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RNA extraction from milk for HPAI surveillance

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We use this protocol and it's working

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Abstract

This is an RNA extraction method that isolates HPAI RNA segments from commercially-available milk samples. The protocol involves processing with a MagMax RNA extraction kit on a Kingfisher instrument. Then, the samples are concentrated using a Zymo Clean and Concentrate kit. The total RNA yields should be suitable for performing qPCR. After trying other methods involving centrifugation, filtration, and trizol, we determined this method to be the easiest and most effective.

Materials

MagMax Wastewater Ultra x96 kit
Zymo RNA Clean and Concentrator-5 kit
ThermoFisher TurboDNase kit
Heat block
Lab plasticware

Troubleshooting

Safety warnings

Performing this protocol with unpasteurized milk from an infected cow might expose the user to active flu virus. Please use pasteurized samples or take appropriate precautions.

RNA extraction

1 Use the MagMax Wastewater Ultra x96 kit[1] (or comparable magnetic bead isolation kit[2-5]) on the ThermoFisher Kingfisher Apex (or comparable instrument). For each sample, load 400 uL of pasteurized milk or heavy whipping cream into the binding plate.

2 Elute in 50 uL of elution solution or nuclease-free water.

Optional Sample Concentration and Cleaning

3 Pool the eluate from 6 extraction replicates of the same sample.

4 Take 200 uL forward into the DNase treatment. Using ThermoFisher TurboDNase, combine 200 uL of pooled eluate, 22 uL 10X TurboDNase buffer, and 2 uL TurboDNase.

5 Incubate at 37 C for 30 minutes.

6 Use a Zymo RNA Clean and Concentrator-5 kit[7] to clean and concentrate the sample. Elute this into 30 uL final volume of nuclease-free water for downstream analysis.

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