ORIGINAL ARTICLE

Value of in-house viral transport medium in breaking the bottlenecks for viral testing in Pakistan

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ABSTRACT

Background: Maintenance of a steady supply of viral transport medium (VTM) for transporting clinical samples to the laboratory for viral testing is critical amid periods of viral outbreak. Hence, we prepared an in-house VTM and validated its capacity to preserve viral nucleic acids.

Material and Methods: We used Phosphate-buffered saline (PBS) supplemented with sterile glycerol and a combination of antibiotics viz. vancomycin, colistin sulphate, amphotericin B and trimethoprim lactate, for our VTM formulation. For stability, antimicrobial efficacy and sterility evaluation, representative samples from each batch were selected. To validate our VTM, we tested clinical nasal swab samples transported in commercially available (*Copan Italia* S.p.A.) and in-house VTM, and compared both the media for viral nucleic acid recovery using Reverse transcriptase-polymerase chain reaction (RT-PCR).

Results: A satisfactory evaluation of in-house VTM in terms of stability, antimicrobial efficacy and sterility was obtained. A total of 239 nasal swab samples were processed in both commercial and PBS VTM, of which 61 (25.5%) transported in commercial VTM were positive compared to 63 (26.4%) transported in PBS VTM. A comparison of Ct values in RT-PCR positive samples from both groups (n=61), showed Ct values of less than 25 in 14.7% samples from PBS VTM compared to 21.3% from commercial VTM. Whereas, more samples from PBS VTM (78.7%) compared to commercial VTM (73.8%) exhibited Ct values of more than 30. Our results showed that PBS VTM exhibited 100% sensitivity, 98.9% specificity, 96.8% positive predictive value and 100% negative predictive value.

Conclusion: Our in-house prepared VTM was successfully validated and offers a readily available, cost-effective, and simpler to prepare alternative for diagnostic laboratories in low resource settings.

Keywords: Viral transport medium, VTM, Viruses, RT-PCR

BACKGROUND

Pakistan is endemic to several viral infections owing to high population growth, varied topography, humid climate, and low levels of awareness and education. These viruses can spread by a variety of routes, such as sexual contact, bloodborne, fecal-oral, vector-borne, respiratory, or airborne contact, as well as transmission after organ transplantation.[1] The viral burden in Pakistan has substantially increased over the past decade because of the COVID-19 pandemic and seasonal Influenza and Dengue outbreaks, leading to an unprecedented rise in morbidity and mortality.[2] Additionally, there is growing concern over the emergence of viruses such as the Crimean-Congo hemorrhagic fever virus, which is endemic to Balochistan province, and the West Nile, Japanese encephalitis, and Chikungunya viruses, which have been reported sporadically throughout the country.[3-6] An already fragile health and diagnostic infrastructure places Pakistan at risk for another viral outbreak in the near future.

In such scenario, the role of viral diagnostics in the early diagnosis of disease is crucial as it enables early treatment and prompt surveillance to identify ongoing outbreaks.[7] The majority of viral diseases are currently diagnosed using genomic techniques that mainly involves the polymerase chain reaction (PCR). To perform PCR with optimal results, it is crucial to maintain the stability of the virus, as it needs to be sufficiently present and preserved in the specimen at the time of testing. In order to achieve this, ensuring a steady supply of the Viral Transport Medium (VTM) is

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critical for transporting the collected specimens to the laboratory. VTM is available in a variety of formulations, each containing a buffered salt solution, a complex supply of protein and/or amino acids, and antimicrobial agents. Its goal is to store the virus for eventual amplification using Nucleic Acid Amplification Test (NAAT) technology and/or viral culture. Although commercially available VTM have been used with success throughout the world, lack of steady supply amid periods of outbreak and concerns over affordability in middle- and lower-income countries, seriously hinder the ability of diagnostic facilities to adequately meet the demands of testing.[8]

Pakistan, a middle-income country, has a limited number of laboratories that provide PCR testing for viral detection[9]. Most of these laboratories are located in the urban areas; and the rural region which comprise 64% of the country's land area, is increasingly dependent on these facilities, further increasing specimen transport time and the necessity for preservation of viral material[10]. To overcome these shortcomings, a VTM that is relatively simple to prepare in a low-resource setting and cost-effective must be developed. Therefore, in order to ensure an adequate supply of the medium during times of shortage, we prepared an in-house VTM and validated its capacity to preserve viral nucleic acids.

MATERIAL AND METHODS

This was a method validation study conducted at the Microbiology and Molecular pathology departments of the Indus Hospital & Health Network, Karachi, Pakistan from 1st May to 30th November, 2022. This study was performed in accordance with the Declaration of Helsinki and was approved by the Institutional Review Board of the Indus Hospital & Health Network (vide reference number IHHN_IRB_2022_01_004).

Patients admitted in the emergency and in-patient departments with signs and symptoms of COVID were included in this study. Sampling technique employed was consecutive (non-probability) sampling. Two nasal swabs were collected at the same time from each patient within 12 hours of admission to the hospital; first swab was transported in the commercial VTM (*Copan Italia* S.p.A.) and second in the in-house prepared VTM. Real-time PCR was performed using the Novel Coronavirus (2019-nCoV) Nucleic Acid Diagnostic Kit (Sansure Biotech Inc.) for the qualitative detection of the ORF1ab and N genes in the nasal swab specimens, and results were compared to validate the efficiency of in-house prepared VTM. Patients who didn't present with signs and symptoms of COVID and those admitted for more than 12 hours in the hospital, were excluded from the study.

VTM formulation & batch preparation: CDC guidelines were followed for VTM preparation.[11] Phosphate-Buffered Saline (PBS) was prepared using the following composition,

- NaCl: 8 g
- KCl: 0.2 g
- Na_2HPO_4: 1.44 g
- KH_2PO_4: 0.24 g
- Distilled water to make 1-liter solution

PBS was autoclaved after preparation and then mixed in a ratio of 1:1 with sterile glycerol. Following antibiotics were added in 1 liter of the prepared medium to inhibit bacterial and fungal growth during the transportation of nasal swabs.

- Vancomycin: 2.0 mg
- Colistin Sulphate: 7.5 mg
- Amphotericin B: 1.0 mg
- Trimethoprim Lactate: 3.0 mg

After preparation, 3 ml of VTM was filled in conical tubes and stored at 4˚C for 48 hours for short-term use. The remaining media was stored at -20˚C for a longer period until required. Throughout the validation process, a total of 19 batches of In-house (PBS) VTM were prepared. Following preparation, representative media from each batch were evaluated for sterility, stability and antimicrobial efficacy.

Quality Control:

- **Sterility evaluation:** In order to assess sterility of the PBS VTM, 1 ml from the representative media was inoculated on the surface of the Sheep blood agar (SBA) plate. The plate was incubated for 48 hours at 37°C ±2°C and examined for any growth at 48 hours, 96 hours, 120 hours, and after one week. The results were documented as 'growth or no growth' against each batch and released for use only when the sterility was verified.
- **Antimicrobial efficacy verification:** For the purpose of ascertaining antimicrobial efficacy of the prepared media, nasal swabs were collected from

volunteers and placed in representative PBS VTM from each batch. These were incubated for 24 hours at 35 °C ±2 °C and inoculated on SBA the next day, which were incubated at 35°C ± 2°C. SBA plates were checked daily for growth for 48 hours. Antimicrobial efficacy of the media was verified if there was no growth on the plates indicating adequate inhibition of nasal flora.

- **Stability testing:** For assessing the potential of the prepared media for preserving the specimen over prolonged periods, a stability check was performed to evaluate the adequate recovery of viral nucleic acids. In order to achieve this, we selected random samples from already reported positive samples which were transported in commercial VTM and in-house VTM and compared both the media for viral recovery at 4 hours (n=17), 24 hours (n=17) and 36 hours (n=4) through Real-Time PCR (RT-PCR).

Patient biodata, data of PCR positivity and cycle threshold (Ct) values of samples transported in both media, commercial and PBS VTM, were documented on a standardized proforma. The data was entered in Microsoft Excel software (Microsoft Excel 2013 {15.0.5553.1000} 32-bit) for the purpose of statistical analysis. Samples reported as PCR positive from both commercial and PBS VTM were compared based on their Ct values. Chi-Square test was used to determine the statistical significance of any association between a range of Ct values and number of positive samples from commercial and PBS VTM. Analytical results are reported as odds ratios and p values. Furthermore, sensitivity, specificity, positive and negative predictive values were computed to assess the overall efficacy of PBS VTM.

RESULTS

Results of the evaluation of representative media from each batch of PBS VTM in terms of stability, antimicrobial efficacy, and sterility were satisfactory. Regarding sterility testing, the media plates inoculated with PBS VTM at 48 hours, 96 hours, 120 hours, and after one week showed no growth. Additionally, antimicrobial assessment revealed no growth after 48 hours, on the media that had been inoculated with the nasal swab specimens. On stability testing, all samples from commercial and in-house VTM showed the same reproducibility of results through RT-PCR at 4 hours and 36 hours intervals. However, we found a discrepancy in commercial VTM at 24 hours interval, where one sample showed false negative result.

A total of 239 nasal swabs were processed in both commercial and PBS VTM, out of which 61 samples (25.5%) transported in commercial VTM were positive whereas, 63 samples (26.4%) transported in PBS VTM turned out positive. Our findings showed that PBS VTM exhibited 100% sensitivity, 98.9% specificity, 96.8% positive predictive value and 100% negative predictive value. Table-I shows the comparison of nucleic acid recovery from commercial and in-house VTM through RT-PCR.

Table-I: Comparison of nucleic acid recovery from commercial and in-house VTM through RT-PCR along with sensitivity, specificity, positive predictive value, negative predictive value and diagnostic accuracy of in-house VTM.

VTM	RT-PCR (n=239)	
	Positive (n %)	Negative (n %)
Commercial	61 (25.5%)	178 (74.5%)
In-house	63 (26.4%)	176 (73.6%)
Sensitivity = 100%		
Specificity = 98.9%		
Positive Predictive Value = 96.8%		
Negative Predictive Value = 100%		
Diagnostic Accuracy = 99.2%		

Table-II: Comparison and association of the Ct values of PCR-positive samples (n=61) with commercial and in-house VTM.

Ct value	Commercial VTM (n=61)	In-house VTM (n=61)	P value	Odds ratio (95% CI)
<25	13 (21.3%)	9 (14.7%)	p=0.35	0.6 (0.3 to 1.6)
25-30	3 (4.9%)	4 (6.6%)	p=0.7	1.4 (0.3 to 6.3)
>30	45 (73.8%)	48 (78.7%)	p=0.5	1.3 (0.6 to 3)

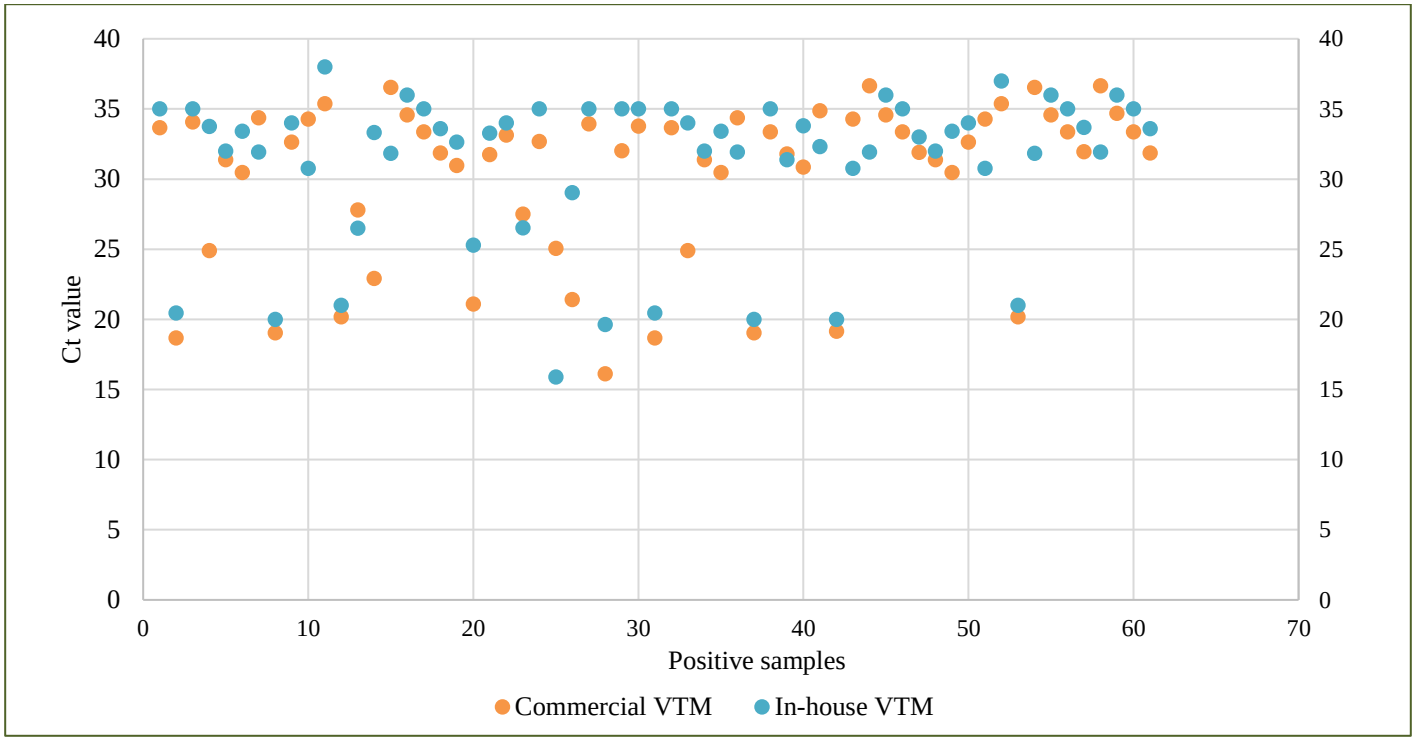

Figure-I: Graphical representation of the Ct values of all PCR-positive samples (n=61) from both commercial and in-house VTM.

A head-to-head comparison of Ct values of samples that exhibited PCR positivity from both groups of VTM (n=61), showed that 14.7% of samples from PBS VTM as compared to 21.3% of samples from commercial VTM had Ct values of less than 25. Whereas, more samples from PBS VTM (78.7%) as compared to commercial VTM (73.8%) exhibited Ct values of more than 30. No statistical significance was noted for any association of a particular group of VTM with a certain of range of Ct values. Comparison and association of the Ct values of PCR-positive samples (n=61) with both commercial and in-house VTM is shown in Table-II. Furthermore, a comparison of the Ct values of all PCR-positive samples (n=61) from both commercial and in-house VTM are graphically represented in Figure-I.

DISCUSSION

A dependable and cost-effective VTM was developed and validated for the proper storage of nasal swab specimens from viremic patients, in our study. There have been many attempts in preparing in-house VTM in the past and clinical laboratories have used several modifications of the commercially available viral media with successful results. An in-house VTM was developed and validated by Peterson *et al*. in a clinical laboratory in USA, to meet the increasing demands of testing during COVID-19 pandemic, using Hanks Balanced Salt Solution (HBSS) supplemented with phenol red, fetal bovine serum (FBS), gentamicin sulfate, and amphotericin B.[8] For validation, positive and negative aliquots spiked with the respective control material from commercially available reference material kit was used. Positive spiked specimens were temporarily kept at room, refrigeration and frozen temperatures because the in-house VTM was intended for use in clinical settings. This was done to ensure that RNA material was still preserved in the VTM for testing at the different temperatures. While this validation study was successful in serving its initial objective, it should be noted that a head-to-head comparison of viral nucleic acid recovery from real clinical samples transported in the in-house and commercially available VTM, was not performed.

Another study from a reference institute in Bangladesh reported the performance evaluation of an in-house VTM prepared from HBSS supplemented with bovine serum albumin (BSA), gentamicin sulfate and fluconazole.[12] RT-PCR was performed on oro-nasal swab samples from 80 random COVID patients, transported in the in-house and commercial VTM. A similar sensitivity and specificity were observed for the in-house VTM as compared to commercial VTM as 15

samples were found to be positive and 65 samples negative from both the media. Prior to validation in clinical samples, both VTM were also compared for RNA recovery from 70 known samples with similar results. While the study compared the recovery of viral nucleic acids from both media in known and clinical samples, it should be highlighted that the validation sample for clinical samples was lower (n=80) as compared to our study (n=239).

Moreover, the in-house VTM in both of the above-mentioned studies was supplemented with either whole FBS or its derivative BSA. Due to rising demand and limited supply, which has caused its price to rise by more than 300% in recent years, FBS doesn't seem to be a cost-effective option for laboratories with fewer resources. Our in-house formulation of VTM with PBS supplemented with sterile glycerol provides an alternative for such low resource settings as it is more readily available, less expensive, and exhibits good uniformity between lots.'[14]

A comparison of Ct values showed that most positive samples transported in our in-house VTM were detected at relatively higher Ct values as compared to commercial VTM, which detected viral RNA at lower Ct values (Table-II & Figure-I). While no statistical significance was observed for this finding, it is probably because of the fact that nasal swab specimens were consecutively sampled from a patient in commercial VTM and then in PBS VTM, which resulted in a lower viral load in the later swab. Nevertheless, it remained within the recommended Ct value (<40).[15] Moreover, two additional samples with Ct values of more than 38 were reported positive with PBS VTM that were reported negative with commercial VTM; these results suggest better preservation of viral nucleic acids in the PBS VTM as compared to commercial VTM, even though it detected more samples at a consistently higher Ct value than commercial VTM.

In this article, we detailed our efforts to create a VTM for locally assembling a specimen collection kit in the event that imported viral media and reagents become scarce due to rising demand and high prices brought on by a potential viral outbreak. We have demonstrated that our in-house VTM is effective in preserving viral nucleic acids under recommended conditions, starting with the VTM development and evaluation and continuing through quality control and validation procedures. Its performance is also comparable to that of the commercial VTM that was examined in this study.

There were few limitations to our study. Firstly, this was a single center study and multi-center studies are required from all over Pakistan to assess the efficacy of our in-house VTM on a wider population. Nonetheless, Indus hospital is a large tertiary care center located in the Korangi district of Karachi that caters to a population of 2.5 million people.[16] Secondly, our study incorporated samples only from COVID patients whereas, samples of viral diseases that are endemic to the region such as Dengue, were not tested. This was an initial validation study of our VTM formulation and we plan to conduct validation of our in-house VTM for several endemic viral diseases in the future. Furthermore, we didn't perform a detailed cost analysis comparing our in-house VTM with commercial VTM, which might diminish its broader applicability. However, the results of this study demonstrate the efficacy of our VTM formulation, and the authors acknowledge that the next logical step is to conduct a cost-analysis to determine its widespread applicability under similar conditions and settings, which they intend to do in the future. Lastly, even though we followed CDC guidelines for our VTM formulation, which prescribes a combination of gentamicin and amphotericin B, we were out of stock for gentamicin due to shortage of supplies. Instead, we employed a combination of vancomycin, colistin, amphotericin B, and trimethoprim, and verified its antimicrobial efficacy with satisfactory results.

CONCLUSION

Our in-house prepared VTM has been validated as a convenient and acceptable alternative for the transportation of samples for viral testing. It offers diagnostic laboratories in low resource settings with a readily available, cost-effective, and simpler to prepare option for routine viral testing as well as in preparing for an impending viral outbreak.

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CONFLICT OF INTEREST

None

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Declared none

AUTHOR CONTRIBUTION

Fareeha Adnan: Conceptualization, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Writing – review & editing, final approval, agreement to be accountable for all aspects of the work

Nazia Khursheed: Conceptualization, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Writing – review & editing, final approval, agreement to be accountable for all aspects of the work

Moiz Ahmed Khan: Formal Analysis, Investigation, Software, Visualization, Writing – original draft, final approval, agreement to be accountable for all aspects of the work

Maira Khan: Data curation, Investigation, Validation, Visualization, Writing – review & editing, final approval, agreement to be accountable for all aspects of the work

Nazia Parveen: Data curation, Investigation, Validation, Visualization, Writing – review & editing, final approval, agreement to be accountable for all aspects of the work

Mariam Ashfaq Khan: Formal Analysis, Investigation, Software, Visualization, Writing – review & editing, final approval, agreement to be accountable for all aspects of the work

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