

Supplementary Online Content

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eMethods

eTable 1. Organisms classified as common bloodstream infection pathogens in children with cancer

eTable 2. Characteristics of 19 included BSI episodes

eTable 3. Quantitative mcfDNA-seq data for each bloodstream infection episode

eTable 4. Blood cultures collected during the early predictive and Predictive Period for each bloodstream infection episode

eTable 5. Effect of frequency of testing on sensitivity of mcfDNA-seq screening for bacterial BSI

eTable 6. Plasma microbial cell-free DNA sequencing results for negative control samples from study participants

eTable 7. Additional bacterial organisms identified during bloodstream infection episodes

eFigure 1. Method of selection of plasma samples for testing as negative controls and BSI episodes

eFigure 2. Method of logical derivation of missing values for estimation of sensitivity

eFigure 3. Pathogen DNA concentration kinetics for each bloodstream infection episode

eFigure 4. Sensitivity of mcfDNA-seq by day prior to bloodstream infection using raw data without imputation

eFigure 5. Sensitivity of predictive mcfDNA-seq for bacterial BSI by frequency of testing using logical derivation to impute missing data

eFigure 6. Sensitivity of predictive mcfDNA-seq for bacterial BSI by frequency of testing using raw data without imputation

eReferences

eAppendix. Range of pathogens identifiable by the Karius test

This supplementary material has been provided by the authors to give readers additional information about their work.

Supplement

1. Supplemental Methods

2. Supplemental Tables

eTable 1. Organisms classified as common bloodstream infection pathogens in children with cancer

eTable 2. Characteristics of 19 included BSI episodes

eTable 3. Quantitative mcfDNA-seq data for each bloodstream infection episode

eTable 4. Blood cultures collected during the early predictive and Predictive Period for each bloodstream infection episode

eTable 5. Effect of frequency of testing on sensitivity of mcfDNA-seq screening for bacterial BSI

eTable 6. Plasma microbial cell-free DNA sequencing results for negative control samples from study participants

eTable 7. Additional bacterial organisms identified during bloodstream infection episodes

3. Supplemental Figures

eFigure 1. Method of selection of plasma samples for testing as negative controls and BSI episodes

eFigure 2. Method of logical derivation of missing values for estimation of sensitivity

eFigure 3. Pathogen DNA concentration kinetics for each bloodstream infection episode

eFigure 4. Sensitivity of mcfDNA-seq by day prior to bloodstream infection using raw data without imputation

eFigure 5. Sensitivity of predictive mcfDNA-seq for bacterial BSI by frequency of testing using logical derivation to impute missing data

eFigure 6. Sensitivity of predictive mcfDNA-seq for bacterial BSI by frequency of testing using raw data without imputation

4. Supplemental References

5. Range of pathogens identifiable by the Karius test

1. Supplemental Methods

Study Design and Ethics

This is the pilot stage of a single-arm prospective observational cohort study. The study was approved by the St. Jude Children's Research Hospital Institutional Review Board before any research procedures were conducted.

Detailed Inclusion and Enrolment Criteria

Participants were younger than 25 years, receiving treatment for relapsed or refractory malignancy at St. Jude, and expected to continue care for at least 7 days. Participants were identified by direct contact with primary clinicians and from patient acceptance notices, and were recruited by study staff. Written informed consent for study participation was obtained from the participant or their legal representative as appropriate, and assent was obtained from participants older than 7 years.

Detailed Off-study Criteria

Study participation was completed upon participant request, loss to follow-up or transfer of care, death of the participant, or at 30 days after HCT, whichever occurred first. Participants with severe gastrointestinal graft-versus-host disease (GVHD) following HCT remained on study until severe GVHD resolved.

Clinical Data and Definitions

Clinical data were collected prospectively from the electronic medical record and from pharmacy and laboratory databases from 30 days before enrollment until 7 days after study completion. BSI episodes were defined according to National Healthcare Safety Network definitions for laboratory-confirmed bloodstream infection.¹ The onset of each BSI was defined as the date of collection of the first positive culture; institutional practice is to collect blood cultures from patients with a new onset fever or suspected systemic infection. The *a priori*-defined Predictive Period comprised the 3 days before BSI onset, and prediction during the period 4-7 days before BSI onset was evaluated as a secondary objective (eFigure 1). Negative control samples were obtained from participants on a day for which no fever or infection was documented within the prior or subsequent 7 days. Participants could contribute multiple BSI episodes and negative control samples, but a minimum of 21 days was required between negative control samples.

Laboratory Procedures

Remnant blood collected in EDTA and available after the completion of clinical testing was stored at 4°C until processed by centrifugation to plasma and stored at -80°C until thawed for testing. Plasma microbial cell-free DNA next generation sequencing (mcfDNA-seq) was performed using the clinical grade Karius Test, in a CLIA/CAP-accredited laboratory (Karius, Inc., Redwood City, CA) as previously described.² Testing with plasma mcfDNA-seq was performed on available samples collected between 7 days before and 4 days after each BSI episode, and 2 negative control samples were added for each BSI episode. Samples were transported to Karius, Inc., overnight on dry ice and were confirmed to be frozen on arrival. BSI samples and negative control samples were sent in the same batch, and the laboratory was blinded to expected results until sequencing was completed and reported.

Reference Database and Quality Control

Reference genomes for *Homo sapiens* and microorganisms (bacteria, viruses, fungi/molds, and other eukaryotic pathogens) were retrieved from the National Center for Biotechnology Information (NCBI) ftp site (NCBI, U.S. National Library of Medicine (NLM), Human Genome: <https://www.ncbi.nlm.nih.gov/genome/guide/human/>, release GRCh38.p7), (NCBI, U.S. NLM, Microbial Genomes: <https://www.ncbi.nlm.nih.gov/genome/microbes/>). Sequence similarities between microorganism references were inspected to identify taxonomic mislabeling and sequence contamination. From the reference genomes passing these quality controls, a subset was selected to maximize sequence diversity. As part of the selection process, NCBI BioSample data (NCBI, U.S. NLM, Biosample: <https://www.ncbi.nlm.nih.gov/biosample/>), were used to ensure the inclusion of reference genomes from both clinical and non-clinical isolates. The final reference genome dataset included over 21,000 reference genomes, containing over 2.7 million sequences. Selected sequences were collected into a single FASTA file and used to generate our microorganism reference BLAST database. A subset of these taxa, including 1251 clinically significant microorganisms, was used as the clinical reportable range. This list is shown in Section 5 of the Supplement.

Clinical Reportable Range (CRR)

The selection of organisms in our clinical reportable range (CRR) was performed as follows. A candidate list was generated by two board-certified Infectious Disease physicians by including (a) DNA viruses, culturable bacteria, additional fastidious and unculturable bacteria, mycobacteria, and eukaryotic pathogens from the standard text [1] and a number of infectious disease references, (b) organisms in the pathogen database referenced in published case reports and (c) reference genomes sequenced from human clinical isolates (as indicated by NCBI's BioSample resource) with publications supporting pathogenicity. Organisms from the above list that were associated with high quality reference genomes, as determined by our reference database QC process (see above), were used to further narrow the range. Finally, organisms at risk of generating common false-positive calls because of sporadic environmental contamination were removed. The sequence database is continuously curated to minimize human cross-reactivity as well as cross-reactivity between pathogens and is screened to mitigate contamination with sequences from human or other organisms.

Sequencing

Plasma samples were thawed, centrifuged at 16,000 rcf for ten minutes, and spiked with a known concentration of synthetic DNA molecules for quality control purposes. Cell-free DNA was extracted from 0.5 mL plasma using a magnetic bead-based method (Omega Biotek, Norcross, GA). DNA libraries for sequencing are constructed using a modified Ovation® Ultralow System V2 library preparation kit (NuGEN, San Carlos, CA). Negative controls (buffer only instead of plasma) and positive controls (healthy plasma spiked with a known mixture of microbial DNA fragments) were processed alongside patient samples in every batch. Samples were multiplexed with other samples and sequenced on an Illumina NextSeq® 500.

Analysis Pipeline

Primary sequencing output files were processed using bcl2fastq (v2.17.1.14) to generate the demultiplexed sequencing reads files. Reads were filtered based on sequencing quality and trimmed based on partial or full adapter sequence. Bowtie2 (version 2.2.4) was used to align the remaining reads against Karius' human and synthetic-molecules references. Sequencing reads exhibiting strong alignment against the human references or the synthetic molecule references were collected and excluded from further analysis. Remaining reads were aligned against Karius' proprietary microorganism reference database using NCBI-blast (version 2.2.30+). A mixture model was used to assign a likelihood to the complete collection of sequencing reads that included the read sequence probabilities and the (unknown) abundances of each taxon in the sample. An expectation-maximization algorithm was applied to compute the maximum likelihood estimate of each taxon abundance. Only taxa whose abundances rejected the null hypothesis of originating from environmental contamination (as calculated from the negative controls) were reported. The quantity for each organism identified was expressed in Molecules Per Microliter (MPM), the number of DNA sequencing reads from the reported organism present per microliter of plasma. The entire process from DNA extraction through analysis was typically completed within 28 hours.

Power and Statistical Considerations

Simon's 2-stage design was applied for the primary objective. The minimal acceptable sensitivity was set at 30% and favorable sensitivity at 50%.³ A favorable sensitivity of 50% was chosen as the approximate efficacy of antibacterial prophylaxis in this population.⁴ An interim analysis was planned after accrual of predictive samples for 15 BSI episodes.

For the estimation of sensitivity and specificity, quantitative results were dichotomized as present or absent. The predictive sensitivity was estimated for the 3-day period before BSI as a unit and separately for each of the 7 days before BSI. The diagnostic sensitivity was estimated from the results of samples collected on the day of BSI onset. Predictive sensitivity was defined as the proportion of episodes for which mcfDNA-seq identified the same organism that was subsequently identified in blood culture. For polymicrobial BSI episodes, the identification by mcfDNA-seq of all organisms grown in culture was required to classify the organism present. The Wilson method was used to calculate 95% confidence intervals for proportions.⁵ For the estimation of sensitivity for each day before BSI onset, logical derivation was used to impute missing values by imputing that missing values between two positive results before day of diagnosis were positive, and negative results preceding negative results were negative, imputation was not used in other circumstances (eFigure 2).

To evaluate the effect on sensitivity of different models of frequency of testing for bacterial BSI (eFigure 5 and eFigure 6), estimated sensitivities were calculated for each day of the week, and minimum and median sensitivities were reported for each model. This analysis assumed that the risk of BSI events is identical for each day and that mcfDNA-seq test results would be available on the day of collection. The models evaluated were: once-weekly testing on Mondays; twice-weekly testing on Mondays and Thursdays; thrice-weekly testing on Mondays, Wednesdays, and Fridays; and testing 5 times per week on each weekday.

Specificity was defined as the proportion of negative control samples in which no bacterial or fungal organisms were identified by mcfDNA-seq. Specificity for common BSI pathogens was an additional *ad hoc* measure defined as the proportion of negative control samples in which no common BSI pathogens were identified by mcfDNA-seq. Common BSI pathogens were defined as any member of a genus that comprised at least 1% of the organisms causing central line-associated BSI in children with cancer between August 2013 and December 2015, according to the previously described Children's Hospital Association Childhood Cancer & Blood Disorders Network BSI database.⁶ (eTable 1) A 3rd degree penalized B-spline curve (PROC SGPLOT/PBSPLINE) was used to analyze possible trends in $\log_{10}(\text{MPM}+1)$ over time; for this analysis, all undetected organisms were assumed to have a DNA concentration of 0 MPM, but no other imputation was performed.

2. Supplemental Tables

eTable 1. Organisms classified as common bloodstream infection pathogens in children with cancer

Organism
Gram positive bacteria
<i>Bacillus spp.</i> ¹
<i>Clostridium spp.</i> ¹
<i>Corynebacterium jeikeium</i>
<i>Enterococcus spp.</i> ¹
<i>Lactobacillus spp.</i> ¹
<i>Rothia spp.</i> ¹
<i>Staphylococcus spp.</i> ¹
<i>Streptococcus spp.</i> ¹
Gram negative bacteria
<i>Citrobacter spp.</i> ¹
<i>Escherichia coli</i> ¹
<i>Klebsiella spp.</i> ¹
<i>Pseudomonas spp.</i> ¹
<i>Stenotrophomonas maltophilia</i> ¹
Fungi
<i>Candida spp.</i> ¹

¹Identified in greater than 1% of episodes of central line associated bloodstream infection in children with cancer (Children's Hospital Association Childhood Cancer & Blood Disorders Network, August 2013 – December 2015)

eTable 2. Characteristics of 19 included BSI episodes

Episode	BSI Organism	Diagnostic Sample	Predictive Sample	Neutropenia
A	<i>Escherichia coli</i>	Yes	Yes	Yes
B	<i>Staphylococcus epidermidis</i>	Yes	No	No
C	<i>S. epidermidis</i>	Yes	No	Yes
D	<i>S. epidermidis</i>	Yes	Yes	No
E	<i>Enterococcus faecium</i>	Yes	Yes	No
F	<i>E. faecium</i>	Yes	Yes	Yes
G	<i>E. faecium</i>	Yes	Yes	No
H	CoNS	Yes	Yes	Yes
I	<i>Rothia mucilaginosa</i>	Yes	Yes	Yes
J	<i>S. epidermidis</i>	Yes	Yes	Yes
K	<i>Corynebacterium jeikeium</i>	Yes	No	Yes
L	<i>E. coli</i>	Yes	Yes	Yes
M	<i>E. coli</i> & <i>R. mucilaginosa</i> *	Yes	Yes	Yes
N	<i>E. coli</i>	Yes	Yes	Yes
O	<i>E. faecium</i>	Yes	Yes	Yes
P	<i>S. epidermidis</i>	Yes	Yes	Yes
Q	<i>Candida krusei</i>	Yes	Yes	Yes
R	<i>Enterococcus gallinarum</i>	Yes	Yes	Yes
S	<i>S. epidermidis</i>	No	Yes	Yes

Diagnostic sample, sample available from day of BSI onset; Predictive sample, sample available within 3 days before BSI onset; CoNS, unspciated coagulase negative *Staphylococcus* sp.; *polymicrobial infection consisting of 2 or more organisms grown from a single blood culture set. *Staphylococcus epidermidis* and CoNS are Gram positive skin commensal organisms often identified as a cause of catheter associated bloodstream infections⁷; *Enterococcus* spp. are Gram positive gastrointestinal flora that are often associated with urinary, intrabdominal or catheter-associated bloodstream infections⁸; *Rothia mucilaginosa* is a Gram positive organism that commonly colonizes oropharyngeal mucosa and causes serious infections, including BSI and meningitis, in immunocompromised patients with mucositis⁹; *Corynebacterium jeikeium* is a Gram positive skin commensal organism that has been associated with serious infections in immunocompromised hosts including BSI, musculoskeletal infection and endocarditis.¹⁰

eTable 3. Quantitative mcfDNA-seq data for each bloodstream infection episode

BSI Episode	Expected organism(s)	Max. MPM in predictive period	MPM at BSI onset
A	<i>E. coli</i>	40,165	18,305
B	<i>S. epidermidis</i>	N/A	149,093
C	<i>S. epidermidis</i>	N/A	91,144
D	<i>S. epidermidis</i>	2,125	84,698
E	<i>E. faecium</i>	1,145,864	9,128,795
F	<i>E. faecium</i>	320,740	187,923
G	<i>E. faecium</i>	10,207	34,756
H	CoNS	ND	ND
I	<i>R. mucilaginosa</i>	8,015	9,213
J	<i>S. epidermidis</i>	20,515	157,456
K	<i>C. jeikeium</i>	N/A	98
L	<i>E. coli</i>	ND	9,280
M	<i>E. coli/R. mucilaginosa</i>	ND/ND	ND/ND
N	<i>E. coli</i>	616	1,023
O	<i>E. faecium</i>	4,760	11,443
P	<i>S. epidermidis</i>	7,890	52,495
Q	<i>C. krusei</i>	ND	ND
R	<i>E. gallinarum</i>	7,903	114,248
S	<i>S. epidermidis</i>	143	N/A

BSI, bloodstream infection; Max. MPM, maximal concentration of pathogen DNA in molecules per microliter; ND, not detected; N/A, sample not available.

eTable 4. Blood cultures collected in the days before each bloodstream infection episode

BSI Episode	Expected organism(s)	Blood culture day	Blood culture result	mcfDNA-seq MPM
A	<i>E. coli</i>	-4	Negative	10,304
B	<i>S. epidermidis</i>	None	N/A	
C	<i>S. epidermidis</i>	None	N/A	
D	<i>S. epidermidis</i>	None	N/A	
E	<i>E. faecium</i>	-6	Negative	No sample
		-4	Negative	ND
		-3	Negative	No sample
		-2	Negative	ND
		-1	Negative	1,145,864
F	<i>E. faecium</i>	-5	Negative	1,476,869
G	<i>E. faecium</i>	-3	Negative	753
		-1	Negative	10,207
H	CoNS	-4	Negative	No sample
		-1	Negative	ND
I	<i>R. mucilaginosa</i>	-6	Negative	No sample
		-5	Negative	ND
		-4	Negative	ND
		-3	Negative	17
		-2	Negative	256
		-1	Negative	8015
J	<i>S. epidermidis</i>	None	N/A	N/A
K	<i>C. jeikeium</i>	None	N/A	N/A
L	<i>E. coli</i>	None	N/A	N/A
M	<i>E. coli/R. mucilaginosa</i>	-6	Negative	ND
N	<i>E. coli</i>	None	N/A	N/A
O	<i>E. faecium</i>	None	N/A	N/A
P	<i>S. epidermidis</i>	-5	<i>S. epidermidis</i> (1 of 2 lumens)	80
		-3	Negative	1108
Q	<i>C. krusei</i>	-6	<i>S. epidermidis</i> (1 of 2 lumens)	ND
		-4	Negative	ND
R	<i>E. gallinarum</i>	-1	Negative	No sample
S	<i>S. epidermidis</i>	None	N/A	N/A

BSI, bloodstream infection; None, no blood culture collected during the 7 days prior to onset of BSI; CoNS, unspeciated coagulase negative *Staphylococcus* spp.; N/A, not applicable; mcfDNA-seq MPM, DNA concentration of the expected BSI pathogen by next generation metagenomic sequencing on day of blood culture; ND, expected BSI pathogen not detected by mcfDNA-seq.

eTable 5. Effect of frequency of testing on sensitivity of mcfDNA-seq screening for bacterial BSI

Testing frequency	Imputed data		Raw data	
	Median sensitivity	(Range)	Median sensitivity	(Range)
Mondays	46.2%	(9.1%, 85.7%)	55.6%	(33.3%, 80%)
Mondays & Thursdays	71.4%	(46.2%, 85.7%)	72.7%	(55.6%, 80%)
Mondays, Wednesdays & Fridays	71.4%	(66.7%, 85.7%)	72.7%	(69.2%, 80%)
Monday through Friday	85.7%	(66.7%, 85.7%)	80%	(69.2%, 80%)

eTable 6. Plasma microbial cell-free DNA sequencing results for bacteria in negative control samples from study participants

Control Sample	Neutropenia	Organisms Identified	MPM	Common BSI Pathogen
1	No	<i>Klebsiella pneumoniae</i>	112	Yes
		<i>Clostridium perfringens</i>	99	Yes
2	No	None		No
3	Yes	None		No
4	No	None		No
5	Yes	None		No
6	No	None		No
7	Yes	<i>Veillonella parvula</i>	41	No
8	Yes	None		No
9	No	None		No
10	Yes	None		No
11	Yes	None		No
12	No	None		No
13	Yes	None		No
14	Yes	None		No
15	Yes	<i>Finnegoldia magna</i>	129	No
16	Yes	None		No
17	No	None		No
18	Yes	None		No
19	Yes	None		No
20	Yes	None		No
21	No	<i>Lactobacillus fermentum</i>	25	Yes
		<i>Helicobacter pylori</i>	38	No
22	Yes	None		No
23	Yes	<i>Neisseria sicca</i>	609	No
24	Yes	None		No
25	Yes	None		No
26	Yes	None		No
27	No	None		No
28	Yes	<i>Escherichia coli</i>	85	Yes
29	No	None		No
30	No	None		No
31	Yes	None		No
32	Yes	None		No
33	Yes	None		No

MPM, concentration of DNA in molecules per microliter.

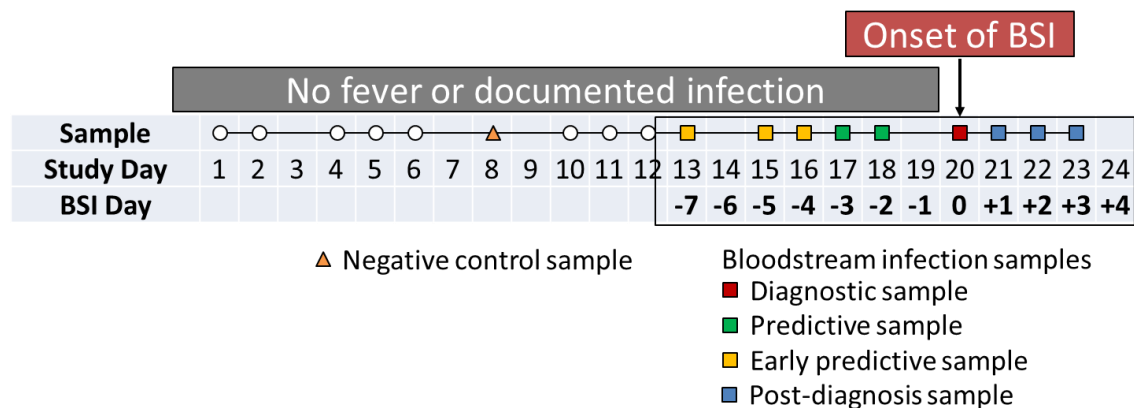
eTable 7. Additional bacterial organisms identified during bloodstream infection episodes

BSI Episode	Expected organism(s)	Additional identified organism	Max. MPM	Effective antibiotic	Antibiotic days
A	<i>E. coli</i>	None	N/A	N/A	N/A
B	<i>S. epidermidis</i>	None	N/A	N/A	N/A
C	<i>S. epidermidis</i>	<i>E. faecalis</i>	2,006	Vancomycin	5
D	<i>S. epidermidis</i>	<i>C. difficile</i>	5,019	Vancomycin	4
E	<i>E. faecium</i>	<i>S. epidermidis</i>	763,462	Vancomycin	3
		<i>S. thermophilus</i>	317	Vancomycin	3
		<i>L. gasseri</i>	2,000	Vancomycin	3
F	<i>E. faecium</i>	<i>S. epidermidis</i>	509,208	Vancomycin	14
G	<i>E. faecium</i>	<i>Alloicoccus otitis</i>	743	Ampicillin	7
		<i>H.parainfluenzae</i>	1,472	Cefepime	4
		<i>L. gasseri</i>	534	Vancomycin	4
		<i>Staphylococcus auricularis</i>	1,273	Vancomycin	4
		<i>Streptococcus oralis</i>	1,411	Vancomycin	4
		<i>Turicella otitidis</i>	454	Vancomycin	4
H	CoNS	<i>E. faecium</i>	8,997	Vancomycin	6
		<i>H.parainfluenzae</i>	4,979	Cefepime	3
		<i>L. acidophilus</i>	81,508	Vancomycin	6
		<i>L. casei</i>	52,189	Penicillin VK	4
		<i>S. thermophilus</i>	161,209	Vancomycin	6
I	<i>R. mucilaginosa</i>	<i>S. thermophilus</i>	18	Vancomycin	9
		<i>S. mitis</i>	47,369	Vancomycin	9
		<i>E. faecium</i>	150	Vancomycin	9
J	<i>S. epidermidis</i>	<i>S. mitis</i>	1,091	Vancomycin	10
K	<i>C. jeikeium</i>	<i>S. epidermidis</i>	182	Vancomycin	3
		<i>Campylobacter showae</i>	46	Meropenem	4
		<i>H.parainfluenzae</i>	42	Meropenem	4
		<i>Pseudomonas pseudoalcaligenes</i>	37	Meropenem	4
L	<i>E. coli</i>	<i>Kingella oralis</i>	67	Ceftriaxone	1
		<i>Solobacterium moorei</i>	68	Meropenem	10

BSI Episode	Expected organism(s)	Additional identified organism	Max. MPM	Effective antibiotic	Antibiotic days
M	<i>E. coli</i> , <i>R. mucilaginosa</i>	<i>S. epidermidis</i>	969	levofloxacin	8
		<i>S. mitis</i>	698	Cefepime	15
		<i>Granulicatella adiacens</i>	28	Vancomycin	12
N	<i>E. coli</i>	<i>Agrobacterium tumefaciens</i>	65	Vancomycin	6
O	<i>E. faecium</i>	<i>F. nucleatum</i>	5,470	Linezolid	11
		<i>H. parainfluenzae</i>	2,476	Cefepime	4
P	<i>S. epidermidis</i>	<i>S. capitis</i>	47	Vancomycin	10
Q	<i>C. krusei</i>	<i>S. capitis</i>	47	Vancomycin	10
R	<i>E. gallinarum</i>	<i>F. nucleatum</i>	465	Metronidazole	11
		<i>Prevotella melaninogenica</i>	220	Metronidazole	11
		<i>L. rhamnosus</i>	2,840	Linezolid	5
S	<i>S. epidermidis</i>	<i>S. mitis</i>	48	Vancomycin	9
		<i>Actinomyces graevenitzi</i>	73	Vancomycin	9
		<i>P. melaninogenica</i>	73	Cefepime	5
		<i>S. thermophilus</i>	138	Vancomycin	9

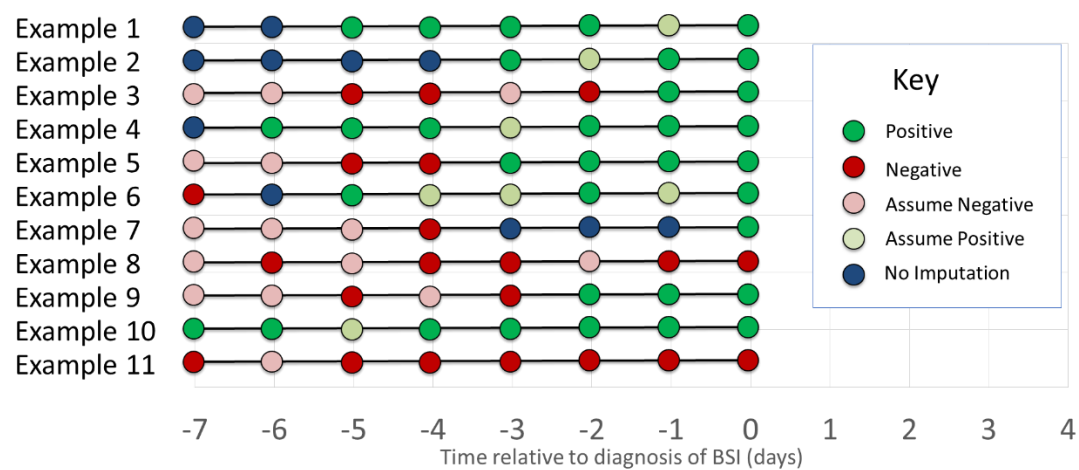
3. Supplemental Figures

eFigure 1. Method of selection of plasma samples for testing as negative controls and BSI episodes

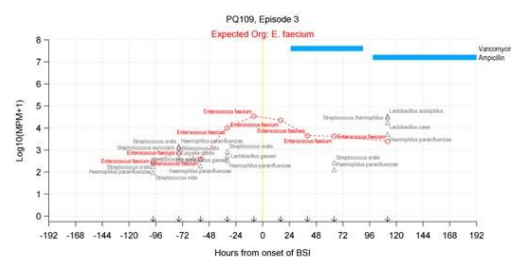
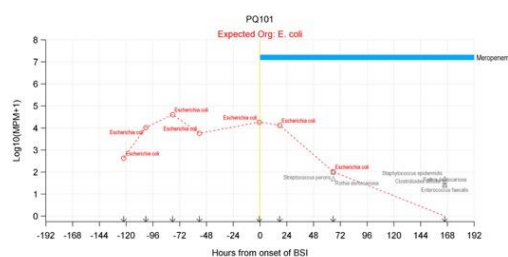


BSI, bloodstream infection. Closed shapes represent available samples for an individual participant; negative control samples were defined as plasma samples obtained when no fever or infection was documented in the prior or subsequent 7 days; BSI samples were defined as plasma samples obtained in the 7 days before or 4 days after the onset of BSI. Samples obtained on the day of onset of BSI were classified as diagnostic samples; samples obtained on the 3 days before the onset of BSI were classified as predictive samples.

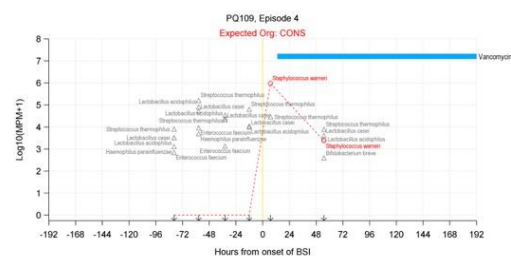
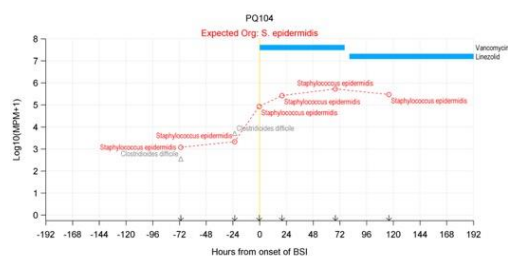
eFigure 2. Method of logical derivation for imputation of missing mcfDNA-seq results



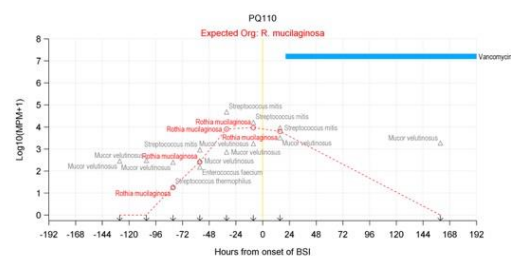
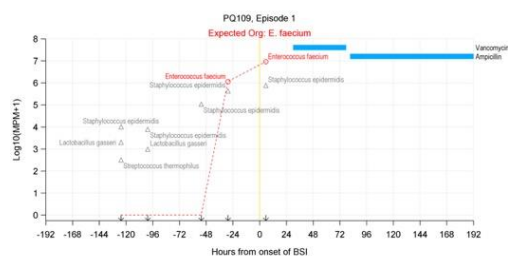
eFigure 3. Pathogen DNA concentration kinetics for each bloodstream infection episode



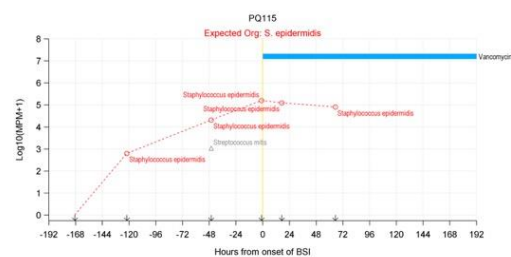
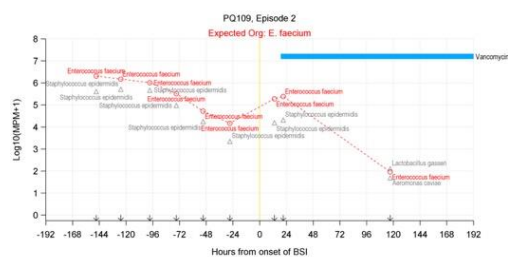
21



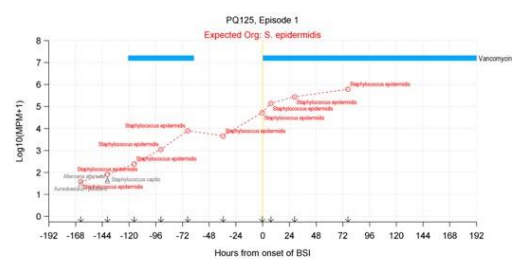
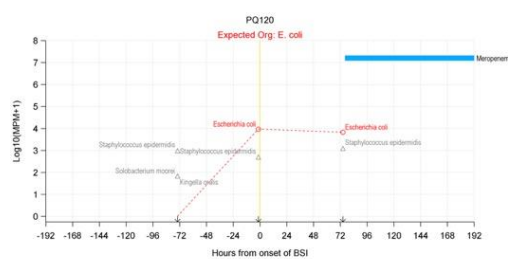
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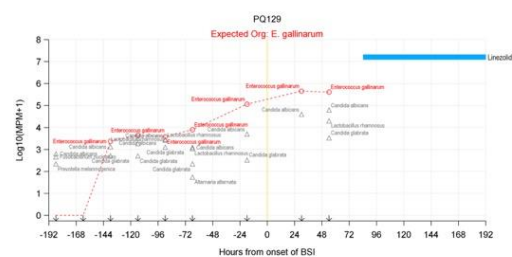
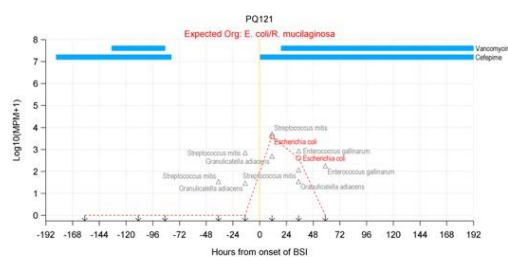
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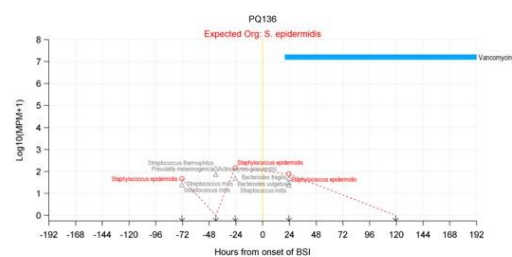
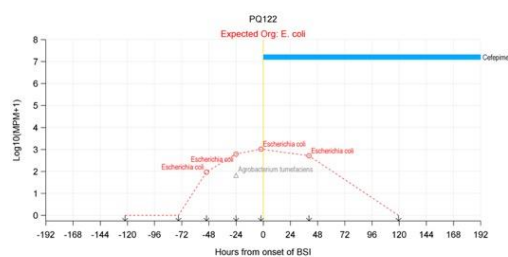
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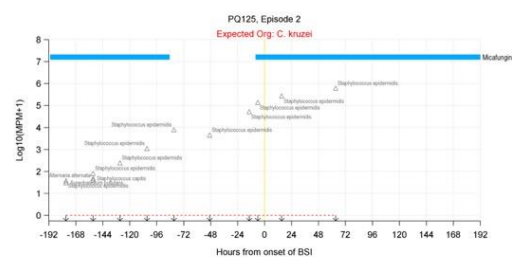
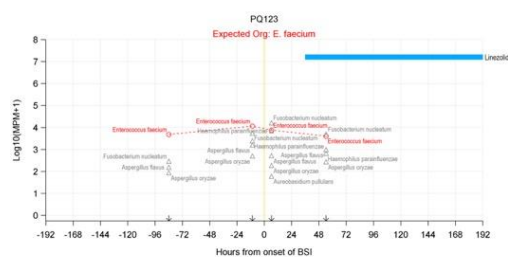
29



30

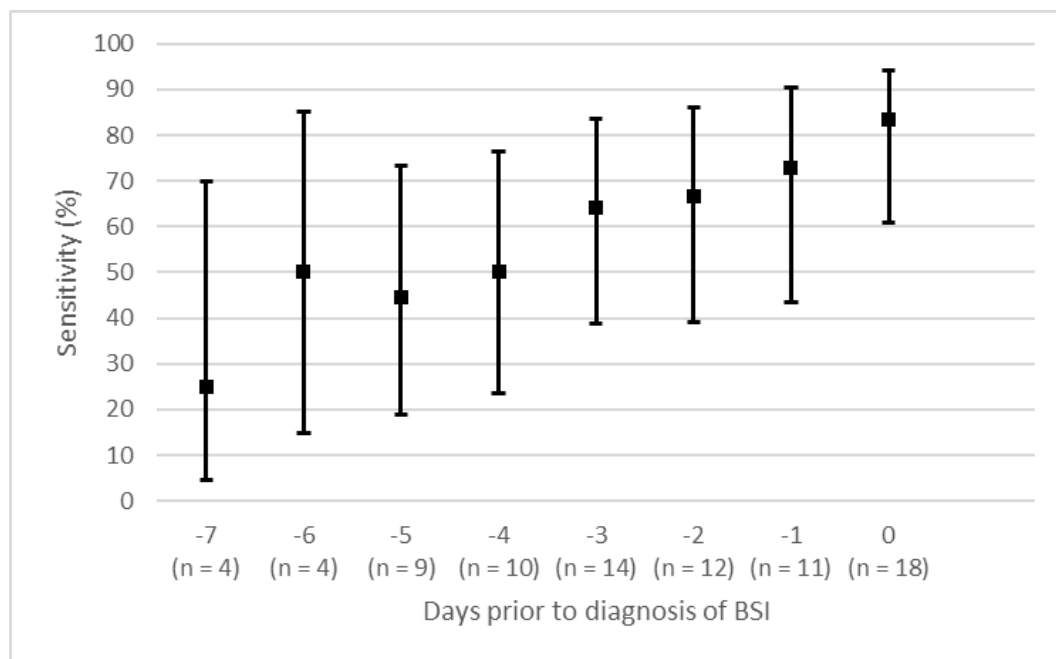


31

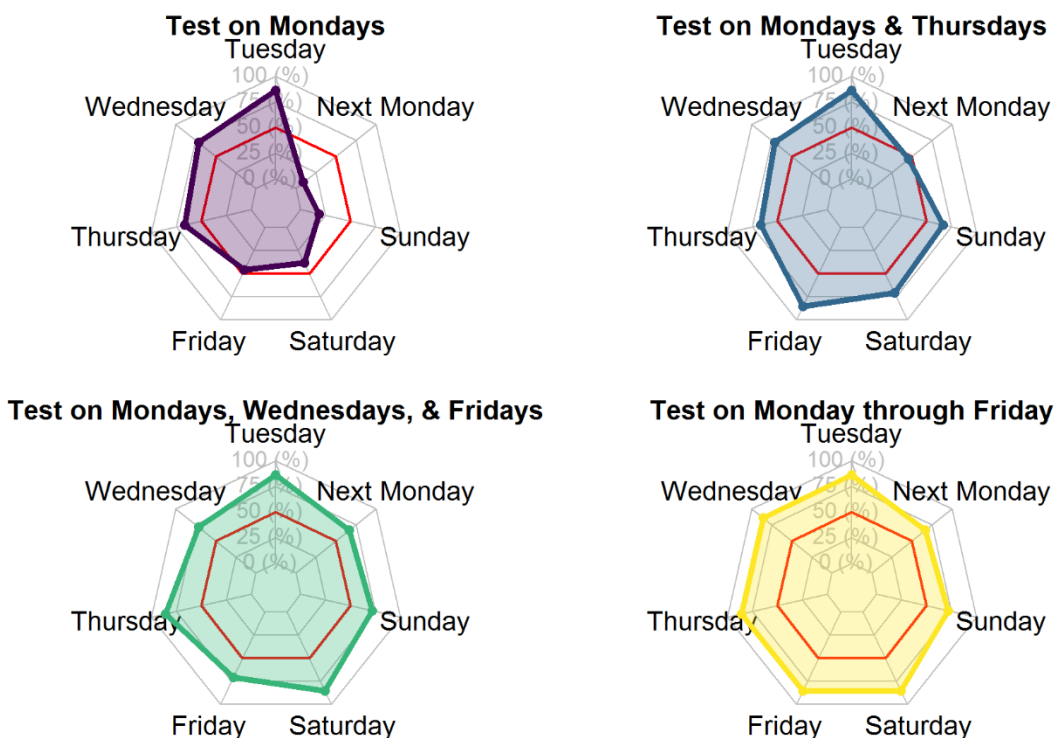


32

eFigure 4. Sensitivity of mcfDNA-seq by day prior to bloodstream infection using raw data without imputation

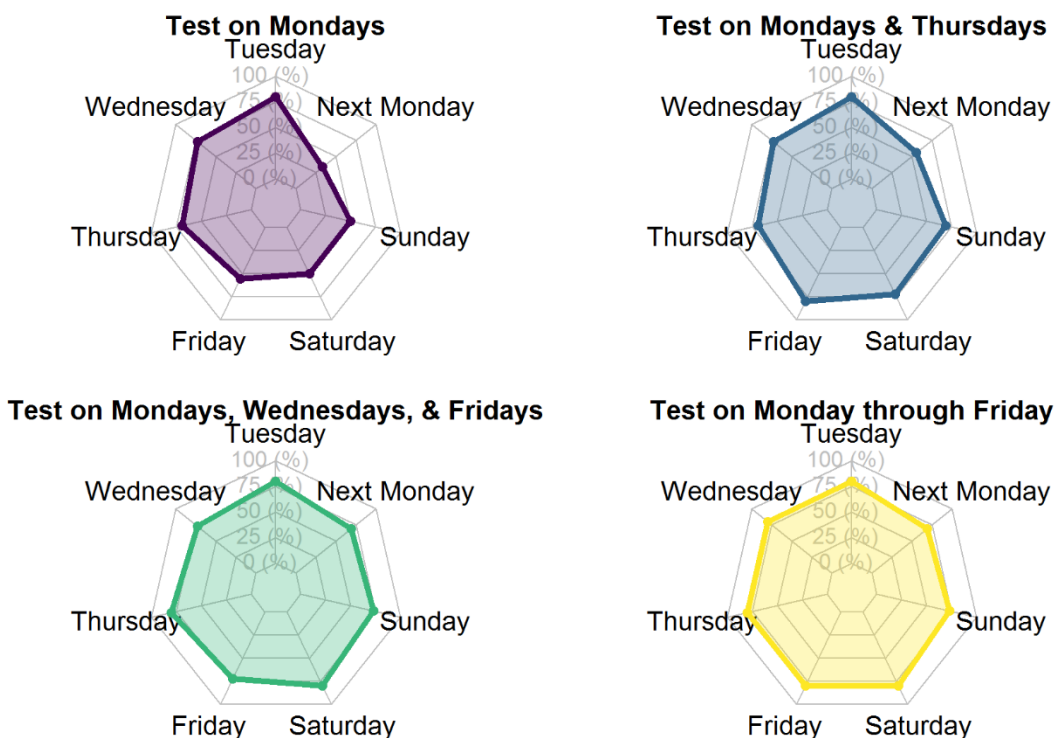


eFigure 5. Sensitivity of predictive mcfDNA-seq for bacterial BSI by frequency of testing



For each frequency of testing model, estimated sensitivities for event in each day of the week were plotted in a radar graph. Larger shaded area indicates higher overall sensitivity. The red line represents favorable sensitivity of 50%. Logical derivation was used to impute values for missing data. Testing thrice weekly was required to keep minimum sensitivity above 50%. BSI, bloodstream infection; mcfDNA-seq, plasma microbial cell-free DNA sequencing.

eFigure 6. Sensitivity of predictive mcfDNA-seq for bacterial BSI by frequency of testing using raw data without imputation



For each frequency of testing model, estimated sensitivities for event in each day of the week were plotted in a radar graph. Larger shaded area indicates higher overall sensitivity. BSI, bloodstream infection; mcfDNA-seq, plasma microbial cell-free DNA sequencing.

4. Supplemental References

1. Centers for Disease Control and Prevention. Bloodstream infection event (central line–associated bloodstream infection and non–central line-associated bloodstream infection). 2017; http://www.cdc.gov/nhsn/PDFs/pscManual/4PSC_CLABSCurrent.pdf. Accessed February 4, 2019, 2019.
2. Blauwkamp TA, Thair S, Rosen MJ, et al. Analytical and clinical validation of a microbial cell-free DNA sequencing test for infectious disease. *Nat Microbiol*. 2019;4(4):663-674.
3. Simon R. Optimal two-stage designs for phase II clinical trials. *Control Clin Trials*. 1989;10(1):1-10.
4. Gafter-Gvili A, Fraser A, Paul M, et al. Antibiotic prophylaxis for bacterial infections in afebrile neutropenic patients following chemotherapy. *Cochrane Database Syst Rev*. 2012;1:CD004386.
5. Wilson EB. Probable inference, the law of succession, and statistical inference. *J Am Stat Assoc*. 1927;22(158):209-212.
6. Gaur AH, Bundy DG, Werner EJ, et al. A prospective, holistic, multicenter approach to tracking and understanding bloodstream infections in pediatric hematology-oncology patients. *Infect Control Hosp Epidemiol*. 2017;38(6):690-696.
7. Yamada K, Namikawa H, Fujimoto H, et al. Clinical Characteristics of Methicillin-resistant Coagulase-negative Staphylococcal Bacteremia in a Tertiary Hospital. *Intern Med*. 2017;56(7):781-785.
8. Bae KS, Shin JA, Kim SK, et al. Enterococcal bacteremia in febrile neutropenic children and adolescents with underlying malignancies, and clinical impact of vancomycin resistance. *Infection*. 2019;47(3):417-424.
9. Ramanan P, Barreto JN, Osmon DR, Tosh PK. *Rothia* bacteremia: a 10-year experience at Mayo Clinic, Rochester, Minnesota. *J Clin Microbiol*. 2014;52(9):3184-3189.
10. Dinleyici EC, Yargic ZA, Bor O, Kiremitci A, Durmaz G. Tigecycline treatment of multi-drug-resistant *Corynebacterium jeikeium* infection in a child with relapsing and refractory acute lymphoblastic leukemia. *Pediatr Blood Cancer*. 2010;55(2):349-351.

5. Range of Pathogens Identifiable by the Karius test

Bacteria and Archaea

Methanobrevibacter smithii; Abiotrophia defectiva; Achromobacter denitrificans; Achromobacter insolitus; Achromobacter ruhlandii; Achromobacter xylosoxidans; Acidaminococcus intestini; Acidovorax citrullii; Acinetobacter baumannii; Acinetobacter bereziniae; Acinetobacter calcoaceticus; Acinetobacter haemolyticus; Acinetobacter nosocomialis; Acinetobacter pittii; Acinetobacter radioresistens; Acinetobacter seifertii; Acinetobacter soli; Acinetobacter ursingii; Actinobacillus ureae; Actinomadura latina; Actinomadura madurae; Actinomyces cardiffensis; Actinomyces europaeus; Actinomyces georgiae; Actinomyces gerencseriae; Actinomyces graevenitzii; Actinomyces israelii; Actinomyces massiliensis; Actinomyces meyeri; Actinomyces neuii; Actinomyces odontolyticus; Actinomyces oris; Actinomyces timonensis; Actinomyces turicensis; Actinomyces urogenitalis; Actinomyces viscosus; Aerococcus christensenii; Aerococcus sanguinicola; Aerococcus urinae; Aerococcus urinaehominis; Aerococcus viridans; Aeromonas caviae; Aeromonas enteropelogenes; Aeromonas hydrophila; Aeromonas schubertii; Aeromonas veronii; Aggregatibacter actinomycetemcomitans; Aggregatibacter aphrophilus; Aggregatibacter segnis; Agrobacterium tumefaciens; Alcaligenes faecalis; Alloioococcus otitis; Alloscardovia omnicolens; Anaerobiospirillum succiniciproducens; Anaerococcus hydrogenalis; Anaerococcus lactolyticus; Anaerococcus prevotii; Anaerococcus tetradius; Anaeroglobus geminatus; Anaplasma phagocytophilum; Arcanobacterium bernardiae; Arcanobacterium haemolyticum; Arcanobacterium pyogenes; Arcobacter butzleri; Arcobacter cryaerophilus; Arcobacter skirrowii; Atopobium parvulum; Atopobium rimae; Atopobium vaginae; Aureimonas altamirensis; Bacillus anthracis; Bacillus cereus; Bacillus circulans; Bacillus coagulans; Bacillus glycinifermentans; Bacillus licheniformis; Bacillus megaterium; Bacillus pumilus; Bacillus sphaericus; Bacillus subtilis; Bacillus thuringiensis; Bacteroides caccae; Bacteroides distasonis; Bacteroides eggerthii; Bacteroides forsythus; Bacteroides fragilis; Bacteroides merdae; Bacteroides ovatus; Bacteroides stercoris; Bacteroides thetaiotaomicron; Bacteroides uniformis; Bacteroides vulgatus; Bartonella alsatica; Bartonella bacilliformis; Bartonella birtlesii; Bartonella bovis; Bartonella clarridgeiae; Bartonella doshiae; Bartonella elizabethae; Bartonella grahamii; Bartonella henselae; Bartonella koehlerae; Bartonella quintana; Bartonella rattaustraliani; Bartonella rochalimae; Bartonella schoenbuchensis; Bartonella taylorii; Bartonella tribocorum; Bartonella vinsonii; Bergeyella zoohelcum; Bifidobacterium adolescentis; Bifidobacterium breve; Bifidobacterium dentium; Bifidobacterium longum; Bifidobacterium scardovii; Bordetella bronchialis; Bordetella bronchiseptica; Bordetella flabilis; Bordetella hinzii; Bordetella holmesii; Bordetella parapertussis; Bordetella pertussis; Bordetella petrii; Bordetella trematum; Borrelia burgdorferi; Borrelia crocidurae; Borrelia duttonii; Borrelia hermsii; Borrelia hispanica; Borrelia mayonii; Borrelia miyamotoi; Borrelia parkeri; Borrelia persica; Borrelia recurrentis; Borrelia turicatae; Borreliella afzelii; Borreliella garinii; Brevibacillus brevis; Brevibacillus laterosporus; Brevibacterium casei; Brevundimonas diminuta; Brevundimonas vesicularis; Brucella abortus; Brucella canis; Brucella melitensis; Brucella suis; Budvicia aquatica; Burkholderia ambifaria; Burkholderia anthina; Burkholderia cenocepacia; Burkholderia cepacia; Burkholderia contaminans; Burkholderia gladioli; Burkholderia glumae; Burkholderia mallei; Burkholderia multivorans; Burkholderia pseudomallei; Burkholderia pyrrocinia; Burkholderia stabilis; Campylobacter coli; Campylobacter concisus; Campylobacter corcagiensis; Campylobacter cuniculorum; Campylobacter curvus; Campylobacter fetus; Campylobacter gracilis; Campylobacter hominis; Campylobacter hyointestinalis; Campylobacter iguaniorum; Campylobacter jejuni; Campylobacter lari; Campylobacter mucosalis; Campylobacter showae; Campylobacter sputorum; Campylobacter upsaliensis; Campylobacter ureolyticus; Capnocytophaga canimorsus; Capnocytophaga cynodegmi; Capnocytophaga gingivalis; Capnocytophaga granulosa; Capnocytophaga haemolytica; Capnocytophaga ochracea; Capnocytophaga sputigena; Cardiobacterium hominis; Cardiobacterium valvarum; Catabacter hongkongensis; Cedecea neteri; Cellulomonas flavigena; Chlamydia psittaci <Chlamydophila psittaci>; Chlamydia trachomatis; Chlamydophila pneumoniae; Chromobacterium haemolyticum; Chromobacterium violaceum; Chryseobacterium gleum; Chryseobacterium indologenes; Citrobacter amalonaticus; Citrobacter braakii; Citrobacter farmeri; Citrobacter freundii; Citrobacter koseri; Clostridium baratii; Clostridium bifementans; Clostridium butyricum; Clostridium clostridioforme; Clostridium difficile; Clostridium haemolyticum; Clostridium innocuum; Clostridium neonatale; Clostridium novyi; Clostridium paraputrificum; Clostridium perfringens; Clostridium sordellii; Clostridium tetani; Comamonas kerstersii; 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*Peptoniphilus coxii*; *Peptoniphilus duerdenii*; *Peptoniphilus harei*; *Peptoniphilus indolicus*; *Peptoniphilus lacrimalis*; *Peptoniphilus rhinitidis*; *Peptostreptococcus anaerobius*; *Peptostreptococcus stomatis*; *Photobacterium damsela*; *Photobacterium damselae*; *Photobacterium luminescens*; *Plesiomonas shigelloides*; *Pluralibacter gergoviae*; *Porphyrimonas asaccharolytica*; *Porphyrimonas gingivalis*; *Prevotella bivia*; *Prevotella buccae*; *Prevotella buccalis*; *Prevotella corporis*; *Prevotella denticola*; *Prevotella disiens*; *Prevotella intermedia*; *Prevotella loeschei*; *Prevotella melaninogenica*; *Prevotella nigrescens*; *Prevotella oralis*; *Prevotella oris*; *Propionibacterium acidifaciens*; *Propionibacterium granulosum*; *Propionibacterium namnetense*; *Propionibacterium propionicum*; *Proteus mirabilis*; *Proteus vulgaris*; *Providencia alcalifaciens*; *Providencia rettgeri*; *Providencia stuartii*; *Pseudomonas aeruginosa*; *Pseudomonas alcaligenes*; *Pseudomonas fluorescens*; *Pseudomonas fulva*; *Pseudomonas luteola*; *Pseudomonas mendocina*; *Pseudomonas mosselii*; *Pseudomonas oryzae*; *Pseudomonas protegens*; *Pseudomonas pseudoalcaligenes*; *Pseudomonas putida*; *Pseudoramibacter alactolyticus*; *Psychrobacter cryohalolentis*; *Rahnella aquatilis*; *Ralstonia insidiosa*; *Ralstonia mannitolilytica*; *Ralstonia pickettii*; *Raoultella ornithinolytica*; *Raoultella planticola*; *Rhizobium pusense*; *Rhodococcus equi*; *Rhodococcus erythropolis*; *Rhodococcus fascians*; *Rhodococcus rhodochrous*; *Rickettsia akari*; *Rickettsia amblyommatis*; *Rickettsia australis*; *Rickettsia canadensis*; *Rickettsia conorii*; *Rickettsia felis*; *Rickettsia helvetica*; *Rickettsia honei*; *Rickettsia japonica*; *Rickettsia massiliae*; *Rickettsia monacensis*; *Rickettsia parkeri*; *Rickettsia prowazekii*; *Rickettsia raoultii*; *Rickettsia rickettsii*; *Rickettsia sibirica*; *Rickettsia slovaca*; *Rickettsia typhi*; *Riemerella anatipestifer*; *Roseobacter denitrificans*; *Roseomonas cervicalis*; *Roseomonas fauriae*; *Roseomonas gilardii*; *Roseomonas mucosa*; *Rothia aeria*; *Rothia dentocariosa*; *Rothia mucilaginosa*; *Salmonella bongori*; *Salmonella enterica*; *Sanguibacteroides justesenii*; *Serratia ficaria*; *Serratia fonticola*; *Serratia liquefaciens*; *Serratia marcescens*; *Serratia odorifera*; *Serratia plymuthica*; *Serratia rubidaea*;

Shewanella algae; Shewanella putrefaciens; Shigella boydii; Shigella dysenteriae; Shigella flexneri; Shigella sonnei; Siccibacter turicensis; Slackia exigua; Sneathia sanguinegens; Solobacterium moorei; Sphingobacterium spiritivorum; Staphylococcus agnetis; Staphylococcus argenteus; Staphylococcus arlettae; Staphylococcus aureus; Staphylococcus auricularis; Staphylococcus capitis; Staphylococcus caprae; Staphylococcus carnosus; Staphylococcus caseolyticus; Staphylococcus chromogenes; Staphylococcus cohnii; Staphylococcus condimenti; Staphylococcus epidermidis; Staphylococcus equorum; Staphylococcus gallinarum; Staphylococcus haemolyticus; Staphylococcus hominis; Staphylococcus hyicus; Staphylococcus lentus; Staphylococcus lugdunensis; Staphylococcus pasteuri; Staphylococcus pettenkoferi; Staphylococcus pseudintermedius; Staphylococcus saprophyticus; Staphylococcus schleiferi; Staphylococcus sciuri; Staphylococcus simiae; Staphylococcus simulans; Staphylococcus succinus; Staphylococcus vitulinus; Staphylococcus warneri; Staphylococcus xylosus; Stenotrophomonas acidaminiphila; Stenotrophomonas maltophilia; Streptobacillus moniliformis; Streptococcus agalactiae; Streptococcus anginosus; Streptococcus canis; Streptococcus constellatus; Streptococcus cricetus; Streptococcus cristatus; Streptococcus dentisani; Streptococcus dysgalactiae; Streptococcus equi; Streptococcus equinus; Streptococcus ferus; Streptococcus gallolyticus; Streptococcus gordonii; Streptococcus hyovaginalis; Streptococcus infantarius; Streptococcus infantis; Streptococcus iniae; Streptococcus intermedius; Streptococcus lutetiensis; Streptococcus macacae; Streptococcus macedonicus; Streptococcus massiliensis; Streptococcus mitis; Streptococcus mutans; Streptococcus oralis; Streptococcus parasanguinis; Streptococcus pasteurianus; Streptococcus peroris; Streptococcus pneumoniae; Streptococcus porcinus; Streptococcus pseudopneumoniae; Streptococcus pyogenes; Streptococcus rattus; Streptococcus salivarius; Streptococcus sanguinis; Streptococcus sobrinus; Streptococcus suis; Streptococcus thermophilus; Streptococcus tigurinus; Streptococcus uberis; Streptococcus vestibularis; Streptomyces cattleya; Streptomyces somaliensis; Sutterella wadsworthensis; Tatumella ptyseos; Terrisporobacter othiniensis; Treponema pallidum; Tropheryma whipplei; Tsukamurella paurometabola; Turicella otitidis; Ureaplasma parvum; Ureaplasma urealyticum; Veillonella dispar; Veillonella montpellierensis; Veillonella parvula; Vibrio albensis; Vibrio alginolyticus; Vibrio cholerae; Vibrio fluvialis; Vibrio furnissii; Vibrio harveyi; Vibrio metschnikovii; Vibrio mimicus; Vibrio navarrensis; Vibrio parahaemolyticus; Vibrio vulnificus; Weissella virosa; Weissella confusa; Weissella paramesenteroides; Wohlfahrtiimonas chitiniclastica; Wolbachia pipientis; Xanthomonas axonopodis; Xylanimonas cellulossilytica; Yersinia enterocolitica; Yersinia frederiksenii; Yersinia intermedia; Yersinia kristensenii; Yersinia pestis; Yersinia pseudotuberculosis; Yersinia ruckeri; Yokenella regensburgei

Fungi and Molds

Absidia glauca; Absidia repens; Acremonium chrysogenum; Acremonium furcatum; Actinomucor elegans; Alternaria alternata; Alternaria arborescens; Alternaria brassicicola; Annaliia algerae; Apophysomyces elegans; Apophysomyces trapeziformis; Apophysomyces variabilis; Aspergillus aculeatus; Aspergillus arachidicola; Aspergillus bombycis; Aspergillus brasiliensis; Aspergillus calidoustus; Aspergillus campestris; Aspergillus candidus; Aspergillus carbonarius; Aspergillus chevalieri; Aspergillus clavatus; Aspergillus cristatus; Aspergillus fischeri; Aspergillus flavus; Aspergillus fumigatus; Aspergillus glaucus; Aspergillus hancockii; Aspergillus lentulus; Aspergillus luchuensis; Aspergillus nidulans; Aspergillus niger; Aspergillus nomius; Aspergillus novofumigatus; Aspergillus ochraceoroseus; Aspergillus oryzae; Aspergillus parasiticus; Aspergillus persii; Aspergillus rambellii; Aspergillus ruber; Aspergillus sclerotiorum; Aspergillus sojae; Aspergillus steynii; Aspergillus sydowii; Aspergillus taichungensis; Aspergillus terreus; Aspergillus thermomutatus; Aspergillus tubingensis; Aspergillus turcosus; Aspergillus udagawae; Aspergillus ustus; Aspergillus versicolor; Aspergillus wentii; Aspergillus westerdijkiae; Aureobasidium melanogenum; Aureobasidium namibiae; Aureobasidium pullulans; Aureobasidium subglaciale; Basidiobolus meristosporus; Beauveria bassiana; Beauveria rudraprayagi; Bipolaris papendorfii; Blastomyces dermatitidis; Blastomyces percursor; Byssoschlamys spectabilis; Candida aaseri; Candida albicans; Candida apicola; Candida arabinofementans; Candida auris; Candida boidinii; Candida bracarenensis; Candida carpophila; Candida castellii; Candida dubliniensis; Candida duobushaemulonii; Candida ethanolica; Candida famata; Candida glabrata; Candida haemulonis; Candida homilientoma; Candida intermedia; Candida ipomoeae; Candida kefyri; Candida kipukae; Candida krusei; Candida lusitaniae; Candida nivariensis; Candida orthopsilosis; Candida parapsilosis; Candida pseudohaemulonii; Candida psychrophila; Candida sojae; Candida sorbophila; Candida sorboxylosa; Candida succiphila; Candida tanzawaensis; Candida tenuis; Candida tropicalis; Candida utilis; Candida versatilis; Capronia semi-immersa; Ceratocystis adiposa; Ceratocystis albifundus; Ceratocystis eucalypticola; Ceratocystis fimbriata; Ceratocystis manginecans; Ceratocystis platani; Cercospora fijiensis; Chaetomium globosum; Chaetomium thermophilum; Chrysosporium queenslandicum; Cladophialophora bantiana; Cladophialophora carrionii; Cladophialophora immunda; Cladophialophora psammophila; Cladophialophora yegresii; Cladosporium cladosporioides; Coccidioides immitis; Coccidioides posadasii; Cokeromyces recurvatus; Colletotrichum acutatum;

Colletotrichum falcatum; *Colletotrichum fiorinae*; *Colletotrichum gloeosporioides*; *Colletotrichum godetiae*; *Colletotrichum graminicola*; *Colletotrichum higginsianum*; *Colletotrichum incanum*; *Colletotrichum nymphaeae*; *Colletotrichum orbiculare*; *Colletotrichum salicis*; *Colletotrichum simmondsii*; *Colletotrichum sublineola*; *Colletotrichum tofieldiae*; *Conidiobolus coronatus*; *Conidiobolus incongruus*; *Coniosporium apollinis*; *Corynespora cassicola*; *Cryptococcus bacillisporus*; *Cryptococcus bestiolae*; *Cryptococcus dejecticola*; *Cryptococcus deuterogetti*; *Cryptococcus fagi*; *Cryptococcus gattii*; *Cryptococcus neoformans*; *Cryptococcus pinus*; *Cryptococcus skinneri*; *Cryptococcus tetragattii*; *Cunninghamella*; *Curvularia lunata*; *Cyphellophora europaea*; *Debaryomyces fabryi*; *Diaporthe ampelina*; *Diaporthe aspalathi*; *Diaporthe longicolla*; *Emmonsia crescens*; *Emmonsia parva*; *Encephalitozoon cuniculi*; *Encephalitozoon hellem*; *Encephalitozoon intestinalis*; *Encephalitozoon romaleae*; *Enterocytozoon bieneusi*; *Exophiala alcalophila*; *Exophiala aquamarina*; *Exophiala calicioides*; *Exophiala dermatitidis*; *Exophiala mesophila*; *Exophiala oligosperma*; *Exophiala sideris*; *Exophiala spinifera*; *Exophiala xenobiotica*; *Filobasidium wieringae*; *Fonsecaea erecta*; *Fonsecaea monophora*; *Fonsecaea multimorphosa*; *Fonsecaea nubica*; *Fonsecaea pedrosoi*; *Fusarium agapanthi*; *Fusarium asiaticum*; *Fusarium avenaceum*; *Fusarium circinatum*; *Fusarium culmorum*; *Fusarium euwallaceae*; *Fusarium fujikuroi*; *Fusarium graminearum*; *Fusarium hostae*; *Fusarium langsethiae*; *Fusarium mangiferae*; *Fusarium meridionale*; *Fusarium nygamai*; *Fusarium oxysporum*; *Fusarium pininemorale*; *Fusarium poae*; *Fusarium praegraminearum*; *Fusarium proliferatum*; *Fusarium pseudograminearum*; *Fusarium sambucinum*; *Fusarium solani*; *Fusarium temperatum*; *Fusarium udum*; *Fusarium verticillioides*; *Geotrichum candidum*; *Hanseniaspora uvarum*; *Hansenula fabianii*; *Histoplasma capsulatum*; *Kluyveromyces lactis*; *Lachancea kluyveri*; *Lachancea lanzarotensis*; *Lachancea thermotolerans*; *Lachancea waltii*; *Leptosphaeria maculans*; *Lichtheimia corymbifera*; *Lichtheimia ramosa*; *Lodderomyces elongisporus*; *Lomentospora prolificans*; *Macrophomina phaseolina*; *Madurella mycetomatis*; *Malassezia caprae*; *Malassezia cuniculi*; *Malassezia dermatis*; *Malassezia equina*; *Malassezia furfur*; *Malassezia globosa*; *Malassezia japonica*; *Malassezia nana*; *Malassezia obtusa*; *Malassezia pachydermatis*; *Malassezia slooffiae*; *Malassezia sympodialis*; *Malassezia yamatoensis*; *Memnoniella echinata*; *Metarhizium acridum*; *Metarhizium album*; *Metarhizium anisopliae*; *Metarhizium brunneum*; *Metarhizium guizhouense*; *Metarhizium majus*; *Metarhizium rileyi*; *Metarhizium robertsii*; *Metschnikowia bicuspidata*; *Metschnikowia fructicola*; *Microsporum canis*; *Microsporum gypseum*; *Mortierella alpina*; *Mortierella elongata*; *Mortierella verticillata*; *Mucor ambiguus*; *Mucor circinelloides*; *Mucor indicus*; *Mucor irregularis*; *Mucor velutinosus*; *Myceliophthora thermophila*; *Nakaseomyces bacillisporus*; *Nakaseomyces delphensis*; *Nakazawaea peltata*; *Naumovozyma dairenensis*; *Nectria haematococca*; *Neofusicoccum parvum*; *Nosema apis*; *Nosema bombycis*; *Nosema ceranae*; *Ochroconis constricta*; *Ochroconis gallopava*; *Ogataea methanolica*; *Ogataea parapolyomorpha*; *Ogataea polymorpha*; *Ophiostoma novo-ulmi*; *Ophiostoma piceae*; *Paecilomyces hepiali*; *Paracoccidioides brasiliensis*; *Paracoccidioides lutzii*; *Penicillium antarcticum*; *Penicillium brasilianum*; *Penicillium capsulatum*; *Penicillium carneum*; *Penicillium coprophilum*; *Penicillium decumbens*; *Penicillium digitatum*; *Penicillium expansum*; *Penicillium flavigenum*; *Penicillium freii*; *Penicillium griseofulvum*; *Penicillium islandicum*; *Penicillium italicum*; *Penicillium janthinellum*; *Penicillium marneffeii*; *Penicillium nalgiovense*; *Penicillium nordicum*; *Penicillium occitanis*; *Penicillium oxalicum*; *Penicillium paneum*; *Penicillium paxilli*; *Penicillium piceum*; *Penicillium pinophilum*; *Penicillium purpurogenum*; *Penicillium roqueforti*; *Penicillium rubens*; *Penicillium sclerotiorum*; *Penicillium steckii*; *Penicillium subrubescens*; *Penicillium verruculosum*; *Penicillium vulpinum*; *Phaeoacremonium minimum*; *Phanerochaete carnosae*; *Phanerochaete chrysosporium*; *Phellinus noxius*; *Phialophora attae*; *Phoma herbarum*; *Phycomyces blakesleeanae*; *Pichia anomala*; *Pneumocystis carinii*; *Pneumocystis jirovecii*; *Pneumocystis murina*; *Pseudozyma hubeiensis*; *Purpureocillium lilacinum*; *Pyrenochaeta lycopersici*; *Pyrenochaeta mackinnonii*; *Ramichloridium mackenziei*; *Rasamsonia emersonii*; *Rhizoctonia solani*; *Rhizomucor miehei*; *Rhizomucor pusillus*; *Rhizomucor variabilis*; *Rhizopus delemar*; *Rhizopus microsporus*; *Rhizopus oryzae*; *Rhizopus stolonifer*; *Rhodotorula graminis*; *Rhodotorula mucilaginosa*; *Rhodotorula toruloides*; *Rhytidhysterium rufulum*; *Saccharomyces cerevisiae*; *Saksenaea oblongispora*; *Saksenaea vasiformis*; *Scedosporium apiospermum*; *Scedosporium aurantiacum*; *Scedosporium boydii*; *Scedosporium dehoogii*; *Schizophyllum commune*; *Sporopachydermia quercuum*; *Sporothrix brasiliensis*; *Sporothrix globosa*; *Sporothrix insectorum*; *Sporothrix pallida*; *Sporothrix schenckii*; *Stachybotrys chartarum*; *Stachybotrys chlorohalonata*; *Stemphylium lycopersici*; *Syncephalastrum monosporum*; *Syncephalastrum racemosum*; *Talaromyces amestolkiae*; *Talaromyces atrovirens*; *Talaromyces cellulolyticus*; *Talaromyces leycettanus*; *Talaromyces stipitatus*; *Talaromyces wortmannii*; *Thermoascus crustaceus*; *Thermomyces lanuginosus*; *Thielavia terrestris*; *Torulaspora delbrueckii*; *Trachipleistophora hominis*; *Trichoderma asperellum*; *Trichoderma atroviride*; *Trichoderma gamsii*; *Trichoderma hamatum*; *Trichoderma harzianum*; *Trichoderma longibrachiatum*; *Trichoderma parareesei*; *Trichoderma reesei*; *Trichoderma virens*; *Trichophyton*; *Trichophyton benhamiae*; *Trichosporon asahii*; *Trichosporon coremiiforme*; *Trichosporon cutaneum*; *Trichosporon faecale*; *Trichosporon guehoae*; *Trichosporon inkin*; *Trichosporon oleaginosus*; *Trichosporon ovoides*; *Trichosporon porosum*; *Ustilago cynodontis*; *Ustilago esculenta*; *Ustilago*

hordei; Ustilago maydis; Ustilago trichophora; Valsa mali; Verticillium alfalfae; Verticillium dahliae; Verticillium longisporum; Verticillium tricorpus; Vittiforma corneae; Volvariella volvacea; Wallemia ichthyophaga; Wallemia mellicola; Wickerhamomyces ciferrii; Yarrowia deformans; Yarrowia keelungensis; Yarrowia lipolytica

Other Eukaryota

Acanthamoeba; Ancylostoma ceylanicum; Ancylostoma duodenale; Angiostrongylus cantonensis; Angiostrongylus costaricensis; Anisakis simplex; Ascaris; Babesia divergens; Babesia microti; Balamuthia mandrillaris; Blastocystis hominis; Brugia malayi; Clonorchis sinensis; Cryptosporidium hominis; Cryptosporidium muris; Cryptosporidium parvum; Cryptosporidium ubiquitum; Cyclospora cayetanensis; Dirofilaria immitis; Dracunculus medinensis; Echinococcus granulosus; Echinococcus multilocularis; Echinostoma caproni; Entamoeba histolytica; Enterobius vermicularis; Fasciola hepatica; Giardia lamblia; Hymenolepis nana; Leishmania aethiopica; Leishmania amazonensis; Leishmania braziliensis; Leishmania donovani; Leishmania infantum; Leishmania major; Leishmania mexicana; Leishmania panamensis; Leishmania tropica; Loa loa; Naegleria fowleri; Necator americanus; Onchocerca volvulus; Opisthorchis viverrini; Plasmodium cynomolgi; Plasmodium falciparum; Plasmodium knowlesi; Plasmodium ovale; Plasmodium vivax; Pythium insidiosum; Schistosoma haematobium; Schistosoma japonicum; Schistosoma mansoni; Strongyloides stercoralis; Taenia asiatica; Taenia saginata; Taenia solium; Toxocara canis; Toxoplasma gondii; Trichinella; Trichomonas vaginalis; Trichuris trichiura; Trypanosoma brucei; Trypanosoma cruzi; Wuchereria bancrofti

Viruses

Adeno-associated dependoparvovirus A; Adeno-associated dependoparvovirus B; Alphapapillomavirus 1; Alphapapillomavirus 10; Alphapapillomavirus 11; Alphapapillomavirus 14; Alphapapillomavirus 2; Alphapapillomavirus 3; Alphapapillomavirus 4; Alphapapillomavirus 5; Alphapapillomavirus 6; Alphapapillomavirus 7; Alphapapillomavirus 8; Alphapapillomavirus 9; Betapapillomavirus 1; Betapapillomavirus 2; Betapapillomavirus 3; Betapapillomavirus 4; Betapapillomavirus 5; BK polyomavirus; Cowpox virus; Cytomegalovirus (CMV); Epstein-Barr virus (EBV); Gammapapillomavirus 1; Gammapapillomavirus 10; Gammapapillomavirus 11; Gammapapillomavirus 13; Gammapapillomavirus 14; Gammapapillomavirus 15; Gammapapillomavirus 16; Gammapapillomavirus 17; Gammapapillomavirus 19; Gammapapillomavirus 2; Gammapapillomavirus 3; Gammapapillomavirus 4; Gammapapillomavirus 5; Gammapapillomavirus 6; Gammapapillomavirus 7; Gammapapillomavirus 8; Gammapapillomavirus 9; Herpes B virus; Herpes simplex virus type 1 (HSV-1); Herpes simplex virus type 2 (HSV-2); Human adenovirus A; Human adenovirus B; Human adenovirus C; Human adenovirus D; Human adenovirus E; Human adenovirus F; Human bocavirus; Human herpesvirus 6A; Human herpesvirus 6B; Human herpesvirus 7; Human papillomavirus; Human papillomavirus 132-like viruses; Human papillomavirus type 136; Human papillomavirus type 140; Human papillomavirus type 154; Human papillomavirus type 167; Human parvovirus; Human polyomavirus 6; Human polyomavirus 7; JC polyomavirus; Kaposi sarcoma-associated herpesvirus; KI polyomavirus; Merkel cell polyomavirus; Molluscum contagiosum virus; Monkeypox virus; Mupapillomavirus 1; Mupapillomavirus 2; Nupapillomavirus 1; Orf virus; Porcine circovirus 1; Porcine circovirus 2; Primate bocaparvovirus 1; Pseudocowpox virus; STL polyomavirus; Tanapox virus; Torque teno virus; Torque teno virus 1; Torque teno virus 10; Torque teno virus 12; Torque teno virus 14; Torque teno virus 15; Torque teno virus 16; Torque teno virus 19; Torque teno virus 2; Torque teno virus 25; Torque teno virus 26; Torque teno virus 27; Torque teno virus 28; Torque teno virus 3; Torque teno virus 4; Torque teno virus 6; Torque teno virus 7; Torque teno virus 8; Trichodysplasia spinulosa-associated polyomavirus; Vaccinia virus; Varicella-zoster virus (VZV); Variola virus; WU Polyomavirus; Yaba monkey tumor virus