Chapter 1

General Introduction
Respiratory tract infections: burden of disease

Acute respiratory tract infections (RTIs) are a leading cause of morbidity and mortality worldwide (1). Every year nearly 3 million children die of acute lower RTIs, such as pneumonia and bronchiolitis (2). Upper RTIs, popularly known as the ‘common cold’, are the most common acute illnesses within the industrialised world. Adults encounter on average two to six colds per year. In young children infection rates are even higher, with an average of five to eight common cold episodes annually. Symptoms can be as mild as just a runny nose, but complications such as asthma exacerbations, otitis media, and pneumonia may develop (3).

Despite the usual self-limiting nature of RTIs, their economic burden on society is enormous. In the United States, the common cold syndrome alone is responsible for about 22 million days of absence from school and 20 million days of absence from work, resulting in lost productivity and medical visits (4). Following this, RTIs are the commonest acute problem dealt with in primary health care (5).

Etiology of respiratory tract infections

Although bacteria are historically considered the main pathogens causing severe RTIs, the importance and contribution of viruses is increasingly recognized. Recent studies suggest more than half of RTIs are caused by viruses, even in severely ill and mechanically ventilated pneumonia patients (6-8). Common respiratory viruses causing RTIs include rhinoviruses (RV), human coronaviruses (HCoV), influenza viruses, respiratory syncytial viruses (RSV), human metapneumovirus (hMPV), parainfluenza viruses (PIV), and adenoviruses (9-11). Several other respiratory viruses have been identified more recently, such as human bocavirus (HBoV), and the respiratory polyomaviruses WU and KI, of which the precise clinical relevance remains under debate, and new viruses continue to emerge (12-14). A brief overview of the characteristics of the most important respiratory viruses is shown in Table 1.

Epidemiology, transmission, and pathogenesis

The epidemiology of respiratory viruses varies considerably, but seasonal patterns can be recognized in temperate climates and less so in (sub)tropical regions. The number of respiratory viruses increases in autumn, is fairly high throughout winter, and decreases in spring (15). RSV and influenza viruses are well-known for their ability to cause seasonal outbreaks, especially in the extremes of the age spectrum and in high-risk individuals, such as immunocompromised patients or patients with chronic underlying cardiac, pulmonary or metabolic comorbidities (4, 16-18).
Respiratory viruses can be transmitted directly from person-to-person through aerosols, respiratory droplets or hand contact, and/or indirectly from contaminated environmental surfaces. Respiratory viruses enter the body through the upper respiratory tract and replicate mainly in respiratory mucosa and respiratory lymphoid tissues. The pathogenesis of viral RTIs likely differs per causative agent and is not yet fully understood for most, but in all depends on the interplay between virus, host and environment. Factors influencing disease manifestations and severity are the virulence of the infecting virus strain, innate and adaptive host immune responses, underlying comorbidities and host genetic factors (19-22).

**Treatment, prevention and control**

Treatment options for viral RTIs are limited and are still primarily based on supportive care. Symptomatic treatment, e.g. nasal decongestants and paracetamol, is often sufficient for mild infections. In the hospital setting, however, fluid replacement, extra oxygen and eventually mechanical ventilation might be necessary (23). Currently, virus-specific treatment is only available against influenza virus infections. Two classes of antivirals are commercially available for influenza viruses: neuraminidase inhibitors, e.g. oseltamivir and zanamivir, and M2-channel blockers, but due to the high prevalence of resistance for this latter class its use is currently not recommended (24). A number of therapeutic drugs are under development for other respiratory viruses (25). For example for rhinoviruses, the most frequently detected respiratory virus in patients with RTI symptoms and known for its contribution to acute asthma and COPD exacerbations, several antiviral drugs are under evaluation, such as the capsid binder vapendavir (26).

At the moment, influenza is the only vaccine-preventable respiratory virus, although influenza vaccines need to be reformulated periodically due to antigenic drift (27). For RSV, passive immunisation with palivizumab is offered for specific risk groups, such as prematurely born children. New antiviral approaches, including nanobodies, broadly neutralizing monoclonal antibodies, and host-targeted therapeutic approaches, i.e. therapeutics targeted at host cellular pathways required for viral replication, are currently in development, but so far treatment remains largely supportive (28-30).

Infection control of viral RTIs is thus largely based on prevention. Infection control measures, such as respiratory hygiene, cough etiquette and masking and separation of persons with respiratory symptoms, are needed to prevent the spread of respiratory viruses (31, 32).
Diagnostic methods for detecting respiratory viruses

Although certain respiratory viruses are associated with specific syndromes, e.g. RSV bronchiolitis, clinical signs and symptoms related to respiratory viruses infections generally overlap. To confirm the etiological agent responsible for the RTI a laboratory diagnosis is required. Accurate diagnosis of the RTI is important for patient management and infection prevention (33).

At the moment, five general approaches are used for the laboratory detection of viruses: microscopy, culture, detection of viral antigens or nucleic acids, and detection of antibodies against the organism (34). The history of virus detection began in 1898, when Martinus Beijerinck, a Dutch microbiologist, introduced the word ‘virus’ for an infectious agent causing disease in tobacco plants. The identification of this pathogen is now acknowledged as the foundation of the field of virology (35).

With the introduction of the electron microscope in 1933 it was for the first time possible to visualize virus particles. For clinical purposes the technique is currently outdated because of its limited sensitivity, but it is still in use in research settings to study viral structure. Furthermore, it can have a role in detecting new and unusual virus outbreaks. In 2003, for example, examinations of respiratory samples by electron microscopy resulted in the discovery of Severe Acute Respiratory Syndrome (SARS)-coronavirus (36).

Cell culture has long been considered as the gold standard for detecting respiratory viruses. Viruses are intracellular pathogens requiring host cells for replication. Therefore, in vitro cell culture systems have been developed to facilitate replication. After inoculation with a respiratory sample, the cell culture is incubated for approximately one week and afterwards examined for cytopathogenic effects by light microscopy, suggesting viral replication. The process of cell culture accelerated with the development of so called ‘shell vial spin amplification cultures’. With this technique, the virus is centrifuged on to a thin cell layer, and labelled with fluorescent monoclonal antibodies against viral antigens. Viral growth can then be detected by fluorescence. In mixed cell culture systems several cell lines are combined in one culture and multiple viruses that require different cells for growth can be isolated (34). Cell culture remains a useful approach for virus detection, but has nowadays become mainly a research tool (37).

Several other laboratory techniques make use of viral antigen detection. With Direct Fluorescent Antibody (DFA) staining, cells from nasopharyngeal samples are directly tested for the presence of an antigen with a fluorescent-labelled antibody. These antibodies are incubated with the
sample to allow antigen-specific binding. Excess and unbound antibodies are washed away and areas in which antigens are present can be visualized using a fluorescence microscope (38).

Serology is historically the mainstay of virus diagnostics. It monitors the immune systems’ specific antibody response to viral antigen exposure. Antibodies are produced after the onset of viral illness and can be detected using serological diagnostic techniques such as hemagglutination inhibition tests, enzyme immunoassays, complement fixation, and neutralization tests (38). However, for detection of respiratory viruses these methods are not regarded as clinically relevant diagnostic tools in daily practice as it usually requires acute and convalescent serum samples to detect rises in antibody levels (39).

With the introduction of highly sensitive molecular techniques such as polymerase chain reaction (PCR) a new standard for respiratory virus detection has been set. Nucleic-acid based amplification techniques use primers and probes directed at unique, conserved regions of a viral genome. They are highly specific and bind only to complementary DNA or RNA sequences (38). Advances in technology have allowed for the development of multiplex PCR tests in which several different viruses can be identified in a single test.

All the above-mentioned techniques have their drawbacks. The isolation of respiratory viruses in culture or serological diagnosis requiring paired blood specimens are slow and labour-intensive. DFA is faster, but requires technical expertise as well and is less sensitive than culture. Because of its high sensitivity, high specificity and its ability to also detect viruses that are difficult to culture or simply do not grow at all in cultured cells, PCR has become the reference method for detection of respiratory viruses (40).

However, also PCR techniques require trained laboratory personnel and specialized equipment that is not always directly available resulting in relatively high costs and long turn-around times. Although more rapid and user-friendly PCR devices have been developed (41), rapid antigen detection tests (RADTs) are often used as a simple, cheaper, and time-saving alternative for virus detection.

RADTs are immunoassays that can detect viral antigens in respiratory specimens, such as membrane-based enzyme immunoassays, lateral flow immunochromatography tests, and optical immunoassays. These assays are, like DFA, based on an antigen-antibody reaction, but as the antibody is not fluorescence-labelled but enzyme-labelled, no fluorescence microscopy is required (38, 42). They have the potential to be carried out near the patient at the point-of-care,
such as the emergency department or a general practitioners’ office, and thus have the ability to function as so-called point-of-care tests.

**Point-of-care tests**

Point-of-care tests (POCTs) are defined as rapid, easy-to-use tests carried out near the patient by non-laboratory-trained personnel. PCR-based POCTs are in development, but at the moment RADTs are most often used as POCTs as they are faster (with results typically available within 30 minutes), less expensive, easy-to-use and thus accessible to staff without laboratory training, compared to the other laboratory techniques.

There is an increasing interest in POCTs as according to several cost-effectiveness evaluations the use of these tests could lead to a more effective utilization of healthcare resources (43, 44). With the rapid identification of viral pathogens causing RTI, unnecessary additional testing, such as X-rays, blood count and cultures, and antibiotic prescription may be avoided (43, 45). Besides, prompt viral diagnosis may lead to rapid implementation of infection control measures, early administration of antiviral medication and a decrease in duration of hospital stay resulting in reduced health care costs. POCTs can offer clinicians the ability to manage patients’ expectations for antibiotics and to encourage patients to self-care when suffering from a self-limiting condition (46).

However, the clinical feasibility of POCTs is only sporadically evaluated in clinical settings. Most of the publications regarding POCTs are retrospective studies or studies where the test is used in a laboratory setting. Results are therefore difficult to extrapolate to daily clinical practice. Because of the potentially promising influence of POCTs on patient care, well designed clinical validation studies are needed. An important aim of this thesis is therefore to evaluate the use and diagnostic accuracy of POCTs for respiratory viruses in both the hospital setting and in primary health care.

**Outline of this thesis**

In Part 1 of this thesis, we describe the development and evaluation of POCTs for respiratory viruses. Chapter 2 provides an overview of all commercially available POCTs for the detection of respiratory viruses in patients with RTIs. In chapter 3, we evaluate the use of a POCT for influenza viruses and RSV in a pediatric hospitalized population. Chapters 4 and 5 describe the development of two new viral targets, respectively HBoV and CoV, for a novel POCT. In chapter 6, we determine the diagnostic accuracy and clinical feasibility of a POCT in a primary health care practice.
In Part 2, we focus on the epidemiology of respiratory viruses, in particular rhinoviruses. RVs are frequently detected respiratory viruses that may cause mild common cold symptoms, but can also lead to more severe RTIs. The large number of RV types, classified into species A, B and C, hampers clear insights in the epidemiology and clinical significance of each RV type. Chapter 7 investigates the prevalence of RV types in a hospitalized patient population. In chapter 8, we describe the clinical, virological and epidemiological characteristics of RV infections in young children with mild or no symptoms compared to children with moderate to severe symptoms in order to explore possible associations between different characteristics of RV infections and clinical outcome.

Laboratory testing for acute RTIs is not routinely performed in primary health care. The etiology of RTIs is therefore usually unknown and although RTIs are mainly of viral origin, the contribution of different respiratory viruses is uncertain. The purpose of chapter 9 is to increase our insight into the epidemiology of respiratory viruses in a primary health care setting and to evaluate the accuracy of the local general practitioners in their clinical diagnosis of influenza virus infection compared to most sensitive PCR techniques. The summary and general discussion on the main findings of this thesis and recommendations for future studies is presented in chapters 10 and 11.
### Table 1. Virological characteristics of common respiratory viruses

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<th>Virus</th>
<th>Family</th>
<th>Genome</th>
<th>Envelope</th>
<th>Characteristics</th>
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| Rhinovirus             | Picornaviridae | RNA    | No       | - Rhinovirus species A, B and C  
- >160 types  
- Main pathogen responsible for the 'common cold', but can also elicit more severe disease |
| Influenza virus        | Orthomyxoviridae | RNA    | Yes      | - Influenzavirus A, B and C  
- Influenza A is associated with seasonal epidemics and pandemics, influenza B only with seasonal epidemics |
| Respiratory syncytial virus | Paramyxoviridae | RNA    | Yes      | - RSV subtype A and B  
- Most important cause of acute lower RTIs in children |
| Parainfluenza virus    | Paramyxoviridae | RNA    | Yes      | - Parainfluenzavirus type 1, 2, 3 and 4  
- Known for its role in croup |
| Human metapneumovirus  | Paramyxoviridae | RNA    | Yes      | - Relatively novel virus (discovered in 2001) with symptoms similar to RSV |
| Human coronavirus      | Coronaviridae | RNA    | Yes      | - 6 genotypes: 229E, OC43, SARS-CoV, NL63, HKU1, MERS-CoV. |
| Human bocavirus        | Parvoviridae | DNA    | No       | - Human Bocavirus type 1, 2, 3 and 4  
- Mainly type 1 responsible for respiratory illness |
| Adenovirus             | Adenoviridae | DNA    | No       | - Besides respiratory symptoms a major cause of keratoconjunctivitis |
REFERENCES


