



## Virology

Influenza B false-positive results by rapid molecular tests Alere™ i  
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## ABSTRACT

The Alere-i™ Influenza A&B (Abbott), a nicking endonuclease amplification reaction test, has recently been improved in order to deliver results in a few minutes. Our field observation highlights two problems with this new version: improper interpretation of a test as valid despite improper reagent hydration and falsely influenza B positive results. We advise users of the new system to check reagent hydration prior to reporting a result and to systematically confirm positive influenza B results.

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## Note

Rapid molecular tests are excellent tools for the detection of influenza A or B strain RNA and serve as excellent tools optimizing hospital infection control and patient management (Busson et al., 2017). These tests demonstrate good sensitivity and specificity (Hassan et al., 2018; Valentin et al., 2019). The Alere-i™ Influenza A&B (Abbott), a nicking endonuclease amplification reaction test, has recently been improved in order to deliver results in a few minutes (US Food and Drug Administration marketing clearance). Since the recent implementation of this technique, we have identified a significant number of false-positive results for influenza B using nasopharyngeal swabs eluted in viral transport media (Virocult®, Sigma).

The first case was identified in a 25-year-old man admitted to the ICU for a coma. A sample was sent to the French National Centre for Respiratory Viral Infections where both RT-PCR and culture were negative for influenza. In order to investigate this false positive, we used positive and negative controls included in the kit and test samples obtained from the platform and lab bench to search for potential cross contamination. We also evaluated the sterile transport medium to search a nonspecific reaction, but all RT-PCR results were negative. The manufacturer's inspection of the platform revealed no problems.

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In the subsequent few days, 3 new samples were positive for influenza B. To confirm these results, we performed new tests on the Alere-i™ platform and with the Xpert® Flu A/B Assay on the Xpert® instrument (Cepheid). All tests were negative regardless of which instrument was used. During an investigation into potential sources of error, we observed an insufficient fixation of the transfer cartridge on the test base for 2 out of 3 Alere samples, resulting in improper hydration of lyophilized reagents and leading to a false-positive result. We tried to reproduce this manipulation error to generate a false positive but only obtained invalid results, underlining the random nature of the problem. Technicians were given general instruction to ensure the transfer cartridge was correctly positioned during the procedure. We also set up a systematic visual control procedure for proper reagent hydration prior to reporting any result.

We modified our procedure as described and communicated this information to another microbiology department that had been using the Alere-i™ Influenza test for 1 month. For each positive result in influenza B, we performed control by the same technique, and in instances of discordance, confirmation tests were performed using the Xpert® Flu A/B Assay. This process revealed 2 additional false positives in our laboratory and 5 in the other laboratory (1.66%; 11/611 tests) despite proper handling and validity of the test procedure. For each false-positive sample, the control with the Alere I Influenza A&B remained negative. Because of this problem with influenza B false positives, we confirmed all previous Alere-i™ positive A flu results with a separate RT-PCR test.

**Table 1**  
Specificity and positive predictive value for influenza B.

Alere™ i Influenza A/B : Flu B		RT-PCR	
		Pos	Neg
Alere i	Pos	1	11
	Neg	0	649
	Total	1	660

Specificity : 649/660 (98,3%), PPV: 1/11 (8,3%).

The lack of specificity of the Alere-i™ Influenza A & B test had previously been described in the literature but only in the context of samples which were positive for both A and B types despite being positive for one strain or the other (Chaplin and Flores-Cortez, 2015). Our field observation highlights 2 problems. The first is improper interpretation of a test as valid and influenza B positive despite improper reagent hydration; the platform should not generate a result when the reagent is not properly hydrated. Additionally, 1.66% of samples were falsely positive influenza B with this new system. The manufacturer's specifications indicate a 98.3% specificity (95% CI: 97.4–98.9%) and positive predictive value (PPV) of 82.8% (99/111) to 97.6% (123/126). We observed a similar specificity but a very low PPV of 8.3% (1/12) (Table 1).

During our root cause investigation, we initially suspected a problem related to mishandling, the platform itself, or a batch of reagent, but our multicentric study suggests a problem with the new platform's software. False-positive results were obtained only for influenza B, and platform controls were consistently negative. We advise users of the new system to check reagent hydration prior to reporting a result and to systematically confirm positive influenza B results on the same platform or using another technique. These data were transmitted to Abbott® to urge them to investigate these problems.

## Declarations

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### Competing interests

None declared.

### Ethical approval

Not applicable.

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