Comparison of the Coris Influ A + B K-SeT® and BD Veritor Flu A + B® for rapid detection of influenza viruses in respiratory samples from 3 consecutive flu seasons in Belgium

Comparison of the Coris Influ A+B K-SeT® and BD Veritor Flu A+B® for rapid detection of influenza viruses in respiratory samples from three consecutive flu seasons in Belgium

Sarah Vandamme[1], Shauni Van Cleempoel[1], Mindy Michiels[1], Herman Goossens[1], Hilde Jansens[1], Veerle Matheeussen[1]

Author affiliation: [1] Laboratory of Clinical Microbiology, Antwerp University Hospital, Wilrijkstraat 10, 2650 Edegem, Belgium

1. Introduction

Influenza is a contagious respiratory illness predominantly caused by influenza viruses A and B. Substantial morbidity and mortality can be attributed to seasonal influenza epidemics worldwide. In Europe, the flu seasons of 2014 to 2017 resulted in an excess of 122 deaths per 100 000 people [1]. Especially vulnerable populations such as pregnant women, the extremes of age, immunocompromised patients and patients with chronic kidney or heart disease, have a high risk of complications e.g. pneumonia, bacterial superinfection and death [2].

The rapid laboratory diagnosis of influenza significantly decreases the (mis)use of antibiotics and overuse of laboratory and radiographic testing while prompting infection-control measures, ultimately leading to decreased healthcare costs [3, 4, 5]. Several methods for influenza detection are currently available such as rapid antigen tests, also known as rapid influenza diagnostic tests (RIDT), and molecular tests. RIDTs have demonstrated a relatively good specificity but lower sensitivity compared to molecular tests [6-8]. Still, they remain the test of choice in many laboratories due to the short turn-around-time (TAT), simplicity in assay procedure and low cost [8]. Among RIDTs the Veritor Flu A+B® (Becton Dickinson) is a chromatographic immunoassay which has proven to be a reliable and fast test [3, 9, 10]. Molecular tests are considered as the gold standard, yielding highly specific and sensitive results [3]. Newly developed sample-in-result-out molecular systems such as GeneXpert®, Cobas Liat® or Alere i® are less technically demanding and have shorter TATs than the “old school” RT-PCR assays requiring manual or (semi-)automated extraction and amplification steps. Yet molecular tests are expensive and are not readily available in every laboratory or outpatient setting [11].

This study evaluates the clinical performance and user friendliness of a new commercially available RIDT, the Influ A+B K-SeT® (Coris BioConcept) in comparison with the established Veritor Flu A+B® for the detection of influenza viruses in nasopharyngeal aspirates (NPA). These NPA specimens were collected during 3 consecutive influenza seasons to challenge the robustness of the assays in detecting different influenza subtypes. Subsequently, the impact on the RIDT performance using fresh versus frozen specimens was evaluated. A commercially available RT-PCR (FTD FLU/HRSV®, Fast Track Diagnostics) was used as reference method. To our knowledge this is the first study that assesses the above mentioned characteristics of both antigen assays compared to RT-PCR in a diagnostic laboratory setting.

2. Materials and methods

2.1 Clinical samples

Nasopharyngeal aspirates (NPA) were obtained by nasopharyngeal wash using a syringe with saline water to recover an NPA of approximately 1 ml. Samples were sent to the microbiology laboratory for routine influenza diagnostics using the FTD FLU/HRSV® RT-PCR assay (Fast Track Diagnostics) as part of the clinical work-up of patients with influenza-like illness in a tertiary hospital (Antwerp University Hospital) during 3 consecutive flu seasons (2014-2017). One-hundred-ninety-eight of these samples were randomly selected to be analysed by the RIDTs. Samples from flu season 2014-2015 (n=57), 2015-2016 (n=63) and 2016-2017 (n=78) were stored at -80°C for two years, -20°C for one year and 4°C respectively until analysis. Samples were obtained mostly from children under the age of 6 (n=152, i.e. 77.0%) but also patients older than 65 years (n=13, i.e. 6.6%) were included since the extremes of ages are the most vulnerable patients.

2.2 Influenza detection techniques

2.2.1. Real-time PCR

A commercial kit, FD FLU/HRSV® from Fast-Track Diagnostics, was used as reference test. The kit is capable of detecting RSV and influenza virus A and B simultaneously in multiple types of respiratory specimens, such as NPA, nasal and throat swabs, bronchoalveolar lavage fluid and sputum. The assay was performed according to the manufacturer's instructions. In short the RNA was extracted using the NucliSENS® easyMAG® (bioMérieux) semi-automated extractor. An internal extraction control (brome mosaic virus) was added to each sample before extraction. After extraction, the RNA-extract was transformed to cDNA and amplificated by a real-time one-step PCR on the Lightcycler®480 (Roche).

2.2.2 BD Veritor System for Rapid Detection of Flu A+B/RSV®

The Veritor Flu A+B/RSV® is an immunochromatographic assay containing murine monoclonal antibodies targeting influenza A or B antigens. Specimens suitable for analysis are NPA, nasopharyngeal swabs and bronchoalveolar lavage fluid. The assay and quality controls were performed according to the manufacturer's instructions. In short, 300 µL of NPA was added to a prefilled reagent tube containing 100 µL detergent solution. After vortexing thoroughly, three drops of the mixture was dispensed into the sample well of the reagent strip and incubated for 10 minutes at room temperature. Following incubation, the reagent strip was interpreted by a compact automatic reader which generated a negative, positive or invalid result after 10 seconds.

2.2.3. Influ A+B K-SeT® (Coris, Bioconcept)

The Influ A+B K-SeT® is an immunochromatographic assay containing monoclonal antibodies targeting the nucleoprotein antigens of influenza A or B and colloidal gold particles. Specimens suitable for analysis are NPA and nasopharyngeal swabs. The assay and quality controls were performed according to the manufacturer's instructions. In short, 7 drops of extraction buffer were added to 200 µL of NPA followed by thorough vortexing. One hundred µL of this mixture was added to the sample well of the cassette and incubated for 15 minutes at room temperature. Following

incubation, the reagent strip was interpreted visually by a lab technician, assisted by a second technician in case of doubt.

2.3 Statistical analysis

The results of the two antigen tests were divided into the following categories: true positive (TP), true negative (TN), false positive (FP) and false negative (FN) with RT-PCR as the gold standard. Subsequently the performance characteristics, i.e. sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV), were calculated for the two antigen tests and expressed as a 95% confidence interval (CI). Furthermore, test agreement was compared using kappa concordance. To visualize the results of the antigen tests in relation to the cycle treshold (Ct) values of the RT-PCR assay a Whisker-box plot was used and means were compared using Student's T test. A p-value <0.05 was considered statistically significant. Statistical analysis software consisted of Microsoft Office Excel® 2016 software (Microsoft Corporation, USA) and MedCalc® v.17.5.5 (MedCalc Software Ltd., Belgium).

3. Results

Among the 198 nasopharyngeal aspirates, 53 (27%) were positive for influenza A and 9 (5%) for influenza B by FTD FLU/HRSV® RT-PCR (Table 1), resulting in an overall influenza positivity rate of 32%. The median age in the population positive for influenza A or B was 2 years (1 – 5 years 95% CI). The proportion of influenza positive patients per age group was as follows: 26% of children under the age of 6, 45% of patients between 6 and 65 years of age and 38% of patients older than 65 years. There were 9 invalid RIDT-results: 6 for Influ A+B K-Set® (3 PCR positive, 3 PCR negative) and 3 for Veritor Flu A+B® (1 PCR positive, 2 PCR negative). Hence the number of eligible samples for data analysis was 192 for Influ A+B K-SeT® and 195 for Veritor Flu A+B®. The overall performance characteristics for both RIDTs compared to RT-PCR are depicted in Table 2. Of the PCR-positive samples (n=62), the Influ A+B K-SeT® detected 43/51 (84.3%) influenza A and 5/8 (62.5%) influenza B while the Veritor Flu A+B® detected 47/52 (90.4%) influenza A and 7/9 (77.8%) influenza B, resulting in overall sensitivities of 81.4 and 88.5% for Influ A+B K-SeT® and Veritor Flu A+B® respectively. Influ A+B K-SeT® missed 8 influenza A and 3 influenza B positive samples (NPV 92.3%) in contrast to Veritor Flu A+B® which missed 5 influenza A and 2 influenza B positive samples (NPV 94.9%). The Influ A+B K-SeT® false negatives had RT-PCR Ct values ranging from 22.66 to 28.92, which was not significantly different from the Ct values of the Veritor Flu A+B® false negatives ranging from 25.89 to 28.92 (p=0.2). For both RIDTs, true positive samples had a significantly lower Ct value compared to false negative samples as shown in figure 1. Influ A+B K-SeT® generated two and Veritor Flu A+B® three false positive influenza A results, resulting in specificities of 98.5 and 97.8% and PPVs of 96.0 and 94.7% respectively. Concordantly both RIDTs achieved very good inter-rater agreement with RT-PCR as demonstrated by a kappa value of 0.83 (0.75 – 0.92 95% CI) for Influ A+B K-SeT® and 0.88 (0.81 – 0.95 95% CI) for Veritor Flu A+B®. In spite of comparable performance characteristics, there were 8 discrepant results between the Influ A+B K-SeT® and the Veritor Flu A+B®. Seven samples were positive with Veritor Flu A+B® but negative with Influ A+B K-SeT, of which 2 were negative and 5 positive by RT-PCR. These RIDT discordant true positive samples (mean Ct 24.57, 22.31 – 26.83 95% CI) did not show a significant difference (p=0.8) in Ct values compared to the RIDT concordant true

positives (mean Ct 22.83, 22.04 – 23.62 95% CI). One sample was detected positive by Influ A+B K-SeT®, but not by Veritor Flu A+B® and was confirmed by RT-PCR as positive (Ct value 25.89).

Some variables may have an effect on the robustness of the assays studied. The majority of the samples in this study were kept frozen until analysis, which might have affected the performance characteristics. Table 3 shows the number of correct results (true positives + true negatives) for the two storage conditions compared to RT-PCR for both assays. There was no significant difference between fresh or frozen samples nor between the two assays in terms of correct results. Also the age of the study population did not have a significant influence on the performance of either assay. Twenty-five samples of adult patients (>18 years old) were all correctly classified by Veritor Flu A+B® compared to RT-PCR. Twenty-three adult samples were tested with Influ A+B K-SeT® of which only two samples showed false negative results compared to RT-PCR. The cause of the discordancy was most likely the flocculent condition of the samples as opposed to the patients' age (both >65 years old).

Regarding user friendliness, the processing time and ultimately turn-around-time are important. The overall processing time of the Influ A+B K-SeT® and Veritor Flu A+B® was 17 and 12 minutes for a single specimen respectively, with a hands-on time for both around 2 minutes. To assure the shortest turn-around-time, unambiguously positive or negative results are desired as opposed to invalid results. The sample's condition is pivotal to obtain reliable results: ideally, it is clear and easily aspirated. There were 9 invalid results, 3 with the Veritor Flu A+B® and 6 with the Influ A+B K-SeT®. Revision of these samples consistently showed viscous and/or flocculent NPA's. In compliance with this observation, samples which were clear or even haemolytic or cloudy but easy to aspirate had no invalid results in this study.

4. Discussion

On-site diagnosis of influenza by point-of-care (POC) tests helps to decrease prescription of antimicrobials, requests for blood cultures and chest radiography, ultimately leading to reduced healthcare costs [13]. Rapid antigen tests for influenza are very useful as a POC test due to their short TATs (15 – 30 minutes), low cost and ease of use [3] although they do not approach the diagnostic accuracy of molecular methods.

In this study, the Influ A+B K-SeT® (Coris, Bioconcept) and Veritor Flu A+B® (BD) were compared with FTD FLU/HRSV® (Fast-Track Diagnostics) for detecting influenza A and B viruses in clinical samples. The overall sensitivity and negative predictive value of the Veritor® system were higher than the Influ A+B K-SeT®. The specificity and positive predictive value were high for both assays with a small advantage for the Influ A+B K-SeT®. These are critical performance characteristics affecting the patient's management. It is important to identify the infected patients in need of antiviral therapy and infection-control measures whilst restricting their use of antimicrobials and preventing unnecessary hospitalisation. Our results for Veritor Flu A+B® are comparable to the claim of the manufacturer and other previously published studies, who reported overall sensitivities ranging from 70.7 to 98.1% and specificities ranging from 94.0 to 100.0% compared to RT-PCR [3, 9, 10, 14-16]. Studies using nasopharyngeal swabs found lower sensitivities (median 82.4%) than our study which used solely NPA [10, 14-17]. No studies are available to evaluate the performance characteristics that we obtained for Influ A+B K-SeT®, which were lower than those claimed by the manufacturer (Coris BioConcept), i.e. 100% for all parameters [18]. It is important to note that they used

immunofluorescence as reference method. Yet, several studies showed that PCR assays were significantly more sensitive than immunofluorescent assays for diagnosis of viral respiratory infections [19].

Regarding the robustness of both RIDTs for the different influenza subtypes and lineages, no definite conclusion can be made. According to the Belgian national reference centre for influenza, the flu seasons 2014-2015 and 2016-2017 were characterized by a predominance of influenza A (mainly subtype H3N2) and little influenza B (mainly Yamagata lineage). Flu season 2015-2016 was marked by an equal prevalence of influenza A (mainly subtype pdmH1N1) and influenza B (mainly Victoria lineage) [12]. Table 1 demonstrates the distribution of samples over the three flu seasons and their influenza status based on the FTD FLU/HRSV® RT-PCR reference test. Good performance characteristics were obtained in this study spanning the mentioned flu seasons. This mirrors the reality of receiving different influenza strains, yet for these study samples confirmatory typing was not obtained. The majority of samples has been frozen prior to analysis though fresh samples are recommended by the manufacturers to assure the best performance. We compared the correct results of both assays obtained with frozen versus fresh samples and observed no difference. We conclude that one freeze-thaw cycle does not affect the performance of both RIDTs. Age on the other hand might affect the performance of RIDTs as it has been shown to be better in children compared to adults, potentially due to higher viral loads and longer viral shedding in children [20]. According to our findings, age does not seem to have a significant influence on the performance of either assay. This has to be interpreted cautiously given the sample size. Discordances in our study were most likely explained by the flocculent condition of the samples as opposed to the patients' age. Both assays have difficulties analysing viscous or flocculent samples, leading to invalid results due to absence of a reaction at the quality control position. A possible solution would be to dilute the sample to obtain a sufficiently liquid sample which is easy to aspirate and which can distribute itself properly along the test strip. The resulting performance is uncertain as the sensitivity can be compromised by decreasing the viral load.

Other criteria for RIDTs such as the user-friendliness and TAT are also of importance, particularly considering the use in an outpatient setting. The Veritor Flu A+B® performs better on these aspects than the Influ A+B K-SeT®, being five minutes faster and easy to interpret when using the digital reader, which eliminates subjective, visual interpretation. However, in any case an inspection of the test strip is needed to check the absence of abnormalities that might interfere with correct reading [21]. By contrast, the Influ A+B K-SeT® requires interpretation by the test operator which can be challenging especially when test lines are very faint. According to the manufacturer's instruction any (weak) red to purple line at the test line position should be considered a positive result. The kit insert warns not to mistake a faint shadow, which can occur as result of the drying process, as a positive result [21]. These interpretation rules are prone to inter-individual variability and misdiagnosis [11]. In our study, each reagent strip was judged by the same laboratory technician, assisted by a second technician in case of doubt. In settings where this immunochromatographic assay would be used by multiple test operators more variation might be expected.

Our study indicates that RIDTs have a good performance in comparison to RT-PCR and show robustness regarding their results for several subtypes of influenza type A. Nonetheless, molecular POC assays are emerging as a worthy competitor, providing high sensitivity and multiple pathogen detection. The most pronounced disadvantage is the cost of such assays. RIDTs on the other hand are very easy to use, quick and more affordable. In settings where molecular tests are not readily available, an RIDT can be of great value despite its lower sensitivity [10].

5. Conclusion

In summary, both RIDTs performed well in detecting influenza virus A and B in nasopharyngeal aspirates compared to RT-PCR as reference method, with a higher sensitivity for the Veritor Flu A+B® test. Visual result interpretation of the Influ A+B K-SeT® requires trained lab technicians, while the digital reader of the Veritor® system minimizes operator errors. To our knowledge this is the first study assessing the performance characteristics, robustness and user friendliness of the assays mentioned in a diagnostic laboratory setting.

6. Acknowledgements

Influ A+B K-SeT® tests were kindly provided by Coris Bioconcept.

References

1. Vestergaard, L.S., et al., *Excess all-cause and influenza-attributable mortality in Europe, December 2016 to February 2017.* Euro Surveill, 2017. **22**(14). https://doi.org/10.2807/1560-7917.ES.2017.22.14.30506.
2. Centers for Disease Control and Prevention. *People at High Risk of Developing Flu–Related Complications*. https://www.cdc.gov/flu/about/disease/high_risk.htm, 2018 (accessed 9 April 2018)
3. Hassan, F., et al., *Comparison of the BD Veritor System for Flu A+B with the Alere BinaxNOW influenza A&B card for detection of influenza A and B viruses in respiratory specimens from pediatric patients.* J Clin Microbiol, 2014. **52**(3): p. 906-10. https://doi: 10.1128/JCM.02484-13.
4. Woo, P.C., et al., *Cost-effectiveness of rapid diagnosis of viral respiratory tract infections in pediatric patients.* J Clin Microbiol, 1997. **35**(6): p. 1579-81.
5. Petric, M., L. Comanor, and C.A. Petti, *Role of the laboratory in diagnosis of influenza during seasonal epidemics and potential pandemics.* J Infect Dis, 2006. **194 Suppl 2**: p. S98-110. https://doi:10.1086/507554.
6. Weinberg, A. and M.L. Walker, *Evaluation of three immunoassay kits for rapid detection of influenza virus A and B.* Clin Diagn Lab Immunol, 2005. **12**(3): p. 367-70. https://doi: 10.1128/CDLI.12.3.367-370.2005.
7. Hurt, A.C., et al., *Performance of six influenza rapid tests in detecting human influenza in clinical specimens.* J Clin Virol, 2007. **39**(2): p. 132-5. https://doi:10.1016/j.jcv.2007.03.002.
8. Vemula, S.V., et al., *Current Approaches for Diagnosis of Influenza Virus Infections in Humans.* Viruses, 2016. **8**(4): p. 96. https://doi:10.3390/v8040096.
9. Dunn, J., et al., *Prompt detection of influenza A and B viruses using the BD Veritor System Flu A+B, Quidel(R) Sofia(R) Influenza A+B FIA, and Alere BinaxNOW(R) Influenza A&B compared to real-time reverse transcription-polymerase chain reaction (RT-PCR).* Diagn Microbiol Infect Dis, 2014. **79**(1): p. 10-3. https://doi: 10.1016/j.diagmicrobio.2014.01.018.
10. Leonardi, G.P., et al., *Comparison of the Sofia and Veritor direct antigen detection assay systems for identification of influenza viruses from patient nasopharyngeal specimens.* J Clin Microbiol, 2015. **53**(4): p. 1345-7. https://doi: 10.1128/JCM.03441-14.
11. Jonckheere, S., et al., *Multicenter evaluation of BD Veritor System and RSV K-SeT for rapid detection of respiratory syncytial virus in a diagnostic laboratory setting.* Diagn Microbiol Infect Dis, 2015. **83**(1): p. 37-40. https://doi:10.1016/j.diagmicrobio.2015.05.012.

12. WIV-ISP, *Influenza: Results of the surveillance of influenza and influenza-like illness in Belgium.* https://epidemio.wiv-isp.be/ID/diseases/Pages/Influenza.aspx, 2018 (accessed 30 April 2018).
13. Bonner, A.B., et al., *Impact of the rapid diagnosis of influenza on physician decision-making and patient management in the pediatric emergency department: results of a randomized, prospective, controlled trial.* Pediatrics, 2003. **112**(2): p. 363-7. https://doi:10.1542/peds.112.2.363.
14. Ryu, S.W., et al., *Comparison of three rapid influenza diagnostic tests with digital readout systems and one conventional rapid influenza diagnostic test.* J Clin Lab Anal, 2018. **32**(2). https://doi:10.1002/jcla.22234.
15. Mese, S., et al., *Analytical performance of the BD veritor system for rapid detection of influenza virus A and B in a primary healthcare setting.* BMC Infect Dis, 2016. **16**: p. 481. https://doi:10.1186/s12879-016-1811-9.
16. Nam, M.H., et al., *Clinical performance evaluation of the BD Veritor System Flu A+B assay.* J Virol Methods, 2014. **204**: p. 86-90. https://doi:10.1016/j.jviromet.2014.04.009.
17. Reynders, M., et al., *Prospective evaluation of Coris Influ-A&B Respi-Strip and of BinaxNOW Influenza A&B assay against viral culture and real-time PCR assay for detection of 2009 pandemic influenza A/H1N1v in Belgian patients.* Acta Clin Belg, 2012. **67**(2): p. 94-8. https://doi:10.2143/ACB.67.2.2062638.
18. Coris BioConcept, Gembloux, Belgium. *In vitro Rapid Diagnostic Test for the detection of Respiratory Influenza A and B Viruses in nasopharyngeal secretions v.02 [package insert].* https://www.e-labeling.eu/COR58121/38897/EN#remarkPopup, 2016 (accessed 9 April 2018).
19. Kuypers, J., et al., *Comparison of real-time PCR assays with fluorescent-antibody assays for diagnosis of respiratory virus infections in children.* J Clin Microbiol, 2006. **44**(7): p. 2382-8. https://doi:10.1128/JCM.00216-06.
20. Cruz, A.T., et al., *Performance of a rapid influenza test in children during the H1N1 2009 influenza a outbreak.* Pediatrics, 2010. **125**(3): p. e645-50. https://doi:10.1542/peds.2009-3060.
21. Becton, Dickinson and Company, Maryland, USA. *BD Veritor™ System For Rapid Detection Flu A+B v. 2016-05 [package insert].* https://www.bd.com/documents/.../DS_POC_Veritor-system-flu-AB-lab-kit_MU_EN.pdf, 2016 (accessed 9 April 2018).

Figures and tables

Influenza season	N° of tested samples	Influenza A positive	Influenza B positive	Influenza A/B negative
2014-2015	57	32 (56%)	-	25 (44%)
2015-2016	63	7 (11%)	9 (14%)	47 (75%)
2016-2017	78	14 (18%)	-	64 (82%)
Total	198	53 (27%)	9 (4%)	136 (69%)

Table 1: Number of samples tested by RT-PCR and percentages of positive and negative results per season

	INFLUENZA A		**INFLUENZA B**	
	Influ A+B K-SeT®	**Veritor Flu A+B®**	**Influ A+B K-SeT®**	**Veritor Flu A+B®**
	(n=192)	**(n=195)**	**(n=192)**	**(n=195)**
True positives	43	47	5	7
False negatives	8	5	3	2
True negatives	139	140	184	186
False positives	2	3	0	0
Sensitivity	84.3	90.4	62.5	77.8
(% [95% CI])	(71.4 - 93.0)	(79.0 - 96.8)	(24.5 - 91.5)	(40.0 - 97.2)
Specificity	98.6	97.9	100.0	100.0
(% [95% CI])	(95.0 - 99.8)	(94.0 - 99.6)	(98.0 - 100.0)	(98.0 - 100.0)
PPV	95.6	94.0	100.0	100.0
(% [95% CI])	(84.4 - 98.8)	(83.6 - 98.0)	(46.3 - 100.0)	(56.1 - 100.0)
NPV	94.6	96.6	98.4	98.9
(% [95% CI])	(90.2 - 97.0)	(92.4 - 98.5)	(96.2 - 99.3)	(96.5 - 99.7)

Table 2: Performance characteristics of the Veritor Flu A+B® and Influ A+B K-SeT® with reference RT-PCR

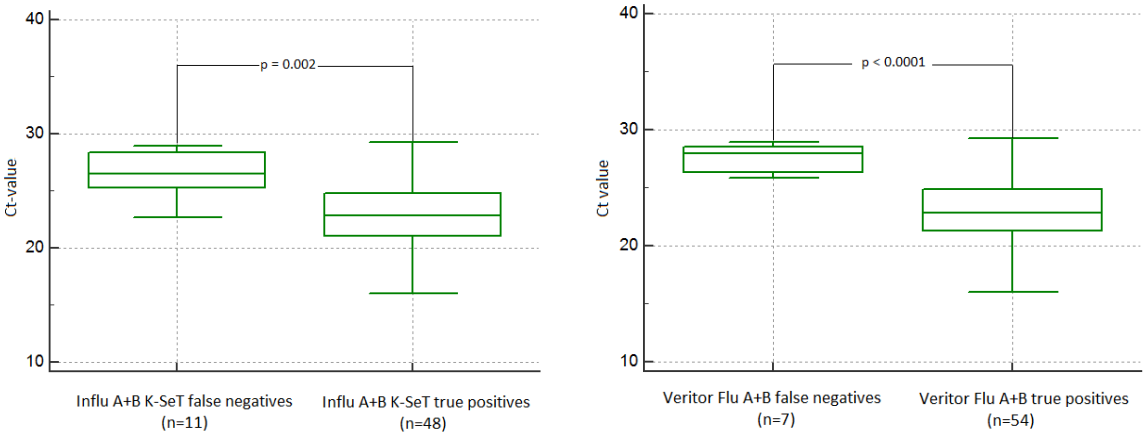

Fig.1: Whisker-Box plots comparing PCR Ct values of Influ A+B K-SeT® true positive and false negative samples (left panel) and Veritor Flu A+B® true positive and false negative samples (right panel).

Correct results / interpretable results (%)

compared to RT-PCR

	Fresh	Frozen
Influ A+B K-SeT®	70 / 76 (92%)	109 / 116 (94%)
Veritor Flu A+B®	72 / 76 (95%)	113 / 119 (95%)

Table 3: Agreement among testing of fresh versus frozen specimens

203