Viral detection with GenoTube Livestock swabs in a wild bird surveillance study

Introduction

The Applied Biosystems™ GenoTube Livestock Swab is a noninvasive device for sample collection (liquid swab) and transportation. The GenoTube swab is easy to use and can be handled by novice users. Once the sample is collected, it is conserved by a fast-drying desiccant that helps ensure a high-quality sample. Once dried, the collected samples can be stored and transported at room temperature without refrigeration, making transportation simple and cost-effective without compromising sample quality. In the laboratory, the samples are easily reconstituted and can be used for numerous analyses and tests. A variety of different sample types can be taken with the GenoTube swab, including nasal, oral, blood, cloacal, and fecal.

Here we compare the use of GenoTube swabs and standard cotton swabs to obtain cloacal samples from wild birds. Poultry diseases cause significant economic losses in poultry industries worldwide. Avian adenovirus and astrovirus are two of the viral pathogens that cause severe diseases in domestic and wild birds. Wild birds are reported to play an important role in the evolution, maintenance, and spread of avian viral pathogens. Therefore, a complete and reliable solution—from sample collection to pathogen detection—ensures effective implementation of surveillance for these viruses in wild birds.

Materials and methods

- Applied Biosystems™ MagMAX™-96 Viral RNA Isolation Kit
- Applied Biosystems™ MagMAX™ Express-96 Deep Well Magnetic Particle Processor
- Applied Biosystems™ 7500 Fast Real-Time PCR System
- Applied Biosystems™ AgPath-ID™ One-Step RT-PCR Reagents

Cloacal swabs from 40 wild birds were collected using GenoTube swabs or standard cotton swabs. The samples were processed, and nucleic acid was extracted using the MagMAX-96 Viral RNA Isolation Kit and the MagMAX Express-96 system.

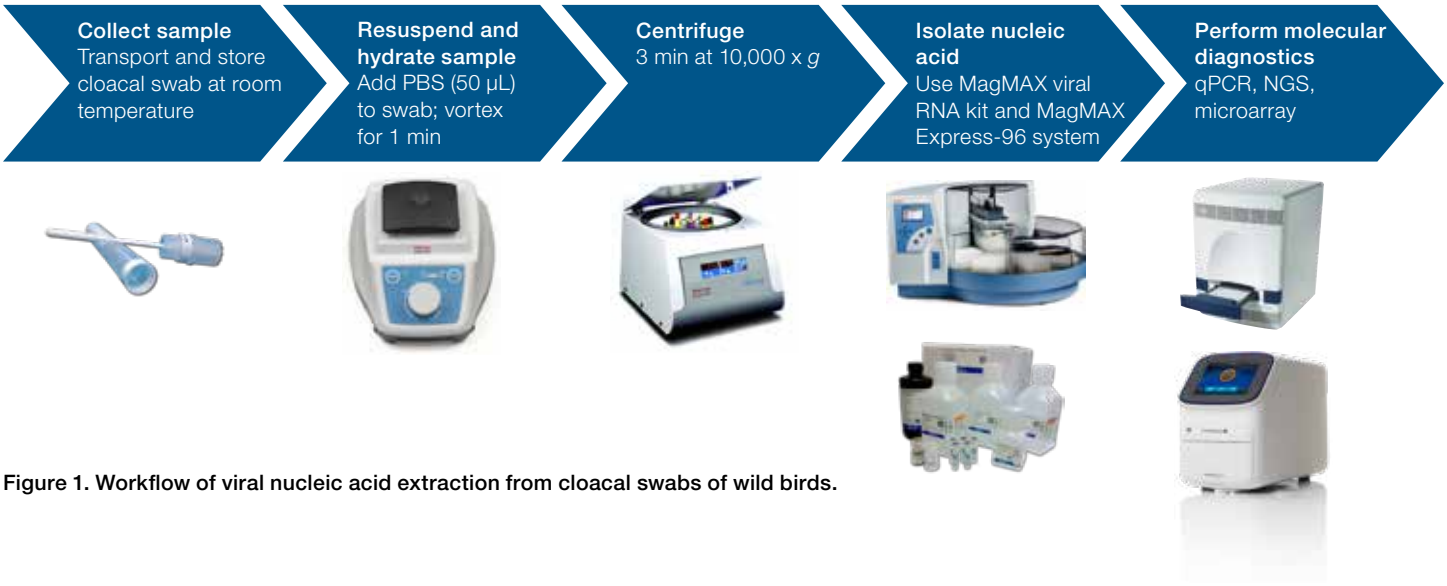

Figure 1. Workflow of viral nucleic acid extraction from cloacal swabs of wild birds.

Table 1. Comparison of PCR results from using GenoTube and cotton swabs.

	Adenovirus		Astrovirus	
Total number of birds sampled = 40	**Positive**	**Negative**	**Positive**	**Negative**
GenoTube swab	16	24	2	38
Average C_t ± SD	30.9 ± 6.5	≥45	37.4 ± 0.2	≥45
Standard cotton swab	6	34	0	40
Average C_t ± SD	32.5 ± 5.7	≥45	NA	≥45

Cloacal swab extraction with MagMAX-96 Viral RNA Isolation Kit

The nucleic acid extraction workflow using the MagMAX-96 Viral RNA Isolation Kit on cloacal samples is shown in Figure 1. The 40 cloacal samples collected using the GenoTube swabs and standard cotton swabs were reconstituted in 50 µL phosphate-buffered saline (PBS), and used for extraction on the MagMAX Express-96 system. The extracted nucleic acid was stored at –70°C and evaluated for viral pathogens by qPCR.

Adenovirus and astrovirus detection by qPCR

Extracted nucleic acid samples were tested for viral pathogens (adenovirus and astrovirus) by qPCR. Results (average C_t values) from using GenoTube swabs and normal cotton swabs were compared.

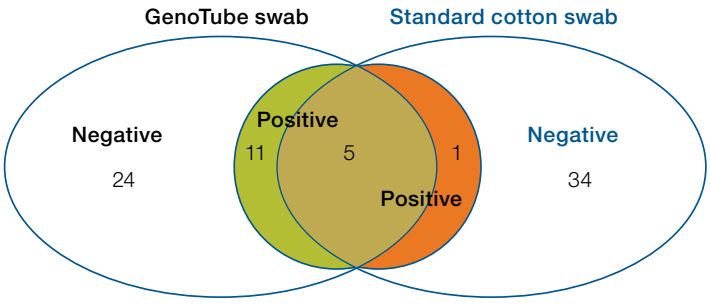

Figure 2. Venn diagram showing results for adenovirus detection using GenoTube swabs vs. cotton swabs in cloacal samples from wild birds. Positive samples are represented in the colored circles. Negative samples are shown in the white areas.

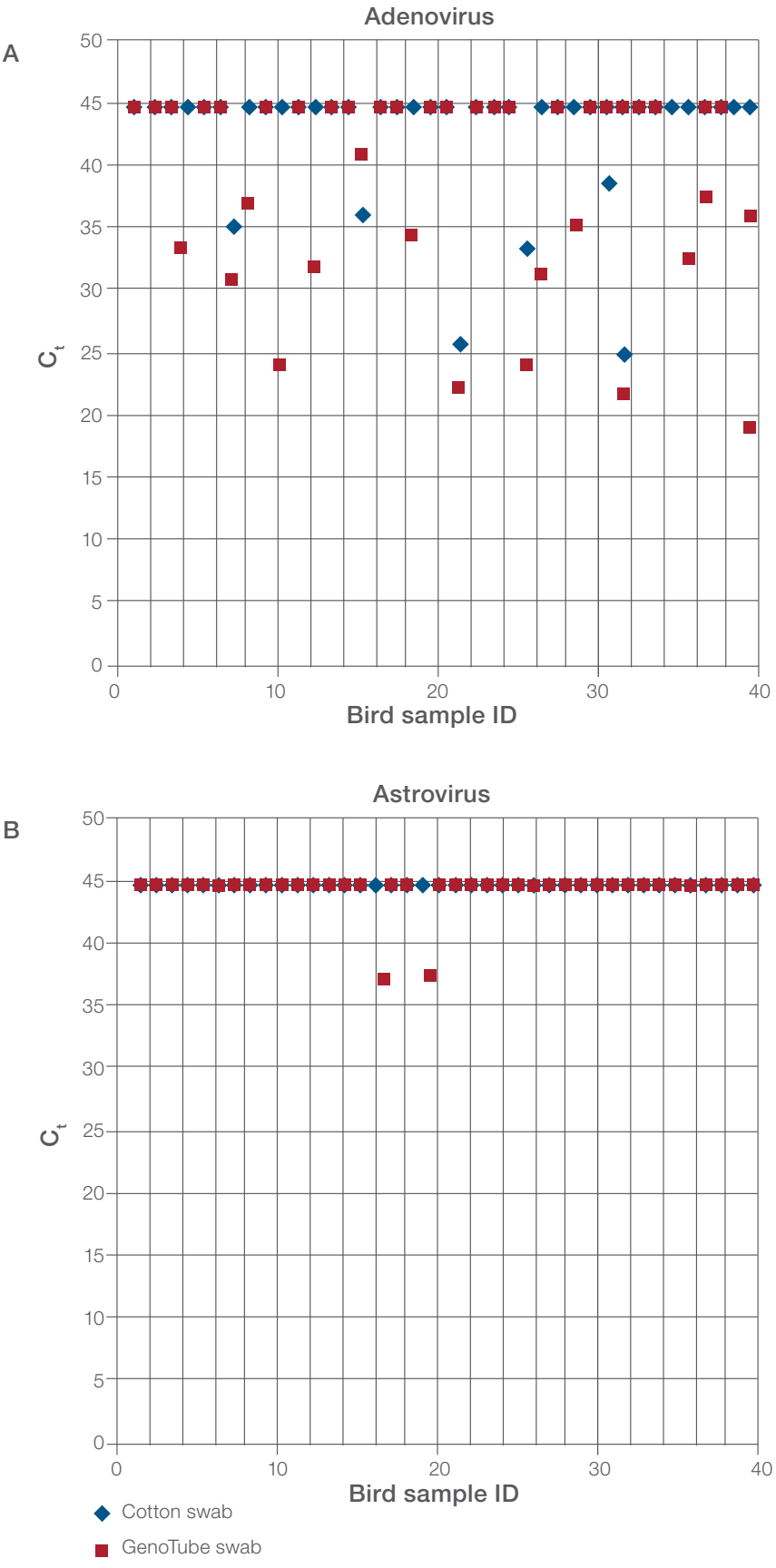

Figure 3. Detection of (A) adenovirus and (B) astrovirus by qPCR in cloacal samples of wild birds using GenoTube swabs vs. cotton swabs.

Results

A comparison of PCR results from samples obtained with GenoTube swabs and cotton swabs is shown in Table 1. The totals of positive detection for adenovirus and astrovirus from GenoTube swabs were 16/40 and 2/40, respectively, which are higher than from using standard cotton swabs (6/40 and 0/40, respectively). These results suggest that sampling using GenoTube swabs improves the sensitivity of the method, compared to the use of cotton swabs (Figures 2 and 3).

Conclusion

This study demonstrates improved conservation of samples when using GenoTube swabs for collection and transportation of cloacal samples from wild birds. Fast drying of samples using the GenoTube swabs is thought to have a positive effect on nucleic acid stability and yields. The yields of nucleic acid extracted from GenoTube swabs were higher than those obtained from cotton swabs, resulting in better sensitivity. GenoTube swabs offer convenience, ease of use, and reliable results.

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Ordering information

Product	Quantity	Cat. No.
MagMAX-96 Viral RNA Isolation Kit*	100 reactions	AM1836
MagMAX CORE Nucleic Acid Purification Kit**	100 reactions	A32700
	500 reactions	A32702
GenoTube Livestock Swab**	1 piece	9062010
AgPath-ID One-Step RT-PCR Reagents†	100 reactions	AM1005
	500 reactions	4387424
	1,000 reactions	4387391

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