894 (0.761-0.960) | 0.980 (0.878-0.999) | 0.951 (0.822-0.992) | 0.933 (0.807-0.983) | 0.955 (0.833-0.992) |
| | | **PPV (95% CI)** | 0.667 (0.497-0.804) | 0.468 (0.324-0.618) | 0.600 (0.434-0.747) | 0.579 (0.409-0.733) | 0.722 (0.546-0.852) | 0.568 (0.411-0.713) | 0.600 (0.434-0.747) | 0.610 (0.445-0.754) |
| | **RAPIDS Validation (n=312)** | **Prevalence (95% CI)** | 0.292 (0.134-0.512) | | | | | | | |
| | | **Sensitivity (95% CI)** | 1 (0.561-1) | 0.714 (0.303-0.949) | 1 (0.561-1) | 0.429 (0.118-0.798) | 0.286 (0.051-0.697) | 1 (0.561-1) | 0.714 (0.303-0.949) | 0.857 (0.420-0.992) |
| | | **Specificity (95% CI)** | 0.235 (0.078-0.502) | 0.647 (0.386-0.847) | 0.176 (0.047-0.442) | 0.588 (0.335-0.806) | 0.588 (0.335-0.806) | 0.588 (0.335-0.806) | 0.176 (0.047-0.442) | 0.471 (0.239-0.715) |
| | | **NPV (95% CI)** | 1 (0.396-1) | 0.846 (0.537-0.973) | 1 (0.310-1) | 0.714 (0.420-0.904) | 0.667 (0.387-0.870) | 1 (0.655-1) | 0.600 (0.170-0.927) | 0.889 (0.507-0.994) |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | **PPV (95% CI)** | 0.350 (0.163-0.591) | 0.455 (0.181-0.754) | 0.333 (0.155-0.569) | 0.300 (0.081-0.646) | 0.222 (0.039-0.598) | 0.500 (0.268-0.732) | 0.263 (0.101-0.514) | 0.400 (0.175-0.671) |
| **OD at 24hrs in Definite Viral Infection** | **Discovery (n=595)** | **Prevalence (95% CI)** | 0.176 (0.118-0.253) | | | | | | | |
| | | **Sensitivity (95% CI)** | 0.667 (0.447-0.836) | 0.542 (0.332-0.738) | 0.583 (0.369-0.772) | 0.542 (0.332-0.738) | 0.583 (0.369-0.772) | 0.542 (0.332-0.738) | 0.583 (0.369-0.772) | 0.417 (0.228-0.631) |
| | | **Specificity (95% CI)** | 0.714 (0.620-0.794) | 0.670 (0.574-0.754) | 0.679 (0.583-0.762) | 0.634 (0.537-0.721) | 0.696 (0.601-0.778) | 0.696 (0.601-0.778) | 0.643 (0.546-0.730) | 0.804 (0.716-0.870) |
| | | **NPV (95% CI)** | 0.909 (0.824-0.957) | 0.872 (0.779-0.931) | 0.884 (0.792-0.940) | 0.866 (0.768-0.928) | 0.886 (0.797-0.941) | 0.876 (0.786-0.934) | 0.878 (0.783-0.937) | 0.865 (0.781-0.922) |
| | | **PPV (95% CI)** | 0.333 (0.208-0.485) | 0.260 (0.151-0.406) | 0.280 (0.167-0.427) | 0.241 (0.139-0.379) | 0.292 (0.174-0.443) | 0.277 (0.161-0.429) | 0.259 (0.154-0.399) | 0.312 (0.167-0.501) |
| | **RAPIDS Validation (n=312)** | **Prevalence (95% CI)** | 0.154 (0.027-0.463) | | | | | | | |
| | | **Sensitivity (95% CI)** | 1 (0.198-1) | 0.500 (0.095-0.905) | 1 (0.198-1) | 1 (0.198-1) | 1 (0.198-1) | 1 (0.198-1) | 1 (0.198-1) | 0.500 (0.095-0.905) |
| | | **Specificity (95% CI)** | 0.273 (0.073-0.607) | 0.818 (0.478-0.968) | 0.182 (0.032-0.522) | 0 (0-0.321) | 0.636 (0.316-0.876) | 0.455 (0.181-0.754) | 0 (0-0.321) | 0.909 (0.571-0.995) |
| | | **NPV (95% CI)** | 1 (0.31-1) | 0.900 (0.541-0.995) | 1 (0.198-1) | NA (NA-NA) | 1 (0.561-1) | 1 (0.463-1) | NA (NA-NA) | 0.909 (0.571-0.995) |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | **PPV (95% CI)** | 0.200 (0.035-0.558) | 0.333 (0.018-0.875) | 0.182 (0.032-0.522) | 0.154 (0.027-0.463) | 0.333 (0.060-0.759) | 0.250 (0.045-0.644) | 0.154 (0.027-0.463) | 0.500 (0.095-0.905) |
| **OD at 0hrs** | **Discovery (n=595)** | **Prevalence (95% CI)** | 0.225 (0.193-0.261) | | | | | | | |
| | | **Sensitivity (95% CI)** | 0.799 (0.719-0.861) | 0.560 (0.471-0.644) | 0.746 (0.662-0.816) | 0.724 (0.639-0.796) | 0.799 (0.719-0.861) | 0.731 (0.647-0.802) | 0.657 (0.569-0.735) | 0.582 (0.494-0.666) |
| | | **Specificity (95% CI)** | 0.746 (0.703-0.785) | 0.764 (0.722-0.801) | 0.696 (0.652-0.738) | 0.735 (0.692-0.775) | 0.705 (0.661-0.746) | 0.681 (0.636-0.723) | 0.753 (0.710-0.791) | 0.764 (0.722-0.801) |
| | | **NPV (95% CI)** | 0.927 (0.895-0.951) | 0.856 (0.818-0.888) | 0.904 (0.868-0.932) | 0.902 (0.866-0.929) | 0.923 (0.889-0.948) | 0.897 (0.859-0.926) | 0.883 (0.846-0.912) | 0.863 (0.825-0.894) |
| | | **PPV (95% CI)** | 0.478 (0.411-0.545) | 0.408 (0.337-0.483) | 0.417 (0.354-0.482) | 0.443 (0.376-0.511) | 0.440 (0.377-0.505) | 0.400 (0.339-0.464) | 0.436 (0.367-0.507) | 0.417 (0.346-0.491) |
| | **RAPIDS Validation (n=312)** | **Prevalence (95% CI)** | 0.244 (0.198-0.296) | | | | | | | |
| | | **Sensitivity (95% CI)** | 0.908 (0.814-0.959) | 0.368 (0.263-0.487) | 0.842 (0.736-0.912) | 0.632 (0.513-0.737) | 0.618 (0.499-0.725) | 0.632 (0.513-0.737) | 0.566 (0.447-0.677) | 0.697 (0.580-0.795) |
| | | **Specificity(95% CI)** | 0.309 (0.252-0.373) | 0.894 (0.846-0.929) | 0.424 (0.360-0.490) | 0.763 (0.702-0.814) | 0.703 (0.640-0.760) | 0.708 (0.644-0.764) | 0.822 (0.766-0.867) | 0.695 (0.631-0.752) |
| | | **NPV (95% CI)** | 0.912 (0.823-0.961) | 0.815 (0.761-0.859) | 0.893 (0.817-0.941) | 0.865 (0.810-0.907) | 0.851 (0.792-0.897) | 0.856 (0.797-0.901) | 0.855 (0.800-0.896) | 0.877 (0.819-0.919) |

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|---|---|---|---|---|---|---|---|---|---|---|
| | | **PPV (95% CI)** | 0.297 (0.240-0.361) | 0.528 (0.388-0.665) | 0.320 (0.257-0.390) | 0.462 (0.364-0.562) | 0.402 (0.313-0.497) | 0.410 (0.321-0.505) | 0.506 (0.396-0.615) | 0.424 (0.337-0.516) |
| | **EUCLIDS Validation (n=362)** | **Prevalence (95% CI)** | 0.556 (0.503-0.607) | | | | | | | |
| | | **Sensitivity (95% CI)** | 0.965 (0.926-0.985) | 0.625 (0.554-0.692) | 0.965 (0.926-0.985) | 1 (0.977-1) | 0.975 (0.939-0.991) | 0.915 (0.865-0.948) | 1 (0.977-1) | 0.675 (0.605-0.738) |
| | | **Specificity(95% CI)** | 0.044 (0.019-0.092) | 0.769 (0.694-0.830) | 0.144 (0.095-0.210) | 0.006 (0-0.040) | 0.212 (0.154-0.286) | 0.331 (0.260-0.411) | 0.006 (0-0.040) | 0.756 (0.681-0.819) |
| | | **NPV (95% CI)** | 0.500 (0.268-0.732) | 0.621 (0.549-0.688) | 0.767 (0.573-0.894) | 1 (0.055-1) | 0.872 (0.718-0.952) | 0.757 (0.637-0.848) | 1 (0.055-1) | 0.651 (0.577-0.718) |
| | | **PPV (95% CI)** | 0.558 (0.504-0.611) | 0.772 (0.698-0.832) | 0.585 (0.529-0.638) | 0.557 (0.504-0.609) | 0.607 (0.552-0.661) | 0.631 (0.572-0.686) | 0.557 (0.504-0.609) | 0.776 (0.705-0.834) |
| **OD at 0hrs in Definite Bacterial Infection** | **Discovery (n=595)** | **Prevalence (95% CI)** | 0.318 (0.223-0.429) | | | | | | | |
| | | **Sensitivity (95% CI)** | 1 (0.845-1) | 0.815 (0.613-0.930) | 0.926 (0.742-0.987) | 0.889 (0.697-0.971) | 0.926 (0.742-0.987) | 0.963 (0.791-0.998) | 0.852 (0.654-0.951) | 0.926 (0.742-0.987) |
| | | **Specificity (95% CI)** | 0.776 (0.644-0.871) | 0.603 (0.466-0.727) | 0.672 (0.535-0.786) | 0.845 (0.721-0.922) | 0.776 (0.644-0.871) | 0.741 (0.607-0.844) | 0.741 (0.607-0.844) | 0.724 (0.589-0.83) |
| | | **NPV (95% CI)** | 1 (0.902-1) | 0.875 (0.724-0.953) | 0.951 (0.822-0.992) | 0.942 (0.831-0.985) | 0.957 (0.843-0.993) | 0.977 (0.865-0.999) | 0.915 (0.787-0.972) | 0.955 (0.833-0.992) |

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|---|---|---|---|---|---|---|---|---|---|
| | **PPV (95% CI)** | 0.675 (0.508-0.809) | 0.489 (0.339-0.640) | 0.568 (0.411-0.713) | 0.727 (0.542-0.861) | 0.658 (0.486-0.799) | 0.634 (0.469-0.774) | 0.605 (0.435-0.755) | 0.610 (0.445-0.754) |
| **RAPIDS Validation (n=312)** | **Prevalence (95% CI)** | 0.292 (0.134-0.512) | | | | | | | |
| | **Sensitivity (95% CI)** | 1 (0.561-1) | 0.714 (0.303-0.949) | 1 (0.561-1) | 0.429 (0.118-0.798) | 0.714 (0.303-0.949) | 1 (0.561-1) | 0.714 (0.303-0.949) | 0.857 (0.420-0.992) |
| | **Specificity(95% CI)** | 0.118 (0.021-0.377) | 0.765 (0.498-0.922) | 0.235 (0.078-0.502) | 0.882 (0.623-0.979) | 0.176 (0.047-0.442) | 0.529 (0.285-0.761) | 0.588 (0.335-0.806) | 0.588 (0.335-0.806) |
| | **NPV (95% CI)** | 1 (0.198-1) | 0.867 (0.584-0.977) | 1 (0.396-1) | 0.789 (0.539-0.930) | 0.600 (0.170-0.927) | 1 (0.629-1) | 0.833 (0.509-0.971) | 0.909 (0.571-0.995) |
| | **PPV (95% CI)** | 0.318 (0.147-0.549) | 0.556 (0.227-0.847) | 0.350 (0.163-0.591) | 0.600 (0.170-0.927) | 0.263 (0.101-0.514) | 0.467 (0.223-0.726) | 0.417 (0.165-0.714) | 0.462 (0.204-0.739) |
| **EUCLIDS Validation (n=362)** | **Prevalence (95% CI)** | 0.636 (0.408-0.82) | | | | | | | |
| | **Sensitivity (95% CI)** | 0.929 (0.642-0.996) | 0.786 (0.488-0.943) | 0.929 (0.642-0.996) | 1 (0.732-1) | 0.786 (0.488-0.943) | 1 (0.732-1) | 1 (0.732-1) | 0.857 (0.562-0.975) |
| | **Specificity(95% CI)** | 0.375 (0.102-0.741) | 0.625 (0.259-0.898) | 0.125 (0.007-0.533) | 0.125 (0.007-0.533) | 0.500 (0.215-0.785) | 0 (0-0.402) | 0 (0-0.402) | 0.625 (0.259-0.898) |
| | **NPV (95% CI)** | 0.750 (0.219-0.987) | 0.625 (0.259-0.898) | 0.500 (0.095-0.905) | 1 (0.055-1) | 0.571 (0.202-0.882) | NA (NA-NA) | NA (NA-NA) | 0.714 (0.303-0.949) |

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|---|---|---|---|---|---|---|---|---|---|---|
| | | **PPV (95% CI)** | 0.722 (0.464-0.893) | 0.786 (0.488-0.943) | 0.650 (0.409-0.837) | 0.667 (0.431-0.845) | 0.733 (0.448-0.911) | 0.636 (0.408-0.820) | 0.636 (0.408-0.820) | 0.800 (0.514-0.947) |
| **OD at 0hrs in Definite Viral Infection** | **Discovery (n=595)** | **Prevalence (95% CI)** | 0.176 (0.118-0.253) | | | | | | | |
| | | **Sensitivity (95% CI)** | 0.792 (0.573-0.921) | 0.542 (0.332-0.738) | 0.750 (0.529-0.894) | 0.833 (0.618-0.945) | 0.833 (0.618-0.945) | 0.792 (0.573-0.921) | 0.708 (0.488-0.866) | 0.750 (0.529-0.894) |
| | | **Specificity (95% CI)** | 0.670 (0.574-0.754) | 0.679 (0.583-0.762) | 0.705 (0.611-0.786) | 0.589 (0.492-0.680) | 0.688 (0.592-0.770) | 0.661 (0.564-0.746) | 0.598 (0.501-0.688) | 0.723 (0.629-0.802) |
| | | **NPV (95% CI)** | 0.938 (0.854-0.977) | 0.874 (0.781-0.932) | 0.929 (0.847-0.971) | 0.943 (0.853-0.982) | 0.951 (0.872-0.984) | 0.937 (0.852-0.976) | 0.905 (0.809-0.958) | 0.931 (0.850-0.972) |
| | | **PPV (95% CI)** | 0.339 (0.222-0.479) | 0.265 (0.154-0.413) | 0.353 (0.228-0.500) | 0.303 (0.199-0.430) | 0.364 (0.241-0.505) | 0.333 (0.217-0.472) | 0.274 (0.172-0.404) | 0.367 (0.238-0.517) |
| | **RAPIDS Validation (n=312)** | **Prevalence (95% CI)** | 0.154 (0.027-0.463) | | | | | | | |
| | | **Sensitivity (95% CI)** | 1 (0.198-1) | 0.500 (0.095-0.905) | 1 (0.198-1) | 1 (0.198-1) | 0.500 (0.095-0.905) | 1 (0.198-1) | 0.500 (0.095-0.905) | 0 (0-0.802) |
| | | **Specificity(95% CI)** | 0.273 (0.073-0.607) | 0.818 (0.478-0.968) | 0.182 (0.032-0.522) | 0.727 (0.393-0.927) | 0.545 (0.246-0.819) | 0.727 (0.393-0.927) | 0.909 (0.571-0.995) | 0.818 (0.478-0.968) |
| | | **NPV (95% CI)** | 1 (0.310-1) | 0.900 (0.541-0.995) | 1 (0.198-1) | 1 (0.598-1) | 0.857 (0.420-0.992) | 1 (0.598-1) | 0.909 (0.571-0.995) | 0.818 (0.478-0.968) |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | **PPV (95% CI)** | 0.200 (0.035-0.558) | 0.333 (0.018-0.875) | 0.182 (0.032-0.522) | 0.400 (0.073-0.830) | 0.167 (0.009-0.635) | 0.400 (0.073-0.830) | 0.500 (0.095-0.905) | 0 (0-0.802) |
| | **EUCLIDS Validation (n=362)** | **Prevalence (95% CI)** | 0.438 (0.268-0.621) | | | | | | | |
| | | **Sensitivity (95% CI)** | 1 (0.732-1) | 0.571 (0.296-0.812) | 1 (0.732-1) | 1 (0.732-1) | 1 (0.732-1) | 1 (0.732-1) | 1 (0.732-1) | 0.786 (0.488-0.943) |
| | | **Specificity(95% CI)** | 0 (0-0.219) | 0.889 (0.639-0.981) | 0 (0-0.219) | 0 (0-0.219) | 0.111 (0.019-0.361) | 0.444 (0.224-0.687) | 0.444 (0.224-0.687) | 0.611 (0.361-0.817) |
| | | **NPV (95% CI)** | NA (NA-NA) | 0.727 (0.496-0.884) | NA (NA-NA) | NA (NA-NA) | 1 (0.198-1) | 1 (0.598-1) | 1 (0.598-1) | 0.786 (0.488-0.943) |
| | | **PPV (95% CI)** | 0.438 (0.268-0.621) | 0.800 (0.442-0.965) | 0.438 (0.268-0.621) | 0.438 (0.268-0.621) | 0.467 (0.288-0.654) | 0.583 (0.369-0.772) | 0.583 (0.369-0.772) | 0.611 (0.361-0.817) |

**eTable 7: Performance of disease-class signature in identifying definite bacterial vs definite viral in different age groups.**

| | RAPIDS Discovery | | RAPIDS Validation | | EUCLIDS Validation | |
|---|---|---|---|---|---|---|
| **Age group** | **Number (%)** | **AUC (95% CI)** | **Number (%)** | **AUC (95% CI)** | **Number (%)** | **AUC (95% CI)** |
| < 1 year | 156 (26%) | 0.929 (0.871 - 0.988) | 43 (14%) | 0.912 (0.773 - 1.000) | 99 (27%) | 0.945 (0.897 - 0.993) |
| 1 – 5 years | 217 (36%) | 0.917 (0.853 - 0.980) | 159 (51%) | 0.901 (0.835 - 0.966) | 154 (43%) | 0.908 (0.830 - 0.986) |
| 5 – 10 years | 119 (20%) | 0.908 (0.799 - 1.000) | 56 (18%) | 0.976 (0.924 - 1.000) | 58 (16%) | 0.729 (0.274 - 1.000) |
| > 10 years | 103 (17%) | 0.954 (0.903 - 1.000) | 54 (17%) | 0.987 (0.955 - 1.000) | 51 (14%) | - |
| All samples | 595 | 0.935 (0.905 - 0.966) | 312 | 0.941 (0.906 - 0.977) | 362 | 0.909 (0.850 - 0.969) |

**eTable 8: Performance of disease-severity signature in identifying presence of organ dysfunction at time of sampling in different age groups.**

| | RAPIDS Discovery | | RAPIDS Validation | | EUCLIDS Validation | |
|---|---|---|---|---|---|---|
| **Age group** | **Number (%)** | **AUC (95% CI)** | **Number (%)** | **AUC (95% CI)** | **Number (%)** | **AUC** |
| < 1 year | 156 (26%) | 0.828 (0.737 - 0.918) | 43 (14%) | 0.758 (0.609 - 0.906) | 99 (27%) | 0.759 (0.662 - 0.856) |
| 1 – 5 years | 217 (36%) | 0.821 (0.736 - 0.907) | 159 (51%) | 0.752 (0.632 - 0.873) | 154 (43%) | 0.829 (0.759 - 0.899) |
| 5 – 10 years | 119 (20%) | 0.919 (0.865 - 0.973) | 56 (18%) | 0.762 (0.570 - 0.954) | 58 (16%) | 0.746 (0.615 - 0.876) |
| > 10 years | 103 (17%) | 0.873 (0.792 - 0.954) | 54 (17%) | 0.806 (0.692 - 0.920) | 51 (14%) | 0.721 (0.574 - 0.867) |
| All samples | 595 | 0.852 (0.809 - 0.895) | 312 | 0.775 (0.712 - 0.838) | 362 | 0.775 (0.727 - 0.823) |

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