ORIGINAL ARTICLE e-ISSN: 2349-0659 p-ISSN; 2350-0964

Comparison of Use of Concentrated Saliva with Serial Dilution Method in Determination of Candida Colonies in Oral Samples

Nitish Bhat[1]*, Rubeena Anjum[1], Kalpna Thakur[2], Nandini Bhardwaj[3]

Abstract

Fungal infections have been commonly observed in the oral cavity with the most common being *Candida albicans* but with time, new species of candida have been observed and isolated from the oral cavity. Identification of new strains is necessary not only because of their ability to cause infection but also due to their susceptibility toward antifungal agents. The use of concentrated saliva or serial dilution method has been used for these culture tests, with each method having its own advantage and disadvantage. Hence, the aim of the study was to quantitatively analyze effectiveness of both these methods on samples from the oral cavity.

Keywords: Candida, Conc. saliva method, Oral fungal infections, Serial dilution, Susceptibility

Asian Pac. J. Health Sci., (2022); DOI: 10.21276/apjhs.2022.9.4.09

Introduction

The term Candida originates from the Latin word candid, meaning white.[1] *Candida albicans* is considered the main opportunistic pathogenic yeast for being the most frequently isolated species in humans.[2] Many candida species have been isolated but in the oral cavity, *C. albicans* remains most prevalent and accounts approximately for over 80% of all oral yeast isolates.[1] More than one candida species is estimated to occur in approximately 10% of oral samples. In recent years, the ability to detect non-albican species has become increasingly important.[3]

Candida has been isolated by various methods including many molecular methods, but the most commonly used methods are the culture tests. For culture test from the oral cavity, concentrated saliva can be used or serial dilution of the sample can be done. Most common method among these two is use of concentrated saliva as its procedure is simple but serial dilution method has been also used. Main objective of using serial dilution method is to estimate the concentration (number of colonies, organisms, bacteria, or viruses) of an unknown sample by counting the number of colonies cultured from serial dilutions of the sample, and then back track the measured counts to the unknown concentration.[4] Therefore, the aim was qualitative analysis of the two methods and to check their importance for various tests performed in the oral cavity.

Methodology

The study was done in April–May 2017. The patients were pre-informed about the procedure. Patients without any evident oral lesions were chosen for the study. Patients between the age group 20 and 40 years were included in the study. Method for collecting the sample for both the techniques was the same. Samples were collected by oral rinse method, subjects were instructed to rinse with 10 ml of saline and expectorate in the sample containers provided to them.

Further procedure was done in a laminar air flow chamber. For the concentrated rinse method, 100 µL was taken directly from the samples using a micropipette and samples were inoculated on preformed agar plates and incubated for 48 h at 37°C in the incubator.

[1]Department of Oral and Maxillofacial Pathology and Oral Microbiology, Indira Gandhi Govt. Dental College, Jammu, Jammu and Kashmir, India.

[2]Department of Oral Pathology and Microbiology, H.P. Government Dental College and Hospital, Shimla, Himachal Pradesh, India.

[3]Department of Oral Pathology and Microbiology, Himachal Institute of Dental Sciences, Paonta Sahib, Himachal Pradesh, India.

Corresponding Author: Nitish Bhat, Department of Oral and Maxillofacial Pathology and Oral Microbiology, Indira Gandhi Government Dental College, Jammu, Jammu and Kashmir, India. E-mail: nitishbhat04@gmail.com

How to cite this article: Bhat N, Anjum R, Thakur K, Bhardwaj N. Comparison of Use of Concentrated Saliva with Serial Dilution Method in Determination of Candida Colonies in Oral Samples. Asian Pac. J. Health Sci., 2022;9(4):38-42.

Source of support: Nil

Conflicts of interest: None

Received: 12/01/2022 **Revised:** 19/03/2022 **Accepted:** 15/04/2022

For serial dilution method, remaining sample was taken and centrifuged at 1700 rpm for 10 min. Now, the supernatant was discarded and sediment material was carried with pipette. The suspension was serially diluted (10-fold) with a solution of phosphate buffered saline. The samples were then inoculated on a pre-formed agar plate using spread plate method. These plates were then incubated at 37°C for 48 h in an incubator.

All isolation procedures were carried out on solid media. The agar plates used for both methods were Hi-Chrome differential agar plates. The growth appeared in 48 h as different colored, smooth, and pasty colonies. The results were then qualitatively analyzed between the two methods.

Results

After 48 h, culture plates from both the methods were observed. Both methods showed colonies of candida as in different colors, smooth, and pasty colonies. The candida growth on these culture

plates showed variety of candida colony species including *C. albicans*, *Candida glabrata*, *Candida tropicalis,* and *Candida krusie*.

The plates with concentrated saliva showed an increased number of candida colonies. The growth is extensive and overlap of the colonies is seen on these culture plates. Mostly, *C. albicans* colonies are seen followed by *C. tropicalis*, *C. glabrata*, and *C. krusie* [Graph 1]. Due to overlap of colonies, it is difficult to count the colonies under colony counter [Figure 1]. The technique is comparably simple and easy to perform and does not consume much time.

The culture plates made using the serial dilution method also showed growth of candidal colonies [Figure 2]. The colonies were distant from each other which made them easily countable under a colony counter. The procedure is also not that much complicated but it requires more time and effort than the concentrated rinse method. Although the colonies were easily distinguishable but in this method, other species of candida which were present in lower concentration in the oral cavity did not appear on the culture plates, only the candida species with the highest percentage appeared mostly on the culture plates.

Both the techniques yielded positive results for cultivating of candida colonies with their own advantages and disadvantages. Some of the variables have been compared between both the methods as shown in Table 1.

Discussion

Candida is a strictly opportunistic species and neither systemic or the superficial forms of candida infections could be initiated in the

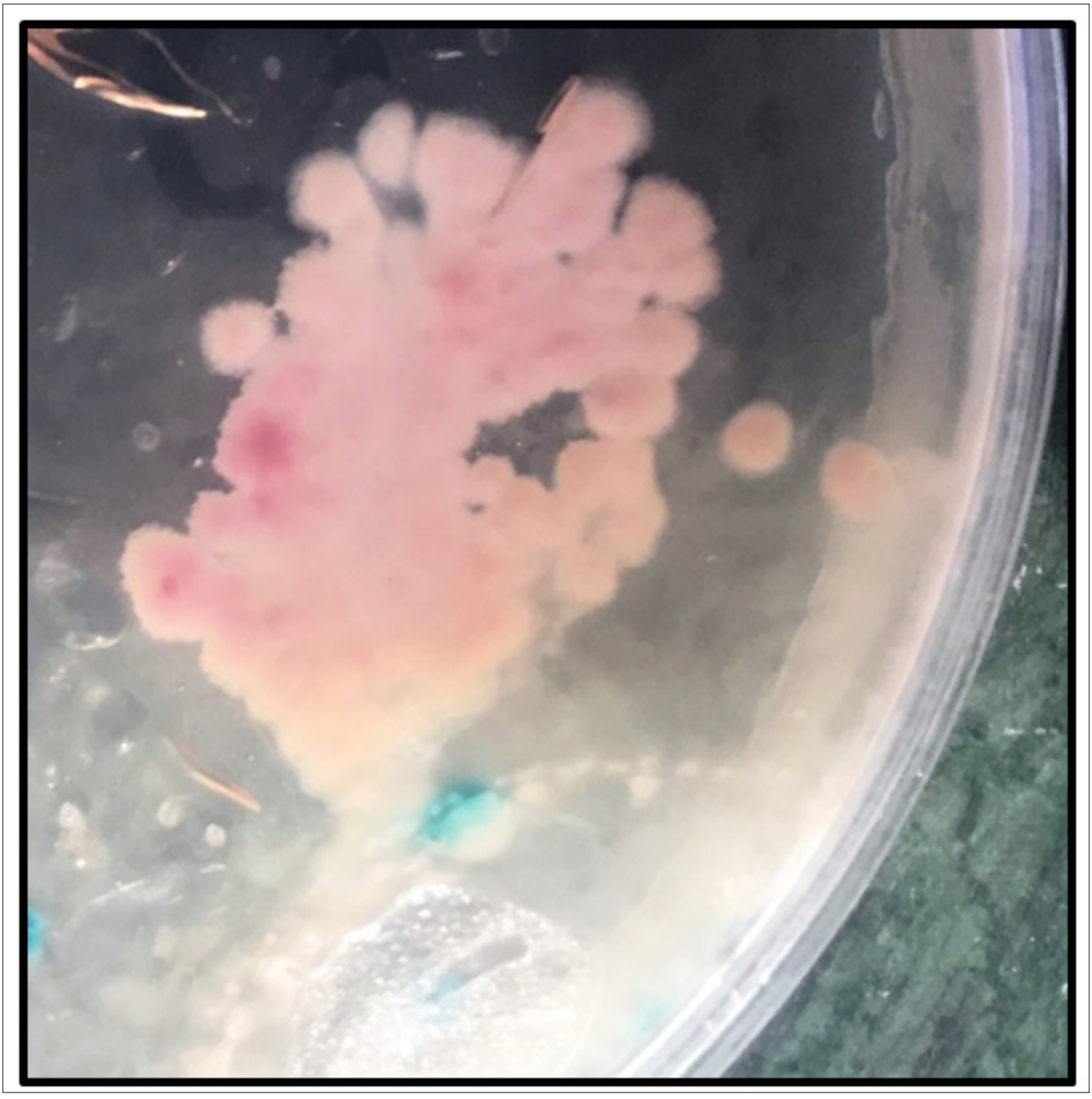


Figure 1: Colonies on Hi-Chrome agar plate observed with concentrated saliva

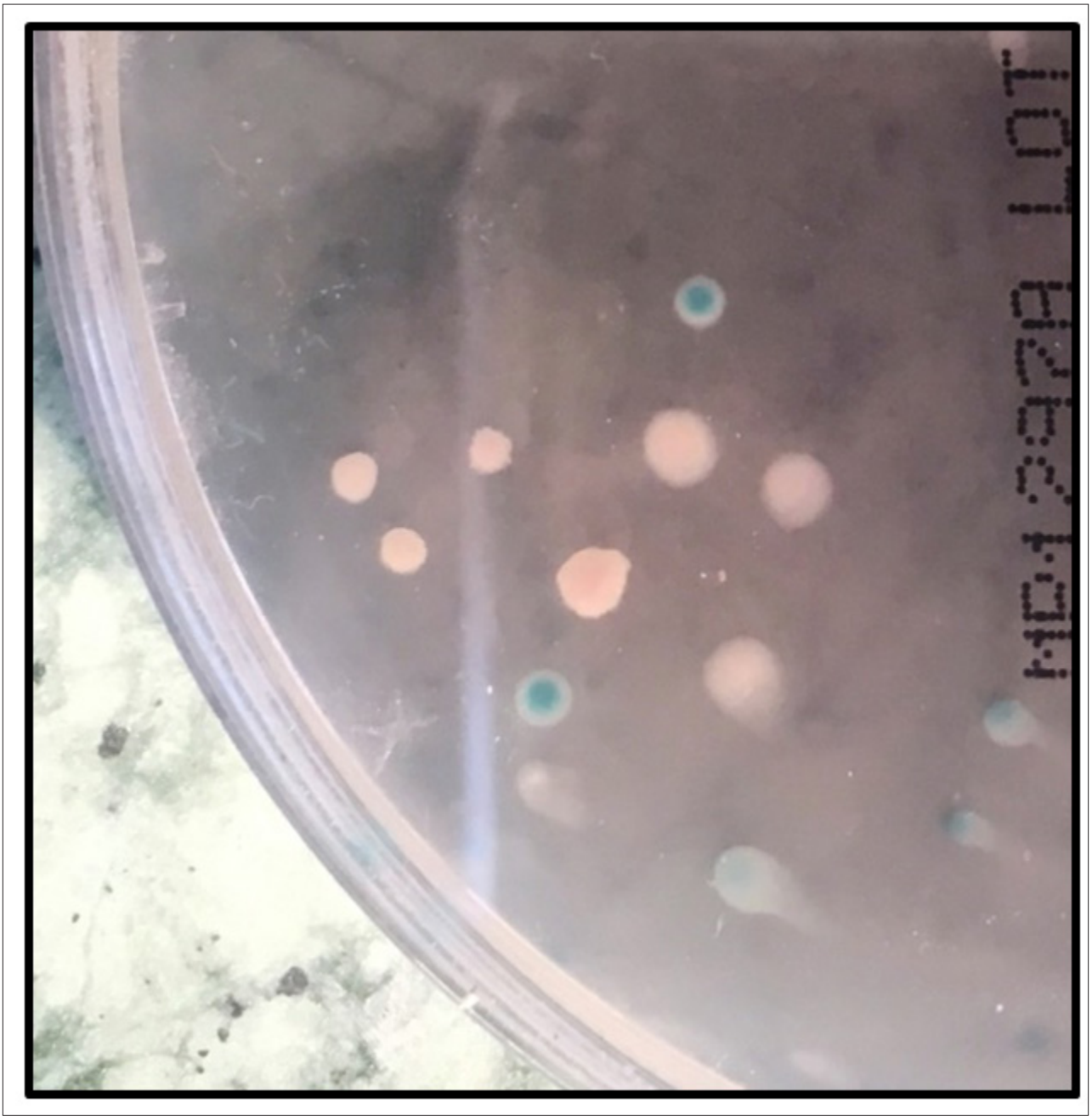


Figure 2: Colonies on Hi-Chrome agar plate observed with serial dilution method

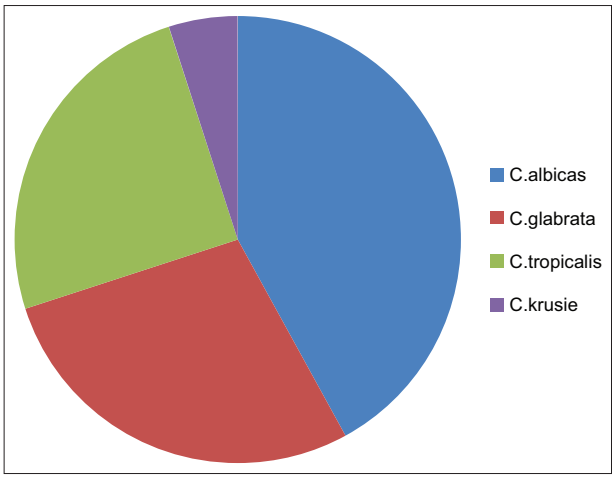
absence of underlying pathology.[5] *C. albicans* is the most common etiologic agent in candidiasis but other species have emerged as important opportunistic pathogens, such as *C. tropicalis*.[6] In diagnosing candidal infections of the oral cavity a smear can be used in differentiating between yeast and hyphal forms but the method is less sensitive than cultural methods, as the species of candida is difficult to differentiate on smear.[1] Various molecular techniques have also been used, including multilocus sequence typing, pulsed-field gel electrophoresis, duplex polymerase chain reaction (PCR), restriction fragment length polymorphisms, random amplified polymorphic DNA, length heterogeneity PCR, and microsatellites.[7] These techniques are accurate,[8,9] sensitive, and specific[10,11] but have certain limitations like DNA isolated from fungi need to be of suitable quality,[12] the potential contamination with clinically irrelevant fungal species, they require technical skills and expensive equipments, they are not available in all laboratories.[13]

Culture methods have been used time and again in the isolating the fungal strains as the method is simple to use. In this study, we used spread-plating as it offers several advantages over pour-plating, these include avoidance of aerobic organisms getting trapped inside agar medium, flexibility in handling, less interfering effects on temperature sensitive organisms, the surface enumeration of colony forming units, and the easy selection of distinct colony types.[14-16]

The most frequently used primary isolation medium for candida is SDA[17] which, although permitting growth of candida,

Table 1: Differences between two methods

S. No.	Variables	Concentrated rinse method	Serial dilution
1.	Overlap of colonies	Yes	No
2.	Countable colonies	No	Yes
3.	Identification of species with lower concentrations	Yes	No
4.	Procedure	Simple	Complicated than the other method
5.	Time taken	Less	More



Graph 1: Various Candida species observed

suppresses the growth of many species of oral bacteria due to its low pH. Incorporation of antibiotics into SDA will further increase its selectivity.[18] On the other hand, the chromogenic medium CHROM-agar is available for the isolation and presumptive identification of *C. albicans*, based on the pigmentation of the developing colonies, which is due to different enzyme activities from candida species. This medium shows different color colonies for *C. albicans* (green), *C. tropicalis* (metallic blue, with a pink halo), and *C. krusei* (pink with velvety appearance). In our study, we also obtained different forms of candida species from the oral cavity including *C. albicans*, *C. glabrata*, *C. tropicalis,* and *C. krusie*.

Samples for the culture from oral cavity have been generally used in concentrated form. The saliva is collected and spread directly over the culture plates with the use of spreaders. This method is easy to use, often shows a number of candida species when used with differentiating agars. Drawbacks include difficulty to count the colonies of fungi due to uneven and overlapping growth of the colonies.

Serial dilution, also called limiting dilution series, is a standard laboratory procedure;[19] the objective of the serial dilution method is to estimate the concentration (number of colonies, organisms, bacteria, or viruses) of an unknown sample by counting the number of colonies cultured from serial dilutions of the sample, and then back track the measured counts to the unknown concentration. Serial dilution techniques are routinely used in hospitals, public health, virology, immunology, and microbiology.[20] Estimation of colony forming units (cfu) through serial dilution plating on a nutrient medium forms the most widely accepted method for monitoring cultivable bacteria and yeasts in different spheres of microbiology.[21] In our study, the results obtained by serial dilution showed a better distribution of fungal colonies over the culture plates, it made the colonies easily countable under a colony meter. Few drawbacks of this technique as summed above included the increased time taken for the procedure, the inability to detect candida species present in lower concentration makes it difficult to detect any new species of candida in the oral samples.

Conclusion

Concentrated rinse method is the method of choice for identification of different species and identifying any new species of candida in the oral cavity with the disadvantage of making the colonies difficult to be counted under a colony counter whereas the serial dilution can be used if the colonies need to be counted by the colony meter but only the species with the largest number will appear by this method.

References

1. Raju SB, Rajappa S. Isolation and identification of Candida from the oral cavity. ISRN Dent 2011;2011:487921.
2. Souza MN, Ortiz SO, Mello MM, Oliveira FM, Severo LC, Goebel CS. Comparison between four usual methods of identification of *Candida* species. Rev Inst Med Trop Sao Paulo 2015;57:281-7.
3. Williams DW, Lewis MA. Isolation and identification of *Candida* from the oral cavity. Oral Dis 2000;6:3-11.
4. Hedges AJ. Estimating the precision of serial dilutions and viable bacterial counts. Int J Food Microbiol 2002;76:207-14.
5. Soysa NS, Samaranayake LP, Ellepola AN. Antimicrobials as a contributory factor in oral candidosis – A brief overview. Oral Dis 2008;14:138-43.
6. Punithavathy PM, Nalina K, Menon T. Antifungal susceptibility testing of Candida tropicalis biofilms against fluconazole using calorimetric indicator resazurin. Indian J Pathol Microbiol 2012;55:72-4.
7. Ieda S, Moriyama M, Takeshita T, Maehara T, Imabayashi Y, Shinozaki S, *et al.* Molecular analysis of fungal populations in patients with oral candidiasis using internal transcribed spacer region. PLoS One 2014;9:e101156.
8. Eggimann P, Bille J, Marchetti O. Diagnosis of invasive candidiasis in the ICU. Ann Intensive Care 2011;1:37.
9. Estrada-Barraza D, Dávalos Martínez A, Flores-Padilla L, Mendoza-De Elias R, SanchézVargas LO. Comparison between conventional methods, ChromAgar Candida and PCR method for the identification of Candida species in clinical isolates. Rev Iberoam Micol 2011;28:36-42.
10. Meletiadis J, Arabatzis M, Bompola M, Tsiveriotis K, Hini S, Petinaki E, *et al.* Comparative evaluation of three commercial identification systems using common and rare bloodstream yeast isolates. J Clin Microbiol 2011;49:2722-7.
11. Zaitz C, Campbell I, Marques AS, Ruiz LR, Framil VM. Compêndio de Micologia Médica. 2th ed. Rio de Janeiro: Guanabara Koogan; 2010.
12. Gosiewski T, Jurkiewicz-Badacz D, Sroka A, Brzychczy-Włoch M, Bulanda M. A novel, nested, multiplex, real-time PCR for detection of bacteria and fungi in blood. BMC Microbiol 2014;14:144.
13. Szabó Z, Tóth B, Kovács M, Kardos G, Maráz A, Rozgonyi F, *et al.* Evaluation of the new Micronaut-Candida system compared to the API ID32C method for yeast identification. J Clin Microbiol 2008;46:1824-5.
14. Hoben HJ, Somasegaran P. Comparison of the pour, spread, and drop plate methods for enumeration of *Rhizobium* spp. in inoculants made from presterilized peat. Appl Environ Microbiol 1982;44:1246-7.
15. Messer JW, Rice EW, Johnson CH. Total viable counts. Spread plate technique. In: Robinson RK, Batt CA, Patel PD, editors. Encyclopedia of

Food Microbiology. Vol. 3. London: Academic Press; 2000. p. 2159-60.
16. Wise K. Preparing Spread-Plate Protocols; October 09, 2006. Available from: http://www.microbelibrary.org/library/laboratory-test/3085-preparing-spread-plates-protocols. [Last accessed on 2015 Apr 28].
17. Odds FC. Sabouraud('s) agar. J Med Vet Mycol 1991;29:355-9.
18. Marsh PD, Martin M. Oral fungal infections. In: Oral Microbiology. Edinburgh, UK: Churchill Livingstone; 2009. p. 166-79.
19. Zelterman D, Tulupyev A, Heimer R, Abdala N. Statistical design for a small serial dilution series. Stat Med 2010;29:411-20.
20. Ben-David A, Davidson CE. Estimation method for serial dilution experiments. J Microbiol Methods 2014;107:214-21.
21. Messer JW, Johnson CH. Total viable counts. Pour plate technique. In: Robinson RK, Batt CA, Patel PD, editors. Encyclopedia of Food Microbiology. Vol. 3. London: Academic Press; 2000. p. 2154-8.