CAT
Critically Appraised Topic

Titel: Influenza and RSV-infections: current standard in accurate, time- and cost-efficient diagnosis.

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CLINICAL BOTTOM LINE

We aimed to define an optimal diagnostic strategy in influenza and RSV infections. We addressed this question from two perspectives: 1) We identify the most accurate, time-efficient and cost-efficient diagnostic tests available for influenza and RSV diagnosis. 2) We define the role of influenza and RSV testing in hospital settings and propose the desired test characteristics of the most appropriate test.

What is the most accurate, time-efficient and cost-efficient diagnostic test available?
Nucleic acid amplification tests (NAAT) have better diagnostic performance characteristics than traditional methods (immunoassays, viral culture, viral serology). **Newer rapid NAATs (Roche Cobas Liat, GeneXpert Xpress Flu, Alere i) are user-friendly, highly sensitive and have brief turn-around-times** (< 30 minutes), compared to semi-rapid NAATs (TAT < 3 hours) and classical RT-PCR assays, **but are significantly more expensive. Cost-efficiency of these assay depends on their clinical impact.**

What is the clinical impact of influenza and RSV testing?
Given the dubious therapeutic efficacy of influenza antivirals, **viral testing for directing antiviral therapy, is in our practice, a minor and questionable indication**. RSV testing might be useful for specific populations, where ribavirin therapy might be beneficial. **No definitive benefit on directing antibiotic therapy** has been demonstrated in high-quality trials in children and adults with influenza, although some studies suggest a minor benefit. No RCTs have been performed using the new point-of-care (POC) NAATs. **A decrease in the use of chest X-rays** was demonstrated in the pediatric Emergency department, with rapid antigen tests. No impact was seen on other technical investigations. In high-quality trials, **viral testing does not have an impact on hospital admission rate**. Inconsistent results were obtained for length-of-stay (LOS). One high-quality trial reported a **benefit in guiding isolation measures in influenza infections**. Inconsistent results were obtained for an impact on length of time in the Emergency department. **Testing for influenza and RSV is useful for guiding isolation measures. There is currently a lack of high-quality trials examining the clinical impact of the newly introduced sensitive point-of-care NAATs on patient management.**

What is the optimal diagnostic strategy in influenza and RSV infections?
A highly sensitive test is required to rule out the diagnosis, in order to direct appropriate isolation precautions. **The GeneXpert Xpress and Roche Cobas Liat, in addition to many standard core lab PCR assays**, meet this criterium. Rapid (TAT < 30 minutes) or semi-rapid NAATs (TAT < 3h) are preferred, given the importance of a timely test result to guide isolation measures. **Two tier testing using immunoassays in a first step, and confirmatory testing with a (semi-)rapid NAAT in a second step, might be still be time-efficient, and possibly more cost-efficient in children.**

Although viral testing does not seem to be cost-effective, considering the therapeutic, diagnostic and outcome impact, testing has a crucial role in guiding isolation measures to prevent nosocomial outbreaks, and therefore we suggest that viral testing is cost-effective.
The added value of the newly developed and more expensive rapid NAATs as opposed to semi-rapid lab assays present in many central laboratories, is yet uncertain.

Conclusion
The use of one-step rapid or semi-rapid NAATs, or two-tier testing (including use of an immunoassay) are the preferred diagnostic strategies, where their use in timely guidance of isolation measures is the best supported test indication. The added value and cost-effectiveness of rapid NAATs in terms of impact on isolation and clinical management is yet to be determined in high-quality trials.

CLINICAL/DIAGNOSTIC SCENARIO

Influenza and Respiratory Syncytial Virus (RSV) are leading causes of respiratory tract infections requiring hospitalization (1–3). The identification of influenza virus (and to a lesser extent RSV) has traditionally been considered to be clinically useful and cost-effective, using rapid and low-cost immunoassays (immunochromatographic or immunofluorescence-based) (4,5). However, traditional tests are limited by their suboptimal sensitivity and/or laborious testing procedure, necessitating second line testing with slower PCR-based assays (6). Recently, rapid and highly accurate nucleic-acid amplification tests (NAATs) for influenza and RSV identification have become available, offering highly sensitive results within 30 minutes. These assays offer a sample-to-result configuration, facilitating continuity during irregular hours. Their significantly higher cost per test has brought the question of clinical impact and cost-effectiveness to attention again, particularly as these often are not reimbursed (as in the Belgian healthcare system). A benchmark (table 1) among nine Belgian hospital laboratories of the BILULU study group revealed a significant heterogeneity in diagnostic workflows for influenza and RSV identification. This group encourages harmonization among its members through evidence-based study of the literature, and through exchanging knowledge and experiences. In this context, we defined the optimal diagnostic strategy in influenza and RSV infections, based on a literature search and on user data of our lab and collaborating labs. We addressed this question from two perspectives: 1) We identified the most accurate, time-efficient and cost-efficient diagnostic tests presently available for influenza and RSV identification. 2) We defined the role of influenza and RSV testing in hospital settings and proposed the desired test characteristics of the required test.

Table 1. Influenza/RSV diagnostic tests in the nine[1] BILULU hospitals.

	First line	Second line[1]
1	BD Max BioGX (Becton Dickinson) GeneXpert Flu/RSV (Cepheid)	Biofire FilmArray (bioMérieux)
2	BD Veritor (Becton Dickinson) Alere i (Alere)	Luminex xTAG RPP (Luminex Corporation)
3	BD Veritor Custom multiplex PCR	
4	GeneXpert Flu/RSV Alere RSV antigen test (Alere)	Custom multiplex PCR
5	Quidel Sofia (Quidel Corporation) Custom multiplex PCR	
6	BD Veritor Custom multiplex PCR	
7	Quidel Sofia Direct immunofluorescence Viral culture	
8	BD Veritor	Custom multiplex PCR (external lab)
9	BD Veritor GeneXpert Flu/RSV	Luminex xTAG RPP

[1] Concerns tests that are used within a two-tier workflow, when first line tests are negative.

QUESTION(S)

The assessment of the evidence for diagnostic testing of influenza and RSV is here considered according to the approach proposed by Price (Fig. 1) (7).

1. **Technical test performance**
   - 1.1. **Question 1**: What pre-analytical factors influence the identification of influenza and RSV?
   - 1.2. **Question 2**: What analytical factors influence the identification of influenza and RSV?
2. **Diagnostic test performance**
   - 2.1. **Question 3**: What are diagnostic performance characteristics of influenza- and RSV- tests?
3. **Clinical test impact**
   - 3.1. **Question 4**: What is the clinical impact of identification of influenza and RSV on therapy decisions?
     - 3.1.1. What is the clinical impact of influenza identification on antiviral therapy?
     - 3.1.2. What is the clinical impact of RSV identification on antiviral therapy?
     - 3.1.3. What is the clinical impact of influenza and RSV identification on to antibiotic therapy?
   - 3.2. **Question 5**: What is the clinical impact of identification of influenza and RSV on use of other technical investigations?
   - 3.3. **Question 6**: What is the clinical impact of identification of influenza and RSV on patient health outcome?
   - 3.4. **Question 7**: What other benefits does influenza and RSV testing offer?
4. **Organizational impact**
   - 4.1. **Question 8**: What is the organizational impact of identification of influenza and RSV?
5. **Cost effectiveness**
   - **5.1. Question 9: Is influenza and RSV testing cost-effective?**
   - **5.2. Question 10: What is the optimal diagnostic strategy in influenza and RSV infections?**

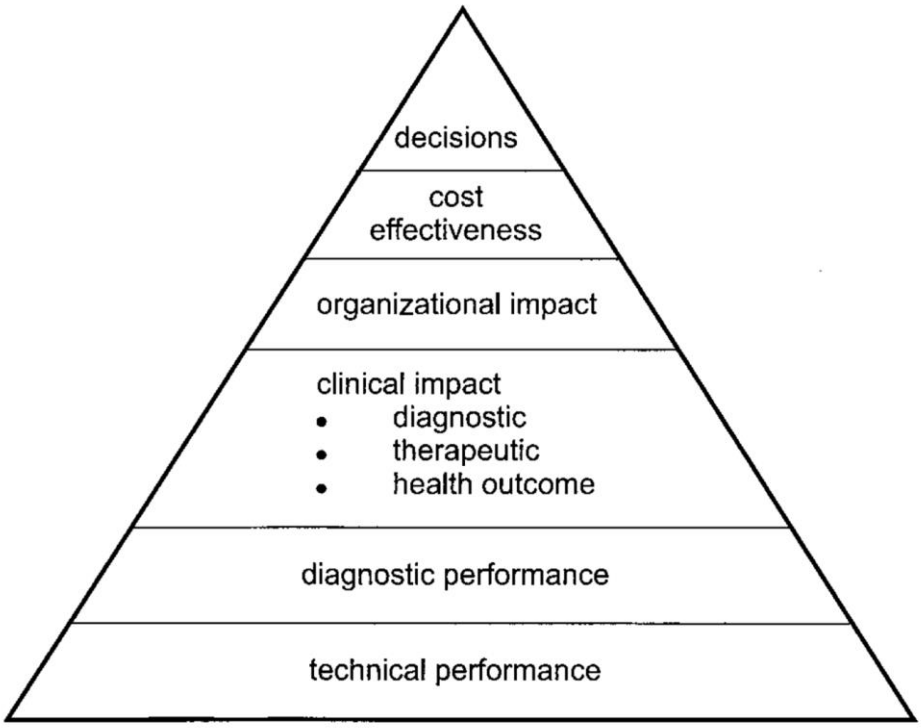

Fig. 1. Evidence of performance designed to facilitate decision-making.
Adapted from Price, 2000.

SEARCH TERMS

1. **Cochrane library Reviews:** "Influenza", "RSV", "respiratory viral"
2. **SumSearch Guidelines:** "Inf", "RSV", "respiratory viral"
3. **Pubmed:**
   - 3.1. **Clinical impact of influenza/RSV diagnostic tests**
     - 3.1.1. (impact OR effect OR benefit) AND (Inf OR RSV OR respiratory viral) AND (rapid OR testing OR diagnosis)
   - **3.2. Cost effectiveness of influenza/RSV testing**
     - 3.2.1. "cost effectiveness (rapid or multiplex OR PCR) AND (viral OR influenza OR RSV) NOT (hepatitis OR HIV)"
   - 3.3. **Performance of rapid NAATs**
     - 3.3.1. Liat[Title/Abstract]; filter 2017/05/21 to 2018/02/04
     - 3.3.2. alere[Title/Abstract]; filter 2017/05/21 to 2018/02/04
     - 3.3.3. ((Xpress OR GenXpert OR GeneXpert OR Xpert) AND (flu* OR influenza OR RSV))

RELEVANT EVIDENCE/REFERENCES

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APPRAISAL

1. Technical test performance

1.1. Question 1: What pre-analytical factors influence the detection of influenza and RSV?

We provide a brief overview of pre-analytical factors we identified that may affect test performance.

1.1.1 Patient-related and biological factors

A variety of factors may influence viral load and consequently test sensitivity in influenza-infected patients. Important variables include certain patient's characteristics and the moment of sampling during the course of infection. The Infectious Diseases Society of America (IDSA) guidelines recommend collection of specimens for influenza as soon as possible after illness onset, and set five days as a limit for useful detection in immunocompetent patients. Longer viral shedding (weeks to months) can be observed in immunocompromised patients, even in the absence of fever or respiratory symptoms, allowing a longer time-frame for diagnosis (8). For influenza, patient-related factors associated with higher viral load include younger age, male sex, and more comorbidities (9). The dynamics of viral shedding in influenza infections varies significantly between healthy individuals (10), and between body sites of one individual, that is, between left and right nostrils of infected patients (11). In one study population spanning three influenza seasons (2007-2010), no significant differences in dynamics of influenza viral load were detected between vaccinated and unvaccinated patients (9). Treatment with oseltamivir has been shown to reduce virus isolation by culture with up to 50% (12). In RSV, lower viral loads have been associated with elderly age and reinfection (13,14). In children with RSV infection, one study reported a significant fall in sensitivity of viral detection, starting from day six from illness onset (15). The impact of viral load on the sensitivity of different diagnostic tests, is further discussed under 2.1.2.

1.1.2 Interfering factors

The use of swabs with a wood shaft has been discouraged because it may interfere with RT-PCR and other molecular assays (16). Other authors showed that the use of cotton swabs with a wooden shaft does not affect test performance when using a Nucleic Acid Sequence Based Amplification (NASBA) assay for detection (17).

1.1.3 Sample stability

There seems to be a minimal impact of time between sample collection and analysis on diagnostic yield. One study paradoxically demonstrated a higher diagnostic yield for a panel of respiratory viruses in samples that were analyzed only after seven days at ambient temperature (18). A study that examined the stability of detectable RSV virus (using NASBA) did not find a significant decrease in diagnostic yield even after 15 days at room temperature (17). These studies have used highly sensitive central lab assays for detection however, and a more significant impact may exist for other assays. Manufacturers have stipulated different recommendations for conditions and duration of time from specimen collection to analysis, which are found in the respective product leaflets.

1.1.4 Sample collection

In an online resource on influenza specimen collection, the U.S. Centers for Disease Control (CDC) list five methods: the nasopharyngeal swab, the nasal/nasopharyngeal aspirate, the nasopharyngeal wash, the deep nasal swab and combined nasal and throat swabs (19). Nasopharyngeal sampling techniques are

considered the most sensitive by the IDSA 2009 guidelines (8). Since the (naso-)pharyngeal aspirates and washes require a suctioning apparatus, these are often considered cumbersome, especially in outpatient settings (20). In our centre, the "flocked" nasopharyngeal swab has presently become the sampling method of choice for all patients. In 2006, the development of flocked nasopharyngeal swabs (the sampling area consisting of nylon fibers) was shown to increase harvesting of epithelial cells, and thus offer a possible sensitivity benefit over classical nasopharyngeal rayon swabs (consisting of cellulose fibers) (21). In 2011, flocked "deep nasal" swabs (or midturbinate swabs — inserted about 5 cm intranasally) were similarly shown to offer better harvesting over nasopharyngeal rayon swabs (22), with potentially less patient discomfort. Recently, in a population of 484 patients, flocked midturbinate swabs were shown to cause significantly less patient discomfort, with only a minimal decrease in sensitivity for influenza detection, compared to flocked nasopharyngeal swabs (20). "Superficial" nasal swabs (flocked and foam variants) were confirmed to result in significantly lower sensitivity in that study (an effect less pronounced in children, however). These findings suggest that the midturbinate swab offers the best balance between patient discomfort and diagnostic sensitivity, in adults. As (superficial) nasal swab specimens have previously also demonstrated good sensitivity in children for influenza infection (8,23), the added value of midturbinate swabs is yet to be demonstrated. For RSV detection in children, flocked nasopharyngeal swabs were slightly less sensitive than a nasopharyngeal aspirate (100% versus 92,3%) (24). The use of (superficial) nasal swabs in children was shown not to be adequate for sensitive detection of the virus (23).

Alternative specimen collection techniques include the use of sputum, facial tissues, or the nasosorption technique. In patients able to produce sputum, detection of influenza is more sensitive if sputum is used, as opposed to nasopharyngeal swabs (25). Similarly, other deep specimens (bronchoalveolar lavage, endotracheal aspirate) have been shown to offer a sensitivity benefit over upper respiratory tract samples, particularly in immunocompromised and ventilated patients (8,26). Although inexpensive and child-friendly, facial tissues demonstrated inadequate sensitivity for individual diagnostics of viral respiratory viruses (27). Nasosorption is a newly developed technique, using an absorptive material that is brought into contact with the nasal mucosa for 30 seconds. A recent preliminary study demonstrated good sensitivity for RSV detection, compared to nasopharyngeal swabs (28).

1.2. Question 2: What analytical factors influence the identification of influenza and RSV?

Test parameters such as (im)precision, lower limit of detection, analytical specificity, accuracy, within-run and between-run variation have been compiled in the U.S. Food and Drug Administration (FDA) 510(k) reports (29). As all tests mentioned here have been shown to comply to required quality standards, the test performance for these parameters will not again be reviewed here.
A broader discussion of sensitivity/specificity and turn-around-time is provided under 2.1.

2. Diagnostic test performance

2.1. Question 3: What are diagnostic performance characteristics of influenza- and RSV-tests?

2.1.1. Overview

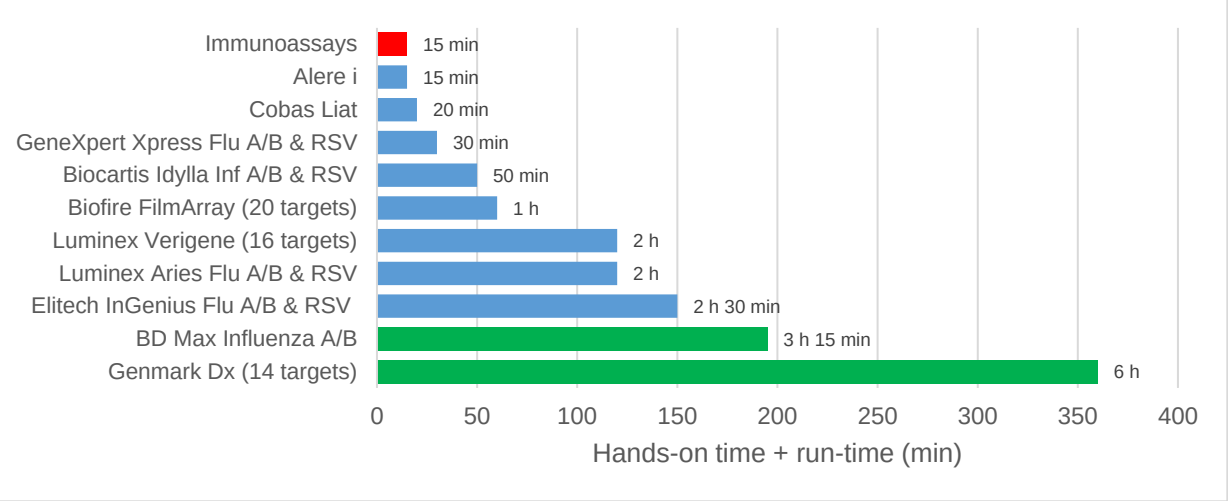
A literature search was performed to identify the diagnostic performance characteristics of different influenza and RSV testing platforms. A benchmark of currently used platforms in the nine different BILULU laboratories was performed (table 1). Since traditional techniques such as viral culture and immunofluorescence assays are largely outdated and obsolete in current laboratory practice, these were not considered in this study. Viral serology, similarly, will not be reviewed here, given its limited availability, its time-consuming nature and therefore its very limited role in clinical management (30).

The most frequently used tests in our survey of Belgian hospital laboratories, are immunoassays and nucleic acid amplification tests (NAATs) (table 1). Since 2011, two novel classes of rapid influenza testing assays have become available, with purported better diagnostic performance: digital immunoassays (DIAs), and rapid NAATs (6). In DIAs, antigen detection is performed digitally, instead of visually by a test operator, as is the case with the classical immunochromatographic tests. Examples of DIAs include the BD Veritor (Becton Dickinson, Franklin Lakes, New Jersey, U.S.) system, and the Quidel Sofia system (Quidel Corporation, San Diego, California, U.S.). Rapid NAATs offer reduced time to result by the use of a modified RT-PCR or amplification technology. "Rapid" is defined here as providing a test result in less than 30 minutes. Examples include the Roche Cobas Liat (Hoffman-La Roche, Basel, Switzerland) the Alere i (Alere Inc., Waltham, Massachusetts, U.S.), the GeneXpert Xpress Flu/RSV cartridges (Cepheid Inc., Sunnyvale, California, U.S.). All of these offer the possibility of simultaneous detection of influenza and RSV viruses with a single cartridge in one test run. Semi-rapid NAATs are here defined as providing test result within three hours. Examples include the Idylla respiratory panel (seven targets) (Biocartis, Mechelen, Belgium), the Biofire FilmArray (twenty targets) (bioMérieux, Marcy-L'Etoile, France), the Verigene (sixteen targets) (Luminex Corporation, Austin, Texas, U.S.), the

Aries Flu A/B & RSV assay, and ELITech InGenius Flu A/B & RSV kits (ELITechGroup, Lincoln, Rhode Island, U.S.). Other RC-PCR central lab systems include the BD Max (influenza A/B assay) (Becton Dickinson) and the Genmark Dx (fourteen targets) (Genmark Diagnostics, Inc., Carlsbad, California, U.S.), among others. Given the established performance of the central lab PCR assays, our literature study was focused on the newly introduced rapid assays.

An overview of approximations of the preparatory hands-on time plus run-time of different tests is provided in figure 2, based on the product inserts of manufacturers.

Fig. 2. Approximate hands-on time plus run-time of influenza and RSV assays.



2.1.2. Sensitivity and specificity of influenza and RSV assays

The performance of rapid influenza tests that offer a test result within 30 minutes, was systematically reviewed by Merckx et al. (excluding the GeneXpert system) (6). Pooled sensitivities and specificities for classical antigen-based rapid influenza diagnostic tests (RIDTs), DIAs and rapid NAATs are shown in table 2. While specificities of all three test classes approached 100%, pooled sensitivities for DIAs and NAATs are significantly higher than those for RIDTs, the latter not exceeding an overall sensitivity of 55%. NAATs as a class offer a sensitivity of over 90%, making these tests useful for ruling out disease, if the test result is negative (corresponding to LR < 0,1 given a specificity of >99%). DIAs offer a sensitivity of around 80%. Subgroup analysis reveals significantly higher sensitivity in children versus adults. This is particularly pronounced for RIDTs and DIAs, compared to NAATs, and for influenza B compared to influenza A. This discrepancy was attributed to purported longer and heavier viral shedding in influenza A infections, and in pediatric infections (6).

Table 2. Pooled sensitivities and specificities of different influenza test classes for influenza A and B. Age-specific subgroup analysis. Adapted from Merckx et al., 2017 (6).

Table 2. Overall and Subgroup Analyses of Pooled Rapid Test Accuracy Estimates for Influenza A and B, by Index Test Type*

Index Test Type	Influenza A		Influenza B	
	Pooled Sensitivity (95% CrI), %	Pooled Specificity (95% CrI), %	Pooled Sensitivity (95% CrI), %	Pooled Specificity (95% CrI), %
Overall				
Traditional RIDTs (94 influenza A studies; 30 influenza B studies)	54.4 (48.9 to 59.8)	99.4 (99.1 to 99.7)	53.2 (41.7 to 64.4)	99.8 (99.7 to 99.9)
DIAs (18 influenza A studies; 17 influenza B studies)	80.0 (73.4 to 85.6)	98.3 (97.4 to 98.9)	76.8 (65.4 to 85.4)	98.7 (97.5 to 99.4)
Rapid NAATs (12 influenza A studies; 12 influenza B studies)	91.6 (84.9 to 95.9)	99.2 (98.6 to 99.7)	95.4 (87.3 to 98.7)	99.4 (98.9 to 99.8)
Difference in sensitivities, overall				
Traditional RIDTs vs. DIAs	**−25.5 (−33.4 to −17.0)**	-	**−23.5 (−37.9 to −7.7)**	-
Traditional RIDTs vs. rapid NAATs	**−37.1 (−44.2 to −28.6)**	-	**−41.7 (−54.0 to −28.5)**	-
DIAs vs. rapid NAATs	**−11.5 (−19.5 to −2.9)**	-	**−18.2 (−30.6 to −6.9)**	-
Subgroup analyses†				
Study population (age)‡				
Traditional RIDTs				
Children (31 influenza A studies; 9 influenza B studies)	61.2 (55.0 to 67.2)	99.2 (98.5 to 99.7)	65.7 (45.3 to 80.5)	99.6 (99.2 to 99.8)
Adults (23 influenza A studies; 5 influenza B studies)	42.6 (34.8 to 50.9)	99.5 (98.6 to 99.8)	33.2 (19.9 to 50.7)	99.9 (99.4 to 100)
Difference in RIDT sensitivity: children vs. adults	**18.5 (8.4 to 28.3)**	-	**31.8 (6.1 to 52.6)**	-
DIAs				
Children (11 influenza A studies; 11 influenza B studies)	87.6 (81.8 to 92.2)	98.1 (96.4 to 99.1)	82.5 (71.2 to 90.2)	98.8 (95.6 to 99.7)
Adults (8 influenza A studies; 7 influenza B studies)	75.4 (66.6 to 82.6)	96.7 (94.7 to 98.0)	57.0 (39.5 to 71.6)	98.8 (97.5 to 99.5)
Difference in DIA sensitivity: children vs. adults	**12.1 (3.1 to 22.1)**	-	**25.3 (6.9 to 44.7)**	-
Rapid NAATs				
Children (4 influenza A studies; 4 influenza B studies)	90.2 (79.2 to 95.8)	99.0 (96.8 to 99.8)	95.9 (82.9 to 99.2)	99.5 (98.2 to 99.9)
Adults (4 influenza A studies; 4 influenza B studies)	87.4 (71.1 to 95.6)	98.0 (93.2 to 99.5)	75.7 (51.8 to 90.7)	99.3 (97.8 to 99.8)
Difference in NAAT sensitivity: children vs. adults	2.7 (−10.7 to 19.7)	-	**19.5 (1.0 to 43.7)**	-

CrI = credible interval; DIA = digital immunoassay; NAAT = nucleic acid amplification test; POC = point of care; RIDT = rapid influenza diagnostic test.
* Differences in pooled sensitivity estimates between groups that did not include the null (0%) in its 95% CrI are in boldface.
† For subgroups that contained ≥3 studies per stratum by index test type.
‡ Data from studies performed in ≥85% adult or ≥85% pediatric populations or from studies of mixed-age populations that provided data for the adult and pediatric subgroups.

Merckx et al. performed a subgroup analysis of sensitivities and specificities of different commercial brands of DIAs and NAATs. Results are shown in Table 3. In their meta-analysis Merckx et al., found that industry sponsored studies yielded significantly higher sensitivities than non-sponsored studies, which suggests this factor might need to be taken into account as a potentially significant source of bias.

In the class of direct immunoassays, the BD Veritor seems to be more sensitive than the Quidel Sofia, with a combined sensitivity for influenza A and B of over 80%.

We performed an in-house evaluation of the sensitivity of the BD Veritor Flu A+B system, with a resulting sensitivity of 64,8% in adults (n =162), and 87,3% (n = 126) in children (minus 18 years), compared to the Luminex xTAG RVP as a reference). For the BD Veritor RSV kit, we found sensitivities of 39,1% (n = 23) in adults, and 68,2% (n = 195) in children, compared to Luminex xTAG RPP.

In the class of the NAATs, sensitivity of the Roche Cobas Liat approached 100%, while the Alere i assay had a combined influenza A/B sensitivity of around 85%. The authors cautioned that only one adult trial was included for the Cobas Liat, whereas three adult studies were included for the Alere i. This might have resulted in a proportionally greater number of samples with lower viral load in the Alere i study group, and thus lower sensitivity. Additionally, the Alere i is currently marketed with cartridges that offer improved influenza B sensitivity, which were not used in the studies evaluated in the meta-analysis of Merckx et al.

We performed an additional literature study to identify recently published studies evaluating the Alere i and Cobas Liat that were not yet included in Merckx' review. A summary of the current literature on the performance of the GeneXpert Xpress cartridge is also provided.

Table 3. Pooled sensitivities and specificities of different commercial brands of DIAs and NAATs for influenza/RSV diagnosis. Adapted from Merckx et al., 2017 (6).

Index Test Type	Influenza A		Influenza B	
	Pooled Sensitivity (95% CrI), %	Pooled Specificity (95% CrI), %	Pooled Sensitivity (95% CrI), %	Pooled Specificity (95% CrI), %
DIAs				
Sofia (12 influenza A studies; 11 influenza B studies)	77.8 (68.8 to 85.4)	98.5 (97.4 to 99.2)	73.5 (55.8 to 86.1)	98.0 (95.4 to 99.1)
Veritor (6 influenza A studies; 6 influenza B studies)	83.0 (73.4 to 90.1)	97.5 (95.5 to 98.7)	80.0 (68.8 to 88.2)	99.5 (98.8 to 99.8)
Difference in DIA sensitivity: Sofia vs. Veritor	−5.1 (−16.4 to 6.9)	-	−6.4 (−25.8 to 10.4)	-
Rapid NAATs				
Alere (7 influenza A studies; 7 influenza B studies)	84.4 (75.3 to 90.9)	98.9 (97.7 to 99.6)	86.6 (69.0 to 95.3)	99.1 (98.1 to 99.7)
Liat (5 influenza A studies; 5 influenza B studies)	97.1 (92.9 to 98.9)	99.4 (98.4 to 99.8)	98.7 (95.6 to 99.7)	99.5 (98.7 to 99.9)
Difference in NAAT sensitivity: Alere vs. Liat	**−12.4 (−21.9 to −4.9)**	-	**−11.8 (−29.5 to −2.8)**	-

CrI = credible interval; DIA = digital immunoassay; NAAT = nucleic acid amplification test; POC = point of care; RIDT = rapid influenza diagnostic test.
* Differences in pooled sensitivity estimates between groups that did not include the null (0%) in its 95% CrI are in boldface.
† For subgroups that contained ≥3 studies per stratum by index test type.
‡ Data from studies performed in ≥85% adult or ≥85% pediatric populations or from studies of mixed-age populations that provided data for the adult and pediatric subgroups.

For the Roche Cobas Liat, we identified three studies published since Merckx' review. Results are shown in table 4. All three studies demonstrated near 100% sensitivity for influenza, with a core lab RT-PCR assay as a reference standard. Two of these studies included at least a significant proportion of adult patients. Only one study assessed performance of RSV detection, with a reported sensitivity of 96,8%. Of note, all three studies were sponsored by Roche and co-authored by Roche employees.

First author	Year	Pathogen	N (positive/total)	Age	Sensitivity	Specificity
Gibson (31)	2017	Influenza A/B and RSV	N = 595/1656	All ages (age distribution not specified)	Inf A: 99.6% Inf B: 99.3% RSV: 96.8%	Inf A: 97,5% Inf B: 99,7% RSV: 98,8%
Young (32)	2017	Influenza A/B	N = 47/87	≥ 18 years	Inf A:100% Inf B: 94,4%	Inf A: 98,3% Inf B: 100%
Melchers (33)	2017	Influenza A/B	N = 56/121	Not reported	Inf A: 96% Inf B: 100%	Inf A: 100% Inf B: 100%

Table 4. Studies evaluating Roche Cobas Liat for influenza/RSV detection, published from 2017/05/21 to 2018/02/04

For the Alere i, we identified three recently published studies (see table 5). Young et al., in a Roche-sponsored comparison of Cobas Liat and Alere i, found a sensitivity of the Alere i of 55,2% of influenza A, and of 72,2% for influenza B, in an adult population (32). Chen et al., on the contrary, reported an influenza A sensitivity of 97,4% and influenza B sensitivity of 81,5%, in a mixed-age population (34). The Alere i has yielded lower sensitivity if performed on frozen samples, for which it was not validated (K.D., personal communication, february 2018). However, Young exclusively used fresh samples, and Chen et al., used around 18% (24/134) frozen samples. Differing sensitivity due to sample viral load (related to patient age) might thus have been a more significant factor. Other factors possibly influencing viral load and/or sensitivity (such as time of sampling during disease course), were not recorded in any of both studies.

In a small validation of the Alere i (partly performed in our center and in Heilig Hart Ziekenhuis Lier), we found a sensitivity of 78,6% for either influenza A or B (n = 14), in fresh samples from adults, compared to the Luminex xTAG RVP.

In an Alere-sponsored study, Schnee reported a sensitivity of 93% for detection of RSV in children.

First author	Year	Pathogen	N (positive/total)	Age	Sensitivity	Specificity
Chen (34)	2018	Influenza A/B and RSV	N = 105/134 (110 fresh, 24 frozen)	All ages	Inf A: 97,4% Inf B: 81,5%	Inf A: 100% Inf B: 99,1%

Schnee (35)	2017	RSV	N = 229/533 (fresh)	< 18j	93%	96%
Young (32)	2017	Influenza A/B	N = 47/87 (fresh)	≥ 18j	Inf A: 55,2% Inf B: 72,2%	Inf A: 98,3% Inf B: 97,1%

Table 5. Studies evaluating Alere i for influenza/RSV detection, published from 2017/05/21 to 2018/02/04

In a search of the literature (not restricted according to publication date), we found three studies that examined performance of the GeneXpert Xpress Inf A/B & RSV assay. Study characteristics are shown in table 6. Sensitivities approached 100% for both RSV and influenza A/B, in an age-mixed study population. The studies of Cohen and Ling were sponsored by Cepheid, the manufacturer of GeneXpert.

In our evaluation of the GeneXpert Xpress system we found a sensitivity of 97,9% (n = 143) in adults and children, with the Luminex xTAG RVP as a reference. The higher sensitivity of the xTAG assay probably relates to its separate (non-integrated) extraction procedure, and the sample dilution (1:2) required for the GeneXpert system.

First author	Year	Pathogen	N (positive/total)	Age	Sensitivity	Specificity
Chen (34)	2018	Influenza A/B	N = 105/134	All ages	Inf A: 100% Inf B: 96,3%	Inf A: 100% Inf B: 100%
Ling (36)	2017	Influenza A/B and RSV	N = 50/100	All ages	Inf A: 100% Inf B: 97,8% RSV: 100%	Inf A: 100% Inf B: 100% RSV: 100%
Cohen (37)	2018	Influenza A/B and RSV	N = 680/2435	All ages	Inf A: 100% Inf B: 100% RSV: 97,1%	Inf A: 95,2% Inf B: 99,5% RSV: 99,6%

Table 6. Studies evaluating the GeneXpert Xpress system for influenza/RSV detection.

3. Clinical impact

The clinical impact of viral testing was assessed in a literature study. Search terms that were used are listed under the heading "Search terms". In total, 42 studies were found that assessed the clinical impact of viral testing. Results of high quality trials (randomized controlled trials or meta-analyses) were systematically appraised in this text. Lower quality studies that were often cited in the literature, or were exemplary for their methodology, were also considered.

3.1. Question 4: What is the clinical impact of identification of influenza and RSV on therapy decisions?

Respiratory tract infections may be bacterial or viral in origin. Clinical presentation of different etiological agents may overlap, and patients may present with bacterial/viral coinfections, complicating selection of appropriate anti-infectious therapy (38,39). We reviewed the evidence for the impact of viral testing on directing treatment decisions; i.e. on the initiation or withholding of antiviral agents or antibiotics, when these are clinically indicated.

3.1.1. What is the clinical impact of identification of influenza on antiviral therapy?

The main antivirals that are used in influenza infections are the neuraminidase-inhibitors oseltamivir (Tamiflu, Roche), zanamivir (Relenza, GlaxoSmithKline), Peramivir (Rapivab, BioCryst Pharmaceuticals), and the M2 protein inhibitors (or adamantanes), amantadine (Symmetrel) and rimantadine (Flumadine). Neuraminidase-inhibitors have activity against both influenza A and B viruses. The M2 protein inhibitors only have activity against influenza A, although the widespread H3N2 and H1N1pdm09 subtypes are almost universally resistant. As the only available oral neuraminidase-inhibitor, oseltamivir has become the most widely used antiviral in the treatment and chemo-prophylaxis of seasonal influenza (8). It is the only influenza antiviral currently marketed in Belgium (40).

Guidance in the decision whether to start antiviral therapy or not, has been an important indication for targeted diagnosis and even typing of infective strains (8,41,42). Indications for influenza testing in the 2009 Infectious Diseases Society of America (IDSA) guidelines roughly correspond to indications for antiviral therapy, i.e. concerning patients at high-risk of complications and hospitalized patients (see Appendix A) (8). Because of the importance that has classically been attributed to this indication, and in light of the controversy that has recently arisen over the benefit/harm balance of antivirals, we have provided a concise literature study and assessment of current use of oseltamivir in our center, in Appendix B (43). Briefly, according to Cochrane review authors, neuraminidase inhibitors have "small and non-specific effects on reducing time to alleviation of influenza symptoms," an uncertain impact on reducing the number of influenza complications, and an increase of the risk of adverse effects (nausea,

vomiting, psychiatric effects, renal events) (43). In a systematic review by the same group of observational data, a benefit on mortality could not be demonstrated (44). According to the Belgian Centre for Chemotherapeutical Information (BCFI), based on these studies, these agents have a very limited role in the treatment of influenza infections (40), that is not further specified.

A query at our center revealed that a very small proportion of hospitalized patients with a suspected or confirmed influenza infection were treated with oseltamivir in the 2017-2018 season (18/407; 4,4%). These numbers seem to suggest that clinicians at our institution deem the clinical effect of oseltamivir among patients hospitalized with influenza to be small.

The use of neuraminidase-inhibitors to shorten the time to alleviation of symptoms in otherwise healthy patients, was also proposed in the IDSA guidelines as a possible indication for therapy. Guidelines on the use of antivirals for this indication differ, as the American Association for Pediatrics (AAP) recommends considering the use antivirals in all healthy children (45), while, for example, Dutch guidelines do not recognize a benefit of antiviral therapy over symptomatic therapy in healthy persons (46,47) and other authoritative sources also are reserved in the use of antivirals in this indication (48). Additionally, some cost-effectiveness studies found empiric antiviral treatment to be more cost-effective than test-guided treatment in children during influenza season (49).

In conclusion, it seems that the clinical impact of targeted influenza diagnosis in directing antiviral treatment is, at least in our Belgian setting, small at the most, given the unclear clinical role and limited use of these agents, even among seriously ill patients.

3.1.2. What is the clinical impact of identification of RSV on to antiviral therapy?

According to guidelines on RSV management, most cases of pediatric RSV infections can be diagnosed clinically, with little consequences of diagnostic testing on clinical management (including use of antibiotics and ribavirin) (50). Indeed, these guidelines advice against the routine use of ribavirin in RSV infections in children. However, ribavirin might have a role in the treatment of RSV infections in special patient populations, such as immunosuppressed children or adults (51). Therefore, there might be a role of directed RSV testing for guiding therapy with ribavirin in these populations. However, an extensive assessment of the clinical use of this agent in these specific populations, and its relative importance in diagnostic testing is beyond the scope of this paper. We conclude that viral testing for RSV to direct the use of ribavirin therapy is of minor importance in most patients.

3.1.3. What is the clinical impact of identification of influenza and RSV on to antibiotic therapy?

Given the scarcity of data on the impact of lab testing on antibiotic use in RSV infections, the evidence of the impact of viral testing for influenza and RSV infections will be combined. The lack of data on a reduction of antibiotic use by RSV testing, may reflect the low use of antibiotics in clinical practice in these patients. Indeed, serious concurrent bacterial infections in RSV-infected children are believed to be low (but not negligible) (50,52). Guidelines currently advise against the use of antibiotics in RSV infections, "except in cases in which there is clear, documented evidence of a secondary bacterial infection" (50). Additionally, since RSV infection may be differentiated clinically from a bacterial respiratory infection in most cases (cfr. 3.1.1.), a lab-confirmed diagnosis of RSV would probably not result in a decreased use of empiric antibiotics.

The 2016 IDSA guidelines on implementing an antibiotic stewardship program recommend the use of rapid respiratory viral testing to reduce the use of inappropriate antibiotics. This concerns a "weak recommendation", based on low-quality evidence (53). A Cochrane review was published in 2014, focused specifically on the use of rapid viral tests in the pediatric emergency department (i.e. tests providing results while the patient is in the emergency department) (52). It was concluded that the evidence was insufficient, although "promising in support of using rapid viral testing to reduce inappropriate antibiotic prescriptions." Of eight available randomized controlled trials, half were excluded due to methodological issues. Of note, one major study was excluded from the analysis because it included children with underlying illness. The proportion of children with an underlying illness was however nearly equal (4,6% versus 5%) in the influenza positive intervention group (as determined using an immunochromatographic test, the Quidel Quickvue influenza) and a control group with a clinical diagnosis of influenza-like illness. This study reported a reduction of -29,2% in antibiotic use ($p = 0,0003$) (54). Of the four trials that were included in the analysis (55–58), only one demonstrated a significant reduction in antibiotic use in the group allotted to receiving a rapid optical immunoassay (fluOIA of Biostar) rapid testing and tested positive, compared to a control group with a clinical diagnosis of influenza (reduction of 24,5% to 7,3%, $p < 0,001$) (55). The other included trials demonstrated a statistically insignificant trend, or no effect.

We conducted a literature search to identify additional studies investigating the use of viral testing on antibiotic use (RCTs and observational studies). We differentiated studies that provided a rapid result (reported time to result of less than an hour — a relatively arbitrary limit), from studies with a longer time to result. As for the rapid testing group, one RCT was identified, that assessed the impact of use of the point-of-care (POC) Quidel Quickvue immunochromatographic test versus a custom RT-PCR assay (mean time to result > 20-30h) and viral culture (mean turn-around time of > 9 days) in an elderly population (> 65 years) and in persons 18-64 years of age with underlying chronic heart or lung disease (59). This study was conducted in the emergency departments and wards of a university center, in patients with an acute cardiopulmonary syndrome. The endpoint was time from admission to cessation of antibiotics. In 120 randomized influenza positive patients, no statistically significant difference was observed between the three groups, although the number of included patients was noted to be small.

We found a larger number of observational studies investigating rapid viral tests (time to result < 1h) (n = 13). These studies had been almost exclusively conducted in ambulatory practices or acute care settings, and mainly reported on initiation of antibiotic therapy. Some of these reported potentially significant reductions in antibiotic use (60,61). Study design was highly heterogenous between studies, and was of low methodological quality in many of these. Examples of poor study design include pre-/post-intervention studies (suffering from potentially inadequately controlled cohorts) (60,62), questionnaire-based studies (assessing in a single patient cohort the physician-reported (but not necessarily actual), test impact on clinical management) (61,63), and studies limited to assessing impact of test result (positive versus negative) instead of test result versus no testing (64,65). Six of fourteen studies were retrospective in nature (64,66–70).

As for studies that employed influenza tests with a reporting time longer than 1 hour, three RCTs (71–73) and one quasi-randomized study were found (74), in addition to seven observational studies (4,75–80).

Several studies examined the hypothesis that viral testing might reduce the duration of antibiotic treatment, in cases where clinicians have already initiated antibiotic therapy, while results of viral testing are pending. Wishaupt et al., in a group of 583 children, randomized to early communication (12-36 hours) or late communication (4 weeks) of the test result of multiplex PCR, did not find a difference in duration of antibiotic treatment, if initiated (71). Unexpectedly, a statistically significant increase (41,6% versus 27,4%; p < 0,000) in initiation of antibiotic therapy in the intervention group (n = 298) as compared to the control group was found. The cohorts were controlled for severity of illness (although a statistically insignificant trend toward higher C-reactive protein values and more X-rays was noted in the intervention group). Oosterheert et al. found no difference in duration of antibiotic treatment after initiation of antibiotic therapy, in 107 patients randomized to multiplex PCR (mean time to result 30 hours), or conventional viral culture (time to result not reported)(72). In a recent RCT in the Lancet, Brendish et al., found no difference in initiation of antibiotic therapy in 720 patients randomized to point-of-care (POC) multiplex PCR (Biofire FilmArray, mean turn-around-time of 2,3 hours) or routine clinical care (with use of custom multiplex PCR at discretion of physician; mean TAT of 37 hours). Mean duration of antibiotic treatment was equal between both groups, although a significantly larger proportion of single-dose or brief (< 48 hours) antibiotic treatment was found in the intervention group (risk difference 6,9% and 7,8%; p = 0,0010 and p = 0,0047, respectively) (73). Andrews et al. found a significant difference in total antibiotic prescribing decisions (start, stop, escalate, de-escalate, continue antibiotics and remain off antibiotics) between a cohort that underwent rapid multiplex PCR testing (Biofire FilmArray, mean TAT 19h), and routine RT-PCR testing (mean TAT 39,5h). However, the proportion of patients receiving antibiotics was similar in both arms, as was duration of antibiotic therapy and time to initiation of antibiotics (74).

In conclusion, randomized-controlled trials suggest a small or inconsistent decrease in antibiotic prescription rate with rapid immunoassays in the pediatric emergency department. RCTs evaluating the use of rapid and semi-rapid assays in adult populations found equal rates of antibiotic prescriptions and duration of antibiotic treatment between intervention and control groups. No RCTs have been conducted so far evaluating the new POC nucleic-acid amplification assays, that combine high sensitivity and brief time to result (< 30 min).

Discrepant results might in part reflect varying practices among clinicians in the management of proven influenza infections, concerning the use of precautionary antibiotics for possible concurrent bacterial infections. The use of empiric antibiotics in children with a proven viral infection is largely based on clinical judgment, and to our knowledge, no influenza or RSV clinical management guidelines provide formal criteria for assessing the need of empiric antibiotics in proven viral infections (8,45). We hypothesize therefore that a lack of uniformity among clinicians in antibiotic use in viral infections, might in part explain the observed inconsistent results. The use of an antimicrobial stewardship program, implemented together with viral testing, might result in more significant reductions in inappropriate antibiotic use in proven influenza or RSV infection (81).

3.2. Question 5: What is the clinical impact of identification of influenza and RSV on the use of other technical investigations?

Doan et al., in a Cochrane review of four RCTs in the pediatric emergency department, found a significant decrease in the number of chest radiographs, in patients testing positive for influenza with immunoassays (RR 0.77, 95% CI 0.65 to 0.91) (52). No significant impact was demonstrated on the use of blood tests and urine investigations. We found no RCTs in other patient populations examining the impact of viral testing on these endpoints. Some lower-quality observational studies of rapid tests (< 1 h time to report) did report significant decreases in one or more of these endpoints in different patient groups (61–63,65,67,82).

We conclude that there is sufficient evidence to support the use of rapid influenza testing to reduce the number of chest radiographs (but no other technical investigations) in children. An impact in adults for chest X-ray or other technical investigations has not been demonstrated in high quality trials.
We are not aware of any studies assessing the impact of rapid viral testing for RSV on the number of technical investigations (see 3.1.2).

3.3. Question 6: What is the clinical impact of identification of influenza and RSV on patient health outcome?

We searched the literature for data regarding the impact of viral testing on health outcome in the general population. We considered hospital admission, hospital length-of-stay (LOS), and mortality/adverse outcome as endpoints.

Five RCTs (of which three employed rapid antigenic tests) investigated hospital admission as an endpoint (54,57,59,71,73). None demonstrated a significant difference in hospital admission rates between patients receiving influenza testing and controls. A small prospective study in influenza-positive pregnant women in the Emergency department, found significantly decreased rates of admission, in the group with a POC diagnosis using the GeneXpert Flu assay (n = 45), compared to off-site RT-PCR (75).

Five RCTs reported on LOS (59,71–74). Only one of these (Brendish et al.) demonstrated a mean reduction in length of stay of 1,1 day (-2,2 to -0,3; p 0,0443) (73). Two retrospective studies found no decrease in LOS (69,80), and three pre-/post intervention observational studies found decreases in LOS ranging from -0,2 days (78), to -0,9 days (4) and -5,3 days (5).

Two RCTs (59,73) reported on mortality rates. No significant difference was found between cohorts.

In conclusion, all high-quality evidence disproves a potential impact of viral testing on the rate of hospital admissions, and three of four RCTs report no impact on LOS. The use of viral testing does not seem to affect patient outcome.

3.4. Question 7: What other benefits might influenza and RSV testing offer?

In a recent narrative review on indications for viral testing in children, a potential for psychological benefits for patient, caregiver and/or clinician was mentioned (30). Viral testing might often be ordered to provide a potentially unambiguous explanation for symptoms, and might ease anxiety in the patient or physician. No studies have examined the benefit of obtaining a reassuring diagnosis, as compared to the potential discomfort associated with viral testing, and associated costs. In a study of children with a viral infection, parental dissatisfaction was associated with a mismatch between parental expectations and the physician's recommendations (83). Therefore, adequate communication of a clinical diagnosis, might be more important than seeking to obtain a specific lab-confirmed etiological diagnosis (30). Additionally, the authors remarked that a degree of diagnostic uncertainty is inherent to the medical profession (30).

3.5. Question 8: What is the organizational impact of identification of influenza and RSV?

Both the U.S. Centers for Disease Control (CDC) and the Dutch Landelijke Coördinatie Infectieziektenbestrijding (LCI) recommend the use of droplet precautions for influenza and RSV infected patients (46,84). The CDC recommends contact precautions for RSV infection, in addition to droplet isolation. Cohorting of patients with the same respiratory pathogen is often practiced for influenza and RSV (84), and has been shown to be an effective measure in preventing nosocomial RSV transmission (85). Recently, an etiological role of different coinfection viruses (such as Human Metapneumovirus) in confirmed RSV infections has become apparent, which has led to some authors questioning the benefit of

cohorting RSV-infected patients (86). Some studies have investigated the organizational impact of rapid viral testing on patients on triaging patients in the emergency department, timely institution of isolation precautions and cohorting patients. Endpoints include time in the Emergency department (until discharge or admission), start of isolation depending on test result, and duration of (empiric) isolation.

One RCT found a significant reduction in the time in the Emergency department (-20%), using a rapid antigenic test (mean time to result not reported) (55). In another RCT using a direct immunofluorescence assay (time to reporting 30-150 min), Doan et al. found no difference between cohorts (58). One prospective study, examining the introduction of the Alere i, found a significant reduction in time spent in the Emergency room (6.06 h versus 4.15 h, P = 0.03) (62).

In a prospective study in children aged <2 years with respiratory symptoms and/or suspected bronchiolitis, Mills et al., quantified the time the patients spent in Emergency department cubicles, versus time in cohorting areas (87). The impact of a rapid RSV antigen-test was determined by quantifying time spent in cohorting areas (after the patient tested RSV positive), during a time span of 24h (which is the time-to-result using conventional PCR- or immunofluorescence-based tests in that hospital). In this manner, the POC test was shown to allow 183 children to be admitted directly to the cohorting area, thus saving a total of 568,5 "cubicle-free" days, which equals about five cubicles being left free for each day of the four-month study period.

Brendish et al. reported no difference in total use of isolation between a POCT multiplex PCR group, and a control group. However, a significantly shorter time to isolation (difference –0,5 days; p = 0,0071) and to de-isolation (-2,1 days; p = 0,0057) was found (73). Nicholson et al. reported the number of isolations in a rapid testing and control cohorts, although no conclusions could be drawn since the number of included isolated patients was too low (59). No other RCTs reported on this endpoint.

In conclusion, viral testing might or might not reduce the time spent in the Emergency department. There is good evidence from one trial that multiplex PCR viral testing reduces the time to isolation and de-isolation in influenza-infected patients. Data from an observational trial suggests a significant impact of rapid RSV testing on patient triaging and cohorting in the Emergency department.

3.6. Question 9: Is influenza and RSV testing cost-effective?

Nicholson et al. performed a cost-effectiveness assessment in three cohorts, randomized to a rapid near-patient immunochromatographic test, a multiplex PCR assay (median time to reporting 29,2 hours), and viral culture (median time to reporting for influenza 629,6 hours), respectively (59). No statistically significant differences in the distributions of total costs, or Quality-Adjusted Life Years (QALYs) gained were found. The near-patient group had the highest gain in QALYs, but this gain was not offset by its higher cost at the thresholds of willingness to pay.

Two retrospective pre-/post intervention studies reported that direct immunofluorescence assays were cost-effective, mainly through a reduction in LOS (4,5) (cfr. 3.3). Given the more robust evidence against a significant benefit in LOS in RCTs (cfr. 3.3), we believe the findings in these studies are not valid.

Nelson reported that influenza testing using multiplex PCR (Biofire FilmArray) was the most cost-effective strategy in children presenting with influenza-like illness, compared to in-house singleplex RT-PCR, direct-fluorescent antibody testing, and rapid antigen tests (Quidel Quickvue Influenza A + B) (88). Diagnostic performance of different tests was assessed, and laboratory utilization, antibiotic and antiviral prescribing was derived from Bonner's 2003 randomized controlled trial (55). Estimates of cost-savings were calculated based on presumed decreases in ICU stay in patients treated with antivirals, and reduced risk of influenza complications and mortality with antiviral treatment. In our assessment of the literature, we considered the evidence for an impact of rapid influenza testing on antibiotic utilization and laboratory tests to be presently undetermined or insignificant (cfr. 3.1). At present there is no convincing evidence that treatment with antivirals significantly decreases morbidity or mortality in influenza-infected patients (43,44). Therefore, we believe that this study and similar ones do not provide a correct assessment of cost-effectiveness of viral testing.

We are not aware of studies that have examined cost-effectiveness of viral testing through its impact on guiding isolation precautions, and in preventing nosocomial outbreaks. Hospital outbreaks of influenza are associated with high morbidity and mortality, closure of wards, and excess healthcare expenditures (89). In our experience, viral testing is decisive in guiding isolation measures, particularly during peak season, when due to high patient influx resources for "empiric" isolation are under pressure. Based on our experience, and on the available evidence for a benefit of viral testing in directing isolation measures (cfr. 3.5), we believe that viral testing can be cost-effective in its crucial "hospital-hygienic" role.

We aimed to define an optimal diagnostic strategy in influenza and RSV infections. We addressed this question from two perspectives: 1) We aimed to identify the most accurate, time-efficient and cost-efficient diagnostic tests available for influenza and RSV diagnosis. 2) We sought to define the role of influenza and RSV testing in hospital settings and to propose the desired test characteristics of the most appropriate test.

What is the most accurate, time-efficient and cost-efficient diagnostic test available?
Nucleic acid amplification tests (NAAT) have better diagnostic performance characteristics than traditional methods (immunoassays, viral culture, viral serology). **Newer rapid NAATs (Roche Cobas Liat, GeneXpert Xpress Flu, Alere i) are user-friendly, highly sensitive and have brief turn-around-times** (< 30 minutes), compared to semi-rapid NAATs (TAT < 3 hours) and classical RT-PCR assays, **but are significantly more expensive. Cost-efficiency of these assay depends on their clinical impact.**

What is the clinical impact of influenza and RSV testing?
Given the dubious therapeutic efficacy of influenza antivirals, we believe that **viral testing for directing antiviral therapy, is in our practice, a minor and questionable indication**. RSV testing might be useful for specific populations, where ribavirin therapy might be beneficial. **No definitive benefit on directing antibiotic therapy** has been demonstrated in high-quality trials in children and adults with influenza, although some studies suggest a minor benefit. No RCTs have been performed using the new point-of-care (POC) NAATs. **A decrease in the use of chest X-rays** was demonstrated in the pediatric Emergency department, with rapid antigen tests. No impact was seen on other technical investigations. In high-quality trials, **viral testing does not have an impact on hospital admission rate**. Inconsistent results were obtained for length-of-stay (LOS). One high-quality trial reported a **benefit in guiding isolation measures in influenza infections**. Inconsistent results were obtained for an impact on length of time in the Emergency department. We conclude that **testing for influenza and RSV is useful for guiding isolation measures. There is currently a lack of high-quality trials examining the clinical impact of the newly introduced sensitive point-of-care NAATs on patient management.**

What is the optimal diagnostic strategy in influenza and RSV infections?
A highly sensitive test is required to rule out the diagnosis, in order to direct appropriate isolation precautions. **Negative likelihood ratios of <0,1 (corresponding to a sensitivity of ≥90,1% if specificity is 99%) are useful for ruling out disease with a negative test result** (6,90). **The GeneXpert Xpress and Roche Cobas Liat, in addition to many standard core lab PCR assays**, seem to meet this criterium. Rapid (TAT < 30 minutes) or semi-rapid NAATs (TAT < 3h) are preferred, given the importance of a timely test result to guide isolation measures. We set 3 hours as a time limit, as this broadly falls within the proposed "four-hour rule" (maximum time patients should spend in the ED) (91), and because a benefit for isolation measures was still demonstrated with an assay with a mean TAT of 2,4 hours (73). **Two tier testing using immunoassays in a first step, and confirmatory testing with a (semi-)rapid NAAT in a second step, might be still be time-efficient, and possibly more cost-efficient in children.**

Although viral testing does not seem to be cost-effective, considering the therapeutic, diagnostic and outcome impact, testing has a crucial role in guiding isolation measures to prevent nosocomial outbreaks, and therefore we suggest that viral testing is cost-effective.
The added value of the newly developed and more expensive rapid NAATs as opposed to semi-rapid lab assays present in many central laboratories, is uncertain. We hypothesize that their added benefit may lie in their briefer runtime and possibly in point-of-care applications, obviating the need of sample transport and trained test operators at the central lab (and thus simplifying diagnostic workflow). It should be remarked that many core lab assays now also offer a sample-to-result configuration, however.

Conclusion
The use of one-step rapid or semi-rapid NAATs, or two-tier testing (including use of an immunoassay) are the preferred diagnostic strategies, where their use in timely guidance of isolation measures is the best supported test indication. The added value and cost-effectiveness of rapid NAATs in terms of impact on isolation and clinical management is yet to be determined in high-quality trials.

TO DO/ACTIONS

1. **For implementation of (new, expensive) assays in hospitals group purchase, validation, and implementation may be beneficial.**
2. **The added value and cost-effectiveness of rapid NAATs in terms of impact on isolation and clinical management is yet to be determined in high-quality trials. The literature concerning clinical impact of influenza/RSV testing is yet to be systematically reviewed.**

ATTACHMENTS

Appendix A: 2009 IDSA indications for antiviral testing and treatment (Harper et al., 2009)

Table 7. Persons who should be tested for influenza. Adapted from Harper et al., 2009 (8).

During influenza season, testing should occur in the following persons if the result will influence clinical management
Outpatient immunocompetent persons of any age at high risk of developing complications of influenza (e.g., hospitalization or death) presenting with acute febrile respiratory symptoms, within 5 days after illness onset, when virus is usually being shed
Outpatient immunocompromised persons of any age presenting with febrile respiratory symptoms, irrespective of time since illness onset, because immunocompromised persons can shed influenza viruses for weeks to months
Hospitalized persons of any age (immunocompetent or immunocompromised) with fever and respiratory symptoms, including those with a diagnosis of community-acquired pneumonia, irrespective of time since illness onset
Elderly persons and infants presenting with suspected sepsis or fever of unknown origin, irrespective of time since illness onset
Children with fever and respiratory symptoms presenting for medical evaluation, irrespective of time since illness onset
Persons of any age who develop fever and respiratory symptoms after hospital admission, irrespective of time since illness onset
Immunocompetent persons with acute febrile respiratory symptoms who are not at high risk of developing complications secondary to influenza infection may be tested for purposes of obtaining local surveillance data
At any time of the year, testing should occur for the following persons
Health care personnel, residents, or visitors in an institution experiencing an influenza outbreak who present with febrile respiratory symptoms, within 5 days after illness onset
Persons who are epidemiologically linked to an influenza outbreak (e.g., household and close contacts of persons with suspected influenza, returned travelers from countries where influenza viruses may be circulating, participants in international mass gatherings, and cruise ship passengers), who present within 5 days after illness onset

Table 8. Patients at high risk for complications from influenza who should be considered for antiviral therapy. Adapted from Harper et al., 2009 (8).

Unvaccinated infants aged 12–24 months
Persons with asthma or other chronic pulmonary diseases, such as cystic fibrosis in children or chronic obstructive pulmonary disease in adults
Persons with hemodynamically significant cardiac disease
Persons who have immunosuppressive disorders or who are receiving immunosuppressive therapy
HIV-infected persons
Persons with sickle cell anemia and other hemoglobinopathies
Persons with diseases that requiring long-term aspirin therapy, such as rheumatoid arthritis or Kawasaki disease
Persons with chronic renal dysfunction
Persons with cancer
Persons with chronic metabolic disease, such as diabetes mellitus
Persons with neuromuscular disorders, seizure disorders, or cognitive dysfunction that may compromise the handling of respiratory secretions
Adults aged >65 years
Residents of any age of nursing homes or other long-term care institutions

NOTE. Although sufficient data do not exist to precisely define the extent of increased risk of influenza in these different groups of patients, there are data to suggest that the highest risk of both mortality and serious morbidity (e.g., hospitalization) occurs for severely immunocompromised patients (e.g., hematopoietic stem cell transplant patients) and very elderly (age, >85 years) residents of nursing homes; infants aged <24 months also have high hospitalization rates but lower case-fatality rates than do the other 2 groups. Data are from [3, 5].

Appendix B: brief assessment of the clinical efficacy and use of neuraminidase-inhibitors

The clinical efficiency of antiviral therapy in influenza infections has been a matter of considerable controversy recently. A 2014 Cochrane review by Jefferson and collaborators concluded that treatment with oseltamivir and zanamivir has small effects on reducing the time to alleviation of influenza symptoms in adults (both 10%), but no significant effect on reducing either hospitalizations or serious influenza complications (43). A significant reduction of pneumonia was observed for treatment with oseltamivir (but not zanamivir), although this effect was questioned by the reviewers since it concerned radiologically unconfirmed, investigator-mediated, and patient self-reported pneumonia. In children,

treatment with oseltamivir achieved a reduction of time to first influenza symptom alleviation of 29 hours, but offered no benefit for all other endpoints. For zanamivir treatment in children, no significant effect was achieved for the aforementioned endpoints, although there was insufficient data to evaluate its effect on serious influenza complications. This review was published in 2014, and was the first to access the over 60% of data of phase III treatment trials of oseltamivir that were previously not made public. The authors alleged that earlier literature studies (including an earlier version of their Cochrane review) has thus been significantly influenced by publication bias in favor of the clinical efficacy and safety of neuraminidase inhibitors, particularly regarding the ability of oseltamivir to reduce complications of influenza (43). Providing slightly better results, a 2015 Roche-sponsored meta-analysis using the same dataset, reported a small but significant reduction in hospitalizations for any cause (risk difference -1.2%) besides a reduction in (radiologically unconfirmed) lower respiratory tract complications requiring antibiotics (risk difference -3.8%), and a 21% shorter time to alleviation of all symptoms for oseltamivir (92). Nevertheless, this study was repeatedly criticized for a refusal on the part of the authors to make public its study protocol (93–95). A potential benefit on mortality was not assessed in any of both meta-analyses, as there was a statistically insufficient number of fatal events in all available Randomized-Controlled Trials (RCTs). In a 2016 paper, the Jefferson group conducted a systematic review of observational studies of the 2009 H1N1 pandemic (almost all involving oseltamivir) and concluded that oseltamivir had no protective effect on mortality among hospitalized patients (44).

At present, the main indications for antiviral treatment for influenza in both the current 2018 U.S. Centers for Disease Control (CDC) recommendations and the 2009 Infectious Diseases Society of America (IDSA) guideline include hospitalized patients with suspected or confirmed influenza, and outpatients with high risk of influenza complications (8,96). The IDSA guidelines, notably dating back to 2009, have graded the evidence for their recommendation as "good evidence from non-randomized trials." (8) Recognizing the lack of evidence from RCTs in hospitalized patients, the CDC acknowledged that its 2018 recommendation is based on expert opinion and retrospective studies (96). The Belgian Centre for Chemotherapeutical Information (BCFI) has questioned the use of oseltamivir for any therapeutic indication, mostly based on findings of the Cochrane review (40). In a recent (controversial) communication by public health authorities in the U.K., the use of antivirals was recommended in all patients with suspected flu, pending results of viral testing (97). These divergent recommendations illustrate the ongoing controversy over the therapeutic efficacy and benefit/harm balance of these agents.

We queried our laboratory information system database for the number of unique lab-confirmed cases of influenza infection for all influenza seasons from 2009 to April 2018 (as diagnosed with antigenic tests, digital immunoassays, and/or nucleic-acid amplification techniques). The number of patients that were registered as having received treatment with oseltamivir was retrospectively assessed. Of note, the number of oseltamivir treated patients in the seasons of 2009 to 2012 is an underestimation of the total number of patients having received oseltamivir treatment in this period, as our database did not contain a limited number of patients treated within a compassionate-use program of Roche, running from 20/1/2010 to 30/6/2013. The proportion of hospitalized patients treated with oseltamivir has decreased over the years, and presently represents around 5% of the hospitalized population that was diagnosed with influenza infection (see Fig. 3 and Table 9).

Fig 3. Oseltamivir use in hospitalised patients with confirmed or suspected influenza infection in AZ Imelda Bonheiden use from 2009 to april 2018.

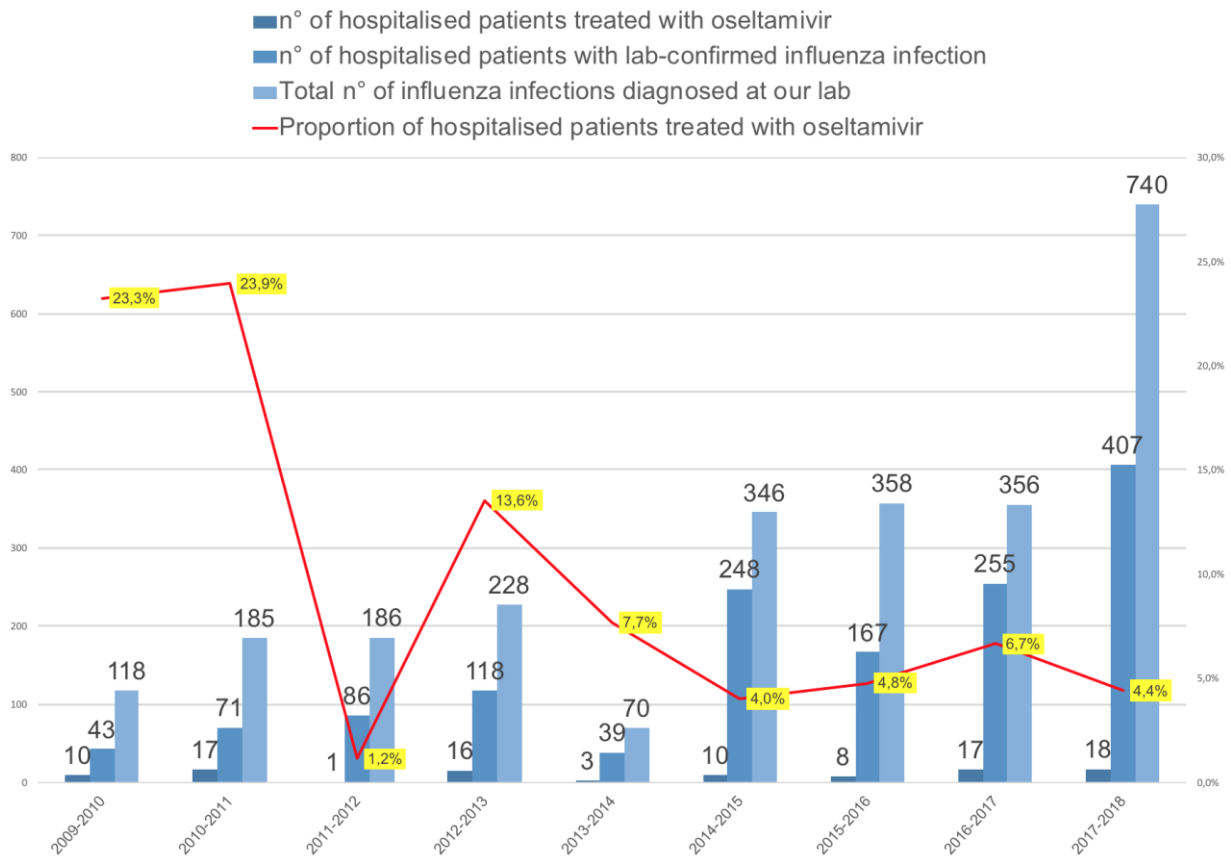


Table 9. Oseltamivir use in hospitalised patients with confirmed or suspected influenza infection in AZ Imelda Bonheiden use from 2009 to april 2018.

	n° of hospitalised patients treated with oseltamivir	n° of hospitalised patients with lab-confirmed influenza infection	Total n° of influenza infections diagnosed at our lab	Proportion of hospitalised patients treated with oseltamivir
2009-2010	10	43	118	23,3%
2010-2011	17	71	185	23,9%
2011-2012	1	86	186	1,2%
2012-2013	16	118	228	13,6%
2013-2014	3	39	70	7,7%
2014-2015	10	248	346	4,0%
2015-2016	8	167	358	4,8%
2016-2017	17	255	356	6,7%
2017-2018	18	407	740	4,4%