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Diagnostic approach to respiratory infections

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Respiratory infection is one of the most common organ specific infectious diseases worldwide and is the fourth leading cause of death in Hong Kong. The incidence is expected to increase as a result of the aging population and an increase in number of patients with immunocompromised condition. Emerging and re-emerging pathogens would also contribute to the surge in cases. With the indiscriminate use of antibiotics and the stifled pace of new antibiotics development, the problem of antibiotics resistance will definitely jeopardize the management of patients with respiratory infection. The role of microbiological laboratory in the diagnosis of respiratory infection is relatively limited and controversial as a result of ample drawbacks and limitations of the diagnostic tests. Nowadays, many international guidelines on the management of respiratory infection adopt an empirical treatment approach with little regard to the diagnostic methods (1,2,3). This review will cover the diagnostic approach for acute pharyngitis, acute epiglottitis, acute bronchitis and community acquired pneumonia (CAP).

Acute pharyngitis

Most cases of acute pharyngitis are caused by viruses. However, it is important to rule out bacterial causes in some patients, especially for group A beta-hemolytic Streptococcus (GAS) which may be present in 5-15% of adults and 30% of children complaining of sore throat. Antimicrobials are often prescribed for patients with acute pharyngitis because of patients' expectation and the physician's worry about the potential risk of GAS associated rheumatic fever and glomerulonephritis. The diagnosis of GAS is thus very crucial in this respect. Unfortunately no gold standard test exists so far to give a reliable and quick result. Throat culture is usually not sensitive and fast enough to be recommended as routine test. It also fails to distinguish acute infection from carrier state. Antibodies testing like the anti-streptolysin O titre cannot provide "real-time" results. Although rapid streptococcal antigen test is widely advocated in the western world, it is less adopted in Hong Kong possibly because of its cost and its variable sensitivity (58-96%) and specificity (63-100%) (4). As a result, the diagnosis of GAS is usually based on the patients' symptoms. Actually clinical criteria for the diagnosis of GAS have reasonable diagnostic accuracy. The Centor clinical criteria for the diagnosis of GAS encompass tonsillar exudates, tender anterior cervical lymphadenopathy or lymphadenitis, absence of cough, and history of fever (5). The presence of more than three of these criteria has sensitivity and specificity of 75%, positive predictive value of 40-60% and negative predictive value of 80% when compared to throat culture for pharyngitis (5,6). The use of rapid streptococcal antigen test to confirm a clinical diagnosis may also increase the accuracy.

Acute epiglottitis
Acute epiglottitis is a medical emergency. It is common in children and is rarely seen in adult and therefore is often misdiagnosed. However, with the introduction of the Hemophilus influenzae conjugated vaccine, frequency in pediatric cases decreases but a steady rise in adult cases is noted in the Western world. A local review in 2001 also observed the same trend although vaccination is not commonly practiced, and the author recommended that the diagnosis should be suspected in all patients with sore throat and dysphagia, especially if the symptoms are out of the proportion to the pharyngeal inflammation. Typical clinical features of acute epiglottitis include dysphagia out of proportion to the signs of pharyngitis, anxiety, leaning forward, drooling of saliva. However, role of microbiological laboratory is limited. Rapid streptococcal antigen test and throat culture may be helpful to exclude bacterial pharyngitis. However, examination of the throat is not recommended in the presence of typical symptoms for epiglottitis and when facility for emergency endotracheal intubation or tracheostomy is not available. Lateral neck radiography and flexible fiberoptic laryngoscopy are most helpful in making a diagnosis.

Acute bronchitis
Acute bronchitis is one of the most common diagnoses in adult clinical practice. Unfortunately no precise definition could be made. It is usually presented with cough for 1-3 weeks, with or without sputum, upper respiratory tract or constitutional symptoms. Like acute pharyngitis, the most common etiology for acute bronchitis is also viral (Influenza A/B virus, parainfluenza virus type 3, respiratory syncytial virus, coronavirus, adenovirus, rhinovirus), while each of Mycoplasma pneumoniae, Chlamydia pneumoniae and Bordetella pertussis / parapertussis may explain about 5-10%. Again the diagnosis of acute bronchitis is largely clinical. Sputum microscopy and culture are not helpful. Role of rapid antigen tests for respiratory viruses (usually enzyme immunoassays (EIA) and immunofluorescence (IF)) is limited, especially for influenza viruses since the sensitivities of these tests are equivalent to that of clinical judgment when that influenza strain is circulating in the community. Viral culture and antibodies for viruses cannot provide “real-time” diagnosis. Nevertheless, especially during this post-Severe Acute Respiratory Syndrome (SARS) and post-avian flu period, rapid antigen tests are considerably important to rule out common respiratory viruses because of the indistinguishable initial clinical features between acute bronchitis, SARS and avian flu. The sensitivities and specificities of the EIA antigen tests for influenza could vary from 37 to 100% and 63 to 100% respectively, depending on the methodologies of the tests and the nature of the samples obtained.

The specimen for antigen tests should be obtained early, preferably in the first 3 days of illness. Type of samples could be nasopharyngeal aspirate, throat/nasal swab, tracheal aspirate, bronchoalvoelar lavage or sputum. The samples should be transported to the laboratory as soon as possible and should be placed at 4°C if delay is expected and at -70°C if culture is not performed within 4 days.

An interesting topic that is worth mentioning is the acute exacerbation of chronic bronchitis. Although bacteria, especially Streptococcus pneumoniae & Hemophilus influenzae, are usually considered to be the etiological agents for the exacerbation, respiratory viruses could also contribute to a significant number of cases (30-40%). Viral studies could be performed, unfortunately it has no bearing to the overall management of the patients involved, except for the suspicion of SARS or avian flu as mentioned above. Sputum microbiology and culture are of limited value since 25% of upper airway of patients with chronic obstructive pulmonary disease (COPD) is colonized with bacterial pathogens. Study has shown that identifying new strains of H. influenzae, M. catarrhalis or S. pneumoniae was associated with a significantly increased risk of an exacerbation (7). However, the molecular method applied in this study could not be generalized, and the empirical use of antibiotics for the exacerbation of COPD may make the identification of pathogen less important, although this management approach is still controversial.

Community acquired pneumonia (CAP)
American Thoracic Society (ATS) (2) and the Infectious Diseases Society of America (IDSA) (1) guidelines emphasize using the history and physical examination to aid in the selection of non-microbiological diagnostic tests for assessment of the severity of illness and as a guide to empirical antibiotic choices. They also recommend chest radiology for all patients with CAP. Unfortunately, ideal laboratory tests for most pathogens do not yet exist and the extent of investigation for CAP has not been standardized.

a. Sputum Gram stain
The IDSA guideline recommends sputum microscopy and culture for hospitalized CAP patients whereas the ATS guideline does not. Although controversy exists in the evaluation of expectorated sputum, its roles in the interpretation of specimens obtained by invasive procedures, or identification of organisms that do not normally colonize the upper airway like Mycobacterium tuberculosis and pathogenic fungi, are not questioned. Pitfalls exist for expectorated sputum include:

1. Many patients, especially severely debilitated ones, are unable to expectorate sputum or produce sputum specimen that is unacceptable.
2. Trained personnel are required for sputum collection and interpretation.
3. Criteria for interpretation of specimens are not standardized.
4. Diagnostic accuracy is mainly available for Streptococcus pneumoniae and atypical pathogens are not identified.

Despite these, the most widely adopted criteria for interpretation of sputum Gram stain requires less than 10 squamous epithelial cells and more than 25 polymorphonuclear leukocytes per low-power (10x) field. Moreover, predominant bacterial morphotype (>75%) may be useful in guiding pathogen-orientated antibiotics therapy. A meta-analysis of 12 studies reported by Reed et al revealed that the sensitivity and specificity for sputum Gram stain, using sputum culture as reference, varied widely from 11% to 100% and from 15-100% respectively (9). Therefore the result of a sputum Gram stain should be interpreted with care and has full consideration of the clinical setting.

b. Sputum culture
The accuracy of sputum culture is also influenced by the same factors that affect Gram stain. Moreover, the contamination of the sputum sample with flora from upper respiratory tract will also decreases the diagnostic value since these organisms often grow more readily than the common respiratory pathogens. Therefore, in the interpretation of the culture result, one should compare with the Gram stain so as to improve the precision of detection. Diagnostic information from culture can also be enhanced by using saline-washed sputum specimens. Despite these approaches, the sensitivity of sputum culture remains low and varies widely (from 20-79%). In view of this, sputum culture should be reserved for use in patients with severe CAP, failed medical treatment, significant immunocompromised state and a sputum culture from a purulent specimen that is processed correctly.

c. Blood culture
Blood culture has been suggested in both ATS and IDSA guidelines for hospitalized patients because of its high specificity. However, the diagnostic yield is low (from 5-16%). Since bacteraemia is associated with higher mortality in CAP, it should be considered, especially for patients presenting with fever and signs of sepsis. It should also be done properly under aseptic technique and before the administration of antimicrobials.

d. Serological studies
Serologic studies are usually reserved for atypical pathogens including Mycoplasma pneumoniae, Chlamydia pneumoniae and Legionella pneumophila, since they are responsible for a significant proportion of etiological organisms for CAP. However, the diagnosis is usually made retrospectively since a 4-fold rise in titre is required (Table 1). Therefore the ATS and IDSA

However, she presented again with progressive right knee pain and swelling about two weeks after the arthroscopy. Examination of the knee and diagnostic tap showed similar findings to the initial admission. This time blood was taken for inflammatory markers in addition to routine work-up. Erythrocyte sedimentation rate (ESR) was 99mm/hr (3-28) and C-reactive protein was 64mg/L (<5). White cell count was 9.6x10^9/L (3.7-9.2). An ultrasound guided synovial biopsy of the right knee showed evidence of non-specific acute inflammation and culture was again negative. Surgical exploration of the knee joint was done under general anaesthesia in view of persistent symptoms and elevated inflammatory markers. A large defect occupying the whole femoral condyle was seen during operation which was filled with sequestrum and small amount of pus. Sequestrectomy was done and gentamicin beads were inserted into the condylar defect. Microscopic examination of the synovium specimen showed heavy infiltration of acute and chronic inflammatory cells. Small amount of multi-nucleated giant cells were also present. Abundant fungal hyphae with septation and acute angle branching were seen. Tissue culture grew Aspergillus fumigatus. Latex agglutination for galactomannan antigen was negative. MRI of right knee found erosion of the articular surfaces of femoral condyles and tibial plateau accompanied by mild subarticular marrow enhancement. Amphotericin B was started and later switched to oral itraconazole solution (daily dose 400mg) in view of better bone penetration, lesser toxicity and availability of oral form. The inflammation gradually subsided and inflammatory markers (ESR and CRP) returned to normal four weeks after starting itraconazole. The patient experienced no significant side effect from itraconazole and the drug was stopped after a total of nine months therapy. She was well and had no symptom recurrence till follow up at 15 months after treatment.

Literature review
Aspergillus species are ubiquitous saprophytic fungi frequently found in the environment and usually not pathogenic. The clinical spectrum of Aspergillus infections can be divided into three forms: allergic disease, superficial locally invasive disease or, invasive aspergillosis. The invasive form is most commonly seen in patients with immunosuppression, solid organ or bone marrow transplantation, chronic granulomatous disease and malignancy.

Haematogenous dissemination of Aspergillus occurs in immunocompromised patients. The common extrapulmonary sites are the brain, heart, kidney, and gastrointestinal tract. Many other unusual forms of invasive aspergillosis have been reported but bone and joint infections are rare [1]. In a review of vertebral aspergillosis (41 cases), 65.8% of patients were found to have predisposing conditions for opportunistic infections but a significant number (34.1%) did not have any predisposition [2]. The commonly found underlying conditions were impaired granulocyte function (19.5%), chemotherapy for neoplasms or organ transplantation (17%), previous pulmonary infection (19.5%), and steroid use (17%). Aspergillus fumigatus was the most frequently isolated species (70.7%).

Diagnosis of Aspergillus osteomyelitis requires a high index of suspicion. Radiologic studies are non-specific although plain X-ray would have abnormalities most of the time. The definitive diagnosis depends on isolation of the organism in tissue culture and histopathologic examination of the relevant clinical specimen. Special stainings such as periodic acid-Schiff and Grocott-Gomori methenamine-silver nitrate are helpful in visualising the hyphae.

Amphotericin B (initial dose 0.8-1.25 mg/kg/d) has been considered as standard therapy in invasive aspergillosis. But the optimal therapy for Aspergillus osteomyelitis has not been well defined. Although amphotericin B was still the mostly widely used antifungal in cases reported in the literature, it has a potential drawback of poor bone penetration. In a recent review of Aspergillus osteomyelitis in immunocompetent patients (42 cases), the authors found that the overall cure rate was 69% [3]. However, the cure rate was only 14% when amphotericin B was used alone compared to 75% when combined with surgery. Although lipid formulation of amphotericin B has been suggested for use in case of treatment failure or toxicity, it has been demonstrated to be ineffective in a case of spinal osteomyelitis [3]. Itraconazole (initial dose 200mg tds for 4 days then 200mg bd) is superior in terms of bone penetration, safety profile and availability in oral form. The solution preparation, taken with empty stomach, should be used to ensure adequate absorption. Recent case reports suggested that the newer antifungal, voriconazole could be an alternative choice of therapy in immunocompetent patients [4]. Voriconazole is a new triazole with potent in vivo and in vitro activity against a broad spectrum of fungi, including Aspergillus species and is generally well tolerated. Finally, prompt and adequate surgical debridement is of paramount importance and the type and extent of surgery should be individualised. However, the optimal duration of therapy, whether combined with surgery or not, is not well defined. Relapse can occur even after months of therapy especially if the host is immunocompromised.

In human aspergillosis, the common portal of entry is by inhalation, through the gastrointestinal tract and direct inoculation through a skin wound or even the orbits. As haematogenous spread of invasive aspergillosis from another primary site to the knee is unlikely in our case, we believe that the acquisition of Aspergillus was related to the local application of herbal plant extracts to the knee after microscopic injuries imposed by repeated intra-articular injections. Studies showed that Aspergillus was in fact one of the most frequently found fungi contaminating medicinal plant material [5,6]. Injection of contaminated heroin has also been implicated in Aspergillus osteomyelitis.

Although uncommon, Aspergillus osteomyelitis can occur in immunocompetent hosts. This case demonstrated that oral itraconazole when combined with surgery is useful in the first line therapy for Aspergillus osteomyelitis.

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mothers. This was then introduced in stepwise fashion so that both clinicians and laboratorians can cope with the workload. With this change in technology there will be an expected rise in number of detected cases in antenatal clinics.

Other problems, however, remain including a reactive or equivocal treponemal test in an asymptomatic patient with no previous history of syphilis. False positive reactions of the FTA-ABS are well documented to occur in Lyme Disease, systemic lupus erythematosus, other autoimmune diseases, and in the elderly (4, 5, 6). The treponemal Western blot (TWB) has been evaluated as an alternative confirmatory test and in diagnostic problems areas, especially congenital syphilis. Using densiometric quantitation and spreadsheet normalization, amongst 84 defined reactive and 105 defined non- reactive sera, three candidate test determinants had included the 47 kDa, 17 kDa, and 15.5 kDa bands. Follow- up TWB of clinically diagnosed cases showed that previously untreated patients with primary or secondary syphilis were more likely to have decreased TWB reactivity than those treated patients with latent symptoms (7).

Much effort has been made to detect and differentiate T. pallidum subspecies pallidum using different genetic methods. When polymerase chain reaction (PCR) amplification and restriction endonuclease digestion of PCR products from laboratory strains and clinical specimens were used to develop a molecular subtyping scheme for T. pallidum, two genes exhibiting intrastrain variability were identified as potential targets for strain differentiation: the acidic repeat protein (arp) gene, and a member of the treponemal pallidum repeat (tpr) gene family (8). Amongst 63 isolates studied, 12 different subtypes resulted with most (54.2%) having arp genes with 14 repeats. The other 11 subtypes with 7 to 21 repeats accounted for 2% to 14% of isolates. MseI restriction digestion of PCR product from a member of the tpr gene family resulted in 7 restriction fragment length polymorphism patterns. By combining the two systems, 16 subtypes were observed amongst 46 isolates.

More recently, a sensitive and specific PCR was developed with primers designed based on DNA polymerase I gene (polA) that has characteristics of high cysteine content, low homology with similar regions of known microorganisms, and presence of 4 insertions in the gene (9). Detection limit was 10-25 organisms on gel, and sensitivity increased by 1 log when fluorescence-labeled amplicons are detected. Sensitivity was claimed to be 85.8%, and specificity 95.7%. It would be very interesting to keep these in view, especially the robustness and sensitivity, when further development of these tests with coverage on more extensive heterogeneous population is performed.

Because of obvious social and legal implications associated with detection of a sexually transmitted disease, quality assurance of laboratory test results is of paramount importance. Over the years, public health laboratories at national levels have undertaken to run various quality assurance programs that included blinded panel testing for VDRL/ RPR, TPHA/ TPPA, and FTA-ABS. With rapid development of various molecular tests, there is an obvious urgent need for evaluation, standardization, and quality assurance to ensure interpretability and comparability of laboratory test results.

In Hong Kong, the Syphilis Laboratory, Public Health Laboratory Centre of Department of Health, organized a pilot run of Syphilis Serology Laboratory Quality Assurance Program in late 2003. After a half-day Syphilis workshop organized for participants, a second run was completed in June 2004, and the number of voluntary participants increased from 9 to 11 laboratories. Analysis of preliminary results showed that there was least difficulty in distinguishing between positive and negative cases using non-treponemal tests, in the limited number of test samples. Some variations were, however, observed in quantitative titres reported, which means caution should be exercised in interpreting these results across laboratories. Continuous improvement for participating laboratories remains a central role for this program, and, with the introduction of new technologies in the field, much enhancement can be anticipated in near future.

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Aspergillus osteomyelitis

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Case report

Invasive aspergillosis is uncommon in immunocompetent hosts. Aspergillus osteomyelitis or arthritis is a rare disease, even in immunocompromised patients. We reported a rare case of Aspergillus osteoarthritis of the knee joint and distal femur successfully treated by combination of surgical debridement and itraconazole in an otherwise immunocompetent patient.

A 64-year-old lady was admitted for right knee pain and swelling in August 2002. She had no significant past health of noted. Three months earlier, she suffered repeated episodes of minor right knee sprain during work. She did not report injury with open wound though. She received a few intra-articular injections from a general practitioner for the presumable diagnosis of degenerative osteoarthritis of the knee joint. And in addition to that she applied some herbal plant extracts to the knee for pain relief.

On admission the general condition of the lady was satisfactory and afebrile. Her right knee was warm and swollen. X-ray showed degenerative changes and multiple intra-articular loose bodies compatible with osteoarthritis. White cell count was $8.7x10^9$/L (3.7-9.2). Chest X-ray was normal. A diagnostic knee tap was done in which a large number of white blood cells, predominantly polymorphs, were seen under the microscope. Gram smear, acid-fast staining and culture were all negative. Arthroscopy showed multiple loose bodies inside the right knee accompanied by severely damaged soft tissues. The symptoms improved after arthroscopic removal of the loose bodies and synovectomy.

guidelines suggest empirically treatment if atypical pathogens are suspected. The specificities for these tests are generally quite good, despite a moderate sensitivity. They are usually reserved for sicker patients, patients presenting with atypical or extrapulmonary symptoms, with protracted course or failed medical treatment. Although IgG antibodies detection is available for SARS with overall sensitivity of more than 90% after at least 3 weeks of onset, it is not useful as a strategy for rapid diagnosis.

Table 1 -- Serologic diagnosis in community-acquired pneumonia

Organism	Diagnostic Results	Sensitivity (%)	Specificity (%)
L. pneumophila	4x increase or titer ≥1:256	40– 60	96–99
M. pneumoniae	4x increase in CF	30–70	>90
	Specific IgM	75–90	>90
	Cold agglutinins ≥1:64	50–60	?
Chlamydia species	4x increase in CF or titer ≥1:64	10–100	?
	4x increase in MIF or IgM ≥ 1:16, or IgG ≥ 1:512	40–95	>80
SARS-Coronavirus	4x increase in indirect IF at 28 days	93%	?
	Indirect ELISA titre ≥ 1:10 at week 3	100%	?

CF = Complement fixation; MIF = microimmunofluorescence; IF: immunofluorescence; ELISA: enzyme-linked immunosorbent assay

e. Antigen studies

Antigen studies can provide a rapid diagnosis and is less affected by prior antibiotic treatment. However, confusion may arise sometime since it cannot distinguish live from dead pathogens, and clinician may misinterpret the result as treatment failure. Nowadays, two antigen tests are relatively widely studied and adopted, namely urine antigen tests for Legionella pneumophila type 1 and Streptococcus pneumoniae. The sensitivity of the urinary enzyme immunoassay (EIA) for Legionella pneumophila type 1 has been found to be 70% and specificity of almost 100% for community-acquired pneumonia. The urinary antigen test, a rapid immunochromatographic assay, for the diagnosis of bacteremic pneumococcal infections in hospitalized adult patients has a sensitivity of 87% and specificity of 97%.

f. Molecular studies

Nucleic acid amplification tests have been developed to offer rapid and accurate detection for those pathogens that are difficult to culture. They have excellent sensitivity and specificity but is costly, expertise-requiring and time-consuming. Two commercial nucleic acid amplification tests for direct detection of Mycobacterium tuberculosis from respiratory samples are available, the AMTDT (Gen-Probe Inc., San Diego, Calif.) and the Amplicor and COBAS (Roche Molecular, Branchburg, N.J.) tests. Optimal protocols for detection of other pathogens have yet to be established. Certain conditions have to be fulfilled and considered before the generation of meaningful results: optimum specimen type, internal inhibition control, analytical and clinical sensitivity and specificity, and reproducibility. RT-PCR for SARS-coronavirus RNA could be detected in serum with sensitivity 80% at day 1 & 42% at day 14. The sensitivity of RT-PCR in nasopharyngeal aspirate sample at 14 day is 68% & that of stool sample at 14 day is 97%. However, detection of SARS coronovirus RNA has to be confirmed by a second PCR assay by using a second aliquot of the specimen or a different set of primers (1). Molecular studies are also available for atypical pathogens in some reference laboratory for research purpose.

g. Invasive procedures

Invasive procedures used for CAP carry different degree of risks and usually include thoracocentesis, transtracheal aspiration, fiberoptic bronchoscopy, transthoracic needle aspiration, open lung biopsy and thoracoscopic lung biopsy. They are thus commonly reserved for patients with severe CAP, immunocompromised state, failed medical treatment and non-resolving pneumonia.

h. Utilization of diagnostic tests

The extent of diagnostic evaluation should depend on the severity of patients with CAP, patients' immune status and medical background and response to treatment. The cost, availability and the turnaround-time of the tests should also be considered. Based on these factors, a simple and general guideline for the use of diagnostic tests is recommended by Smith (8) and is illustrated in the following table (Table 2).

Table 2 -- Diagnostics tests for community-acquired pneumonia (8)

Patient Category	Test							
	Chest Radiograph	*CBC, Differential counts & blood Chemistries*	*Pulse Oximetry ABG*	*Blood Culture*	*Sputum Gram Stain and Culture*	*Urinary Legionella Antigen*	*Serologic Studies*	*Invasive Procedures*
Ambulatory	X	X						
Hospitalized	X	X	X	X				
Severe CAP	X	X	X	X	X	X	?	?
Immunocom-promised	X	X	X	X	X	X	?	?
Treatment failure	X	X	X	X	X	X	?	?

? = may be appropriate depending on clinical picture; CBC = complete blood count; ABG = arterial blood gases; CAP = community-acquired pneumonia.
X = recommended
Adopted from Smith PR. What diagnostic tests are needed for community-acquired pneumonia? Med Clin N Am 2001;85:1381–96.

Summary

Respiratory infections are the commonest infection with significant mortality and morbidity worldwide. The role of microbiological laboratory in the diagnosis of these infections remains controversial in general. Empirically treatment is usually recommended for acute pharyngitis, acute bronchitis and CAP not requiring hospitalization. For patients requiring hospitalization, appropriate diagnostic tests are suggested despite their limitations. For severely ill or immunocompromised patients, more extensive and invasive investigation may be considered.

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Practical diagnostic virology

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Viral infections are commonly encountered in the daily clinical practice, especially in the out-patient setting. In the majority of cases, clinical diagnosis is sufficient, and laboratory investigations are not required. Nevertheless, in a number of situations, the virology laboratory may yield additional information for management purposes. In order to ensure cost-effective use of the diagnostic virology service, it is useful to familiarize with various aspects of virology testing.

Indications for use of the virology laboratory service

There are three major indications for utilization of the virology laboratory service. The first is to obtain information for patient management. Antiviral therapy may be guided by laboratory confirmation of the aetiological agent especially when clinical presentation is non-specific, such as for human immunodeficiency virus (HIV) infection and diseases related to the herpes group of viruses in immunocompromised hosts. Procedures may be undertaken such as termination of pregnancy in primary rubella infection in the first trimester. The second indication is for public health and infection control purpose, where measures can be implemented on confirmation of the diagnosis. In a confirmed dengue infection, pest control measures are activated for vector control. In a food-borne outbreak of norovirus infection, the incriminated food needs to be identified, and infection control measures implemented to prevent further spread of the outbreak. Thirdly, the virology laboratory plays an important role in delineating epidemiological characteristics of viruses, in support of setting of health policies, such as unlinked anonymous HIV screening to determine disease burden, and immunity screening for measles in evaluation of the vaccination programme.

Choice of test and timing of specimen

Virology tests are broadly classified into three categories: direct detection, virus culture and serology.

Direct detection tests include those that could detect the whole virus or any of its components, mainly viral antigens or nucleic acids. The electron microscope could visualize viral particles directly. It is however insensitive for diagnosis in general. In the practical situation, only specimens with at least 10^6 viral particles per ml, such as rotavirus in diarrhoeal stool specimens and herpes group viruses in vesicular fluid specimens, could yield positive results. Regarding viral antigen detection, tests are available for a wider range of agents. For respiratory virus infections, direct detection of antigens of a panel of viruses in nasopharyngeal specimens could be performed. Rotavirus antigens could be detected in diarrhoeal stool. As for nucleic acid detection, the most commonly used method is polymerase chain reaction (PCR)-based assays. Examples include detection of norovirus RNA in diarrhoeal stool and herpes simplex virus DNA in the cerebrospinal fluid (CSF) of patientswith encephalitis. PCR-based assays are highly sensitive when appropriate specimens are tested, and the specificity is good in laboratories where precautions against cross-contamination are taken. Direct detection tests are useful for clinical specimens obtained early in the course of illness during the viral replication phase, and usually yield results within 1 to 2 days. They are particularly useful when a rapid result is required and where the virus is difficult to culture. Apart from diagnostic application, quantitative detection of viral nucleic acids, such as HIV RNA and hepatitis B virus (HBV) DNA, now forms part of the standard protocol for monitoring response to antiviral therapy.

Viruses are obligate intracellular agents, and unlike bacteria, could not be cultured in inanimate medium such as agar. Virus isolation is performed usually by inoculation of cell cultures. As in the case for direct detection tests, specimens for virus culture should be obtained in the early phase of the illness before viral shedding is limited by the host immune response. Virus culture may take 1 to 2 weeks to yield a positive result. On detection of cytopathic effects (CPE) in cell cultures, identification tests are performed to confirm the identity, and as appropriate, type and subtype of the agent. Despite the time required to yield a result, virus culture is still regarded as the gold standard in viral diagnosis due to its specific nature and the high sensitivity when appropriate specimens could be obtained. Nevertheless, not all viral agents are readily cultivable, such as diarrhoeal viruses including rotavirus and norovirus, such that other assays are employed for these infections.

The third category of tests is serology for virus antibody detection. This is an indirect method for viral diagnosis since it relies on the host immune response, and is often retrospective in nature due to the time required for the development of antibodies after acquisition of an infection. Nevertheless, serology tests are still clinically useful in certain situations. Detection of IgM antibodies usually signifies an acute infection. In some cases such as measles and dengue, IgM antibodies would not be detectable until 5 to 7 days after onset of infection. In other situations, IgM antibodies may already be present on clinical presentation, such as in hepatitis A and erythema infectiosum caused by parvovirus B19. Apart from IgM detection, acute infections could be diagnosed by "viral titre" determination. Such testing requires paired serum specimens at least 1 to 2 weeks apart to detect seroconversion or a 4-fold or greater increase in antibody titres. Technically, a variety of methods are employed, such as the complement fixation test (CFT), as appropriate for different viral agents. Due to the retrospective nature, determination of viral titres is often performed only for documentation of the diagnosis rather than for management purpose. In addition, serology results need to be interpreted with caution in patients who have received blood products or vaccination recently, since a false positive antibody response may occur in such cases. In chronic infections such as HIV and hepatitis C virus (HCV) carriage states, the presence of antibodies in a single serum specimen could confirm the diagnosis. Another application of serology testing is the determination of immunity to a viral agent, such as rubella IgG screening in pregnancy to determine the need for post-partum immunization.

Specimen collection and transport to the laboratory

For direct detection and virus isolation, specimens from the site of infection are required. For example, in respiratory infections, nasopharyngeal aspirates are preferred, since these could be processed to obtain a cell deposit for direct viral antigen detection, and have a higher sensitivity than swab specimens for direct detection tests and viral culture. In other occasions, additional specimens from other sites can be more easily available and provide the diagnosis. In patients with viral meningitis and myocarditis, where enterovirus infection is one of the important differential diagnoses, stool specimens should be obtained for viral culture. For direct detection tests, specimens should be placed in a clean container without preservatives, while culture specimens should be placed in viral transport medium to preserve the viability of the virus, although fluid specimens such as CSF or urine specimens could be sent in a plain sterile container. Specimens for serology testing should be placed in plain bottles without any preservatives or anticoagulants. All specimens should be properly labeled to match the information on the request form, packed and transported to the laboratory at 4°C within the same day. In some special situations, such as for HIV RNA load assay, the specimen needs to arrive at the laboratory within 6 hours of collection to ensure the integrity of the RNA.

Practical scenarios

A 2-year-old boy presented with fever and ulcers in the mouth for three days. Together with physical findings of vesicles on the hands and feet, a clinical diagnosis of hand, foot and mouth disease was made. Further history revealed that the 5-year-old sister had a similar but milder illness one week ago, as had a few other children in the same kindergarten. With the possibility of an outbreak of Enterovirus type 71 (EV 71) infection, a nasopharyngeal aspirate (NPA) and stool specimen were collected in viral transport medium in the out-patient setting, and the suspected outbreak was reported to the Department of Health. Infection control advice was given at the kindergarten, and NPA and stool specimens were obtained from symptomatic cases. After four days, an enterovirus was isolated from the NPA specimen of the boy. The isolate was confirmed to be EV 71 one day later, and the stool specimen also yielded EV 71 the next day. Since the patient presented early, viral cultures on upper respiratory and stool specimens are the appropriate tests. Reliable direct detection tests specific for EV 71 are not routinely available, and serology tests are retrospective and do not have a role in diagnosis of acute hand, foot and mouth disease.

A 25-year-old man presented with fever, severe headache and bone pains for two days. He recently came back from a 2-week stay in Indonesia, and the illness onset was on the day of return. Dengue fever was suspected and complete blood picture revealed platelet counts of 76 x 10^6/ml. On consultation with a Clinical Virologist, a blood sample was sent for virology investigations. The sample was negative for Dengue IgM antibodies, but positive for Dengue RNA by reverse transcription (RT)-PCR, yielding a Dengue type 4 result. The case was notified to the Department of Health for initiation of mosquito control actions. In acute dengue fever, IgM antibodies are reliably detected only at least 5 days after illness onset. Within the first week of the illness, RT-PCR on serum samples could confirm the diagnosis and yield serotype information. Viral culture is too time-consuming and cumbersome for routine diagnosis.

Virology consultation

Virology is a specialized and rapidly advancing field. With the emergence of novel diseases as exemplified by SARS and the development of new technologies, consultation with the clinical virologist would enhance various aspects of investigation and management of viral infections. Advice is given on the appropriate tests to perform in specific clinical situations, optimum collection and transport of specimens, and interpretation of results for patient management and further testing. Interpretation of virology results requires the consideration of the clinical presentation, specimen type and time of collection, testing method, subsequent clinical course and any therapeutic intervention. Regular communication with the clinical virologist will ensure the cost-effective use of laboratory resources to maximize patient benefit.

Update on laboratory investigations for syphilis

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Reported incidences of syphilis depend on disease transmission within given populations, and the extent to which interactions between available health services and these populations limit disease transmission (1, 2). But the extent to which available statistics reflect the incidence of syphilis also depends on case- finding efforts, and variations in social factors that affect interaction between infected individuals and health services (3).

Because of the protean clinical manifestations of different stages of syphilis, laboratory tests remain the cornerstone in the diagnosis. A combination of non-treponemal and treponemal serological tests is essential for confirming or defining all stages of syphilis.

Three major areas of recent development in the laboratory investigations for syphilis are important from the public health perspective: firstly, use of enzyme immunoassay (EIA) as a population- based screening tool for disease control; secondly, molecular tools for detection and subtyping of Treponema pallidum; and thirdly, laboratory quality assurance activities to safeguard accuracy of test results.

Because of intrinsic properties of the T. pallidum spirochaete which preclude traditional culture detection, antibody detection has been the only effective method for confirmation of diagnosis for many years. This can be done by either using non-treponemal tests, viz: Venereal Disease Reference Laboratory (VDRL), rapid plasma reagin (RPR); or treponemal tests, viz: Treponema pallidum haemagglutination assay (TPHA), Treponema pallidum particle agglutination (TPPA), and the fluorescent treponemal antibody absorption (FTA-ABS) test. In the last decade, many laboratories have moved to screening for syphilis with a specific treponemal test which eliminates the problems of prozone, biological false positives, and low sensitivity in latent syphilis associated with screening with a non- treponemal test.

In a study done in Public Health Laboratory Centre using EIA as primary screening tool for mothers attending antenatal clinics, it was found that out of 4595 consecutive sera obtained from antenatal clinics over a 3-month period, VDRL detected 17 out of 50 syphilis cases (sensitivity 34%, positive predictive value 89.5%), while 2 cases out of 4595 were false positives (specificity 99.9%, negative predictive value, 99.3%). On the other hand, EIA detected all 50 (sensitivity 100%, positive predictive value 80.6%), while 12 were probable false positives (specificity 99.7%, negative predictive value 100%). Because of the significantly improved sensitivity of EIA in our local setting, it was decided that EIA be used as screening for all antenatal