applied biosystems

Evaluation of GenoTubes for transport, storage and extraction of nucleic acids from Porcine Oral Fluids

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ABSTRACT

Porcine Oral fluids are currently transported and stored in a refrigerated environment, which adds to the cost of shipping and also has a significant environmental impact. In this experiment, we attempt to use GenoTubes to transport Oral Fluids from the producer to the diagnostic lab.
The GenoTube is a non-invasive nasal swab for collection of DNA from the nostril of cattle, horses and other animals. With its proprietary drying system, it enables easy collection of animal diagnostic samples. Due to fast drying, the collected sample is conserved and helps send a high quality sample to the lab. Once dried, collected samples can be transported and stored without refrigeration, which makes transportation simple and cost effective. In the lab the samples can be easily reconstituted and used for numerous analyses.
Thirteen oral fluid samples (North American PRRSV positive and negative) were acquired and tested. They were processed using the standard protocol described using the MagMAX Pathogen RNA/DNA kit (cat# 4462359 www.thermofisher.com) as well as using GenoTubes. The Spuren, Evidence and Livestock (cat# 9062010 www.thermofisher.com) GenoTubes were evaluated during this experiment.

INTRODUCTION

The GenoTube is a non-invasive nasal swab for collection of DNA from the nostril of cattle, horses and other animals. The GenoTube is easy to use and can be handled by even novice users. With its proprietary drying system, it enables easy collection of animal diagnostic samples. Due to fast drying, the collected sample is conserved and helps send a high quality sample to the lab. Once dried, collected samples can be transported and stored without refrigeration, which makes transportation simple and cost effective. In the lab the samples can be easily reconstituted and used for numerous analyses. A variety of different sample types can be taken with the GenoTube Livestock, including: nasal swabs, saliva, blood, and feces samples.
In this experiment, we are evaluating the use of GenoTubes with Oral Fluid samples from pigs. The Oral Fluids are collected with the help of a cotton rope hung in the pig pen. The pigs chew on the rope and deposit Oral Fluids that are then collected in a container and shipped to the diagnostic lab.
The samples are shipped and stored at refrigerated temperatures to prevent fungal growth. However, the storage is a financial burden on the lab.
Using the GenoTube to ship and store Oral Fluid samples, we eliminate the need for shipping and storing these samples at refrigerated temperatures. Instead, these samples can be shipped and stored at room temperature until they're ready for use.

MATERIALS AND METHODS

Thirteen oral fluid samples of unknown status were acquired from Iowa State University's veterinary diagnostic lab. These samples were shipped on ice packs and immediately processed to ensure freshness.
Three types of Genotubes were evaluate (Fig 1.). This was done to determine which one of the three would provide the most comparable results to the control process of Oral Fluid extraction using the MagMAX Pathogen RNA/DNA kit protocol for extraction of nucleic acids from Oral Fluids (Fig 2).

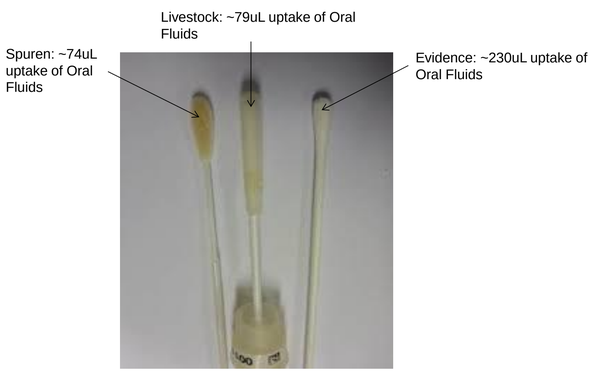
Run in n=1 replicates, Oral fluids were also processed the normal way as a positive control (Fig 3.) Sample preparation was performed on the KingFisher Flex magnetic particle purification system (www.thermofisher.com) using the Pathogen RNA/DNA kit (Cat# 4462359, www.thermofisher.com) high volume protocol.
Swabs were dipped in the Oral Fluid samples and swirled around for 5 seconds. They were then placed in desiccant tube and dried for 24 hours at room temperature to simulate overnight shipping. After 24 hours, the swab heads were broken off, and swabs were resuspended in 1000 uL of Lysis Concentrate with Xeno (20K copies/rxn) and CarrierRNA (2uL/rxn). Tubes were vortexed for 5 seconds and then left standing for 5 minutes.

After 5 minutes, 600 uL of lysate was removed and used as sample. In the sample plate, 600 uL lysate, 350 uL Isopropanol and 20 uL bead mix was added and mixed. The samples were further purified using 2 washes of 300 uL Wash 1 and 2 washes of 450 uL Wash 2. The purified nucleic acid was eluted in 90 uL of Elution Buffer. Elution was processed with the VetMAX NA and EU PRRSV reagents (cat# 4468465, www.thermofisher.com) on the ABI 7500 Fast Real Time PCR instrument (Cat # 4351107, www.thermofisher.com).

A 5 day stability study was also performed (Fig 4.) to determine how long a sample can provide reliable results when stored on a GenoTube for up to 5 days at room temperature. The sample preparation and PCR amplification and analysis was performed in the same manner as the experiment above.

RESULTS

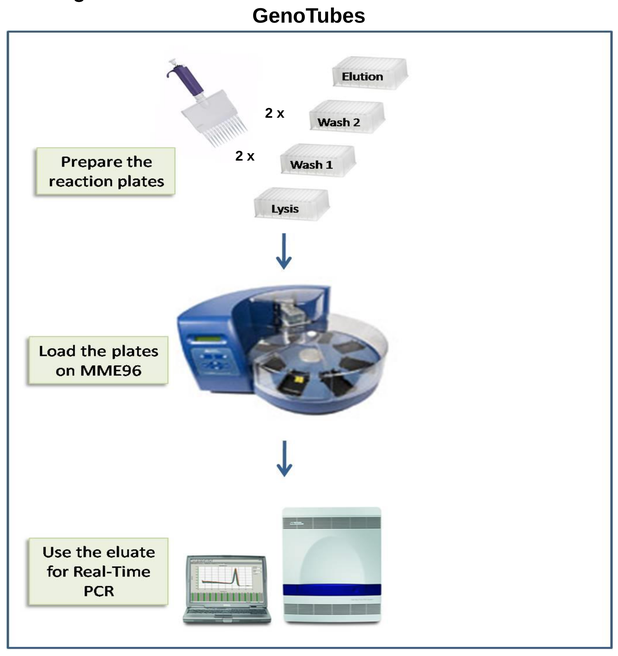
Figure 1. GenoTube Types



Three types of GenoTubes were used in this set of experiments. These were compared to determine which would be best for recovery of pathogen RNA from Oral Fluid samples.

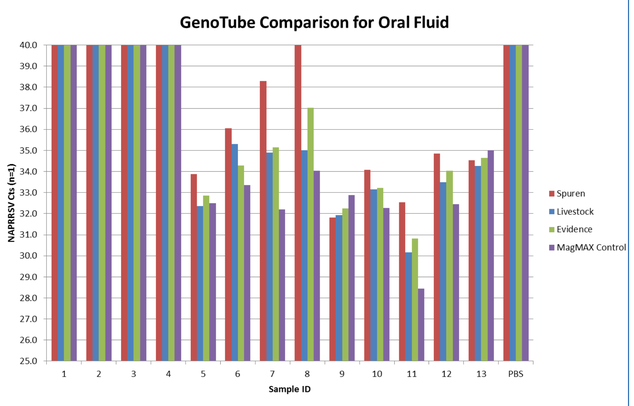
To measure uptake by the swab heads, sample volumes were measured before and after the swab was dipped into the sample. The difference between the sample volume before and after the dipping determined the volume that was absorbed by the swab head. The amount of Oral Fluid taken up by the swab was thought to factor in to the final yield of the sample.

Figure 2. Workflow for nucleic acid extraction from GenoTubes



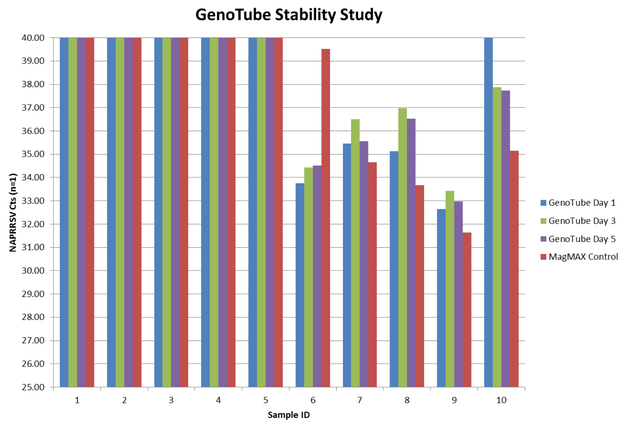
- Swabs were dipped in Oral Fluids and swirled around for 5 seconds
- They were then placed in desiccant tube and dried for 24 hours at room temperature.
- Sample prep was performed on the KF Flex using the Pathogen RNA/DNA high volume protocol.
- After 24 hours, the swab heads were broken off, and swabs were resuspended in 1000 uL of Lysis Concentrate with Xeno (20K copies/rxn) and CarrierRNA (2uL/rxn)
- Tubes were votexed for 5 seconds and then left standing for 5 minutes.
- After 5 minutes, 600 uL of lysate was removed and used as sample.
- Reagent volumes: 600 uL lysate, 350 uL Isop and 20 uL bead mix. Wash 1 = 300 uL, Wash 2 = 450 uL., Elution = 90 uL.
- Elution was processed with the VetMAX NA and EU PRRSV reagents on the ABI 7500 Fast.

Figure 3. GenoTube Comparison for Oral Fluids



Thirteen oral fluid samples (North American PRRSV positive and negative) were acquired and tested. They were processed using the standard protocol described using the MagMAX Pathogen RNA/DNA kit (cat# 4462359 www.thermofisher.com) as well as using GenoTubes. Results are discussed in the conclusion.

Figure 4. GenoTube Room Temperature Stability Study



A 5 day stability study was performed to determine if the samples stored for 1 day, 3 days and 5 days using GenoTubes would give similar results.

10 Oral Fluid samples (5 positive and 5 negative for NAPRRSV) were acquired and processed using the control MagMAX protocol to set a baseline, and GenoTubes. Each Genotube sample was divided into 3 and designated Day 1, Day 3 or Day 5.

All GenoTubes were swirled in the sample for up to 10 seconds and then stored in their desiccant tubes for either 1 day, 3 days or 5 days and then processed using the GenoTube MagMAX protocol discussed in the previous experiment.

Results show that the samples are stable at room temperature for up to 5 days and there is very little degradation during storage. Sample 6 seems to be an outlier for the MagMAX control and will be ignored.

Figure 5. Drying Time for GenoTube Livestock Swab

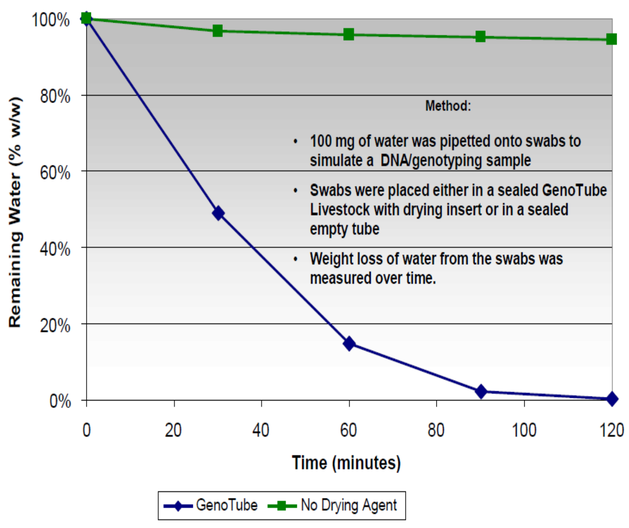


Figure 6. Drying Insert in the GenoTube Livestock Tube

GenoTube swabs were tested to determine how fast they dry.
100 mg of water was deposited onto the swab head and the tube was weighed.

The tube was then inserted into the drying insert and weighed again every 20 minutes. The graph (Fig 5.) shows that the water was completely absorbed by the drying agent (Fig 6.) in 2 hours.

The benefits of drying are as follows:

- Fast drying has a positive Effect on the DNA Yield
- Drying within 4 hours yields up to 8 times more DNA compared to drying within 25 hours
- Active drying unlike passive drying is independent from the climatic conditions
- Air drying is no alternative as the likelihood of confusion and contamination are too great

Figure 7. Shelf Life for GenoTube Livestock Swab

The GenoTube Livestock product has a shelf life of 3 years, after which, the desiccant loses it's ability to dry the sample effectively.

CONCLUSIONS

The GenoTube Livestock is a non-invasive tool to collect nucleic acid from Oral Fluids. The GenoTube is easy to use and can be handled by anyone. After the swab is placed back in the tube, the sample is dried and the nucleic acid in the sample is stabilized. The tube can be transported and stored under normal storage conditions (room temperature) without refrigeration. Nucleic acid contained in the sample remains stable for years. The amount of nucleic acid is sufficient for testing with real time PCR using the ABI 7500 Fast RT-PCR machine.

Sample preparation using the GenoTube can be performed using the protocol described in the Materials and Methods section of this poster.

Based on the data, the best GenoTube type for transport and storage of Oral Fluids is the Livestock GenoTube. The results in Fig. 3 show the Livestock swab as generating the best results compared to the Spuren and Evidence swabs. It is not quite comparable to the MagMAX control method of isolation due to the fact that the control uses 300 uL of Oral Fluids as a starting sample, thus increasing the amount of potential target that could be recovered during isolation of nucleic acid.

The stability study (Fig 4.) shows that samples stored in the GenoTube can remain fairly stable for up to 5 days. Some degradation of signal is observed, but overall, the sensitivity (# of positive vs. negative calls) remains intact. Sample 10 had an issue with the control method, but further testing (data not shown) revealed it to be a positive sample.

REFERENCES

1. **The importance of drying performance for the preservation of DNA**
Alex M Garvin1, Ralf Holzinger1, Florian Berner2, Walter Krebs2, Bernhard Hostettler3, Elges Lardi3, Christian Hertli3, Roy Quartermaine3, Christoph Stamm3 1 Confarma France SARL, Zone Industrielle Canal d'Alsace, 68490 Hombourg, France. 2 ZHAW Zurich University of Applied Sciences, Institute of Chemistry and Biological Chemistry, Einsiedlerstrasse 31, 8820 Wädenswil, Switzerland. 3 Prionics AG, Wagistrasse 27a, 8952 Schlieren, Switzerland.

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TRADEMARKS/LICENSING

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