Australian Government

Department of Health and Ageing

Australia and New Zealand Horizon Scanning Network

ANZHSN

AN INITIATIVE OF THE NATIONAL, STATE AND TERRITORY GOVERNMENTS OF AUSTRALIA AND THE GOVERNMENT OF NEW ZEALAND

National Horizon Scanning Unit
Horizon scanning prioritising summary

Volume 14, Number 1:

Primer and probe set for the diagnosis of avian influenza

September 2006

AHTA

Adelaide Health Technology Assessment

[add ISSN]
[add Publications Approval Number]

Electronic copies can be obtained from http://www.horizonscanning.gov.au

Enquiries about the content of this summary should be directed to:

HealthPACT Secretariat
Department of Health and Ageing
MDP 106
GPO Box 9848
Canberra ACT 2606
AUSTRALIA

The production of this *Horizon scanning prioritising summary* was overseen by the Health Policy Advisory Committee on Technology (HealthPACT), a sub-committee of the Medical Services Advisory Committee (MSAC). HealthPACT comprises representatives from health departments in all states and territories, the Australia and New Zealand governments; MSAC and ASERNIP-S. The Australian Health Ministers' Advisory Council (AHMAC) supports HealthPACT through funding.

This *Horizon scanning prioritising summary* was prepared by Tom Sullivan and Janet Hiller from the National Horizon Scanning Unit, Adelaide Health Technology Assessment, Discipline of Public Health, Mail Drop 511, University of Adelaide, South Australia, 5005.

PRIORITISING SUMMARY

REGISTER ID: **000226**

NAME OF TECHNOLOGY: **PRIMER AND PROBE SET FOR THE DIAGNOSIS OF AVIAN INFLUENZA**

PURPOSE AND TARGET GROUP: **RAPID IN VITRO DETECTION OF HIGHLY PATHOGENIC AVIAN INFLUENZA (ASIAN LINEAGE) IN HUMANS**

STAGE OF DEVELOPMENT (IN AUSTRALIA):

- ☒ Yet to emerge
- ☐ Experimental
- ☐ Investigational
- ☐ Nearly established
- ☐ Established
- ☐ Established *but* changed indication or modification of technique
- ☐ Should be taken out of use

AUSTRALIAN THERAPEUTIC GOODS ADMINISTRATION APPROVAL

- ☐ Yes ARTG number
- ☒ No
- ☐ Not applicable

INTERNATIONAL UTILISATION:

COUNTRY	LEVEL OF USE		
	Trials Underway or Completed	Limited Use	Widely Diffused
United States		✓	

IMPACT SUMMARY:

This prioritising summary investigates the effectiveness and value of a real-time reverse transcription-polymerase chain reaction primer and probe set for the rapid diagnosis of highly pathogenic avian influenza (Asian lineage) in humans.

BACKGROUND

Avian influenza is a viral infection of birds caused by type A strains of the influenza virus. Current fears of a human pandemic of avian influenza relate to the highly pathogenic H5N1 strain of the virus. In 2003, the H5N1 virus began to circulate in birds throughout south-east Asia, and by April 2006, had spread to more than 50 countries in an outbreak unprecedented in its geographical size (WHO 2006a). Incidences of H5N1 in birds have now been reported in countries throughout Asia, in Africa, Europe and the Middle East. At present, the H5N1 strain remains for the most part a disease confined to birds. Transmission of the disease from birds to humans remains extremely rare, requiring close contact between the two species. While an estimated 150 million birds have died from H5N1 since its initial outbreak in 2003, only 238 human cases have been laboratory-confirmed (WHO 2006b). In those humans that do contract the virus however, H5N1 causes severe illness and often death. Symptoms may

include fever, diarrhoea, vomiting, abdominal pain, chest pain, pneumonia, and multiple organ dysfunction (WHO 2006c).

Despite the low incidence of H5N1 in humans to date (no cases have been reported in Australia), the risk of a human pandemic of avian influenza remains significant. If H5N1 develops an ability to spread efficiently among humans through a process of reassortment or adaptive mutation, the virus will have met all the conditions necessary for a pandemic to occur. Given the severity of the H5N1 virus in humans, a pandemic would have extensive worldwide health consequences. In Australia, it has been estimated that an avian influenza pandemic for which there were no appropriate methods of vaccination or treatment could result in between 2.6 and 7.5 million people seeking medical aid, 58,000 to 148,000 hospitalisations and 13,000 to 44,000 deaths (DoHA 2005).

In addition to the development and stockpiling of appropriate vaccines and antiviral drugs, the recent outbreak of avian influenza has highlighted the need for a rapid and accurate diagnostic test for the virus (Ng et al 2005). Such a test would play an important role not only in the surveillance of the disease in the early stages of outbreak, but also in the timely recommendation of antiviral therapy for those patients found to have the virus (who would otherwise not have been prescribed antiviral drugs). Conventional diagnostic tools such as virus isolation or serologic testing require anywhere between 2-days and 2-weeks for results to be obtained, thus limiting their usefulness for these purposes. In the USA, the Centers for Disease Control and Prevention (CDC) have recently developed a real-time reverse transcription-polymerase chain reaction (RT-PCR) primer and probe set capable of diagnosing novel influenza A viruses in respiratory specimens or viral cultures in less than two hours. The primer and probe set consists of two primer pairs, three labelled probes and an inactivated virus control. The primers FluA2 and FluA3 target two distinct regions of the hemagglutinin (HA) gene which are only present in cases of H5N1 influenza (FDA 2006). The detection of both targets indicates a positive result for H5N1, while the detection of neither target indicates a negative result. If only one of the targets is detected, the result is reported as being equivocal for H5N1. An equivocal outcome may be the result of contamination, failure of one of the primer/probe pairs to react, inhibition or non-specific reactivity (FDA 2006). Such equivocal results may dictate the need for further testing, or alternatively require interpretation in the context of additional clinical and epidemiological information.

CLINICAL NEED AND BURDEN OF DISEASE

Between December 2003 and August 2006, the World Health Organization registered a total of 238 laboratory-confirmed human cases of H5N1 across ten different countries. One hundred and thirty nine of these laboratory-confirmed cases died from the disease, representing a case-fatality rate of 58 per cent (WHO 2006b). In patients infected with avian influenza, clinical deterioration is rapid. Following an incubation period of approximately seven days, severe symptoms including respiratory problems develop within three to five days. In patients who have died from the virus, the median duration from the onset of illness until death has been estimated to be just nine days (WHO 2006a).

DIFFUSION

The real-time RT-PCR primer and probe set was approved by the Food and Drug Administration (FDA) in February 2006 following an expedited review process that lasted less than two weeks. The FDA's expedited review process enables new technologies with important public health benefits to be available for use as soon as possible.

In the United States, testing with the primer and probe set has been restricted to laboratories that are members of the Laboratory Response Network (LRN). The restriction was imposed so that only laboratories with experienced personnel, appropriate safety measures and proper mechanisms for communicating with public health programs could use the technology.

COMPARATORS

Among the many alternative methods for detecting influenza, virus isolation, serology and rapid diagnostic tests are the most commonly used (Ng et al 2005). Virus isolation, which involves isolation of a virus in cell culture, is considered to be the gold standard for influenza diagnosis due to its accuracy and ability to distinguish between subtypes of influenza virus. The results of virus isolation can usually be obtained in 24 to 72 hours. Serology is generally used as an alternative to virus isolation when the direct identification of a virus is not feasible. Also capable of distinguishing between different strains of influenza, the results of serology are usually obtained in around two weeks. Finally, rapid diagnostic tests such as the Directigen Flu A+B (Decton Dickinson) or ZstatFlu8 (ZymeTx) can detect influenza viruses usually within an hour. While these tests are rapid and simple, subtyping of influenza viruses is not possible.

EFFECTIVENESS AND SAFETY ISSUES

At this stage, only a small number of studies have evaluated the diagnostic properties of the real-time RT-PCR primer and probe set. In a small-scale study, Ng et al (2005) investigated the sensitivity of real-time RT-PCR by analysing 28 archived respiratory samples from 18 human cases of H5N1 (level III-2 diagnostic evidence). All the samples were laboratory-confirmed as H5N1 positive using virus isolation as the gold standard of measurement. The authors found that real-time RT-PCR successfully classified all 28 samples as positive for H5N1. To test for cross-reactivity, real-time RT-PCR was also applied to samples of respiratory syncytial virus, rhinoviruses, enteroviruses, and strains of influenza virus other than H5N1. None of the samples tested positive using real-time RT-PCR, indicating that the test was highly specific for H5N1 RNA.

Prior to submitting their application to the FDA in 2006, the CDC investigated the diagnostic performance of the real-time RT-PCR primer and probe set in detecting varying concentrations of the H5N1 virus (level III-2 diagnostic evidence). Samples were obtained by spiking simulated respiratory specimens (swabs or liquid) with human cellular material and titered amounts of H5N1 virus control material. At a concentration of 400 $TCID_{50}$/mL, all 36 investigated samples returned a positive result for H5N1. At a lower concentration of 40 $TCID_{50}$/mL, 47 out of 55 samples (85.5%) returned a positive result, while 5/55 (9.1%) returned an equivocal result. At a very low concentration of 4 $TCID_{50}$/mL, real-time RT-PCR still returned a positive result in 32 out of 36 samples and an equivocal result in three of the

remaining four samples. Of the 72 control samples that were investigated in the study, 71 returned a negative result while the remaining sample was classified as equivocal.

In their FDA application, the CDC present data pertaining to the ability of real-time RT-PCR to detect laboratory-confirmed human cases of the H5N1 virus. In total 22 specimens from 10 laboratory-confirmed human cases were presented in their analysis. Specimens were obtained using either throat swabs (8 in total), nasal swabs (8 in total) or alternative means (6 in total). Of the 22 specimens analysed, real-time RT-PCR returned a positive test result on 10 occasions and an equivocal result on a further nine occasions. All laboratory-confirmed cases reported at least one positive test result when multiple specimens were considered.

To demonstrate levels of cross-reactivity in their FDA application, the CDC also tested the real-time RT-PCR primer and probe set using banked respiratory specimens from individuals with influenza-like illness. A total of 203 H5N1 negative samples were analysed in the study, the primer and probe set returning a negative result on 201 occasions (99%). The remaining two samples in the study were classified as equivocal by the primer and probe set.

COST IMPACT

At this stage, the real-time RT-PCR primer and probe set has not received marketing approval from the TGA. As a result, the cost of the kit is currently unknown. The primer and probe set uses standard RT-PCR equipment currently available in most pathology laboratories, and therefore is unlikely to attract any additional costs.

ETHICAL, CULTURAL OR RELIGIOUS CONSIDERATIONS

No issues were identified/raised in the sources examined.

OTHER ISSUES

Previous experience with H7N7 avian influenza suggests that neuraminidase inhibitors and M2 inhibitors, classes of antiviral drugs currently used to treat seasonal influenza, may be effective in treating human cases of H5N1 avian influenza (Gubareva et al 1995). The efficacy of these treatments, particularly neuraminidase inhibitors, depends on the length of time between symptom onset and the administration of treatment. In the treatment of seasonal influenza, neuraminidase inhibitors have been shown to reduce the duration of viral replication and improve survival provided they are administered within 48 hours following symptom onset (WHO 2005). Although clinical data are limited, the successful treatment of H5N1 avian influenza is also likely to require the rapid administration of antiviral drugs following symptom onset. In the early stages of an outbreak of H5N1 avian influenza, the ability to diagnose the disease in less than two hours using the real-time RT-PCR primer and probe set may therefore offer considerable benefits in patient management. In addition to the timely recommendation of antiviral drugs, the primer and probe set may be useful in quickly ruling out avian influenza in patients exhibiting symptoms uncharacteristic of seasonal influenza, thereby avoiding unnecessary exposure to antiviral drugs.

CONCLUSION:

An outbreak of H5N1 influenza in Australia remains a possibility. In the occurrence of an outbreak, the control and treatment of the disease will require a multi-faceted approach. In

addition to vaccines and antiviral drugs, rapid methods for disease diagnosis could offer a number of benefits including improved disease surveillance and management of patients. On current evidence, the real-time RT-PCR primer and probe set appears to be a relatively accurate and cheap option for the rapid diagnosis of H5N1 influenza. At a time however when worldwide interest in avian influenza is high and cases of the disease have yet to appear in Australia, it may be prudent to monitor such methods of rapid disease diagnosis during further outbreaks overseas.

HEALTHPACT ACTION:

Technologies relating to the surveillance and treatment of avian influenza are currently managed by the Office of Health Protection. It is likely this group would have identified this technology and any further avian influenza diagnostic tests. It is therefore recommended that the technology be archived.

SOURCES OF FURTHER INFORMATION:

DoHA (2005). *Australian Management Plan for Pandemic Influenza*, Department of Health and Ageing, Canberra.

FDA (2006). *510(k) Denovo Decision Summary: Influenza A/H5 (Asian lineage) Virus Real-time RT-PCR Primer and Probe Set*, Food and Drug Administration.

Gubareva, L. V., Penn, C. R. & Webster, R. G. (1995). 'Inhibition of replication of avian influenza viruses by the neuraminidase inhibitor 4-guanidino-2,4-dideoxy-2,3-dehydro-N-acetylneuraminic acid', *Virology*, 212 (2), 323-330.

Ng, E. K., Cheng, P. K. et al (2005). 'Influenza A H5N1 detection', *Emerg Infect Dis*, 11 (8), 1303-1305.

WHO (2005). 'Avian influenza: frequently asked questions', *Wkly Epidemiol Rec*, 80 (44), 377-384.

WHO (2006a). 'Epidemiology of WHO-confirmed human cases of avian influenza A(H5N1) infection', *Wkly Epidemiol Rec*, 81 (26), 249-257.

WHO (2006b). *Cumulative Number of Confirmed Human Cases of Avian Influenza A/(H5N1) Reported to WHO* [Internet]. Available from: http://www.who.int/csr/disease/avian_influenza/country/cases_table_2006_08_14/en/index.html [Accessed 24/08/2006].

WHO (2006c). 'Avian influenza fact sheet (April 2006)', *Wkly Epidemiol Rec*, 81 (14), 129-136.

LIST OF STUDIES INCLUDED

Total number of studies	
Level III-2 Diagnostic evidence	2

SEARCH CRITERIA TO BE USED:

Influenza A Virus, H5N1 Subtype

Influenza, Human/*epidemiology/mortality

Influenza, Human/*mortality/*virology

Population Surveillance