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Application of next generation sequencing technology on contamination monitoring in microbiology laboratory

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1. Introduction

The prevention and control of contamination caused by cultured viruses, bacteria and other infectious materials are the most important part in biosafety management in the laboratory [1–3]. Microbiological contaminations in laboratories have been frequently reported in instruments [4], rooms [5,6], and the operators [7,8]. These contaminations are mainly from the performance of experiments, for example, bacteria/virus isolation and culture, gene amplification, and clinical samples preparation. As such, it is important to establish an effective and unbiased method to evaluate and monitor the risk of lab contaminations.

The potential contamination in the environment of a microbiology lab is very complicated, as usually the source and background of contaminates is unknown. However, it is hard to conduct effective contamination surveillance in a common laboratory due to the methodology limitation of contaminant detections. The isolation of microbial species is difficult because of its tedious work and poor sensitivity. Molecular tests such as PCR is sensitive, however it can only detect the gene of known pathogens. It is desired to develop a comprehensive technology which can detect the potential contaminates of unknown resources, characterized by culture-independency and high sensitivity.

The development of next-generation sequencing (NGS) technology provided a culture-independent method to obtain microorganisms genome information with high sensitivity [9,10], and has been widely used to detect the pathogenic microorganisms [11–13]. In this study, we evaluated the performance of NGS as a promising tool on the surveillance and risk assessment of potential lab contaminations. Frequently used microbiology laboratories which included biosafety laboratory level I (BSL-I) and II (BSL-II) were investigated in our study. Various sites were inspected in different rooms. We aimed to establish an unbiased and effective evaluating technique involving NGS to monitor the laboratory contamination.

2. Materials and methods

2.1. Sample collection

Four rooms were investigated in the study. Room 1 is a BSL-II used for viral isolation and culture. Room 2 is a BSL-II, used for pre-treatment of respiratory samples from patients with respiratory tract infections and also used for nucleic acid extraction. Room 3 is a BSL-I, where a negative-
Scientific question
This study investigated the performance of next generation sequencing (NGS) technology in monitoring laboratory microbiological contamination.

Evidence before this study
Contamination is a big issue in microbiology laboratories, while it is hard to conduct effective contamination surveillance in a common laboratory due to the lack of proper methodology. The isolation of microbial species is difficult which needs tedious lab work and has low sensitivity. Although molecular tests such as PCR are sensitive, only limited number of known microbes could be detected simultaneously.

New findings
A holistic assessment of the biological background in the environment of a microbiology laboratory was conducted through NGS technology in this study. In general, the richness of viral and bacterial sequences is positively associated with the frequency of laboratory activities in the room and on the bench. The data indicated that viral reads were mainly from experimental activities, materials and environment. All types of bacteria seem to be derived from the external environment. The gene fragments from exogenous microorganisms have a great impact on the deep sequencing results, especially for the clinical samples with low microbial biomass. Application of the NGS technique on biosafety monitoring would enable us to disentangle the background noise presenting in the sequencing data, which in turn help to distinguish true contamination caused by microorganisms from false positives.

Significance of the study
The study demonstrated that NGS could be a promising approach in monitoring the contamination from different sources in the lab, suggesting its promising usage in monitoring and assessing the risk of potential laboratory contamination.

3. Results

3.1. Data overview
The quality of nucleic acids measured by OD 260/280 was 1.8–2.2 for all samples. Similar quantity of RNA was yielded from H1–H9 samples (88.8–127.2 ng, median of 108.80 ng) (Figure 1A), which indicated similar biomass in all sampled locations. As expected, the amount of nucleic acids from H0 was ultralow (below the detection limit) and all nucleic acids were used to construct the sequencing library. After sequencing and data filtering, a range from 3,046,082 to 6,000,548 reads from H1–H9 samples were considered for genome mapping. Alpha diversity (observed genera/species and Shannon index) calculation and principal coordinate analysis (PCoA) (based on abundance weighted Jaccard distance) were performed with Qiime [17] (v1.9.1). Mann-Whitney test was performed with GraphPad prism 7.

Table 1
| Room no. | Laboratory function                                      | Biosafety level | Biosafety cabinet | Bench | Centrifuge rotor |
|---------|-----------------------------------------------------------|-----------------|-------------------|-------|-----------------|
| 1       | Viral isolation and culture                               | II              | H1                | H2    | H3              |
| 2       | Clinical samples pretreatment and nucleic acids extraction| II              | H4                | H5    | H6              |
| 3       | Nucleic acids templates amplification                    | I               | H7*               |       |                 |
| 4       | Molecular biological and immunological experiment        | I               |                   | H8    | H9              |

* H7 is a negative-pressure hood used for nucleic acid template adding for PCR.
reads from these samples could be classified. The samples from H0 (480,074 reads) and H9 (1,354,249 reads) had dramatically low amount of reads, and only 15.76% and 23.58% of reads were successfully aligned and classified to a certain taxonomic rank (Figure 1B). The data obtained from the positive control was consistent with the real-time PCR results. The reads of known pathogen, HCoV 229E accounted to 72.4% of all microbial reads, thus verified the reliability of NGS procedure.

3.2. Detections of viral sequences

As the major activities in the laboratory involved respiratory viruses, we first analyzed the viral reads in the data. The results demonstrated that viral sequences were in a relatively low abundance (0.01 ± 0.02%), with the viral reads ranged from 64 to 733 (median of 148). The number of viral species ranged from 8 to 34 (median of 12) in different samples including H0. The distribution of virus species in each sampling site was shown in Figure 2. The samples collected from Room 4, which was used for molecular biological and immunological experiments, had the highest number of viral species (15 species). The three most abundant viruses were cercopithecine herpesvirus 5 (CeHV-5) (23% reads, observed at 7 sites), influenza A viruses (IFVA) (16%, 8 sites) and lactococcus phage 936 sensu lato (8%, 2 sites).

Due to the high sensitivity of the NGS method, viral reads that derived from routine laboratory work were identified, such as IFVA, human enterovirus 71 viruses, HCoV HKUI and NL63 (seen in Figure S1). These reads were mainly from Room 2 and Room 4. Among these, reads of IFVA accounted for the highest proportion. They were detected in 6 sites except the biosafety cabinet. However, the obtained gene sequences were all close to the primer positions for viral genes amplification (Figure 3), as PCR tests were performed on the viral positive clinical samples within a month before sampling.

The sequences of bovine diarrhea virus were only found on the biosafety cabinet in Room 1, which was used for viral isolation and culture experiments (Figure 2). The existence of bovine diarrhea viral nucleic acids in fetal bovine serum (FBS) was further confirmed by PCR methods (data not shown) [18], which may represent contamination of bovine serum used for cell culture as reported also in previous studies [18–21]. The reads of CeHV-5 were identified at all sampling sites except H2, H3 and H9. This virus was previously isolated from the tissue of rhesus macaque [22], and had been detected in the cell line obtained from the kidney of rhesus macaque [23]. There are two rhesus macaque kidney cell lines, LLC-MK2 and Vero cells, used in the lab for virus isolation during the study period. However, we did not find CeHV-5 nucleic acids in these cultured cells by PCR. There were also viral sequences aligned to human mastadenovirus, polyomavirus, hepatitis virus, human metapneumovirus and rotavirus, but their read numbers and covered lengths were very limited, thus were likely to be false positives (Figures 2 and S1).

3.3. Diversity and composition of the sequences of biological substances

The alpha diversity of genera measured by observed genera and Shannon index did not differ significantly among H1-H9 (Figures 4A,
However, when going down to the species level, H2 and H8 showed much higher diversity than other samples except for the negative control (Figures 4C, 2D). Besides H2 and H8, another sample collected from the operating bench, H5, also had considerable higher alpha diversity, indicating a generally high diversity of biological substances on the bench. It is noteworthy that H0 had high evenness of biological substances background, as shown by the high Shannon index at both the genus and species levels (Figure 4B, D), which might reflect that the contamination from reagents and/or environment is more severe for low biomass samples.

A tight clustering of H1, H2 and H3, which were all taken from the viral culture room (Room 1), was highlighted on PCoA plot that based on weighted Jaccard distance at the genus level (Figure 4E). This suggests a considerable impact of the physical distance/boundary on the spread of biological substances.

Of all the biomass sequences classified to the domain level, Bacteria is the most abundant in all samples (77.76 ± 12.53%), followed by fungi and all other Eukaryota, accounting for 5.89 ± 4.61% and 16.32 ± 12.21% of the classified reads, respectively. The amount of Archaea (0.01 ± 0.01%) and Viruses (0.01 ± 0.02%) is relatively low.

Zooming into the species level, the top 20 abundant ones include four species belonging to the phylum Actinobacteria of Bacteria, twelve species belonging to the phylum Proteobacteria, two species belonging to the phylum Chordata of Eukaryota, and two species belonging to the phylum Streptophyta of Eukaryota. For the Bacteria domain, Micrococcus luteus (M. luteus) is outstandingly abundant, accounting for 58.88 ± 31.72% of the reads classified to the species level. This result is reasonable because M. luteus distributes widely in soil, water, dust and air, and it is part of the normal flora of human skin, mouth, mucosae, oropharynx and upper respiratory tract [24]. The rest of most abundant bacteria includes both gram-positive (Actinobacteria) and gram-negative (Proteobacteria) bacteria, which originate from a diverse range of environments: Escherichia coli, Sphingomonas, Pseudomonas putida and Variovorax paradoxus are frequently observed in soil and water; Cutibacterium acnes is a human skin-associated organism; Moraxella catarrhalis and Acinetobacter baumannii are common...
Figure 4. Diversity and compositions of identified bacteria. (A–D) Alpha diversity calculated by (A) number of species, (B) Shannon index at the genus level, (C) number of species and (D) Shannon index at the species level. (E) Principal coordinate analysis (PCoA) plot based on the weighted Jaccard distance between genera. In A–E, the numbers of reads were rarefied to 200,000 and 100,000 per sample at the genus level and species level, respectively. (F) Heatmap showing the top 20 most abundant species. The abundances were normalized by the number of reads assigned to the species level for each sample. NC, negative control.
opportunistic pathogens. Ralstonia had been identified as a common contaminant of DNA extraction kits or PCR reagents [25].

For the Eukaryota domain, the abundances of human-originated reads in H1 (10.26%) and H6 (7.93%) were higher than that in other samples (below 2.15%). They were possibly derived from personnel entering the rooms for equipment maintenance at that time. Additionally, H6 had a dramatically high percentage of mouse-originated reads (12.9%) compared to that in other samples (below 0.01%). This could be associated with the isolation of mouse lymphocytes at that location. Regarding plant-originated biological substances, Gossypium hirsutum, known as upland cotton and often present in clothes, had a much higher percentage in H8 (4.89%) than in other samples (below 0.05%, Figure 4F), suggesting a greater impact of human activity on the operating bench in the BSL-1 room. The relative abundance of Populus trichocarpa, namely California poplar, was generally higher in the BSL-1 rooms with ordinary pressure system comparing with that in the BSL-2 rooms with negative pressure system (1.01 ± 1.32% vs 0.12 ± 0.27%, p = 0.17, Mann-Whitney test, Figure 4F). This indicates that the environmental contamination could be affected by the season, considering that the samples were taken in April, when poplar blossomed in Beijing.

4. Discussion

NGS technology is an effective approach for laboratory management and microbe monitoring [26]. In this study, we carried out a holistic assessment of the biological background in the environment of a microbiology laboratory. In general, the diversity of viral and bacterial sequences is positively associated with the frequency of laboratory activities in the room and on the bench.

We are more concerned about the distribution of viruses in this microbiology laboratory, where respiratory viruses-related experiments were performed regularly. Firstly, all the viral reads obtained from the environment samples were compared to the positive control. It showed the HCoV-229E reads dominant in the positive control was only detected in H1 and H4 samples with less than 10 reads. The two sampling sites were biosafety cabinet bench in Room 1 and 2, used for virus culture and clinical samples treating, indicating an occasional contamination from samples or nucleic acids. No other samples showed positive on HCoV 229E, which helped to ensure us the quality control of NGS procedure in this study. There are viral reads in the negative control (H0) mapping to CeHV-5, IFVA and flock house virus, which similar to the distribution in H7, the PCR hood used for adding nucleic acids templates, indicating a residual nucleic acids contamination. Viral reads related to the experiments performed in recent one month were also identified in these environment samples. In a summary, we considered these viral sequences were mainly from three parts. (1) Experimental activities. The obtained short viral reads are mainly mapped to the terminal gene region or the PCR primers targeting regions (see Figure 3). No complete viral genome was identified even in samples collected from viral isolation and culture sites. Moreover, the reads of human mastadenovirus, polyomavirus, enterovirus, and human betahepesvirus 5, were found only in samples collected from bench, centrifuge rotor (H8, H9) in BSL-1 and PCR hood (H7), correlated to the experiments performed in these sites. Of note, since RNA were preferentially amplified in our protocol, DNA contamination (e.g., PCR products) level should be underestimated, thus we are presenting a lower boundary of DNA contamination. The results indicated these viral reads were mainly from the gene amplification products or residual degraded nucleic acids. As the viral reads related to the viruses used in recent one month could be detected, the residual DNA fragments could help to trace experimental activities within a month. (2) Experimental materials. For example, the fetal bovine serum was confirmed to be contaminated by the bovine viral diarrhea virus [18–21]. Besides that, the reverse transcriptase which derived from murine leukemia virus [27–29], and the recombinant protein expression system which involves the use of viral vectors are potential sources of contamination [30–32]. (3) Environment. Reads of the tobacco mosaic virus and cucumber mosaic virus were found in most of the samples except negative control and the samples collected from biosafety cabinets in BSL-II (H1 and H4). As reported, the two kinds of viruses are widely distributed in water and soil [33,34]. The hepatitis C virus and rotavirus, not used in the lab, were found in samples from the centrifuge rotor (H6) and the bench in BSL-I with less than ten reads. Whether these viral reads were from environment or not need to be investigated further.

The largest part of biomass in our data is contributed by bacteria, of which M. luteus is the most prevalent. All types of bacteria seem to be derived from the external environment [24]. Room 4 has the most abundant microbial species, as frequent molecular biological experiments and immunology related experiments occurred in this room. A large number of microbial sequences in all centrifuge rotors were found, which emphasized the necessity of routine clean and disinfection for this equipment. The gene fragments from exogenous microorganisms have a great impact on the deep sequencing results, especially for the clinical samples with low microbial biomass, such as cerebrospinal fluid, blood, and bronchoalveolar lavage fluid [25,35–38]. Application of the NGS technique on biosafety monitoring would enable us to disentangle the background noise presenting in the sequencing data, which in turn help to distinguish true microorganisms from false positives. Meanwhile, laboratory contamination should be suspected when obtaining the complete genome sequence of cultured viruses, or abnormally enrichment of specific microbes.

5. Conclusions

NGS method was capable of monitoring the nucleic acids contaminations from different sources in the lab, demonstrating its promising utility in monitoring and assessing the risk of potential laboratory contamination. The risk of contamination from reagents, remnant DNA and environment should be considered in data analysis and results interpretation.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jsbseal.2019.02.003.

Acknowledgements

This study was supported by CAMS Innovation Fund for Medical Sciences (2016-ICM-1-014, 2017-ICM-3-017), the National Major Science & Technology Project for Control and Prevention of Major Infectious Diseases in China (2017ZX10103004, 2018ZX10305409, 2018ZX10301401, 2018ZX1072401), and Fondation Mérieux. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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