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MINISTERSTVO ŠKOLSTVÍ, MLÁDEŽE A TĚLOVÝCHOVY
OP Vzdělávání pro konkurenceschopnost
INVESTICE DO ROZVOJE VZDĚLÁVÁNÍ

Název: **Methods for the detection of influenza virus**

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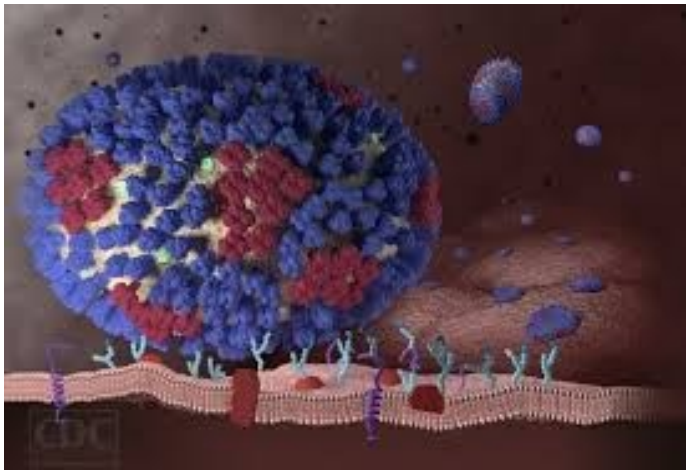
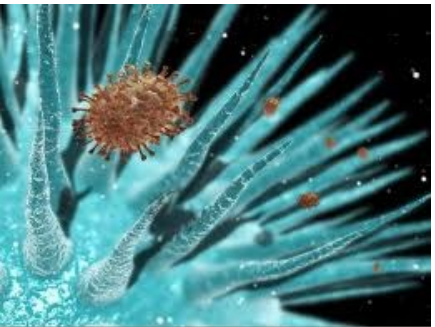
Název projektu: Partnerská síť centra excelentního bionanotechnologického výzkumu

NanoBioMetalNet

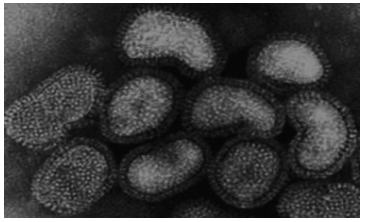
CONTENT



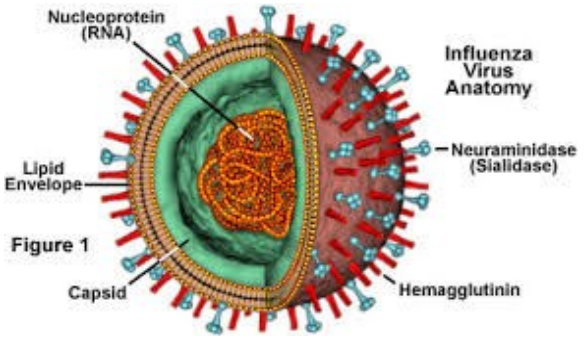
- Basic about influenza
- Standard methods for viruses detection
- PCR and real time PCR – as the most commonly used method
- Isolation and identification of influenza nucleic acid
- Isolation and detection of influenza protein (hemagglutinin)



Basic about influneza



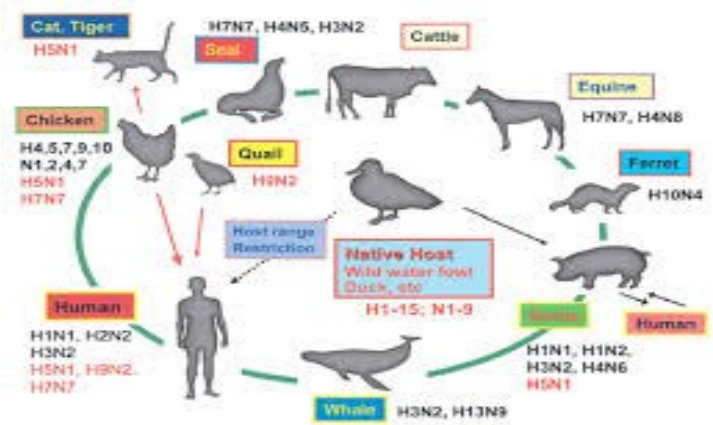
- An infectious respiratory and febrile disease
- Waterfowl are natural reservoir, also transmitted to mammals and humans
- Transmition by droplet infection



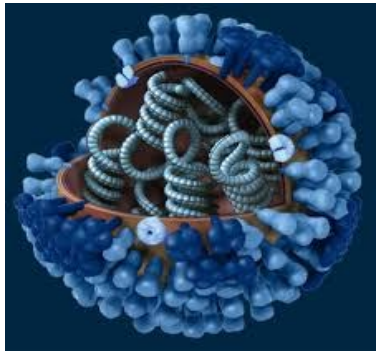
- Caused by ss (-) RNA viruses, family Orthomyxoviridae
- Influenza A, B and C (differ in host range, structure and virulence)

  type A is most virulent and cause seasonal epidemic and pandemic

  type B and C causes epidemics exceptionally
- type A can be subtyped based on combination of antigens Hemagglutinin (HA) and neuraminidase (NA)
- Influenza nucleic acid (RNA) and antigens (HA and NA) are main targets for methods for detection



Methods for the influenza virus detection



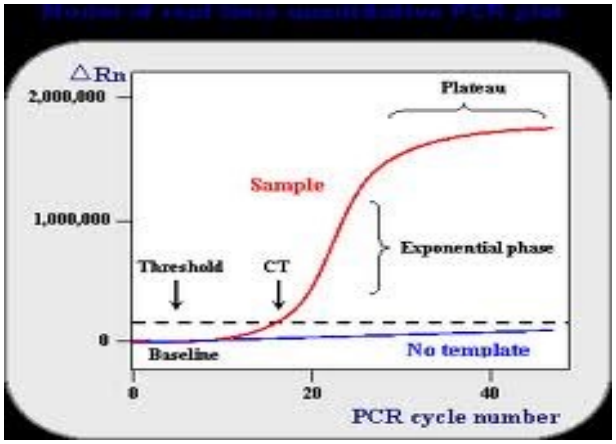
Diret methods – detection of pathogenic agents (or its nucleic acid, antigen….)

- Cell cultivation, cultivation on embrionated eggs, useing of laboratory animals
- PCR and real time PCR
- Imunological methods (ELISA, FISH, western blot)

Indiret methods – detection of immune response on pathogenic agents (antibody)

Serological methods (ELISA)

PCR and real time PCR

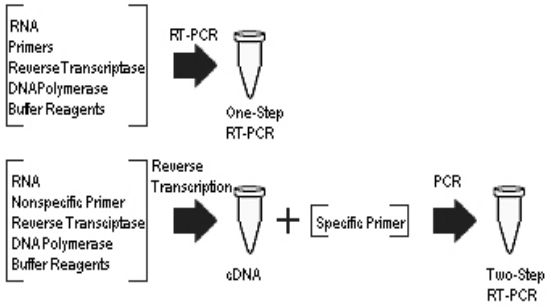


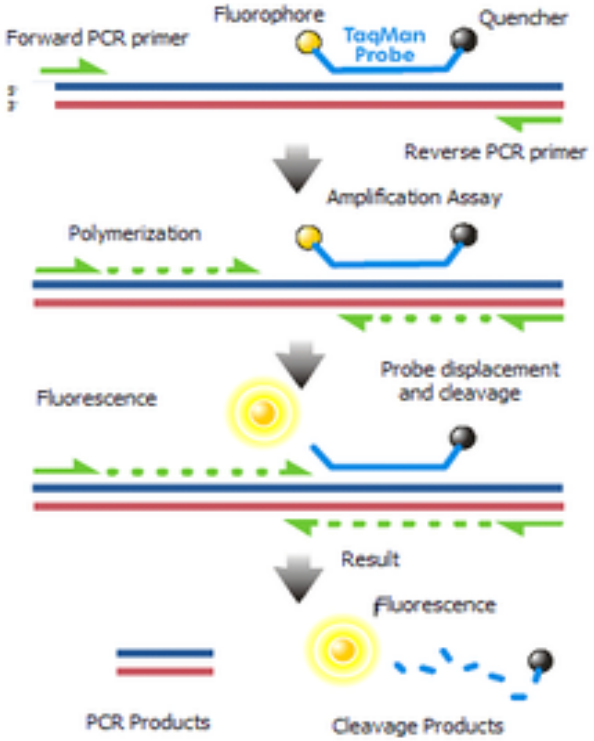
In RT-PCR, theRNA template is first converted into a complementary DNA (cDNA) using a reverse transcriptase. The cDNA is then used as a template for amplification using PCR. RT-PCR is currently the most commonly used method of RNA detection. The use of RT-PCR for the detection of RNA transcript has revolutionalized the study of gene.

real time PCR is currently the most

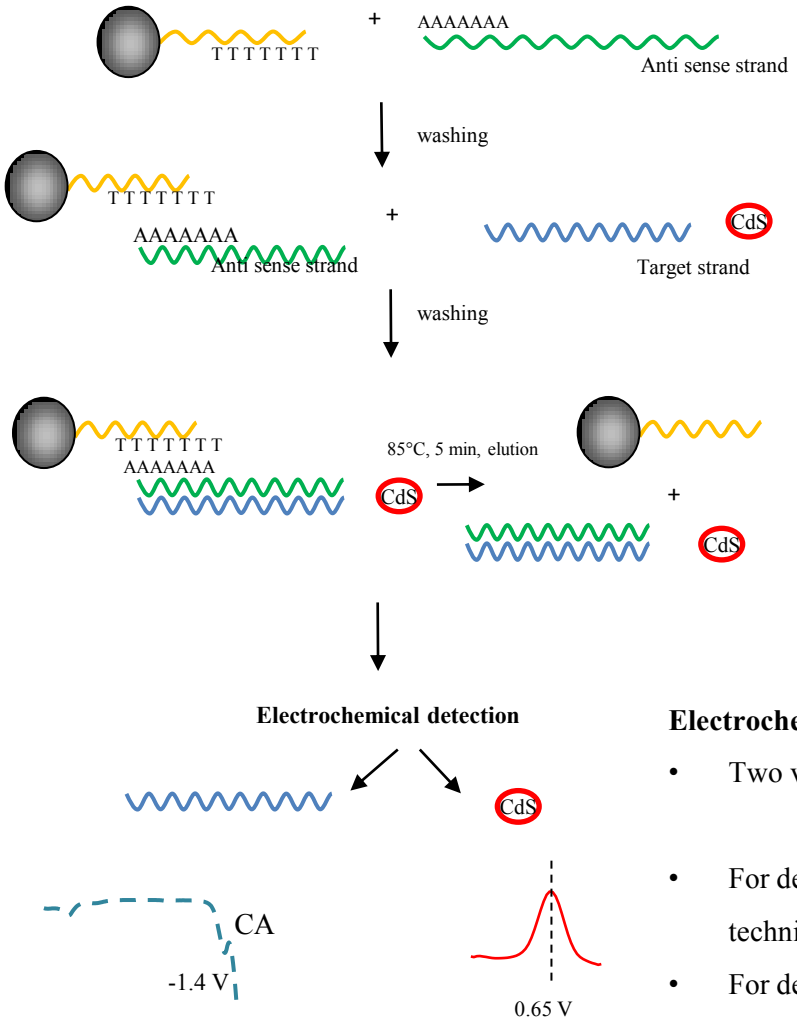
Process of detection:

- Isolation of RNA from samples
- Enzyme-transcription into cDNA
- Amplification of specific strins of influenza viruss
- Amplification is monitored in real time



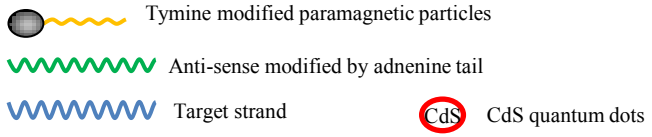


Isolation and identification of influenza nucleic acid



Isolation of target influenza sequence using paramagnetic particles

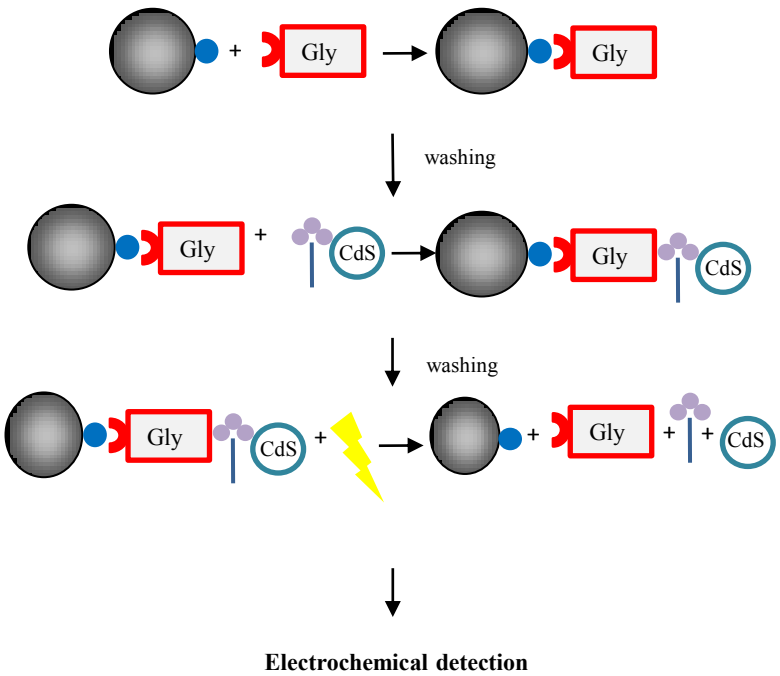
- Binding of adenine modified anti sense strand on tymine modified paramagnetic particles (based complementary of base pairs)
- Washing
- Binding of target strand-CdS complex on anti sense strand (based on complementary of base pairs)
- Washing
- Elution of isolated target sequence-CdS complex



Electrochemical detection of isolated target strand-CdS complex

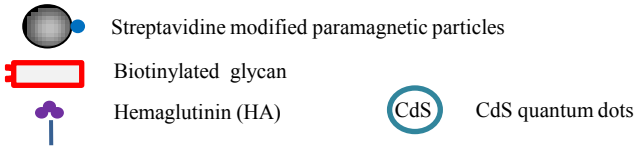
- Two voltammetry methods were used
- For detection of nucleic acid was used sqare wave voltametry, coupled with adsorptive transfer technique (acetate buffer - electrolyte)
- For detection of Cd (from CdS label) was used differential pulse voltammetry coupled with anodic stripping voltammetry (acetate buffer - electrolyte)

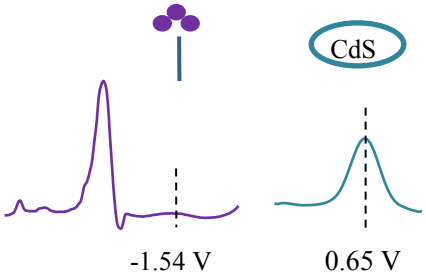
Isolation and identification of influenza hemagglutinine



Isolation using paramagnetic particles

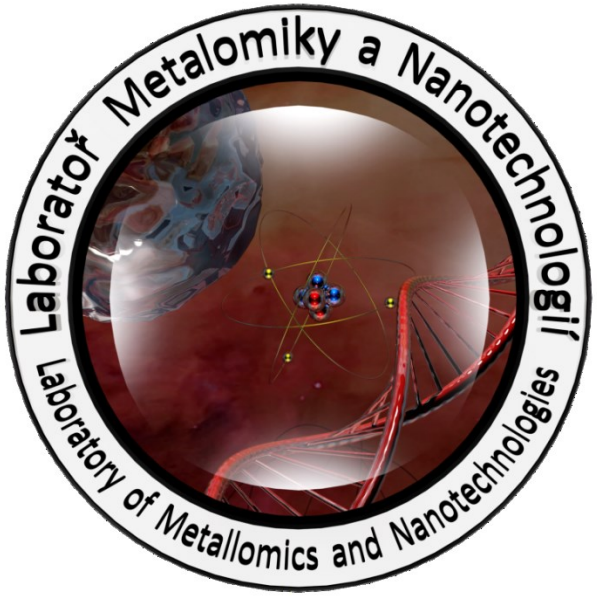
- Binding of biotinylated glycan on streptavivdin modified paramagnetic particles (based on biotin-streptavidin afinity)
- Washing
- Binding of HA-CdS complex on glycan (based on HA-glycan afinity)
- Washing
- Sonication and destroying of isolated glycan-HA-CdS complex





Electrochemical detection of isolated HA-CdS complex

- Two voltammetry methods were used
- For detection of HA was used differential pulse voltametry, coupled with adsorptive transfer technique (with brdicka supporting electrolyte)
- For detection of Cd (from CdS label) was used differential pulse voltammetry coupled with anodic stripping voltammetry (with acetate buffer supporting electrolyte)



Acknowledgements

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Thank you for your attention

Reg.č.projektu: CZ.1.07/2.4.00/31.0023

Název projektu: Partnerská síť centra excelentního bionanotechnologického výzkumu

NanoBioMetalNet