Background
The rapid and specific detection of influenza virus during the respiratory season is critical for optimal patient management. Antigen-based testing is often used at the point of care to obtain the best turn-around time. However, these screening tests vary in their test performance and the ability to detect both influenza type A and B efficiently. Clinical decisions are sometimes made on the results of antigen screening tests alone without adherence to the recommendation of confirmation by cell culture or a molecular method. Many detection methods are available, including cell culture, direct immunofluorescence, and molecular tests. Cell culture is considered the “gold standard”, but has the distinct disadvantage of longer turn-around times compared to molecular methods. Likewise, molecular amplification has sometimes been referred to as the “platinum standard”. This study compared antigen screening to a real-time PCR-based commercial assay in order to determine antigen test efficiency during the 2007/2008 respiratory season. Statistics were also provided by the Department of Health from to further support the efficiency of antigen testing as compared to molecular amplification.

Materials and Methods
Specimens (n = 190) consisted of throat and nasopharyngeal swabs submitted during the 2007/2008 respiratory season in Hawaii for influenza virus antigen testing (BD Directigen EZ Flu A+B). Additional data (n = 1,071) from the Hawaii Department of Health, State Laboratory Division was included in this work. All specimens were further tested using a real-time RT-PCR method. Nucleic acid extraction was accomplished using the MagNA Pure LC RNA Isolation Kit (Roche). Real-time RT-PCR amplification and detection was performed using the Cepheid real-time PCR influenza A/B assay in conjunction with the SmartCycler II real-time PCR instrument (Cepheid).

Results
The incidence of influenza virus type A and type B virus infection was 17.9% (34/190) and 11.0% (21/190) respectively in the patient sampling. Influenza virus was detected using RT-PCR in 28.9% (55/190) of the specimens. Alternatively, antigen testing (BD Directigen EZ Flu A+B) detected 7.9% (15/190) influenza type A and 1.0% (2/190) influenza type B. Influenza virus was detected using antigen testing in 4.9% (9/190) of the specimens. Compared to RT-PCR testing, the antigen screening assay missed detecting influenza type A and influenza type B in 55.9% (19/34) and 90.5% (19/21) of the cases respectively. Testing for combined influenza type A and type B virus from the Hawaii Department of Health revealed an influenza antigen sensitivity and specificity of 34.7% and 100% respectively compared to RT-PCR.

Conclusions
These results indicate a much lower test performance for the antigen assays (e.g. BD Directigen EZ Flu A+B) than expected. By comparison, RT-PCR testing demonstrated a higher incidence of influenza virus types A and B in the population sampled. These results support the use of RT-PCR as the preferred method for the diagnosis of influenza virus infection.

DISCUSSION AND CONCLUSIONS
1. These results show a much lower test performance for the antigen assay (e.g. BD Directigen EZ Flu A+B) than expected (e.g. Sensitivity for Flu A in adults 63–90% depending upon the specimen source). Additional antigen test performance is cited in the references.
2. As expected, in the respiratory season the specificity is favorable, but considering the low sensitivity of antigen testing the test should be used with an awareness of the usefulness in the particular clinical setting.
3. Antigen testing is a screen and a negative test should be confirmed by either cell culture or RT-PCR (i.e. data supported by this work.)