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Nucleic Acid Amplification Test In Pulmonary Diseases

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ABSTRACT

Nuclei as a cell nucleus have been found since the 18th century. This discovery was then developed for the need for methods for diagnosing pathogenic organisms of an infectious disease at that time. The aim of this study is to review the principle of nucleic acid amplification test and describe the existing evidence regarding the role of the test in respiratory diseases. The study use the keywords, such as nucleic acid, diagonsis and nucleic acid amplification test, to invesitage relevant studies in PubMed and Google Scholar database. We found that nucleic acid-based testing techniques have evolved to adapt to the need for early and rapid detection and rely on a high level of accuracy. This continuously developed technology can replace conventional test methods. Conventional examination is known to take longer time so the definitive therapeutic might be delayed. However, there is still an obstacle, namely the cost of tests quite expensive because of the standard procedure that must be strictly followed, otherwise result might not be valid. The technique from the beginning needs to be carried out following the procedure, starting with the preparation of tools, how to take samples, how to deliver the samples to the laboratory, and how to process and analyze data. This procedure is necessary to minimize false positive or negative results. As a conclusion, nucleic acid assays is beneficial in pulmonary infectious diseases diagnosis, however it is high-cost and not recommended for treatment evaluation in pulmonary infection.

INTRODUCTION

Friedrich Miescher identified the nucleic acids in the year of 1868 using an isolation technique from the leucocytes. The molecules were later known as nuclein. A nucleic acid is a macromolecule made up of many smaller molecular units. These small units called nucleotides are chemically linked together in a chain (Csako, 2006). Deoxyribonucleic acid (DNA) is a molecular unit consist of four types of small size molecules described as nucleotide bases, namely cytosine (C), adenine (A), thymine (T), and guanine (G).

DNA polymerase is an enzyme capable of building new copies of DNA in the shape of nucleic acid molecules. Scientists have utilized DNA polymerase through a Polymerase Chain Reaction (PCR) assay (Stellato et al., 2008).

POLYMERASE CHAIN REACTION (PCR)

The beginning of the development of PCR examination was the discovery of thermostable polymerized DNA extracted from a bacterium that grows in hot springs called *Thermus aquaticus*.

This polymerase is resistant to the heating and cooling required in PCR assays and allows amplification. PCR examination requires four main components. They are nucleotide triphosphate, DNA-polymerase that is stable in various temperature, gene-specific primers, and the target DNA for amplification. The source DNA is obtained by isolating genomic DNA from the cell samples and tissues or performing ribonucleic acid (RNA) materials reverse transcription (Jocefczuk et al., 2011).

The first step of PCR examination is separating the double strands (dsDNA) by incrasing the temperature of specimen material to 900 C. The second is making base-specific annealing from primers to complementary DNA strands by cooling. The third is heating the mixed sample material until it reaches a temperature of 720 C. This temperature is the best temperature for Taq DNA polymerase process (Wang et al., 2019).

Based on DNA amplification and manipulation, PCR assays extend molecular biology investigations. The desired genome is then subject to amplification from genomic DNA and incorporated in plasmids or other vectors for further study. PCR assay is known to have a role in diagnosing diseases. PCR enables gene mutations identification by genome sequencing. Thus, it helps to recognize diseases such as cancer (Pavsic et al., 2015).

THE ROLE OF NUCLEIC ACID-BASED EXAMINATIONS FOR TUBERCULOSIS DIAGNOSIS

The rapid molecular test for Mycobacterium tuberculosis detection is a molecular examination using the semi-quantitative Real-Time PCR Assay (RT-PCR) principles with the target of the rpoB gene. This assay processes the rpoB gene material by DNA automatic extraction in single-use bullets. The assay technique is using repeated-amplification of the target DNA and fluorimetric detection (Kurniawan et al., 2016).

Van Rie A et al (2013) examined suspected tuberculosis with negative Acid-fast bacilli (AFB). While the sensitivity and specificity of AFB staining were 27% and 99%, the RT-PCR method revealed a 67% sensitivity and 99% specificity, respectively. Moreover, AFB also has an advantage in the faster results (in 2 hours), thus allowing to start the therapy on the same day or the next day (Pavsic et al., 2015).

Examination with the RT-PCR method has a high enough sensitivity value. It can be an early detection tool to capture patients suffering from pulmonary tuberculosis. Meanwhile, a high specificity value can determine whether a person has pulmonary tuberculosis or not. The RT-PCR method can be used as an early detection tool and as a determinant of the diagnosis of pulmonary tuberculosis, allowing immediate therapy (Khan et al., 2022).

There was a study getting one sputum specimen with a positive result of the GeneXpert *Mycobacterium tuberculosis* (MTB) RT-PCR method, but the result of Lowenstein Jensen culture was MTB negative (true negative). It is probably because the GeneXpert RT-PCR method can detect DNA of dead MTB bacteria in sputum specimens, causing positive MTB detection results. In sputum culture, bacteria did not grow maybe because the number of living MTB bacteria in the sputum was less than 50-100 bacteria/ml sputum (He et al, 2024).

The United States Food and Drug Administration of America approved the two rapid diagnostic tests in the midle of 1990s, they are the direct MTB amplification using a Gen-Probe and MTB nucleic acid amplification examination in patients with positive smear resu 7|Journal Of Health Science the method to ive AFB sputum results (Laraque et al., 2009). In Indonesia, nucleic acid amplication was started to be routinely performed in 2014.

In a medium-sized study, nucleic acid amplification assays used other than airways specimens, such as pleura, lymph nodes, bone marrow, peritoneum, and synovial fluid for MTB rapid diagnosing. The sensitivity is quite good, namely 64 - 68%, specificity 97 - 100%, negative predictive value 87.5 - 99.2%, and positive predictive value 81.5 - 100%. A study shows a lower sensitivity rate of pleural biopsy

material, namely 52.6% (Laraque et al., 2009). Hughes Ralphs et al (2011) conducted a study on the cost-effectiveness of the MTB RT-PCR examination in establishing the diagnosis of tuberculosis. The study concluded that RT-PCR examination of MTB in high prevalence regions would be more useful in target populations with a higher likelihood of tuberculosis.

THE ROLE OF NUCLEIC ACID-BASED EXAMINATIONS FOR THE DIAGNOSIS OF LUNG MALIGNANCY

Molecular examination of lung cancer has become the standard. Thoracic oncologists today faced other difficulities regarding how to choose examination materials from tissue or fluid biopsies (Abdayem et al., 2021). The term liquid biopsy reflects to the examination of circulating tumor DNA within the plasma (ctDNA). The liquid biopsy also means include the circulating cell-free RNA (cfRNA), the circulating cell-free DNA (cfDNA), the circulating extracellular tumor proteins, vesicles, platelets, and metabolites (Liu et al., 2020).

After cell death or apoptosis, cfDNA is released into the bloodstream. The concentration of cfDNA within plasma is correlated positively to the numbers of cfDNA detached by the tumor and indirectly proportional to the DNA elimination rate through renal clearance. cfDNA assays in brain metastases patients with have low level sensitivity (Rossi et al., 2013).

THE ROLE OF NUCLEIC ACID-BASED EXAMINATIONS FOR THE LUNG MYCOSIS DIAGNOSIS

Aspergillosis diagnosis is a challenge because current methods have poor specificity and sensitivity and require a long time. Specimen culture and histopathological examination of sterile airway specimens are the standards for establishing the diagnosis (Lass-Florl, 2019). Nucleic acid-based assays such as PCR targeting fungal DNA showed better sensitivity and specificity, especially if the culture results were negative (Table 1) (Buchheidt et al., 2017).

Table 1. Various of molecular examination and antigen in invasive aspergillosis

Methods	Indications	Benefits	Limitations
Galactomannan (GM)	Early detection. Two serum specimens every week, positivity index more than 0,5. Bronchoalveolar [illegible]AL), positivity [illegible]e 0,5-1	High risk patient detection. Adult and child consistent neutropenia > 1 is sign of theraphy failure.	No non-neutropenia patient data available. Initial antifungal medication lowered the sensitivity.
Lateral device flow assay (LFA)	Immunochromatographic identification using dipstick assay	Same sensitivity and specificity with GM. Requiring shorter process in the examination than the GM with serum specimen.	Not yet available commercially and hard to interpret dipstick assay in several occasions.
PCR	Immunocompromise patient DNA detection. Specimen sample: blood, BAL, and tissue	Rapid early detection. High sensitivity, and high negative predictive value	More expensive. Fungi DNA extraction technique is hard and collected from complex specimens

From: Lass-Florl C. How to make a fast diagnosis in invasive aspergillosis. Med Mycol. 2019;57:S155-S160.

THE ROLE OF NUCLEIC ACID-BASED EXAMINATIONS FOR VIRUS DIAGNOSIS

Viral culture examination is the standard and still important in diagnosing viral lung diseases. Related to the development of molecular-based technology, this examination shows many advantages compared to culture examination. The biggest advantage is the shorter inspection time until the results come out. The doctor can start therapy within two to 24 hours of the examined specimen. Another advantage is good sensitivity and specificity. Some essays are very sensitive that they need only less number of tested specimen for nucleic acids. Some viruses, such as Influenza and Respiratory Syncytial Virus, can be detected after clinical signs and symptoms improve (Beck et al., 2010).

Ussualy there are several main phases to the inspection process. The first step is extraction of nucleic acid to obtained genetic molecules from the host cells and the microorganism cells. The second step is amplification to amplify some parts of the pathogen genome. The third step is to detect the genetic material that has been amplified on the cartridge to determine a positive or negative result (Zhang et al., 2020).

THE ROLE OF NUCLEIC ACID-BASED EXAMINATIONS FOR BACTERIAL DIAGNOSIS

Streptococcus pneumoniae is higly prevalent etiology of community acquired lung parenchymal infection in children and adults. However, definitive level of diagnosis is a challenge because the gold-standard culture of sputum specimens for potential colonization of oropharyngeal bacteria and blood cultures are less sensitive (Murdoch, 2003). Antigen tests using urine specimens show high but low sensitivity in children. Several articles conclude that PCR testing is not recommended hence the high false-positive rate (Mothershed, et al., 2006).

Mycoplasma pneumoniae bacteria are difficult to culture and take a long time. Therefore, finding these bacteria is often based on serological tests and IgM antibodies with better sensitivity and specificity than serological tests. Study suggests a positive association between serological tests and PCR results. Nasopharyngeal, oropharynx, and lower airway specimens show good sensitivity (Mothershed, et al., 2006).

Legionella bacteria can infect the lower respiratory tract and cause community pneumonia. Several studies on the role of PCR in establishing a diagnosis using sputum specimens have shown better sensitivity than sputum culture. But the difficulty is that most sufferers experience dry cough symptoms. Examination of urine specimens can be a supporting examination of urine antigens (Murdoch, 2003).

Chlamydia pneumoniae can be cultured in special media but requires a long time and low sensitivity. Most of the findings of these bacteria depend on serological examination using microimmunofluorescence. Serological testing requires the convalescent and acute and serum specimens. This explain why the diagnosis can only be established retrospectively. PCR examination showed lower sensitivity than specimen culture and the specificity value was hard to determine because there

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were few standard standards for comparison (Murdoch, 2003).

Table 2. PCR assay in bacteria causing pneumonia

Pathogens	Gene Targets	Specimen Samples	Results
Streptococcus pneumoniae	Autolysin, pneumolysin	Nasopharynx, sputum, urine, serum, plasma, white blood cells, TNA	Sensitivity is varied at blood specimen
Mycoplasma pneumoniae	ATPase operon, P1 adhesion, 16S rRNA	Sputum, nasopharynx, TNA, BAL	Sensitivity is better than the specimen culture, nasopharynx is more preferred specimen sample
Legionella	16S rRNA, 5S rRNA, *mip*	Serum, sputum, ETA, BAL, nasopharynx, serum, white blood cells, urine	Same sensitivity as the lower respiratory track specimen culture
Chlamydia pneumoniae	MOMP, fragmen clone Pst, 16S-23S spacer rRNA, 16S rRNA, 60 kDA protein, 53 kDA protein,	Nasopharynx, BAL, oropharyngeal swab, oropharynx, sputum	Better sensitivity than the specimen culture
Chlamydia psittaci	MOMP, 16S rRNA, *gseA*	Sputum, nasopharynx, blood, lung tissue	Not evaluated
Pneumocystis carinii	Mitochondria rRNA, 5S rRNA, 18S rRNA, dihyrofolate reductase, thmidylate sintase, MSG	Lung tissue, sputum, nasopharynx, BAL, oropharynx, ETA, blood, serum	Useful for *P.carinii* pneumonia suspicion with negative cytology examination result

From: Murdoch DR. Nucleic acid amplification tests for the diagnosis of pneumonia. Clin Infect Dis. 2003;36(9):1162-70.

The standard method of *Pneumocystis carinii* pneumonia (PCP) diagnosis is staining of cells in bronchoalveolar lavage (BAL) specimens or sputum specimens by immunofluorescence staining, such as Toluidine blue, Methenamine silver, or Giemsa (Duan et al., 2022). Some studies showed that PCR provided better sensitivity and specificity than cytology. Establishing a diagnosis is not a problem like other causes of pneumonia because the timing of both cytology and PCR is almost the same (Chotiprasitsakul et al., 2020). PCR may be preferable to testing sputum specimens in individuales with a significant clinical risk of a history of *Human Immunodeficiency Virus* (HIV). PCR assay in bacterial pneumonia are described in Table 2.

CONCLUSION

Nucleic acid-based testing may be an option for specific diseases and infections. It plays a significant role in lung cancer. This examination helps provide the best treatment options that enhance the quality improvement and survival rates. Nucleic acid assays in pulmonary infectious diseases benefit in accuracy and time required to obtain results. It is advantageous for the patient to receive definitive therapy. However, it also has several limitations. Nucleic acid-based testing is classified as a high-cost assay since advanced technology is involved. Also, using the essay as a treatment evaluation is not recommended.

Conflict of interest declaration

The authors have no conflict of interest.

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