Chapter 11

General Discussion
Respiratory viruses are thought to be responsible for the vast majority of respiratory tract infections and account for more episodes of respiratory tract illness than any other microbial pathogens (1). Rapid identification of viral respiratory tract infections has several advantages. Prompt diagnosis may reduce unnecessary additional testing, avoid inappropriate prescription of antibiotics, lead to rapid implementation of infection control measures, and early administration of antiviral medication (2, 3). As a result, a decrease in hospital stay and a reduction in health care costs might be accomplished (4). However, making both a rapid and accurate viral diagnosis is challenging. In the next paragraphs, the current challenges with rapid testing that emerged from this thesis will be addressed and the implications for future research and clinical practice discussed.

### CHALLENGE 1. Limited virus detection range and disappointing sensitivity of rapid tests

Clinical signs and symptoms of respiratory viruses overlap (5). The etiological diagnosis therefore requires a syndromic approach as multiple viruses might be the cause of the infection. The results of chapter 9 demonstrated that based on clinical symptoms alone influenza cases are often missed and non-influenza cases are incorrectly diagnosed as influenza. To correctly and timely identify patients with a respiratory virus, rapid diagnostic tests - preferably tests capable of detecting multiple pathogens - are needed. Unfortunately, the number of rapid tests capable of detecting multiple viruses is limited. Our systematic review showed that most rapid tests have the possibility to either detect influenza viruses or RSV. In addition, their diagnostic sensitivity is often unsatisfactory low, with an overall pooled sensitivity of 61.1% for rapid tests for influenza. Sensitivity was even lower when including only high quality studies. Lower-than-expected sensitivities for rapid tests were demonstrated as well in chapter 3 and 6.

### Unmet need: Rapid, sensitive and specific multiplex test platforms

Rapid, sensitive and specific multiplex rapid tests are needed. More sensitive molecular assays that have the ability to detect a broad range of viral and bacterial pathogens in a short time span are currently in development. At the moment, fully automated molecular methods which require minimal hands-on-time are commercially available (6). A major drawback of these newer diagnostic devices are the high costs and the low sample throughput (7, 8), which limits their potential for direct ‘point-of-care’ use. Molecular methods have the advantage that they are highly sensitive, but the interpretation of molecular test results is not always straightforward. Often the question remains whether high sensitivity provides more reliable information about the true etiology of a given infection as PCR detects viral nucleic acids regardless of the presence...
of viral antigens or replicating infectious virus (9). The presence of viral DNA or RNA does not always reflect acute disease since positive PCR results may represent a previous infection or asymptomatic carriage. Furthermore, with PCR often more than one respiratory virus is detected and the contribution of the positive result to disease severity is not always clear. Quantification of viral load might help in determining the clinical relevance of the infection, but at the moment clinically relevant cut-off values for respiratory viruses in randomly collected samples are limited (10). Finally, as with all molecular methods, there is a danger of contamination and mistakes in the pre-analytical phases (9). Also in the upcoming years, non-molecular rapid tests will therefore still be of importance in clinical patient management. Their possible role in identifying clinically relevant infections, as proposed in chapters 4 and 5, should be further examined as no large clinical studies with rapid tests comparing asymptomatic and symptomatic infections are available.

As already mentioned in chapter 6, not only direct viral testing, but also analysis of the host response to infection might help in correctly diagnosing respiratory tract infections (11). Various laboratory tests that aid in differentiating viral from bacterial infections are currently in use, such as white blood cell count, C-reactive protein (CRP) and procalcitonin concentrations. However, effects of these tests on antibiotic prescription rates are inconsistent (12). Recently, the combination of CRP, tumour necrosis factor-related, apoptosis-inducing ligand, and interferon gamma-induced protein-10 has been investigated (13), and new approaches towards monitoring the host response in viral and bacterial infections, such as gene expression profiling, continue to evolve (14-19). Although results are promising, and assays in which the detection of viruses, bacteria, and host responses are combined might provide new diagnostic opportunities, there is still a long road ahead before routine use of these newer devices in clinical practice. First, results of these assays should be confirmed in larger studies with different patient populations (20).

**CHALLENGE 2. Collecting the correct sample for diagnosing respiratory tract infections**

A second difficulty of accurately diagnosing respiratory tract infections is collecting the correct sample. To identify the cause of the respiratory tract infection lower respiratory tract samples, e.g. sputum or broncho-alveolar lavages, are the preferred sample type. However, these are difficult to obtain, especially in children and the most commonly used samples today are therefore upper respiratory tract samples, such as nasal swabs and aspirates. The problem
with upper respiratory tract samples is that the virus detected might be from a resolving upper respiratory tract infection and not the cause of the lower respiratory tract infection.

**Unmet need: Improved, standardized methods for respiratory sample collection**

Respiratory virus detection highly depends on the type of sample collected, the time of collection after the onset of clinical symptoms, and the transport and storage of the sample before testing. Several sampling methods are currently approved for rapid tests, such as nasal swabs and nasopharyngeal aspirates (21). At the moment, nasopharyngeal flocked swabs are recommended for detecting respiratory viruses (22), but improved, standardized methods for reliable respiratory sample collection for diagnosing respiratory tract infections are warranted, and the search for better sample collection methods for lower respiratory tract infections should continue.

**CHALLENGE 3. Lack of well-designed diagnostic test accuracy studies**

To determine the diagnostic performance of a rapid test diagnostic test accuracy studies are needed. Our systematic review showed that the same rapid test has different sensitivity and specificity estimates in different diagnostic accuracy studies. Diagnostic accuracy calculations are extremely sensitive to the design of the study and are influenced by many factors, such as virus prevalence, time from disease onset to sample collection, sample type, quality of the collected sample, and age of the patient (23, 24). A remarkable finding of the quality assessment of the studies included in our review was that many of the study characteristics influencing the risk of bias were not clearly reported or simply missing.

Another major finding was that although all rapid tests studied in this review were designed to be performed by non-laboratory trained personnel at the point-of-care, many studies were not evaluated at the point-of-care. The setting or the personnel that performed the test was rarely properly described. The actual diagnostic performance of the rapid tests in the setting of daily clinical practice might therefore be different than the accuracy estimates reported in the studies.

**Unmet need: High quality diagnostic test accuracy studies performed at the point-of-care**

To increase their quality, diagnostic test accuracy studies should be reported according to the Standards for Reporting Diagnostic accuracy studies (STARD-guidelines) (25). Despite that
these guidelines have already been developed in the beginning of the 2000s, adherence to the STARD is still moderate and should be encouraged (26).

Besides, laboratory validation studies of rapid tests are not sufficient to evaluate the diagnostic accuracy and use of rapid tests. As shown in chapter 3, the implementation of POCTs requires novel strategies. For implementation of a rapid test, logistic difficulties must be overcome and it is therefore important to provide sufficient training moments for personnel for an adequate operation of the rapid tests and interpretation of the test results. In July 2015, a general practitioners’ guideline was published in the Netherlands on how to deal with POCTs in general practice as also in the field of general practice there is a clear trend towards point-of-care testing (27). One of the main messages from this guideline underscored the rise in numbers of available POCTs and introduction of these tests in primary health care settings without proper evaluation and implementation studies being performed. In chapter 6 we therefore not only evaluated the diagnostic performance, but also the clinical feasibility of a rapid test. Our study is one of the few that addresses this item and hopefully a stimulant for other researchers to take this important topic into account as well when evaluating a new test.

**CHALLENGE 4. Lack of clinical impact studies from both the doctor’s and the patients perspective**

Despite the strict inclusion criteria for our systematic review, we could still include 125 articles that evaluated the accuracy of one or more rapid tests. On the contrary, the number of studies evaluating the clinical impact of rapid test results on patient management is limited (28-31). Besides, in recent years the concept of patient-centered care has received attention. Patient-centered care underscores the importance of better understanding the experience of illness and the patients’ needs (32). In chapter 6 we showed that patients highly appreciated the availability of rapid test for respiratory viruses in primary health care. To our knowledge, ours is the only study in which the patient’s perspective on rapid testing was addressed.

**Unmet need: Randomized controlled trials to determine the impact of point-of-care tests**

In addition to high quality diagnostic accuracy studies performed at the point-of-care there is a need for studies that evaluate the clinical impact of rapid tests (33). Several studies did report on the association of rapid tests on prescription of antibiotics, initiation of antiviral therapy, implementation isolation measures, and/or additional testing. Some reported a positive influence of rapid testing on clinical outcome (31, 34), but others did not report any significant
differences compared to routine diagnostics. However, many of these studies were retrospective studies (35) or studies in which the turn-around-time of the evaluated test was still quite long (36).

Only with well-designed randomized controlled trials, the true impact of rapid tests on clinical patient management can be assessed. We therefore encourage the planning of randomized controlled trials that assess the clinical relevance of rapid tests of terms of clinically relevant outcomes, such as use of antibiotics, prescription of antiviral medication, use of additional ancillary testing, and length of hospital stay. Cost-effectiveness evaluations are an important component of these studies. Furthermore, for optimal patient management, to encourage self-management and reduce the number of unnecessary doctor visits it is important to know the needs and views of patients themselves with regard to rapid testing and how this should be implemented.

**RAPID DIAGNOSTICS TO PAINT THE EPIDEMIOLOGICAL PICTURE**

Despite the limitations of the current rapid tests addressed in the previous paragraph and the challenges that are faced with point-of-care testing, faster and more accurate virus diagnostics are warranted. As we have shown in chapters 6 and 9, viruses could not be detected in almost half of the patients with a respiratory tract infection in primary health care. Similar results were described in a recent study by Jain *et al* (2015), in which in nearly two-third of the hospitalized patients no disease-causing pathogen could be identified in those with radiologically confirmed lower RTI (37). Improved rapid diagnostics can aid to bridge this gap in knowledge and increase our insight in the epidemiology of respiratory viruses. For example, with the introduction of PCR also non-culturable viruses, e.g. rhinovirus C and human bocavirus 1 were more easily identified. This has contributed significantly to our understanding of the viruses causing respiratory tract infections. As shown in chapters 4, 7 and 8, the general view of these viruses as just 'common cold' viruses has been adjusted since especially rhinoviruses are increasingly recognized as important respiratory pathogens capable of causing severe lower respiratory tract infections as well (38, 39).

In addition, not only will enhanced detection of respiratory viruses increase our insight in the epidemiology, it might also give direction to development of antiviral therapy as exemplified for RVs (40-42). The availability of rapid, sensitive, and inexpensive diagnostic tests that can detect respiratory pathogens might boost the interest of pharmaceutical companies in drug
development as the disease spectrum of the different viruses becomes more clear and targeted therapy can become a realistic option.

However, in the near future a complete epidemiological picture is not yet feasible and a substantial proportion of the respiratory tract infections will probably remain undiagnosed. In the absence of a detectable pathogen, host-targeted approaches - such as the above mentioned host protein-based assays and gene expression profiling - might help in differentiating viral from bacterial infections.

**FUTURE PERSPECTIVE: CATCHING THE COMMON COLD?**

The studies presented in this thesis focused on two themes: 1) development and evaluation of rapid tests for the detection of respiratory viruses; 2) epidemiology and clinical relevance of respiratory viruses, in particular rhinoviruses. In the era of emerging respiratory viruses, there is a growing need for rapid, sensitive and specific identification of these viral pathogens. Rapid tests have the potential to fulfil these needs, but one should be aware of their current limitations in diagnostic performance and pathogen identification. The development of improved rapid tests, preferably tests that can detect both respiratory viruses and bacteria in combination with host response markers, should therefore be encouraged. Before these tests become commercially available, high quality evaluation studies are required. These evaluation studies should not only focus on diagnostic accuracy, i.e. if the new test correctly identifies the pathogen and pathogen-specific host response, but also on the interpretation, implementation, and impact of the rapid test on clinical patient management (43). This thesis showed that point-of-care testing involves more than just performing a rapid test at the point-of-care. Implementation of point-of-care testing requires novel strategies for logistics and organization and for successful implementation of rapid tests in the clinic high quality evaluation studies at the point-of-care are required. Furthermore, studies on the impact of rapid tests should not only be measured from the doctor’s perspective, but also from the patient’s perspective.
REFERENCES


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