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Molecular detection and typing of influenza viruses: Are we ready for an influenza pandemic?

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ABSTRACT

Background: We cannot predict when an influenza pandemic will occur or which variant of the virus will cause it. Little information is currently available on the ability of laboratories to detect and subtype influenza viruses including the avian influenza viruses.

Objectives: To assess the ability of laboratories to detect and subtype influenza viruses.

Study design: In 2006 QCMD distributed an External Quality Assessment panel for the molecular detection and haemagglutinin subtyping of influenza viruses to 87 laboratories in 34 countries Worldwide, which were given 6 weeks to return results. These data were analysed to assess laboratory performance.

Results: Influenza virus positive panel samples were correctly identified by 35–98% of laboratories. The correct haemagglutinin subtype was reported by 32–87% of laboratories that detected the virus: incorrect subtyping results included the reporting of avian influenza viruses as human strains and *vice versa*. Twelve laboratories reported false positives with some avian influenza viruses reported.

Conclusions: These data suggest that improvements are needed in the molecular detection of influenza viruses and influenza virus A haemagglutinin subtyping. Only rapid and accurate identification of circulating pandemic influenza virus will ensure that the maximum time is available for intervention.

1. INTRODUCTION

An influenza pandemic is inevitable but we cannot predict when it will occur or which variant of the virus will be the cause (Rezza, 2004, Webby and Webster, 2003).

Influenza virus A (H5N1) is currently of most concern. This avian influenza virus was first reported to infect humans in Hong Kong in 1997 where there were 18 confirmed cases and a mortality rate of 33% (Claas et al., 1998). To-date influenza virus A (H5N1) has been the causal agent in 317 human cases Worldwide with a mortality rate of 60%

(http://www.who.int/csr/disease/avian_influenza/country/cases_table_2007_06_29/en/index.html).

Little information is available on the proficiency of laboratories Worldwide to accurately detect and subtype influenza viruses including the avian influenza viruses, which are considered as a possible cause of future pandemics in humans. One of the best ways of assessing this ability is through participation in external quality assessment (EQA) programmes ([Meijer et al., 2005], [Templeton et al., 2006] and [Valette and Aymard, 2002]). An EQA study was organised by Quality Control for Molecular Diagnostics (QCMD) (<http://www.qcmd.org>) in collaboration with the European Influenza Surveillance Scheme (EISS) and the European Society for Clinical Virology (ESCV). The aim of this EQA study was to assess the ability of laboratories Worldwide to detect and haemagglutinin subtype influenza viruses using molecular technologies.

2. METHODS

The QCMD Influenza virus Haemagglutinin Typing Proficiency Programme panel consisted of 12 samples containing inactivated influenza viruses of the A and B type (of which the A type viruses comprised haemagglutinin subtypes of human and avian origin) and two negative samples. All panel samples were in 0.5 ml of Dulbecco's modified Eagle's medium/10% Foetal Calf Serum except for influenza virus A (H1N2) and B, which were in 0.5 ml of Viral Transport Medium (Table 1). The panel materials were inactivated by heat and/or irradiation. The materials were analysed by four independent laboratories, recognised as proficient in influenza virus testing using molecular methods, prior to distribution to participating laboratories.

[TABLE 1]

Laboratories had been invited to participate during early 2006. The randomised EQA panel was distributed on dry ice by QCMD in May 2006 (along with instructions on how the panel samples were to be processed) to 87 laboratories in 34 countries Worldwide (Europe, n = 78; Asia, n = 4; Africa, n = 3; Oceania, n = 2). Laboratories were given 6 weeks to test the panel samples using their routine molecular diagnostic tests for influenza virus and to return their results to QCMD via an online data collection system. They were asked to return qualitative data (presence/absence of influenza virus) and the full haemagglutinin subtype in alpha-numeric format. Laboratories were asked to submit a single dataset for each assay performed. Where laboratories returned multiple datasets (reporting on multiple assays) these data were combined resulting in a single set of results for each laboratory. These results were analysed in order to assess the performance of laboratories in the correct detection, typing and haemagglutinin subtyping of influenza viruses.

3. RESULTS

From the 87 laboratories registered for the EQA study, 60 (69%) laboratories returned a total of 114 datasets (average of two datasets each). Sixteen datasets were removed from the analysis because they reported all panel samples as negative or 'not determined' (meaning that neither a positive or negative result was reported), leaving the total number of datasets analysed at 98.

Seven (11.6%) laboratories used commercial assays, 43 (71.7%) used in-house assays and 10 (16.7%) used a mixture of both.

Influenza virus positive panel samples were correctly identified by 35–98.3% of laboratories (mean = 70.1%). The correct type and haemagglutinin subtype was reported by 78–95.7% (mean = 87.6%) and 31.7–87% (mean = 55%) of laboratories that detected the virus. The percentage of laboratories correctly detecting influenza virus was directly proportional to the viral load of the panel samples, with the percentage of laboratories that correctly subtyped the influenza A-type viruses being much less (Table 1).

Two avian influenza viruses were represented in the EQA panel (A (H5) and A (H7)). The influenza virus A (H5) panel sample of highest viral load (approximately 9600 copies/ml) was correctly subtyped by 87.0%

(n = 40/46) of laboratories that detected influenza virus. One laboratory incorrectly reported that the panel sample contained influenza virus A (H1). Five laboratories detected the virus but reported no subtype. The influenza virus A (H7) panel sample (approximately 4500 copies/ml) was correctly subtyped by 31.7% (n = 13/41) of laboratories that detected the virus: five laboratories incorrectly reported that the panel sample contained other influenza viruses (A H3 (n = 2), A H5 (n = 1), A H3 and B (n = 1), B (n = 1)). Twenty-three laboratories detected an influenza virus in the panel sample but reported no subtype.

Two currently circulating human influenza virus A subtypes were represented in the EQA panel (A H1 and A H3). The influenza virus A (H1) panel sample (approximately 188,000 copies/ml) was correctly subtyped by 54.2% (n = 32/59) of laboratories that detected the virus. Five laboratories incorrectly reported that the panel sample contained other influenza viruses (A H3 (n = 1), A H5 (n = 1), A H1 and H5 (n = 2), A H1 and B (n = 1)). Twenty-two laboratories detected the virus but reported no subtype. The influenza virus A (H3) panel sample of highest viral load (approximately 20,000 copies/ml) was correctly subtyped by 51.7% (n = 30/58) of laboratories that detected the virus: four laboratories incorrectly reported that the panel sample contained other influenza viruses (A H1 (n = 1), A H5 (n = 1), A H1 and H5 (n = 1), A H3 and H5 (n = 1)). Twenty-four laboratories detected the virus but reported no subtype.

The two influenza virus negative panel samples were incorrectly reported 15 times. There were 13 (10.8%) false positive results (reported by 12 laboratories) and two 'not determined' results (reported by two laboratories). Three laboratories reported that these panel samples contained influenza virus A (H5) (Table 1).

4. DISCUSSION

Rapid and accurate detection of candidate pandemic influenza virus strains currently circulating in humans is absolutely necessary for the surveillance and control of pandemic influenza (Li et al., 2004). Incorrect diagnosis of a pandemic strain, as a currently circulating influenza virus, would delay intervention and reduce the likelihood of successful containment. The incorrect diagnosis of a candidate pandemic strain, in a case of non-pandemic influenza, would also have serious consequences for the country involved.

This study clearly demonstrated that while laboratories generally showed competence in detecting the presence of influenza virus many did not report the correct haemagglutinin subtype of influenza virus, either of the subtypes currently circulating in humans (influenza virus A H1 or A H3) or of the subtype considered as a risk for a future human pandemic (influenza virus A H5). Incorrect subtyping of influenza viruses was common and included the improper reporting of avian influenza viruses as human strains and vice versa. In addition, this study clearly showed that the ability of laboratories to correctly detect and subtype influenza viruses dropped rapidly with decreasing viral load. These observations may be explained by an incomplete match of the primers and/or probes used for the full range of influenza virus A haemagglutinin subtypes represented in the panel.

False positivity was also high with some avian influenza viruses reported. This may have been the result of contamination occurring in the laboratories concerned. There were no false positive results reported by the independent testing laboratories.

Laboratories must improve their performance in the sensitive detection and correct subtyping of influenza viruses so that inaccuracies in diagnosis can be avoided. This will only be achieved if laboratories have access to the most recent information on circulating strains and proper international reference materials for avian influenza viruses. Laboratories must also monitor their performance regularly and participation in international EQA programmes is an important part of this.

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TABLE

Table 1
Summary of the results returned by laboratories ($n=60$)

Sample content ^a	Stock dilution	Viral load copies/ml ^b	Correct detection		Correct type (A or B) reported ^{c,d}		Correct subtype reported ^c	
			<i>n</i>	%	<i>n</i>	%	<i>n</i>	%
Influenza virus A, H1	1.0×10^{-2}	188,000	59	98.3	53	89.8	32	54.2
Influenza virus A, H3	2.0×10^{-5}	20,000	58	96.7	51	87.9	30	51.7
Influenza virus A, H3	2.0×10^{-6}	2,200	46	76.7	39	84.8	17	37.0
Influenza virus A, H3	1.0×10^{-6}	1,200	39	65.0	33	84.6	16	41.0
Influenza virus A, H3	2.0×10^{-7}	200	21	35.0	18	85.7	7	33.3
Influenza virus A, H5	1.0×10^{-3}	9,600	46	76.7	44	95.7	40	87.0
Influenza virus A, H5	2.0×10^{-4}	1,600	42	70.0	38	90.5	31	73.8
Influenza virus A, H5	1.0×10^{-4}	1,340	40	66.7	37	92.5	27	67.5
Influenza virus A, H5	1.0×10^{-4}	1,460	38	63.3	33	86.8	25	65.8
Influenza virus A, H5	2.0×10^{-5}	130	29	48.3	26	89.7	18	62.1
Influenza virus A, H7	4.0×10^{-5}	4,500	41	68.3	32	78.0	13	31.7
Influenza virus B	1.0×10^{-2}	22,717	46	76.7	39	84.8		
Negative	—	—	53	88.3				
Negative	—	—	52	86.7				

^a The strains used in the panel were as follows. Influenza virus A (H1N2) and B were clinical isolates collected from patients during the 2004–2005 influenza season. Influenza virus A (H1N2) was closely related to A/New Caledonia/20/99 and influenza virus B belonged to the Yamagata lineage. Influenza virus A (H3N2) was A/Netherlands/565/05. Influenza virus A (H5N1) was A/Hong Kong/213/03 and influenza virus A (H7N3) was A/Mallard/Netherlands/12/00.

^b Quantification of influenza virus A was performed using the Invitrogen Superscript One step qRT-PCR Kit on the Applied Biosystems 7500 Real-time PCR machine. Quantification of influenza virus B was performed using an in-house TaqMan Real-time PCR assay on the Applied Biosystems 7700 Real-time PCR machine. The influenza virus A and B quantitative assays were performed on different systems and may not be comparable.

^c The percentage of correct type and subtype results were calculated against the number of laboratories that correctly detected the virus.

^d Participant laboratories were not explicitly requested to report the influenza virus type (A or B). This may be a confounding factor in the reported difference in correct detection of influenza virus and correctly reported influenza virus type.