

# NOVEJŠE DIAGNOSTIČNE METODE V VETERINARSKEM LABORATORIJU

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Sodobni diagnostični in raziskovalni laboratoriji imajo na voljo opremo in znanje za izvajanje klasičnih metod v kombinaciji z molekularnimi in serološkimi ter nekaterimi drugimi metodami. Povečevanje števila vzorcev in krajšanje razpoložljivega časa od prejema vzorca do diagnoze ter hiter tehnološki razvoj stalno sili laboratorije k nadgrajevanju in optimizaciji postopkov. Med novejšimi diagnostičnimi metodami se najhitreje razvijajo molekularne metode, ki temeljijo na izolaciji nukleinskih kislin in pomnoževanju tarčnega odseka. V primerjavi s klasičnimi so molekularne metode običajno hitrejše, predvsem pa omogočajo analizo tudi tistih mikrobov, ki jih v laboratorijskih pogojih ne moremo gojiti oz. rastejo zelo počasi. Med molekularnimi metodami je najbolj uveljavljena metoda PCR in njene različice, npr. digitalni PCR kot ena zadnjih, ter sekvenciranje. Po prvi generaciji sekvenciranja po Sangerju se v zadnjem desetletju vse bolj uveljavlja sekvenciranje naslednje generacije (NGS), ki ponuja najbolj celovite diagnostične in raziskovalne odgovore. Metoda NGS postaja vedno bolj cenovno dostopna in se lahko izvaja tudi v okviru posebej specializiranih laboratorijev, seveda ob ustrezni računalniški podpori. Pri masovnem paralelnem sekvenciranju namreč pridobimo velike količine podatkov o odčitanih nukleotidnih zaporedjih, ki imajo uporabno vrednost šele po ustrezni analizi s specializiranimi orodji bioinformatike. Večina teh programov se poganja v operacijskem sistemu Linux, na voljo pa so različni komercialni paketi, ki jih zaznamuje raznolikost, potreba po predznanju uporabnikov in nezanemarljiv finančni vložek. Med novejšimi metodami v rutinsko uporabo prihaja tudi masna spektrometrija MALDI-TOF, ki temelji na analizi celičnih proteinov in je cenovno dostopna metoda za hitro in rutinsko identifikacijo bakterijskih izolatov v kliničnih mikrobioloških laboratorijih. Uveljavljajo se tudi metode, ki omogočajo hkratno analizo velikega števila izraženih lastnosti. Med te uvrščamo fenotipske mikromreže, ki so v osnovi avtomatizirani biokemijski testi na mikrotitrskih ploščah za sledenje skoraj 2000 različnih fenotipov mikrobnih celic, ter avtomatizirane multiparametrične serološke teste. Vpeljava najnovejših metod laboratoriju zagotavlja konkurenčnost na trgu ter hitrejše pridobivanje najbolj zanesljivih in ultimativnih odgovorov na diagnostična ali raziskovalna vprašanja.

Ključne besede: molekularne metode; sekvenciranje naslednje generacije (NGS); masna spektrometrija MALDI-TOF

## New diagnostic methods in veterinary laboratories

Modern diagnostic and research laboratories have the equipment and expertise to perform analyses by traditional methods in combination with molecular and serological methods, in addition to some others, that are forced to upgrading and optimization due

to the increasing number of samples, high time-to-result demand and rapid technological development. Molecular methods, based on the isolation of nucleic acids, are most rapidly developing among the modern diagnostic methods. In comparison with conventional methods, molecular methods are faster and, most importantly, enable also the analysis of microbes that cannot grow under laboratory conditions or grow very slowly. Among molecular methods, PCR with its variants, like digital PCR as one of the latest, and sequencing are the most commonly used. After sequencing of the first-generation (Sanger), the next-generation sequencing (NGS) has been gaining popularity in the last decade as offering the most comprehensive diagnostic and research answers. NGS is becoming more affordable and can be implemented also in smaller laboratories, with appropriate information-technology support. Namely, with the massive parallel sequencing a large number of data is collected, having a practical value only after proper analyses with the specialized bioinformatic tools. Most of these programs are powered by the Linux operating system and various commercial packages are available, characterized by their versatility, need for user-knowledge and considerable financial investment. Among the newer methods, the MALDI-TOF mass spectrometry, based on the analysis of cell proteins, is also coming into the routine use. It is a method that is affordable for rapid and routine identification of bacterial isolates in clinical microbiological laboratories. In addition, methods that enable simultaneous analysis of a large number of expressed characteristics are also being promoted. These include the phenotypic microarrays, which are basically the automated biochemical tests performed in microtiter plates for tracking nearly 2000 different phenotypes of microbial cells, and the automated multiparametric immunoassay systems. The introduction of the latest laboratory methods ensures competitiveness to the laboratory at the market and more rapid acquisition of the most reliable and ultimate answers to the diagnostic or research questions.

Key words: molecular methods; next-generation sequencing (NGS); MALDI-TOF mass spectrometry