evropský sociální fond v ČR
EVROPSKÁ UNIE
MINISTERSTVO ŠKOLSTVÍ, MLÁDEŽE A TĚLOVÝCHOVY
OP Vzdělávání pro konkurenceschopnost
INVESTICE DO ROZVOJE VZDĚLÁVÁNÍ

Electrochemistry rapid as method for identification influenza viruses mutation by microarrays

Name:

Author: Miguel Ángel Merlos Rodrigo

Date: 15.1.2014

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

Název projektu: Partnerská síť centra excelentního bionanotechnologického výzkumu

NanoBioMetalNet

Electrochemistry rapid as method for identification influenza viruses mutation by microarrays

1. Introduction:

Influenza, antiviral drug and mutations of sequence in influenza viruses (resistance of antiviral drugs) and electrochemical method for detection mutations in influenza viruses

2. Material and Method:

Designed of experimental and arrays chip

3. Results

4. Conclusion

Electrochemistry rapid as method for identification influenza viruses mutation by microarrays

Introduction

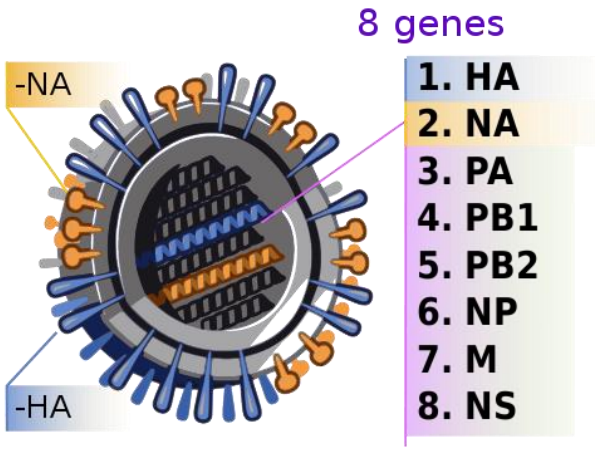
Electrochemistry rapid as method for identification influenza viruses mutation by microarrays

1. Introduction:

Influenza is an infectious disease caused by RNA viruses of the family Orthomyxoviridae.

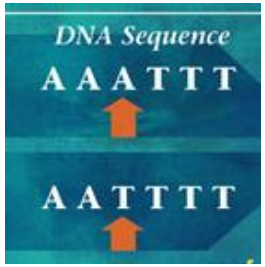
Influenza is considered to be one of the life threatening infectious diseases.

The genome of the influenza virus encodes 8 genes:



Antiviral inhibitors have become an important alternate means of containing the spread of influenza. The current antivirals are mainly against the neuraminidase (such as zanamivir and oseltamivir) and the protein M 2 (such as adamantanes).

However, mutations in the influenza viruses induce resistance to antiviral drug.



Electrochemistry rapid as method for identification influenza viruses mutation by microarrays

1. Introduction:

New molecular techniques are required urgently for the rapid detection of the mutation in the sequence of influenza viruses

CombiMatrix ElectraSense™

CombiMatrix ElectroSense™: Microarray

MicroArray is a new technology to show the expression of genes.

MicroArray is a hybridization of a nucleic acid sample (target) to a very large set of oligonucleotide probes inside on the chip.

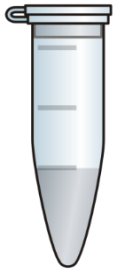
Array Reader

Chip

CombiMatrix ElectroSense™: Microarray and hybridization

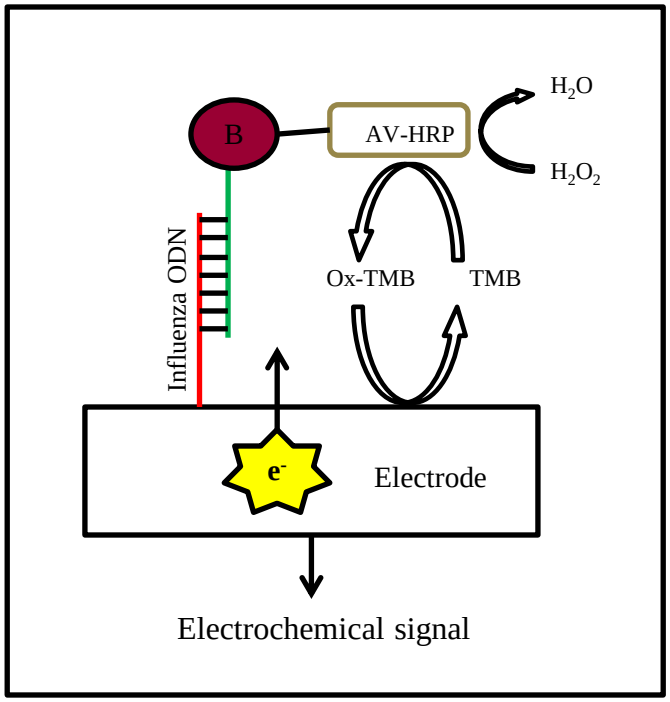
Surface of chip

Oligonucleotides probes of influenza viruses inside of the chip



Samples of RNA Influenza viruses

CombiMatrix ElectroSense™: Electrochemical detection



B Biotin

AV-HRP
horseradish peroxidase

TMB
(3, 3', 5, 5'–tetramethylbenzidene)

- The approach is based on the detection of redox active chemistries (such as horseradish peroxidase (HRP) and the associated substrate TMB) proximal to specific microarray electrodes.

- Microarray probes are hybridized to biotin-labeled targets.

- The HRP-streptavidin conjugate binds to biotin, and enzymatic oxidation of the electron donor substrate then occurs.

- The detection current is generated due to electro-reduction of the HRP reaction product, and it is measured with the CombiMatrix ElectraSense™ Reader.

Material and Method

Electrochemistry rapid as method for identification influenza viruses mutation by microarrays

2. Material and method:

New chip (arrays with 2000 oligonucleotides probes)

Viruses	Genes	GenBank accession number	Sequence
Seasonal A(H1N1)	neuraminidase	CY076797	5'-TCCTCATAATGAAATTGGG-3'
Pandemic A(H1N1)	neuraminidase	GQ377078	5'-TCCTCATAGTGATAATTAGG-3'
H2N2	neuraminidase	CY125896.1	5'-TAGCAATTGCAGGGATCCTA-3'
H3N8	neuraminidase	HM589207.1	5'-TGGATCAAGAGGCCATGTTT-3'
A(H3N2)	neuraminidase	GQ259082	5'-ACACATAAGGTTCTCTTGT-3'
H8N8	neuraminidase	JQ433881.1	5'-GTTGAAATCAGTTTCAATGG-3'
Seasonal A(H3N1)	neuraminidase	CY053327.1	5'-ACCGTTAAAGACAGAAGC C-3'
HPAI A(H5N1)	neuraminidase	EU265982.1	5'-AGGGAAAGTAGTTAAATCAG-3'
H7N7	neuraminidase	AY340079.1	5'-CCAGAAACATTCTGAGGACT-3'
H8N2	neuraminidase	NC_004909.1	5'-TACATGATAGGAGTCCCCATT-3'
Seasonal A(H1N1)	hemagglutinine	JN017181.1	5'-AGAGAAGAAATAGATGGGGTA-3'
Pandemic A(H1N1)	hemagglutinine	JX025498.1	5'-ACCCAAAGCTCAGCAAATC-3'
H2N2	hemagglutinine	CY125918.1	5'-TGTGTGCACATACAATGCTG-3'
H3N8	hemagglutinine	HM589205.1	5'-ATCAACACTAAATCAGAGGTC-3'
A(H3N2)	hemagglutinine	JX844665.1	5'-TTTTGTTGAACGCAGCAAAG-3'
H8N8	hemagglutinine	JQ433879.1	5'-GACCTTAAAAGCACTCAAGC-3'
Seasonal A(H3N1)	hemagglutinine	CY053325.1	5'-GGGAATACACCACCCTAATT-3'
HPAI A(H5N1)	hemagglutinine	EU265981.1	5'-CTCCAGAATATCCATACAAAA-3'
H7N7	hemagglutinine	AY339459.1	5'-GAAACGGTGGAACGACCAAA-3'
H8N2	hemagglutinine	NC_004908.1	5'-GTAGTGCAATGTCAGACTGA-3'
Seasonal A(H1N1)	nucleocapsid protein	CY087027.1	5'-ATAGACGGAAAATGGATGAG-3'
Pandemic A(H1N1)	nucleocapsid protein	JX025388.1	5'-GACGACTAATCCAGAATAGC-3'
H2N2	nucleocapsid protein	CY125897.1	5'-AGAGAGTAAATGGAAAGTGGA-3'
H3N8	nucleocapsid protein	HM589206.1	5'-AATGAGAAAGCGCAACC-3'
A(H3N2)	nucleocapsid protein	CY113840.2	5'-GATTATGAAGGCGGTTGAT-3'
H8N8	nucleocapsid protein	JQ433880.1	5'-CTTCAGTCTTATCAGACCAAA-3'
Seasonal A(H3N1)	nucleocapsid protein	CY053328.1	5'-GCGAATAATGGAGAAGACG-3'
HPAI A(H5N1)	nucleocapsid protein	EU265985.1	5'-ACATATCAGAGAACCAGAGC-3'
H7N7	nucleocapsid protein	AY340425.1	5'-CTAATTCGGATAAAGCCG-3'
H8N2	nucleocapsid protein	AF255743.1	5'-GGAAGGTTGATCCAGAAC-3'
Seasonal A(H1N1)	matrix protein 1	CY087025.1	5'-ACTATACAAGAAGCTCAAAG-3'
Pandemic A(H1N1)	matrix protein 1	JX025390.1	5'-ATGAAAACAGAATGGTGCTG-3'
H2N2	matrix protein 1	CY125895.1	5'-TTAAGAGGGAGATAACATTC-3'
H3N8	matrix protein 1	HM589208.1	5'-CTTTCTCCAAAATGCCCTAA-3'
A(H3N2)	matrix protein 1	CY113838.2	5'-AACATGTGAACAGATTGCTG-3'
H8N8	matrix protein 1	JQ433882.1	5'-TACAGGAAGCTTAAAAGGGA-3'
Seasonal A(H3N1)	matrix protein 1	CY053326.1	5'-TCCTGTCACCTCTGACTA A-3'
HPAI A(H5N1)	matrix protein 1	EU265984.1	5'-ACTGCAGCGTAGACGTTTT-3'
H7N7	matrix protein 1	GU053111.1	5'-CCCACTAATCAGGCATGAA-3'
H8N2	matrix protein 1	AJ278646.1	5'-CAAGAAGCTGAAGAGGGAAA-3'
Seasonal A(H1N1)	matrix protein 2	CY087025.1	5'-TCGCTTTAAATACGGTTTGAA-3'
Pandemic A(H1N1)	matrix protein 2	JX025390.1	5'-TCATTGGGATCTTGCACCT-3'
H2N2	matrix protein 2	CY125895.1	5'-AACACGGTCTGAAAAGAGGG-3'
H3N8	matrix protein 2	HM589208.1	5'-ATCATTGGGATCTTGCACTT-3'
A(H3N2)	matrix protein 2	CY113838.2	5'-TTCAAACACGGTCTGAAAAGA-3'
H8N8	matrix protein 2	JQ433882.1	5'-GCCTTAAATACGGTTTGAA-3'
Seasonal A(H3N1)	matrix protein 2	CY053326.1	5'-TATCATTGGGATCTTGCACT-3'
HPAI A(H5N1)	matrix protein 2	EU265984.1	5'-TCGCCTAAATACGGTTTGAA-3'
H7N7	matrix protein 2	GU053111.1	5'-CGCCTTAAATACGGTTTGAA-3'
H8N2	matrix protein 2	AJ278646.1	5'-CTTCAAATGCATTTATCGTC-3'
Seasonal A(H1N1)	polymerase PB1	CY125673.1	5'-AAGATGATGACCAACTCCCA-3'
Pandemic A(H1N1)	polymerase PB1	HQ240702.2	5'-AGTGGAATGAAAATCAAAATC-3'
H2N2	polymerase PB1	CY125900.1	5'-TGGATGAGAATCAAAATCCT-3'
H3N8	polymerase PB1	HM589203.1	5'-GGATAAAGAGGAAATGGAAAT-3'
A(H3N2)	polymerase PB1	CY112971.2	5'-TGAATCTTGGGCAAAAGAAA-3'
H8N8	polymerase PB1	JQ433877.1	5'-GATGACTAATTCACAAGACA-3'
Seasonal A(H3N1)	polymerase PB1	JF758819.1	5'-AGCATTGACACTGAACACTA-3'
HPAI A(H5N1)	polymerase PB1	EU265987.1	5'-AGAAAATGGTACACAAGGAA-3'
H7N7	polymerase PB1	AY340083.1	5'-AATTACTGGAGACAACACCA-3'
H8N2	polymerase PB1	NC_004911.1	5'-AGTAAGAGCATGAAGCTACG-3'
Seasonal A(H1N1)	polymerase PA	CY125671.1	5'-AAACCAGACTATTCACCATAA-3'
Pandemic A(H1N1)	polymerase PA	JQ433878.1	5'-ATTCACTGGAGGAGATGG-3'
H2N2	polymerase PA	CY125923.1	5'-AGAACATTCTTTGGATGGAA-3'
H3N8	polymerase PA	HM589204.1	5'-GAAAGCCAACAAGATAAATC-3'
A(H3N2)	polymerase PA	CY113858.2	5'-CAGAGCCACTGAGTACATAAT-3'
H8N8	polymerase PA	JQ433878.1	5'-AGCCGAAGAGACAATTGAAG-3'
Seasonal A(H3N1)	polymerase PA	CY053330.1	5'-TGCATTGAGGCAAGCTTT-3'
HPAI A(H5N1)	polymerase PA	EU265986.1	5'-GGGCAAGCTTTCTCAAATGT-3'
H7N7	polymerase PA	AY342418.1	5'-ACAACACCACGCCCTCT-3'
H8N2	polymerase PA	NC_004912.1	5'-CTGAAATTAAGCATTGAGGAC-3'
Seasonal A(H1N1)	nuclear export protein (nep)	CY125670.1	5'-GATTGAAGAAGTGAGACACAA-3'
Pandemic A(H1N1)	nuclear export protein (nep)	HQ240288.2	5'-TGGTTAATTGAAGAAATGCGG-3'
H2N2	nuclear export protein (nep)	CY125898.1	5'-ACGGAAAATGGCGAGACAA-3'
H3N8	nuclear export protein (nep)	HM589209.1	5'-TGATTGAGGAAGTACGACATA-3'
A(H3N2)	nuclear export protein (nep)	CY103967.1	5'- AGAACAGTTAGGTCAAAAGT-3'
H8N8	nuclear export protein (nep)	JQ433883.1	5'-TGGCTGATTGAAGAAGTGC-3'
Seasonal A(H3N1)	nuclear export protein (nep)	CY053329.1	5'-ACCTCCACTCCCTCCAAAA-3'
HPAI A(H5N1)	nuclear export protein (nep)	CY069818.1	5'-GCTGATTGAAGAAGTACGACA-3'
H7N7	nuclear export protein (nep)	GU053125.1	5'-AAGTGCGACATAGGTTGAAG-3'
H8N2	nuclear export protein (nep)	FJ793288.1	5'-TGGCTGATTGAAGAAGTGC--3'

The sequences were used for probe design Genbank® number (the NIH genetic sequence database) of genes. A total of 80 oligonucleotide probes were synthesized and printed onto the CombiMatrix CustomArrayTM 4×2K microarray support to generate the prototype chips for the different influenza viruses

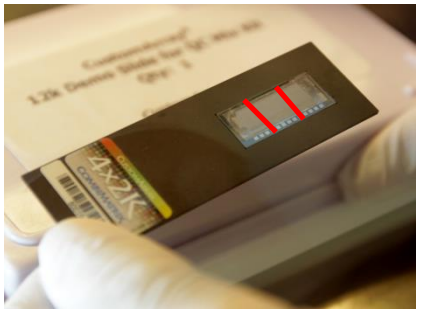


Table 1. The Summary of sequences of the selected specific microarray probes and number assigned in the microarray.

Electrochemistry rapid as method for identification influenza viruses mutation by microarrays

2. Material and method:

Viruses	Genes	GeenBank accession number	Oligonucleotides probes with one mutations
Seasonal A(H1N1)	neuraminidase	CY076797	5'-TCCTCATAATAAAAATTGGG-3'
Pandemic A(H1N1)	neuraminidase	GQ377078	5'-TCCTCAAAGTGATAATTAGG-3'
H2N2	neuraminidase	CY125896.1	5'-TAGCAATGCAGGGATCCTA-3'
H2N8	neuraminidase	HM589207.1	5'-TGGATCAAAAGG CCATGTTT-3'
A(H3N2)	neuraminidase	GQ293082	5'-ACACATAAAGTTCTCTTGT-3'
H3N8	neuraminidase	JQ433881.1	5'-GTTGAAACAGT TTCAATGG-3'
seasonal A(H5N1)	neuraminidase	CY053327.1	5'-ACCGTTAAAACAGAAGCC-3'
HPAI A(H5N1)	neuraminidase	EU263982.1	5'-AGGGAAAATAGTTAAATCAG-3'
H7N7	neuraminidase	AY340079.1	5'-CCAGAAAAATTCTGAGGACT-3'
H9N2	neuraminidase	NC_004909.1	5'-TACATGAAA GGAGTCCCCAT-3'
Seasonal A(H1N1)	hemagglutinine	JN017181.1	5'-AGAGAAAAATAGATGGGGTA-3'
Pandemic A(H1N1)	hemagglutinine	JX625498.1	5'-ACCCAAAACTCAGCAAATC-3'
H2N2	hemagglutinine	CY125918.1	5'-TGTGTGGAATATACAATGCTG-3'
H2N8	hemagglutinine	HM589205.1	5'-ATCAACACAAAATCAGAGGTC-3'
A(H3N2)	hemagglutinine	JX844665.1	5'-TTTTGTTGAAAGCAGCAAAG-3'
H3N8	hemagglutinine	JQ433879.1	5'-GACCTTAAAAACACTCAAGC-3'
seasonal A(H5N1)	hemagglutinine	CY053325.1	5'-GGGAATAAACCACCCTAA T-3'
HPAI A(H5N1)	hemagglutinine	EU263981.1	5'-CTCCAGAAAATGCATACAAAA-3'
H7N7	hemagglutinine	AY338459.1	5'-GAAACGGTGGAACGAAAAAA-3'
H9N2	hemagglutinine	NC_004908.1	5'-GTAGTGCAAAGTCAGACTGA-3'
Seasonal A(H1N1)	nucleocapsid protein	CY087027.1	5'-ATAGACGAAAAATGGATGAG-3'
Pandemic A(H1N1)	nucleocapsid protein	JX625388.1	5'-GACGACTAAACCAGAATAGC-3'
H2N2	nucleocapsid protein	CY125897.1	5'-AGAGAGAAAATG GAAAGTGGA-3'
H2N8	nucleocapsid protein	HM589206.1	5'-AATGGAGAAAACGCAACC-3'
A(H3N2)	nucleocapsid protein	CY113840.2	5'-GATTATAAAGGGCGG TTGAT-3'
H3N8	nucleocapsid protein	JQ433880.1	5'-CTTCAGTCTTATCAAACCAAA-3'
seasonal A(H5N1)	nucleocapsid protein	CY053328.1	5'-GGCG AAAATGGAGAAGACG-3'
HPAI A(H5N1)	nucleocapsid protein	EU263985.1	5'-ACATATCAGAAAACGAGAGC-3'
H7N7	nucleocapsid protein	AY342425.1	5'-CTAATTCGGATGAAAAAGCG-3'
H9N2	nucleocapsid protein	AF255743.1	5'-GGAAAGTTGATCCAGAAC-3'
Seasonal A(H1N1)	matrix protein 1	CY087025.1	5'-ACTATACAAAAAGCT CAAAAG-3'
Pandemic A(H1N1)	matrix protein 1	JX625390.1	5'-ATGAAAAAAGAATGGTGCTG-3'
H2N2	matrix protein 1	CY125895.1	5'-TTAAAAAGGGAGATAACATTC-3'
H2N8	matrix protein 1	HM589208.1	5'-CTT TGTCAAAAATGCCCTTAA-3'
A(H3N2)	matrix protein 1	CY113838.2	5'-AACATGTGAAAAGATTGCTG-3'
H3N8	matrix protein 1	JQ433882.1	5'-TACAGGAAGCTAAAAAGGGA-3'
seasonal A(H5N1)	matrix protein 1	CY053326.1	5'-TCCTGTCACCTCTAACTA A-3'
HPAI A(H5N1)	matrix protein 1	EU263984.1	5'-ACTGCAGCGTAAACGTTT T-3'
H7N7	matrix protein 1	GU053111.1	5'-CCCACTAAACAGGCATGAA-3'
H9N2	matrix protein 1	AJ278646.1	5'-CAAGAAGCTGAAAAGGGA AA-3'
Seasonal A(H1N1)	matrix protein 2	CY087025.1	5'-TCGCTTTAAAAACGGTTTGAA-3'
Pandemic A(H1N1)	matrix protein 2	JX625390.1	5'-TCATTGGGAACTTGCACCT-3'
H2N2	matrix protein 2	CY125895.1	5'-AACACGGTCTGAAAAAGGG-3'
H2N8	matrix protein 2	HM589208.1	5'-ATCATTGGGAACTTGCACTT-3'
A(H3N2)	matrix protein 2	CY113838.2	5'-TTCAAACACGGTCTGAAAAAA-3'
H3N8	matrix protein 2	JQ433882.1	5'-GCCTTAAAAACGGTTTGAA-3'
seasonal A(H5N1)	matrix protein 2	CY053326.1	5'-TATCATTGGGAACTTGCACT-3'
HPAI A(H5N1)	matrix protein 2	EU263984.1	5'-TCGCCTTAAAAACGGTTTGAA-3'
H7N7	matrix protein 2	GU053111.1	5'-CGCCTTAAAAACGGTTTGAA-3'
H9N2	matrix protein 2	AJ278646.1	5'-CTTCAAAAGCATTTATCGTC-3'
Seasonal A(H1N1)	polymerase PB1	CY125673.1	5'-AAAATG ATGACCAACTCCCA-3'
Pandemic A(H1N1)	polymerase PB1	HQ240702.2	5'-AGTGGAATGAAAAAACAAAATC-3'
H2N2	polymerase PB1	CY125900.1	5'-TGGAATGAGAATAAAAATCCT-3'
H2N8	polymerase PB1	HM589203.1	5'-GGATAAAGAGGAAAAGGAAAT-3'
A(H3N2)	polymerase PB1	CY112971.2	5'-TGAATCTTGGGCAAAAAAAA-3'
H3N8	polymerase PB1	JQ433877.1	5'-GATGACTAATTCAAAAGACA-3'
seasonal A(H5N1)	polymerase PB1	#758819.1	5'-AGCATTGACACTGAAAACTA-3'
HPAI A(H5N1)	polymerase PB1	EU263987.1	5'-AGAAAAAGGTAACACAAAGAA-3'
H7N7	polymerase PB1	AY340083.1	5'-AATTACTGGAGAAACACCA-3'
H9N2	polymerase PB1	NC_004911.1	5'-AGTAAAAGCATTGAAGCTACG-3'
Seasonal A(H1N1)	polymerase PA	CY125671.1	5'-AAAACAGACTATTCACCATAA-3'
Pandemic A(H1N1)	polymerase PA	JQ433878.1	5'-ATTCACTGGAAAAGGAGATGG-3'
H2N2	polymerase PA	CY125923.1	5'-AGAAAATTCTTTGGATGGAA-3'
H2N8	polymerase PA	HM589204.1	5'-GAAAGCCAAAAAGATAAAATC-3'
A(H3N2)	polymerase PA	CY113858.2	5'-CAGAGCCACTGAGTACAAAAT-3'
H3N8	polymerase PA	JQ433878.1	5'-AGGCGAAAAGACAATTGAAG-3'
seasonal A(H5N1)	polymerase PA	CY053330.1	5'-TGCATTGAGGGCAAACTT T-3'
HPAI A(H5N1)	polymerase PA	EU263986.1	5'-GGGCAAGCTTTCTCAAAAGT-3'
H7N7	polymerase PA	AY342418.1	5'-ACAAAACCACGCCCTCT-3'
H9N2	polymerase PA	NC_004912.1	5'-CTGAAAATAAGCATTGAGGAC-3'
Seasonal A(H1N1)	nuclear export protein (nep)	CY125670.1	5'-GATTGAAAAAGTGAGACACAA-3'
Pandemic A(H1N1)	nuclear export protein (nep)	HQ240288.2	5'-TGGTTAATTGAAAAAATGCGG-3'
H2N2	nuclear export protein (nep)	CY125898.1	5'-ACGGAAAAAAGGCGAGAACAA-3'
H2N8	nuclear export protein (nep)	HM589209.1	5'-TGATTGAGGAAATACGACATA-3'
A(H3N2)	nuclear export protein (nep)	CY103967.1	5'- AGAACAGTTAGGTAAAAAGT-3'
H3N8	nuclear export protein (nep)	JQ433883.1	5'-TGGCTGATTGAAAAAGTGC-3'
seasonal A(H5N1)	nuclear export protein (nep)	CY053329.1	5'-ACCTCCACTCCCTCAAAAA-3'
HPAI A(H5N1)	nuclear export protein (nep)	CY098618.1	5'-GCTGATTGAAAAAGTACGACA-3'
H7N7	nuclear export protein (nep)	GU053125.1	5'-AAGTGCGACAAAGGTTGAAG-3'
H9N2	nuclear export protein (nep)	FJ793288.1	5'-TGGCTGATTGAAAAAGTGC-3'

Other part of array have probes specific sequences with one mutations to influenza subtypes

Table 2. Summary of sequences of the selected specific array probes with one mutation in the sequence and number assigned in the microarray.

Electrochemistry rapid as method for identification influenza viruses mutation by microarrays

2. Material and method:

	A(H1N1) Neuroaminidase	HPAI A(H5N1) Matrix protein 1
	Sequence of gen 1	Sequence of gen 38
W/O	5′-TCCTCATAATGAAAATTGGG-3′	5′-ACTGCAGCGTAGACGTTTT-3′
M	5′-TCCTCATAATAAAAATTGGG-3′	5′-ACTGCAGCGTAAACGTTTT-3′
MM	5′-TCCTCATAAAAAAAATTGGG-3′	5′-ACTGCAGCGAAAACGTTTT-3′
MMM	5′-TCCTCATAAAAAAAAATGGG-3′	5′-ACTGCAGCGAAAAAGTTTT-3′
MMMM	5′-TCCTCATAAAAAAAAAAGGG-3′	5′-ACTGCAGCGAAAAAATTTT-3′
MMMMM	5′-TCCTCAAAAAAAAAAAAGGG-3′	5′-ACTGCAGCAAAAAAATTTT-3′
	H2N2 Matrix protein 1	Seasonal A(H1N1) Matrix protein 2
	Sequence of gen 33	Sequence of gen 41
W/O	5′-TTAAGAGGGAGATAACATTC-3′	5′-TCGCTTTAAATACGGTTTGAA-3′
M	5′-TTAAAAGGGAGATAACATTC-3′	5′-TCGCTTTAAAAACGGTTTGAA-3′
MM	5′-TTAAAAGGGAGAAAACATTC-3′	5′-TCGCTTTAAAAAAGGTTTGAA-3′
MMM	5′-TTAAAAGGGAGAAAAATTC-3′	5′-TCGCTTAAAAAAAGGTTTGAA-3′
MMMM	5′-TTAAAAGGGAAAAAAATTC-3′	5′-TCGCTTAAAAAAAAGTTTGAA-3′
MMMMM	5′-TTAAAAGAGAAAAAAATTC-3′	5′-TCGCTTAAAAAAAAATTTGAA-3′
	A(H3N2) Hemaglutinine	Seasonal A(H1N1) polymerase PB1
	Sequence of gen 15	Sequence of gen 51
W/O	5′-TTTTGTTGAACGCAGCAAAG-3′	5′-AAGATGATGACCAACTCCCA-3′
M	5′-TTTTGTTGAAAGCAGCAAAG-3′	5′-AAAATGATGACCAACTCCCA-3′
MM	5′-TTTTGTTGAAAACAGCAAAG-3′	5′-AAAAAGATGACCAACTCCCA-3′
MMM	5′-TTTTGTTGAAAACAGCAAAA-3′	5′-AAAAAAATGACCAACTCCCA-3′
MMMM	5′-TTTTGTTGAAAAAAGCAAAA-3′	5′-AAAAAAAAGACCAACTCCCA-3′
MMMMM	5′-TTTTGTTGAAAAAAGAAAAA-3′	5′-AAAAAAAAAACCAACTCCCA-3′
	HPAI A(H5N1) nucleocapsid	H2N2 polymerase PA
	Sequence of gen 28	Sequence of gen 63
W/O	5′-ACATATCAGAGAACGAGAGC-3′	5′-AGAACATTCTTTGGATGGAA-3′
M	5′-ACATATCAGAAAACGAGAGC-3′	5′-AGAAAATTCTTTGGATGGAA-3′
MM	5′-AAATATCAGAAAACGAGAGC-3′	5′-AAAAAATTCTTTGGATGGAA-3′
MMM	5′-AAAAATCAGAAAACGAGAGC-3′	5′-AAAAAAATCTTTGGATGGAA-3′
MMMM	5′-AAAAAACAGAAAACGAGAGC-3′	5′-AAAAAAATCTTTGGATGAAA-3′
MMMMM	5′-AAAAAAAGAAAACGAGAGC-3′	5′-AAAAAAATCTTTGGATAAAA-3′

Other part of array have probes specific sequences with one, two, three and four mutations to influenza subtypes

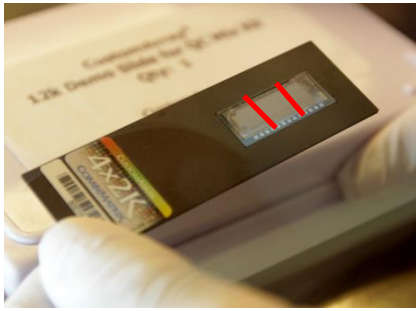
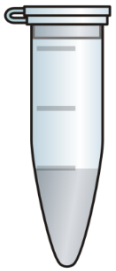


Table 3. Summary of specify sequences of specific probes for different influenza virus with one, two, three and four mutations within the microarray. Selected viruses were A(H1N1) Neuroaminidase (1) , HPAI A(H5N1) Matrix protein 1 (38), H2N2 Matrix protein 1 (33), Seasonal A(H1N1) Matrix protein 2 (41), A(H3N2) Hemaglutinine (15), Seasonal A(H1N1) Polymerase PB1 (51), HPAI A(H5N1) Nucleocapsid (28) and H2N2 Polymerase PA (63). W/O – without mutation, M- one mutations, MM – two mutations, MMM – three mutations and MMMM – four mutations.

Electrochemistry rapid as method for identification influenza viruses mutation by microarrays

Viruses	Genes	GenBank accession number	Sequence
Seasonal A(H1N1)	neuraminidase	CY076797	5'-TCCTCATAATGAAAATTDGG-3'
Pandemic A(H1N1)	neuraminidase	GQ377078	5'-TCCTCATAGTGATATTAGG-3'
H2N2	neuraminidase	CY125896.1	5'-TAGCAATTGCAGGGATCCTA-3'
H3N8	neuraminidase	HM589207.1	5'-TDGATCAAGAGCCATGTTT-3'
A(H3N2)	neuraminidase	GQ289082	5'-ACACATAAGGTTCTCTTG-3'
H5N8	neuraminidase	JQ433881.1	5'-GTTGAAATCAGTTTCAATGG-3'
Seasonal A(H5N1)	neuraminidase	CY053327.1	5'-ACCGTTAAAGACAGAAGC C-3'
HPAI A(H5N1)	neuraminidase	EU260982.1	5'-AGGGAAAGTAGTTAAATCAG-3'
H7N7	neuraminidase	AY340079.1	5'-CCAGAAACATTCTGAGGACT-3'
H8N2	neuraminidase	NC_004909.1	5'-TAGATGATAGGAGTCCCAT-3'
Seasonal A(H1N1)	hemagglutinine	JN017181.1	5'-AGAGAAGAAATAGATGGGGTA-3'
Pandemic A(H1N1)	hemagglutinine	JX625498.1	5'-ACCCAAAGCTCAGCAAATC-3'
H2N2	hemagglutinine	CY125918.1	5'-TGTGTGGACATACAATCCTG-3'
H3N8	hemagglutinine	HM589205.1	5'-ATCAACACTAAATCAGAGGTC-3'
A(H3N2)	hemagglutinine	JX844665.1	5'-TTTTGTTDAACGCAGCAAAG-3'
H5N8	hemagglutinine	JQ433879.1	5'-GACCTTAAAAGCACTCAAGC-3'
Seasonal A(H5N1)	hemagglutinine	CY053325.1	5'-GGGAATACACCACCCTAAT-3'
HPAI A(H5N1)	hemagglutinine	EU260981.1	5'-CTCCAGAATATGCATACAAAA-3'
H7N7	hemagglutinine	AY339459.1	5'-GAAACGGTGGAACGAACAAA-3'
H8N2	hemagglutinine	NC_004908.1	5'-GTAGTGCAATGTCAGACTGA-3'
Seasonal A(H1N1)	nucleocapsid protein	CY087027.1	5'-ATAGACGGAAAATGGATGAG-3'
Pandemic A(H1N1)	nucleocapsid protein	JX625388.1	5'-GACGACTAATCCAGAATAGC-3'
H2N2	nucleocapsid protein	CY125897.1	5'-AGAGAGTAAATGGAAAGTGGA-3'
H3N8	nucleocapsid protein	HM589206.1	5'-AATDGAGAAGACGCAACC-3'
A(H3N2)	nucleocapsid protein	CY118840.2	5'-GATTATGAAGGGCGGTTGAT-3'
H5N8	nucleocapsid protein	JQ433880.1	5'-CTTCAGTCTTATCAGACCAAA-3'
Seasonal A(H5N1)	nucleocapsid protein	CY053328.1	5'-GCGAATAATGGAGAAGACG-3'
HPAI A(H5N1)	nucleocapsid protein	EU260985.1	5'-ACATATCAGAGAACGAGAGC-3'
H7N7	nucleocapsid protein	AY340425.1	5'-CTAATTCGGATGATAAAGCG-3'
H8N2	nucleocapsid protein	AF255743.1	5'-GGAAGGTTGATCCAGAAC-3'
Seasonal A(H1N1)	matrix protein 1	CY087025.1	5'-ACTATACAAGAAGCTCAAAAG-3'
Pandemic A(H1N1)	matrix protein 1	JX625390.1	5'-ATGAAACAGAATTGGTGCTG-3'
H2N2	matrix protein 1	CY125895.1	5'-TTAAGAGGGAGATAACATTC-3'
H3N8	matrix protein 1	HM589208.1	5'-CTTTGTCCAAAATGCCCTTAA-3'
A(H3N2)	matrix protein 1	CY118838.2	5'-AACATGTGAACAGATTGCTG-3'
H5N8	matrix protein 1	JQ433882.1	5'-TACAGGAAGCTTAAAGGGA-3'
Seasonal A(H5N1)	matrix protein 1	CY053326.1	5'-TCCTGTCACCTCTGACTA A-3'
HPAI A(H5N1)	matrix protein 1	EU260984.1	5'-ACTGCAGCGTAGACGTTTT-3'
H7N7	matrix protein 1	GU053111.1	5'-CCCACTAATCAGGCATGAA-3'
H8N2	matrix protein 1	AJ278646.1	5'-CAAGAAGCTGAAGAGGGAAA-3'
Seasonal A(H1N1)	matrix protein 2	CY087025.1	5'-TCGCTTTAAATACGGTTTGAA-3'
Pandemic A(H1N1)	matrix protein 2	JX625390.1	5'-TCATTGGGATCTTGCACCT-3'
H2N2	matrix protein 2	CY125895.1	5'-AACACGGTCTGAAAAGAGGG-3'
H3N8	matrix protein 2	HM589208.1	5'-ATCATTGGGATCTTGCACTT-3'
A(H3N2)	matrix protein 2	CY118838.2	5'-TTCAACACGGTCTGAAAAGA-3'
H5N8	matrix protein 2	JQ433882.1	5'-GCCTTAAATACGGTTTGAA-3'
Seasonal A(H5N1)	matrix protein 2	CY053326.1	5'-TATCATTGGGATCTTGCACT-3'
HPAI A(H5N1)	matrix protein 2	EU260984.1	5'-TCGCCTTAAATACGGTTTGAA-3'
H7N7	matrix protein 2	GU053111.1	5'-CGCCTTAAATACGGTTTGAA-3'
H8N2	matrix protein 2	AJ278646.1	5'-CTTCAATGCATTTATCGTC-3'
Seasonal A(H1N1)	polymerase PB1	CY125673.1	5'-AAGATGATGACCAACTCCCA-3'
Pandemic A(H1N1)	polymerase PB1	HQ240702.2	5'-AGTDGAATGAAAATCAAAATC-3'
H2N2	polymerase PB1	CY125900.1	5'-TDGAATGAGAATCAAAATCCT-3'
H3N8	polymerase PB1	HM589209.1	5'-GGATAAAGAGAGGAAATDGAAAT-3'
A(H3N2)	polymerase PB1	CY112971.2	5'-TGAATCTTDGGCAAAAGAAA-3'
H5N8	polymerase PB1	JQ433877.1	5'-GATGACTAATTCACAAGACA-3'
Seasonal A(H5N1)	polymerase PB1	JF758819.1	5'-AGCATTGACACTGAACACTA-3'
HPAI A(H5N1)	polymerase PB1	EU260987.1	5'-AGAAAATDGTGACACAAAGAA-3'
H7N7	polymerase PB1	AY340083.1	5'-AATTACTDGAGACAACACCA-3'
H8N2	polymerase PB1	NC_004911.1	5'-AGTAAGAGCATGAAGCTACG-3'
Seasonal A(H1N1)	polymerase PA	CY125671.1	5'-AAACCAGACTATTCACCATAA-3'
Pandemic A(H1N1)	polymerase PA	JQ433878.1	5'-ATTCACTGGAGAGGAGATGG-3'
H2N2	polymerase PA	CY125923.1	5'-AGAACATTCTTTGGATDGAA-3'
H3N8	polymerase PA	HM589204.1	5'-GAAAGCCAACAAGATAAAATC-3'
A(H3N2)	polymerase PA	CY113858.2	5'-CAGAGCCACTGAGTACATAT-3'
H5N8	polymerase PA	JQ433878.1	5'-AGGCGAAGAGACAATTGAAG-3'
Seasonal A(H5N1)	polymerase PA	CY053330.1	5'-TGCATTGAGGGCAAACTTT-3'
HPAI A(H5N1)	polymerase PA	EU260986.1	5'-GGGCAAGCTTTCTCAAATGT-3'
H7N7	polymerase PA	AY342418.1	5'-ACAACACCACGCCCTCT-3'
H8N2	polymerase PA	NC_004912.1	5'-CTGAAATTAAGCATTGAGGAC-3'
Seasonal A(H1N1)	nuclear export protein (nep)	CY125670.1	5'-GATTDAAGAAGTGAGACACA A-3'
Pandemic A(H1N1)	nuclear export protein (nep)	HQ240288.2	5'-TGGTTAATTGAAGAAATDCGG-3'
H2N2	nuclear export protein (nep)	CY125898.1	5'-ACGGAAAATGGCGAGAACAA-3'
H3N8	nuclear export protein (nep)	HM589209.1	5'-TGATTGAGGAAGTACGACATA-3'
A(H3N2)	nuclear export protein (nep)	CY103967.1	5'- AGAACAGTTAGGTCAAAAGT-3'
H5N8	nuclear export protein (nep)	JQ433883.1	5'-TGGCTGATTGAAGAAGTGC-3'
Seasonal A(H5N1)	nuclear export protein (nep)	CY053329.1	5'-ACCTCCACTCCCTCCAAAA-3'
HPAI A(H5N1)	nuclear export protein (nep)	CY098518.1	5'-CCTGATTGAAGAAGTACGACA-3'
H7N7	nuclear export protein (nep)	GU053125.1	5'-AAGTGCGACATAGGTTGAAG-3'
H8N2	nuclear export protein (nep)	FJ793288.1	5'-TGGCTGATTGAAGAAGTGC-3'

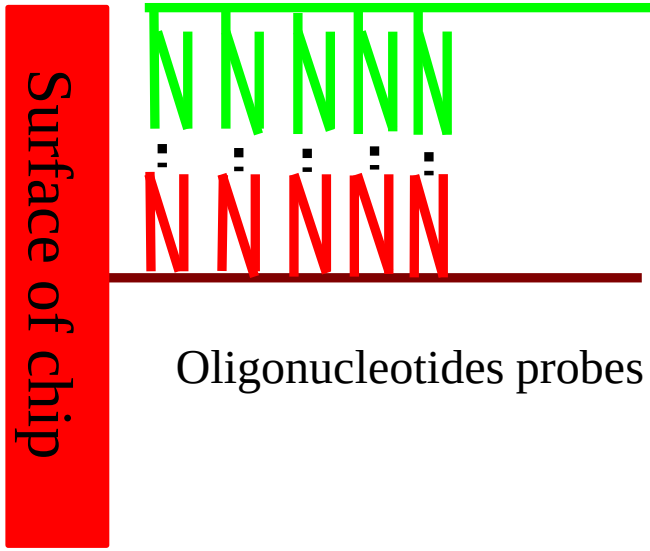
Oligonucleotide complementary targets labeled on the 3' end with biotin was obtained from Metabions International AG (Germany).



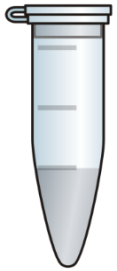
Mix Oligonucleotides 3" biotin complementary sequences of Influenza viruses

Electrochemistry rapid as method for identification influenza viruses mutation by microarrays

2. Material and method:



Oligonucleotides probes of influenza viruses inside of the chip



Mix Oligonucleotides 3” biotin complementary sequences of Influenza viruses

The 3’ Biotin Oligonucleotide probes Mix microarrays were hybridized for 1 h at 40°C in hybridization solution

Results

Electrochemistry rapid as method for identification influenza viruses mutation by microarrays

3. Results:

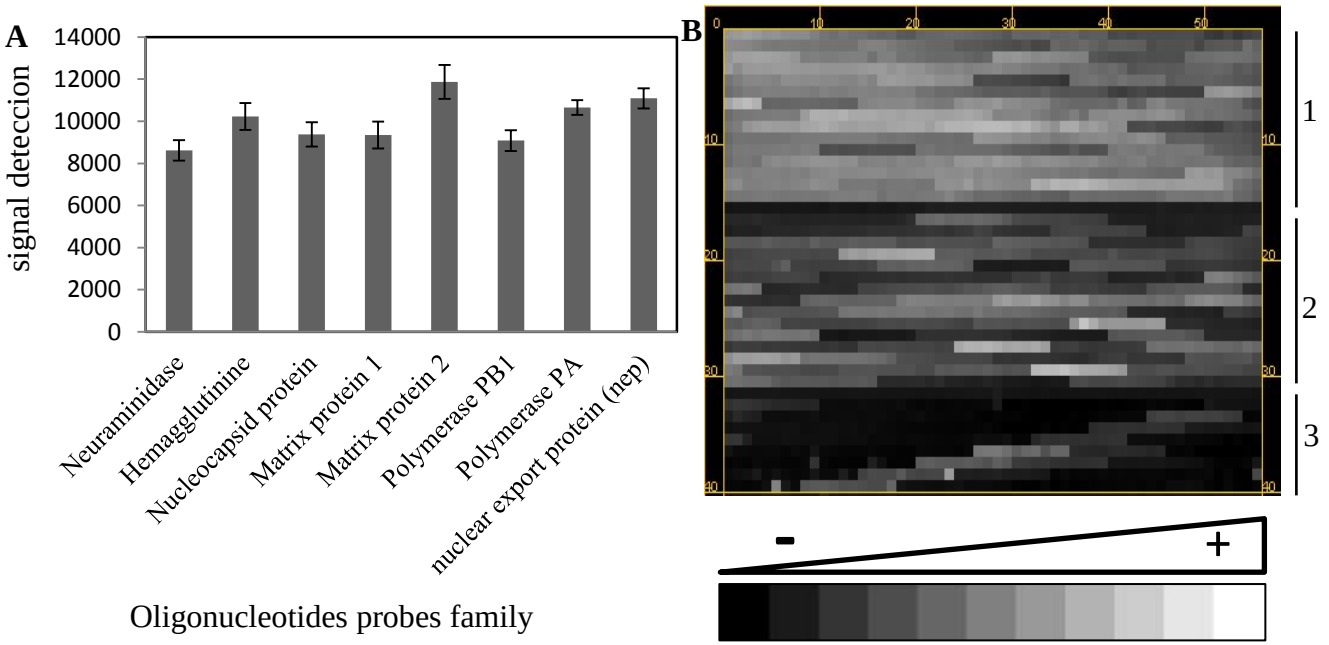


Figure 1. A)The average signal for probes oligonucleotides family and ± error standard after hybridization. **B**) Image of microarray of influenza:**1**) the position and intensity signal of 80 normal oligonucleotide probes, **2**) 80 oligonucleotides probes with one mutation and **3**) 32 oligonucleotides probes with one, two, three and four mutations in the sequences. All probes have 10 repetitions inside on arrays.

The sensitivity of the CombiMatrix influenza detection system was high and the specificity was 100%. Therefore, the prototype CombiMatrix influenza microarray system is an effective method for influenza subtype analysis.

Electrochemistry rapid as method for identification influenza viruses mutation by microarrays

3. Results:

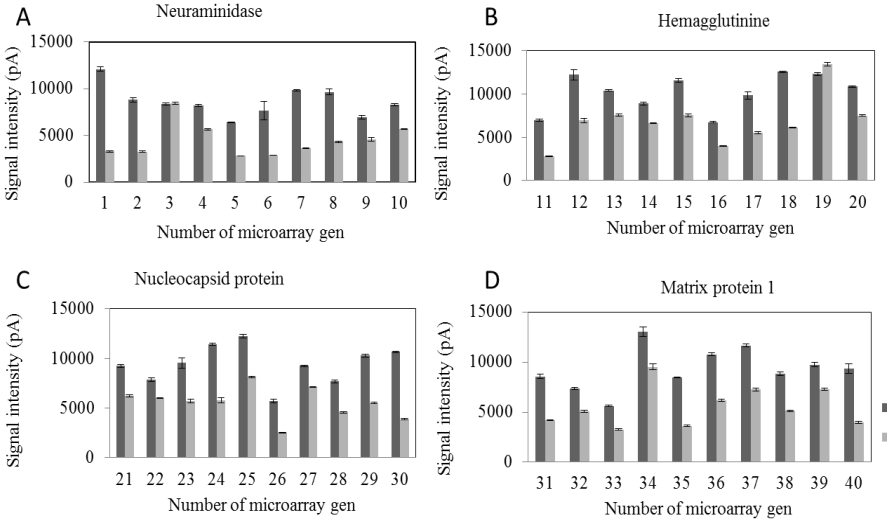


Figure 2. Signal after hybridization of oligonucleotide probes of influenza viruses without and/or one mutation in the sequences of probes. A) neuraminidase, B) hemagglutinine, C) nucleocapsid protein and D) matrix protein 1 genes.

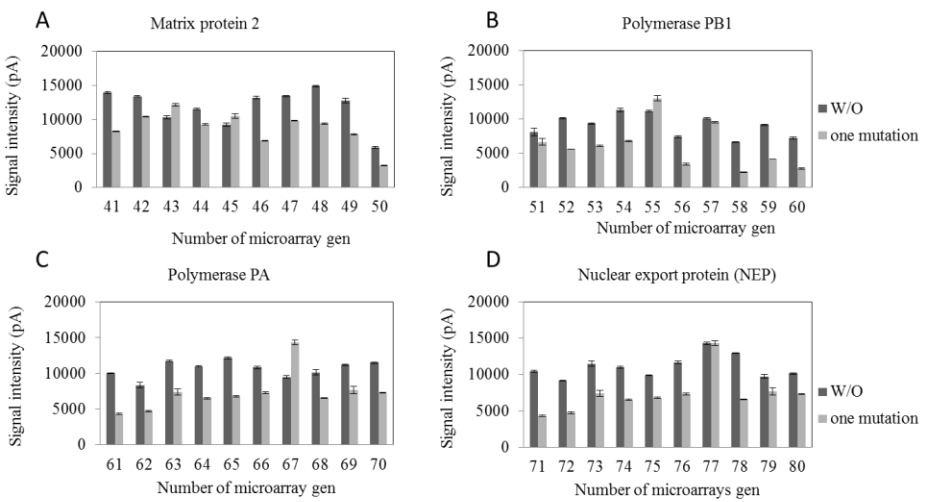
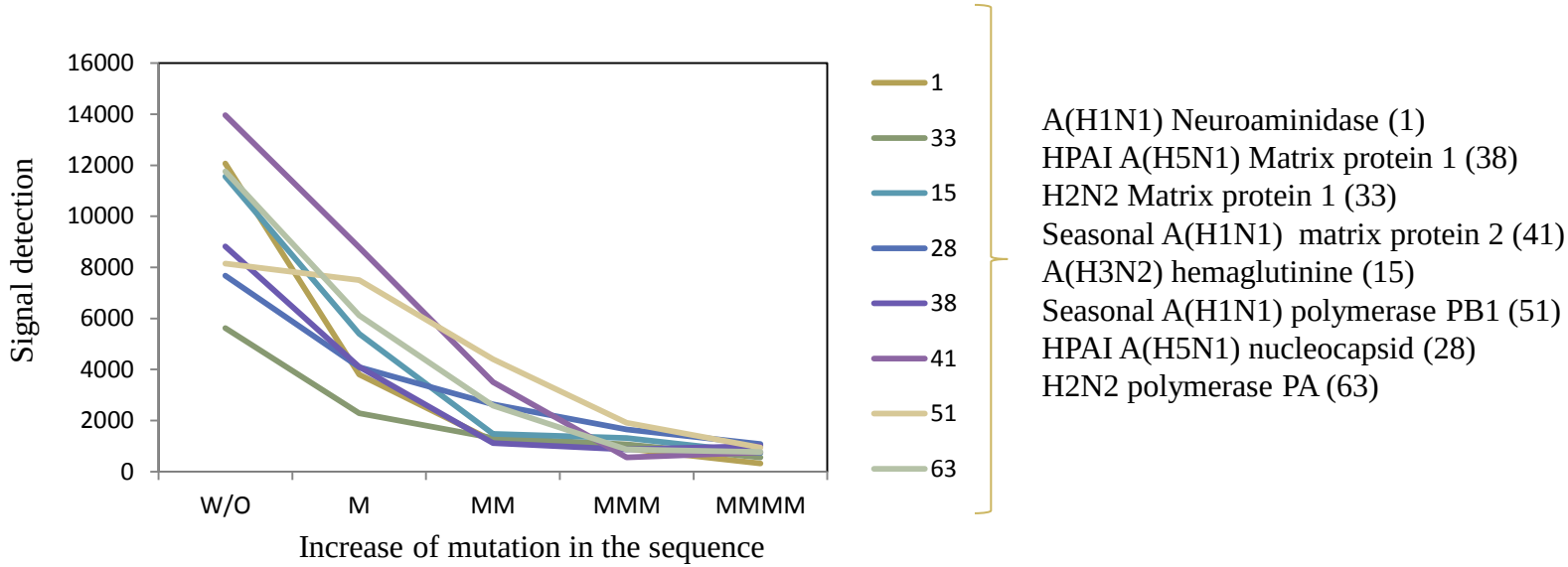


Figure 3. Signal after hybridization of oligonucleotide probes of influenza viruses without (W/O) and/or one mutation in the sequences of probes. A) matrix protein 2, B) polymerase PB1, C) Polymerase PA and D) nuclear export protein (NEP) genes

The results of ours study showed a general decline in the signal when the probes have one mutation in the sequence

Electrochemistry rapid as method for identification influenza viruses mutation by microarrays

3. Results:



The 8 different specific probes of different influenza virus with one, two, three and four mutations in the sequence within the microarray showed a decreased in the signal when increased the number of mutations in the sequences

Electrochemistry rapid as method for identification influenza viruses mutation by microarrays

4. Conclusion:

- The sensitivity of the CombiMatrix influenza detection system was high and the specificity was 100%. Therefore, the prototype CombiMatrix influenza microarray system is an effective method for influenza subtype analysis.

- Clear decreased of signal in electrochemical detection technology show that this novel electrochemical method can be used with high reliability for the detection of one mutation in the sequences of influenza virus.

- These results shown that the CombiMatrix ElectroSense™ influenza is novel method for identification mutations in the sequence of influenza and give us an approximation of the number of mutations suffered influenza virus, because influenza viruses display a high mutation rate and complex evolutionary patterns, inducing antiviral drug resistance.

evropský sociální fond v ČR
EVROPSKÁ UNIE
MINISTERSTVO ŠKOLSTVÍ, MLÁDEŽE A TĚLOVÝCHOVY
OP Vzdělávání pro konkurenceschopnost
INVESTICE DO ROZVOJE VZDĚLÁVÁNÍ

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