

Many zoonotic diseases have birds as their natural hosts (Shiina *et al.* 2004; Burt 2005). For example, waterfowl are the natural hosts

require biosafety precautions. Standard sampling and storage during avian influenza surveillance is bound to the availability of nearby deep freezers and transport of samples is subjected to strict regulations. Analysis can only take place in specialised laboratories. These facts make avian influenza research almost impossible if not conducted within the infrastructure of one of the few big collaborative projects. Hence, important contributions from the many smaller ecological projects may be missed (Bin Muzaffar *et al.* 2006; Cromie *et al.* 2006).

Here, a possible solution for this problem is examined: a method to sample, store and analyse potential AIV containing samples. This method does not require immediate deep freezing. The issue of preserving RNA viruses for later analysis (Munster *et al.* 2009) has been addressed several times already in similar fields (Li *et al.* 2004b; Moscoso *et al.* 2005; Ndunguru *et al.* 2005; Perozo *et al.* 2006; Purvis *et al.* 2006; Inoue *et al.* 2007; Nuchprayoon *et al.* 2007; Picard-Meyer *et al.* 2007; Muthukrishnan *et al.* 2008). The so-called FTA cards® (Whatman®) are used to preserve AIV RNA on a dry storage basis. The chemicals in the FTA (Flinders Technology Associates) card render pathogens inactive upon contact (Rogers & Burgoyne 1997) and transport can be arranged safely with only few further biosafety measures to be taken. FTA cards would therefore also be suitable for working with highly pathogenic strains of AIV. Proof of the potential of this principle is given in this short communication. The basis of this method is the isolation of the RNA followed by a one-step RT-PCR. The establishment of these protocols will be possible in any molecular laboratory, without the need for further biosafety measures. Samples can be mailed by normal postal services. Both sampling and analysis will be available to any molecular ecologist, thereby facilitating further scientific progress. This holds new possibilities for innovative studies in the fields of, for instance, molecular ecology, host-pathogen interactions or ecological immunology.

Methods

Wild Mallard were caught in a duck trap at Ottenby Bird Observatory, Sweden (56°12'N 16°24'E), and cloacal samples were taken for AIV detection. Detailed information about trap d informm24.9(A c4t4.9129 -1.3 TD(

The RNA was eluted into 50 µl elution buffer as provided by the kit. Three 2 mm punches from an untreated FTA card were carried along as negative extraction control; that is, to determine any contamination of the laboratory's tools or devices with AIV material. For RT-PCR detection we used the one-step Access RT-PCR System (Promega) – *i.e.* where reverse transcription into cDNA and PCR amplification is carried out in one tube – following a protocol adjusted from Fouchier *et al.* (2000). Stock solutions of 0.5 µl with 100 mM of the primers M52C and M253R (Fouchier *et al.* 2000) were used in reactions containing 10 µl AMV/*Tfl* 5× buffer, 1 µl dNTPs, 7 mM $MgSO_{4}$bu xtra3R (Fhier

reported where alternative preservation methods were used (< 200 bp in ethanol; Wang *et al.* 2008).

Since it has recently been shown that RNA fragments of > 700 bp in size can be amplified successfully in other systems (Muthukrishnan *et al.* 2008) we assume that storage of avian influenza samples on FTA cards has the potential to be superior to the ethanol fixation method if primers for larger fragments are used. Some studies tested the sensitivity (*e.g.* RNA quantity) required for detection. They reported the detection of a positive signal even after many-fold dilutions (Perozo *et al.* 2006) or for only 0.1 fg of RNA template (Rogers & Burgoyne 2000), and after storage at ambient temperatures for > 2 weeks. Others claim that RNA on FTA cards is stable even after six months of storage under ambient conditions (Rogers & Burgoyne 2000). Whether these methods are applicable under fieldwork conditions remains to be tested. In natural samples like faeces or oral/cloacal swabs there is also the chance that AIV is present in lower concentrations than tested here. This poses the risk of not detecting an avian influenza infection when there actually is one (*i.e.* a false negative). In particular the effects of storage time and temperature, as well as sensitivity at lower concentrations and contamination through faecal material, would need attention in such a systematic test. Recent studies have however shown that PCR is more sensitive than traditional methods, even when AIVs are only present as unviable particles (Runstadler *et al.* 2007). This also makes detection possible when infection is almost cleared by immune response. To this point, cloacal samples were not tested directly in the present study but it seems that the use of FTA cards in large scale AIV sampling may be the means by which the field of AIV ecology can be lifted beyond the constraints of difficult sampling, storage and laboratory facilities.

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