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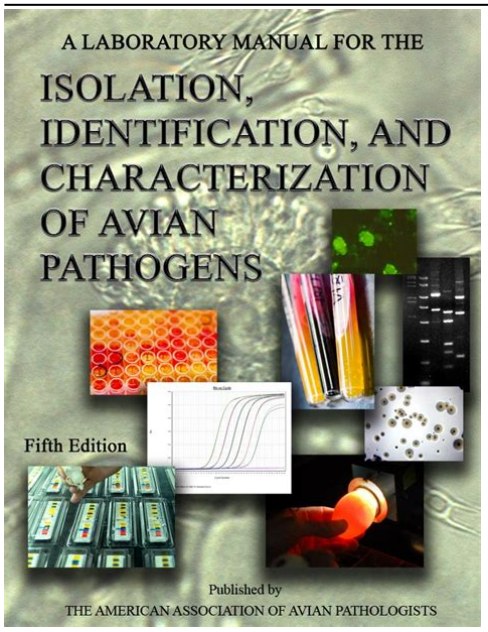

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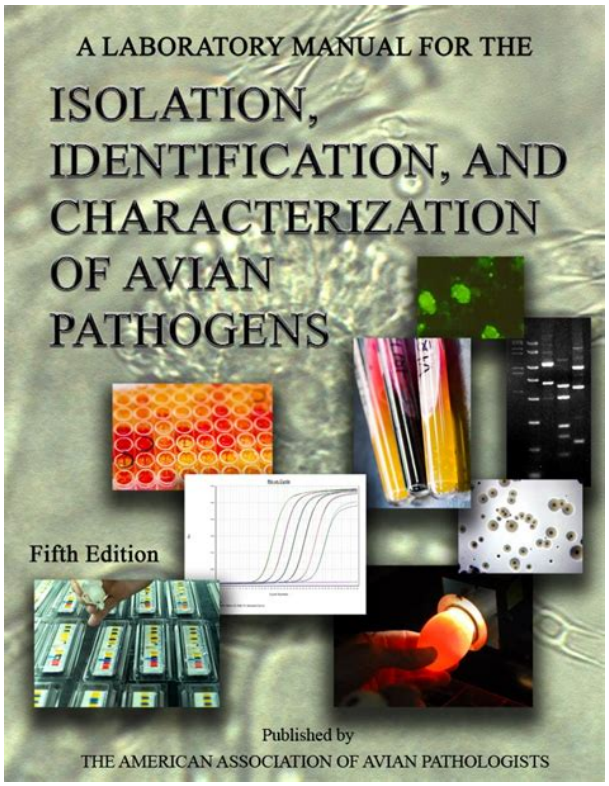

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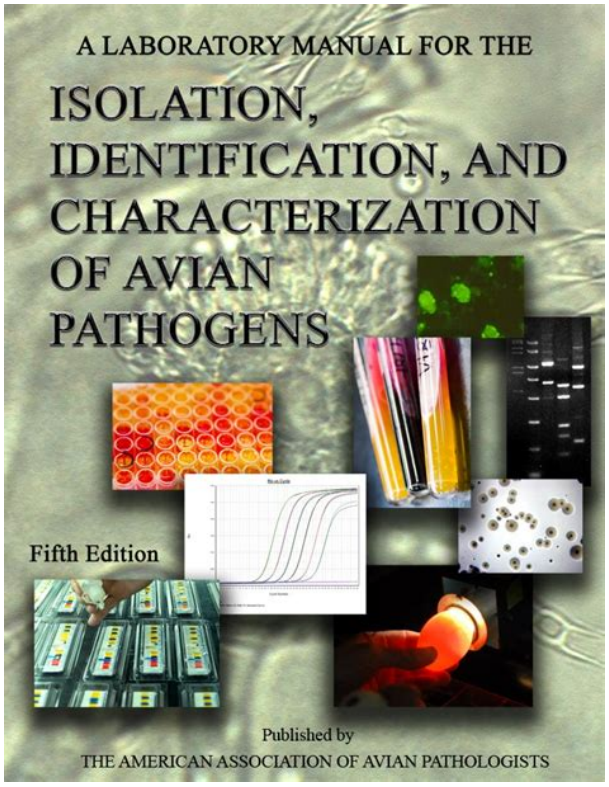

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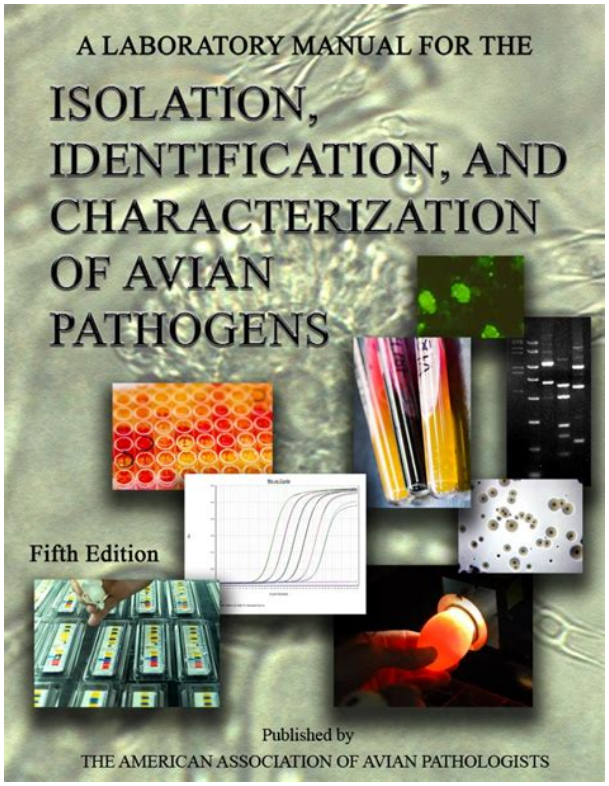

I understand it is a method of active vaccination but I dont understand how it can now be used as a vaccine. Is it the same process used if the viruss DNA is being used. View How can I prepare 10% buffered formalin or Trump's fixative for polyclads fixation. Question 5 answers Asked 3rd Feb, 2017 Alejandro Catala Jimenez I want to know how to prepare those chemicals for fixation of tropical polyclads View What is the best way to check the specificity of a primer pair for PCR amplification of a target sequence. Question 11 answers Asked 9th Nov, 2014 Fizza Akhter Can NCBI blast highlight the specificity of a primer pair for PCR amplification of a desired sequence. What would be the best possible ways to check the primer specificity. View Is there any procedure to store PCR products. Question 29 answers Asked 31st Aug, 2013 Kavin Soni I am planning to do PCR for 100 different seed varieties so it is not possible for me to check PCR products on agarose gel in short period of time so I am seeking protocols to store pcr products. View What is the difference between Emerging Sources Citation Index and Science Citation Index. Discussion 9 replies Asked 14th May, 2020 Saroj Rai What is the difference between Emerging Sources Citation Index ESCI and Science Citation Index SCI. Does ESCI also have an Impact Factor like SCI. View Genetic diversity AFLP Discussion 5 replies Asked 13th May, 2020 Marjan Sadat Hosseini How can I explain it If genetic diversity within population is high 48% but the gene flow is very low 0.229 What is the reason.Thanks a lot in advances. View Is there any quantitative study on Biodiversity restoration during COVID19 lockdown. Question 8 answers Asked 24th Apr, 2020 Abhijit Mitra Today the greatest threat on the planet Earth is the invasion by the tiny particle causing Corona Virus Disease COVID19.

Most people infected with the COVID19 virus usually experience mild to moderate respiratory illness and recover without requiring special and very advanced treatment. Older people and those having medical problems like cardiovascular disease, diabetes, chronic respiratory disease and cancer are more likely to develop serious illness. The best way to prevent and slow down transmission is to be well informed about the COVID19 virus, the disease it causes and how it spreads. One of the best practices to stay safe from infection is by washing hands or using an alcohol based rub sanitizer frequently without touching the face. The COVID19 virus spreads primarily through droplets of saliva or discharge from the nose when an infected person coughs or sneezes, so it is important to practice respiratory etiquette for example, by coughing into a flexed elbow. View Related Publications Fishes A Field and Laboratory Manual on Their Structure, Identification, and Natural

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Mg was recovered from wet charcoal swabs sent through the mail but not from dry or wet plain swabs. Previous article in issue Next article in issue Keywords Mycoplasma gallisepticum Chicken Diagnosis Isolation Recommended articles Citing articles 0 1 Present address ASEAN Poultry Disease Research and Training Centre, Veterinary Research Institute, 59 Jalan Sultan Azlan Shah, 31400 Ipoh, Perak, Malaysia. Citing articles Article Metrics View article metrics About ScienceDirect Remote access Shopping cart Advertise Contact and support Terms and conditions Privacy policy We use cookies to help provide and enhance our service and tailor content and ads. By continuing you agree to the use of cookies. In the present study brain samples were collected from twelve pigeon flocks exhibiting signs of nervousness and diarrhea in a live bird market LVM in Egypt. All collected samples were positive for NDV and negative for avian influenza virus and infectious bronchitis virus. Seven positive samples were subjected for genotypic characterization based upon partial F gene sequencing. However, phylogenetic analysis revealed clustering of all tested strains within subgenotype VIg. Three isolates were assessed for pathogenicity based upon the mean death time MDT, the intracerebral pathogenicity index ICPI and the intravenous pathogenicity index IVPI in chicken. The ICPI and MDT revealed that all tested isolates were of moderate virulence Mesogenic in chickens. Mature chickens showed no clinical signs or death as assessed by IVPI. Pigeon in Egypt reared in free rang system and sold in LBM mostly for restocking, that with extensive infection with PPMV represent a potential threat to chickens. Therefore, strict biosecurity measures and control measures at live bird markets, alongside the development of a vaccine may be required to reduce the risk of PPMV1 outbreaks.

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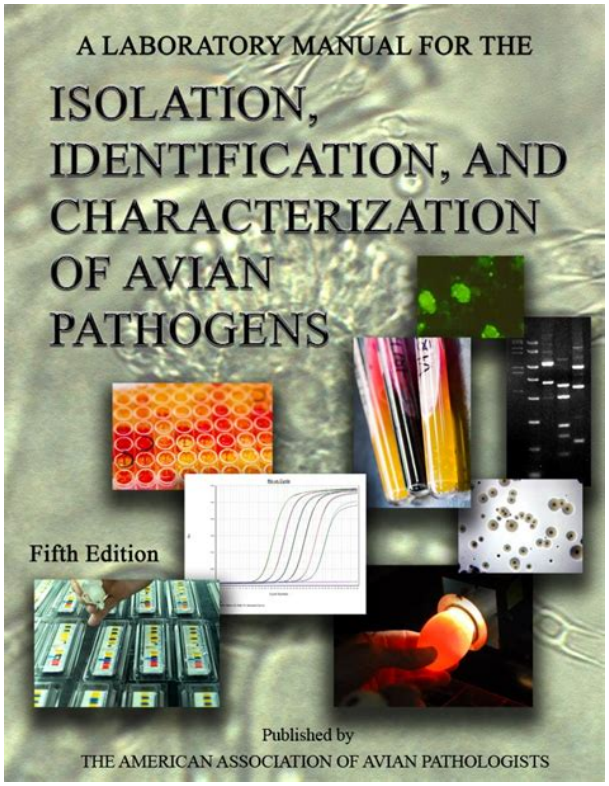
NDV is an enveloped, nonsegmented, negative sense, single stranded RNA virus of approximately 15,186 nucleotides that encodes six structural proteins nucleoprotein NP, phosphoprotein P, matrix M, fusion F, hemagglutinin neuraminidase HN, and the RNA polymerase L Lamb, 1996 . The M, F and HN proteins associated with the viral envelope mediate the entry and release of the NDV.However, passaging of the virus in in chickens has resulted in an increase in virulence and replication based upon ICPI values.In the present study, samples were collected from live pigeon markets in Egypt from pigeons with nervous manifestations and with whitish green diarrhea. All virus isolation and animal experiments were performed in virology unit and biological experiments and research unit at RLQP under strict control biosafety level 3 facilities. The brain was removed under aseptic conditions and placed in viral transport medium VTM and transferred to the laboratory for further investigation. Brains samples were homogenized with in equal volume of phosphate buffer saline. The homogenate was vortexed and centrifuged at 1260xg for 30 min.Genetic material was amplified using Quanti Tect probe RTPCR kit Qiagen, primers and thermal profile as previously described by Wise Suarez et al., 2004 to detect NDV. Furthermore, the extracted RNA was also screened using real time RTPCR for presence of avian influenza AI H5, H7, H9, and avian corona virus using primers and thermal profiles as previously described Slomka Pavlidis et al., 2007 ; Shabat Meir et al., 2010 , Callison Jackwood et al., 2001 . The allantoic fluid was tested for haemagglutination activity by heamagglutination HA and heamagglutination Inhibition HI tests using four HA units according to reference procedure OIE, 2012 . The egg infective dose 50 EID50 was calculated according to Reed and Muench, 1938 . The virulence of three selected isolates Egy.



PPMV1,8 and10 was evaluated using MDT in 9day old embryonated chicken eggs and ICPI in one day old SPF chicks in accordance with reference procedures OIE, 2012 . The cycling conditions were 45. C for 20 min at RT, 95. C for 2 min and 30 cycles of 95. Cfor 30 Sec., 52 C for 45Sec., and 72 C for 45 Sec., followed by 72 C for 10min. PCR products were purified using the QIA quick gel extraction kit Qiagen, USA, according to the manufacturer's instructions. The purified PCR products were sequenced by Applied Bio systems 3130 genetic analyzer Hitachi, Japan u sing Big dye Terminator V3.1 cycle sequencing chemistry PerkinElmer, Foster city, CA, USA according to the manufacturer's instructions. BioEdit v7.0 Hall, 1999 was used for the alignment of nucleotide and predicted amino acid sequences. Sequences representing different NDV genotypes and sub genotype VI were retrieved from the GenBank database and aligned with strains of the present study.Nervous signs and diarrhea were observed in the majority of cases. Septicemia and congested brain were the most frequently observed gross lesions. No correlation between vaccination status and incidence of the disease has been observed. Based upon real time RTPCR results, all collected samples were positive for virulent NDV and negative for AIVH5, H7, H9 and Avian corona virus. Three isolates were selected for further analyses of pathogenicity. The ICPI values of the selected isolates PPMV1, 8 and10 were 1.41, 1.48 and 1.44 respectively and the MDT was 69, 64 and 67 hours respectively. No overt clinical signs were recorded in any of the 10 chickens for each virus throughout 10 days of observation, indicating zero IVPI. These indices revealed that the tested ND viruses were of moderate virulence Mesogenic in chickens Swayne, 1998 . All sequenced viruses bear a multi basic amino acid KRQKR cleavage site at position 112 to 116 and a phenylalanine residue at position 117 Table 1 .

This motif is one of the characteristic motifs of velogenic isolates Liu Wang et al., 2006 . Furthermore, Phylogenetic analysis of the seven viruses, along with sixty nine strains representing different genotype VI NDVs revealed that all tested viruses clustered within the genotype VIg clade Figure 2 . In a previous study, samples from pigeons with nervous manifestations and diarrhea were collected from El Sharkia governorate during 2013 to 2015 and tested for the presence of avian influenza and NDV, in turn confirming the cocirculation of paramyxo and influenza viruses in pigeons Mansour Mohamed et al., 2017 . Isolates of the same genotype have previously been recovered from oropharyngeal and cloacal swabs collected from healthy pigeons in Egypt Sabra

Dimitrov et al., 2017 . Although the viruses are of moderate virulence as assessed using IVPI and MDT they initially caused no clinical signs in chickens, the virulence gradually increased upon passage in chickens. As a result, the potential of PPMV1 to induce losses among chickens must not be underestimated and the virus must be controlled within the pigeon population via improved vaccination and biosecurity measures. Ahmed Samy designed the study, performed the methodology, evaluated the results and wrote the manuscript. Ahmed Abd El Halim performed PCR confirmation. All author read and approved the final version of the manuscript for submission. Transboundary emerging dis. 612 134139. Avian Pathol. 153 487493. Nucleic acids symp. Ser. Avian Pathol. 321 8193. Slovenian Vet. Res. 473. Fields virol. Avian Pathol. 464 367375. Available online at. What are these Abul Hashem, Mohammad Eliyas and A.K.M. Mostafa Anower ResearchersLinks is a member of CrossRef, CrossMark, iThenticate. The simultaneous dotimmunobinding assay gave reproducible results and allowed considerable savings on the cost of reagents compared to liquid ELISA.

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0 resulted in bestmatch identifications that were still concordant at species level with the molecular method used for previous identification. Therefore, MALDITOF MS is a promising tool for reliable identification of avian Mycoplasma spp. Keywords avian mycoplasma, birds, MALDITOF MS, mass spectrometry Mycoplasmas, bacteria belonging to class Mollicutes, are a part of the commensal microbial flora of many avian species, but some of the 27 avian Mycoplasm a species are important pathogens in veterinary medicine. 6 These pathogenic species can cause high economic losses in poultry production. 6 Wild birds can also be affected. 5, 17 Given that mixed infections with Mycoplasm a spp.Hence, these tools can be used to detect pathogenic Mycoplasma spp.We investigated MALDITOF MS for the differentiation and identification of avian mycoplasmas to the species level by creating and evaluating an avian mycoplasma spectral database. We constructed the database from 36 main spectrum profiles MSPs from 23 type and reference strains, 1 live vaccine strain, and 8 clinical isolates of 23 avian Mycoplasma spp., previously identified by molecular

methods Table 1 . Apart from one M. gallisepticum MG live vaccine strain, all strains and isolates used for MSP creation were identified by genusspecific PCR and sequencing LGC Genomics, Berlin, Germany. 18 The MG live vaccine strain was identified by speciesspecific PCR and restriction enzyme analysis as well as by sequencing of the PCR amplicon. 13 The 112 clinical isolates analyzed after the database construction were collected from 10 different avian species Table 2 . Single colony subcultures were performed 3 times to rule out mixed cultures for all 117 isolates. View larger version Table 2. Number and originating bird species of the isolates of Mycoplasma spp.If colony growth on the agar plate was detected, MALDITOF MS analysis was performed on the liquid medium.

In case of discordant results between the previous molecular identification and MALDITOF MS, the isolates were again examined by genusspecific PCR targeting the 16S rRNA gene and the 1623S rRNA intergenic transcribed spacer region IRS and sequencing of the PCR amplicons. 16, 18 The incubated liquid medium was centrifuged to obtain a Mycoplasma pellet. Although 1mL of liquid medium was used for the database construction, 1mL or 2.5mL of liquid medium was used for the MALDITOF MS analysis of the clinical isolates depending on their growth capacities. The liquid medium was centrifuged 8,600.After centrifugation 17,000.For MSP creation, 8 spots per isolate were prepared. The protein extracts of the clinical isolates were spotted twice or, in case of visual nonhomogeneity, up to 4 times. The data were analyzed in the automatic mode Biotyper v.3.1 and compared to the MALDI Biotyper MSP database and the database generated in our study. Spectra were internally calibrated using a bacterial test standard part 255343; Bruker Daltonics. The Mycoplasma MSP database was constructed by assigning the reference peak lists to different reference strains. Three mass spectrum measurements of 8 different spots of protein extract for each strain were obtained. Quality control of the raw mass spectra was performed Flex analysis v.3.4; Bruker Daltonics and included a check for absence of flatline spectra, intrusive peaks, and low matrix background signal. After smoothing, baseline correction, and peak picking, at least 20 spectra were selected for MSP creation. The automated MSP creation functionality of the MALDI Biotyper software calculated a MSP with information about mean peak masses, peak intensities, and peak frequencies. For MALDITOF MS analysis of the isolates, each spot was measured once. Spectra were classified by matching MSPs MALDI Biotyper realtime classification software; Bruker Daltonics with the database mentioned previously.

For 3 of 117 3% isolates, the result was a genuslevel identification and a bestmatch identification of the species that matched the molecular identification. For 2 of 117 2% isolates, no reliable identification was obtained Table 3 . One of these 2 isolates resulted in a bestmatch identification of M. gypis in all MALDITOF MS runs. The 16S rRNA gene and ISR sequences of this isolate were 98% and 97% identical to M. gypis, respectively. The second discordant isolate resulted in different bestmatch identifications e.g., Sphingomonas pseudosanguinis, Citrobacter freundii, Bacillus novalis in each run, with a maximum score of 1.306, including no Mycoplasma species. The sequences of the 16S rRNA gene and the ISR of this isolate were 97% and 95% identical to M. gypis, respectively. Regarding the number of runs needed, the first run resulted in correct species identification with scores of 2.0 for 93 of 117 80% isolates, whereas for 15 of 117 13% a second run, and for 3 of 117 3% a third run was needed. Table 3. Best scores per isolate in the indicated MALDITOF runs. Table 3. Best scores per isolate in the indicated MALDITOF runs. View larger version Given that Mycoplasma colonies are small and often inlaid in the agar, the direct deposition of colonies has been shown to often lead to inaccurate MALDITOF MS results. 14 Therefore, agar medium was used to identify the Mycoplasma growth, but we used broth medium as a source of material for MALDITOF MS analysis. To improve the spectral quality, multiple washing steps were performed on the Mycoplasma pellet to reduce the protein background generated by media contents i.e., porcine serum and yeast. However, washing the pellet also decreases the quantity of protein in the subsequent protein extraction.

14 Given that the approach of generating spectra by applying material directly to the target plate without protein extraction resulted in weak scores, protein extraction was performed prior to MALDITOF MS, as described for other bacteria. 7, 14 The analysis of 3 of 117 3% isolates resulted in a genuslevel identification score of 1.7 but Mycoplasma spp.However, these isolates may also belong to a yettobe described Mycoplasma species with high similarities to M. gypis, given that avian Mycoplasma isolates that cannot be assigned to a described species are found regularly. 9, 12, 13 A further explanation may be the absence of sufficient protein signal in order to create an ideal spectrum to be compared to the database, a situation that has been reported if only small amounts of microbial material can be harvested for protein extraction. 4 To improve protein density, additional processing steps are described for fastidious bacteria, 19 a process not used in our study. An optimized preparation method might improve the results for these isolates. Our avian mycoplasma spectral database was reliable for the identification of avian Mycoplasma isolates, given that all isolates with a score of 2.0 were identified correctly. The results showed high concordance between the MALDITOF MS and the molecular species identification. Even though the MALDITOF MS method is based on culture rather than on direct detection within a clinical sample, it offers the possibility for species identification using just one generic test. This is especially helpful if there is no specific anticipated Mycoplasma species and in the investigation of the presence of Mycoplasma spp.Further expansion of the database and optimization of the preanalytic protocols will improve the accuracy of identifications of avian Mycoplasma spp.

AcknowledgementsWe also thank Ralf Doerr and the technical staff of Institute of Hygiene and Infectious Diseases of Animals, Justus Liebig University Giessen, for technical assistance. Moreover, we thank AniCon Labor Holtinghausen, Germany, as well as Moorgut Kartzfehn von Kameke Bosel, Germany, and Janet M. Bradbury and her team, University of Liverpool, for providing us with Mycoplasma isolates, and the Institute for Terrestrial and Aquatic Wildlife Research ITAW of the University of Veterinary Medicine Hannover Foundation for sampling freeranging pheasants for mycoplasma examination. ORCID iD Liane Baudler References 1. Alatoom, AA. Comparison of direct colony method versus extraction method for identification of grampositive cocci by use of Bruker Biotyper matrixassisted laser desorption ionizationtime of flight mass spectrometry. Biswas, S, Rolain, JM. Use of MALDITOF mass spectrometry for identification of bacteria that are difficult to culture. Bizzini, A. Performance of matrixassisted laser desorption ionizationtime of flight mass spectrometry for identification of bacterial strains routinely isolated in a clinical microbiology laboratory. Bizzini, A. Matrixassisted laser desorption ionizationtime of flight mass spectrometry as an alternative to 16S rRNA gene sequencing for identification of difficulttoidentify bacterial strains. Bradbury, JM, Morrow, C. Avian mycoplasmas. In Poultry Diseases. Bradbury, JM. Recovery of mycoplasmas from birds. In Miles, R, Nicholas, RAJ, eds. Methods in Molecular Biology, Mycoplasma Protocols. Croxatto, A. Applications of MALDITOF mass spectrometry in clinical diagnostic microbiology. Ford, BA, Burnham, CAD. Optimization of routine identification of clinically relevant gramnegative bacteria by use of matrixassisted laser desorption ionizationtime of flight mass spectrometry and the Bruker Biotyper. Goldberg, DR. The occurrence of mycoplasmas in selected wild North American waterfowl. Goto, K.

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