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# Learning RNA Isolation on Oral Swab Sample on Bats in Bandar Lampung Urban Areas

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## Abstract

**Background.** Bats are recognized as important reservoir hosts for various zoonotic viruses, including coronaviruses, posing potential public health risks, particularly in urban environments where human–animal interactions are frequent.

**Aims.** This study aimed to evaluate the effectiveness of RNA isolation from oral swab samples of bats captured in urban areas of Bandar Lampung, Indonesia, as a preliminary step for molecular detection of viral pathogens.

**Methods.** Sampling was conducted using mist nets in the University of Lampung area, followed by oral swab collection and preservation in viral transport medium. RNA isolation was performed through four main stages: lysis, binding, washing, and elution. The quality of RNA was assessed using agarose gel electrophoresis, while the quantity was measured using a Qubit Fluorometer.

**Result.** A total of six bats were successfully sampled, consisting of *Cynopterus brachyotis* and *Cynopterus sphinx*. Qualitative analysis revealed a single thin, luminescent band in all samples, indicating suboptimal RNA integrity. Quantitative results showed RNA concentrations ranging from 0.58 to 2.03 ng/µL, with three samples falling within the acceptable range (1.8–2.0 ng/µL), suggesting adequate RNA purity. Variations in RNA quality and concentration were likely influenced by factors such as incubation duration and sample handling, potentially leading to contamination or degradation.

**Conclusion.** In conclusion, RNA isolation from bat oral swabs in urban areas is feasible; however, protocol optimization is necessary to improve RNA integrity and yield. High-quality RNA is essential for reliable downstream molecular analyses, including viral detection and surveillance of zoonotic diseases.

**Keywords:** bats, RNA isolation, oral swab, zoonosis, urban ecosystem, molecular detection

## INTRODUCTION

Bats are the only mammals that can fly because their front limbs are modified into membranes, or what can be called wings. Bat wings are extensions of the body formed from elastic membranes. These wings help with flight and envelop the body (Lekagul & Mc Neely, 1977). In Indonesia, there are two types of bats found: fruit eaters and insect eaters. In terms of ecology, fruit-eating bats act as pollinators, helping spread seeds. Meanwhile, insectivorous bats act as environmental control agents by eating insects that disturb plants. Bats usually live in forests, caves, trees, hollow trees, and the roofs of buildings. Bats are also often found in urban areas (Prasetyo et al., 2011).

The presence of bats in urban areas allows direct contact with humans. This direct contact poses a risk of zoonosis (Wijayanti, 2021). According to the World Health Organization (2020), zoonoses are diseases transmitted from animals to humans. Bats are known as reservoir animals for various viruses and diseases. The COVID-19 pandemic last December is often associated with the virus in bats. Data collection and early detection of coronavirus in bats have never been conducted in Lampung.

The first step in gene expression is RNA isolation. Many copies of gene sequences in the form of messenger RNA (mRNA) cause high gene activity in tissues or cells (Amanda & Cartealy, 2015). As an initial step in coronavirus detection using predictive protocols, RNA isolation is performed. The main principle of RNA isolation is that the tissue is broken down and extracted to produce a cell extract containing RNA and cellular debris. Then the cell extract is purified to contain cell pellets containing total DNA/RNA (Faatih, 2009). The RNA isolation method is a method used to isolate pure RNA through four stages, namely lysis, binding, washing, and elution. To confirm the presence and purity of nucleic acid in the sample, the RNA isolation results will be tested qualitatively and quantitatively. RNA isolation will later be tested qualitatively and quantitatively using agarose gel electrophoresis and the Qubit Fluorometer.

Although bats are widely recognized as reservoirs of zoonotic viruses, molecular surveillance in urban areas of Lampung remains underdeveloped. Previous studies have emphasized the ecological role of bats and their potential association with zoonotic transmission, yet limited information is available regarding the technical feasibility of RNA isolation from bat oral swab samples in Bandar Lampung. This gap is important because RNA quality and quantity determine the reliability of downstream molecular detection, including

RT-PCR-based viral surveillance. Therefore, this study addresses the need for preliminary methodological data on RNA isolation from urban bat oral swabs as an initial step toward monitoring zoonotic pathogens.

This study provides novel baseline data on RNA isolation from oral swab samples collected from bats captured in urban areas of Bandar Lampung, Indonesia. By combining electrophoresis-based RNA quality assessment and Qubit Fluorometer-based quantification, the study demonstrates the feasibility and limitations of using bat oral swabs for preliminary molecular surveillance of zoonotic viruses.

## METHOD

Sampling of bat oral swabs was conducted in the University of Lampung campus area, and molecular detection was performed at the Biotechnology Laboratory, Lampung Disease Investigation Center. This research was conducted under the supervision of Dr. Elly Lestari Rustiati, M. Sc, funded by PIU HETI University of Lampung and in collaboration with Lampung Disease Investigation Center.

### Life trapping and sample collection

Before sampling, the first step was trapping bats using mist nets. Mist nets were installed between trees where bats were active. Captured bats were orally swabbed using a cotton swab by wiping the bat's mouth thoroughly, and then the cotton swab was inserted into the VTM tube.

### Lysis

A total of 5.6 µl carrier RNA and 560 µl AVL buffer were added to a microtube, and 140 µl of oral swab sample was added. The solution was vortexed for 15 seconds and then incubated at room temperature for 10 minutes.

### Binding

After incubation, 560 µl of absolute alcohol was added to the solution and homogenized for 15 seconds. 630 µl of solution was transferred to the spin column and centrifuged at 10,000 rpm for 1 min at 4-8 °C. The supernatant solution was discarded, and the collection tube was replaced. The remaining solution was given the same treatment as before.

### Washing

This stage uses two buffers as a wash, namely AW1 buffer and AW2 buffer, each of which is up to 500 µl, which is done in stages.

**Elution**

The spin column containing the solution was transferred to a microtube, 60 µl of AVE buffer was added, and then the microtube was centrifuged at 10000 rpm at 4-8 °C for 1 minute. The spin column was discarded, and the microtube containing the isolation results was stored in a freezer at -20 ºC.

**RNA Quality test**

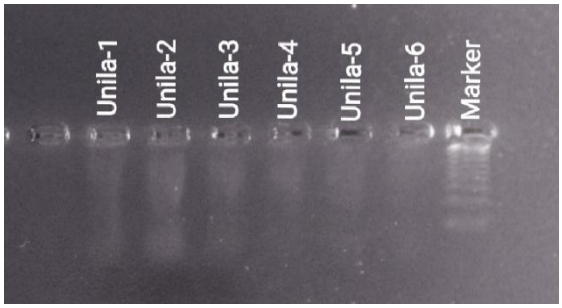
A total of 1 g of agarose powder and 100 mL of TAE buffer were placed in an Erlenmeyer flask and heated in an oven for 3 minutes. Added 10 µl of SYBR dye, then stirred. The solution was placed in a chamber with a comb installed for the wells, and the gel was allowed to harden before the comb was removed. Take as much as 2 µl loading dye and 5 µl sample, then insert into the well. In the last well, the marker is inserted. The chamber was connected to an electric current via a power supply cable at the cathode and anode, with a voltage of 100 V and a current of 400 A for 15 minutes.

**RNA Quantity test**

RNA quantity testing was performed using a Qubit Fluorometer. The kit used is the Invitrogen Kit (QubitTM RNA BR Assay Kit). The working solution and sample were placed in a special microtube, then tested in the Qubit Fluorometer in stages. The quantity test results will be visible on the layer.

## DISCUSSION

Six bats were captured, consisting of five species of *Cynopterus brachyotis* and one species of *Cynopterus sphinx.* The results of the RNA quality test, carried out by electrophoresis (Figure 1), showed a single, faint band. A good RNA quality test shows two bands: 18S and 28S rRNA (Farrel, 1993). The lack of sharpness of RNA band luminescence on agarose gels can be caused by the incubation time and the relatively low concentration of RNA in the isolation results (Wardi et al., 2021).




**Picture 1. RNA Quality Test Results**

**Table 1. RNA Quantity Results**

| Sample | Concentration (ng/µl) |
|---|---|
| Unila 1 | 1,98 |
| Unila 2 | 0,58 |
| Unila 3 | 1,55 |
| Unila 4 | 1,93 |
| Unila 5 | 1,75 |
| Unila 6 | 2,03 |

RNA concentration can affect the results of molecular analysis of a sample. Qualitative test results are not always reliable because several factors can affect them. Quantitative tests were also performed on bat oral swab samples. According to Sambrook and Russell (2001), RNA with a good concentration is in the range of 1.8-2.0. Samples 1, 4, and 6 showed concentrations between 1.8 and 2.0, indicating that the three samples contained good RNA concentrations (Table 1). If the concentration results are lower or higher, the sample still contains contaminants. Protein is a macromolecule consisting of several amino acids that have certain functions. One of the molecules that can cause RNA contamination during RNA isolation is protein. The quantity test using the Qubit Fluorometer is considered more sensitive because it can measure the specific nucleic acid of interest. The working principle of the Qubit Fluorometer is a fluorescent dye that uses a fluorometer with high sensitivity to detect the concentration of the fluorescent dye added to the sample (Pratiwi & Widodo, 2020).

| Aspect | Analysis |
|---|---|
| State of the Art | Current zoonotic disease surveillance recognizes bats as important reservoirs of viral pathogens, including coronaviruses. Molecular detection usually requires high-quality RNA from biological samples such as oral swabs. RNA isolation, followed by qualitative assessment using agarose gel electrophoresis and quantitative measurement using Qubit Fluorometer, is an established preliminary step for downstream viral detection. |
| Research Gap | Early molecular surveillance of bat-borne viruses in the urban areas of Bandar Lampung remains limited. Specifically, there is little baseline information on the feasibility and quality of RNA isolation from bat oral swab samples collected in urban ecosystems. Existing studies often focus on bat ecology or on zoonotic risk in general, but not on the technical quality of RNA extraction from local bat samples as a preparatory step for viral detection. |
| Novelty | This study provides preliminary evidence that RNA can be isolated from oral swabs of urban bats in Bandar Lampung. It reports both qualitative and quantitative RNA profiles from *Cynopterus brachyotis* and *Cynopterus sphinx*, offering baseline methodological data for future molecular surveillance of zoonotic pathogens in Lampung. |

## CONCLUSIONS

Our conclusion is that the RNA quality test on all oral swab samples showed one thin luminescent band. In the quantity test, 3 samples showed good concentration. RNA with good quality and quantity will produce more accurate amplification results

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