False positive influenza A and B detections in clinical samples due to contamination with live attenuated influenza vaccine

Curran et al. (2012) described the detection of inactivated influenza A vaccine (IAV) contamination in clinical samples being sent for respiratory virus PCR testing. The authors concluded that the administration of IAV and clinical sampling in the same room resulted in vaccine strains contaminating surveillance swabs collected from patients with influenza-like illness (ILI)/respiratory illness. Herein we describe our recent similar experience in Scotland where we detected the recently licensed live attenuated influenza vaccine (LAIV) in clinical samples taken from patients with ILI.

Between October and November 2013, the West of Scotland Specialist Virology Centre in Glasgow detected 14 throat swab samples which, using real-time PCR methods (Gunson & Carman, 2011), contained low level [cycle threshold (Ct) >35] influenza positive signals (see Table 1). Some of the samples were positive for a single influenza A or B virus whereas others were positive for >1 influenza virus. Eight of the 14 samples were also positive for non-influenza respiratory viruses. The 14 samples were all collected from individual patients that had been sent to us from 10 different GP practices. All GP practices were participating in the Scottish GP spotter influenza surveillance system (HPS, 2013a) and were situated in six different Scottish Health Boards (including Greater Glasgow and Clyde, Fife, Tayside, Lothian and Ayrshire and Arran). On repeat testing all sample were positive and no negative controls were detected as influenza positive during this time.

At the time of testing, influenza A and B activity was very low and detections were not being reported through the Scottish GP spotter surveillance or in other European countries (PHE, 2013). This and the fact that all the positive detections were very weak and that some contained >1 target suggested to us that these low positive results may not represent ‘true’ influenza infection and may in fact be the result of contamination from recent vaccinations. In Scotland the influenza vaccinations began in October and continued throughout the month. Furthermore, this year differed from previous years in that it was the first time that all 2- and 3-year-old children in Scotland had been offered the LAIV (HPS, 2013b).

We investigated whether these detections were actually due to LAIV by testing the 14 samples using a recently published LAIV specific real-time PCR assay (Shcherbik et al., 2014) (see Table 1). This assay targets the matrix region of the influenza A component of the LAIV. In our hands the assay was highly sensitive with the same end point detection limit as our generic influenza A assay. The assay was also found to be specific for H2N2 when challenged with various influenza A subtypes, water controls (n=48) and other non-influenza respiratory pathogens. Of the 14 samples suspected of containing LAIV seven were positive by the H2N2 specific assay. This included one sample that on the initial influenza screen was weakly positive for influenza B only. Rather than consider this a false positive H2N2 result we believe that this result actually represents a false negative influenza A matrix test result. The failure of the matrix assay to detect this weak sample as positive is not surprising as tests with similar detection limits can often give discrepant results when testing such weakly positive samples. The remaining seven samples were negative. This may have been because the samples contained LAIV contamination at levels beyond the cut off of the LAIV assay. However, we cannot rule out the possibility that these samples contained wild-type virus or perhaps IAV derived virus. To clarify testing these samples were subject to Sanger or next-generation sequencing. However, both methods were unable to detect and therefore sequence the virus. Vaccine history was available for 12 of the 14 patients. Of the 12 patients with a vaccine history, three were given IAV and the remaining nine had not received any influenza vaccination. The two patients without a vaccine history were 29 and 75 year olds and therefore would not have been offered LAIV. Since none of the individuals were given LAIV we can rule out the positives being a result of vaccine replication/shedding post vaccination. The false-positive results may be due to transmission of LAIV from recently vaccinated individuals as previous studies have also shown that live vaccine can be, albeit rarely, transmitted between those given vaccine and close contacts. However, none of the 12 patients had known contact with a child who had been recently vaccinated with LAIV.

We feel the most likely explanation for these results is that the intranasal administration of LAIV allows large amounts of influenza A and B virus to be aerosolised, which in turn can contaminate the local environment. Subsequently, samples taken from patients attending with respiratory infection can then become contaminated with LAIV. To give an indication of the level of influenza virus present in LAIV the control used in this study was an RNA extract of LAIV. This extract was tested and found to have Ct <10. We contacted each of the GP practices and found that all had been carrying out LAIV vaccinations in children on site, and all stated that it was likely that vaccinations and sampling had taken place in the same room. In the paper by Curran et al. (2012), they describe the detection of IAV in environmental samples taken from areas where vaccination clinics had taken place, and they showed that vaccine could be detectable on surfaces for up to 66 days. Environmental sampling was not carried out in this instance but would have been informative.

To conclude, our results highlight those laboratories using highly sensitive real-time PCR methods should be aware of the
risk of LAIV contamination occurring within clinical samples taken at the time of vaccination protocols/roll out. Public health bodies should also be aware of this issue when interpreting laboratory surveillance data. Laboratories should consider using a LAIV-specific assay to confirm all weak influenza A or B positive results, especially those detected concurrently with vaccination programmes. Using such a test will ensure that this type of contamination will not affect public health surveillance data or patient management and will prevent unnecessary laboratory expenses should such results be mistaken for PCR contamination. The results also highlight that services offering influenza vaccinations should take special care to decontaminate the environment prior to sampling patients with respiratory illness.

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Abbreviations: Ct, cycle threshold; IAV, inactivated influenza A vaccine; ILI, influenza-like illness; LAIV, licensed live attenuated influenza vaccine.

