>TruTip® Nova for Hamilton®

Purification of Viral RNA from Saliva using Akonni TruTip® Nova on the Hamilton STARlet Low-Cost, In-Tip Chemistry

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ABSTRACT

The Serology and Diagnostics High Throughput Facility at the University of Ottawa, and Akonni Biosystems developed a simple and efficient TruTip Nova automated extraction method to isolate influenza RNA from OMNIgene DNA + RNA saliva collection kits. Akonni's new in-tip chemistry is paired with the advanced, proven and reliable Hamilton Microlab® STARlet liquid handling workstation without special hardware accessories such as heaters or magnets, nor did the method require changes to the instrument firmware. Viral RNA, extracted from saliva using TruTip Nova, showed comparable (or better) sensitivity to Qiagen spin columns. Repeatability studies demonstrated high precision with low standard deviations and no cross-contamination.

INTRODUCTION

The purification of viral RNA from saliva is the necessary first step to molecular-based testing. Though viral RNA can be isolated from numerous clinical sample types, saliva is emerging as the sample matrix of choice for many research and clinical laboratories. Saliva offers high-quality RNA similar to that obtained from nasopharyngeal swabs, but with a less invasive collection method. Conventional magnetic bead methods require more deck space than is available on the STARlet. Akonni Biosystems developed TruTip Nova to dramatically reduce in-tip chemistry costs and offer an economical means of saving on deck space, pipette tips, instrument motion (wear), and protocol steps (time) as compared to conventional extraction methodologies, which predominantly rely on magnetic beads or spin columns.

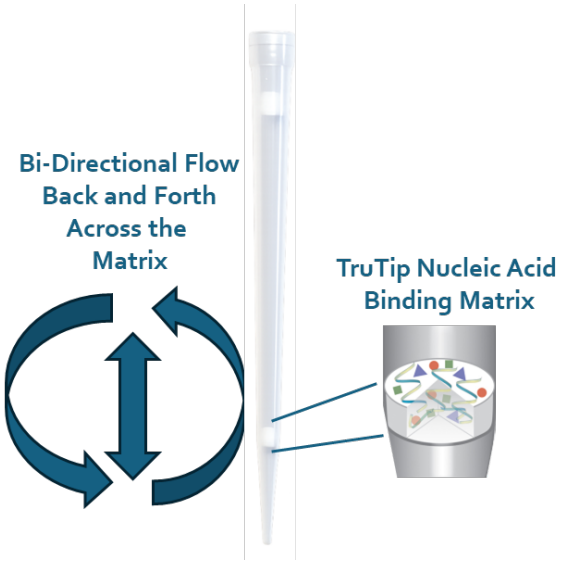


Figure 1. TruTip Nova Diagram

TruTip technology uses a porous binding matrix embedded in a pipette tip (Figure 1), eliminating the need for costly vacuum filtration, centrifugation or magnetic rod systems. The purified sample is free of inhibitors and contaminants and ready for downstream molecular analysis. Automating the extraction process on the Hamilton Microlab STARlet platform offers a dependable, cost-effective solution for high-throughput workloads. The flexible platform can be customized for specific user requirements and workflows. TruTip Nova's economical workflow allows ample remaining deck space to integrate upstream or downstream processes in the same run. In the following studies, we have demonstrated the high precision of TruTip Nova in processing viral RNA from low-volume saliva samples and its advantages. The method developed for these studies allows for multiples of 8 samples up to 96 samples.

Akonni® Biosystems

MATERIALS AND METHODS

Hamilton STARlet equipped as follows (deck layout in Figure 2)**:**

8x 1mL pipetting channels
96-channel Multi-Probe Head
2 x Framed Tip Rack (FTR) Landscape Carrier (Part No. 182085)
4 x Sample Carriers (SMP_CAR_24_A00) (Part No. 173400)
2 x Deep Well Plate Landscape Carrier Carriers (Part No. 182090)
96-Well Tip Adapter (Part No. 182040)

Other Consumables:

12-well Troughs: Axygen® Multiple Well Reagent Reservoir with 12-Channel Trough, High Profile (Corning, cat# RES-MW12-HP-SI)
96-well Plate: Clear V-Bottom 2 mL Deep Well Plate (Fisher Scientific, cat# 07-200-701)
1000µL filter tips: CO-RE® II Conductive Tips (Hamilton, cat# 235905)
Round-bottom low-profile 96-well plate: Round Bottom Microplate With Lid (Fisher Scientific, cat# 07-200-760)
Saliva Tubes: OMNIGene Saliva DNA and RNA Collection (DNA Genotek, cat# OMR-610)

Akonni TruTip Extraction Viral RNA Saliva Kit Consumables:

1 mL Hamilton TruTip Nova tips (rack of 96) (Akonni, cat# 300-11144)
Akonni Lysis and Binding Buffer D
Akonni Wash Buffer D
Akonni Wash Buffer E
Akonni Elution Buffer A2
95% ethanol and acetone (provided by user)

SAMPLE, REAGENT, AND WORKSTATION SETUP

OMNIgene-preserved saliva samples were incubated for 1 hour in a water bath at 50°C, as directed by the manufacturer. All carriers and consumables were placed onto the Hamilton STARlet worktable, as shown in Figure 2. Reagents were added to the reagent troughs. OMNIgene tubes are uncapped and placed in sample carriers on the deck (see Figure 2). If needed, samples can be pre-transferred to a 96-well deep-well plate and placed on the deck in the appropriate position.

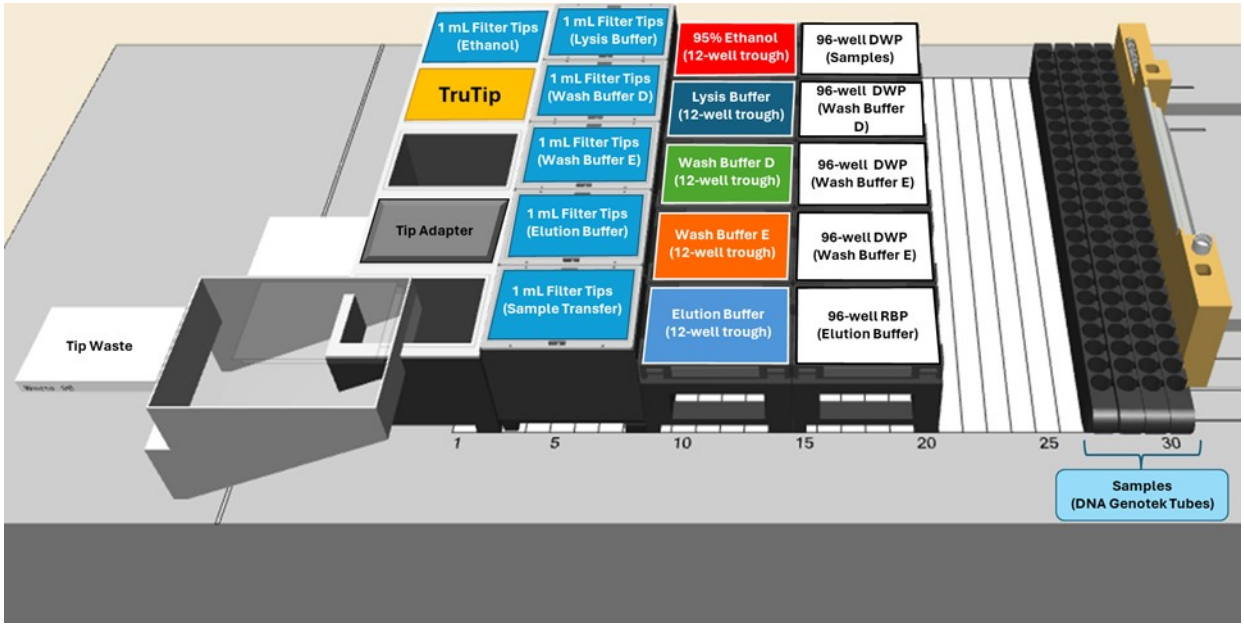
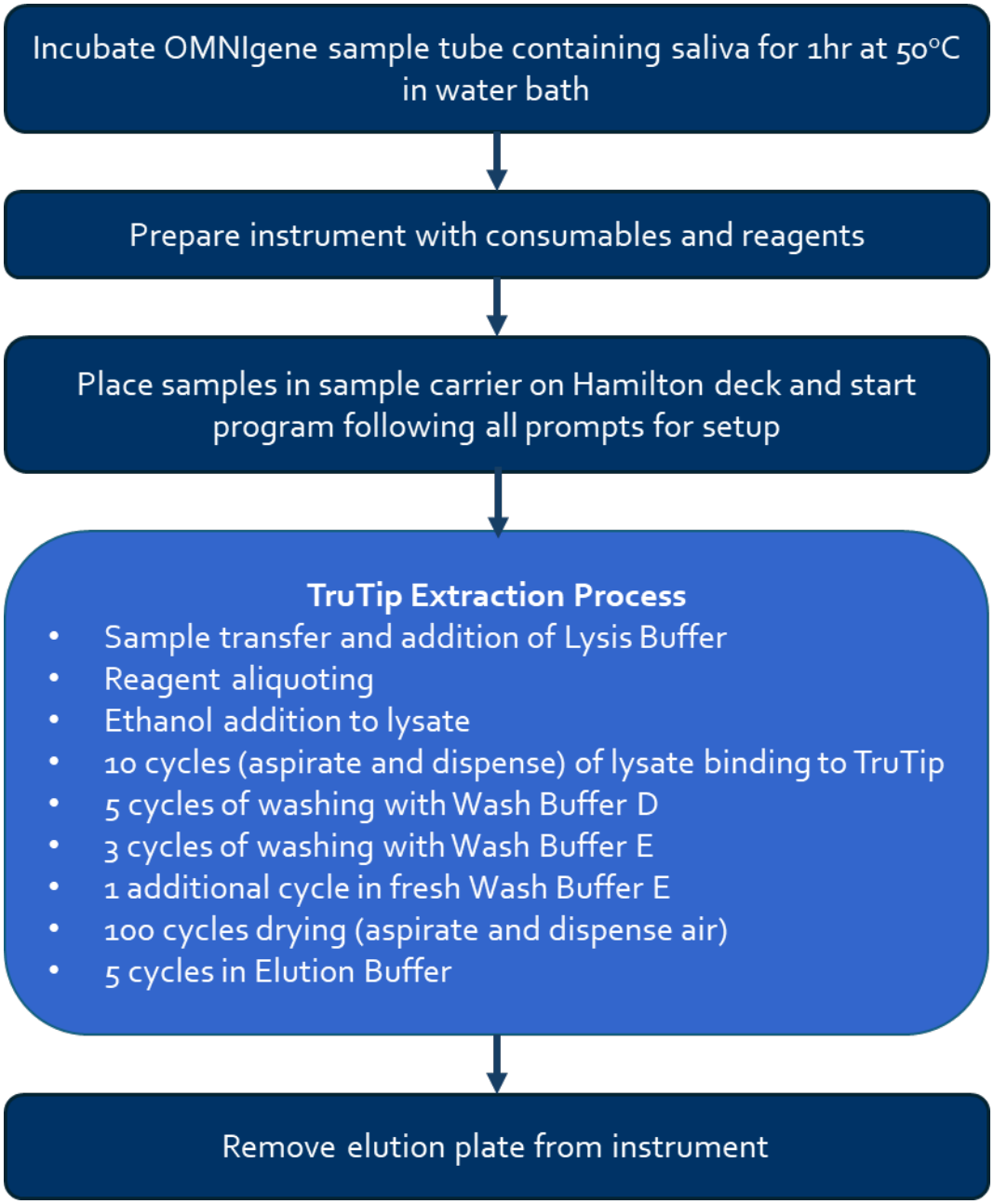


Figure 2. Hamilton STARlet deck layout for the extraction of up to 96 samples. DWP: Deep-well plate, RBP: Round-bottom plate.

WORKFLOW OVERVIEW



RESULTS AND DISCUSSION

Repeatability and Cross-Contamination Susceptibility

Reproducibility using the automated Hamilton TruTip Nova Nucleic Acid Purification from Saliva Kit was compared directly to manual extractions using Qiagen's QIAmp Viral RNA Extraction kit (Qiagen, cat# 52904) using a 140 µL input for both methods. Saliva samples were collected in OMNIgene saliva collection tubes (OMR-610), then pooled and divided into two homogenous replicates. One replicate received no virus (Sample A) and one replicate was spiked with Influenza A H5N1 (strain A/turkey/Ontario/1966) to a titer of 316 $TCID_{50}$/mL (Sample B). Two rounds of automated and manual extractions were performed on individual aliquots of Sample A and Sample B (88 and 96 respective total for automated and 24 and 24 respective total for manual). The second round of automated extractions employed a checkerboard pattern of sample plating to identify potential cross-contamination. Extraction methods were compared via detection of Influenza A Matrix gene and control human RNAseP (RPP30) RNA using real-time reverse-transcriptase PCR assay (Luna® Universal Probe One-Step RT-qPCR Kit, NEB). Figure 3 shows the Influenza A H5N1 RNA recoveries (in blue) and RPP30 recoveries (in red) of the two

RESULTS AND DISCUSSION (CONT'D)

extraction methods from pooled saliva with no false positives detected for any of the Influenza-negative samples. The automated processing time for TruTip for 96 samples was 70-75 minutes compared to approximately 2 hours for 24 samples for the manual QIAmp Viral RNA Extraction kit. The TruTip extraction process resulted in better precision and comparable recovery compared to the QIAmp Viral RNA Extraction Kit.

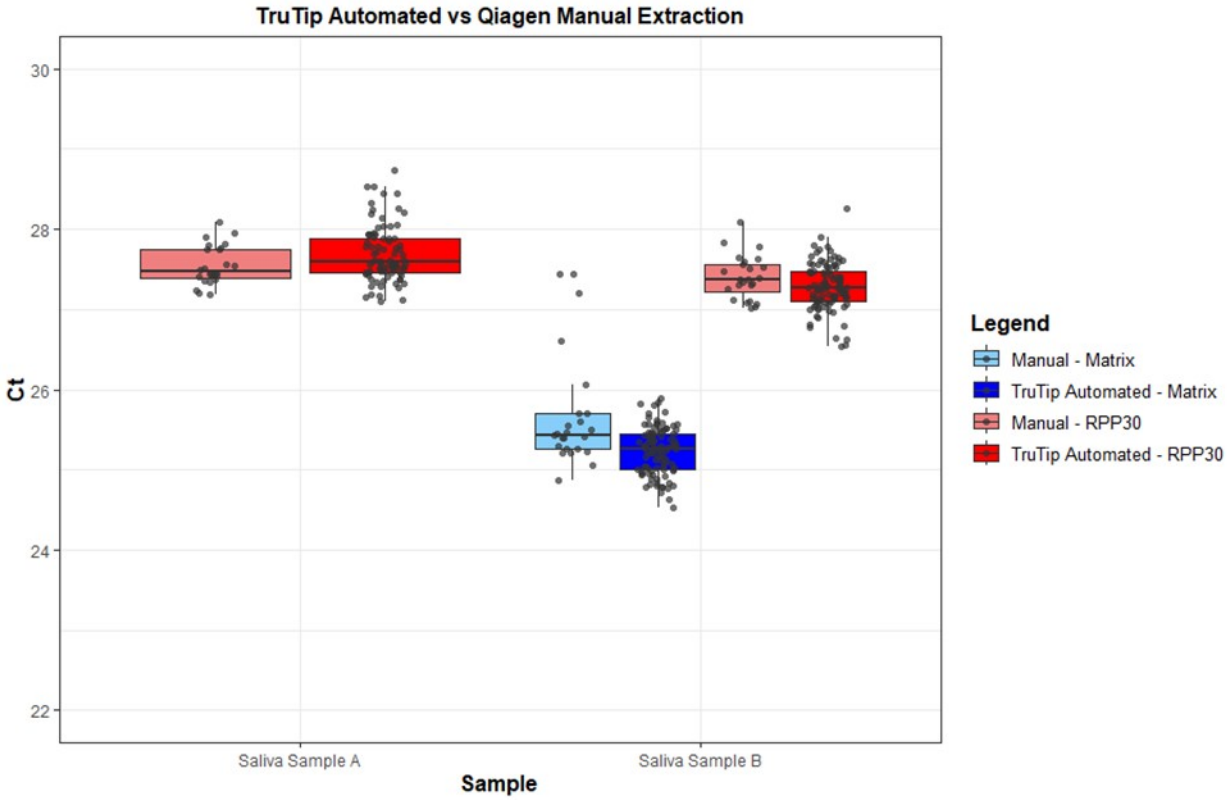


Figure 3. Comparison between TruTip Nova and QIAmp Viral RNA extraction recoveries from pooled saliva without Influenza A H5N1 (Sample A) and with Influenza A (Sample B, Matrix gene). Replicate extractions of 140 µL pooled saliva samples were extracted using TruTip Nova (n=88 for Sample A and n=96 for Sample B) and the QIAmp kit (n=24 for Sample A and n=24 for Sample B). RPP30 is the internal control.

Sensitivity

The sensitivity of these same two methods (TruTip Nova and QIAmp Viral RNA Extraction Kit) were also compared across an Influenza A titer dilution series ranging from 31600 $TCID_{50}$/mL to 0.316 $TCID_{50}$/mL, with extractions of each dilution point run in triplicate for both methods. All dilutions and replicates for the TruTip Nova were run simultaneously on the Hamilton STARlet. While the two methods show comparable performance, at the lowest titer TruTip Nova showed higher concentration (lower Ct) and greater precision than the manual QIAmp Viral RNA Extraction Kit.

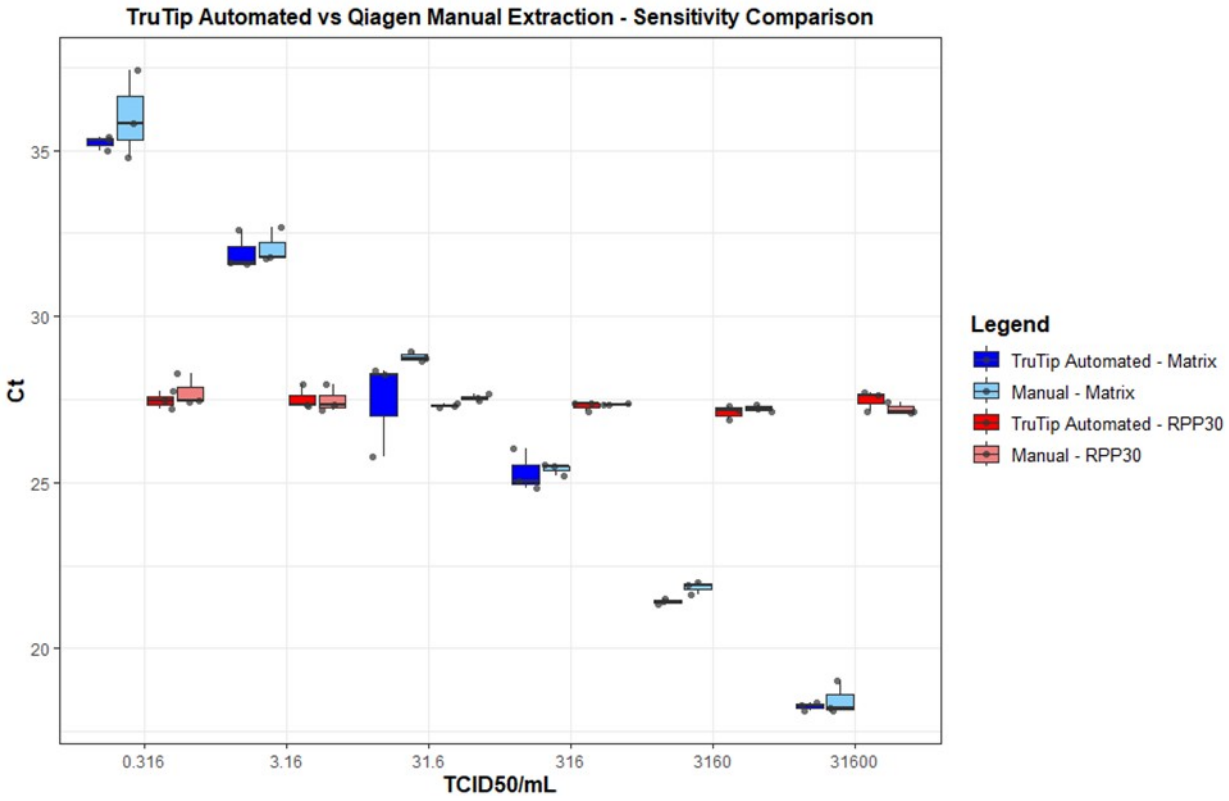


Figure 4. Sensitivity comparison of Akonni's TruTip Nova with QIAmp Viral RNA Extraction Kit. Blue boxes represent the Influenza A H5N1 Matrix gene and the red boxes represent the RPP30 internal control. The data illustrates as good or better performance of the automated TruTip method (particularly at the lowest titer, 0.316 TCID50/mL) compared to the QIAmp Viral Extraction Kit.

Conclusion

This data demonstrates that TruTip Nova can repeatedly isolate viral RNA from saliva at low titers while saving on pipette tips, deck space, instrument motion (wear), and protocol steps (time) using an in-tip chemistry innovation that offers dramatic cost savings. The workflow for high-throughput extraction of viral RNA from saliva is greatly simplified using OMNIgene Collection Kits with TruTip Nova on the Hamilton Microlab STARlet system with extraction of up to 96 samples in less than 75 minutes. This kit is currently released for RUO.

Summary of Significance

⇒ In-tip chemistries offer savings in pipette tips, deck space, instrument wear, and tip exchanges (steps)
⇒ Historically, in-tip chemistries have been more expensive than magnetic bead and spin columns; TruTip Nova changes that paradigm
⇒ TruTip Nova does not require additional hardware accessories or firmware updates
⇒ Despite the competitive cost and simplified and efficient workflow, TruTip Nova offers comparable repeatability and sensitivity to an established method for isolating RNA from saliva
⇒ With fewer tip exchanges and protocol steps, 96 samples can be processed in less than 75 minutes
⇒ Conventional magnetic bead approaches require larger deck space than the STARlet offers for processing 96 samples simultaneously and typically require 2hrs of processing time
⇒ Fully-automated protocol for viral RNA purification from saliva allows users to set up and walk away