



INVESTICE DO ROZVOJE VZDĚLÁVÁNÍ

Electrochemistry rapid as method for identification influenza viruses mutation by microarrays

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Název projektu: Partnerská síť centra excelentního bionanotechnologického výzkumu



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

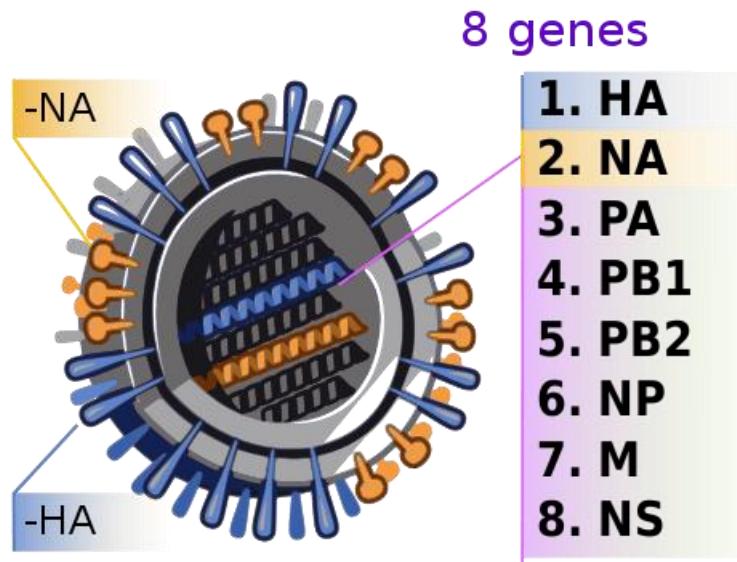
Electrochemistry rapid as method for identification influenza viruses mutation by microarrays

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:



Electrochemistry rapid as method for identification influenza viruses mutation by microarrays

1. Introduction:

The threat of an influenza pandemic virulent, highly transmissible has motivated an escalating research effort to identify the transmissible genotypes of avian influenza viruses that cross over into the human population (avian-human transmission) and sustain human-human transmission.



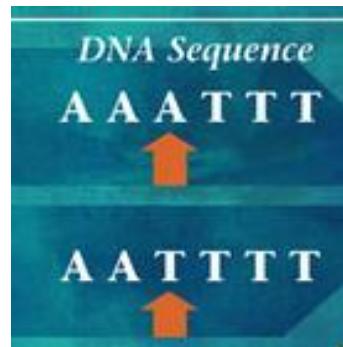
Electrochemistry rapid as method for identification influenza viruses mutation by microarrays

1. Introduction:

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.

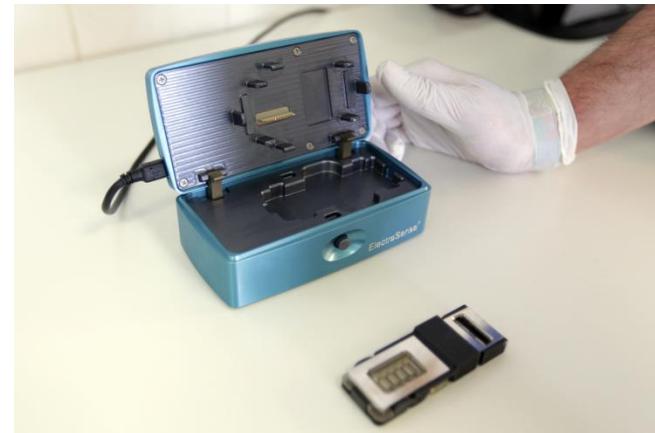


Electrochemistry rapid as method for identification influenza viruses mutation by microarrays

1. Introduction:

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

CombiMatrix ElectraSense™



**Electrochemistry rapid as method for identification influenza
viruses mutation by microarrays**

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	CW76797	5'-TCCTCATATGAAAATTGGC-3'
Pandemic A(H1N1)	neuraminidase	GQ77078	5'-TCCCTATGTGAAATAGCG-3'
HN2	neuraminidase	CY125896.1	5'-TAACAACTTCAGGGCTCTA-3'
HN8	neuraminidase	HMSB207.1	5'-TGGAACGGCAATTCGTT-3'
A(H3N2)	neuraminidase	CQ44665.1	5'-AACATCTTCTGGGAACTT-3'
HN2	neuraminidase	JQ433881.1	5'-CTTGTAACTTCGAAATGCG-3'
Seasonal A(H3N2)	neuraminidase	CY155327.1	5'-ACCGTTAAAGACGAACTC-3'
HN1 (A/H3N2)	neuraminidase	EU26982.1	5'-AGGGAAATGATGTTAATCG-3'
HN7	neuraminidase	AY340079.1	5'-CCAGAAATCTTGAACTGCT-3'
HN2	neuraminidase	NC_004609.1	5'-TACATGATAGAGGTGCCCA-3'
Seasonal A(H1N1)	hemagglutinin	JN017181.1	5'-AGAGAGATAAGTGGGTA-3'
Pandemic A(H1N1)	hemagglutinin	JN023498.1	5'-ACCCAAGACCTCACAGAAC-3'
HN2	hemagglutinin	CY125918.1	5'-TGTGTTGAACTACAATGCG-3'
HN8	hemagglutinin	HMSB205.1	5'-ATCACACTAAATCGAGGTC-3'
A(H3N2)	hemagglutinin	JN034665.1	5'-TGTGTTGAACTACAATGCG-3'
HN2	hemagglutinin	JQ433879.1	5'-GAGCTTAAAGACGAACTC-3'
Seasonal A(H3N2)	hemagglutinin	CY155327.1	5'-GGGAAATACGCCCTTATG-3'
HN1 (A/H3N2)	hemagglutinin	EU26981.1	5'-CTCCAGAAATAATGCAATCAGAA-3'
HN7	hemagglutinin	AY33459.1	5'-GAAGACGTTGACGGAAAC-3'
HN2	hemagglutinin	NC_004609.1	5'-GTAGTGCACATUTGACGAA-3'
Seasonal A(H1N1)	nucleoprotein	CW87927.1	5'-ATAGACGGAAATAGATGAG-3'
Pandemic A(H1N1)	nucleoprotein	JN023588.1	5'-GAGCAGCTTACGAAAGCG-3'
HN2	nucleoprotein	CY125897.1	5'-AGAGGTAAATGGAAAGCG-3'
HN8	nucleoprotein	HMSB206.1	5'-ATGGAGAAAGCGAAAC-3'
A(H3N2)	nucleoprotein	CY113842.0	5'-GATGAAATGGAAAGCGAA-3'
HN2	nucleoprotein	JQ433882.1	5'-GAGCTTAAAGACGAACTC-3'
Seasonal A(H3N2)	nucleoprotein	CY155328.1	5'-GCGAAATAGAGGAAAC-3'
HN1 (A/H3N2)	nucleoprotein	EU26985.1	5'-ACATTCAGAGAACGAGCG-3'
HN7	nucleoprotein	AY340425.1	5'-CTTAAATCGGATATAAGCG-3'
HN2	nucleoprotein	AF255743.1	5'-GGAAGGTTGACGAAAC-3'
Seasonal A(H1N1)	matrix protein	CW87925.1	5'-ATGATCAAGAAAGCTCAAAG-3'
Pandemic A(H1N1)	matrix protein	JN023590.1	5'-ATGAAACAGAGATGGCTCT-3'
HN2	matrix protein	CY125895.1	5'-TTAGAGGGAGATAACTTC-3'
HN8	matrix protein	HMSB208.1	5'-CTTGTCAAAAGTCCTTA-3'
A(H3N2)	matrix protein	CY113842.2	5'-ACCTTGTGAACTGGAAATG-3'
HN2	matrix protein	JQ433882.1	5'-GATGAAATGGAAAGCGAA-3'
Seasonal A(H3N2)	matrix protein	CY155326.1	5'-TCGTTGACCTTCGACTA-3'
HN1 (A/H3N2)	matrix protein	EU26984.1	5'-ACTTCACCGTAGACGTTT-3'
HN7	matrix protein	GU053111.1	5'-CCCCACTTAATAGCGATAA-3'
HN2	matrix protein	ADJ78464.1	5'-CAAGAAGCTTAAAGAGGAA-3'
Seasonal A(H1N1)	matrix protein2	CW87925.1	5'-TCCTTTAAATACGGTTGA-3'
Pandemic A(H1N1)	matrix protein2	JN023590.1	5'-TCATGGGATCTTGACCTC-3'
HN2	matrix protein2	CY125895.1	5'-AACACGGCTTAAAGAGCG-3'
HN8	matrix protein2	HMSB208.1	5'-ATCACTGGATCTTGACCTC-3'
A(H3N2)	matrix protein2	CY113838.2	5'-TAAACATGGAACTGGAAAGA-3'
HN2	matrix protein2	JQ433882.1	5'-GATGAAATGGAAAGCGAA-3'
Seasonal A(H3N2)	matrix protein2	CY155326.1	5'-TCGTTGACCTTCGACTA-3'
HN1 (A/H3N2)	matrix protein2	EU26984.1	5'-TCGTTGACCTTCGACTA-3'
HN7	matrix protein2	GU053111.1	5'-CCCCCTTAATACGGTTGA-3'
HN2	matrix protein2	ADJ78464.1	5'-CTTAAATCCATTTCGTC-3'
Seasonal A(H1N1)	polymerase PB1	CY125873.1	5'-AAGATGATGAGCCAACCTCCAA-3'
Pandemic A(H1N1)	polymerase PB1	HQ240702.2	5'-AGTGGAAAGAAATACATC-3'
HN2	polymerase PB1	CY125900.1	5'-TGGATGAAATCTTCCT-3'
HN8	polymerase PB1	HMSB203.1	5'-GATGAAAGGGAAATGAAAT-3'
A(H3N2)	polymerase PB1	CY112971.2	5'-TGTATCTTGGCCGAAAGAA-3'
HN2	polymerase PB1	JQ433882.1	5'-GATGAAATGGAAAGCGAA-3'
Seasonal A(H3N2)	polymerase PB1	FTF78019.1	5'-GATGAAATGGAAAGCGAA-3'
HN1 (A/H3N2)	polymerase PB1	EU26987.1	5'-AGGATTTGAACTTAAAGCT-3'
HN7	polymerase PB1	AY340083.1	5'-ATTAATCGGAAACACACAA-3'
HN2	polymerase PB1	NC_004911.1	5'-AGTAAGAGATAAAGCTCG-3'
Seasonal A(H1N1)	polymerase PA	CY125871.1	5'-AACACGACTATTCGACATA-3'
Pandemic A(H1N1)	polymerase PA	JQ433878.1	5'-ATTCATCTGAGAGGGAGTG-3'
HN2	polymerase PA	CY125923.1	5'-AGACATCTCTTGATGAGAA-3'
HN8	polymerase PA	HMSB204.1	5'-GAAAGCCAAACAGATAAAATC-3'
A(H3N2)	polymerase PA	CY113838.2	5'-GAGAACCCACTTACATATAT-3'
HN2	polymerase PA	JQ433882.1	5'-GATGAAATGGAAAGCGAA-3'
Seasonal A(H3N2)	polymerase PA	CY155330.1	5'-TGGATGAAAGCGAAAGCTT-3'
HN1 (A/H3N2)	polymerase PA	EU26986.1	5'-GGGAAACCTTTCACAAATG-3'
HN7	polymerase PA	AY340418.1	5'-ACACACACACCCCTCT-3'
HN2	polymerase PA	NC_004912.1	5'-CTGAAATTAAACATTAGAAC-3'
Seasonal A(H1N1)	nuclear export protein (nsp)	CY125870.1	5'-GATGAAAGAGAAATGGACACAA-3'
Pandemic A(H1N1)	nuclear export protein (nsp)	HQ240388.2	5'-TGTGTTAAATGAAAGAAACCG-3'
HN2	nuclear export protein (nsp)	CY125898.1	5'-ACCGGAAAATGGCGGAAACAA-3'
HN8	nuclear export protein (nsp)	HMSB209.1	5'-TGATTTGAGACTCGACATA-3'
A(H3N2)	nuclear export protein (nsp)	CY103987.1	5'-AGACAGTTAGGCTCAAAGAT-3'
HN2	nuclear export protein (nsp)	JQ433882.1	5'-TGTGTTGAACTTAAAGCGAA-3'
Seasonal A(H3N2)	nuclear export protein (nsp)	CY155330.1	5'-ACCTGTTCTCTGAAACAA-3'
HN1 (A/H3N2)	nuclear export protein (nsp)	CY155330.1	5'-GCTGTTAAATGAAAGCTGAA-3'
HN7	nuclear export protein (nsp)	GU053125.1	5'-AGATGACACATGGCTTAAAG-3'
HN2	nuclear export protein (nsp)	EY793288.1	5'-TGCTTBATTGAAAGAACTGCC-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:

Virus	Gene	GenBank accession number	Probes with one mutations
Seasonal A(H1N1)	neuraminidase	CY076796	5'-TCCTCATATAAAATTGGG-3'
Pandemic A(H1N1)	neuraminidase	QO377078	5'-TCCTCAAGTGATAATTACGG-3'
H2N2	neuraminidase	CY125961	5'-TAGCAATGCGAGGATCCTA-3'
H2N8	neuraminidase	HMS89207	5'-TGGATCAAAGGCAATGTT-3'
A(H3N2)	neuraminidase	GQ293082	5'-ACACATAAACGTCTCTGT-3'
HBN8	neuraminidase	JQ433881	5'-GTTGAAAAACAGTTCAATGG-3'
seasonal A(H5N1)	neuraminidase	CY083327	5'-ACCGTAAAACAGAACGCC-3'
HPAI A(H5N1)	neuraminidase	EU263982	5'-AGGAAAATGTTAAATCAG-3'
H7N7	neuraminidase	AY340079	5'-CCAGAAAATTCTGAAGACT-3'
H9N2	neuraminidase	NC_004908	5'-TACATGAAAGAGTCCCCAT-3'
Seasonal A(H1N1)	hemagglutinin	JN017181	5'-AGGAAAATTATGAGATGGGTA-3'
Pandemic A(H1N1)	hemagglutinin	JX625498	5'-ACCAAAACCTACGAAATC-3'
H2N2	hemagglutinin	CY138801	5'-ATGTTAAATGAAATGGG-3'
H2N8	hemagglutinin	HM589205	5'-ACACAAACAGAACAGGTGTC-3'
A(H3N2)	hemagglutinin	DY844665	5'-TTTGTGAAACGCCAACAG-3'
HBN8	hemagglutinin	JQ433879	5'-GACCTTAAACACTAAAGC-3'
seasonal A(H5N1)	hemagglutinin	CY083325	5'-GGGATAACCCACCTAA T-3'
HPAI A(H5N1)	hemagglutinin	EU263981	5'-CTCAAGAATGTCATAAA-3'
H7N7	hemagglutinin	AY338459	5'-GAAACGTGGAACGAAA-3'
H9N2	hemagglutinin	NC_004908	5'-TGTGTCGAAAGCAGCTG-3'
Seasonal A(H1N1)	neurocapsid protein	CY087027	5'-ATGACGAAAATGGATAGC-3'
Pandemic A(H1N1)	neurocapsid protein	JN265388	5'-GACGACTAACAGAATAGC-3'
H2N2	neurocapsid protein	CY125987	5'-AGAGAGAAATGGAAAGTGA-3'
H2N8	neurocapsid protein	HMS89206	5'-AATGGGAGAAACGAAAC-3'
A(H3N2)	neurocapsid protein	CY113840	5'-GATTATAAGGGCGG TTGA-3'
HBN8	neurocapsid protein	JQ433880	5'-CTTACGTCTTACAAACAAA-3'
seasonal A(H5N1)	neurocapsid protein	CY083324	5'-GCAATGAGGAAACGAGACC-3'
HPAI A(H5N1)	neurocapsid protein	EU263985	5'-CAATTCTGGATAAAGCAGC-3'
H7N7	neurocapsid protein	AY342425	5'-GGAATGTTGATCAGAAC-3'
H9N2	neurocapsid protein	AE235743	5'-GGAATGTTGATCAGAAC-3'
Seasonal A(H1N1)	matrix protein 1	CY087025	5'-ACTATACAAAAGCTTAAAAG-3'
Pandemic A(H1N1)	matrix protein 1	JN265390	5'-ATGAAAAGAATGGCTG-3'
H2N2	matrix protein 1	CY125985	5'-TTAAAGGGAGATAACATTG-3'
H2N8	matrix protein 1	HMS89208	5'-CTT TGTCAAAATGCCCTAA-3'
A(H3N2)	matrix protein 1	CY113838	5'-AACATGAAAAGATGCTG-3'
HBN8	matrix protein 1	JQ433882	5'-TACAGGAACCTAAAGGGAA-3'
seasonal A(H5N1)	matrix protein 1	CY083326	5'-TCTTGACCTTACTA A-3'
HPAI A(H5N1)	matrix protein 1	EU263984	5'-ACTGCAAGCTAACGTT T-3'
H7N7	matrix protein 1	GU053111	5'-CCCAACTAAAGGGATGAA-3'
H9N2	matrix protein 1	AE278646	5'-CAAGAAGGAAAGGGA AA-3'
Seasonal A(H1N1)	matrix protein 2	CY087024	5'-TGTGAAATGAAATGGG-3'
Pandemic A(H1N1)	matrix protein 2	JN265390	5'-TCAATTGGAAACTGCACTC-3'
H2N2	matrix protein 2	CY125985	5'-AACACGGCTCTGAAAAGGG-3'
H2N8	matrix protein 2	HMS89208	5'-ATCATTGGAACTTGCCTT-3'
A(H3N2)	matrix protein 2	CY113838	5'-TCAAAACACCGTIGAAAAA-3'
HBN8	matrix protein 2	JQ433882	5'-GCCCTTAAACCGGTGGA-3'
seasonal A(H5N1)	matrix protein 2	CY083326	5'-TATCATTTGGAACTTGACT-3'
HPAI A(H5N1)	matrix protein 2	EU263984	5'-TCCCTTAAACCGGTGAA-3'
H7N7	matrix protein 2	GU053111	5'-GCCCTTAAACCGGTGAA-3'
H9N2	matrix protein 2	AE278646	5'-CTTAAACGATTATCGTC-3'
Seasonal A(H1N1)	polymerase PB1	CY125673	5'-AAAATGACAACTCCCA-3'
Pandemic A(H1N1)	polymerase PB1	HQ240702	5'-AGTGGAAATGAAAACAATC-3'
H2N2	polymerase PB1	CY125981	5'-TGGAAATGAAATAAACCT-3'
H2N8	polymerase PB1	HMS89203	5'-GGGAAATGAAATGGG-3'
A(H3N2)	polymerase PB1	CY113971	5'-TAAATCTTGGGAAAAA AAAA-3'
HBN8	polymerase PB1	JQ433877	5'-GATGACTTAACTTAAAGAAC-3'
seasonal A(H5N1)	polymerase PB1	JF758819	5'-AGGATTGACACTGAAACTA-3'
HPAI A(H5N1)	polymerase PB1	EU263987	5'-AGAAAAGGAAACACAGAA-3'
H7N7	polymerase PB1	AY340083	5'-AAATCTGGAGAAACACCA-3'
H9N2	polymerase PB1	NC_004911	5'-AGAAAAGCATAAGGTCAC-3'
Seasonal A(H1N1)	polymerase PA	CY125671	5'-AAAACAGATTTACCAATA-3'
Pandemic A(H1N1)	polymerase PA	JQ433878	5'-ATCACTTGGAAAGGAGATGG-3'
H2N2	polymerase PA	CY125923	5'-AGAAATCTTGGATGGAA-3'
H2N8	polymerase PA	HMS89204	5'-GAAAGCCC AAAGATAAAATC-3'
A(H3N2)	polymerase PA	CY113858	5'-CAGAGCCATGAGTACAAAT-3'
HBN8	polymerase PA	JQ433878	5'-AGGGGAAAGCAATGAGA-3'
seasonal A(H5N1)	polymerase PA	CY083323	5'-TGTGAAATGAAACCTT GT-3'
HPAI A(H5N1)	polymerase PA	EU263986	5'-GGGAAACTCTTAAAGAAC-3'
H7N7	polymerase PA	AY342418	5'-AGAAAAGGAAACGGT-3'
H9N2	polymerase PA	NC_004912	5'-CTGAAATGAGCATGGAC-3'
Seasonal A(H1N1)	nuclear export protein	CY125670	5'-GTTGAAATGAGGAGACACA-3'
Pandemic A(H1N1)	nuclear export protein	HQ240288	5'-TTGTTAAATGAAATGCGG-3'
H2N2	nuclear export protein	CY125989	5'-ACGAAAAGGGCGAGAACAA-3'
H2N8	nuclear export protein	HMS89209	5'-TGATGAGGAAATCACACATA-3'
A(H3N2)	nuclear export protein	CY103967	5'-AGAACAGTGTAAAAGGT-3'
HBN8	nuclear export protein	JQ433883	5'-TGGCTGATTTAAAAGTGC-3'
seasonal A(H5N1)	nuclear export protein	CY083329	5'-ACCTTACCTCTTCCTAACAA-3'
HPAI A(H5N1)	nuclear export protein	CY098618	5'-CTCTGATTTGAAAAGTACGACA-3'
H7N7	nuclear export protein	GU053125	5'-AAGTCGACAAAGGTGAG-3'
H9N2	nuclear export protein	JF793288	5'-TGCTGATTTGAAAAGTGC-3'

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

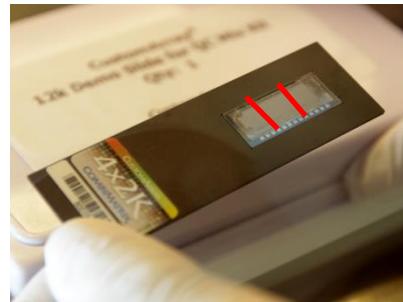


Table 2. Summary of sequences of the selected specific array probes with one mutation in the sequence and number assigned in the microarray.

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'-TCCTCATAATGAAAATGGG-3'	5'-ACTGCAGCGTAGACGTTT-3'
M	5'-TCCTCATAAT AAA ATGGG-3'	5'-ACTGCAGCGTA A ACGTTT-3'
MM	5'-TCCTCATAAA AAA ATGGG-3'	5'-ACTGCAGCG AA ACGTTT-3'
MMM	5'-TCCTCATAAA AAA AAAATGGG-3'	5'-ACTGCAGCG AA AA G TTT-3'
MMMM	5'-TCCTCATAAA AAA AAAAGGG-3'	5'-ACTGCAGCG AA AA AA TTT-3'
MMMMM	5'-TCCTCA AAA AAAAGGG-3'	5'-ACTGCAGC AAA AA AA TTT-3'
	H2N2 Matrix protein 1	Seasonal A(H1N1) Matrix protein 2
	Sequence of gen 33	Sequence of gen 41
W/O	5'-TTAACGGGGAGATAACATTG-3'	5'-TCGCTTAAATACGGTTGAA-3'
M	5'-TTAA A GGGGAGATAACATTG-3'	5'-TCGCTTAA A ACGGTTGAA-3'
MM	5'-TTAA A GGGGAGA A ACATTG-3'	5'-TCGCTTAA A AA G TTTGA-3'
MMM	5'-TTAA A GGGGAGA A AA A TTG-3'	5'-TCGCTTAA A AA AA GGTTGAA-3'
MMMM	5'-TTAA A GGGGAA A AA A ATTG-3'	5'-TCGCTTAA A AA AA GGTTGAA-3'
MMMMM	5'-TTAA A AGAGA A AA A AA A ATTG-3'	5'-TCGCTTAA A AA AA AA AA TTTGA-3'
	A(H3N2) Hemagglutinin	Seasonal A(H1N1) polymerase PB1
	Sequence of gen 15	Sequence of gen 51
W/O	5'-TTTGTTGAAACCGAGCAAAG-3'	5'-AAGATGATGCCAACTCCCA-3'
M	5'-TTTGTTGAA A CCAGCAAAG-3'	5'-AA A TGATGCCAACTCCCA-3'
MM	5'-TTTGTTGAA A ACAGCAAAG-3'	5'-AA AA AGATGCCAACTCCCA-3'
MMM	5'-TTTGTTGAA A ACAGCAA A -3'	5'-AA AA A ATGCCAACTCCCA-3'
MMMM	5'-TTTGTTGAA A ACAGCAA A -3'	5'-AA AA AA AGACCAACTCCCA-3'
MMMMM	5'-TTTGTTGAA A ACAGCAA A -3'	5'-AA AA AA A ACCAACTCCCA-3'
	HPAI A(H5N1) nucleocapsid	H2N2 polymerase PA
	Sequence of gen 28	Sequence of gen 63
W/O	5'-ACATATCAGAGAACGAGAGC-3'	5'-AGAACATTC T TGGATGGAA-3'
M	5'-ACATATCAGAA A ACGAGAGC-3'	5'-AGAA A ATTC T TGGATGGAA-3'
MM	5'-AAATATCAGAA A ACGAGAGC-3'	5'-AAA AA ATTC T TGGATGGAA-3'
MMM	5'-AAA A ATCAGAA A ACGAGAGC-3'	5'-A AAA AA A CTTGGATGGAA-3'
MMMM	5'-AAA A AA C AGAA A ACGAGAGC-3'	5'-A AAA AA A ATCTTGGATGGAA-3'
MMMMM	5'-AAA A AA A AGAA A ACGAGAGC-3'	5'-A AAA AA A ATCTTGGATA AAA -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) Hemagglutinin (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

Virus	Gene	GenBank accession number	Sequence
Seasonal A(H1N1)	neuraminidase	CV076797	5'-TCCTCATTAATGCAAAAATTGGCG-3'
Pandemic A(H1N1)	neuraminidase	GQ277078	5'-TCCTCATTAATGCAAAAATTGGCG-3'
HN2	neuraminidase	HM582208.1	5'-TACCAAATTCGAAAGGAAATCTA-3'
HN3	neuraminidase	HM582207.1	5'-TACCAAATTCGAAAGGAAATCTA-3'
A(H3N2)	neuraminidase	GQ291082	5'-ACACATAAACCTTCCTTGCG-3'
HN5	neuraminidase	JQ433811.1	5'-GTGAAATCACTGTTCAATAGG-3'
Seasonal A(H3N2)	neuraminidase	CV053271.1	5'-ACCTTAAAGACAGAACG-C-3'
HN1	neuraminidase	BZU69821.1	5'-ACGGAAAGTGAAGTAAATCAG-3'
HN7	neuraminidase	AY340079.1	5'-CCAGAACATTTGGAGACGCT-3'
HN2	neuraminidase	NC_004909.1	5'-TACATCTAGAGGAGTCCCAG-3'
Seasonal A(H1N1)	hemagglutinin	JN017181.1	5'-AGCAAAAGAAATAGCTGGGTAA-3'
Pandemic A(H1N1)	hemagglutinin	JN025498.1	5'-ACCCAAAGCTCACGCCAAATC-3'
HN2	hemagglutinin	CY125918.1	5'-TGTTGAGCATACATGCTG-3'
HN8	hemagglutinin	HM582051	5'-ATCAACACTAAATCAGAAGCTG-3'
A(H3N2)	hemagglutinin	JX844651	5'-TTTTTGTGACCCACAAAGG-3'
HN8	hemagglutinin	JQ433879.1	5'-GACCTTAAAGGACCTAAAGC-3'
Seasonal A(H3N1)	hemagglutinin	CV053252.1	5'-GGGATACACCACCTTAT-3'
HN1	hemagglutinin	BZU69811.1	5'-CTCCAGAAATAGCTATAACAA-3'
HN7	hemagglutinin	AY338459.1	5'-GAAACGTTGAAACGAAACAA-3'
HN2	hemagglutinin	NC_004908.1	5'-CTAGTCGAAATGTCAGACCTAA-3'
Seasonal A(H1N1)	nucleoprotein	CW083027.1	5'-ATAGACCGAAATGATGAG-3'
Pandemic A(H1N1)	nucleoprotein	JN025388.1	5'-GACCACTAATCAGAATGCC-3'
HN2	nucleoprotein	CY125917.1	5'-AQAQAACTAAATGAGATGGA-3'
HN8	nucleoprotein	HM582061	5'-AATGAGAAAGGAAACCA-3'
A(H3N2)	nucleoprotein	CT113840.2	5'-GATAATGAAAGGCGGTGAT-3'
HN8	nucleoprotein	JQ433880.1	5'-CTTCAGATTAATCAAGACCAA-3'
Seasonal A(H3N1)	nucleoprotein	CV053232.1	5'-CCGAAATAATGAGAAAGC-3'
HN1	nucleoprotein	BZU69831.1	5'-ACAATACAGAAAGAAATGC-3'
HN7	nucleoprotein	AY342421.1	5'-CTAAATTGGGATATAAACCG-3'
HN2	nucleoprotein	NC_004911.1	5'-GCGGATACGGAAAGACG-3'
Seasonal A(H1N1)	matrix protein 1	CY083023.1	5'-ACTATCAAGAAAGAAAG-3'
Pandemic A(H1N1)	matrix protein 1	JN025390.1	5'-ATGAAACAGAAATGCTCTG-3'
HN2	matrix protein 1	CT125895.1	5'-TDAAGAGCGGAGTAACTTC-3'
HN8	matrix protein 1	HM582081	5'-CTTTCCTCAAAAGCTGCTTA-3'
A(H3N2)	matrix protein 1	CT113838.2	5'-AACATGTGAAAGATGCTG-3'
HN8	matrix protein 1	JQ433882.1	5'-TACAGGAACTCTTAAAGGG-3'
Seasonal A(H3N1)	matrix protein 1	CV053234.1	5'-TCCTGTACCCCTGACTA-A-3'
HN1	matrix protein 1	BZU69841.1	5'-ACTGCACTGTAAGCTGTTT-3'
HN7	matrix protein 1	GU053111.1	5'-CCCACTTAATCAGGATGAA-3'
HN2	matrix protein 1	AD_78646.1	5'-CAAAAGCTGAAAGGGAAA-3'
Seasonal A(H1N1)	matrix protein 2	CY083025.1	5'-TCCTTTAAAATCGGTTGAA-3'
Pandemic A(H1N1)	matrix protein 2	JN025390.1	5'-TCATTGGGATCTTGCAACCT-3'
HN2	matrix protein 2	CY125895.1	5'-AACACCGTGAAGAAAGGG-3'
HN8	matrix protein 2	HM582081	5'-ATCACTGGGATTCCTACT-3'
A(H3N2)	matrix protein 2	CT113838.2	5'-TCTAAAACCGGTCTGAAAGA-3'
HN8	matrix protein 2	JQ433882.1	5'-CCCTTAATAACCGTTGAA-3'
Seasonal A(H3N1)	matrix protein 2	CV053261.1	5'-TATCAATGGGATCTTCCTACT-3'
HN1	matrix protein 2	BZU69841.1	5'-TCCTCTTAATAACGGTTGAA-3'
HN7	matrix protein 2	GU053111.1	5'-CCCTTAAATACGGGTTGAA-3'
HN2	matrix protein 2	AD_78646.1	5'-CTTAACTGGATTATTCGTC-3'
Seasonal A(H1N1)	polymerase PB1	CT125873.1	5'-AAAGATGATGACCAACCTCCA-3'
Pandemic A(H1N1)	polymerase PB1	HQ240702.2	5'-ACTGGAATGAAAATCAACATC-3'
HN2	polymerase PB1	JN025001.1	5'-TGGATGAGAAATCAAAATCT-3'
HN8	polymerase PB1	HM582031	5'-CGATAAAAGAGAAATGAAAT-3'
A(H3N2)	polymerase PB1	CT113871	5'-TGAATCTTGGAAAGAAAGAA-3'
HN8	polymerase PB1	JQ433877.1	5'-GATGTTAACTGAAATGCT-3'
Seasonal A(H3N1)	polymerase PB1	CV053277.1	5'-GATGTTAACTGAAATGCT-3'
HN1	polymerase PB1	BZU69871.1	5'-AGAAAATGCTGAAACAAAGA-3'
HN7	polymerase PB1	AY340083.1	5'-AATTCCTGGAGAACACCA-3'
HN2	polymerase PB1	NC_004911.1	5'-ACTAGACGAACTGAACTGCG-3'
Seasonal A(H1N1)	polymerase PA	CT125871.1	5'-AAACGAGACTCTTCACATA-3'
Pandemic A(H1N1)	polymerase PA	JQ433878.1	5'-ATTCACTGGAAAGGAAATGG-3'
HN2	polymerase PA	CY125923.1	5'-AGAACATCTCTTGGATGGAA-3'
HN8	polymerase PA	HM582041	5'-GAAACCCAAAGAACGATCAAATC-3'
A(H3N2)	polymerase PA	CT113852	5'-CAGACCCACTGAGTACATAT-3'
HN8	polymerase PA	JQ433878.1	5'-AOCCGAAGAACAAATGGAAG-3'
Seasonal A(H3N1)	polymerase PA	CV053330.1	5'-TGCATTTAAGGGCAACCTT-3'
HN1	polymerase PA	BZU69861.1	5'-GCGCAAGCTTCAAAATGTF-3'
HN7	polymerase PA	AY342418.1	5'-ACAAACCAACGCCCTC-3'
HN2	polymerase PA	NC_004912.1	5'-CTGAAATTAAACCTATGAGAC-3'
Seasonal A(H1N1)	nuclear export protein (nep)	CY125670.1	5'-ATTTAGAAAGATGACACAA-3'
Pandemic A(H1N1)	nuclear export protein (nep)	HQ240288.2	5'-TGTTAAATGGAGAAATCGCG-3'
HN2	nuclear export protein (nep)	JN025891.1	5'-ACCGAAAATGCGGACACAA-3'
HN8	nuclear export protein (nep)	HM582091.1	5'-TATTGAGGAACTAACGACATA-3'
A(H3N2)	nuclear export protein (nep)	CT103671	5'-AGAACATGGTAACTGAAATG-3'
HN8	nuclear export protein (nep)	JQ433883.1	5'-TGCTGATTTGAAAGAATGCG-3'
Seasonal A(H3N1)	nuclear export protein (nep)	CV053229.1	5'-ACCTCCACTGCTCAACAA-3'
HN1	nuclear export protein (nep)	CY098818.1	5'-OCTGATTGAAAGAATGCGAA-3'
HN7	nuclear export protein (nep)	GU053125.1	5'-AAGTGGCACATGAGTGAAG-3'
HN2	nuclear export protein (nep)	JF793288.1	5'-TGCGTATTGAAAGAATGCG-3'

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



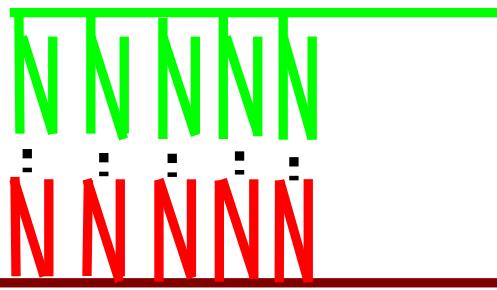
Mix Oligonucleotides 3" biotin complementary sequences of Influenza viruses

Electrochemistry rapid as method for identification influenza viruses mutation by microarrays

2. Material and method:



Surface of chip



Oligonucleotides probes of influenza viruses inside of the chip



Mix Oligonucleotides 3" biotin complementary sequences of Influenza viruses



B

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

**Electrochemistry rapid as method for identification influenza
viruses mutation by microarrays**

Results

Electrochemistry rapid as method for identification influenza viruses mutation by microarrays

3. Results:

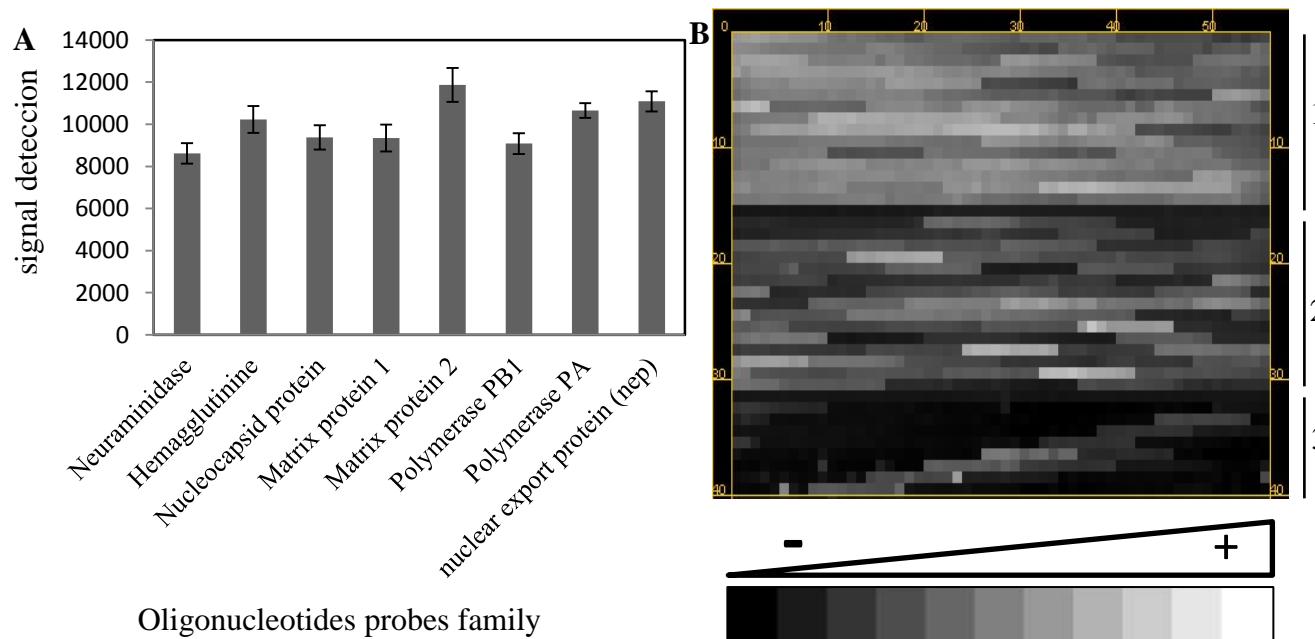
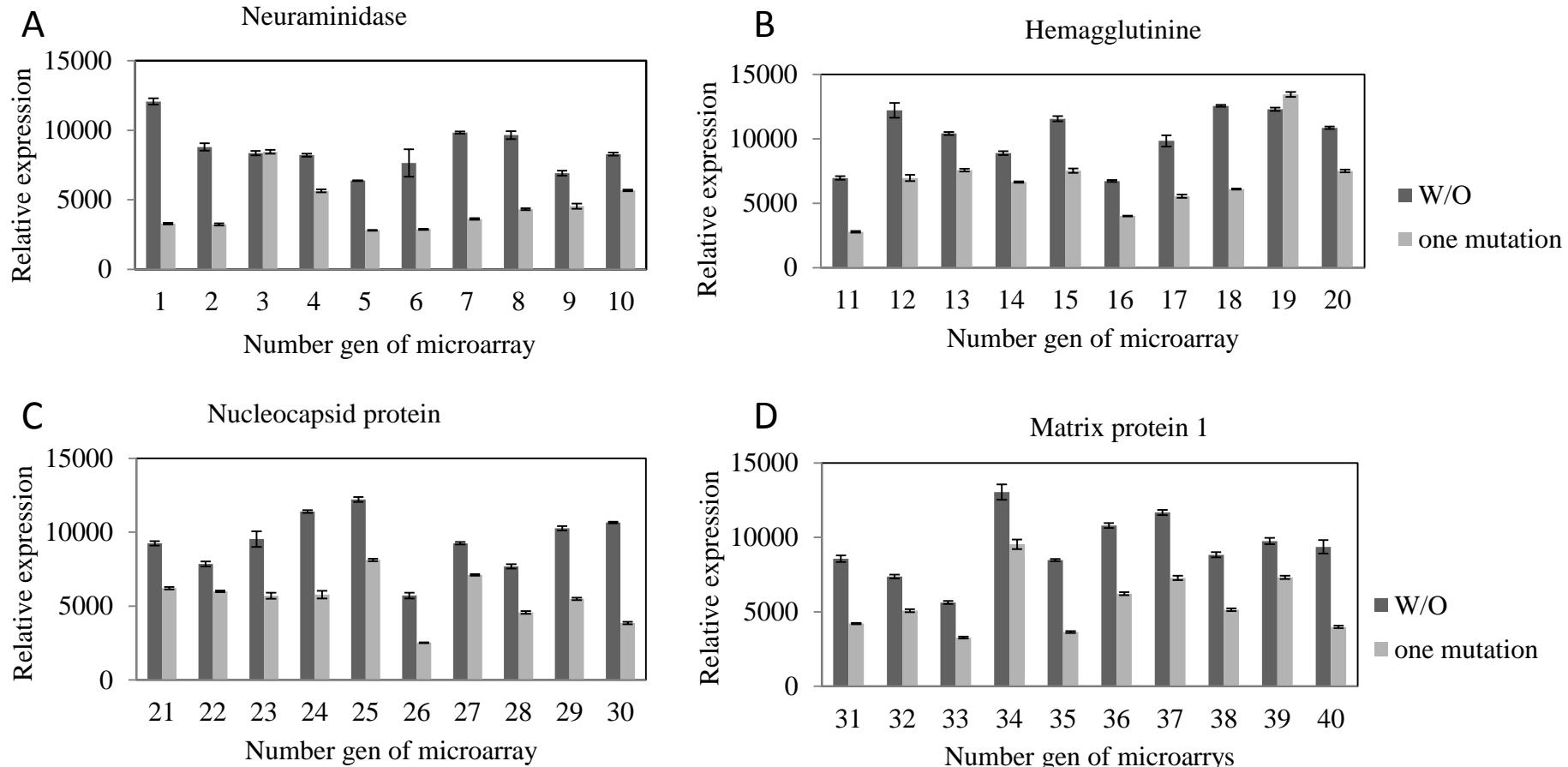


Figure. The average signal for probes oligonucleotides family and \pm error standard after hybridization. B) Image of microarray of influenza: 1) the position and intensity signal of 80 normal oligonucleotide probes, 2) 80 oligonucleotides probes with one mutation and 3) 32 oligonucleotides probes with 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

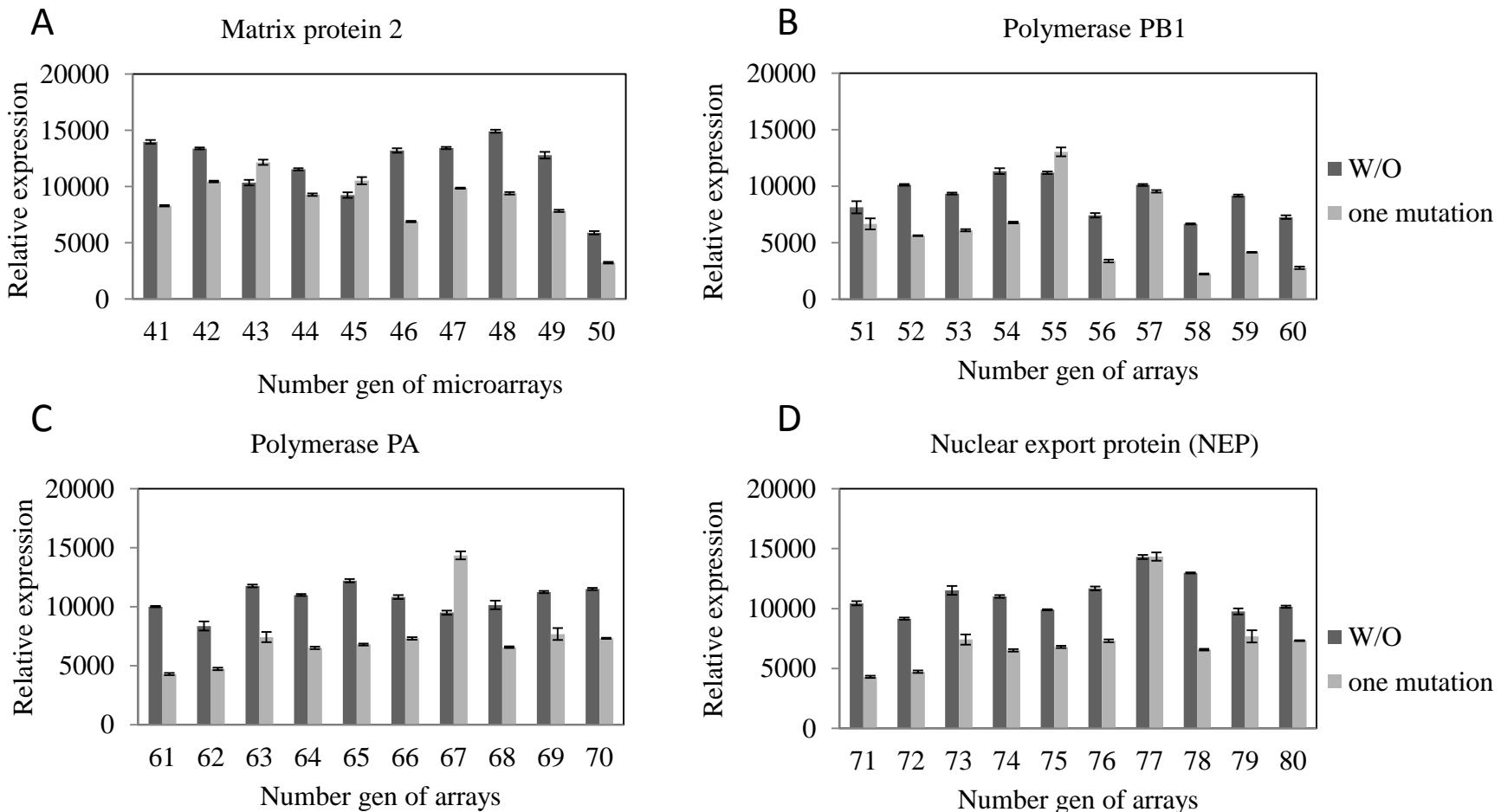
3. Results:



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

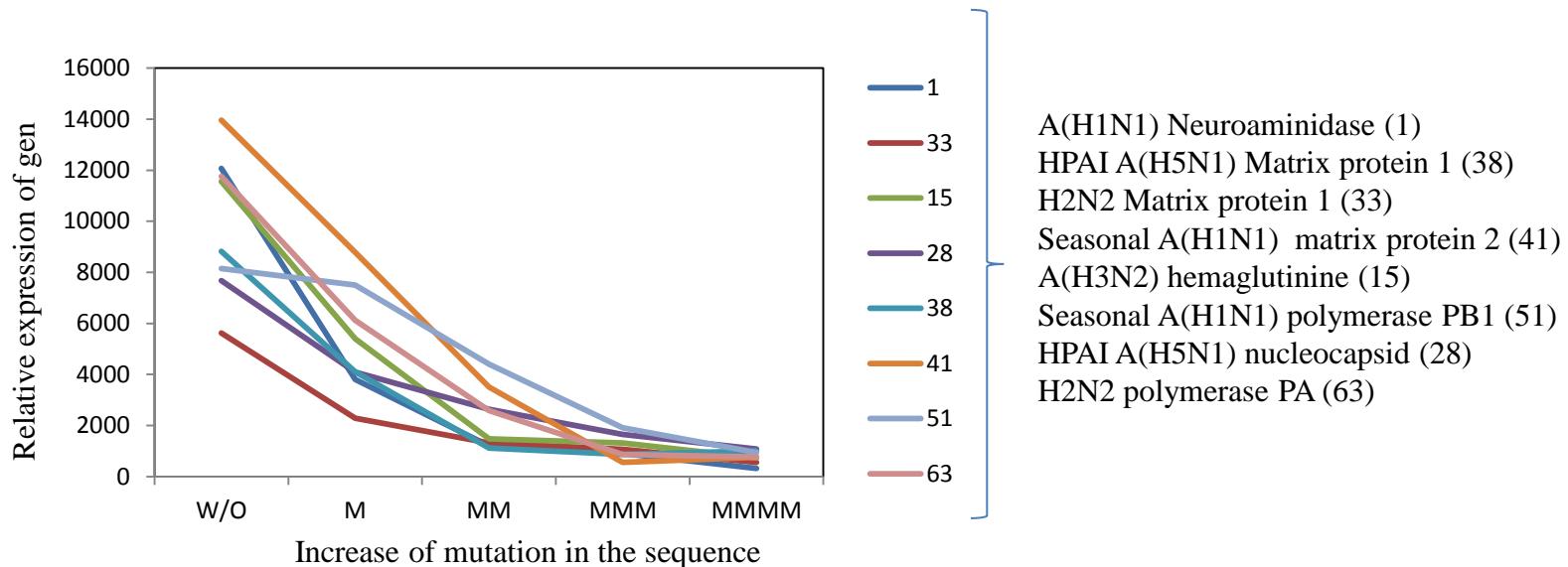
3. Results:



The 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.



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MINISTERSTVO ŠKOLSTVÍ,
MLÁDEŽE A TĚLOVÝCHOVY



OP Vzdělávání
pro konkurenční schopnost

INVESTICE DO ROZVOJE VZDĚLÁVÁNÍ

Thank you for attention

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

Název projektu: Partnerská síť centra excelentního bionanotechnologického výzkumu

