Dry cotton or flocked respiratory swabs as a simple collection technique for the molecular detection of respiratory viruses using real-time NASBA

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ABSTRACT

This paper describes the molecular detection of influenza A, influenza B, respiratory syncytial virus and human metapneumovirus using real-time nucleic acid sequence based amplification (NASBA) from respiratory samples collected on simple dry cotton swabs, non-invasively and in the absence of transport medium. Viral RNA was detectable on dry cotton and flocked swabs for at least 2 weeks at room temperature and was readily extracted using magnetic silica extraction methods. Dry cotton respiratory swabs were matched with traditionally collected respiratory samples from the same patient, and results of traditional laboratory techniques and real-time NASBA were compared for all four viral targets. The results not only showed a significant increase in the detection rate of the viral targets over traditional laboratory methods of 46%, but also that dry swabs did not compromise their recovery. Over two subsequent winter seasons, 736 dry cotton respiratory swabs were collected from symptomatic patients and tested using real-time NASBA giving an overall detection rate for these respiratory virus targets of 38%. The simplicity of the method together with the increased detection rate observed in the study proves that transporting a dry respiratory swab to the laboratory for respiratory virus diagnosis using molecular methods is a suitable and robust alternative to traditional sample types.

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1. Introduction

Respiratory viruses contribute to significant morbidity and mortality in healthy and vulnerable individuals. The introduction of improved antiviral treatments for respiratory viral infection in recent years has meant that rapid diagnosis of respiratory viral infection is vital to ensure patients are treated and managed appropriately (Englund et al., 1996; Boivin et al., 2004; Templeton et al., 2004; Moore et al., 2004; Deiman et al., 2007).

Achieving a rapid result that is both sensitive and specific is challenging. The timing of sample collection in relation to the onset of symptoms together with the quality of the sample is crucial. For example, although direct immunofluorescence allows results to be available within 1 h of sample receipt, compared to collecting a respiratory swab, more invasive sampling is required to collect a nasopharyngeal aspirate or broncho-alveolar lavage. Also, these samples in adult patients can give poor results due to reduced viral shedding and reduced amounts of cellular material when compared to infants (Hall et al., 1976; Englund et al., 1996; Falsey, 2007). More rapid point of care testing for certain targets like influenza and RSV have their place, but these tests are generally less sensitive than traditional laboratory tests and so it is important that negative results from these assays are later confirmed by a more sensitive test (Moore et al., 2006; Dwyer and Sintchenko, 2007).

For the laboratory diagnosis of respiratory viral infection, cell culture has been historically the gold standard. But for successful isolation it is important that the clinical specimen is collected from the patient close to initial symptom onset and be transported under appropriate conditions to the laboratory. For swabs taken from the respiratory tract, this requires the use of virus transport medium. Using traditional cell culture techniques, a positive result may take several days to become positive depending on the quality of the sample and the viral load, but a negative result may not be available for up to 2 weeks. However, recent advances in cell culture techniques, in particular the commercial shell vial techniques, such as ready-cells (R mix) have reduced the time to result to just 48 h (LaSala et al., 2007).

Molecular tests for respiratory virus detection are being used increasingly in routine diagnostic laboratories (Hibbitts and Fox, 2002). Numerous studies have shown that molecular techniques based on conventional PCR or nucleic acid sequence based amplification (NASBA) vastly improves the detection rate for respiratory viruses over traditional laboratory techniques. Using real-time detection of the amplified product, same day results are now a
reality (Englund et al., 1996; Boivin et al., 2004; Templeton et al., 2004; Moore et al., 2004, 2006; Deiman et al., 2007). However, there has been very little work reported on evaluating the most appropriate way of collecting and transporting respiratory samples to the laboratory for molecular testing. The evaluation of new molecular assays is often based on samples received for virus isolation allowing for testing by more than one method and thus a direct comparison of results. By using this approach routinely it means that the sample received might be contaminated with the target virus if processed in a room where cell culture is performed. In an ideal situation, a dedicated respiratory sample for molecular testing would be collected non-invasively; with viral nucleic acid stability guaranteed allowing for accurate detection of the target virus for several days. Dedicated samples for molecular testing would reduce the risk of contamination in laboratories also performing traditional methods for virus detection and the ease of sample collection would facilitate clinical teams in confirming a diagnosis of respiratory infection. Removing the need for virus transport medium would reduce its associated cost, storage requirements and risk of leakage during transportation.

Other simple collection and transportation methods have been described. One of the most effective methods is a dried blood spot collected onto filter paper (Karapanagiotidis et al., 2005). This method was used in the late 1990s for HIV-1 RNA detection (Cassol et al., 1997, Mwaba et al., 2003; Alvarez-Munoz et al., 2005; Li et al., 2005) genotyping (Plantier et al., 2005) and measles surveillance (Plantier et al., 2005) in Africa (El Mubarak et al., 2004). These showed that the integrity of RNA can be maintained for long periods and at varying temperatures (Abe and Konomi, 1998). A more complex, but commonly used approach is to use FTA filter paper, which is impregnated with lyophilised chemicals which lyses both viruses and bacteria rendering them non infectious (Moscoso et al., 2005). Filter paper strips have also been used to collect faecal material which is then fixed prior to sending for molecular testing and sequencing studies (Vilec et al., 2001; Woollants et al., 2004). Recently, a method has been described for collecting respiratory samples for surveillance using swabs fixed in ethanol (Krafft et al., 2005). Similarly, in an outbreak of parainfluenza 3 on a haematology ward at the University Hospital of Wales where nasal samples were collected directly into guanidine thiocyanate lysis buffer for transportation and nucleic acid extraction (Hibbitts et al., 2003).

In the winter season of 2003–2004, a real-time nucleic acid sequence based amplification assay for the detection of influenza A was developed. During the clinical evaluation studies a small number of dry respiratory swabs were received for testing transported in the outer sterile protective cover alone. Rather than discard the swabs as unsuitable for testing, they were broken into lysis buffer on receipt in the laboratory, extracted and tested for influenza as for other samples. In more than one instance influenza A was detected (Moore et al., 2004).

This paper includes three constituent studies that together demonstrate the validity of dry swabs for the detection of respiratory viruses. The first describes a simple RNA stability study comparing the widely available cotton tipped wooden swab and the recently introduced flocked swab designed to increase the surface area of the swab allowing for improved sample collection and yield. The second describes a pilot study comparing dry cotton tipped respiratory swabs with matching nasopharyngeal aspirates or respiratory swabs transported in viral transport medium collected at the same time from the same patient using both real-time NASBA and traditional laboratory techniques and the final part demonstrates how dry cotton respiratory swabs can be used as a simple effective collection technique for routine molecular testing.

2. Methods

2.1. Study to determine viral stability

To demonstrate viral stability, dry sterile cotton tipped, wooden swabs and flocked swabs (Bibby Sterilin, Copan, Italy) were compared using serially diluted viral isolate. RSV was selected for this work as it was considered to be most environmentally labile virus being targeted in the study. RSV positive virus culture supernatant was obtained from the Welsh Specialist Virology Centre and its TCID<sub>50</sub> was calculated to be $1 \times 10^9$ using the method of Reed and Meunch (1938). The virus culture fluid was diluted to $10^{-2}$ into sterile phosphate buffered saline (PBS). To a series of matching cotton and flocked swabs marked day 0 to day 15, $50 \mu l$ of the diluted virus culture fluid was added to the tip of the swab and the swab re-sheathed and allowed to dry at room temperature. After 1 h the swabs marked day 0 were broken into a 0.9 ml Lysis buffer tube (bioMérieux, Marcy l’ Étoile, France), vortexed and left to stand for 10 min before freezing at −80 °C. After 24 h at room temperature, day 1 swabs were processed in the as for day 0. This was repeated on a daily basis until the swabs inoculated on day 15 had been processed. All of the swabs were then defrosted and RNA was extracted from 200 μl of the Lysis buffer as described below for molecular testing by real-time NASBA.

2.2. Comparison study of dry respiratory swabs and matching clinical respiratory specimens

Dry cotton tipped wooden ended swabs were utilised as these were used routinely by all clinicians and available widely on all clinical ward areas. The swab was collected from either the nasal passage or the throat and returned to its original holder for transportation back to the laboratory. Samples were collected from children and adult oncology and haematology patients presenting with respiratory illness and from patients in community outbreaks where respiratory virus infections were suspected.

From one cohort of patients, clinicians were asked to collect two respiratory swabs from either the throat or nasal passage, one was put in virus transport medium for virus isolation in cell culture and molecular testing by real-time NASBA (Moore et al., 2004) and the second sent dry for real-time NASBA only.

In a second patient cohort, nasopharyngeal aspirates were taken following the usual local protocols for rapid virus detection in acutely unwell patients using direct immunofluorescence followed by cell culture. These samples were also tested and by real-time NASBA. In addition a corresponding dry respiratory swab for real-time NASBA testing only was also collected from each patient.

2.3. Dry respiratory swab collection for routine NASBA testing study

Dry cotton respiratory swabs received in the laboratory up to 5 days after collection from the throat or nasopharyngeal passage were accepted for testing by real-time NASBA only. These samples were obtained from a wide range of patient groups including adults and children both hospitalised, attending out-patient clinics and from the community.

2.4. Direct immunofluorescence and cell culture

The routine laboratory methods used for direct immunofluorescence and cell culture have been described previously (Moore et al., 2004, 2006). Briefly, all nasopharyngeal aspirates received were tested by both direct immunofluorescence and then inoculated into cell culture (PLC, MRC5 and Hep-2 cells), whilst all throat
swabs received in virus transport medium were inoculated directly into cell culture. A proportion of the samples were also inoculated into R Mix cell culture (Diagnetics Hybrids inc, Athens, OH) as part of a parallel evaluation following manufacturer’s instructions.

2.5. Real-time NASBA assays

During the 2004–2005 (October–April) season, all samples were tested for four respiratory viruses (influenza A, influenza B, RSV and hMPV) by real-time NASBA; this included the nasopharyngeal aspirate and respiratory swabs received in virus transport medium. This season included all of the samples collected for the pilot study. The following winter season (2005–2006) only dry respiratory swabs were collected and tested for RSV, influenza A and influenza B only.

2.5.1. Influenza A and influenza B molecular testing

Both influenza A and B real-time NASBAs were developed in-house and evaluated against a panel of positive material including the 2005 QCMD respiratory panel (Templeton et al., 2006). The Influenza A assay has been described previously (Moore et al., 2004). The influenza B assay was developed to target the polymerase PA gene (segment 3). The influenza B primer sequences used were P1: 5′ AAT-TCT-AAT-ACG-ACT-CAC-TAT-AGG-GAG-AAG-GCT-ATT-CAA-CAT-CTG-CGT-CCA-TC 3′ (including the T7 promoter sequence) and P2: 5′ ATY-ACT-TCA-TAY-TGT-GCT-CTC-A 3′ together with a specific molecular beacon 5′ FAM-CCA-TGC-CCC-TTG-TCC-TTC-TAA-TGC-TGT-ATA-GGC-ATG-G-DABCYL 3′. During the development of the assay the sensitivity and specificity of the assay on RNA transcripts and virus stock dilutions were shown to be comparable to that of the influenza A assay.

2.5.2. RSV and human metapneumovirus (hMPV) molecular testing

The commercial NucliSens RSV A+B assay and hMPV assay (bioMérieux, Marcy l’ Etiole, France) was used throughout the season. The principle behind these assays has been described previously. (Moore et al., 2006; Manji et al., 2006; Deiman et al., 2007)

2.6. Sample processing for molecular testing

To reduce the effect of inhibitory substances the nasopharyngeal aspirates and samples received in transport medium were either diluted 1:10 in sterile phosphate buffered saline or if repeatedly inhibitory were further treated with DNase I as previously described (Moore et al., 2006).

On receipt in the laboratory the dry cotton swab was broken into a 0.9 ml tube of NucliSens Lysis Buffer. The vial was vortexed and left to stand for 10 min at room temperature. A 200 μl aliquot of Lysis buffer containing either the pre-treated sample or swab was then transferred to a 2 ml NucliSens lysis buffer tube for extraction. The remaining Lysis buffer was then stored frozen at −80 °C.

2.7. Nucleic acid extraction

For each extraction a positive control consisting of a mixture of all four respiratory targets, influenza A (H1N1 and H3N2), influenza B (unknown strain type), RSV and hMPV was used. The influenza A and B and RSV isolates were obtained from The Welsh Specialist Virology Centre. The hMPV control was obtained from The bioMérieux R&D laboratory (Grenoble, France). A negative control was also included in each extraction run.

All samples and controls during the 2004–2005 season were processed as follows; the internal control for the RSV and hMPV was reconstituted by combining the lyophilised spheres for both assays into one tube and reconstituted 220 μl of elution buffer. The vial was vortexed until the buffer was clear and the spheres were fully dissolved. To each 2 ml lysis buffer tube containing sample, 20 μl of the internal control mix was added and the tube vortexed. The lysis buffer was extracted using the NucliSens miniMAG magnetic silica extraction method following manufacturer’s instructions (Moore et al., 2006).

Samples received during the 2005–2006 season were processed in the same way but with addition of just the RSV internal control, which was reconstituted in 550 μl of wash buffer 3 followed by 550 μl of magnetic silica. The nucleic acid extraction was performed on the fully automated bioMérieux NucliSens easyMAG system following the manufacturer’s protocol.

The nucleic acid was eluted from the magnetic silica from either extraction method into 25 μl of elution buffer; this was either amplified immediately or stored at −80 °C.

2.8. Amplification and detection

For each target, a 5 μl aliquot of nucleic acid eluate was used for each reaction. The bioMérieux NucliSens basic kit version 2 reagents along with the NucliSens EasyQ system were used to perform the amplification and detection of the real-time NASBA assays. The manufacturer’s instructions were followed to perform the RSV and hMPV assays (Moore et al., 2006). For the influenza assays optimised conditions were followed as determined during assay development and evaluation (Moore et al., 2004).

For more detailed analysis, the RNA stability assay was performed using the EasyQ analyzer 1.2 software. Unlike other real-time PCR platforms, real-time NASBA reports as fluorescence signal over time, taking 120 reads over 90 min. The threshold level set for the RSV assay wild type signal is 1.2, by using the analyser software it is possible to determine the time at which the signal reaches this threshold and thus becomes positive. The time to positive for both dry cotton and flocked swabs were analysed for each day and plotted graphically to determine whether there was a difference with storage time.

2.9. Statistical analysis

The javastat online statistics package (http://statpages.org/crab2x2.html) was used to determine the kappa statistic for agreement of methodologies, sensitivity, specificity, positive predictive value and negative predictive values. The unpaired difference between the methods used was calculated using Newcombe statistics (Newcombe, 1998).

3. Results

3.1. Virus stability results

RSV RNA could successfully be extracted and detected from all of the swabs. The amount of RNA detected on the cotton tipped swabs decreased towards day 15 with a difference in ‘time to positive’ at day 15 of 8 min in favour of the flocked swab. The consistency in the time to positive results was significantly improved using the flocked swab, which varied very little over the 15 days resulting in a more linear graph over time when compared to the cotton swab (Fig. 1).

3.2. Dry respiratory swabs and matching clinical respiratory specimen study

In total 164 dry respiratory swab results were compared to the results of respiratory swabs received in virus transport medium (56) and nasopharyngeal aspirates (108). Of these, 37 swabs (66%)
were received from patients involved in respiratory illness outbreaks. The results of the comparison work has been summarised in Fig. 2 showing the increased detection rate of the four respiratory viruses using dry cotton swabs and real-time NASBA over the traditional laboratory techniques.

When compared to swabs received in virus transport media for cell culture, the dry cotton swabs yielded 34 positive results using real-time NASBA giving an overall positivity rate of 60% (38% influenza A, 20% influenza B and 2% RSV) compared to just 5% if traditional cell culture was used. The isolation rate increased to 23% if R mix was used which detected more influenza A than traditional cell culture.

Compared to direct immunofluorescence and cell culture on nasopharyngeal aspirates, the dry cotton swabs yielded 25 positive results using real-time NASBA giving an overall detection rate of 24% (11% influenza A, 11% RSV and 2% dual influenza A and RSV) compared 5% for both IF and cell culture. The detection rate again increased to 12% if R mix was used in conjunction with immunofluorescence.

Aliquots of all influenza positive samples were sent to the Influenza Reference Laboratory based at the Centre for Infections, London for further strain analysis. The results obtained confirmed the real-time NASBA results obtained. Further to this, real-time NASBA assays were also later performed on the swabs in virus transport media tested and on the nasopharyngeal aspirates, the results of which showed 100% correlation with the dried swab result, indicating that the virus was present in both samples.

One of the viruses isolated in cell culture was parainfluenza 3 and was removed from the statistical analysis as this virus was not included in the real-time NASBA virus panel.

3.2.1. Statistical analysis

For the comparison between dry cotton swabs and swabs received in virus transport medium, using traditional cell culture as the gold standard, dry respiratory swabs in combination with real-time NASBA gave a sensitivity of 100% (95% CI 35–100) and a specificity of 41% (95% CI 38–41). This translated to a positive predictive value of 59% (95% CI 21–59) and a negative predictive value of 100% (95% CI 94–100). This indicates that real-time NASBA detected all the relevant viruses isolated in cell culture, but that the additional samples giving positive results are considered false positive by comparison with the gold standard. By applying the kappa statistic and removing the need for a gold standard, a better reflection of agreement can be established. The kappa statistic was shown to be 0.047 (95% CI 0.031–0.047) showing a poor agreement between the two testing strategies. Using the Newcombe statistic it is possible to show how one method compares directly with another on the same cohort of samples. Against traditional cell culture; real-time NASBA using dried swabs showed an increased detection rate of 55% (95% CI 39–68) but compared to R mix this was reduced to 36% (95% CI 17–51).

For the comparison between dry cotton swabs and nasopharyngeal aspirates, using the traditional assays in combination as the gold standard, dry respiratory swabs with real-time NASBA detection gave a sensitivity of 100% (95% CI 52–100) and a specificity of 80% (95% CI 74–80). This translated to a positive predictive value of 23% (95% CI 7–23) and a negative predictive value of 100% (95% CI 97–100). The kappa statistic was 0.28 (95% CI 0.060–0.19) showing fair to poor agreement between the two methodologies. Using the Newcombe statistic on immunofluorescence and traditional culture there was a detection rate improvement of 18% (95% CI 8–27) when real-time NASBA is used on dry swabs. However, if R mix was used in combination with IF this changed to 10% (95% CI 1–20).

3.3. Routine testing of dry cotton respiratory swabs

In total, 344 swabs were collected from the period of the 1st October 2004 to the 1st April 2005 and tested for the four viral targets over this season. Positive results from one or more of the four targets were obtained from 127/344 (37%) samples. During the period from 1st October 2005 to 1st April 2006, 392 dry respiratory swabs were taken for routine testing for three respiratory targets. Of these, 150/392 (39%) samples were found to be positive for one or more of the respiratory targets. Of these, 150/392 (39%) samples were found to be positive for one or more of the respiratory targets. Fig. 3 summarises the viruses detected by season, during 2004–2005, hMPV accounted for 2% of all positive results. Dual infections with influenza A and RSV (5%) were detected where the RSV and influenza A seasons crossed in each season.

In patients with underlying malignancies, RSV was the most common virus detected across both seasons. In haematology patients RSV was detected in 18% and 20% of samples collected each year. Clinical symptoms ranged from mild upper respiratory tract infections to severe lower respiratory tract infections often associated with prolonged shedding of virus. Repeat testing of patients demonstrated that prolonged shedding of virus was more commonly associated with RSV than with any of the other viral targets in the study. One patient who had prolonged shedding was a 5-year-old female who had a previous RSV positive result by direct immunofluorescence before molecular screening was performed. Subsequent sampling of this patient showed prolonged shedding...
of RSV over a period of 3 months, which was further confirmed by virus isolation and direct immunofluorescence in matching samples.

Another 15-year-old child initially presented with a cough and coryzal signs following an allograft for anaplastic large cell non Hodgkin’s lymphoma. Her first screen was positive for RSV and she was started on ribavirin therapy. However, her illness progressed to pneumonia despite treatment and four follow up dry cotton respiratory swabs were also shown to be RSV positive. She continued to excrete RSV for a month following treatment before finally clearing the virus. Throughout this time, she remained symptomatic.

Using dry cotton swabs and real-time NASBA the cause of 11 respiratory virus outbreaks was identified. Influenza A was the causative agent in 6/11 (75%) of the outbreaks as defined by two or more samples from the outbreak being found positive. Influenza B was found to be the causative agent in 4/11 (25%) of the outbreaks, including 2 outbreaks in schools. Additionally, RSV was found to be the causative agent of an outbreak in a neonatal unit and also responsible for a protracted outbreak in a haematology ward.

Over the two seasons, nine samples were received from patients who had died following a history of respiratory illness. The only virus detected in these patients was hMPV that was detected in the lung swab of an infant who died of suspected sudden infant death syndrome following a short illness.

3.4. Inhibition rates

An important consideration when processing respiratory samples for molecular testing is inhibitory substances that might be present in the samples. This is a significant problem for samples such as nasopharyngeal aspirates, sputum and broncho-alveolar lavages, which in this study and in previous studies have needed to be pre-treated prior to extraction to reduce inhibition rates (Moore et al., 2006). The work performed using dry respiratory swabs has shown that inhibition rates are negligible (<0.5%) and are rarely encountered using this method.

4. Discussion

As more routine laboratories introduce molecular testing for the detection of respiratory viruses the most appropriate way of collecting respiratory samples to ensure a rapid and sensitive delivery of results also needs to be assessed.

The stability study in this paper demonstrates that nucleic acid from RSV is stable over a long period of time on a dry swab and that the nucleic acid is readily released once the swab is vortexed into the guanidinium based lysis buffer. This means that dry swabs can be sent directly to the laboratory without need of any form of transport medium, or fixative reagent to ensure nucleic acid integrity. These findings are supported by the increased detection rate seen using dry cotton swabs compared to traditional respiratory samples demonstrated in the comparison study.

Viral RNA was extracted successfully from conventional cotton tipped or flocked swab types even after storage at room temperature for 15 days. The results of the flocked swab showed that this type of swab not only kept the RNA stable for prolonged periods of time, but that the amount of nucleic acid released from the swab was highly consistent as demonstrated in the time to positive curve. Whilst cotton tipped swabs can still be recommended on the basis of the study and routine diagnostic work results, the use of flocked swabs in combination with a molecular test such as real-time NASBA will improve the detection rate of respiratory viruses further. This is particularly true if samples are to be transported over a distance to the laboratory or if there was a delay in sample processing.

Over two winter seasons, 736 dry respiratory swabs were collected and tested for up to four respiratory targets. Of these, one or more viral targets were detected in 277 samples (38%). The majority of samples received were from older children and adult patients with an underlying condition in whom rapid respiratory virus detection has in the past been complicated by the difficulties in obtaining good quality clinical samples. Rapid molecular testing has allowed for improved understanding and management of respiratory viral infections in different patient groups. RSV in particular, was shown to be a significant cause of respiratory symptoms and was associated with prolonged shedding of virus in immunocompromised children and adults. By using a non-invasive method of sample collection that is acceptable to both patients and staff, samples can quickly and easily be collected and sent directly to the laboratory for testing. The laboratory can also request repeat samples to be taken from patients for same day testing to monitor the course of infection or to confirm the presence of a virus, particularly in vulnerable patients who may require strict infection control measures and appropriate antiviral treatment.

By further expanding the molecular panel of respiratory viruses it has been shown that the detection rate of respiratory viruses increases further to 60% as retrospective work performed on stored dry respiratory swabs in lysis buffer (unpublished in-house data) has shown that enteroviruses, rhinoviruses, parainfluenza types 1–4 and adenovirus can also be detected from the swabs. This retrospective work also demonstrated that once frozen, the nucleic acid in lysis buffer remains stable for many months.

Although real-time NASBA was the method of choice for detecting the respiratory virus targets, the extracted nucleic acid could be amplified using traditional PCR and reverse transcription-PCR methods. This makes the collection method appropriate for most molecular technologies, although in combination with real-time NASBA a result can be obtained within 4 h. This is particularly useful for same day testing when investigating the cause of severe acute infections and outbreaks.

The disadvantage of this method is the lack of virus for culture, particularly for influenza. It is possible to type a virus using gene sequencing but the role of genetic drift in immune escape can only be determined using neutralisation techniques, which require the live virus. It is also impossible to ascertain whether the virus being detected by a molecular method is still infectious or is non-viable virus at the end of an infection. Symptomatic patients often have respiratory swabs taken that give no virus growth in cell culture or by direct immunofluorescence, but are repeatedly positive by molecular testing using the dry respiratory swab collection method.
All positive results should therefore be taken in context with clinical features.

The application of this simple collection method using dry respiratory swabs has consistently been shown to be sensitive and specific for the detection of a range of respiratory viruses by molecular methodology. It is now used routinely across Wales for the community surveillance of influenza, respiratory virus outbreak investigation, screening of high-risk patients presenting with respiratory illness and also for monitoring the course of the infection. The expansion of the range of viruses being targeted by molecular technology in combination with this method of sample collection will further enhance clinical management of respiratory infections in Wales.

5. Conclusion

Collecting a nasal or throat swab and transporting it dry to the laboratory as a dedicated molecular specimen is a simple and robust method for detecting a wide range of respiratory viral targets.

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