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Short Communication

Development of an inactivated viral transport medium for diagnostic testing in low-resource countries

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Abstract

Viral transport medium (VTM) is crucial for retaining clinical specimens, such as the virus or its genetic material from the mucus of respiratory tract of coronavirus disease 2019 (COVID-19) suspected patients. However, the locally produced VTM in Indonesia lacks the ability to inactivate the virus, risking the safety of diagnostic personnel. The aim of this study was to formulate inactive VTM (iVTM) incorporating chaotropic agents like guanidine salt, along with anionic detergents, chelators, buffers, and surfactants, to inactivate the virus while maintaining RNA integrity. Viral RNA stability in iVTM (pH 4 and pH 6) was evaluated for 30 days at 4°C and 25–28°C. In vitro inactivation test was performed on SARS-CoV-2 isolate (variant B1). The stability test revealed that storing the clinical specimens in iVTM at pH 6 maintained severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) detectability by qPCR for up to 30 days at cold and room temperatures. Stability assessments conducted over a 4-month period (at 25–28°C) on iVTM with a pH of 6 revealed clear appearance, consistent pH stability, no alteration in the solution color, and no indications of bacterial or fungal contamination. Results from an in vitro inactivation assay demonstrated that iVTM pH 6 eliminated SARS-CoV-2 infectivity within just five minutes of contact. These findings suggest that iVTM pH 6 offers a safer and cost-effective alternative for handling and transportation of clinical specimens.

Keywords: Inactivated VTM, SARS-CoV-2, inactivation, diagnostics, RT-qPCR

Introduction

Viral respiratory infections (VRIs) are the most prevalent type of infectious diseases and are a major contributor to illness and death globally. Several large-scale outbreaks and pandemics of VRIs, such as severe acute respiratory syndrome (SARS) in 2003, influenza H1N1 in 2009, Middle East respiratory syndrome (MERS) in 2012, and coronavirus disease 2019 (COVID-19) in 2019 to 2022 emphasize the importance of rapid diagnosis to reduce mortality [1]. Identifying patients suspected of having COVID-19 in the early stage is critical because the patients might be asymptomatic. However, once the symptoms appear it might be too late for treatment and the patients also have spread the virus infecting others. Therefore, an accurate, precise, and fast detection process will be beneficial in taking steps to control the spread of COVID-19 [2,3] and other VRIs. Here, we focused our study to improve the specimen storing process in detection of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the virus that causes COVID-19.

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Received: January 7,2025 - Accepted: July 2, 2025 - Published online: August 1, 2025

For COVID-19 detection, pooled samples containing individual upper respiratory swab (nasopharyngeal swab, oropharyngeal swab, or saliva) and lower respiratory swab (sputum, tracheal aspirate, bronchoscopic brushing, or bronchoalveolar lavage fluid) specimens are recommended [4]. However, samples collection point could be located far away from the testing center, or there could be too many samples for immediate testing, resulting in an extended waiting period from sample collection to testing. The storage duration of samples could decrease virus survivability and the integrity of their genetic material, affecting the test results [5,6]. Hence, viral transport medium (VTM) is crucial to maintain the stability of clinical specimens, supporting the accuracy of COVID-19 diagnosis [7]. However, studies have pointed out the biohazardous potential of using VTM during transport or testing because the virus remains infectious [8]. This led to positive cases of health and laboratory workers from exposure to SARS-CoV-2 from clinical specimens [9-12].

VTM should be appropriately designed to inactivate the virus when a specimen is collected from a patient. In the inactivation process, especially the chemically induced one, the virus will undergo irreversible denaturation of their protein structure [13], eliminating pathogenicity. Chaotropic agents - especially guanidine salts, can inactivate viruses properly without degrading the viral genetic material [14-17]. In addition, other reagents, such as anionic detergents, reducing agents, chelators, buffers, and surfactants, were reported to completely inactivate the virus while maintaining the stability of viral RNA [18]. Therefore, choosing a suitable reagent can improve the quality of the medium formulation.

The storage temperature is also crucial in maintaining genetic material stability. High temperatures are reported to accelerate nuclease enzyme activity and degrade genetic materials [19,20]. According to a study [4], samples containing RNA SARS-CoV-2 generally require storage at low temperatures (2°C to -70°C) to maintain the stability of their biological components and genetic materials. Amid the pandemic, particularly in low resource countries, this has presented a significant challenge due to the substantial effort and expenses involved. Therefore, an alternative solution for room temperature storage is critical.

Hence, the aim of this study was to develop a novel VTM that is safe for specimen handling, processing, and transport while ensuring the stability and preservation of viral nucleic acid for prolonged period, namely the inactivated VTM (iVTM). The iVTM was tested under specific pH conditions (pH 4 and pH 6) and temperature settings (4°C and 25–28°C). Additionally, the study assessed the stability of viral RNA in the medium after a 30-day storage period. The results suggested that the formulated iVTM is considerable for the additional purpose of protecting the diagnostic personnel and reducing cost for providing a cold temperature storage facility.

Methods

Preparation of iVTM

The composition of iVTM is presented in **Table 1**. For preparation of iVTM, the guanidine salt (Guanidine thiocyanate, Sigma-Aldrich, Cat. No. 307394, St. Louis, MO, USA) was added slowly to the nuclease-free water while stirring the mixture on a hot plate (Heidolph, Schwabach, Germany). The stirring speed was set to 150 rpm and the temperature was maintained at 60–65°C to facilitate the dissolving of guanidine salt crystals. Then, 1 M buffer solution (TRIS-HCl, Sigma-Aldrich, Cat. No. T3253, St. Louis, MO, USA) and 0.5 M chelating agent were added, followed by the anionic detergent. The pH was adjusted to 6 or 4, and the total volume was brought up to 50 mL using nuclease-free water. The iVTM solution was then stored at room temperature. Prior to in vitro testing, the iVTM was sterilized by using UV-C light (254 nm) for 60 minutes.

The guanidine salt (4 M) acted as a chaotropic agent for viral inactivation while preserving the integrity of viral RNA, and the anionic detergent (5% v/v) disrupted the viral lipid envelope. The buffer solution (100 mM TRIS-HCl) was used to maintain a stable pH, while the chelating agent (0.1 mM) inhibited nuclease activity by binding divalent metal ions. Nuclease-free water was used as the solvent to prevent contamination, and hydrochloric acid (1 N) was used to adjust the pH of the iVTM to either 4 or 6 for testing.

Table 1. Composition of inactivated viral transport medium (iVTM)

Compound	Final concentration (50 mL)	Percentage of compound in iVTM solution
Guanidine salt	4 M	40% (m/v)
Anionic detergent	5%	5% (v/v)
Buffer solution	100 mM	10% (v/v)
Chelating agent	0.1 mM	0.02% (v/v)

SARS-CoV-2 culture

All experiments involving SARS-CoV-2 were performed in biosafety level 3 laboratory of National Research and Innovation Agency in Cibinong, Indonesia. African green monkey kidney cells, Vero E6 cells, were used for the SARS-CoV-2 culture and the titration of infectious virus in tissue culture infectious dose 50 ($TCID_{50}$) assay. Vero E6 cells were cultured in Dulbecco's Modified Eagle Medium (DMEM; Sigma-Aldrich, Darmstadt, Germany), which already contained L-glutamine (Sigma-Aldrich, Darmstadt, Germany), supplemented with 10% fetal bovine serum (FBS; Gibco, New York, USA) and 100 U/mL antibiotic solution (penicillin-streptomycin; Gibco, New York, USA). The cells were incubated at 37°C and 5% CO_2 atmosphere.

The SARS-CoV-2 isolate from nasopharyngeal swab of an Indonesian patient was used and has been identified as the B1 variant through whole genome sequencing (EPI_ISL_4004658). The SARS-CoV-2 was cultured and propagated in Vero E6 cells, which had attained a 90% confluency level for a period of three days. Following this, the supernatant was collected via centrifugation at 1500 rpm for ten minutes. The virus stocks were stored at -80°C. For working virus stock, Vero E6 cells were infected with multiplicity of infection (MOI) of 0.01.

Viral titration and $TCID_{50}$ assay

The concentration of viable virus was assessed using the $TCID_{50}$ assay, according to the protocol described in a previous study [22]. Vero E6 cells were grown in DMEM supplemented with 10% FBS, seeded into a 96-well plate at a density of 2×10^4 cells per well, and incubated for 24 hours until reaching approximately 80% confluency. The virus was diluted in DMEM containing 2% FBS (1:9 v/v) using a ten-fold serial dilution ranging from 10^{-1} to 10^{-7}. A 100 µL aliquot of each virus dilution was then inoculated into the cells. The cells were incubated at 37°C, 5% CO_2 for 72 hours. Following incubation, the cells were fixed with 100 µL of 4% formaldehyde-PBS (v/v) for 60 minutes. The cells were then stained with 100 µL of 0.5% crystal violet (C.I. 42555, Merck, Darmstadt, Germany) for five minutes. The wells were rinsed twice with 100 µL of 1X PBS. The viral titer of SARS-CoV-2 was calculated using the Reed and Muench method [23].

Stability test of SARS-CoV-2 RNA iVTM

The stabilities of SARS-CoV-2 RNA in iVTM with pH 4 and pH 6 were evaluated for 30 days. For each pH condition, two separate sets of samples were prepared and stored at 4°C and 25°C (room temperature), respectively. Based on the results of this stability test, iVTM with pH 6 (simply called as iVTM pH 6) showed better stability and was therefore used for further tests.

Each treatment was spiked with viable SARS-CoV-2 (1×10^6 $TCID_{50}$/mL) using a volume ratio of 20:1 (iVTM: virus suspension). Viral RNA was analyzed at different time points (0, 1, 7, 14, and 30 days). Each treatment was conducted with three biological replicates, and each biological replicate included four technical replicates. RNA extraction was performed using Viral RNA Mini Kit (QIAGEN GmbH, Hilden, Germany). RT-PCR multiplex assay was performed using the mBioCov-19 RT-qPCR Kit (Bio Farma, Bandung, Indonesia) on CFX 96 Touch Real-Time PCR (Bio-Rad, California, USA). The mBioCoV-19 diagnostic kit is designed to detect *RdRp* and helicase genes of SARS-CoV-2, with RNA extraction was conducted using the QIAamp Viral RNA Kit (QIAGEN GmbH, Hilden, Germany). Amplification was declared positive if both genes were detected with a cycle threshold (CT) value lower than 40.00.

Long-term stability test of iVTM pH 6

Long-term iVTM pH 6 stability was assessed for over 12 months of storage, considering the color/clarity, pH, and microbial contamination test. Physical observation on iVTM pH 6 was carried out by visually observing the color/clarity of the medium. The clarity parameter was determined based on the presence or absence of visually observed particles in iVTM pH 6. The

pH of iVTM pH 6 was subsequently examined at the 0, 4, 9, and 12-month intervals during storage. The microbial contamination test was carried out on fastidious bacteria (Reasoner's 2a Agar medium, 27°C, 168 h), non-fastidious bacteria (Nutrient Agar/NA medium, 27°C, 24 h), coliform bacteria (Eosin Methylene Blue medium, 27°C, 72 h), *Staphylococcus* sp. (Staphylococcal agar, 27°C, 72 h), *Salmonella shigella* (NA medium, 27°C, 72 h), mold (NA medium, 27°C, 72 h), and yeast (NA medium, 27°C, 72 h) [24]. Using the scatter method, 100 µL of iVTM pH 6 was inoculated onto the agar plate. The number of colonies on the agar plates was then counted. Each test was conducted in three replications.

SARS-CoV-2 infection activity test in iVTM pH 6

A total of 100 µL SARS-CoV-2 (7.33×10^6 $TCID_{50}$/mL) was added to 2 mL of inactive VTM pH 6 with a medium/virus ratio of 20:1 (v/v). Viruses were incubated in iVTM for 5, 15, or 30 minutes at room temperature (n=3, for each time point). After the incubation process, samples from each treatment were filtered with Amicon 4-mL 100 kDa (Merck KGaA, Darmstadt, Germany). The samples were then centrifuged at 6000 rpm for five minutes and washed thrice with 2 mL PBS. The filtrate was then resuspended in 2 mL of 2% DMEM FBS. A total of 100 µL/well of filtrate for each treatment sample was infected on a monolayer of Vero E6 cells (2×10^4 cells/well). Cells were incubated for three days in an incubator with a temperature of 37°C and a CO_2 level of 5%. The presence of infectious SARS-CoV-2, previously inactivated in iVTM pH 6, was assessed by observing the development of viral cytopathic effect (CPE) in Vero cells and quantifying the virus titer through the $TCID_{50}$ method. The negative control in the experiment consisted of 100 µL of DMEM added with 2 mL of formulated inactive VTM. The positive control in the experiment consisted of 100 µL (7.33×10^6 $TCID_{50}$/mL) SARS-CoV-2 added to 2 mL DMEM containing 2% FBS.

Validity test of iVTM as a diagnostic component for detection of SARS-CoV-2

A total of 20 individuals having symptoms indicative of COVID-19 was enrolled and the clinical specimen collection was conducted at Jasa Kartini Tasikmalaya Hospital, Tasikmalaya, Indonesia. The protocol for collecting specimens from human subjects was carried out in accordance with the ethical clearance from Preclinical Ethics Committee of Universitas Jendral Ahmad Yani. Each subject went through nasopharyngeal and oropharyngeal swab twice. The nasopharyngeal swab was made from the same nostril to exclude variability of virus titer between the two nostrils. The first clinical specimen was stored in commercial VTM Jun Nuo Viral Transport Medium (Shandong Chengwu Medical, Heze, China), while the second clinical specimen was stored in iVTM created from the optimal formulation, prepared at pH 6 and maintained at room temperature. Hence, in each iVTM or commercial VTM collection tube contained samples from nasopharyngeal and oropharyngeal. The collected samples were then subsequently subjected to RNA extraction and qPCR analysis.

Biosafety statement

All experiments were carried out inside a biosafety level 2 cabinet, within the confines of biosafety level 3 containment at the Research and Innovation Agency, BRIN, Indonesia. These experiments adhered to approved protocols by the Institutional Biosafety Committee.

Data analysis

The Kolmogorov-Smirnov normality test was used to assess data distribution with $p<0.05$ was considered statistically significant. The CT value was analyzed using the Mann-Whitney and two-way analysis of variance (ANOVA) approach. The two-way ANOVA and Kruskal-Wallis were applied to compare the stability of iVTM with positive control. A Mann-Whitney test was used to analyze the iVTM validity test compared to commercial VTM. These data were analyzed using SPSS Statistics 23 (IBM, New York, USA), while GraphPad Prism 8.3.0 for Windows (GraphPad Software, California, USA) was utilized for generating graphs.

Results

iVTM pH 6 is stable for long term storage

This study compared the stability of clinical SARS-CoV-2 specimens stored at room temperature (25–28°C) with those stored at the optimal temperature (4°C). Observations made through physical examination revealed a shift in the color of iVTM pH 4 following seven days of storage under two distinct temperature conditions (**Table 1**). The excess sulfur resulting from the reaction between guanidine salt and HCl led to the yellowing of the medium. Sulfur minerals have the potential to initiate RNA degradation. In contrast, iVTM pH 6 retained its clarity. Even after prolonged storage (30 days), iVTM pH 6 exhibited no discernible variation from its freshly prepared state (**Table 1**). Following a 12-month incubation at room temperature, iVTM pH 6 maintained its excellent clarity, pH remained largely unchanged, and it remained free from bacterial and fungal growth (**Table 2**). Therefore, iVTM pH 6 was chosen as the best formulation and used for further tests.

Table 1. Clarity and color of inactive viral transport medium (iVTM) pH 4 and 6, stored at temperature of 4°C and 25–28°C (room temperature, RT). Observation was made on day 0, 7, 14, and 30

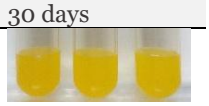
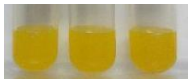
Condition	Observation			
	0 day	7 days	14 days	30 days
iVTM pH 4 Stored temperature 4°C				
iVTM pH 4 Stored temperature RT				
iVTM pH 6 Stored temperature 4°C				
iVTM pH 6 Stored temperature RT				

Table 2. Long term stability test of inactive viral transport medium (iVTM) (pH 6)

Parameters	Specification	Test results			
		Initial	4 months	9 months	12 months
Dosage form	Liquid	Liquid	Liquid	Liquid	Liquid
Color	Clear	Clear	Clear	Clear	Clear
pH solution	6–7	6.76±0.1	6.66±0.1	6.89±0.1	6.86±0.1
Clarity	Free from any particle that visually observed	Clear	Clear	Clear	Clear
Sterility test	Bacterial contamination (presence of bacteria: <30 CFU/mL)	Sterile	Sterile	Sterile	Sterile
	Fungal contamination (presence of fungi: <30 CFU/mL)	Sterile	Sterile	Sterile	Sterile

RNA stability preservation with iVTM pH 6 despite SARS-CoV-2 deactivation

The stability of viral RNA in VTM was evaluated in this study using RT-qPCR, focusing on the relative quantification of helicase and *RdRp* genes. The CT value of helicase and *RdRp* genes increased during storage in iVTM pH 4 (**Figure 1A** and **1B**). The increasing CT values for both genes were faster when the samples were stored at room temperature compared to at 4°C. A significant difference of helicase and *RdRp* CT values between control and samples stored in iVTM pH 6 at 4°C was also observed with a $p<0.05$ (**Figure 1C**) particularly on day 7, likely due to high variability among replicates. This may have influenced significant results without indicating a consistent RNA degradation trend.

Interestingly, the CT values of SARS-CoV-2 RNA in iVTM pH 6 remained stable for 30 days when stored at room temperature (**Figure 1D**) as indicated by a $p>0.05$. Compared to the

positive control, the duration of storage did not exert a noteworthy influence on the stability of the helicase and *RdRp* genes.

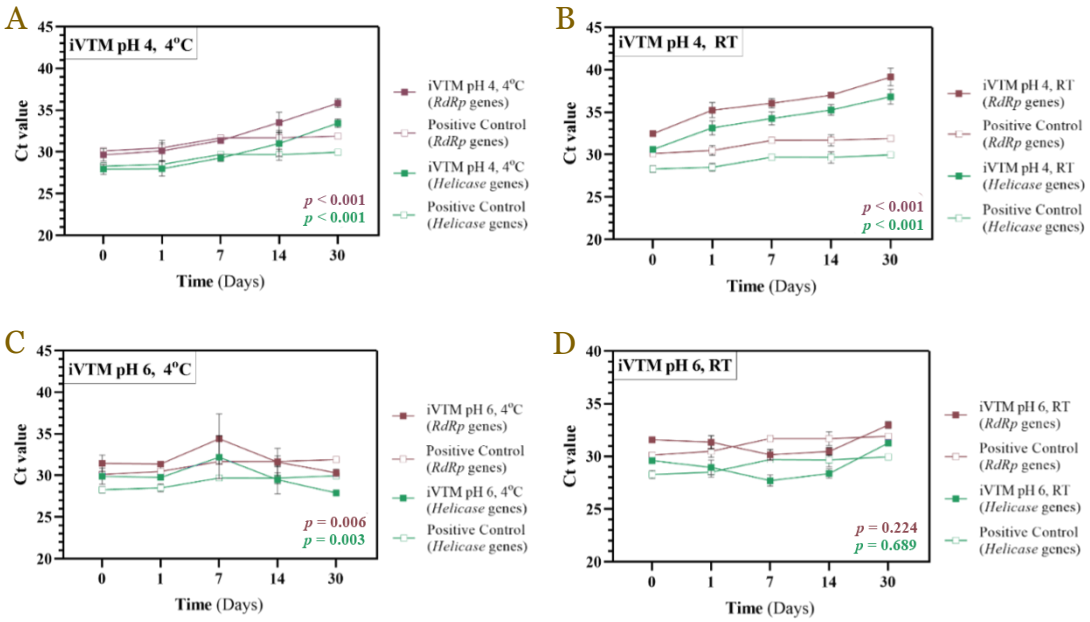


Figure 1. Stability of SARS-CoV-2 RNA in iVTM pH 4 and pH 6. CT values of *RdRp* and helicase genes of SARS-CoV-2 in the positive control and iVTM: (A) pH 4 at 4°C; (B) pH 4 at RT; (C) pH 6 at 4°C; and (D) pH 6 at RT after 0, 1, 7, and 14 days of storage. Full blue box indicates CT value of *RdRp* gene from control, while empty blue box from samples stored in respective iVTM. Full magenta box indicates CT value of helicase gene from control, while empty magenta box from samples stored in respective iVTM. Amplification is considered positive if the detected SARS-CoV-2 marker gene show a CT value less than or equal to 40.00 (≤40.00). A $p<0.05$ (ANOVA, two-way) indicates that the CT value between control and sample are significantly different.

$TCID_{50}$ titration was performed to determine untreated SARS-CoV-2 virus stock, evaluate the virus's infectious activity to Vero cells, and serve as a baseline for assessing residual infectivity of SARS-CoV-2 after inactivation in iVTM. Results of the viral titration are presented in supplement **Table S1** (see **Underlying data**), showing a $TCID_{50}$/mL of 2.29×10^8 with a 95% confidence interval ranging from 1.01×10^8 to 4.81×10^8. Evaluation of iVTM pH 6 efficacies for SARS-CoV-2 deactivation are presented in **Table 3**. The $TCID_{50}$ test results demonstrated that iVTM pH 6 successfully achieved complete inactivation of SARS-CoV-2 after incubation with the virus for 5, 15, or 30 minutes at room temperature.

Table 3. Efficacy of the SARS-CoV-2 deactivation process in inactive VTM pH 6

Medium	Medium/virus ratio (v/v)	Contact period (minutes)	Virus detected with $TCID_{50}$[a]	Detected virus RNA (CT±SD)[b,c]	Interpretation
Inactive VTM	20:1	5	No (0/3)	28.66±0.29	Virus successfully deactivated
		15	No (0/3)	29.65±0.93	Virus successfully deactivated
		30	No (0/3)	27.63±0.69	Virus successfully deactivated

CT: cycle threshold; $TCID_{50}$: tissue culture infectious dose 50; VTM: viral transport medium
[a]$TCID_{50}$ assay's limit of detection is 1 TCID50/mL
[b]Values in parentheses indicate the number of positive samples/number of replications
[c]Gene target is SARS-CoV-2 helicase

The visualization of the TCID50 assay results, showing cytopathic effect (CPE) percentages in Vero cells infected with virus at serially diluted concentrations following exposure to iVTM for 5, 15, and 30 minutes is presented in **Figure 2**. A clear reduction in CPE was observed with decreasing virus concentrations, while no CPE was detected in cells exposed to virus pre-

incubated in iVTM, confirming complete viral inactivation across all tested durations. As expected, the positive control group exhibited significant CPE 72 hours post-infection, while the negative and mock controls showed no visible cytopathic changes (**Figure S1**).

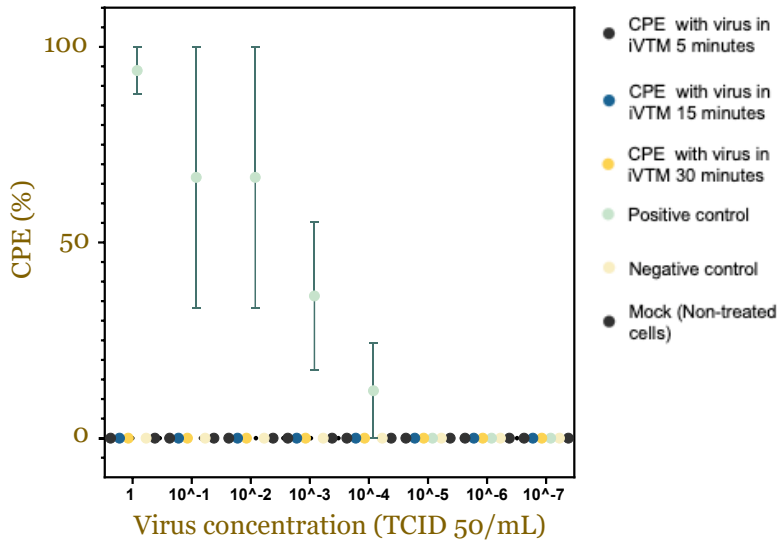


Figure 2. Percentage of cytopathic effect (CPE) observed in Vero E6 cells infected with serial dilutions of SARS-CoV-2 following exposure to iVTM pH 6 for 5, 15, and 30 minutes. The CPE was assessed 72 hours post-infection. Complete inactivation was observed at all tested incubation times, as indicated by the absence of CPE across all virus concentrations. Positive control wells (untreated virus) exhibited high CPE, while negative and mock-treated controls showed no CPE, confirming assay specificity. Error bars represent standard deviations from three biological and eleven technical replicates.

Furthermore, according to RT-qPCR analysis, iVTM pH 6 effectively preserved SARS-CoV-2 RNA as indicated by the CT value of the helicase gene (**Figure 1**). The statistical analysis revealed that there was no significant difference ($p>0.05$) between the CT value of iVTM pH 6 and that of the positive control.

Preliminary validation of iVTM for inactivation of clinical samples containing SARS-CoV-2

In this study, the effectiveness of iVTM (pH 6) as a transport medium for clinical specimens containing SARS-CoV-2 at room temperature storage was investigated. Validation was carried out using RT-qPCR analysis, comparing the CT values of iVTM (pH 6) with those obtained using commercial VTM as the standard. Among the 20 samples collected from suspected COVID-19 patients that tested positive when stored in commercial VTM, all of them also tested positive when stored in iVTM (pH 6) (**Table 4**). This demonstrates the high sensitivity (100%) of iVTM (pH 6) for the tested sample population.

Table 4. RT-qPCR results on clinical specimens collected on Jun Nuo® VTM and iVTM pH 6

RT-qPCR diagnostic test results	Commercial viral transport medium (VTM) n (%)	inactive VTM (iVTM) pH 6 n (%)
SARS-CoV-2 positive	20 (100)	20 (100)
Invalid	0 (0)	0 (0)

Discussion

The storage temperature of clinical specimens in transport medium is one of the factors that can affect the stability of genetic material. High temperatures are reported to accelerate the activity of nuclease enzymes, leading to the degradation of genetic material [19,20]. According to a guideline [4], in an interim guideline, clinical specimens of SARS-CoV-2 RNA require storage conditions at a very low temperature (2°C to -7°C) to maintain the stability of the biological components and their genetic material. These storage conditions must be maintained from the

beginning of the collection, during transportation, and even during long-term storage, prior to conducting clinical specimen testing. However, maintaining low-temperature storage conditions for handling clinical specimens posed a notable challenge for maintaining virus integrity during the COVID-19 pandemic, particularly in a tropical country such as Indonesia.

This study demonstrated that iVTM (pH 6) is a robust medium for the room-temperature storage of clinical specimens, with promising results over a 12-month period. The medium showed no signs of bacterial or fungal contamination and remained stable without significant pH alterations, making it suitable for use in resource-limited settings. In contrast, iVTM (pH 4) was proved less effective for long-term storage, as sulfur-induced RNA degradation was observed, evident by the yellowing of the medium and destabilization of the RNA. These findings highlight the superiority of iVTM (pH 6) over iVTM (pH 4) for preserving the integrity of SARS-CoV-2 RNA at room temperature.

Since SARS-CoV-2 RNA is utilized in RT-qPCR diagnostic testing, maintaining its integrity is crucial to achieving reliable results. Initial validation testing of iVTM revealed that for the sample population used, iVTM's sensitivity (pH 6) is high (100%) in terms of validity. All positive COVID-19 samples tested by RT-qPCR could be correctly identified by the formulation's inactive VTM (pH 6). The iVTM CT value (pH 6) did not significantly affect the CT value of commercial VTM on each detected SARS-CoV-2 gene, according to a statistical study of the relationship between the CT values of positive samples of the two VTM treatments. It can be concluded that the iVTM (pH 6) formulation results are able to show diagnostic results that are not significantly different from commercial VTM.

In the deactivation process, particularly those induced by chemicals, pH stands out as a crucial factor that can influence the stability of viral structural proteins. This phenomenon is likely associated with Histidine H49 and H519 in the SARS-CoV-2 Spike protein. A previous study [13] and [21] has identified these histidines as pivotal switches responsible for inducing alterations in protein conformation in response to shifts in the environmental pH. A study [22] suggested that a pH range of 7.5–8 was favorable for sustaining the viability of SARS-CoV-2. However, a study [23] reported that a pH below 6.8 can trigger conformational changes in the S protein of SARS-CoV-2, potentially leading to denaturation and virus inactivation.

The inactivated VTM formulation used in this study consisted of Guanidine salt, anionic detergents, buffer solution, chelating agent, and nuclease-free water (**Table 1**). Guanidine salt and anionic detergent can be used to inactivate viruses by creating conditions that may break down the SARS-CoV-2 structural protein and reduce the pathogenicity of the virus. The stability of non-covalent bonds in the amino acid chains of protein structures can be disturbed by the chaotropic effect of guanidine salt. Guanidine salt can break protein hydrogen bonds, especially beta sheets (β-sheets) [24,25], disrupt hydrophobic protein interactions [26] and reduce protein disulfide bonds [27]. Guanidine salt in iVTM primarily targets the Spike (S) protein, which facilitates the virus's attachment to the host cell receptor [28]. In addition, anionic detergents destroy the lipid of the virus, especially the enveloped virus group, such as SARS-CoV-2 [29,30]. In more detail, a study [31] explained that the chemical activity of anionic detergents could break the bond interaction between protein-protein, protein-lipids, and lipid-lipid.

Beyond viral inactivation, guanidine salt and a chelating agent work to preserve the integrity of nucleic acids in viral particles. Based on several studies that have been reported, the Guanidine-based viral transport medium, with a pH of around 6–7, could maintain the stability of viral RNA [15,32-34]. As a chaotropic agent, Guanidine can assist in maintaining the integrity of viral nucleic acid by inactivating the nuclease that causes degradation of genetic material [35]. In addition to Guanidine salt, a chelating agent in iVTM also aids in preventing RNA breakdown. By binding to and removing essential cofactors, a chelating agent effectively prevents nuclease activity and preserves the integrity of nucleic acids in biological samples [36]. Therefore, SARS-CoV-2 RNA can be protected from endogenous and exogenous RNase during storage in iVTM (pH 6). In the future, the results of this study may be translated to improve storage conditions for specimens from other viral respiratory infections.

Conclusion

This study demonstrated that iVTM pH 6 is a highly effective VTM for maintaining the stability of SARS-CoV-2 RNA at room temperature. The formulation's ability to preserve RNA integrity without significant alterations in diagnostic performance compared to commercial VTMs highlights its potential for use in resource-limited settings. The robust stability of iVTM pH 6 is attributed to the synergistic effects of its components, particularly guanidine salt and a chelating agent, which collectively inactivate nuclease enzymes and protect RNA from degradation. These results underscore the importance of formulation and especially pH optimization in VTM for ensuring reliable diagnostic outcomes while addressing the safety of the personnels involved in specimen storage and transport, especially in tropical and low-resource regions. This also could be the basis for developing VTM for other clinical specimens, especially those extracted from saliva, nasopharyngeal or oropharyngeal mucus. It is also a viable option to test the compatibility of this formulated iVTM in inactivating the livestock viruses, while preserving their genetic material.

Ethics approval

The protocol for the study using the human samples was approved by the Preclinical Ethics Committee of Universitas Jendral Ahmad Yani, Indonesia (No.02/KEPK/FITKES-UNJANI/II/2022).

Acknowledgments

Certain technical details of this study have been withheld due to ongoing intellectual property registration (P00202214780, submitted date 14 December 2022). The authors acknowledge the support of Directorate of Science and Technology Zones of Institut Teknologi Bandung for facilitating the IP process. The authors would like to thank Biotechnology Research Centre of BRIN (Research and Innovation Agency) and Jasa Kartini Hospital Tasikmalaya. The authors also acknowledge Beti Ernawati Dewi from Faculty of Medicine, Universitas Indonesia, who kindly provided Vero E6 cell line for this study, and to Sugiyono Saputra and his whole genome sequencing team from BRIN for identifying the variant of the virus isolate.

Competing interests

All the authors declare that there are no conflicts of interest.

Funding

This study was funded by LPIK – ITB to KM, through grant LPIK.PN-6-02-2021.

Underlying data

Supplementary data is available at the following link: https://doi.org/10.6084/m9.figshare.29642414.v1.

Declaration of artificial intelligence use

We hereby confirm that no artificial intelligence (AI) tools or methodologies were utilized at any stage of this study, including during data collection, analysis, visualization, or manuscript preparation. All work presented in this study was conducted manually by the authors without the assistance of AI-based tools or systems.

How to cite

Rahmani S, Meitha K, Septiani P, *et al*. Development of an inactivated viral transport medium for diagnostic testing in low-resource countries. Narra J 2025; 5 (3): e2068 - http://doi.org/10.52225/narra.v5i3.2068.

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