EVALUATION OF A METAGENOMIC NEXT-GENERATION SEQUENCING ASSAY WITH A NOVEL HOST DEPLETION METHOD FOR PATHOGEN IDENTIFICATION IN SEPTIC PATIENTS

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ABSTRACT

Background The traditional diagnosis of sepsis has always been based on microbial blood culture (BC). However, BC suffers from (1) long culture cycle, leading to delay in results, and (2) low diagnostic yields. Metagenomic next-generation sequencing (mNGS) has been proposed as an efficient and agnostic option that potentially overcomes these issues. In this study, a mNGS workflow utilizing a novel filter to specifically capture white blood cells and deplete host DNA background, was evaluated against BC results, as well as mNGS without host depletion, for pathogen identification.

Materials and Methods Patients admitted to Taipei Veterans General Hospital (TVGH) with suspected sepsis were recruited to the study approved by the IRB. Blood sample was taken for BC (designated as BC1) before any antibiotic exposure. Upon patient enrolment, blood was taken again and divided in 3 portions with one used for the 2nd BC (BC2). The other two were used for mNGS with one processed with the filter and the other without filtering, to assess the effectiveness of host-depletion by the filter.

Results A total of 50 patients were recruited among which 45 had results for all 4 tests. mNGS with filter had the highest positive rate of 74.4%, followed by mNGS without filter and BC1 (51.1% and 50.0% respectively), while the 2nd BC had the lowest positive rate of 22.0%. Further, mNGS was less sensitive to antibiotics exposure as compared to BC. The overall correlation between samples with vs without filtration (R^2=0.96) confirmed that filtration does not affect microbial composition in a sample. For the BC positive samples, the effect of host depletion by filtration increased microbial target reads/million QC reads from 46 reads to 243 reads on average. Microbial reads enrichment by the filter appeared to be more effective for the samples with lower microbial concentration, thus increasing the test sensitivity over mNGS without filter. Using the 2nd BC results as reference, mNGS with filter and mNGS without filter exhibited sensitivities of 81.8% and 63.6%.

Conclusion The mNGS with filter was able to recover most of the pathogens identified by clinical BC and achieved the highest diagnostic yield. With the clinical implementation to complete the workflow within 24 hours, it has the potential to overcome slow turnaround and low diagnostic yield issues of traditional BC.

INTRODUCTION

Sepsis is a life-threatening condition arising from the human immune response to the infection of the bloodstream [1]. Sepsis can progress into septic shock, organ failure and death if diagnosis and correct treatment are not timely made [2]. Morbidity and mortality rates of blood stream infections (BSIs) are especially high in the intensive care units (ICU) and neonatology units of hospitals [3]. The traditional workflow to diagnose sepsis in clinical microbiology laboratories starts with blood culturing (BC), necessitating between 1 ml and 10 ml of blood per BC bottle, often in automated systems such as the BACTECTM (Becton Dickinson-BD, Maryland, USA) or BacT/Alert (bioMérieux, Marcy l'Etoile, France. A positive BC is followed by Gram staining. Bacterial genus and species identification can be performed on the cultured strain with biochemical assays or MALDI-TOF mass spectrometry (MS). Antibiotics susceptibility testing of the cultured strain is generally performed with microbiological assays.

The short-comings of traditional methods have been expounded [4]: they do not detect viruses and parasites; some bacterial pathogens are difficult to culture; the culture cycle is long, so the results are delayed; and it is difficult to identify mixed infections (due to single colony picking). One of the major shortcomings of traditional methods is its low diagnostic yield (positive rates). In one study [4], the positivity rates of blood, sputum, and BALF using traditional detection methods were 14% (13/90), 38% (34/90), 22% (10/45), respectively. In immunocompromised and immunocompetent groups of patients, 47% (14/30) and 27% (16/60) of patients, respectively, were positive by NGS but negative by traditional detection methods.

Despite the availability of rapid molecular assays, most early antibiotic treatment is still empirical. According to one study [5], approximately 46% of early empirical antibiotic treatment was inappropriate, directly leading to a sepsis-related mortality rate of almost 35%. Approximately 50% of antibiotics administered are unnecessary or too broad-spectrum, which increases the toxicity of drugs and the incidence of bacterial resistance [5]. Therefore, early identification of pathogens is particularly important to enable targeted antibiotic therapy. Several reports have presented the potential advantages of molecular diagnostics over BC, such as a shorter turnaround time, detection of difficult to grow bacteria or detection after prior intake of antibiotics which inhibits bacterial growth [6]. Caliendo et al. reported that molecular diagnosis of BSI can reduce hospitalization rates and length of ICU stays, and decrease mortality due to BSI [7].

In recent years, metagenomic NGS has been proposed as a more efficient and accurate means for pathogen diagnosis and expanded the options of diagnostic strategies for pathogen identification [4] [8] [9] [10]. mNGS is based on the "agnostic" extraction and sequencing of all nucleic acids in a patient's specimen in parallel, so as to obtain the sequence of host and microorganisms. mNGS can be applied to a wide range of specimen types (sputum, throat swab, blood, alveolar lavage fluid, pleural fluid, cerebrospinal fluid, pus, tissue specimens, etc), and it can directly detect nucleic acids in clinical samples without biasness or selectivity. Viruses, bacteria, fungi, and parasites can be detected in an unbiased manner. mNGS could detect and identify multiple pathogens simultaneously.

NGS was used for the clinical diagnosis of neuroleptospirosis in a 14-year-old critically ill boy with meningoencephalitis; this case was the first to demonstrate the utility of metagenomic NGS (mNGS) in providing clinically actionable information, as successful diagnosis prompted appropriate targeted antibiotic treatment and eventual recovery of the patient [11]. Huang et al. showed that in patients with pulmonary infection, the positive-rate of mNGS (88.30%) for pathogen detection in pulmonary infection was much higher than that of traditional detection methods (25.73%), while the specificity

of mNGS (81.16%) was slightly lower than that of traditional detection methods (88.41%) [9]. Cheng and Yu [4] showed that, in a cohort of immunocompromised and immunocompetent sepsis patients, 77 (86%) were positive for 1 or more pathogens using NGS, and 50 (56%) were positive using traditional detection methods.

One widely recognised major hurdle in mNGS of blood samples is the overwhelming presence of human DNA, leading to vast wastage of sequencing real estate [11]. A few methods have been proposed for removing host DNA background, including differential lysis of human cells [12] and methylated human DNA removal [13]. Recently, host cell depletion device such as Devin fractionation filter has been made available for removing human nucleated cells. Incorporating such device into mNGS workflow imposes an efficient way to enrich microorganisms in the sample, to increase the sensitivity of the assay or decrease the total cost of the assay. The objective of this study is to evaluate the efficacy of the Devin fractionation filter in host depletion and enrichment of microbial genomic sequences in the mNGS workflow used in this study. Another objective of this study is to evaluate the clinical performance of the mNGS workflow incorporating Devin filtration, using a cohort of patients with suspected sepsis who had been admitted to Taipei Veterans General Hospital (TVGH).

MATERIALS AND METHODS

Patient samples Blood samples were recruited from patients admitted to Emergency Department of Taipei Veterans General Hospital (TVGH) from April 2021 to September 2022 who were suspected of sepsis. The study has been approved by the Institutional Review Board (Ethics Committee) of TVGH (IRB number, 2021-03-013AC, approval date: March 22, 2021). As illustrated in Fig. 1, Blood sample for routine clinical blood culture was taken upon admission as the 1st blood culture (designated as BC1) which may be followed by antibiotic exposure for most of the cases. Blood sample for this study was then taken after consent was obtained. It was divided in 2 portions. One portion was used for blood culture designated as 2nd blood culture (BC2) while the other portion of approximately 8 mL was used for mNGS.

Host depletion using Devin™ fractionation filter In order to assess the effectiveness of host-depletion by the Devin™ filter, the ~8 mL whole blood sample was further divided into two portions, one processed for host-depletion using the Devin™ fraction filter (Micronbrane Medical, Taiwan), and the other processed without filtering. The Devin™ fractionation syringe filter series exploits the antifouling Zwitterionic coating technology to specifically capture the white blood cells (>99%) in the whole blood, while allowing other blood components to flow through the membrane. The filter was securely attached to a syringe on the one side. Approximately 4 mL of whole blood sample was transferred into the syringe. The syringe plunger was gently pressed down to push the sample through the filter to a 15 mL falcon tube. Both the filtered and unfiltered blood samples were used for the downstream assays.

Sample processing and DNA extraction As illustrated in Fig. 1, ZymoBIOMICS Spike-in Control I (High Microbial Load) (Zymo Research, Irvine, CA) which included two extremophiles bacterial species (*Imtechella halotolerans* and *Allobacillus halotolerans*) was added to all samples including NTC (No Template Control) at the concentration of 10^4 Genome Copies/mL to act as internal reference controls. Both the filtered and unfiltered blood samples were centrifuged at low speed (400g) for 15 min at room temperature to obtain the plasma. The plasma was then centrifuged at high speed (16,000g) to obtain the sample pellet for DNA extraction using Devin™ Microbial DNA Enrichment kit (Micronbrane Medical, Taiwan). For selected samples, the supernatant after the high-speed centrifugation was also obtained for cell-free DNA (cfDNA) extraction using iCatcher® Circulating cfDNA1000 Kit (CatchGene Co., Ltd., Taiwan).

Library construction and NGS Library preparation with DNA extracted from the pellet was carried out using Illumina DNA Prep Library Kit (Illumina, USA), according to manufacturer's instructions. After DNA tagmentation, PCR amplification was carried out using the following conditions: (Top lid at 100°C); 68°C, 3min; 98°C, 3min; 15 cycles of 98°C, 45sec/ 62°C, 30sec/ 68°C, 2min; 68° C, 1min; 10° C, ∞. After amplification, the reaction was purified using Sample Purification Beads and eluted with 21 µl of Resuspension Buffer (Illumina, USA). 20 µl supernatant was transferred to a new Lobind Eppendorf tube and stored at -20°C. The constructed libraries were sent to a service provider for sequencing on Illumina NovaSeq platform to obtain a minimum of 20 million reads per sample at 150bp. NGS Library preparation for cfDNA from plasma were carried out using xGen™ ssDNA & Low-Input DNA Library Preparation Kit (Integrated DNA Technologies, USA) following manufacturer's protocol.

Bioinformatics pipeline and results interpretation All sequencing reads were trimmed for adapter sequences and poor-quality bases (<Q30) using fastp v0.23.2 [14]. Reads mapped to human genome (GRCh38) were performed with bwa 0.7.17 (bwa-mem algorithm) [15]. The remaining reads were aligned to microbial database with bwa 0.7.17. A set of representative genomes for microorganisms

(bacteria, viruses, fungi, protozoa, and other multicellular eukaryotic pathogens) from the NCBI Nucleotide and Genome databases was used for microbial alignment. The final database consisted of about 1400 genomes. For each sample, the % of each microorganism (microbe %) was calculated as the % of the classified reads in the total microbial reads. The absolute classified reads were normalized to one million QC reads named as RPM (Reads per Million) for each microorganism. A non-template control (NTC) was mandated for each reagent batch. The Microbe % and RPM was calculated in the same way for NTC as for the samples. The NTC was used to filter out contaminants from the laboratory and reagents. Sample to NTC ratios were computed using % of reads/total microbial reads (microbe $\%_{SAMPLE}$: microbe $\%_{NTC}$). Microorganisms were kept only if their % were found $\geq$ 5-folds in samples than in controls and their RPM $\geq$ 5. mNGS results were primarily compared with microbial identification results from BC2 (the reference result), since both mNGS and BC2 resulted from the same blood draw. Subsequently, BC1 results as well as other culture results if available were also used for evaluating the overall performance of mNGS. Prior to finalization of read-length of analysis, a subset of 11 BC1 positive samples were subject to analysis based on 100bp, 120bp, and 150bp (Supplemental Table 1). Analysis using various read length did not affect the recovery of positive sequences. As such, 100bp-based analysis was selected to be used in this study as the sequencing and analysis time of 100bp read length is the most feasible condition to finish the whole metagenomic NGS workflow within 24 hours for real clinical application.

RESULTS

Clinical Performance of mNGS with and without host depletion

Total of 50 test subjects (identified by sample number in Table 1) were recruited for this study. These were patients admitted into Emergency Department with suspected sepsis. The microbial culture results from the first blood draw (BC1) were the routine clinical results. This may be followed by antibiotic exposure in most cases. Blood sample for this study (BC2) was then taken after consent was obtained. For testing the efficacy of host depletion, each sample was processed with and without Devin filtration followed by the same DNA extraction, NGS library construction process and sequenced at 150bp. Three cases (#25, #31 and #37) had library preparation failure for both with and without filter samples. Two cases (#27 and #38) failed library preparation failure for the without filter sample only. Hence, the success rate of library prep was 92% (92/100).

As the turn-around time (TAT) is one of the crucial parameters for pathogen detection method, we first evaluated the microorganism classification performance by different sequencing read lengths in the 11 samples having positive culture results for both BC1 and BC2. As shown in Supplementary Table 1, the sequencing analysis results were compared among different read length of 150, 120 and 100bp in both mNGS with and without Devin™ filter. Reads were normalized to million QC reads (RPM) for comparison among samples. Overall, a highly comparable results were observed for different read length among all 11 samples. Most importantly, when applying the tentative cut-off criteria for potential pathogen identification, viz fold change (microbe $\%_{SAMPLE}$: microbe $\%_{NTC}$) ≥ 5 and microbial RPM ≥ 5, the results were unaffected by different read length. Hence, analysis with 100 bp read lengths offers the most efficient option, and was selected to be used in this study. The summary of the reads classification results at 100 bp for all samples was shown in the Supplementary Table 2.

Both fold change (microbe $\%_{SAMPLE}$: microbe $\%_{NTC}$) and individual species RPM (Reads per Million) are direct indicators of the amount of species in the sample. Using BC results as a comparison, setting both indictors at a threshold of ≥ 5 for pathogen calling appeared to be reasonable criteria to achieve good sensitivity and specificity. The microorganisms identified passing these criteria for all samples were listed in Supplementary Table 3. The identification results of all samples were then compared to both the BC1 and BC2 results and summarized in Tables 1 and 2. As shown in Table 1, when comparing to BC2 positive results, mNGS with filtration was able to detect the pathogens consistent with the microbial culture in 9 out 11 samples while mNGS without out filter was able to detect 7 out of 11 samples. Hence the sensitivity of mNGS with or without filtration was 81.8% and 63.6% respectively. Filtration let to higher sensitivity performance through increasing overall number of microbial reads and target species reads in most of the samples. In sample #17, both mNGS methods failed to detect MRSA. In sample #9, both mNGS failed to identify *E. coli* but were able to detect other species passing the criteria set. Further, mNGS (with filtration) provided the highest diagnostic yield (35/47; 74.4%), followed by mNGS (without filtration) (23/45; 51.1%), BC1 (25/50; 50%) and BC2 (11/50; 22%). Out of 12 samples which were positive for BC1 but negative for BC2, mNGS detected pathogen in 8 (with filter) and 4 (without filter) samples consistent with the culture results of BC1 . Hence, this data also suggested that mNGS is less sensitive to antibiotic treatment as compared to culture methods.

In general, mNGS detected more microorganisms per sample as compared to microbial culture (see columns labelled as “Others” in Table 1 and “# Potential Pathogens Identified” in Table 2). Microbial detection by mNGS from blood culture negative samples, where both BC1 and BC2 were negative, is shown in Table 2. Interestingly, amongst the 22 blood culture negative samples (both BC1 and BC2

negative), microbial detection in 5 samples were correlated to additional testing results – mNGS results in 4 samples included a pathogen that correlated with other clinical results; 1 sample was negative for mNGS and had negative results from all routine tests. This suggested that although mNGS detected more pathogens as compared to microbial culture, the additional pathogens called may be correlatable to other clinical results, and may be clinically useful.

Effect of Devin filtration on number and ratio of microbial reads

Although human reads remained the predominant class of sequence from whole blood samples despite the employment of host cell depletion by Devin™ filter, total microbial RPM increased considerably in 38 out of 45 pairs of samples having data from both mNGS with and without filter. For the BC positive samples, the effect of host depletion by filtration increased microbial target reads/million QC reads from 46 reads to 243 reads on average. A plot of the microbial RPM before and after filtration (Fig. 2a) showed that a wide range of enrichment (fold change in microbial RPM upon filtration compared to that without filtration) occurred amongst the samples. Fold-enrichment ranged from less than 1-log to more than 3-log (Fig. 2a, Supplementary Table 2). Particularly, the enrichment of microbial reads appears to be more effective with lower number of microbial reads entering the filter. In a small fraction of samples , enrichment was unsuccessful and number of reads declined slightly upon filtration. The reasons could be various and would be further discussed in the later session. In a fraction of samples, enrichment appeared insignificant, with no enrichment or even decrease in number of microbial reads upon filtration (samples 10, 12, 21, 22, 40, 48, 50). Interestingly, 3/7 of these were negative for BC1 and BC2, and 3/7 of these were negative for BC2, suggesting that there might be no significant level of pathogen content in these samples.

The % of individual species reads (microbial%) was computed by dividing each species reads to the total microbial reads (after removing human and unclassified reads). During infection, microbial% of the pathogen would be higher than that in normal individuals or a pre-defined baseline [16]. As such, we use the fold-enrichment of individual species microbial% in a sample over that in the NTC as one of the criteria to identify potential positive pathogens. Hence, it is important that the process of Devin filtration should not randomly alter the microbial% for each microorganism in a sample. To verify this, the microbial % reads for each microorganism in all samples with filter was correlated with that from the same samples without filter (Fig. 2b). Results showed that there was a tight correlation (R^2=0.96) in microbial % reads with and without filtration, and the correlation was close to 1:1. Hence, the process of Devin filtration does not affect proportion of microbial species in general.

Comparison with cfDNA detection

Although this study was based on the use of gDNA for pathogen detection, a number of mNGS studies employs cfDNA as the target for pathogen detection. The strength and weakness of a cfDNA approach is discussed below. To investigate the correlation between gDNA and cfDNA approaches, cfDNA was harvested from the plasma from selected BC positive samples and subject to mNGS. Results showed that most of the expected pathogens were also detectable by cfDNA (Table 3). In the absence of filtration, the microbial RPM was similar between gDNA and cfDNA which was 280 and 140, respectively. However, with the use of filter, gDNA-based mNGS obtained significantly increased microbial RPM of an average of 2,359, while cfDNA-based method only achieved and average microbial RPM of 95 by filter, which was even slightly less than the process without filter. Hence, gDNA but not cfDNA was amenable to host-depletion and enrichment in microbial sequences. This suggests that signal derived from cfDNA would be closer to baseline noise, imposing a greater tendency for calling false positives.

DISCUSSION

Enhancement of mNGS sensitivity by host depletion

One long-standing challenge of mNGS is the overwhelming presence of host DNA background in human biospecimens especially the whole blood. Although different host depletion methods have been tested, they are not practical in the clinical workflow in addition to widely varied host depletion effect. The host cell/DNA depletion by filtration tested in this study showed significant level of enrichment of microbial reads comparing to the sample processed without filter, especially for the samples with low microbial content. The host depletion indeed enhanced the sensitivity from 63.6% for mNGS without filter to 81.8% to mNGS with filter, suggesting that filtration can enrich microbial content in the gDNA portion of the sample using the process tested in this study. On the other hand, cfDNA was compared to this gDNA based mNGS approached in this study because most of the reported studies were based on cfDNA. No significant enrichment of microbial content in the cfDNA portion was observed between samples with and without filtration. Thus, we demonstrated in this study that gDNA based mNGS was amenable to host-depletion and enrichment in microbial sequences by filtration. With the easy process of filtration together with 100bp sequencing and analysis, it is possible now to establish a clinical applicable workflow can be completed within 24 hours with enhanced sensitivity comparing to other mNGS methods.

Criteria for pathogen identification by mNGS

Another major challenge of mNGS is that currently it lacks consensus of the definitive criteria for distinguishing positive pathogen from background. It's documented that most of the reagents used for mNGS have been found to also introduce foreign DNA during the sequencing process. This phenomenon has been described as "kit-ome", which will seriously confound the sample result [8]. Therefore, it is important to capture the nucleic acid background of the environment and reagents used for sequencing, which can help filter out contaminated background readings during the interpretation of the results. In this study, a template-free control (NTC) was used in the mNGS analysis to set the baseline for making positive calls. We used the tentative cut-off criteria fold change (microbe $\%_{SAMPLE}$: microbe $\%_{NTC}$) ≥ 5 and microbial RPM ≥ 5 for potential pathogen identification to achieve sensitivity as well as remove potential false positive from the background as much as possible. However, the current criteria may miss some potential positive. For expample, in sample 33 of which the urine culture is positive for *Aerococcus urinae*, this species showed significant fold change of microbe $\%_{SAMPLE}$: microbe $\%_{NTC}$) but microbial RPM of 3.18 didn't pass this species as positive. Further refinement of such criteria need to be tested in a much larger sample size. On the other hand, in some of the samples especially those processed at the same time, there were some identified positives may contributed by the process background such as sample 3 and 4, sample 7 and 8, sample 13 and 15. Further refinement of this would be 1) to apply NTC to every batch of sample processing and sequencing; 2) to build a database of all NTCs of the same batch of reagents.

Clinical accuracy and diagnostic yield

Karius offers a commercial cfDNA based mNGS laboratory developed test (LDT) for sepsis diagnosis. The test received CLIA and New York State approvals, with a claimed sensitivity of 92.9% and a claimed specificity of 62.7%. Working on pathogen detection in cerebrospinal fluid (CSF) by mNGS, Miller et al [16] reported a claimed sensitivity of 73% and a claimed specificity of 99%. In some study [17], sensitivity and specificity were not explicitly stated. In this study, a sensitivity of 81.8% was reported.

Karius reported 2.7x more causal pathogens than initial blood culture and 1.3x more causal pathogens than all microbiology tests. Cheng and Yu [4] reported in their study that 77 (86%) were positive for 1 or more pathogens using NGS, compared to 50 (56%) which were positive using traditional detection methods. In this study, the diagnostic yield by mNGS with host depletion by filtration (90%) was significantly higher than clinical microbial culture (56%). Hence, mNGS was able to provide more positive results than conventional pathogen detection.

A previous study showed that the rate of positive mNGS results was constant over the different time points after sepsis developed, while the positivity of blood culture decreased at later time points [10]. In another study, cell-free DNA sequencing could still identify fungus such as *P. jirovecii* or *Aspergillus* species among patients receiving effective antifungal agents [18]. In this study, the second blood culture done after antibiotic treatment had significantly lower sensitivity compared to mNGS, and there were several cases where an organism is detected consistent with BC1 but was negative by BC2. Hence, our findings were consistent with other studies in that mNGS possessed greater sensitivity for those who had blood sampled prior to effective antimicrobial agent exposure, suggesting mNGS could be a more effective detection method for patients undergoing antibiotic treatment.

Future prospects and concluding remarks

The target independent identification of potential pathogen by mNGS has made it a very promising tools for clinical microbiology comparing to other molecular tests. It can detect different microorganisms simultaneously, for example, *Escherichia coli* and *Candida albicans* in sample 43. It can detect microorganisms unculturable by routine culture method, for example, Torque teno virus in sample 41. In conclusion, mNGS was able to [19] identify majority of the organisms detected by microbial culture and showed a good correlation with routine clinical results. In addition, it provided more results, potentially able to assuage the short-coming of poor diagnostic yield of conventional culture. However, the demonstration of clinical utility is required for such test to be applied for clinical diagnostics. Stanford Healthcare set out to determine the real-world clinical utility of the Karius test in a subset of immunocompromised patients [20], with the conclusion that, in 48 cases, positive impact was observed in 6 (7.3%) cases, negative impact observed in 3 (3.7%) cases, and no impact in 71 (86.6%) cases. More studies need to be performed to assess clinical utility in different group of patients.

The other important questions to consider are whether mNGS has the diagnostic accuracy required to complement currently available diagnostics, and how best to integrate mNGS into current testing algorithms. One possibility is to reserve mNGS as last resort for cases for which conventional microbiological testing has failed to provide an answer, or to include mNGS more proximally in testing in parallel to conventional tests. These are currently an active subject of discussion in the microbiology field that needs to be determined empirically.

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Table 1: Summary of mNGS results correlated with clinical microbial culture and reference microbial culture results.

No.	BC Results	BC1	BC2	mNGS With filter		mNGS Without filter	
				BC Positive	Others	BC Positive	Others
BC1 and BC2 positive (11 samples)							
5	Enterococcus faecalis	+	+	Enterococcus faecalis		Enterococcus faecalis	
9	E. coli	+	+	NEGATIVE	6	NEGATIVE	1
10	Proteus mirabilis	+	+	Proteus mirabilis		Proteus mirabilis	
11	Proteus mirabilis	+	+	Proteus mirabilis	2	Proteus mirabilis	3
14	Klebsiella pneumoniae	+	+	Klebsiella pneumoniae	2	Klebsiella pneumoniae	
17	MRSA	+	+	NEGATIVE		NEGATIVE	
42	Escherichia coli	+	+	Escherichia coli	1	NEGATIVE	
43	Escherichia coli & Candida albicans	+	+	Escherichia coli & Candida albicans	2	NEGATIVE	
45	Klebsiella pneumoniae	+	+	Klebsiella pneumoniae	3	Klebsiella pneumoniae	
46	Escherichia coli	+	+	Escherichia coli	5	Escherichia coli	3
49	Escherichia coli	+	+	Escherichia coli	1	Escherichia coli	2
	Sensitivity (%) (Based on BC2)			81.8 (9/11)		63.6 (7/11)	
BC1 positive / BC2 negative (12 samples)							
1	Staphylococcus aureus (MRSA)	+	-	NEGATIVE	9	NEGATIVE	2
6	Proteus mirabilis	+	-	Proteus mirabilis	14	NEGATIVE	
12	Klebsiella pneumoniae	+	-	Klebsiella pneumoniae	3	Klebsiella pneumoniae	3
18	Proteus mirabilis	+	-	Proteus mirabilis		NEGATIVE	
19	Pseudomonas aeruginosa	+	-	Pseudomonas aeruginosa	1	NEGATIVE	
21	Escherichia coli	+	-	NEGATIVE	2	NEGATIVE	
22	Klebsiella pneumoniae	+	-	NEGATIVE		NEGATIVE	
23	Achromobacter species	+	-	Achromobacter insolitus	11	Achromobacter insolitus	6
29	Pseudomonas aeruginosa	+	-	Pseudomonas aeruginosa	1	Pseudomonas aeruginosa	1
30	Escherichia coli	+	-	Escherichia coli	21	NEGATIVE	
36	Escherichia coli	+	-	NEGATIVE		NEGATIVE	
44	Klebsiella pneumoniae	+	-	Klebsiella pneumoniae	4	Klebsiella pneumoniae	1
	Sensitivity (%) (Based on BC1)		39.1	73.9 (17/23)		47.8 (11/23)	
BC1 and BC2 negative (22 samples)							
	Detailed listing in Table 2						
Unsuccessful library prep (5 samples)							
25	negative	-	-	Library prep failure		Library prep failure	
31	Proteus mirabilis; Clostridium perfringens	+	-	Library prep failure		Library prep failure	
37	negative	-	-	Library prep failure		Library prep failure	
27	negative	-	-		10	Library prep failure	
38	Escherichia coli	+	-	Escherichia coli	4	Library prep failure	

Table 2: Potential pathogens identified by mNGS in the 22 blood culture negative samples.

No.	# Potential Pathogen Identified		Clinical diagnosis	Other culture results	Correlation with mNGS
	mNGS W/ filter	mNGS W/O filter			
2	1		*Clostridium difficile*	stool: *Clostridium difficile*	
3	8	2	*Pseudomonas aeruginosa*	urine: *Pseudomonas aeruginosa*	
4	7	3	*Escherichia coli*	urine: *Escherichia coli*	
7	8	1	*Escherichia coli*	urine: *Escherichia coli*	
8	9	11	undetermined	urine: Gram positive cocci in chain	*Streptococcus suis*
13	2	1	*Escherichia coli*	urine: *Escherichia coli*	
15	2	1	*Klebsiella pneumoniae*	blood (in other hospital): *Klebsiella pneumoniae*	
16	9		*Escherichia coli*	urine: *Escherichia coli*	
20			*Clostridium difficile*	PCR (+): *Clostridium difficile*	
24	1	2	undetermined	negative for urine and sputum	
26	3	2	undetermined	negative for urine and sputum	
28	2	1	*Klebsiella pneumoniae*	abscess: *Klebsiella pneumoniae*	*Klebsiella pneumoniae*
32	1		*Escherichia coli*	urine: *Escherichia coli*	
33			undetermined	urine: 1. *Aerococcus urinae*; 2. Gram negative bacilli	
34			undetermined	urine: *Citrobacter freundii*; sputum: *Acinetobacter baumannii* (CRAB)	
35			undetermined	negative for urine and sputum	Negative for mNGS
39	1		*Candida*	urine: Yeast	
40			*Escherichia coli*	urine: *Escherichia coli*	
41	1		undetermined	negative for urine and sputum	Torque teno virus
47	9	4	*Candida*	endo aspirate: *Candida krusei* and *Candida glabrata*	
48	1	2	*Klebsiella pneumoniae*	abscess: *Klebsiella pneumoniae*	*Klebsiella pneumoniae*
50	1	1	*Streptococcus agalactiae*	urine: *Streptococcus agalactiae*	*Streptococcus agalactiae*

Table 3: Comparison between genomic DNA-based mNGS and cell-free DNA-based mNGS in selected samples.

No.	BC Results	Filter	Plasma cfDNA mNGS (Reads/Million QC Reads)						Pellet gDNA mNGS (Reads/Million QC Reads)						
			QC reads	A_halo	I_halo	Human	Microbial	Target	QC reads	A_halo	I_halo	Human	Microbial	Unclassified	Target
9	*Escherichia coli*	+	17,271,416	0	1	678,789	33	10	26,129,510	5,497	4,671	695,456	13,515	280,860	106
		-	19,409,969	1	1	773,167	28	9	24,721,756	520	902	851,295	189	147,094	3
11	*Proteus mirabilis*	+	18,717,399	2	5	891,739	284	0	38,070,697	5,376	7,883	768,171	1,645	216,924	159
		-	17,581,154	6	14	895,122	501	0	49,385,166	1,556	3,195	1,595,623	871	396,393	41
14	*Klebsiella pneumoniae*	+	17,965,891	0	0	874,166	121	102	48,456,495	281	355	555,658	301	443,406	161
		-	14,634,038	0	0	706,821	60	45	27,302,004	2	1	894,517	48	209,804	25
17	MRSA	+	10,598,680	0	0	50,970	49	0	36,865,299	284	312	596,557	65	402,782	1
		-	15,827,810	0	0	931,437	12	1	35,954,996	66	9	1,137,207	28	317,077	1
19	*Pseudomonas aeruginosa*	+	20,592,770	0	0	54,732	109	2	26,843,560	441	488	781,736	42	217,293	13
		-	20,332,529	0	1	154,006	201	4	27,897,183	34	25	841,496	20	158,426	2
23	*Achromobacter species*	+	17,092,585	2	4	776,858	99	79	30,817,981	22,533	15,655	577,856	3,257	380,699	325
		-	14,707,563	0	0	55,473	65	2	36,357,904	2,380	2,515	755,295	697	239,112	229
30	*Escherichia coli*	+	19,799,176	0	0	30,474	89	7	27,963,103	754	1,377	889,600	1,453	106,816	93
		-	20,271,797	1	3	378,187	342	8	26,037,075	29	60	822,646	28	177,236	1
43	*Escherichia coli*	+	12,236,113	0	0	80,867	43	3	28,916,267	1,467	2,810	705,206	453	290,064	28
		-	12,250,956	0	0	8,910	36	3	33,734,655	367	682	1,102,967	135	260,423	5
49	*Escherichia coli*	+	12,066,105	0	0	34,571	26	2	23,940,664	913	1,248	795,679	499	201,662	25
		-	14,780,596	0	2	831,639	34	13	24,586,630	350	697	783,625	505	209,357	39

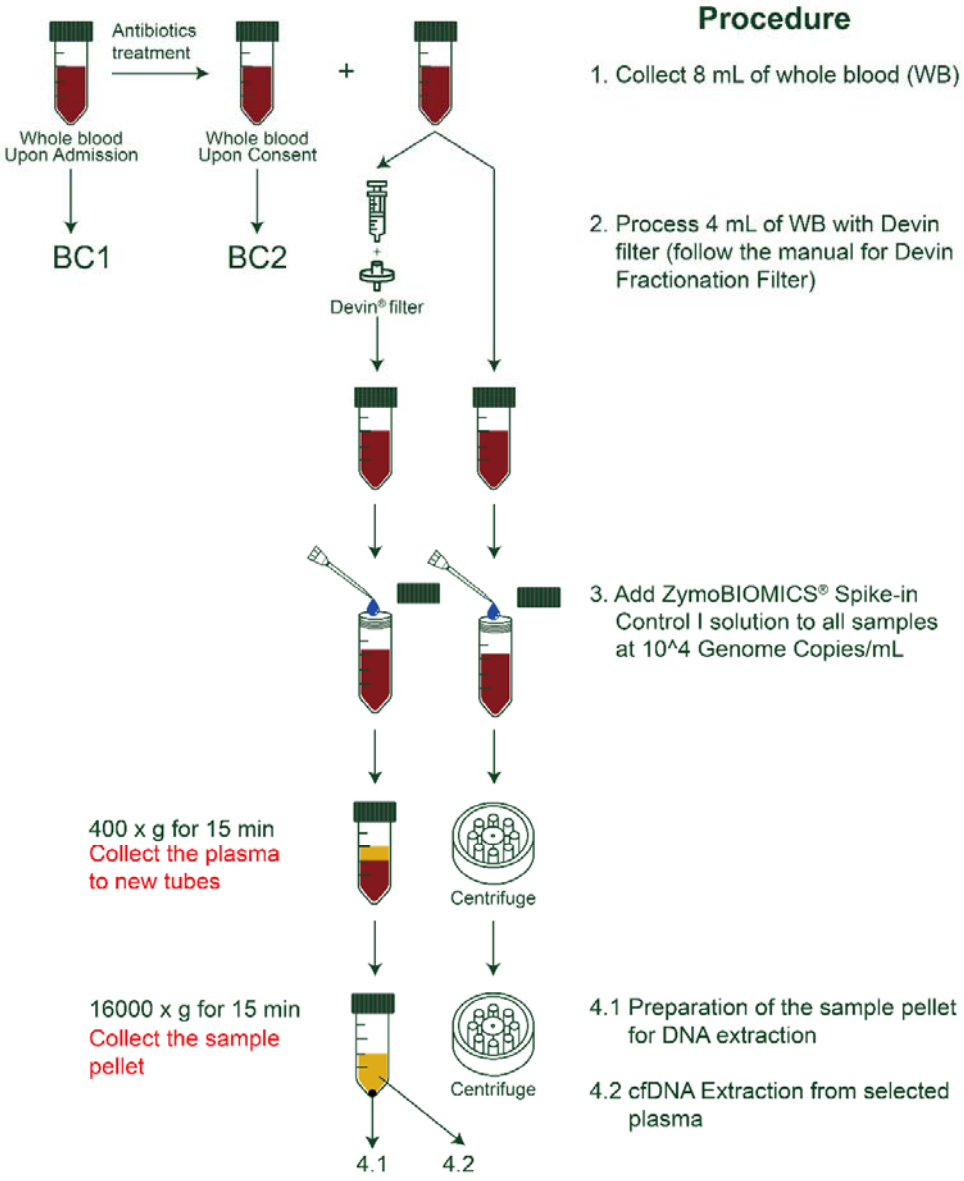
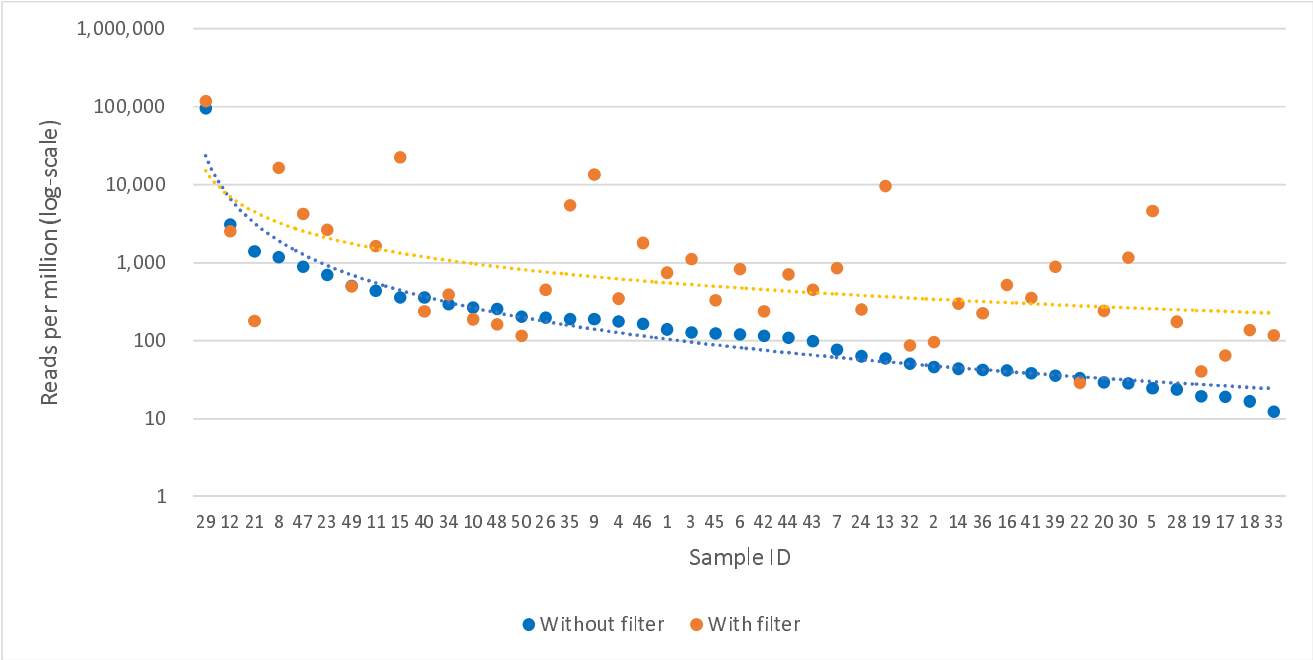


Fig. 1. Experimental plan for this study.

(a)



(b)

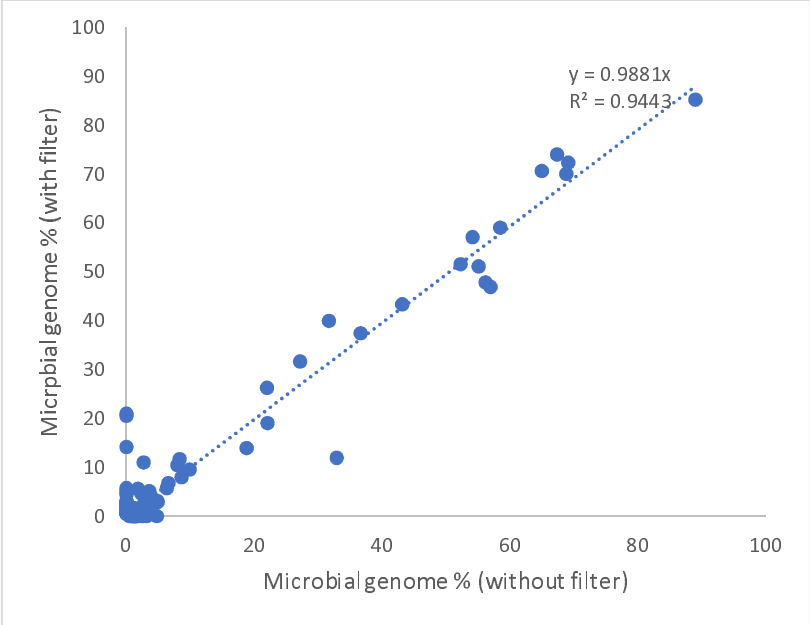


Fig. 2. Comparisons of mNGS results from "With Filter" and "Without Filter" samples. (a) Comparison of sample reads (in Reads per Million) with and without filtration, arranged in order of decreasing reads for "Without Filter" samples. Y-axis (Sample output in RPM) is in log scale. (b) Plot of microbial genome % from "Without Filter" samples vs "With Filter" samples.

Supplemental Table 1. Comparison of target reads from analyses based on 100bp, 120bp and 150bp read lengths.

			mNGS w/ filter (Reads/Million QC Reads)							mNGS w/o filter (Reads/Million QC Reads)						
No.	BC Results (BC1+BC2+)	Length	QC reads	A_halo	I_halo	Human	Microbial	Unclassified	Target	QC reads	A_halo	I_halo	Human	Microbial	Unclassified	Target
5	Enterococcus faecalis	150bp	29,415,977	53,454	11,599	472,560	884	461,503	762	33,552,849	10,817	3,886	769,101	128	216,069	82
		120bp	29,608,326	53,159	11,538	468,783	873	465,647	754	33,917,148	10,755	3,865	762,573	123	222,684	81
		100bp	30,624,819	51,268	11,119	448,627	835	488,151	720	33,650,802	10,820	3,885	747,721	121	237,454	81
9	Escherichia coli	150bp	25,939,257	5,614	4,732	717,892	16,798	254,963	116	24,539,226	525	910	880,994	240	117,331	3
		120bp	26,055,023	5,555	4,708	711,711	15,364	262,661	115	24,648,590	523	907	875,436	216	122,919	3
		100bp	26,129,510	5,497	4,671	695,456	13,515	280,860	106	24,721,756	520	902	851,295	189	147,094	3
10	Proteus mirabilis	150bp	37,795,374	273	421	500,427	202	498,676	77	21,311,225	34	47	746,414	258	121,703	105
		120bp	37,990,451	272	420	496,703	195	502,410	77	21,406,413	34	47	740,732	246	127,406	104
		100bp	38,146,610	270	416	488,233	189	510,892	76	21,471,857	34	47	719,489	232	148,740	104
11	Proteus mirabilis	150bp	37,801,114	5,446	7,981	798,519	2,076	185,978	163	49,097,239	1,571	3,225	1,655,603	1,095	339,272	41
		120bp	37,964,488	5,418	7,949	790,765	1,880	193,988	162	49,273,381	1,564	3,213	1,641,589	994	351,674	41
		100bp	38,070,697	5,376	7,883	768,171	1,645	216,924	159	49,385,166	1,556	3,195	1,595,623	871	396,393	41
14	Klebsiella pneumoniae	150bp	47,771,606	286	362	580,520	312	418,520	168	27,124,936	2	1	925,780	50	179,538	26
		120bp	48,173,290	284	359	572,212	306	426,839	164	27,232,493	2	1	918,524	47	186,256	26
		100bp	48,456,495	281	355	555,658	301	443,406	161	27,302,004	2	1	894,517	48	209,804	25
17	MRSA	150bp	36,397,462	289	317	618,042	76	381,275	1	35,652,507	66	9	1,175,811	26	276,966	1
		120bp	36,671,574	287	315	611,499	70	387,829	1	35,835,593	66	9	1,168,009	26	285,749	1
		100bp	36,865,299	284	312	596,557	65	402,782	1	35,954,996	66	9	1,137,207	28	317,077	1
42	Escherichia coli	150bp	23,900,937	134	29	874,048	308	125,480	7	27,046,543	848	997	960,831	150	139,349	5
		120bp	24,088,163	133	29	865,630	280	133,927	6	27,046,543	845	992	956,568	149	138,731	5
		100bp	24,212,385	132	29	839,711	240	159,888	6	27,332,019	842	988	929,766	128	173,861	5
43	Escherichia coli & Candida albicans	150bp	28,522,975	1,491	2,860	738,941	559	256,148	30	33,366,587	370	690	1,144,729	153	213,782	5
		120bp	28,761,517	1,481	2,839	729,079	514	266,088	29	33,366,587	369	687	1,139,650	152	212,834	5
		100bp	28,916,267	1,467	2,810	705,206	453	290,064	28	33,734,655	367	682	1,102,967	135	260,423	5
45	Klebsiella pneumoniae	150bp	18,920,723	144	19	748,746	355	250,737	247	27,717,908	71	145	959,664	140	169,515	108
		120bp	19,058,784	143	19	742,607	344	256,887	245	27,879,711	71	145	953,435	138	177,299	107
		100bp	19,151,162	142	19	723,721	332	275,785	242	27,980,656	70	143	927,259	141	204,210	106
46	Escherichia coli	150bp	33,714,921	3,121	1,260	729,035	1,872	264,711	1,246	21,397,594	29	17	750,749	144	121,037	100
		120bp	33,998,045	3,101	1,252	720,682	1,841	273,124	1,210	21,560,856	28	17	747,034	144	127,506	94
		100bp	34,189,349	3,073	1,240	697,513	1,798	296,376	1,149	21,665,310	28	17	728,305	144	147,872	92
49	Escherichia coli	150bp	23,784,973	926	1,265	828,916	657	168,236	27	24,406,342	356	707	830,553	623	162,346	42
		120bp	23,880,506	920	1,259	820,887	583	176,352	26	24,516,514	353	703	818,939	569	174,077	40
		100bp	23,940,664	913	1,248	795,679	499	201,662	25	24,586,630	350	697	783,625	505	209,357	39

Supplemental Table 2. Sample reads from all samples.

No.	BC1 Results	BC1	BC2	mNGS w/ filter (Reads/Million QC Reads)						mNGS w/o filter (Reads/Million QC Reads)					
				QC reads	A_halo	I_halo	Human	Microbial	Unclassified	QC reads	A_halo	I_halo	Human	Microbial	Unclassified
1	Staphylococcus aureus (MRSA)	+	-	26,874,647	284,561	272,359	258,754	746	183,579	43,224,176	30,098	42,845	739,796	140	187,120
2	negative	-	-	26,186,906	11,233	10,953	691,594	96	286,124	28,118,220	4,705	3,987	757,768	46	233,494
3	negative	-	-	27,738,717	215,429	273,669	308,884	1,112	200,906	23,447,427	41,580	53,692	719,329	129	185,270
4	negative	-	-	28,547,723	103,619	224,762	343,899	348	327,372	29,211,386	21,550	49,031	597,688	177	331,554
5	Proteus mirabilis	+	-	27,883,257	247,432	294,721	312,346	4,639	140,862	32,394,909	1,018	759	837,179	25	161,019
6	Enterococcus faecalis	+	+	30,624,819	51,268	11,119	448,627	835	488,151	33,650,802	10,820	3,885	747,721	121	237,454
7	negative	-	-	23,902,590	2,463	2,324	930,937	852	63,425	33,735,056	109	120	852,806	77	146,888
8	negative	-	-	4,584,817	9,610	17,023	877,372	16,560	79,435	26,743,891	313	313	901,470	1,178	96,726
9	E. coli	+	+	26,129,510	5,497	4,671	695,456	13,515	280,860	24,721,756	520	902	851,295	189	147,094
10	Proteus mirabilis	+	+	38,146,610	270	416	488,233	189	510,892	21,471,857	39	54	828,388	267	171,252
11	Proteus mirabilis	+	+	38,070,697	5,376	7,883	768,171	1,645	216,924	49,385,166	779	1,600	798,754	436	198,431
12	Klebsiella pneumoniae	+	-	38,659,564	63	76	619,828	2,529	377,504	34,975,786	139	282	687,604	3,111	308,864
13	negative	-	-	25,353,467	8,470	8,386	763,949	9,690	209,505	25,368,316	53	44	847,812	60	152,031
14	Klebsiella pneumoniae	+	+	48,456,495	281	355	555,658	301	443,406	27,302,004	2	0	809,979	44	189,975
15	negative	-	-	25,631,842	13,736	14,578	683,575	22,436	265,675	37,609,174	632	648	824,551	360	173,810
16	negative	-	-	31,733,417	2,568	2,046	721,508	520	273,358	39,535,385	157	154	821,625	42	178,022
17	MRSA	+	+	36,865,299	284	312	596,557	65	402,782	35,954,996	46	6	781,915	19	218,014
18	Proteus mirabilis	+	-	28,052,179	824	1,198	702,840	137	295,000	27,209,478	16	22	847,032	17	152,913
19	Pseudomonas aeruginosa	+	-	27,246,168	436	482	754,048	40	244,994	27,897,183	34	25	841,496	20	158,426
20	negative	-	-	29,232,228	452	826	539,168	243	459,310	26,756,735	141	146	855,335	29	144,348
21	Escherichia coli	+	-	20,165,261	494	725	574,594	180	424,008	22,092,098	166	435	740,207	1,407	257,786
22	Klebsiella pneumoniae	+	-	39,491,616	248	182	859,433	29	140,108	33,406,603	90	148	870,375	33	129,353
23	Achromobacter species	+	-	31,387,305	22,129	15,356	556,667	2,641	403,207	36,357,904	2,380	2,515	755,295	697	239,112
24	negative	-	-	35,345,932	469	488	810,182	251	188,609	55,344,131	113	80	874,802	63	124,942
25	negative	-	-												
26	negative	-	-	34,689,495	193	307	770,246	449	228,805	15,497,865	61	55	141,362	199	858,324
27	negative	-	-	28,608,412	23,675	20,143	748,024	8,028	200,130						
28	negative	-	-	27,280,309	791	1,072	786,959	176	211,002	30,130,081	16	14	845,505	24	154,441
29	Pseudomonas aeruginosa	+	-	28,857,811	73,190	48,709	559,774	117,810	200,517	29,346,400	18,548	25,655	744,089	95,734	115,974
30	Escherichia coli	+	-	28,158,790	745	1,362	855,347	1,166	141,380	26,037,075	29	60	822,646	28	177,236
31	Proteus mirabilis; Clostridium perfringens	+	-												
32	negative	-	-	29,656,175	60	48	676,036	87	323,769	29,328,723	116	100	863,037	51	136,696
33	negative	-	-	32,398,145	49	2	826,248	118	173,582	29,586,937	9	2	855,433	12	144,544
34	negative	-	-	29,975,987	2,338	1,747	777,967	390	217,557	19,380,173	235	200	854,605	294	144,666
35	negative	-	-	30,750,148	23,912	18,841	690,882	5,450	260,914	33,075,784	530	376	860,051	190	138,853
36	Escherichia coli	+	-	32,270,957	694	716	783,895	225	214,470	21,735,684	52	37	873,070	42	126,799
37	negative	-	-												
38	Escherichia coli	+	-	19,794,833	373	537	649,344	1,740	348,006						
39	negative	-	-	25,584,865	761	669	843,909	887	153,774	26,214,472	58	45	858,255	36	141,606
40	negative	-	-	30,626,048	1,911	1,558	669,674	240	326,617	30,511,094	858	903	729,191	358	268,691
41	negative	-	-	25,914,287	359	120	876,129	356	123,037	32,895,717	93	137	857,240	39	142,493
42	Escherichia coli	+	+	24,212,385	132	29	839,711	240	159,888	27,332,019	761	894	840,972	116	157,257
43	Escherichia coli & Candida albicans	+	+	28,916,267	1,467	2,810	705,206	453	290,064	33,734,655	269	500	808,287	99	190,846

44	Klebsiella pneumoniae	+	-	28,530,594	3,477	2,916	588,260	714	404,633	27,115,403	248	244	785,449	109	213,950
45	Klebsiella pneumoniae	+	+	19,151,162	142	19	723,721	332	275,785	27,980,656	62	126	819,262	125	180,425
46	Escherichia coli	+	+	34,189,349	3,073	1,240	697,513	1,798	296,376	21,665,310	32	19	831,051	165	168,733
47	negative	-	-	22,696,014	3,126	4,740	715,638	4,254	272,243	26,658,180	1,198	1,488	798,436	893	197,986
48	negative	-	-	30,225,187	117	176	793,952	162	205,593	25,692,243	178	275	888,051	257	111,238
49	Escherichia coli	+	+	23,940,664	913	1,248	795,679	499	201,662	24,586,630	352	701	787,931	507	210,508
50	negative	-	-	22,923,655	25	31	596,744	117	403,083	27,452,004	237	486	700,539	204	298,533

Supplemental Table 3. Complete list of potential pathogens Identified by mNGS (Fold change (% Microbialsample/% MicrobialNTC >=5 and RPM >=5)

No.	BC1	BC2	Tax	mNGS W/ filter			mNGS W/O filter		
				reads	RPM	fold change(H)	reads	RPM	fold change(H)
BC1 and BC2 positive									
5	+	+	***Enterococcus faecalis***	20660	719.5	535.19	2680	80.83	437.62
9	+	+	*Kytococcus sedentarius*	4640	179.4	6.81			
			Micrococcus luteus	74201	2868.91	11.08			
			Roseomonas mucosa	17486	676.08	22.24			
			Staphylococcus cohnii	7955	307.57	9.56			
			Staphylococcus lugdunensis	1764	68.2	6.35			
			Staphylococcus warneri				161	6.52	37.50
			Streptococcus suis	5933	229.39	5.87			
10	+	+	***Proteus mirabilis***	2915	76.47	463.58	2559	119.19	512.10
11	+	+	*Bacteroides uniformis*	559	14.88	16.11	744	15.1	62.35
			Micrococcus luteus				2085	42.32	5.10
			Proteus mirabilis	6064	161.42	110.98	1003	20.36	53.37
			Streptococcus suis	1327	35.32	7.40	374	7.59	6.06
14	+	+	*Acinetobacter johnsonii*	3515	72.59	68.67			
			Klebsiella pneumoniae	7804	161.15	74.18	630	23.08	72.79
			Klebsiella quasipneumoniae	268	5.53	13.48			
42	+	+	***Escherichia coli***	144	5.95	22.43			
43	+	+	*[Candida] haemuloni*	163	5.66	607.12			
			Candida albicans	163	5.66	4148.67			
			Escherichia coli	803	27.89	55.54			
			Escherichia fergusonii	156	5.42	55.79			
45	+	+	*Escherichia coli*	237	12.38	7.87			
			Klebsiella pneumoniae	4636	242.11	100.86	2609	93.26	103.56
			Klebsiella quasipneumoniae	138	7.21	15.89			
			Klebsiella variicola	162	8.46	48.73			
46	+	+	*Escherichia albertii*	2520	74.03	204.32	138	6.37	193.00
			Escherichia coli	39294	1154.28	135.12	2285	105.47	135.53
			Escherichia fergusonii	8592	252.39	262.34	458	21.14	241.22
			Shigella boydii	393	11.54	52.78			

			Shigella flexneri	3525	103.55	433.57	225	10.39	477.36
			Shigella sonnei	564	16.57	2096.91			
49	+	+	*Acinetobacter bereziniae*				964	39.25	20.29
			Escherichia coli	592	24.78	10.48	976	39.74	16.54
			Escherichia fergusonii				204	8.31	30.70
			Pseudomonas oryzihabitans	132	5.53	22.70			
BC1 positive / BC2 negative									
1	+	-	*Brucella abortus*	170	14.28	5.77			
			Brucella melitensis	166	13.94	5.40			
			Klebsiella michiganensis	145	12.18	9.30			
			Klebsiella oxytoca	379	31.83	8.29	206	5.14	14.92
			Pseudomonas mendocina	112	9.41	5.90			
			Streptococcus infantarius	118	9.91	34.38			
			Streptococcus macedonicus	155	13.02	30.40			
			Streptococcus suis	1269	106.57	22.10	626	15.62	36.08
			Streptococcus thermophilus	146	12.26	20.11			
6	+	-	*Corynebacterium accolens*	6170	483.3	25.07			
			Corynebacterium amycolatum	18317	1434.8	952.84			
			Corynebacterium aurimucosum	3870	303.14	45.80			
			Corynebacterium diphtheriae	2593	203.11	103.20			
			Corynebacterium jeikeium	27278	2136.72	438.80			
			Corynebacterium minutissimum	1824	142.88	63.70			
			Corynebacterium simulans	2683	210.16	35.53			
			Corynebacterium striatum	4599	360.25	118.42			
			Corynebacterium urealyticum	2425	189.95	238.46			
			Dermabacter hominis	7404	579.97	984.83			
			Facklamia hominis	3222	252.38	1607.16			
			Finegoldia magna	2473	193.71	17.20			
			Proteus mirabilis	2937	230.06	26.03			
			Staphylococcus haemolyticus	1791	140.29	5.43			
			Staphylococcus hominis	2149	168.33	12.65			
12	+	-	*Klebsiella aerogenes*	868	22.46	18.87	937	26.8	18.30
			Klebsiella pneumoniae	84638	2189.62	119.93	92709	2651.78	118.01
			Klebsiella quasipneumoniae	3628	93.86	27.21	3798	108.64	25.59

			Klebsiella variicola	2916	75.44	57.13	3207	91.73	56.44
18	+	-	***Proteus mirabilis***	257	9.18	76.73			
19	+	-	***Pseudomonas aeruginosa***	352	12.93	133.71			
21	+	-	*Paracoccus sanguinis*	143	7.1	6.60			
			Roseomonas mucosa	133	6.6	16.47			
23	+	-	***Achromobacter insolitus***	9901	327.73	208.63	8337	230.43	574.30
			Cutibacterium namnetense				329	9.09	15.66
			Klebsiella michiganensis	760	25.16	25.97			
			Klebsiella oxytoca	1885	62.39	21.55	363	10.03	13.57
			Pseudomonas mendocina	541	17.91	8.29			
			Pseudomonas pseudoalcaligenes	886	29.33	8.45	220	6.08	6.86
			Streptococcus infantarius	686	22.71	65.16			
			Streptococcus macedonicus	906	29.99	61.57			
			Streptococcus suis	7796	258.05	49.17	1515	41.87	31.24
			Streptococcus thermophilus	1038	34.36	74.98	204	5.64	48.17
			Vibrio fluvialis	8116	268.65	5.11			
			Vibrio furnissii	447	14.8	5.12			
29	+	-	***Pseudomonas aeruginosa***	3200435	126299.38	662.24	2759959	98397.1	691.09
30	+	-	*Brevundimonas diminuta*	444	15.8	11.29			
			Brevundimonas vesicularis	339	12.06	9.31			
			Corynebacterium accolens	264	9.4	357.29			
			Corynebacterium afermentans	363	12.92	356.55			
			Corynebacterium aurimucosum	1128	40.14	4906.86			
			Corynebacterium freneyi	850	30.25	976.68			
			Corynebacterium minutissimum	610	21.71	12382.67			
			Corynebacterium riegelii	806	28.68	10.00			
			Corynebacterium simulans	165	5.87	1004.80			
			Corynebacterium urealyticum	7136	253.96	14485.93			
			Corynebacterium ureicelerivorans	298	10.61	255.61			
			Cutibacterium namnetense	488	17.37	17.93			
			Escherichia albertii	143	5.09	32.98			
			Escherichia coli	2424	86.26	66.86			
			Escherichia fergusonii	417	14.84	59.48			
			Hafnia paralvei	340	12.1	6902.00			

			Janibacter indicus	158	5.62	16.73			
			Micrococcus luteus	213	7.58	11.87			
			Oligella urethralis	539	19.18	10.00			
			Shigella flexneri	232	8.26	1766.00			
			Stenotrophomonas maltophilia	505	17.97	5.18			
			Yersinia enterocolitica	196	6.98	39.65			
38	+	-	*Escherichia albertii*	351	17.75	77.20			
			Escherichia coli	18065	913.44	475.05			
			Escherichia fergusonii	1920	97.08	261.08			
			Shigella boydii	254	12.84	819.33			
			Shigella flexneri	613	31	4449.00			
44	+	-	*[Candida] haemuloni*	163	5.75	390.49			
			Escherichia coli	488	17.21	21.71	137	5.05	41.94
			Klebsiella pneumoniae	4241	149.6	85.54	855	31.55	118.66
			Klebsiella quasipneumoniae	189	6.67	17.32			
			Klebsiella variicola	152	5.36	25.57			
BC1 and BC2 negative									
2	-	-	*Streptococcus suis*	270	10.54	37.41			
3	-	-	*Agrobacterium tumefaciens*	570	40.22	5.91			
			Brucella abortus	858	60.54	18.94			
			Brucella canis	510	35.99	17.37			
			Brucella melitensis	838	59.13	17.73			
			Brucella suis	864	60.97	16.15			
			Ochrobactrum anthropi	3829	270.19	17.53	259	12.21	12.12
			Ochrobactrum intermedium	3214	226.79	16.73	241	11.36	12.82
			Rhizobium pusense	441	31.12	5.21			
4	-	-	*Brevundimonas diminuta*	176	9.18	9.03			
			Brucella abortus	210	10.95	14.41			
			Brucella canis	118	6.15	12.49			
			Brucella melitensis	196	10.22	12.89			
			Brucella suis	217	11.32	12.61	141	5.19	15.72
			Ochrobactrum anthropi	998	52.05	14.20	539	19.85	14.71
			Ochrobactrum intermedium	790	41.2	12.78	446	16.43	13.85
7	-	-	*Klebsiella michiganensis*	251	10.55	15.86			

			Klebsiella oxytoca	574	24.13	12.37			
			Pseudomonas mendocina	177	7.44	9.19			
			Pseudomonas pseudoalcaligenes	312	13.12	6.85			
			Streptococcus infantarius	176	7.4	50.52			
			Streptococcus macedonicus	184	7.73	35.55			
			Streptococcus suis	2093	87.98	35.92	200	5.93	26.79
			Streptococcus thermophilus	200	8.41	27.14			
8	-	-	*Acinetobacter johnsonii*	2491	558.18	9.34	841	31.47	7.60
			Aeromonas caviae				154	5.76	5.11
			Janibacter indicus				568	21.25	9.30
			Klebsiella michiganensis	656	147	11.12	366	13.69	14.94
			Klebsiella oxytoca	1525	341.72	8.81	859	32.14	11.96
			Pseudomonas mendocina	557	124.81	7.75	292	10.93	9.79
			Pseudomonas pseudoalcaligenes	921	206.38	5.42	465	17.4	6.60
			Streptococcus infantarius	512	114.73	39.41	253	9.47	46.92
			Streptococcus macedonicus	622	139.38	32.22	323	12.09	40.32
			Streptococcus suis	8084	1811.46	37.19	3112	116.44	34.50
			Streptococcus thermophilus	754	168.96	27.43	409	15.3	35.86
13	-	-	*Acinetobacter johnsonii*	179947	7219.22	208.54	762	30.04	143.67
			Acinetobacter ursingii	3148	126.29	12.40			
15	-	-	*Acinetobacter johnsonii*	415001	16662.62	205.45	8943	238.09	188.27
			Acinetobacter ursingii	7351	295.15	12.37			
16	-	-	*Bacteroides stercoris*	198	6.27	17.58			
			Bacteroides uniformis	196	6.21	21.44			
			Bacteroides vulgatus	529	16.75	48.69			
			Corynebacterium simulans	3323	105.2	344.76			
			Corynebacterium striatum	231	7.31	46.60			
			Enterococcus faecalis	593	18.77	23.81			
			Finegoldia magna	332	10.51	18.09			
			Janibacter indicus	215	6.81	6.72			
			Staphylococcus haemolyticus	910	28.81	21.62			
24	-	-	*Streptococcus suis*	502	14.22	29.53	354	6.4	52.70
			Vibrio fluvialis				469	8.48	6.97
26	-	-	*Cutibacterium granulosum*	278	8.02	140.60			

			Cutibacterium namnetense	7449	214.84	577.49	1728	111.51	677.83
			Streptococcus suis	495	14.28	16.62	82	5.29	13.93
27	-	-	*Escherichia coli*	1760	64.34	6.94			
			Klebsiella michiganensis	1786	65.29	22.03			
			Klebsiella oxytoca	4076	149	16.82			
			Pseudomonas aeruginosa	2870	104.92	8.79			
			Pseudomonas mendocina	1094	39.99	6.05			
			Pseudomonas pseudoalcaligenes	1864	68.14	6.42			
			Streptococcus infantarius	1443	52.75	49.47			
			Streptococcus macedonicus	1945	71.1	47.71			
			Streptococcus suis	17574	642.45	40.01			
			Streptococcus thermophilus	2385	87.19	62.18			
28	-	-	*Klebsiella pneumoniae*	1846	67.79	157.91	221	7.34	126.23
			Stenotrophomonas maltophilia	147	5.4	10.31			
32	-	-	*Klebsiella pneumoniae*	300	10.12	47.57			
39	-	-	*Cutibacterium namnetense*	274	10.72	14.57			
41	-	-	*Torque teno virus*	1393	53.78	3436.34			
47	-	-	*Corynebacterium afermentans*	5408	240.17	186.11	427	16.06	59.6179402
			Corynebacterium glucuronolyticum	617	27.4	28.26			
			Corynebacterium riegelii	1194	53.03	32.99			
			Corynebacterium striatum	735	32.64	25.36			
			Corynebacterium timonense	1487	66.04	23.36			
			Corynebacterium ureicelerivorans	10607	471.06	185.36	639	24.03	45.3040911
			Dermabacter hominis	620	27.53	110.49			
			Janibacter indicus	1516	67.33	8.10			
			Lactobacillus crispatus	474	21.05	15.64	191	7.18	25.57
			Mycobacterium chelonae				171	6.43	8.38
48	-	-	*Acinetobacter radioresistens*				269	10.47	20.31
			Klebsiella pneumoniae	2374	78.57	67.25	2678	104.28	56.09
50	-	-	*Streptococcus agalactiae*	1253	54.66	9153.42	3192	116.36	11108.06